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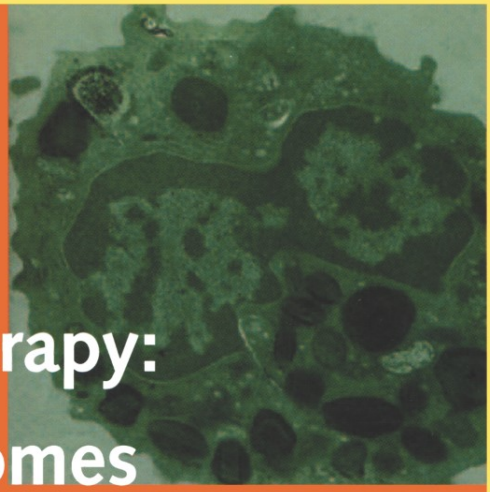

Antirheumatic Therapy: Actions and Outcomes

Richard O. Day
Daniel E. Furst
Piet L.C.M. van Riel
Barry Bresnihan

Editors

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Editors

Richard O. Day
Department of Clinical Pharmacology and Toxicology
St. Vincent's Hospital Sydney
Victoria Street
Darlinghurst, Sydney, NSW 2010
Australia

Piet L.C.M. van Riel
University Hospital Nijmegen
546 Dept. of Rheumatology
P.O. Box 9101
6500 HB Nijmegen
The Netherlands

Daniel E. Furst
Carl M. Pearson Professor of Rheumatology
University of California at Los Angeles
1000 Veteran Ave Rm 32–59
Los Angeles, CA 90025-1670
USA

Barry Bresnihan
Department of Rheumatology
St Vicent's University Hospital
Elm Park
Dublin 4
Ireland

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Contents

List of contributors

Kristin Bird, Medical University of South Carolina, Division of Rheumatology, Suite 912, 96 Jonathan Lucas St, Charleston, SC 29425, USA; e-mail: bird@musc.edu

Beau Bothwell, University of California at Los Angeles, The David Geffen School of Medicine, Division of Rheumatology, 1000 Veteran Avenue, Room 32-46, Los Angeles, CA 90025-1670, USA

Barry Bresnihan, Department of Rheumatology, St Vincent's University Hospital, Elm Park, Dublin 4, Ireland; e-mail: b.bresnihan@svcpc.ie

Ernest Choy, Sir Alfred Baring Garrod Clinical Trials Unit, Academic Department of Rheumatology, King's College, London, UK; e-mail: ernest.choy@kcl.ac.uk

Ben A.C. Dijkmans, Jan van Breemen Institute, Dr Jan van Breemenstraat 2, 1056 AB Amsterdam, The Netherlands, and Department of Rheumatology, Vrije Universiteit Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands: e-mail: BAC.Dijkmans@vumc.nl

Daniel E. Furst, Carl M. Pearson Professor of Rheumatology, University of California at Los Angeles, 1000 Veteran Avenue, Room 32-59, Los Angeles, CA 90025-1670, USA; e-mail: defurst@mednet.ucla.edu

Garry G. Graham, Department of Physiology and Pharmacology, School of Medical Sciences, University of New South Wales, Sydney NSW 2052, Australia; e-mail: g.graham@unsw.edu.au

Cees J. Haagsma, Twenteborg Hospital, PO Box 7600, 7600 SZ Almelo, The Netherlands; e-mail: c.haagsma@zgt.nl

Boulos Haraoui, CHUM, Hôpital Notre-Dame, 1560 Sherbrooke est, Montréal, Québec H2L 4M1, Canada

Alberta Hoi, Centre for Inflammatory Disease, Monash University, Monash Medical Centre, Melbourne, Australia; e-mail: alberta.hoi@med.monash.edu.au

David Kane, School of Clinical Medical Sciences (Rheumatology), 4th Floor, Cookson Building, University of Newcastle, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK; e-mail: d.j.kane@ncl.ac.uk

Arthur Kavanaugh, Division of Rheumatology, Allergy and Immunology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0943, USA; e-mail: Akavanaugh@ucsd.edu

Edward C. Keystone, The Rebecca MacDonald Centre for Arthritis and Autoimmune Disease, The Joseph Wolf Lebovic Building, Mount Sinai Hospital, 60 Murray Str., 2nd Floor, Toronto, Ontario, Canada M5G 1X5;
e-mail: edkeystone@mtsinai.on.ca

John R. Kirwan, Academic Rheumatology Unit, Bristol Royal Infirmary, Lower Maudlin Street, Bristol, BS2 8HW, UK; e-mail: john.kirwan@bristol.ac.uk

Geoffrey Littlejohn, Department Rheumatology, Level 3, Block E, Monash Medical Centre, 246 Clayton Rd, Clayton, Melbourne, Victoria, Australia, 3168;
e-mail: geoff.littlejohn@med.monash.edu.au

Andreas Maetzel, Division of Clinical Decision Making, Department of Health Policy, Management and Evaluation, University Health Network, University of Toronto, 200 Elizabeth Street EN6-232A, Toronto, Ontario, Canada M5G 2C4;
e-mail: andreas.maetzel@utoronto.ca

James R. O'Dell, Section of Rheumatology and Immunology, Department of Internal Medicine, Internal Medicine Residency Training Program, 983025 University of Nebraska Medical Center, Omaha, NE 68198-3025, USA

Harold E. Paulus, UCLA School of Medicine, Division of Rheumatology, Los Angeles, CA 90095, USA

Mark G. Perry, Academic Rheumatology Unit, Bristol Royal Infirmary, Lower Maudlin Street, Bristol, BS2 8HW, UK; e-mail: mark.perry@bristol.ac.uk

Kevin Pile, James Cook University Department of Medicine, Bag 8, Institute of Medicine, Townsville Hospital, PO Box 670, Townsville, Queensland 4814, Australia, e-mail: kevin.pile@jcu.edu.au

Rolf Rau, Ev. Fachkrankenhaus Ratingen, Rosenstr. 2, 40882 Ratingen, Germany; e-mail: rrau@uni-duesseldorf.de

Maria E. Suarez-Almazor, Baylor College of Medicine, Houston Center for Quality of Care and Utilization Studes, Michael E. DeBakey VA Medical Center, 2002 Holcombe (152), Houston, TX 77030, USA; e-mail: mes@bcm.tmc.edu

Daniel H. Solomon, Division of Pharmacoepidemiology and Pharmacoeconomics (DHS), Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital, Harvard Medical School, 1620 Tremont Street Suite 3030, Boston, MA 02120, USA; e-mail: dhsolomon@partners.org

Zuhre Tutuncu, Division of Rheumatology, Allergy and Immunology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0943, USA; e-mail: ztutuncu@ucsd.edu

Alan Tyndall, Department of Rheumatology, Felix-Platter Spital, 4012 Basel, Switzerland; e-mail: alan.tyndall@fps-basel.ch

Arno W.R. van Kuijk, Deaprtment of Clinical Immunology and Rheumatology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, and Jan van Breemen Institute, Dr Jan van Breemenstraat 2, 1056 AB Amsterdam, The Netherlands; e-mail: a.w.vankuijk@amc.uva.nl

Jacob M. van Laar, Department of Rheumatology, Leiden University Medical Center, POB 9600, 2300 RC Leiden, The Netherlands; e-mail: j.m.van_laar@lumc.nl

Preface

Our goal for this book is to examine the contemporary therapy of rheumatoid arthritis (RA) from the increasingly important perspective of impact upon quality of life, costs and long-term health outcomes. For too long the focus has been on short-term, symptomatic, and surrogate indicator outcomes. Yet RA is a life-long disorder with the majority of impact on an individual patient many years following onset. Further, even in the short-term, researchers and rheumatologists have tended to emphasize measurements of disease activity such as joint counts, ESR and physician's opinion as to the amount of disease activity present. It is only relatively recently that measures of structural damage, quality of life and impact on broad domains of health have been given increasing emphasis. Also, the significance of early treatment of RA in order to optimise long-term outcomes has a relatively short history [1]. We have been focussed on the disease processes as surrogates for long-term outcomes. Until the short-term process measures are validated as surrogates of long-term effects we should also turn our attention to outcomes of disease and the impact of our management on those outcomes [2].

In our view, this book is especially timely. We are at the dawn of a revolution in the management of RA and other complex immunological inflammatory disorders because their molecular, genetic and environmental mechanisms are being unravelled. In the process, we are revealing a substantial number of novel and significant targets for pharmacotherapy. The outstanding success of tumour necrosis factor (TNF) inhibition is an early example of the power of precise targeting of relevant mechanisms [3]. Yet, the increasing numbers of therapeutic options available to clinicians and patients poses a dilemma – these products of the molecular and genetic revolution are presently extremely expensive and powerful. It is now more urgent than ever to understand where these new therapies "fit" into the therapeutic armamentarium. It is even more important to understand which patients are most likely to benefit from aggressive therapy or specific therapies [4–6]. Linked to this need is the increasing demand to know what therapies deliver in terms of value to the patient, both short and long term. Payers such as Health Maintenance Organisa-

tions (HMOs) and government agencies are particularly interested in "incremental cost benefits". In other words, what "extra" does this medicine offer and what is the "increment" in outcomes "worth"? Thus, the science of pharmacoeconomics is developing rapidly in sophistication and also significance, at multiple levels.

Payers, including individual sufferers, are less likely to want to purchase therapies that do not deliver health benefits to them as individuals and organisations. Herein lies a dilemma. Organisations buying health services on behalf of patients necessarily must rely on controlled trials with strict entry and exclusion criteria in order to assess the "value" of an intervention. This model is sometimes difficult to manage and defend as RA has a wide range of manifestations, severities, and time-courses between individuals. However, an important trend that is increasingly likely to impact on the treatment of RA is our ability to identify the mechanisms of RA in individuals and match those against the mechanisms of action and pharmacokinetics of the antirheumatic treatments we have and are developing. As "targetting" becomes more prevalent, physicians and rheumatologists will need to have greater skills and knowledge about disease and drug mechanisms.

A further need for the modern rheumatologist is the increasing prevalence of co-morbidities in our patients. For example, the revelation that uncontrolled inflammation of RA is an independent cardiovascular risk factor behoves rheumatologists to assess cardiovascular risks in their patients and to ensure that hypertension, diabetes, hyperlipidaemia, obesity and smoking are being dealt with and that inflammation is controlled [7]. A consequence is that polypharmacy is an increasing issue facing rheumatologists and our patients as medicines are being used more widely in primary and secondary prevention. An immediate concern relates to drug interactions and adverse reactions, requiring additional knowledge about a wide range of medicines we use.

We hope that this book will assist rheumatologists, physicians and researchers gain important insights into the modern treatment of RA. Chapters have been organised to address mechanisms of action briefly emphasizing the strength of evidence that the mechanisms actually apply in humans *in vivo*. The applicable clinical pharmacology of value to the practising physician is presented and, where appropriate, linked to the probability of achieving acceptable efficacy and tolerability. Efficacy data presented focuses most on structural (imaging), functional and quality of life measures. Authors were asked to rely most on recent, long-term studies and high quality economic studies where available.

Toxicity concerns are increasing in the light of the experience with biologics and other highly effective treatments now available to treat RA. Can comprehensive data-bases assist in establishing the true toxicity profiles of potent new agents such as the biologics? [8]. Particular attention has been paid to ranking drugs and their toxic effects to assist the clinician in their selection of treatment options [9].

A perennial need is for high quality, evidence-based, authoritative and contemporary guidelines for monitoring RA therapies and where available, these are high-

lighted. Increasingly, it is difficult to know which treatments to select in individual patients and, notably, which combinations might be optimal at the various stages of the patient's illness [10]. This matter is addressed and where possible, is based on high quality clinical trial evidence. We hope that this book will truly add a different perspective to the use of antirheumatic therapy.

September 2004

Richard O. Day
Daniel E. Furst
Piet L.C.M. van Riel
Barry Bresnihan

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Medicinal chemistry of the disease modifying anti-rheumatic drugs

Garry G. Graham

Department of Physiology and Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia and Department of Clinical Pharmacology, St Vincent's Hospital, Sydney, NSW 2010, Australia

Introduction

The disease modifying anti-rheumatic drugs (DMARD) and the corticosteroids constitute a great variety of chemical compounds and, as is the case with all drugs, the chemical properties of the DMARD and corticosteroids are important aspects of their pharmacology. In this chapter, the medicinal chemistry of the various DMARD is discussed. The coverage includes the chemical factors that affect their handling by the body. In addition to the DMARD discussed in this book, the chemistry of penicillamine is also described. Although the use of penicillamine as an anti-rheumatic drug has declined in recent years, thiol compounds, such as penicillamine, are still of great interest because of their antioxidant activity and potential activities in inflammatory states.

Antimalarials (chloroquine and hydroxychloroquine)

At least three antimalarial drugs have anti-rheumatic activity in man. The first to be discovered, accidentally, was mepacrine (quinacrine). This drug is no longer used, but the discovery of its anti-rheumatic activity was followed by the successful testing of chloroquine and hydroxychloroquine for the treatment of rheumatoid arthritis (RA) (see the chapter by Bothwell and Furst). Hydroxychloroquine is now generally preferred to chloroquine because of its lesser toxicity.

These two antimalarials are very lipid soluble bases, as is indicated by their octanol/water partition coefficient (P) of the un-ionised forms. For chloroquine, the logarithm of the partition coefficient between octanol and water ($\log P$) is 4.72, while the octanol/water partition coefficient of hydroxychloroquine ($\log P = 3.85$) is lower because of hydrogen bonding of water to the hydroxyl group (Fig. 1). It should be noted that the lipid solubility of drugs is usually quoted as the partition between octanol and water because octanol has similar lipid-like characteristics to

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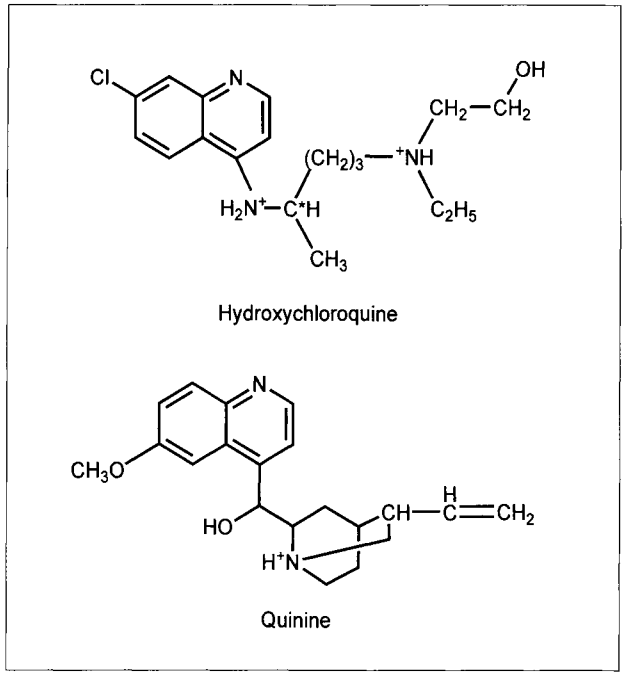

Figure 1

Structures of hydroxychloroquine and quinine showing the major form of each drug in aqueous solution

The pKa values of hydroxychloroquine are 8.3 and 9.7 [1] and therefore the major form of hydroxychloroquine at pH 7.4 is the di-cation (two positive charges per molecule). The pKa values of quinine are 4.1 and 8.5 [2] and it follows that the mono-cation shown is the major form present at pH 7.4. The chiral centre in hydroxychloroquine is shown (*). There are four chiral centres in quinine, which is only one of the 16 possible isomers.

cell membranes. A high octanol/water partition coefficient, together with a molecular weight in the range of 200–400, is considered to predict ready passive diffusion through cell membranes.

In contrast to most basic drugs, chloroquine and hydroxychloroquine contain two basic nitrogen moieties, which are both highly ionized at pH 7.4 (Fig. 1) [1, 2]. Thus, they are di-cations at physiological pH values. At pH 7.4, the neutral species accounts for less than 0.05% of both antimalarials (Fig. 1). However, the un-ionised forms of both drugs are so lipid soluble that they still partition preferentially into octanol at pH 7.4. Thus, the logarithm of the distribution coefficients between octanol and buffer (log D) for chloroquine and hydroxychloroquine are 0.96 and 0.66 at pH 7.4, respectively (i.e., their partition coefficients are 9.1 and 4.6) [1].

Like many other drugs whose neutral forms are highly lipid soluble, it is probable that both antimalarials diffuse easily through cell membranes in the un-ionised form and, consequently, they are well absorbed from the gastrointestinal tract and distributed throughout the body. Carrier mediated transport, through a carrier, such as Pgh-1, is also possible [1].

Both chloroquine and hydroxychloroquine achieve high tissue concentrations. For example, the volume of distribution of chloroquine is about 190 L/kg [3]. The di-cation forms of chloroquine and hydroxychloroquine (Fig. 1) are considered to cause the very high tissue levels of these drugs through binding firstly to tissue nucleic acids as well as proteins, which are mostly negative at physiological pH values. Trapping in the acidic contents of tissue lysosomes may also be important in producing the very high tissue concentrations of the antimalarials [4, 5] and may also be involved in the mechanism of the anti-rheumatic action of the antimalarials (see the chapter by Bothwell and Furst). The old antimalarial, mepacrine, which has antirheumatic activity, is also largely a di-cation at pH 7.4. Another antimalarial, amodiaquine, and its metabolite desethyl metabolite, also exist largely as the di-cation at physiological pH values but there are no reports of their anti-rheumatic activity.

The high tissue binding of the antimalarials is the cause of their long terminal half lives of elimination. Thus, the terminal half life of chloroquine is about 3 weeks [3]. Many other drugs are bases (e.g., antidepressants, antipsychotics, antihistamines and narcotic analgesics) but these drugs have only one basic nitrogen and, consequently, are mono-cations at physiological pH values. These basic drugs have lower volumes of distribution and shorter half lives of elimination. Quinine contains two basic nitrogens but the major form at pH 7.4 is the mono-cation (Fig. 1). Very little di-cation is present at pH 7.4. Its volume of distribution is about 1.8 L/kg and half life of elimination is about 11 h. Both are far lower than the corresponding pharmacokinetic parameters of chloroquine and hydroxychloroquine.

The long half lives of elimination of chloroquine and hydroxychloroquine indicates that they should accumulate slowly during treatment and, consequently, their treatment may be improved by initial high doses. This has been tested. The usual daily dose of hydroxychloroquine is 400 mg from the start of treatment but daily doses of 400, 800 and 1,200 mg have been used over the first 6 weeks of a clinical trial with a small increase in the number of patients responding at the higher initial doses of hydroxychloroquine [6].

Both chloroquine and hydroxychloroquine have chiral centres and therefore exist as two enantiomers. The anti-rheumatic activity and toxicity of the two enantiomers may differ but there is no information on this. There are, however, known differences between the pharmacokinetic behaviour of the two enantiomers. During long-term therapy, the plasma concentrations of the R-hydroxychloroquine are about 60% greater than the plasma concentrations of the S enantiomer, indicating a smaller clearance of the R enantiomer [7]. However, the unbound proportions of

the R-hydroxychloroquine in plasma are nearly twice those of the S enantiomers [8] and, consequently, the concentrations of unbound R enantiomer are approximately three times as great as the concentrations of the unbound S enantiomer. The same pattern is seen with chloroquine.

Azathioprine

Azathioprine is a synthetic cytotoxic purine that is used as an immunosuppressant to prevent rejection of transplanted tissues and for the treatment of several diseases including several tumours, RA and inflammatory bowel disease. Azathioprine is metabolised to 6-mercaptopurine, which then undergoes several metabolic reactions which lead both to its activation and to loss of activity (Fig. 2) [9] (see the chapter by Kane and Bresnihan). 6-mercaptopurine is activated by its metabolism to 6-thioinosine monophosphate (thioinosinic acid), a reaction catalysed by hypoxanthine guanine phosphoribosyl transferase (HPRT). This step is then followed by the production of the 6-thioguanine nucleotides.

A major pathway of inactivation of 6-mercaptopurine is through the activity of the enzyme xanthine oxidase; and, not surprisingly, treatment with the xanthine oxidase inhibitor, allopurinol, substantially decreases the formation of the inactive thiouric acid. Consequently, the dosage of azathioprine and 6-mercaptopurine should be reduced by about 75% in patients taking allopurinol.

A second pathway of metabolism of 6-mercaptopurine is through the enzyme, thiopurine methyltransferase (TPMT), which is under genetic control [10]. About 85% of patients have normal or high activity of the enzyme, while about 1 in 300 individuals are homozygotes for the abnormal enzyme and have negligible inactivating capacity. Consequently, bone marrow toxicity is very common in the homozygotes due to their high levels of the active thioguanine nucleotides. If genetic analysis is available, these patients should not receive azathioprine or 6-mercaptopurine. The heterozygotes have intermediate activity of TPMT and should receive lower than usual doses of azathioprine. Overall, optimal dosage of these drugs could be determined in the light of the known activity of the TPMT in an individual patient, although this is still uncommon in clinical practice.

The activity of TPMT can be determined by both phenotyping the activity of the enzyme and also by genotyping [11, 12]. The morbidity from azathioprine is reduced significantly by either phenotypic or genotypic analysis. Even economic costs are reduced because the analysis decreases the incidence of severe myelotoxicity, and therefore saves on the substantial costs of treatment of myelotoxicity. In the treatment of inflammatory bowel disease, the levels of the active metabolites in red blood cells can be used to monitor treatment with azathioprine. Thus, levels of 6-thioguanine nucleotides between 235 and 450 pmol/8 $\times$ 10^8 red blood cells [13] appear optimal, but it is not known if this range is optimal for the treatment of RA.

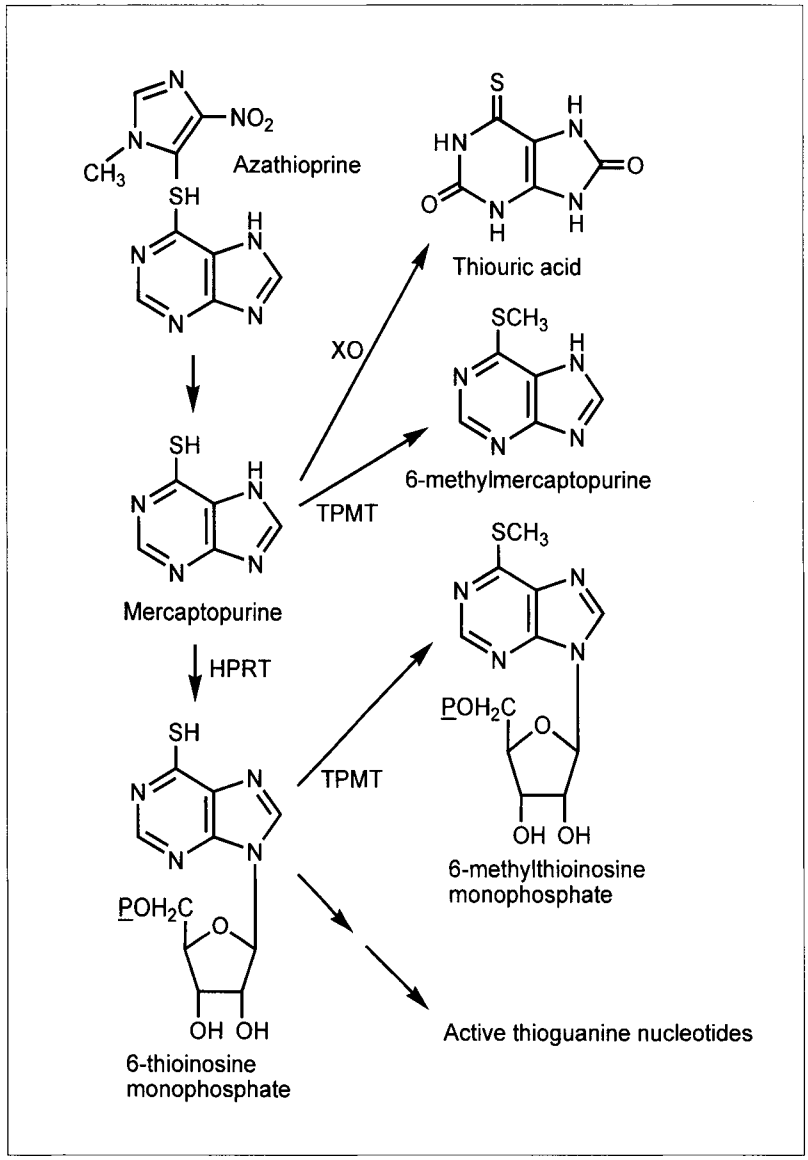

Figure 2
Structure and metabolism of azathioprine
Azathioprine is activated by its metabolism to 6-mercaptopurine and ultimately to the active thioguanine nucleotides. Thiopurine methyltransferase (TPMT) is usually considered to produce inactive metabolites but 6-methylthioinosine monophosphate (6-methylthioinosinic acid) may cause feedback inhibition of the de novo *synthesis of purines [9]. Xanthine oxidase (XO) catalyses the production of inactive metabolites, the final product being 6-thiouric acid. HPRT = hypoxanthine phosphoribosyltransferase,* P = *phosphate.*

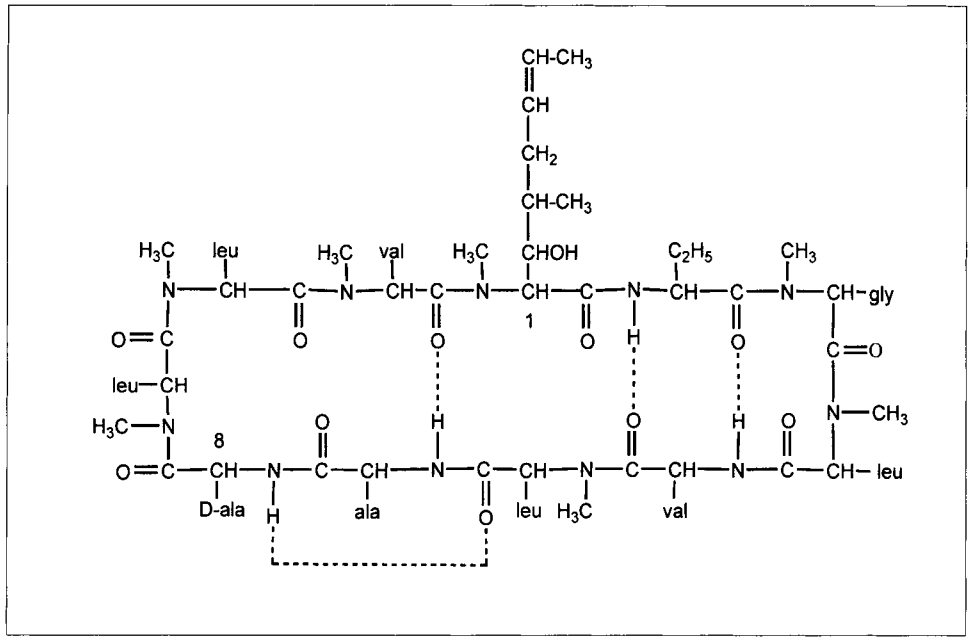

Figure 3
Structure of cyclosporin
It contains 11 amino acids with hydrophobic side chains, apart from a hydroxyl on the unique amino acid at position 1. The amino acid at position 8 is D-alanine but all other amino acids have the normal L configuration. Hydrogen bonding of the peptide chain is either totally internal (broken lines) or prevented by N-methylation of the amide nitrogens. Amino side chains are: leu = leucine, val = valine, ala = alanine.

Cyclosporin

Cyclosporin is a fungal antimetabolite that has profound effects on the immune response. It is efficacious in the treatment of RA but has a low therapeutic index (see the chapter by van Kuijk and Dijkmans. Cyclosporin is a small polypeptide but, unlike most other peptides, is very lipophilic because all the amino acid side chains are hydrophobic and hydrogen bonding of the peptide chain is either totally internal or prevented by N-methylation of the amide nitrogens (Fig. 3). The lipophilic nature of cyclosporin makes it difficult to formulate into a product that dissolves sufficiently in the gastrointestinal tract to yield reliable absorption. The most recent changes in the formulation are capsules and a liquid preparation that emulsify on dilution with water. The half life of elimination is about 6 h but the drug is most commonly administered twice daily. Unlike most peptides, but because

of its non-polar nature, cyclosporin is metabolised by the hepatic cytochrome P450, most significantly by cytochrome P450 3A4. A variety of other drugs induce or inhibit cytochrome P450 3A4 and cyclosporin is therefore subject to a large number of clinically important drug interactions.

The immunosuppressant, tacrolimus (FK506), also has anti-rheumatic activity [14]. Tacrolimus contains aliphatic carbon chains together with carbocyclic and heterocyclic cyclic structures but no amino acids. It is therefore totally different in structure to cyclosporin.

Despite the contrasting structures, the mechanism of action of cyclosporin and tacrolimus are very similar. The protein phosphatase, calcineurin is the common target of both cyclosporin and tacrolimus, but the two immunosuppressants bind initially to different receptors. Cyclosporin binds to cyclophilin A, while tacrolimus binds to FK506 binding protein. However both complexes, cyclosporin–cyclophilin A and tacrolimus–FK506 binding protein, bind to same surface of calcineurin [15]. The result is similar immunosuppressive activity of the two structurally dissimilar drugs. Similar anti-rheumatic activity is anticipated.

Gold complexes

All gold drugs contain gold in the oxidation state I, i.e., Au(I). However, the Au^+ ion does not exist in aqueous solution and the gold drugs are all complexes, most commonly with thiol groups (also known as sulphydryl groups, i.e., compounds containing the –SH group). Two types of gold complexes are available. The most widely used are the water soluble complexes, such as sodium aurothiomalate (Fig. 4). More lipid soluble complexes of gold have also been tested and one such complex, auranofin, is now marketed.

Sodium aurothiomalate

Sodium aurothiomalate is the most widely studied gold complex, and one of the few complexes still available (see the chapter by Rau). Sodium aurothiomalate is highly water soluble largely due to the ionisation and of the carboxyl groups in the thiomalate ligands at physiological pH values. Its low lipid solubility, together with the large molecular weight makes aurothiomalate poorly absorbed and of low efficacy when administered orally, and it is only administered by intramuscular injection.

As is the case with some of the thiol compounds, such as penicillamine, the thiomalate ligand in aurothiomalate is a chiral compound [16]. In this case, however, thiomalate is present as the racemate. However, the stereochemistry of the thiomalate ligands is considered to have little influence on the anti-rheumatic activity of aurothiomalate because the ligands of all the gold complexes are not perma-

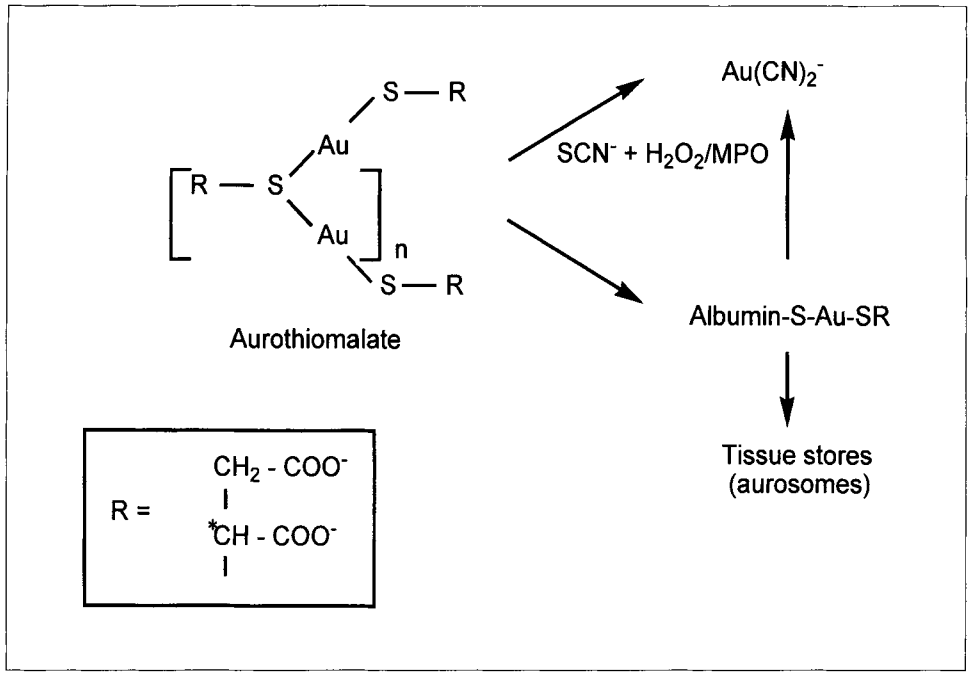

Figure 4
Schematic structure and metabolism of aurothiomalate
Aurothiomalate is a polymeric compound which consists of about 8–10 units of sodium thiomalate, which is complexed to Au(I) with the chain terminated by an additional thiomalate residue. Gold is present in plasma mainly as complexes with albumin and endogenous thiols (RS^-). Thiomalate contains a chiral centre, as shown in the inset. Aurothiomalate and albumin complexes are converted to aurocyanide ($Au(CN)_2^-$) by myeloperoxidase (MPO) during the oxidation of thiocyanate (SCN^-) by hydrogen peroxide (H_2O_2).

nently bound to the gold. The thiomalate ligands exchange readily with endogenous ligands, such as the thiol containing compounds albumin and glutathione, and, also, with cyanide. *In vivo*, the thiomalate is separated from the gold and it is probable that the major species in plasma is albuminS-Au-thiol, where the thiol is an endogenous compound such as glutathione or cysteine (Fig. 4).

Total gold disappears from plasma with a half life of about 5 days after dosage [17] but, while much of the gold is excreted in urine, some accumulates in macrophages as aurosomes, which are membrane-bound bodies probably derived from lysosomes [18] (see the chapter by Rau).

A significant aspect of the pharmacology of the gold complexes is that aurothiomalate is metabolised by myeloperoxidase and lactoperoxidase in the presence of

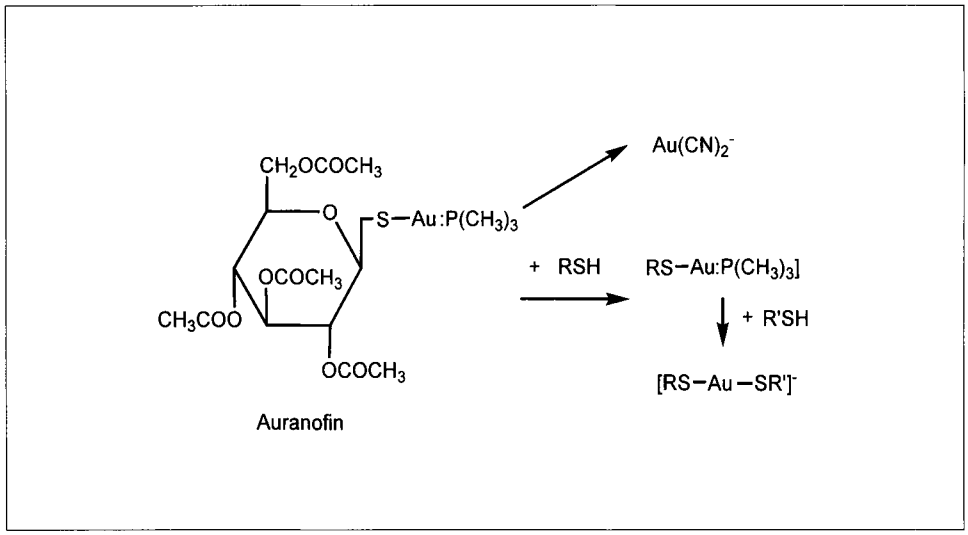

Figure 5
Structure and outline of metabolism of auranofin
The phosphine and thiol ligands of auranofin are largely displaced by endogenous thiols. Aurocyanide ($Au(CN)_2^-$) has also been detected in plasma during treatment with auranofin and is presumably formed by the action of myeloperoxidase.

hydrogen peroxide and thiocyanate to generate aurocyanide ($Au(CN)_2^-$) (Fig. 5) [19]. Myeloperoxidase is an important enzyme in the oxidative burst of neutrophils and monocytes, while lactoperoxidase is present in milk and saliva. Aurocyanide potently inhibits the oxidative burst of neutrophils and also of the proliferation of lymphocytes and may mediate many of the anti-rheumatic and adverse effects of the gold complexes [19]. Aurocyanide is extremely stable with a stability constant of the order of 10^{38} [20]. Although aurocyanide may mediate the cellular effects of the gold complexes, it is too toxic for systemic use.

Auranofin

Auranofin is a mixed gold complex, the ligands being a thiol, tetraacetylthioglucose, and triethylphosphine (Fig. 5). About 25% of the gold content of an oral dose is absorbed from the gastrointestinal tract, although the circulating form is not known. As is the case with aurothiomalate, the two ligands in auranofin are removed in the body. The tetraacetylthioglucose ligand is the less strongly bound and, consequently, is displaced before the triethylphosphine ligand [21].

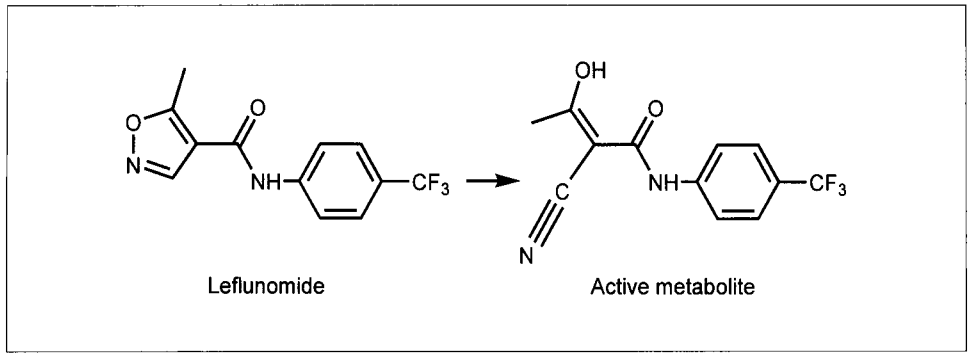

Figure 6
Structures of leflunomide and its active metabolite which is produced by hydrolysis of the parent drug

Gold (III) complexes

The Au(III) complexes are very strong oxidising agents in aqueous solution but interest in the biological effects of Au(III) complexes has also been revived because they can be formed by the action of HOCl, the product of myeloperoxidase [22].

Leflunomide

Leflunomide is an inactive prodrug that is converted almost completely to an open chain metabolite, which is the active form in a variety of immunological diseases including RA (Fig. 6) (see the chapter by Hoi and Littlejohn). Leflunomide is only administered orally and undergoes almost complete first pass metabolism to the active metabolite. This compound has a half life of elimination of about 15–18 days [23]. This is a problem in the use of leflunomide and more rapidly eliminated analogues of the active metabolite are being sought.

The long half life of elimination of the active metabolite has been considered in the design of dosage schedules of leflunomide. A half life of elimination of about 15–18 weeks indicates that the metabolite accumulates significantly for 2–3 months if a constant dosage schedule is used. In order to achieve therapeutic levels of the drug rapidly, a loading dose schedule is frequently administered followed by smaller maintenance doses. The maximal loading dose schedule is not, however, always used. A constant dosage allows the slow accumulation of the active metabolite while, at the same time, the patient may develop resistance to minor, although still

distressing adverse reactions, such as nausea and diarrhoea, which often lead to discontinuation of the drug.

The active metabolite of leflunomide is excreted in bile and the long half life of elimination is probably due to the development of enterohepatic cycling. This can be interrupted by dosage with cholestyramine, which binds the metabolite very strongly and the half life of elimination decreases to about 1 day. Consequently, cholestyramine is administered if there are significant side effects to leflunomide or if the patient wishes to become pregnant.

Methotrexate

Methotrexate is a close analogue of folic acid (Fig. 7) (see the chapter by Pile). Several analogues have been tested as cytotoxic drugs in the treatment of tumours but methotrexate is the only antifolate that is used in the treatment of rheumatic diseases. Methotrexate is a hydrophilic ionised drug at physiological pH indicating that it should not diffuse readily through cell membranes. Its log *P* value is quoted as −1.8 [2]. It is, however, transported into and out of cells by membrane carriers.

Methotrexate contains a glutamate moiety and, after entering the cell, up to six glutamates are added (Fig. 7). This polyglutamation maintains a low intracellular concentration of methotrexate leading to a very high accumulation of polyglutamated methotrexate. This material cannot be transported extracellularly unless hydrolysed to the monoglutamate level by polyglutamate hydrolase. The polyglutamation of methotrexate effectively increases its intracellular life and enhances its enzyme inhibitory potency.

Penicillamine

Penicillamine is an active DMARD but its use in RA is now limited because of the lack of response in a substantial numbers of patients and the development of side effects. Nevertheless compounds such as penicillamine, which contain thiol groups, have many biological interactions and are being widely investigated, particularly for their antioxidant activity (Fig. 8).

Penicillamine is a dimethylated analogue of the amino acid, cysteine, but contains the unnatural D configuration that contrasts with the L configuration of the amino acids, which are present in proteins. Thus, penicillamine is dimethyl-D-cysteine. Its pKa values are 1.8 (carboxyl group), 7.9 (ammonium group) and 10.5 (thiol group). Therefore, like other amino acids, it is largely present at blood pH as the zwitterion (Fig. 8) and has a very low octanol/water partition coefficient.

The important functional group in penicillamine is the thiol group (-SH). The structures of several thiol compounds of anti-inflammatory interest are shown in

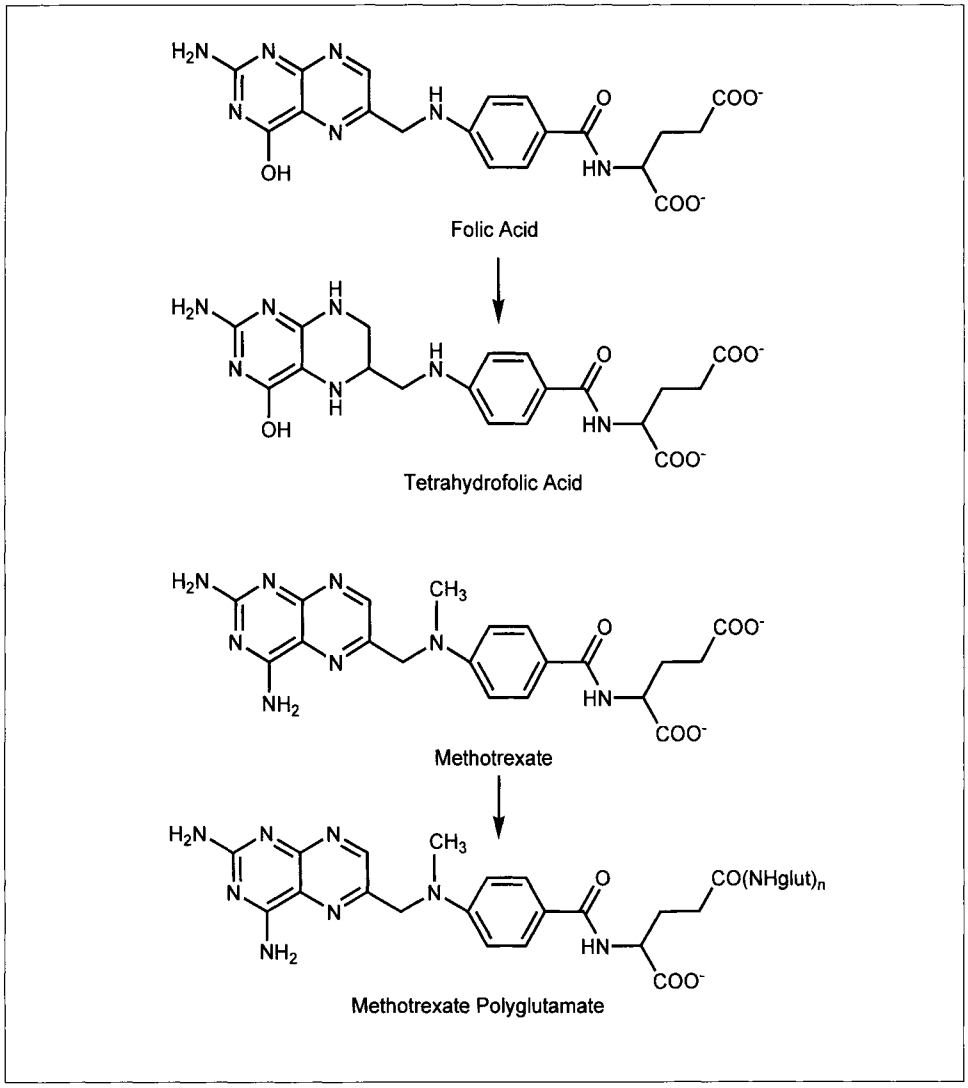

Figure 7
Comparative structures of methotrexate and folic acid
Folic acid is only active as cofactor for one carbon transfers after reduction to tetrahydrofolic acid. Both folic acid and methotrexate form polyglutamates in cells, as is shown for methotrexate.

Figure 8. In contrast to penicillamine, acetylcysteine and bucillamine contain the normal L configuration of amino acids. Tiopronin is a derivative of the symmetrical amino acid, glycine, but the side chain contains a chiral centre and the material

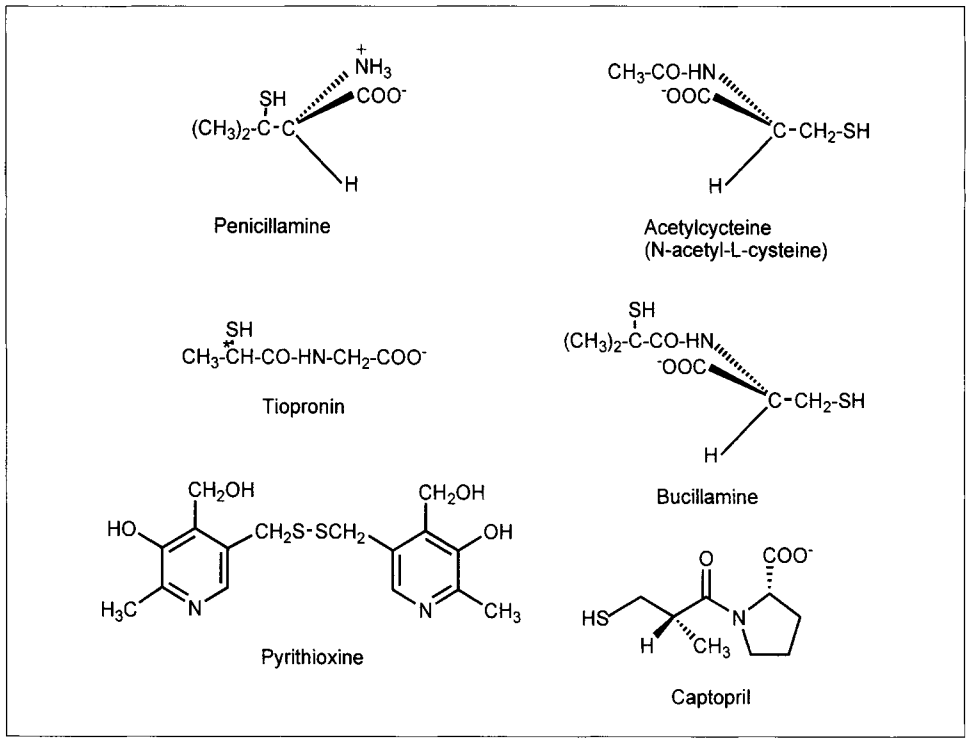

Figure 8
Structures of penicillamine and other thiol compounds with anti-rheumatic activity
Penicillamine has the unnatural D configuration in contrast to the L configuration of the naturally-occurring amino acids. Tiopronin is a derivative of the symmetrical amino acid, glycine, but contains a chiral centre (*) in the thiol side chain and is available as the racemate. Captopril contains two chiral centres. It is a derivative of the natural amino acid, L-proline but contains an additional chiral centre in the thiol side chain. It is available as a single isomer.

which has been examined is a racemic mixture of the two enantiomers. Bucillamine differs from the other thiol compounds in that it contains two thiol groups. Unlike the other thiol compounds in Figure 8, thiopyridoxine and its disulphide, pyrithioxine, are aromatic compounds.

In man, DMARD activity is shown by nearly all of the listed thiol compounds including bucillamine [24], thiopronine (tiopronin, thiola), thiopyridoxine and pyrithioxine [25], and captopril (Fig. 8). The anti-inflammatory activity of captopril has been correlated with its thiol group as another angiotensin converting enzyme (ACE) inhibitor (pentopril) does not contain a thiol group and does not have DMARD activity [26]. Acetylcysteine did not show anti-rheumatic activity in RA

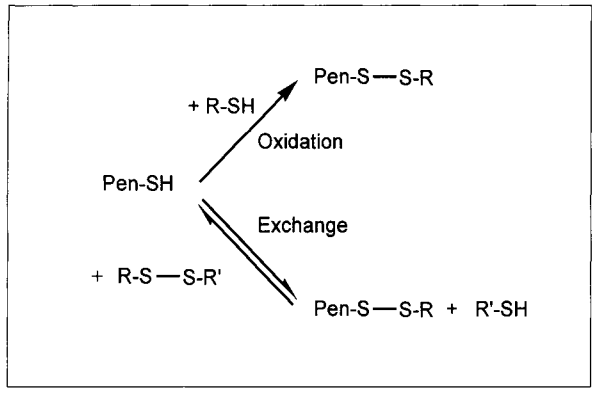

Figure 9
Potential oxidative and exchange reactions of penicillamine (Pen-SH) with an endogenous thiol (R-SH) and an endogenous disulphide (R-S-S-R'). Other thiol compounds should undergo the same general reactions.

although the patients tested were refractory to other treatments [27]. Acetylcysteine does, however, suppress collagen-induced arthritis in mice [28]. Acetylcysteine is well known for its mucolytic activity and also as an antidote to overdoses of paracetamol. Another related compound is levamisole, which is a cyclic sulphide that is hydrolysed to a thiol compound. Levamisole is, however, no longer used because of its induction of leucopenia.

The reactivity of penicillamine and other thiols generally includes their ability to be oxidised to disulphides with other thiol compounds (Fig. 9). A clinical example of the value of the production of disulphide formation is the use of penicillamine and thiopronine in cystinuria [29]. Penicillamine, for example, forms a disulphide with cysteine that is much more soluble than cysteine and thus does not precipitate in the renal tract. Penicillamine and other thiol drugs also form disulphides with a cysteine residue in serum albumin. These are commonly the major forms of the thiols in blood during treatment with penicillamine.

The thiol group is acidic, and although the pKa values are generally high. The oxidation of the thiol group generally increases with decreasing pKa values and thus penicillamine (pKa 10.5) should be less readily oxidised than cysteine (pKa 8.5) or the important intracellular thiol compound, glutathione (pKa 9.2) [30]. The oxidative reactions of thiol compounds are mediated by the thiolate anion (RS^-) and, in oxidative reactions, the thiolate anions lose an electron to become thiyl radical ($RS^{\cdot}$), which is reactive because of its unpaired electron. These free radicals and other reactive species, such as superoxide, which can be formed during the oxidation of thiols, are probably responsible for the adverse reactions produced by thiol compounds.

Another general feature of penicillamine and other thiols is their ability to form complexes with a variety of metal ions. The formation of copper complexes makes penicillamine useful in the treatment of Wilson's disease. In this disease, the excessive deposition of copper causes liver cell damage. Penicillamine is also useful in the treatment of poisoning with compounds containing arsenic, lead or mercury.

Thiopyridoxine appears to differ from the other thiol compounds in several regards. It does not form a mixed disulphide with cysteine in a simple chemical system and does not increase the urinary excretion of copper [25]. However, both thiopyridoxine and the disulphide, pyrithioxine, have similar anti-rheumatic and side effects to penicillamine and the other aliphatic thiols.

Sulfasalazine

Sulfasalazine is the diazo conjugate of sulphapyridine and mesalazine (aminosalicylate) (Fig. 10). It was developed in order to provide the combination of an antibacterial drug (sulphapyridine) and an anti-inflammatory compound (mesalazine [31]). Sulfasalazine is poorly absorbed from the small intestine but metabolised in the reducing environment of the large intestine where the two components, sulphapyridine and mesalazine, are released. The sulphapyridine is largely absorbed in the large intestine in contrast to the poor absorption of mesalazine and the unchanged sulfasalazine [32, 33] (see the chapter by Haagsma).

Sulfasalazine is also used widely for the treatment of ulcerative colitis where mesalazine is the active moiety. In order to remove the toxicity of sulphapyridine, particularly its haematological effects, several other preparations have been used in the treatment of ulcerative colitis and can be usefully contrasted with sulfasalazine. These include the enteric coated mesalazine and olsalazine, which is a dimer in which two aminosalicylate residues are linked through a diazo bond. In a similar fashion to sulfasalazine, the azo bond of olsalazine is cleaved in the large intestine with the resultant release of two molecules of mesalazine (Fig. 10).

In the treatment of RA, the active moiety is unclear. In two studies, sulphapyridine decreased the disease activity of patients with RA [34, 35]. This indicates that sulphapyridine may be the active component of sulfasalazine. The cause of the apparent activity of sulphapyridine is unknown. The obvious question is whether the activity of sulphapyridine is due to its antibacterial activity. In order to answer this question, the anti-rheumatic activity of another sulphonamide, sulphamethoxazole, has been evaluated. Unfortunately, the results are conflicting with two studies showing anti-rheumatic activity of sulphamethoxazole and one study showing no effect [35]. In contrast to the effect of sulphapyridine, olsalazine, the prodrug which yields only mesalazine in the large intestine (Fig. 10), showed suppression of the symptoms of ankylosing spondylitis in a small open study [36]. The overall con-

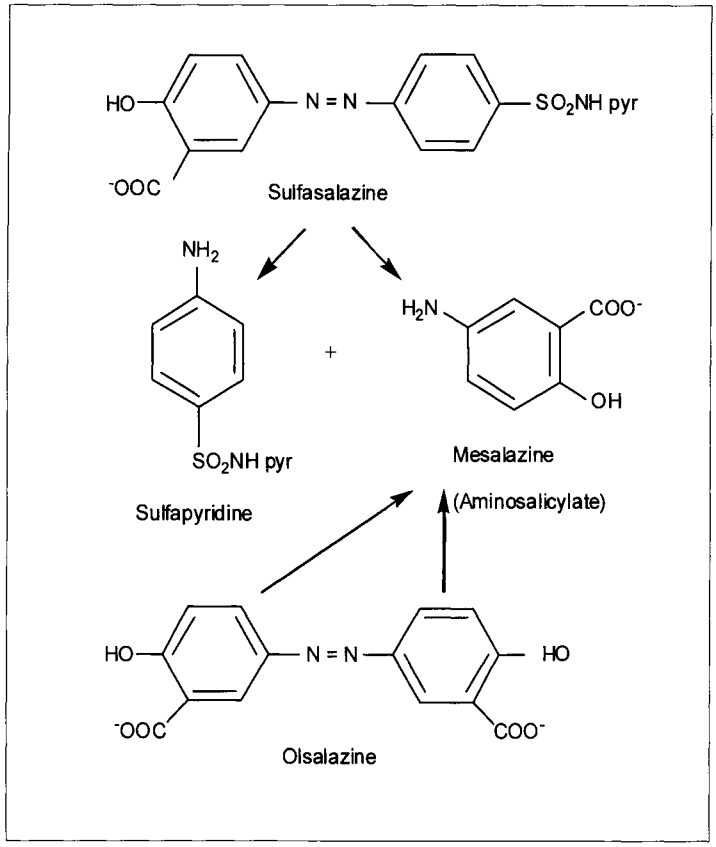

Figure 10
Structure and initial reductive metabolism of sulfasalazine and olsalazine (pyr = pyridine residue). Reduction of the azo bond of sulfasalazine within the large intestine yields sulphapyridine and mesalazine (aminosalicylate) while the reductive metabolism of olsalazine yields only mesalazine.

clusions are that the active species of sulphasalazine has not been identified clearly and that the mode of action of the sulphapyridine, if indeed it is the active metabolite, is unknown.

Tetracyclines

Several tetracycline antibiotics have been used in the treatment of RA but minocycline is the only tetracycline whose use is supported by double-blind clinical trials (see the chapter by Bird and O'Dell). The nomenclature of the tetracyclines should

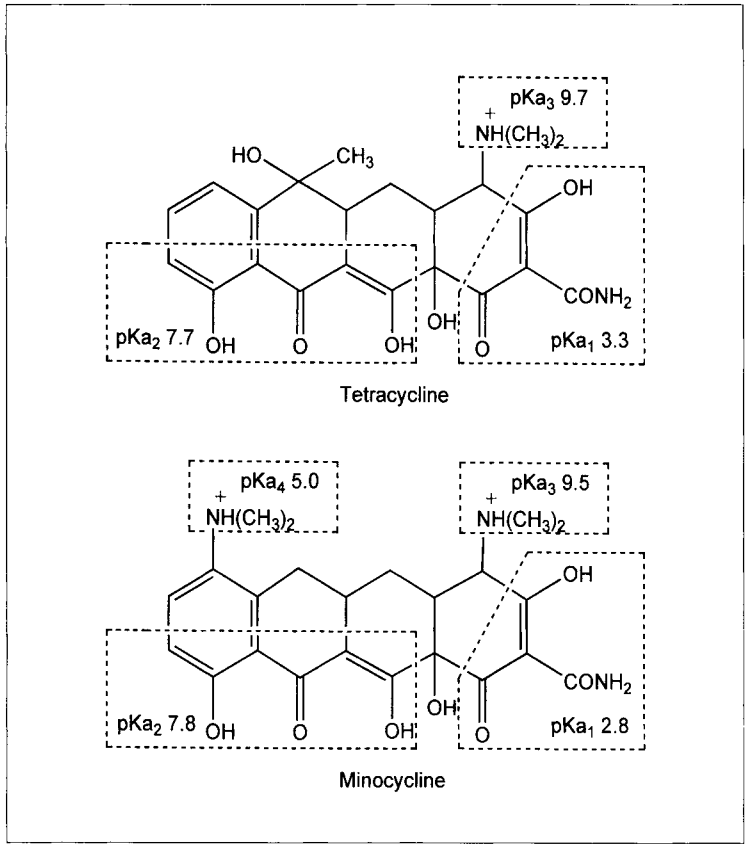

Figure 11

Structures and ionisation of tetracycline and minocycline [37]

At pH 7.4, the major forms of both compounds are zwitterions with a negative charge from ionisation of the tricarbonylmethane region (pKa_1 values of 3.3 (tetracycline) and 2.8 (minocycline)) and a positive charge of the dimethyl ammonium groups (pKa_3).

be noted at this point. The term, tetracyclines, is applied to the group of antibiotics, while tetracycline is a specific compound.

Minocycline is a semi-synthetic tetracycline that contains two dimethyl amino groups as opposed to the single dimethylamino group in tetracycline (Fig. 11). Tetracycline and doxycycline have not been found to have anti-rheumatic activity but further work would be required to disprove their clinical utility. There are no animal studies that may indicate potential differences in the anti-rheumatic activity of the various tetracyclines. There is considerable knowledge about the relationship between the chemical structures of the tetracyclines and their antibiotic activity [37] but this is not case with their anti-rheumatic activity.

Minocycline and tetracycline are taken up well by mammalian cells. This is indicated because their volumes of distribution of both antibiotics, relative to the unbound concentrations in plasma, are both about 5 L/kg. The mechanism of the cellular uptake of the tetracyclines is, however, not well understood. Tetracyclines diffuse through model lipid barriers by passive diffusion, but this is surprising because they are zwitterions at physiological pH values and therefore should have very little lipid solubility [37]. Minocycline is stated to be more likely to undergo passive diffusion through cell membranes because of it is higher lipid solubility. Thus, the log P of minocycline is quoted to be 1.6 indicating that minocycline is nearly 400 times as great as the partition coefficient of tetracycline ($\log P = -1$) [38]. Unfortunately, the solvent and conditions are not stated. Increased lipid solubility of minocycline is consistent with the loss of the hydroxyl group of tetracycline (Fig. 11) but magnitude of the difference between the quoted partition coefficients is surprising. There is also evidence that tetracyclines are taken up by bacterial and mammalian cells by carrier transport mechanisms [37, 39].

The value of minocycline may come, in part, because its half life of elimination (about 16 h) is about 60% longer than the half life of elimination of tetracycline [3]. The longer half life of minocycline indicates relatively stable plasma concentrations when it is administered twice a day. A further advantage of minocycline is that its oral absorption is little affected by food [40]. By contrast, the oral absorption of tetracycline is greatly diminished by food as well as calcium and magnesium salts.

Corticosteroids used in rheumatic diseases

A large number of naturally-occurring corticosteroids have been isolated, while an even larger number are purely synthetic. The anti-inflammatory activity of the corticosteroids is associated with their metabolic effects on the metabolism of glucose, proteins and lipids, i.e., their glucocorticoid activity. In the remainder of this section, the term, corticosteroid, will be used to describe compounds that have predominately glucocorticosteroid activity.

Corticosteroids are used for the treatment of a variety of diseases, and several routes of administration are used; but only four routes are used in the treatment of rheumatic diseases. These are the oral route, intra-articular injection, intramuscular and intravenous injection (see the chapter by Kirwan and Perry).

Oral corticosteroids

Prednisolone is the most widely used oral corticosteroid for the treatment of RA. Its structure is very similar to the naturally occurring hormone hydrocortisone (also known as cortisol) (Fig. 12). Prednisolone binds to the glucocorticoid receptor more

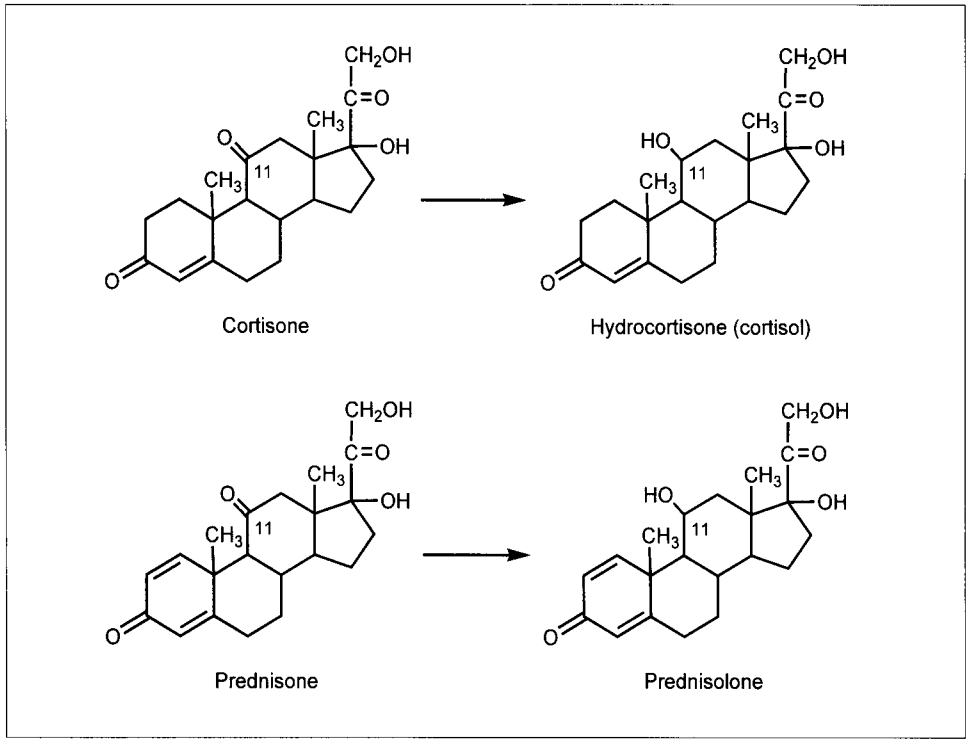

Figure 12
Reductive metabolism of 11-keto corticosteroids cortisone and prednisone to therapeutically active 11-hydroxy corticosteroids

strongly than hydrocortisone and is also metabolised more slowly than hydrocortisone. The result is that prednisolone is approximately four times as potent as hydrocortisone *in vivo* [41].

Prednisone is an analogue of cortisone and, like cortisone, has very low activity and is reduced to the active drug. In this case, prednisone is reduced to prednisolone (Fig. 12). Prednisolone possesses some mineralocorticoid activity, which is shown at high doses. Thus at high doses, it may cause retention of sodium and water with the concomitant loss of potassium in urine. Methylprednisolone has similar activity to prednisolone but methylprednisolone has slightly greater glucocorticoid and lesser mineralocorticosteroid activity [41].

Dexamethasone and betamethasone are also used for their anti-rheumatic effects. Both are very potent and highly selective for the glucocorticoid receptor. However, their biological half lives are estimated to be in the range 36–72 h. By contrast, the half lives of prednisolone and methylprednisolone are in the range of 12–36 h. These shorter biological half lives of prednisolone and methylprednisolone

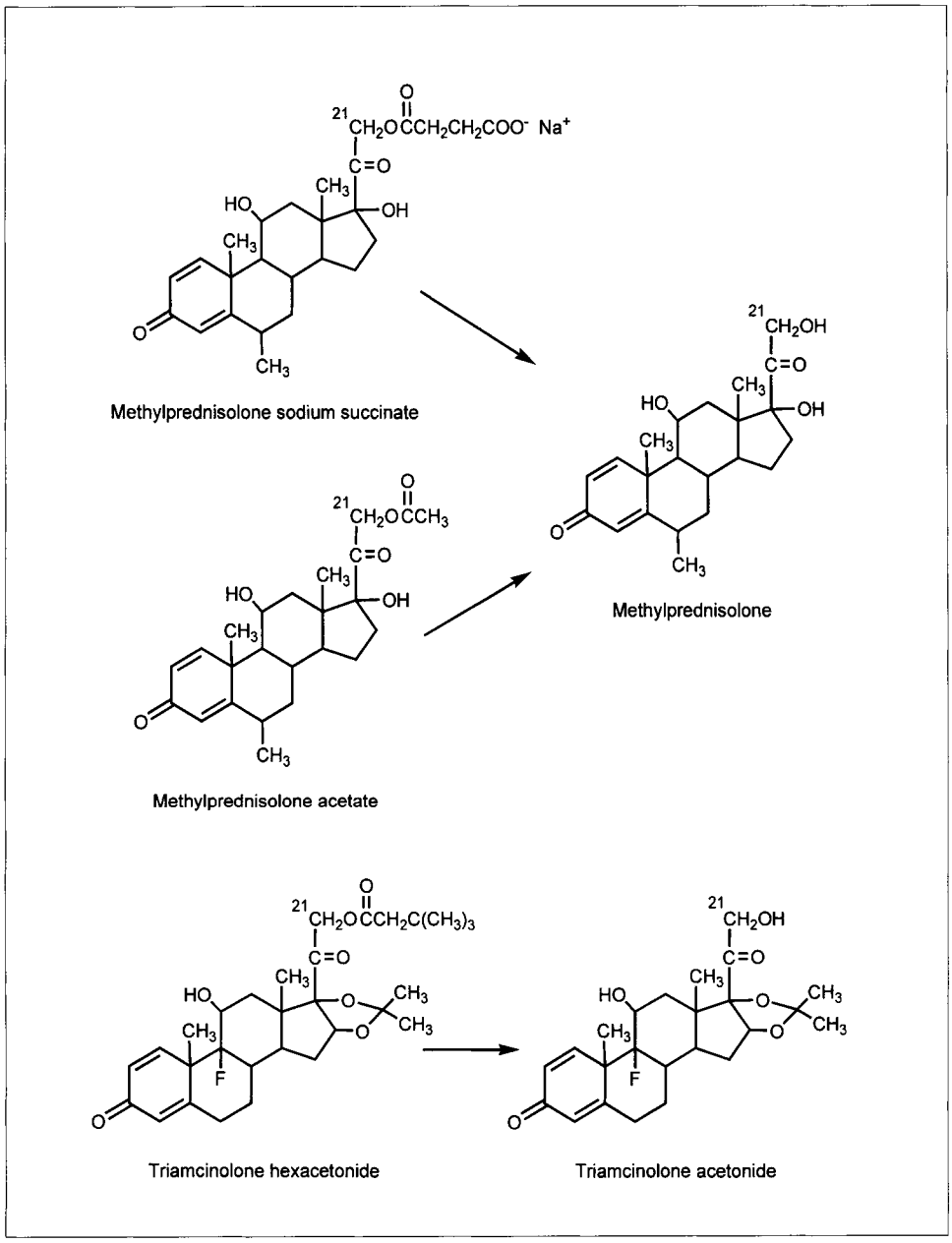

Figure 13
Hydrolytic metabolism of inactive 21-esters to active corticosteroids
Methylprednisolone hemisuccinate is water soluble and is administered by intravenous injection while methylprednisolone acetate and triamcinolone hexacetonide are used in depot intra-articular injections.

are considered to make them preferable to the longer acting corticosteroids, despite having some mineralocorticosteroid activity, because the shorter acting steroids produce less suppression of the pituitary–adrenal axis.

Several corticosteroids, including dexamethasone, betamethasone, triamcinolone and triamcinolone acetonide have a fluoro substituent in the B ring of the steroid system. Fludrocortisone, which is widely used as its acetate ester, also has a fluoro substituent in the same position and has greater glucocorticoid activity than hydrocortisone. The mineralocorticoid activity of fludrocortisone is increased to an even greater degree and it is used a mineralocorticoid [41].

Intra-articular injections

It is common in rheumatological practice to inject suspensions of corticosteroids into joints which are particularly inflamed. The most widely used corticosteroids for intra-articular injection are methylprednisolone acetate and triamcinolone hexacetonide (Fig. 12). Both are prodrugs, since the esterification at C21 prevents glucocorticoid activity. Once in solution, the ester group at the C21 position is hydrolysed rapidly to release the steroids containing the free primary alcohol at C21. Thus, the active steroids are methylprednisolone and triamcinolone acetonide. The reason for the use of the esters is that they are less water soluble and, more importantly, even more slowly soluble in the aqueous environment of joints than the active steroids. Therefore, the esters provide more sustained local effects than the active steroids (Fig. 13). The acetal structure in triamcinolone acetonide (Fig. 13) is metabolically stable and, furthermore, triamcinolone acetonide has a short half life elimination. This makes the depot of triamcinolone hexacetonide (Fig. 13) a useful corticosteroid for local use because of the subsequent relatively low systemic exposure of the active steroid.

The ester steroids have inherent slow solubilities in aqueous environments. However, this may not be the only reason for their sustained effect. From animal studies, the suspended corticosteroid in synovial fluid is incorporated into a fibrin-like mass [42]. This probably serves to slow the rate of dissolution even further.

Intravenous corticosteroids

In acute cases many diseases, including RA, corticosteroids are administered by intravenous injection. However, the corticosteroids are only sparingly soluble in water and therefore the active corticosteroids cannot be used intravenously. Their aqueous solubility is, however, increased greatly by conversion of the C21 primary alcohol group to the phosphate and hemisuccinate esters (Fig. 13). As their sodium salts, these esters are soluble in water and can be administered intravenously. These esters are also inactive but, like other C21 esters, are hydrolysed rapidly to the active steroids *in vivo*.

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Targeting DMARD therapy

Maria E. Suarez-Almazor

Baylor College of Medicine, Houston Center for Quality of Care and Utilization Studies, Michael E. DeBakey VA Medical Center, 2002 Holcombe (152), Houston, TX 77030, USA

Introduction

Medical advances in the past few years have opened an exciting era in the treatment of rheumatoid arthritis (RA). For the first time, physicians and their patients are faced with a variety of therapeutic choices in the management of RA and must make decisions about specific disease-modifying antirheumatic drugs (DMARDs), including potential combinations of different agents. A careful balance of short-term and long-term benefits and risks must be considered. In addition, financial and societal issues play a role in these already complex therapeutic decisions with increasing frequency. The cost of healthcare has skyrocketed in the past decades. Policymakers, physicians and their patients are faced everyday with decisional conflicts coupled with constraints in health budgets around the world, that must balance not only health outcomes, but also economic costs to patients, providers and society at large. These considerations are particularly salient when assessing the therapeutic benefits of the newly developed biologic agents which are substantially more costly than traditional DMARDS, but which could be cost-effective if they reduce long-term damage and disability.

When making therapeutic decisions, physicians must follow defined steps including the following: (i) identifying alternative options; (ii) ascertaining potential benefits and risks by examining the available evidence; (iii) summarizing and integrating the evidence; (iv) considering individual clinical traits; (v) assessing patient preferences; (vi) evaluating the health system environment; and (vii) considering societal benefits and costs at large. Almost any therapeutic decision includes these considerations, albeit often, the final conclusions are based on heuristics or clinical intuition because of the difficulties of weighing all the pros and cons implicated in clinical decisions, which invariably involve multi-attribute choices. Empirical research has shown that the human brain cannot process the complexity of these multi-attribute problems as rationally as would be desired, and, in order to be efficient, the brain

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Table 1 Considerations in evidence-based clinical practice for DMARD indications

External evidence (scientific literature)	Internal evidence (patient traits)
• **Patient population**	• **Genetic and biologic**
Disease severity	Baseline risk for poor outcomes
Risk	Baseline risk for toxicity
Psychosocial issues	Differences in response
• **Intervention**	• **Psychosocial**
Logistic issues	Access
Acceptability	Compliance
• **Outcome measures used**	• **Epidemiologic and clinical**
• **Length of follow-up**	Sociodemographics (age)
	Disease severity
	Comorbidities
	Markers of prognosis
	Risk of adverse events

solves complex decision-making through heuristics and biases. This has resulted in substantial unexplained practice variation and inappropriate prescribing across almost every disease studied, often increasing costs with no apparent benefit [1]. It is therefore imperative to identify mechanisms to combine the various sources of available information to efficiently target therapy in patients and to obtain maximum benefits with the lowest possible health and economic costs.

In the following sections, we will review the role of evidence-based medicine and that of individual considerations in clinical decision-making, and the potential economic implications of individually targeting effective therapy in RA.

Evidence-based medicine

Evidence-based medicine can be defined as the explicit and rational use of the best available evidence for healthcare decision-making in individual patients. These decisions should balance the external evidence obtained from high-quality studies and the internal evidence as it relates to the patient's individual traits and preferences [2–4]. Table 1 lists the various factors that must be taken into account in evidence-based decision-making, as they relate to DMARD therapy in RA. On one hand, the evidence obtained from the literature must be appraised with attention

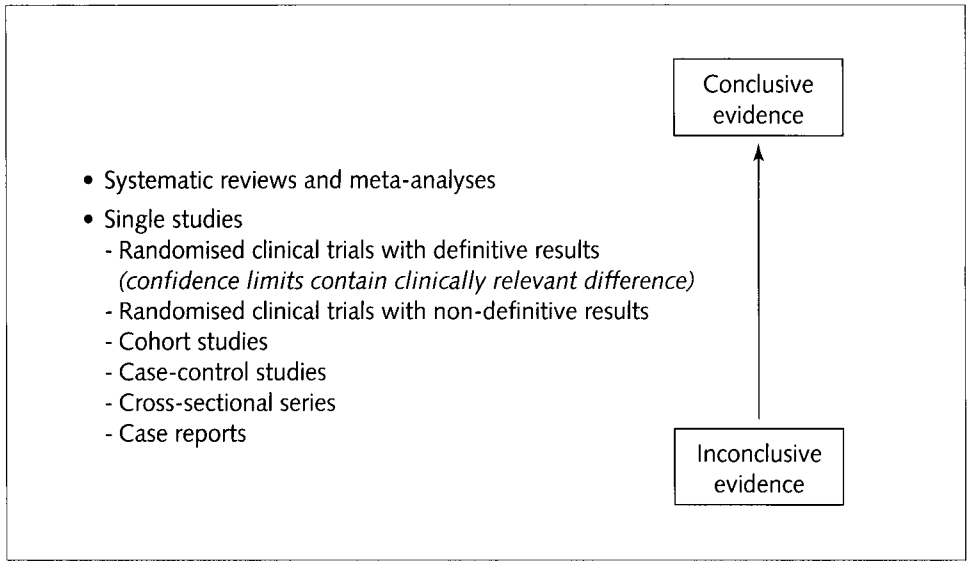

Figure 1
Hierarchy of evidence

to scientific rigor and clinical applicability. On the other hand, the individual traits and preferences of the patients must be considered to maximize gains and benefits and minimize risks and costs. External scientific evidence provides clinicians with an understanding of efficacy and effectiveness in groups of patients considered together, but the final benefit–cost ratio must be individually assessed for each patient.

External evidence

Hierarchy of scientific rigor

The most scientifically rigorous evidence for therapeutic efficacy is obtained from comprehensive systematic reviews of randomised clinical trials (RCTs), with or without meta-analysis (Fig. 1) [3–6]. Systematic reviews methodically and critically synthesize evidence from all available sources, using a systematic, unbiased approach. The next level of evidence is provided by individual RCTs. Observational studies are next in methodological rigor, with cohort studies being less subject to bias than case-control designs. Finally, small case series and case reports, although the most frequently published type of study only provide anecdotal evidence, which

is mostly useful for generating hypotheses. In evidence-based decision-making, data from both RCTs and observational studies must be considered. While RCTs provide the much-needed rigor obtained through experimental trial design, they often are not generalizable to the population of patients at large. Patients in RCTs are highly selected and have few comorbidities, and the length of follow-up in the trial is short. In addition, the comparator in DMARD trials is often placebo, or continuing drug therapy with the agent that has failed. These strategies are different from therapeutic decision-making in "real life". Observational studies, particularly cohorts, may be more subject to bias, but they provide data on unselected populations, often possessing greater length of follow-up (crucial to evaluating risk, particularly infrequent but severe adverse events), and portray a realistic view of effectiveness in the community at large. Auranofin, for instance, was efficacious in placebo-controlled RCTs, but was subsequently shown to be considerably less effective than other agents when used in everyday clinical practice.

Clinically useful outcome measures

Two major domains have to be assessed in the evaluation of the progression and outcome of RA: disease activity and disease damage. Several measures are currently used to assess disease activity (e.g., swollen joints), damage (e.g., radiographic changes), and outcomes that are a result of both disease activity and damage (e.g., functional impairment). The American College of Rheumatology (ACR) and OMERACT (Outcome Measures for Rheumatology Clinical Trials) have standardized which measures should be used in clinical trials: number of swollen and tender joints, physician and patient global assessments, functional status, acute phase reactants (ESR, CRP), and radiographic damage [7, 8]. These measures, when presented as aggregated means and standard deviations, are only useful in assessing overall efficacy in a group, but are inadequate to inform individual decision-making in terms of probabilistic outcomes. For example, a mean reduction of three swollen joints does not indicate what the probability is that a given patient will have 2, 4, 6 or 8 swollen joints after 6 months of therapy. The ACR has developed composite measures of improvement that can inform patients and physicians about the probability of improvement [9]: For an ACR20 response, improvement occurs with at least a 20% reduction in the number of tender and swollen joints, and a 20% improvement in three or more of the following: pain, functional status, acute phase reactants, physician global assessment, and patient global assessment; ACR50 and ACR70 responses, respectively, require a minimum of 50% or 70% improvement in these same measures. Similar criteria for improvement have been developed by the European League against Rheumatism (EULAR) [10].

An additional measure that helps make trial results more understandable for practicing clinicians and their patients, and can be helpful in daily clinical practice

decision-making, is the number needed to treat (NNT). The NNT is the estimated number of patients that have to be treated with an intervention in order to prevent one additional bad outcome, or to gain one additional good outcome. It is calculated as the inverse of the absolute risk reduction (ARR) [NNT = 1/ARR] [4, 11, 12]. The ARR is the difference in the rate of outcomes between the experimental treatment group and the control group. The NNT is calculated from a dichotomous outcome, in this case improvement *versus* no improvement [11, 12]. Similarly, the number needed to harm (NNH) is the number of patients who have to be treated for an adverse event to occur; i.e., how many patients will not develop the event for each patient who does. Low NNTs denote high efficacy (few patients need to be treated to obtain improvement in one patient). High NNHs indicate low toxicity (many patients can be treated for a single adverse event to occur). The advantage of NNTs and NNHs is that they can be used to compare effects across drugs from different trials. Improvement measures are only valid if they are adjusted to the background placebo effects. For instance, 60% of patients on either drug A or B can achieve ACR20 responses in a trial; however, the efficacy of the drug will be quite different if the ACR20 placebo response for drug A is 40%, and for drug B 20%. NNTs and NNHs take background placebo effects into account and are more appropriate for clinicians to comparing drugs tested in different trials, than just evaluating ACR improvement responses.

Starting DMARD therapy

One of the initial questions clinicians face is when to start DMARDs in a patient with newly developed RA, and how much of a delay in the onset of therapy has longer-term deleterious effects. There is ample evidence sustaining the beneficial effects of DMARD therapy in patients with RA. The major question is not whether to administer DMARDs, but when to initiate treatment and with which drug. About 10% of patients with polyarthritis experience a short illness that resolves and remains largely quiescent, and early treatment could unnecessarily expose them to adverse effects [13]. The rationale for early onset of DMARD therapy is well supported by clinical trials and cohort studies, and ACR guidelines for the management of RA state that the majority of patients with newly diagnosed RA should be started on DMARD therapy within 3 months of diagnosis [14].

Most RCTs comparing commonly used DMARDs to placebo have shown efficacy of the study drug, but in general, the follow-up in these trials is less than 12 months. Conceivably, patients receiving placebo for a short period of time could "catch-up" to patients treated earlier. Several studies have attempted to answer this question by examining the longer-term effects of lags in DMARD initiation, even for short periods of time [15, 16]. These studies have examined the long-term effects (> 12 months) of delayed therapy in patients receiving placebo. In most cases, the

delay was 6–12 months, and once the trial was completed, patients were initiated on DMARDs. One of the largest studies, including 440 patients with variable disease duration, showed that a delay in the onset of gold therapy was associated with a decrease in physical function after 5 years [17]. Another prospective follow up of 119 patients with early RA, originally included in a RCT of hydroxychloroquine *versus* placebo, found that a 9-month delay in instituting DMARD treatment had a significant detrimental effect after 3 years on pain and global well-being in the original placebo group [18]. In a trial comparing minocycline to a placebo, all participants were given DMARDs at the end of the 3 month study. After a 4 year follow up, eight patients who originally received minocycline were in remission compared to one in the placebo group ($P = 0.02$) [19]. As can be seen, these delayed effects occurred for DMARDs that are considered to be of only moderate efficacy. Studies using a more aggressive approach have shown more dramatic effects. In the COBRA combination RCT [20]. 155 patients with early RA were randomised to receive combined step-down prednisolone plus methotrexate and sulfasalazine, or sulfasalazine alone. The combined treatment group improved significantly at 28 weeks, but at 56 weeks, after prednisolone and MTX were discontinued, disease activity was comparable in both groups. Radiographic progression remained significantly lower in the combined group. A follow up study of the trial [21] showed that the patients initially randomized to the combination therapy still had lower rates of radiographic progression at 5 years, despite discontinuation of the COBRA regime. An additional trial, comparing treatment with a single DMARD to a combination regime in 195 patients [22], reported that a delay in therapy was the only significant predictor for remission in patients receiving a single drug; this was not observed in those receiving combination therapy, suggesting that aggressive therapy may be necessary for patients in whom DMARD onset has been delayed.

Deciding which DMARD: efficacy and effectiveness

Efficacy studies are RCTs designed to demonstrate whether a drug can work in selected populations, in controlled environments. Because of differences in patient characteristics, study design, and placebo response rates, it is difficult to compare trials of different drugs. Systematic reviews and meta-analyses can assist in these comparisons by pooling the data, while controlling for confounders and adjusting for placebo effects. The few systematic reviews that have compared various traditional DMARDs have not shown dramatic differences among the drugs [23–26]. In clinical practice, the choice of an initial DMARD often remains a matter of personal preference. Although the efficacy of methotrexate and sulfasalazine appears to be similar in RCTs [27, 28], methotrexate remains the preferred choice in DMARD-naïve patients in the US, while sulfasalazine therapy is more widespread in Europe. In general, using indirect comparisons, no major differences are

observed between traditional DMARDs when adjusting for placebo effects with measures such as NNT. Only biologic agents appear to offer some advantages over other drugs in short-term studies. These benefits seem more pronounced in patients with longer disease duration who have failed other therapies. For DMARD-naïve patients, although some significant differences have been observed in the rate of radiological progression when comparing traditional DMARDs with biologic agents, other clinical differences are small [29, 30]. Table 2 shows NNTs for selected RCTs of DMARDs and biologic agents [25, 31]. The table is not comprehensive and only provides data on a few selected trials as illustrations, but as can be seen, even with biologic therapies at least half of the patients with RA do not show a minimal clinically relevant response (ACR 20) that can be attributed to the drug: a NNT of 2 is interpreted as only one out of every two patients improving because of treatment.

While clinical trials assess efficacy, observational studies evaluate effectiveness, not only if a drug can work, but also if it indeed works when used in the community at large. Cohort studies also provide the best evidence for assessment of toxicity. Long-term comparative cohort studies of traditional DMARDs have shown that methotrexate is the most effective drug in terms of discontinuation rates [25, 32–34]. More patients stay on methotrexate than on any other DMARDs, although after 5 years, approximately half of them have discontinued treatment. No sufficient data has been gathered so far to adequately evaluate the long-term effects of biologic agents compared to other DMARDs, although several countries and institutions have established registries which will provide much-needed information within the next few years.

Individual considerations

It is clear that the treatment of RA has significantly improved in the past decade. Patients are treated earlier and more aggressively, and new drugs are being developed at a fast pace. Despite these advances, the majority of patients continue to progress, and unfortunately, we are still unable to explain why some individuals respond to a given drug while others do not. Careful consideration of benefits and risks is necessary to target treatment, to maximize efficacy with minimum toxicity, and if possible to control costs. Up until now, it has been much easier to identify patients at risk for toxicity than patients at risk of therapeutic failure because of lack of efficacy. It is clear that one of the major determinants of risk for any drug therapy is comorbidity, but this topic goes beyond the scope of this Chapter; suffice it to state that the risk–benefit ratio of any intervention must be carefully considered on the basis of the patient's baseline risk for complications. In addition, careful attention should be given to possible interactions and pharmacokinetics when combinations of drugs are to be used [35].

Table 2 Number needed to treat (NNT) for selected clinical trials

Author (reference)	Active Treatment	Comparator	Criteria for NNT	NNT (95% CI)
Single DMARDs				
Esdaile et al [102]	HCQ	Placebo	Paulus criteria at 36 weeks	4.6 (2.5–25.6)
Smolen et al [103]	SSZ	Placebo	ACR20 at 24 weeks	3.7 (2.5–6.7)
			ACR 50 at 24 weeks	
Strand et al [104]	MTX	Placebo	ACR20 at 12 months	5 (3.2–11.1)
			ACR50 at 12 months	6.7 (4.3–12.5)
			ACR70 at 12 months	20 (NS)
Williams et al [105]	MTX	Placebo	Paulus criteria at 18 weeks	2.9 (2.1–4.9)
Osiri et al [106]	Leflunomide	Placebo	ACR20 at 12 months	3.9 (2.7–5.5)
			ACR20 at 12 months	3.9 (2.9–5.6)
			ACR20 at 12 months	6.3 (4.5–11.1)
DMARD Combinations				
Boers et al [20]	Combined step-down prednisolone + MTX + SSZ	SSZ	ACR20 at 28 weeks	4.4 (2.6–12.5)
Ferraccioli et al [107]	Combined step-up MTX + CsA + SSZ	SSZ	ACR20 at 18 weeks	1.5 (1.2–2.0)
Dougados et al [28]	Combined step-up SSZ + MIX	SSZ	ACR20 at 52 weeks	20 (NS)
Haagsma et al [27]	Combined SSZ + MTX	SSZ	ACR20 at 52 weeks	25 (NS)
		MTX	ACR20 at 52 weeks	16.7 (NS)
Biologic Agents				
Early RA				
Bathon et al [30]	Etanercept 25mg	MIX	ACR20 at 52 weeks	5 (3.4–8.3)
			ACR20 at 52 weeks	20 (NS)
			ACR20 at 52 weeks	33 (NS)
Genovese et al [108]	Etanercept 25mg	MIX	ACR20 at 2 weeks	7.7 (NS)

Table 2 (continued)

Author (reference)	Active Treatment	Comparator	Criteria for NNT	NNT (95% CI)
Established RA				
Maini et al [109]	Infliximab 3 mg/kg q 8 wks + MTX	*MIX*	ACR20 at 30 weeks	3.3 (2.3–6.3)
			ACR20 at 30 weeks	4.5 (3.1–9.1)
			ACR20 at 30 weeks	12.5 (6.7–50)
Lipsky et al [110]	Infliximab 3 mg/kg q 8 wks + MIX	MIX	ACR20 at 54 weeks	4 (2.6–8.3)
			ACR20 at 54 weeks	12.5 (6.7–100)
Weinblatt et al [111]	Adalimumab 40 mg + MTX	MIX	ACR20 at 24 weeks	1.9 (1.5–2.6)
			ACR20 at 24 weeks	2.1 (1.6–3.0)
			ACR20 at 24 weeks	4.5 (2.9–10)
Moreland et al [112]	Etanercept 25 mg	Placebo	ACR20 at 6 months	1.6 (1.4–2.0)
			ACR20 at 6 months	2.2 (1.8–3.0)
			ACR20 at 6 months	5.6 (3.7–11.1)
Weinblatt et al [113]	Etanercept 25 mg + MTX	MIX	ACR20 at 24 weeks	2.2 (1.6–40)
			ACR20 at 24 weeks	2.8 (2.0–45)
			ACR20 at 24 weeks	6.7 (3.8–20.0)
Cohen et al [114]	Anakinra 1 mg/kg/d + MTX	MIX	ACR20 at 24 weeks	5.3 (2.7–50)
			ACR20 at 24 weeks	5.0 (3.1–143)
			ACR20 at 24 weeks	10.0 (5.3–50)

NS = not significant; MTX = methotrexate; SSZ = sulfasalazine; CsA = cyclosporine

Adapted from Suarez-Almazor ME, Osiri M, Emery P, (2004) Ottawa Methods Group. Rheumatoid arthritis. In: Tugwell P, Shea B, Boers M, Brooks P, Simon LS, Strand V et al (eds) Evidence-based rheumatology. BMJ Publishing Group, London, 243–314

This section will describe those factors that have been associated with either prognosis or response to therapy, which could be considered when targeting specific DMARD therapy to individual patients.

Prognostic factors

Several clinical and laboratory parameters have been associated with prognosis in RA in follow-up studies of inception cohorts [36–58]. Clinical characteristics associated with poor outcomes include persistent symmetrical polyarthritis at onset, rapid development of radiographic erosions, and systemic manifestations such as subcutaneous nodules or vasculitis. Laboratory and genetic markers include positive rheumatoid factor (RF), anti-cyclic citrullinated peptide antibody (anti-CCP), and presence of the HLA–DR4 shared epitope. A number of other genetic markers have been associated with disease severity in a few studies, but the evidence remains inconclusive. Unfortunately, the predictive value of these factors, alone or in combination, remains low, and it is challenging to predict the individual disease course or the response to treatment on the basis of baseline characteristics. Of the various measures, the most predictive ones remain the clinical parameters of progression derived from observation, such as persistent synovitis, and the development of early erosions. Single laboratory parameters associated with prognosis are not discriminative enough. For instance, most studies evaluating RF show increased relative risks or odds ratios ranging between 2 and 6, which denotes a moderate association, but can result in important misclassification for prognostic groups. In addition, the association is largely based on radiographic damage, and is weaker when clinical outcomes are considered. Similarly, the relationship between the shared epitope and outcome is largely variable across disease groups, and its predictive value in unselected populations of RA patients is low [59]. Visser et al. developed a clinical predictive model for early inflammatory arthritis on the basis of seven clinical and laboratory parameters including morning stiffness for more than an hour, three or more swollen joints, metatarsophalangeal (MTP) pain, erosions, RF, and anti-CCP [41]. The model prospectively discriminated between self-limiting, persistent non-erosive and erosive arthritis after 2 years of follow-up. Nevertheless, the variables in this model are clinically accessible, and it would be interesting to know if this predictive algorithm performs significantly better than physicians' judgment and *gestalt*.

Predictors of therapeutic response

As difficult as predicting prognosis in RA may be, even more challenging is predicting the response to a given drug before the treatment has been initiated. Unfor-

tunately, the best marker of response remains observation through trial and error. In general, for most patients, response to a DMARD occurs within the first months of therapy, and it is unlikely that a patient will respond favorably if there has been no benefit after a trial of a few months. Although not sufficiently predictive, some markers have been associated with the likelihood of response. Rheumatoid factor is not conclusively related to clinical response to DMARDs, but observational cohort studies, in which invariably all patients are treated, show that patients with positive RF have worse outcomes, especially radiological, which indirectly suggests that response to DMARDs is weaker in seropositive patients. The same arguments can be used for anti-CCP, although additional long-term data is needed.

Pharmacogenetics

The recent growth in the field of pharmacogenetics offers exciting opportunities [60]. Drug advances in the treatment of RA in the past decade have been mostly dependent on the development of biologic agents which target specific paths in the pathogenesis of the disease, as reviewed in other Chapters. Although biologic therapies appear to be more effective than traditional DMARDs, many patients still do not respond to these agents. It is also unclear whether patients who do not respond to agents with specific targets (e.g., anti-tumor necrosis factor, –TNF-α) may obtain benefit with therapies aimed towards other cytokines or biologic markers. Ideally, if it were possible to identify those patients who may respond to given therapies, or develop toxicities, through the analysis of haplotypes and polymorphisms, tailor-made drug therapy could be administered to maximize benefits and minimize risks.

The most studied genes in RA are those in the HLA region, yet the role of the shared epitope in therapeutic response remains somewhat debatable. In the MIRA trial comparing minocycline to placebo, an interaction between the presence of the shared epitope and treatment group was observed [61]. In the group treated with minocycline, no differences were observed in radiological progression between DR4 positive and negative patients. However, in the placebo group, a gradient was observed, with increased radiological damage according to the allele dose (none, heterozygous, and homozygous). In a trial comparing the combination of methotrexate, sulfasalazine, and hydroxychloroquine with methotrexate alone [62], a differential response was also observed. Patients with the shared epitope had a better response to combination therapy compared to methotrexate alone, which accounted for most of the positive results of the trial, while negative patients has similar responses to either treatment. In a study investigating the effect of early *versus* delayed DMARD therapy in cohorts of patients with early RA, including patients originally involved in the COBRA trial [20], the investigators assessed the

relationship between having a shared epitope allele, and the effect of delaying DMARD therapy [63]. In the cohort study, the presence of shared epitope alleles did not affect the radiological progression of patients treated early with DMARDs, but in those with delayed treatment, it was associated with a higher damage score. In the COBRA follow-up, the combination treatment group had a lower rate of radiographic progression regardless of the shared epitope status, but for those treated with sulfasalazine alone, increased progression was observed in those carrying the allele [64]. These findings suggest that there is a window of opportunity for aggressive treatment, after which having shared epitope alleles decreases the response to therapy.

Increasingly, markers for response to anti-TNF-α therapy are being investigated, with receptor polymorphisms being the logical candidates. In one study, patients who responded to anti-TNF-α therapy showed different frequencies of specific polymorphisms: Responders more often carried TT genotypes (*versus* GG and GT) than non-responders, but the difference was only moderate (38 *versus* 11%) [65]. In other studies, different genotypes have been associated with response [66, 67]. Combination of alleles related to interleukin-10 (IL10) and transforming growth factor (TGFB1) have been related to non-responsiveness [66]. Differences in response have also been observed for haplotypes combining different HLA alleles and TNF and DS6 microsatellites [67]. Although the data is scarce, the shared epitope does not appear to have to an effect in the efficacy of TNF blockade [67].

Genetic markers have also been associated with response to and toxicity of methotrexate in RA. Specific polymorphisms in thymidylate synthetase and 5,10-methylenetetrahydrofolate reductase, enzymes involved in folate metabolism, are increased in patients with beneficial responses, and/or toxicities who receive methotrexate [68, 69]. The relative risks, however, are small, ranging between 1.5 and 2, which limits the predictive usefulness of this marker in clinical practice.

The field of pharmacogenetics is rapidly evolving and brings hope that new findings may be applicable to the clinical management of patients with RA. As single genes and polymorphisms are identified, they may not be highly predictive on their own. However, combinations of genetic markers, disease activity parameters, and clinical features may eventually result in useful algorithms to tailor specific therapies to individual patients.

Patient preferences and shared decision-making

Patient preferences and individual tolerance of risk are crucial components in the choice of DMARD therapy. Increasingly, patients want to be active participants in decision-making processes about their health. Shared decision-making requires that patients become informed, gain an understanding of potential harms and benefits, and ultimately make choices consistent with their personal values. A study in

patients with RA showed that 89% of them wanted full disclosure about therapeutic options and potential risks, but the study did not specifically examine preferences for shared decisions [70]. The Centers for Disease Control and Prevention defines informed decision-making as occurring when the individual: a) understands the nature of the disease; b) understands the clinical service and its likely harms and benefits; c) considers his or her preferences as appropriate; d) participates in the decision at a personally desirable level, and e) either elects to make a decision consistent with his or her values, or defers the decision to a later time [71].

Unfortunately, extensive empirical evidence shows that individuals have difficulty processing probabilistic information about risks and benefits. The framing of the alternative choices may have a major impact on their decisions, not necessarily reflecting informed, rational preferences. How individuals "trade-off" multiple uncertain outcomes, such as risk and benefits, in their decisions remains unclear, although various models have been proposed. Compensatory strategies trade-off low values in some attributes for high values in others. It is likely that different people use different strategies. A major determinant of preferences is how the problem is presented in relation to potential gains or losses. In a classical experiment by Tversky and Kahneman, respondents were presented with two differently worded scenarios, but with identical expected outcomes [72]. The problem stated that an Asian disease was expected to kill 600 people. In the first scenario, respondents had to choose between program A, which would save 200 people, or program B, with which there was a one-third probability that 600 people would be saved, and two-thirds probability that no people would be saved. Most respondents (72%) were risk averse, and chose program A. When the framing was modified to reflect losses, the results shifted. Respondents were asked to choose between program C, with which 400 people would die, or program D, with one-third probability that nobody would die and two-thirds probability that 600 would die. In this frame, 78% of respondents were risk seeking and chose program D. Fraenkel et al. have shown that patients with RA are typically risk adverse [73–75]. In one study, 66% of patients refused to accept a risk of cancer of 1 in 100,000 persons for a hypothetical treatment [74]. Although hypothetical choices may differ from real life decisions, these studies show that framing of DMARD alternatives with an emphasis on benefits or an emphasis on risks, may influence patient preferences.

Decision aids have been proposed and developed to promote informed, shared decision-making [76, 77]. These aids use a variety of media, from self-directed educational booklets, to interactive, computer-based decision support systems. The purpose of these aids is to provide information and assist in therapeutic decisions following a systematic approach that presents multi-attribute alternatives in a more understandable, graphic way. These decision aids are increasingly being used in research, and the ultimate goal is that they become integrated into clinical practice in the near future.

The economic perspective

Rheumatoid arthritis has major socioeconomic impacts for the patient and for society at large because of its long-term disability and its prevalence in the population. The lifetime cost of RA has been estimated between US$61,000 and US$122,000 [78–80]. Annual direct costs originated specifically through health services utilization range between $3,000 and $10,000 [80–83]. Most studies have shown that the highest component of direct costs, up to 50–60%, can be attributed to hospital admissions, although only a minority of the patients are ever hospitalized [80, 81, 84, 85]. Drug therapy costs constitute 25–40% of the direct costs, and include primarily DMARD costs [80, 81, 83, 86]. Nevertheless, in RA, indirect costs from work disability and productivity losses exceed direct costs, comprising 50–75% of the total costs [80, 87–90]. In the first 5 years of disease, work disability reaches 15–30%, progressively increasing thereafter [91, 92]. In addition, employed patients with RA miss on average three workdays per month [91, 93]. Disability and disease activity are the major determinants of costs in this disease [82, 90, 94].

Most of the published costing studies were conducted before the development and marketing of biologic agents. These drugs are more expensive, and it has been estimated that the annual cost of therapy for a RA patient receiving biologic agents is US$19,000, compared to US$6,000 in patients receiving traditional DMARDS [95]. Yet, if these therapies show greater effectiveness in the longer-term, they could potentially become cost-effective by decreasing the total costs of RA, which are driven primarily by long-term disability [96, 97].

Economic evaluations of biologic agents have been based on the results of clinical trials, which are not always generalizable to the population of patients at large. No study has evaluated the potential implications of administering targeted therapy to subgroups of patients, but given the cost of therapy, if those patients who would benefit the most can be identified and treated, the potential for cost-savings would be considerable.

The future

As medical decisions become more complex and offer more alternatives, informed choices based on careful probabilistic considerations and critical thinking become operationally challenging for policymakers, physicians, and patients. Physicians largely base their decisions on clinical experience (clinical heuristics). Because of the inherent biases in this approach, policymakers and professional organizations have encouraged the use of clinical practice guidelines to maximize effectiveness, efficiency, and quality of care. Yet guidelines are often disliked by clinicians, who feel they provide "cookbook" advice with little attention to the individual patient. With the exponential growth of computational methods and informatics, predictive tools

to be used for individual patients can become a reality. Real-time computer-based decision-support systems for medical decision-making, such as neural networks and nomograms, may become standard in clinical settings. Neural networks are artificial intelligence tools that are becoming increasingly widespread across a number of disciplines [98–100]. They are particularly well suited to the needs of health decision-making, because they perform multifactorial analyses well, and medical decisions are seldom made on the basis of a single factor; rather, they are almost always made in situations where multiple factors must be weighed against each other. With the increasing number of medical databases and the identification of new biologic markers, "smart computing" in decision-making is becoming a reality. Nomograms are devices based on algorithms and mathematical modeling, which predict probabilities of outcomes. They are increasingly being developed to provide decision-support using friendly interfaces, such as personal computers or personal digital assistants (PDAs). Several examples of nomograms to assist decision-making about interventions with high risk–benefit ratios have been published in oncology [101]. To provide evidence-based decisional support, several conditions must be met: scientific validity of the data used in the model; statistical robustness of the predictive model; incorporation of uncertainty parameters in the decision processes; inclusion of patient preferences; and user-friendly interfaces. This field is evolving, largely influenced by mathematical and computational advances in decision-making. Today, these advances support systems unthinkable a decade ago. We forecast that these developments will lead health decisions in the future, and will open exciting opportunities to combine clinical and genetic data, in addition to careful consideration of patient preferences, and will assist us in effectively and efficiently targeting DMARD therapy in patients with RA.

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Pharmacoeconomic properties of disease-modifying antirheumatic drugs

Andreas Maetzel[1] *and Daniel H. Solomon*[2]

[1]Division of Clinical Decision Making, Dept. of Health Policy, Management and Evaluation (AM), University Health Network, University of Toronto, 200 Elizabeth Street EN6-232A, Toronto, Ontario, Canada M5G 2C4; [2]Division of Pharmacoepidemiology and Pharmacoeconomics (DHS), Division of Rheumatology, Immunology, and Allergy (DHS), Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02120, USA

General concepts

The discipline of pharmacoeconomics has gained in importance in recent decades as countries and healthcare payers face pressures to justify investments in healthcare innovations. This situation has largely resulted from a coincidence of a demographic shift towards an increasingly elderly population and the development of effective and much more expensive therapies based in biotechnological advances. Many of these therapies improve health and may increase productivity and possibly prevent costly complications. Consequently health expenditures, previously considered "expenses", are now more frequently referred to as "investments" to maintain a healthy and productive workforce [1]. Demonstration of "value for money" has become an important milestone for therapies on their way to rapid, and reimbursed, adoption.

Pharmacoeconomic evaluation uses the tools of decision analysis to build an evaluative framework to explicitly consider all data that are of relevance for those involved in the decision-making processes. Any statistical software, such as SAS®, S-Plus®, or high-level programming languages can be used to build an evaluative framework and run simulations. More user-friendly and intuitive applications have been developed for representing the outcomes associated with decisions. Software packages include, among others, DATA Pro® by Treeage (www.treeage.com), Decision Maker (http://infolab.umdnj.edu/windm), or Crystal Ball® (www.decisioneering.com), a Microsoft Excel® add-in.

In day-to-day healthcare, decision-making processes generally involve an "informal" consideration of relevant and available information, including that from randomized controlled trials and observational studies, knowledge of physiological and technological processes, knowledge of the particular patient, and precious knowl-

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edge taught by experience and through exposure to master clinicians. Decision analysis expands this into a "formal" representation of such information by numerically addressing the uncertainty inherent in both evidence and knowledge [2–6]. The consequences of decisions can be examined in a decision tree or an influence diagram, where probabilities are assigned to intermediate and final consequences and values are associated with the outcomes. In the final steps of a decision analysis, the values attached to each individual outcome are multiplied with the probabilities of the paths leading to each outcome and the resulting expected (weighted) values for the paths are added to obtain the expected value of the relevant clinical strategy. Clinical decision analysis stops here and considers the decision with the "better" expected value as the one to recommend, or as the "preferred" decision.

Cost-effectiveness analysis further expands on decision analysis and engages in a comparison of the expected "costs" of each decision and their expected "effectiveness" represented by the expected values discussed below. Typically, cost effectiveness looks at the additional health benefits (effectiveness) that can be bought when spending more money on an intervention that costs more than standard practice. The resulting cost-effectiveness ratio gives an explicit account of whether a new intervention provides "value for money" and may assist decision-makers in formulating conditions for regulatory approval and reimbursement by payers.

Any value system can be used to determine the final outcomes as long as the values matter to the subject whose perspective is represented by the decision tree. For example, patients and clinicians are foremost interested in hard clinical outcomes, such as a cure, life-years gained, deaths avoided, or clinical remission. Patients and clinicians may additionally be interested in disease severity, as described for example by a 100 cm visual analogue scale (VAS) or scores on some disease-specific questionnaire. Policymakers are generally interested in costs and, for reasons of equity and distributive justice, in society's valuation of the health states in some general terms – for example generic quality of life as perceived for certain disease states by the general population.

Expressing health benefits as natural units has some advantages, as these are understandable to many, including decision-makers; examples in rheumatoid arthritis (RA) include: remission, responder defined as improvement of greater than 50% in American College of Rheumatology (ACR) composite criteria, a patient achieving full productivity, or a serious adverse event. For the purpose of most pharmacoeconomic evaluations, health benefits are aggregated into a single quality of life measure, to capture both positive and negative events in one measure. A crucial advantage of this representation of health is the ability to describe a disease state in terms of its "full health" equivalent with a generic numbering system that can be applied to many diseases, thus making benefits more comparable. For example, patients report that, for them, spending 10 years with RA would be equivalent to spending 8 years in full health, i.e., 10 years with RA are worth approximately eight Quality-Adjusted Life-Years (QALYs) [7]. In other words, patients with RA, on

average, would be willing to give up 2 years of a 10-year life expectancy to avoid living with RA. These values can be derived either from surveying patients directly (clinician or patient perspective) or by asking non-affected individuals how they feel about typical patient health states (societal, i.e., policymaker's perspective). Gains in QALYs are then generally used to summarize the effects of new interventions. For example, a new intervention may increase a patient's quality of life by a tenth of a QALY, that would be 36.5 quality-adjusted life-days, or a bit more than a month of full-health equivalent gained when compared to life with the standard intervention.

For illustration purposes, suppose a new intervention with an annual cost of US $16,000 has been evaluated and the cost savings and health gains occurred as outlined in Table 1. The cost-effectiveness of the new intervention, for a 1-year period and from the perspective of society, would be $10,000 divided by 0.25, or $40,000 per patient achieving remission. With health gains expressed in terms of QALYs, the cost-effectiveness would be $10,000 divided by 0.1, which is $100,000 per QALY gained. The budget of a health plan is not affected by improvements in productivity, thus the perspective changes slightly and the cost-effectiveness would be $12,000 divided by 0.1 or $120,000 per QALY gained.

In the example shown in Table 1, achieving a remission in one out of four patients, or a QALY gain of 0.1, is clinically extremely relevant. But is the new intervention worth the extra cost? There is very little guidance on the choice of appropriate thresholds for cost-effectiveness analyses. A value of $50,000 per QALY is often cited as a threshold below which therapies can be considered cost-effective [8], however, persistence of this same threshold to date shows that it has not kept pace with inflation [9]. Moreover, many therapies are reimbursed with thresholds that far exceed $50,000 per QALY. Experience with decision-makers shows that reimbursement decisions are largely driven by the clinical gains achieved with a new treatment [10, 11]. Pharmacoeconomic considerations will continue to play a role in the decision-making process when the clinical benefit is equivocal and the cost-effectiveness ratio exceeds $100,000 per QALY gained, which some may consider being at the margin of acceptability. Reimbursing a new drug based on a higher cost-effectiveness ratio, for example $200,000 per QALY, may require proof of strong clinical benefit in populations of need, such as patients with rare diseases or diseases for which no powerful therapies were available up to this point.

Pharmacoeconomic evaluation of DMARDs in RA

As discussed above, explicit pharmacoeconomic evaluation is performed on the basis of a schematic representation of the disease, i.e., a simulation model that permits evaluation of various treatment and decision scenarios. Two commonly used representations are shown in Figure 1 and Figure 2. Most disease models represent the disease by using descriptors, such as health or disease status, including func-

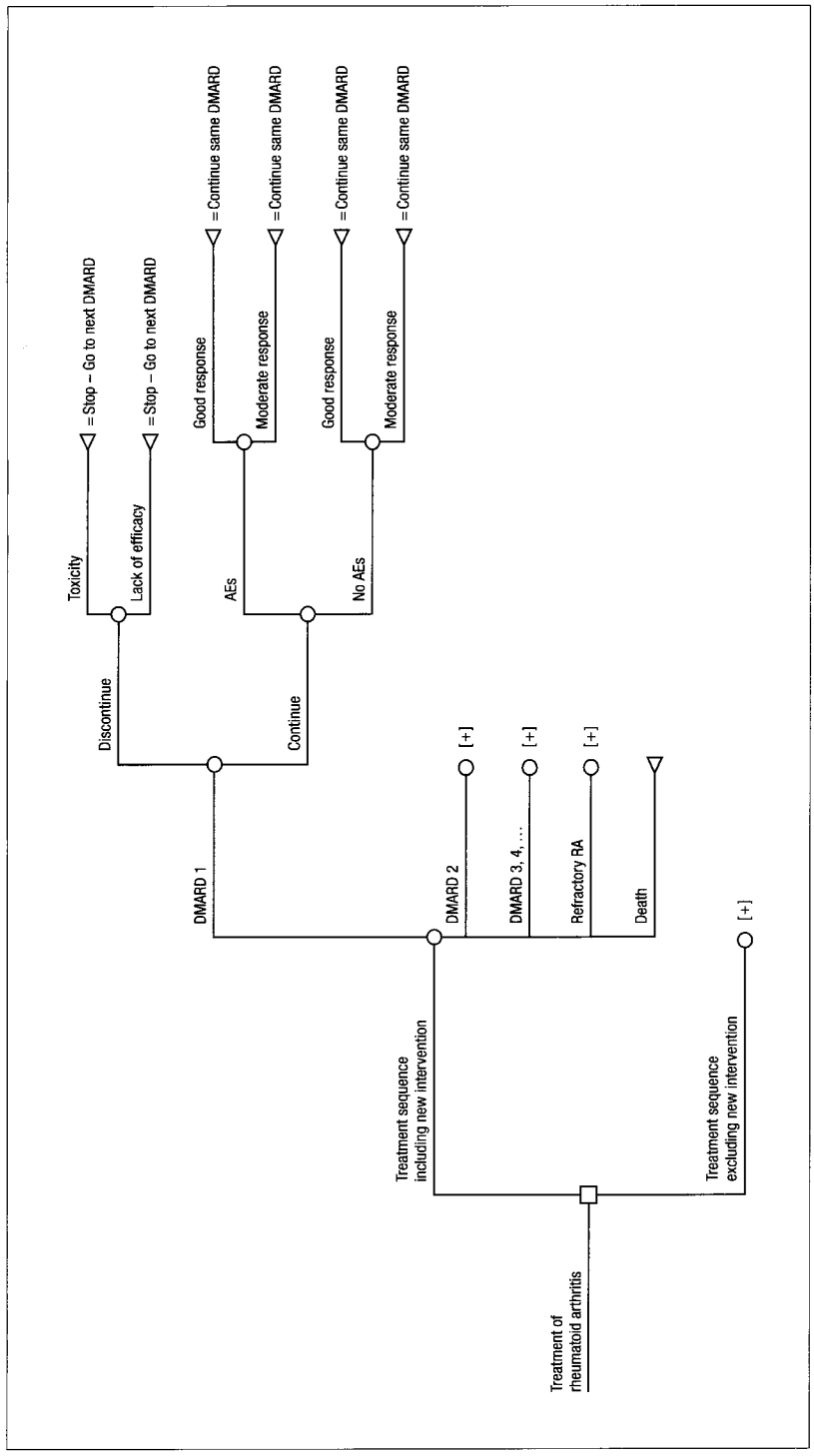

Figure 1
Possible representation of the course of RA including a sequence of DMARD regimens
Good/Moderate response can be qualified according to ACR criteria for 20%/50% and 70% improvement, or according to WHO/ILAR classification of good/moderate response. (AE = adverse event; DMARD = disease-modifying anti-rheumatic drug)

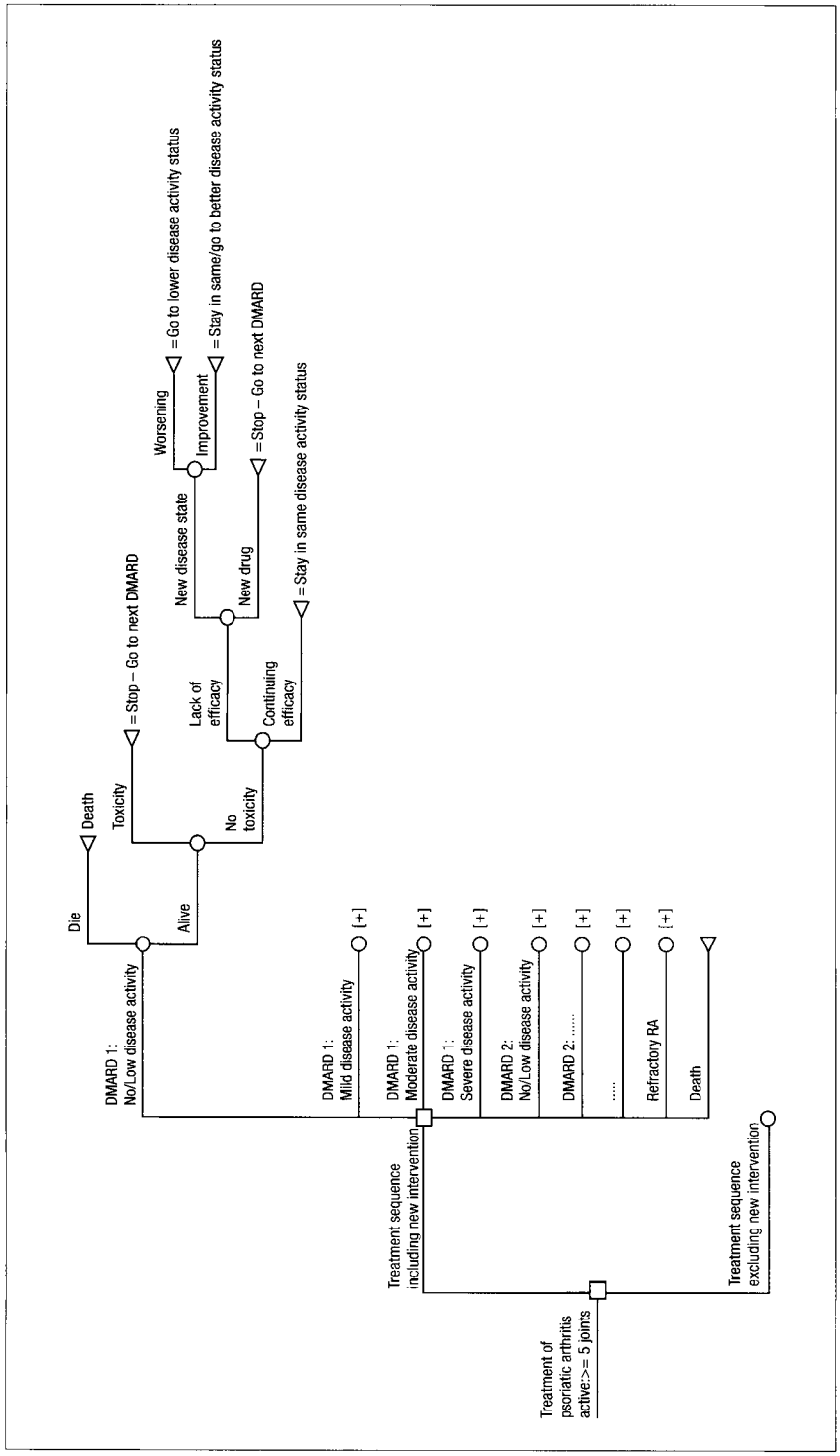

Figure 2
Possible representation of the course of RA including a sequence of DMARD regimens
Disease activity can be classified according to health assessment questionnaire or other health status indices of relevance for RA.

Table 1 - Example cost-effectiveness data of a 1-year intervention

	New Intervention	Standard Intervention	Difference
Costs			
Medical care costs (except drug)	$3,000	$5,000	–$2,000
Drug costs	$16,000	$1,000	$15,000
Non-medical care costs	$1,000	$2,000	-$1,000
Healthplan Total:	*$20,000*	*$8,000*	*$12,000*
Productivity costs	$3,000	$5,000	–$2,000
Societal Total:	*$23,000*	*$13,000*	*$10,000*
Effectiveness			
% of patients in remission	25%	0%	25%
quality-adjusted life-years (QALYs)	0.9	0.8	0.1

*cost savings are negative, expenses positive

$$\text{Cost-Effectiveness ratio: } \frac{\text{(Cost New – Cost Standard)}}{\text{(QALY New – QALY Standard)}}$$

$$\text{Healthplan perspective: } \frac{(\$20{,}000 - \$8{,}000)}{(0.9 - 0.8)} = \frac{\$12{,}000}{0.1} = \$120{,}000 \text{ / } 1 \text{ QALY}$$

$$\text{Societal perspective: } \frac{(\$23{,}000 - \$8{,}000)}{(0.9 - 0.8)} = \frac{\$10{,}000}{0.1} = \$100{,}000 \text{ / } 1 \text{ QALY}$$

tional status, degree of response to treatment, partial and full remission, and on/off-therapy status necessitating switches in therapy, due to lack of efficacy, or serious adverse events. Less often considered are events such as the need for reconstructive surgery or rare events, including deaths attributable to the disease. A certain level of abstraction is required in representing a complex disease, such as RA, in a disease simulation model. Model design, the combination of evidence from a variety of sources, and the extrapolation that usually comes with disease simulation, can lead to subtle variations among disease models and consequently differences in results [10].

Standard methods for pharmacoeconomic evaluations were recommended by the US Public Health Service Panel on Cost-Effectiveness in Health and Medicine [12]. These standards were recently refined for the evaluation of interventions in RA (Tab. 2) [13, 14]. Key take-home messages of the recommendations are to perform pharmacoeconomic evaluations with certain sets of assumptions regarding the time horizon, extrapolations, choice and inclusion of costs and effectiveness variables, choice and quantitative description of comparator, choice of therapeutic strategies and description of the population chosen for the analysis (Tab. 2). Recommendations for the critical appraisal of economic evaluations were also published [15, 16] (Tab. 3) and it is recommended to get acquainted with the basic methodological principles of pharmacoeconomic analysis to approach such studies with an appreciation of the underlying methodologies.

Of particular importance for RA models is the fact that therapy is continuous and that patients often require changes of DMARD regimens. Biologic response modifiers, such as TNF-antagonists, are generally not approved as first-line agents, but clearly have a role in patients who fail methotrexate, for instance. Thus, the incremental cost-effectiveness of new DMARDs can be evaluated as the only DMARD [17, 18] or as part of a sequence of DMARDs [7, 19]. When evaluated within a sequence, the cost-effectiveness ratio is a property of the sequence, for example the one including TNF-antagonists *versus* one excluding them. It may therefore be influenced by the combination and place of the other DMARDs and clearly by the way the evaluated DMARD is modeled to change the disease course.

The disease itself can be represented by relative events, such as response to therapy and improvement in health status that comes with it. However, it has been recognized that absolute representations of the course of the disease, in terms of disability indices, such as scores in the health assessment questionnaire (HAQ) [20], radiological indices or in terms of disease activity scores, such as the DAS28 [21], are more suitable to model the disease course over time. The HAQ and DAS28 have been well described and are now used more often in pharmacoeconomic evaluations [17–19]. It is questionable, though, whether either index is suitable to model long-term projections of the influence of new DMARDs over the course of the disease. Most likely, both measures are more directly related to changes in disease activity, rather than structural changes. Structural changes either captured by radiological

Table 2 - Reference case recommendations for economic evaluations in RA (adapted from [13], with permission

Methodological element	Recommendation
1. Study horizon	Trial based analysis, minimum 1 year; Model based analyses, minimum 5–10 yrs
2. Duration of therapy	Continuous
3. Extrapolation beyond trial duration	Report clinical trial data alone and extrapolate (model) using a synthesis of evidence from observational studies, trials, and other sources with sensitivity analysis (minimize use of expert opinion)
4. Modeling beyond therapy	No additional benefit or harm after therapy is stopped
5. Synthesis of comparisons where head-to-head trials do not exist	Synthetic comparisons by using relative effects from controlled trials
6. Clinical outcome measures	Joint count, pain by VAS, physical measure of function (e.g., HAQ), measure of inflammation (CRP/ESR), HRQoL, Toxicity (report adverse events with patients as the unit of analysis)
7. Mortality	Hazard rates for mortality from observational studies
8. Valuation of health states (e.g., QALY)	Patients' values for clinical choices, general population's values for health policy decisions
9. Resource utilization	Include all associated direct medical and non-medical costs in the analysis, but report indirect costs (productivity losses) separately. When estimating mean costs in the presence of censoring due to discontinuation of therapy, adjust using appropriate statistical methods to allow for unequal exposure to risk of resource use
10. Discontinuation of therapy	Use discontinuation rates from trials, adjusted using observational data
11. Therapeutic sequence	Include modeling of most commonly used therapeutic sequence with sensitivity analysis to consider other strategies
12. Population risk stratification	Include clear definition of underlying population including low and high risk groups

indices or by phenotypic descriptions of disease expression have not been employed in disease models of RA. The relationship of disease activity measures to structural progression may be important to establish in order to provide valid long-term pro-

Table 3 - Users' guides for economic analysis of clinical practice (adapted from JAMA (1997) 277: 1802–1806.

Are the results valid?
- Did the authors provide a full economic comparison of health care strategies?
- Were the costs and outcomes properly measured and valued?
- Are estimates of costs and outcomes related to the baseline risk in the treatment population
What were the results?
- What were the incremental costs and outcomes of each strategy?
- Do incremental costs and outcomes differ between subgroups?
- How much does allowance for uncertainty (sensitivity analyses) change the results?
Will the results help in caring for my patients?
- Are the treatment benefits worth the harms and costs?
- Could my patients expect similar health outcomes?
- Could I expect similar costs?

jections of the course of the disease. These also need to be reconciled with real-life experiences, which often document effectiveness at a fraction of that observed in randomized clinical trials [22, 23]. The establishment of registries with standardized documentation of disease outcomes will go a long way towards reconciling real-life experiences with findings from randomized clinical trials. ((Tab. 4))

Economic sequelae of rheumatoid arthritis

Pharmacoeconomic evaluations attempt to evaluate to what degree new agents are able to offset the cost of caring for the disease. An understanding of the cost of illness (COI) is therefore important to bring perspective to the pharmacoeconomic value of new agents. Several COI studies have been conducted over the past 20 years to estimate the annual costs of RA (Fig. 3) [24]. The results of such studies are needed to inform policymakers about the size of the potential economic impact that a disease may have at a national level. Obtaining accurate estimates, however, may be hampered by methodological difficulties pertaining to: 1) disease definition and sampling of patients for studies; 2) comprehensiveness of data capture; 3) attribution of costs to target disease and other comorbid conditions, and; 4) valuation of productivity losses.

Table 4 - Components of the cost of rheumatoid arthritis

Types of cost	Definition	Examples
Direct medical costs	Resources directly related to the care of rheumatoid arthritis	Costs for drug treatments, laboratory tests, visits to physicians or nurses, hospitalizations, surgical procedures, durable medical equipment, rehabilitation services
Direct non-medical costs	Resources related to non-medical issues arising because of rheumatoid arthritis	Costs of child care during a physician visit or hospitalization
Productivity costs*	Resources related to lost wages because of rheumatoid arthritis	Costs of disability (temporary, partial, or permanent), costs of missed work because of treatments

**These costs are sometimes termed "indirect" costs.*

Many COI studies of arthritis conditions have relied on national surveys to estimate the costs of disease; however several recent COI studies in RA have used consecutive samples recruited from healthcare providers. Yelin and Wanke used the University of California at San Francisco RA Panel Study, which followed 1,156 patients with RA recruited from random samples of Northern California rheumatologists [25]. These patients were followed for 14 years; 511 of these patients provided information for the economic evaluation in 1996. Patients underwent a comprehensive interview process to recall the use of health resource during a year prior to the interview. Overall, annual 1996 medical costs totalled $8,500 dollars, of which $5,900 were incurred for RA. Newhall-Perry and colleagues recruited 150 consecutive patients with new onset RA (< 1 year) through the Western Consortium of Practicing Rheumatologists [26]. Patients' HAQ score was identical to the average HAQ score reported for the RA panel survey patients at baseline. In this study, direct annual costs (1994 dollars) were estimated to be $4,400, of which $2,400 were incurred for RA. In a recent systematic review, the mean annual direct costs of patients with RA were found to be $5,800 (1996 US dollars) [24], a figure between the estimates of the studies cited above. Estimates for the proportion of total medical costs attributable to RA vary from 55–70% [27]. Thus, national forecasts of the total economic burden of RA need to account for the role of comorbidities among the total costs.

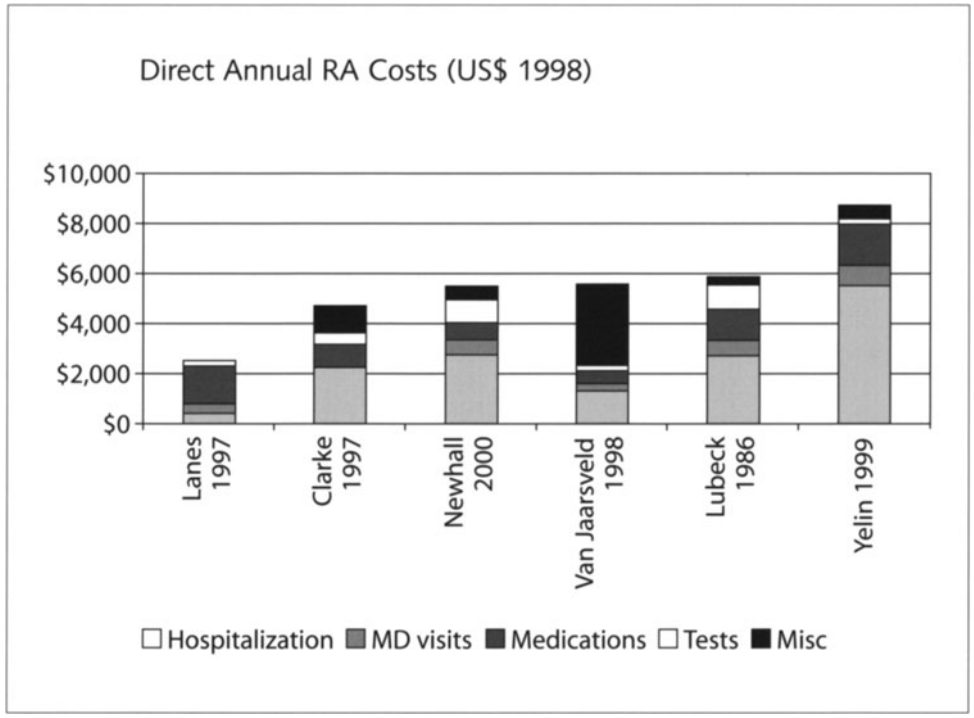

Figure 3
Direct medical costs of rheumatoid arthritis

Indirect costs are highly dependent on the estimation methods and, on average, are similar to direct costs [24]. Estimation of indirect costs is controversial, and current methods give insufficient weight to the potential economic gains that could be achieved by curing diseases that mainly affect women [28]. However, dollar valuations of these potential improvements in productivity are "potential" rather than "actual" costs to society. Counting "actual" costs is methodologically challenging – a difficulty that is partially responsible for the widespread recommendation that indirect costs be excluded from economic evaluations [12]. An alternative method for estimating "actual" indirect costs – the friction cost approach – only includes productivity costs during the period that is needed to restore the initial production level [29], generally at a fraction of 20% or less of fully valuated costs. Thus, decision-makers need to be aware of the potential variation in total direct and indirect RA costs, and the fraction of the costs that may be offset by the high acquisition costs of new interventions.

The costs of drug treatment for rheumatoid arthritis

The annual costs of DMARDs have slowly increased since the introduction of injectable gold in the early 1960s. Annual acquisition costs (excluding administration) increased from ~$500 with gold, methotrexate and sulfasalazine to ~$3,500 for leflunomide and cyclosporine, to up to $25,000 for the TNF-blocking agents (Tab. 5). Table 5 also shows the tremendous diversity in drug prices by country.

Cost-effectiveness analyses for rheumatoid arthritis drug treatment

Several cost-effectiveness analyses published in the last few years have examined recently introduced DMARDs: TNF-blocking agents and leflunomide (Tab. 6).

The 6-month cost-effectiveness (1999 US dollars) of the TNF-blocking agent etanercept, either in combination with methotrexate or alone, was compared to triple therapy (methotrexate combined with hydroxychloroquine and sulfasalazine), combination of methotrexate and cyclosporine, continuation of methotrexate alone despite failure, or no DMARD [12]. The incremental cost per patient achieving ACR core set 20% improvement was calculated as well as the incremental cost per weighted ACR 20%, 50% and 70% improvement. Both triple therapy and etanercept + methotrexate combination therapy emerged as the favoured treatment strategies. While triple therapy was associated with a cost-effectiveness ratio of $6,300/$3,100 (direct costs/total costs) per weighted ACR70 responder gained compared to treatment without any DMARD, the cost-effectiveness ratio of etanercept + methotrexate combination therapy was $36,300/$34,800 (direct costs/total costs) per weighted ACR70 responder gained compared to triple therapy. The analysis was short-term only and biased slightly against triple therapy, from an efficacy endpoint perspective; QALYs were not used as outcomes. In a similarly conducted analysis, the authors estimated the cost-effectiveness of treatment options for methotrexate-naive patients [30]. The cost-effectiveness ratio of etanercept was $42,900/$40,800 (direct costs/total costs) per weighted ACR70 responder gained over treatment with methotrexate.

Two manufacturer-sponsored economic evaluations examined the cost-effectiveness of infliximab in combination with methotrexate compared to methotrexate therapy alone in initial methotrexate failures based on the Anti-TNF Trial in Rheumatoid Arthritis with Concomitant Therapy (ATTRACT) [17, 18]. In both studies, HAQ-based disability levels reached at the 54-week time point of the ATTRACT trial were used to project the disability progression, including increased mortality, for the patient's lifetime [17] or for 10 years [18]. In the base case of both analyses, patients were assumed to take infliximab 3 mg/kg every 8 weeks for 54 weeks and then to resume their normal DMARD regimens. Improvements in HAQ

Table 5 Costs of selected drugs for rheumatoid arthritis, by country

	Methotrexate	Inj. Gold	Sulfasalazine EN	HCQ	CyA	LEF	Infliximab	Infliximab	Etanercept
Mean dosage	15mg/week	50mg/inj.	2g/day	400mg/day	200mg/day	20mg/day	3mg/kg	5mg/kg	2.5mg x 2/week
Unit	2.5mg tablet	50mg vial	500mg tablet	200mg tablet	100mg caps.	20mg tabl.	100mg vial	100mg vial.	25mg syr.
# of units/year	312	28	1460	730	730	365	20	35,8	104
Canada									
Price per unit	$0,63	$16,99	$0,11	$0,52	$3,88	$9,59	$940,00	$940,00	$165,00
Yearly price	$198	$476	$158	$383	$2.833	$3.500	$18.800	$33.652	$17.160
Germany									
Price per unit	€ 0,55	€ 26,48	€ 0,38	€ 0,29	€ 5,55	€ 4,03	€ 797,70	€ 797,70	€ 268,43
Yearly price	€ 171	€ 741	€ 551	€ 208	€ 4.048	€ 1.472	€ 15.954	€ 28.558	€ 27.917
US									
Price per unit	$3,56	$18,22	$0,34	$1,10	$6,17	$10,12	$691,61	$691,61	$163,33
Yearly price	$1.112	$510	$495	$800	$4.501	$3.695	$13.832	$24.760	$16.986
UK									
Price per unit	£0,11	£9,36	£0,08	£0,08	£2,54	£1,55	£451,20	£451,20	£81,25
Yearly price	£36	£262	£110	£55	£1.857	£566	£9.024	£16.153	£8.450
Netherlands									
Price per unit	€ 0,20	n/a	€ 0,12	€ 0,29	€ 3,29	€ 2,51	€ 653,98	€ 653,98	€ 132,30
Yearly price	€ 63	-	€ 181	€ 212	€ 2.402	€ 917	€ 13.080	€ 23.412	€ 13.759

Canada:http: //www.gov.on.ca/health/english/program/drugs/odbf/odbf_mn.html (infliximab: http://www.ramq.gouv.qc.ca/crc/pro/listmed/pdf/listemed.pdf, calculated for 75kg average weight)
Germany: Red Book 2002 (set pharmacy prices, hospitals may negotiate better prices)
UK: British National Formulary (http://bnf.vhn.net/home/) as of May 2002
US: 2002 Drug Topics Red Book

Table 6 - Base case cost-effectiveness analyses of drug treatments for rheumatoid arthritis

Author (ref)	Population	Treatment of Interest	Comparator Treatment	Time Horizon	Incremental Cost-Effectiveness Ratio, direct costs only (1998/1999 US$)
Choi [32]	Methotrexate failures	Triple therapy	No DMARD	6 months	$6,300 per weighted ACR70%
		Etanercept + Methotrexate	Triple therapy	6 months	$36,300 per weighted ACR70%
Choi [30]	DMARD naive	Methotrexate/Sulfasalazine	No DMARD	6 months	$3,700/$1,500 per weighted ACR70%
		Etanercept	Methotrexate	6 months	$40,800 per weighted ACR70%
Wong [17]	Methotrexate failures	Infliximab + MTX	MTX alone (placebo)	lifetime	$30,500 per QALY
Kobelt [18]	Methotrexate failures	Infliximab + MTX	MTX alone (placebo)	10 years	$39,300 per QALY
Maetzel [7]	DMARD naive	DMARDS incl. leflunomide	DMARDs excl. leflunomide	5 years	$72,000 per QALY
Brennan [19]	Methotrexate failures	DMARDs incl. etanercept	DMARDs excl. etanercept	lifetime	$26,000 per QALY

Legend: € converted to US$ using purchasing power parities (2003 OECD)

scores, reached with infliximab by the end of week 54, were maintained during model extension, when patients were off infliximab. The US-based analysis was modelled with data supplied by the Arthritis Rheumatism, and Aging Medical Information System (ARAMIS). For this analysis, quality of life adjustments for disability levels were inferred from the patient's assessment of global health. At the end of week 54, patients assessed their global disease status to be 0.509 (methotrexate) compared to 0.621 (infliximab + methotrexate). The QALY gain of 0.11, which correspond to > 40 days of full health equivalence, may be accentuated as this measure is not anchored by immediate death and perfect health. One year treatment with infliximab was found to increase quality-adjusted life expectancy by 0.29 QALYs (i.e., 106 days of full health equivalence). The European evaluation was modelled based on the experience of two cohorts of patients with early RA, one UK and one Swedish. Consistent with the US-based evaluation, quality-adjusted life expectancy was modelled to increase by 0.298 (UK) or 0.248 (Sweden) QALYs at the end of the 10-year period. The cost-effectiveness ratios of $30,500 per QALY (1998 US dollars) for the US model, or €3,440 (Sweden)/€34,800 (UK) may underestimate the cost-effectiveness ratio associated with infliximab, if indeed HAQ levels in the infliximab group quickly returned to those in the control group after cessation of infliximab therapy.

Another pharmacoeconomic evaluation of TNF antagonists estimated the cost-effectiveness of etanercept monotherapy in patients failing two DMARDs, according to the British Society for Rheumatology guidelines [19]. The authors modelled progression in HAQ scores over patients' lifetimes and linked HAQ scores to values in the EQ-5D indirect utility index. An initial gain of –0.84 in HAQ with etanercept was matched with gains between –0.35 and –0.52 for the other three DMARDs modelled (intramuscular gold, leflunomide and cyclosporin) and semi-annual progressions in HAQ-scores of 0.0075 for etanercept, *versus* 0.017 for the other three DMARDs. The authors calculated a baseline cost-effectiveness ratio of UK£16,330 per QALY, which would translate into ~ $26,000 per QALY. The analysis was criticized for its overly optimistic assumption in improvements in HAQ scores, which were observed in the supporting clinical trials but which are rarely matched in practice-based observational studies [22].

An economic evaluation of adding leflunomide within a 5-year time horizon to a conventional strategy of DMARDs was performed by one of the authors (AM) on behalf of Aventis, the manufacturer of leflunomide [7]. The conventional DMARD-strategy modelled methotrexate followed by combination with sulfasalazine and hydroxychloroquine, followed by injectable gold salts and low-dose cyclosporine. The evaluation was done from a Canadian public payer's perspective over a 5-year time horizon. This analysis showed that leflunomide would cost approximately $14,000 (1999 US dollars) per year of ACR20 response gained and $72,000 per QALY gained. Adding leflunomide to a conventional DMARD regimen resulted in ~ $1,200 additional direct medical costs, because only patients failing methotrexate

combination therapy were modelled to receive leflunomide. However, the strategy including leflunomide resulted in comparatively small quality of life gains, 0.02 QALYs, i.e., 6.2 days of full health equivalence.

Gaps in the current evidence base and conclusions

As drug treatments for RA evolve, so does the field of pharmacoeconomics. The methods are constantly being refined and gaps in the evidence base filled in. Our ability to more accurately estimate the "value for money" of new treatments depends on continued work in several areas outlined in [14]. While the medical costs have been examined by many, the non-medical costs have not been well studied. Future work on non-medical costs has been aided by the formation of a number of longitudinal RA cohorts that are collecting economic information.

Many of the clinical trials examining new agents, such as leflunomide or biologic therapies, use "partial responders" with an inadequate response to their current DMARD as the eligible study population. The disadvantages of using partial responders have been outlined by others [31]. Since partial responders have a very low likelihood of improvement, the relevant clinical decision in patients with inadequate response to one DMARD is not whether to continue the ineffective DMARD, but whether to start new treatment A or new treatment B.

Finally, long-term follow up of patients taking biologics and leflunomide will provide for more precise estimates of the relative rates of their potential beneficial and adverse effects. For example, it is possible that such newer agents may result in greater longevity through reducing important comorbidities, such as acute myocardial infarctions and osteoporotic fractures. On the other hand, these newer drugs may result in significant increases in rare side effects, such as atypical infections and uncommon cancers. The clinical and economic implications of such beneficial and adverse outcomes will help clarify the role of these agents. While the published data suggest that biologic therapies have incremental cost-effectiveness ratios that seem quite favorable, not all new agents look like a "good buy" for the healthcare system. As the portion of healthcare budgets devoted to drugs continues to grow, prescribing physicians will be increasingly asked to make hard decisions about which medications to prescribe to which patients. The science of pharmacoeconomics helps payers, clinicians, patients, and society at large to understand the value of a given medication.

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Future molecular targets

Edward C. Keystone[1] *and Boulos Haraoui*[2]

[1]University of Toronto, Ontario, Canada, and Université de Montréal, Montréal, Canada The Rebecca MacDonald Centre for Arthritis and Autoimmune Disease, The Joseph Wolf Lebovic Building, Mount Sinai Hospital, 60 Murray St., 2nd Floor, Toronto, Ontario, M5G 1X5, Canada; [2]CHUM, Hôpital Notre-Dame, 1560 Sherbrooke est, Montréal, Québec H2L 4M1, Canada

Introduction

Over the past 20 years, biotechnology has pioneered the development of genetically engineered therapies that target specific aspects of the immune response. However, biologic agents are frequently immunogenic, requiring complex and labor intensive production processes and parenteral administration, resulting in high costs which limit both availability and access to treatment.

To avoid these difficulties, small molecules were developed that also selectively target pathogenic elements of the immune response. Synthetic molecules are easier and less costly to manufacture, orally administered, non-immunogenic and therefore more amenable for widespread therapeutic use than biologic agents. The rationale leading to development of the majority of these synthetic agents stems from the concept that proinflammatory molecules, including adhesion molecules, cytokines and proteolytic enzymes, are generated *via* intracellular signaling pathways utilizing enzymes and kinases that can be selectively inhibited by specific chemical moieties. It is expected that these small molecule synthetic agents will gradually supplant use of biologic agents for the treatment of rheumatoid arthritis (RA) over the next 10–15 years.

The inflammatory response observed in RA locally within the synovium depends on a diverse array of cell surface molecules, soluble factors and enzymes that recruit cellular elements to the synovium and activate gene expression to further amplify the inflammatory process. Generation of these mediators, as well as the cellular responses to them, requires transduction of an extracellular signal for gene activation. Signal transduction is initiated as a consequence of binding of a ligand to its specific cell surface receptor. Following binding, the receptors cluster into dimers or trimers of the ligand with subsequent recruitment of cytoplasmic signaling proteins to the ligand receptor complexes. These receptor associated factors are a family of proteins that initiate an intracytoplasmic cascade of enzymes (mainly kinases) that

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act as intracellular signaling molecules or switches [1–3]. Upon activation of a series of these kinases within the cytoplasm, transcription factors are generated that translocate to the nucleus to bind the promoter regions of genes containing appropriate recognition sequences to initiate gene transcription. Messenger RNA (mRNA) is generated by this process with subsequent translocation of the mRNA transcripts to cytoplasmic ribosomes for translation into protein. Generation of proteins, soluble mediators, surface molecules, and enzymes may be selectively inhibited by targeting any step in the intracellular signaling process.

Transcription factor families as targets

Transcription factors are central to modulating the process of gene transcription. Genes, for many of the inflammatory mediators, contain signal recognition sequences (response elements) for transcription factors. As a consequence, inhibition of a limited number of key transcription factors may affect many inflammatory mediators. Key transcription factors required for the expression of a substantial number of mediators include: (1) activator protein 1 (AP-1); (2) nuclear factor κB (NF-κB); (3) nuclear factor of activated T cells (NF-AT); and (4) signal transducer and activator of transcription (STATs). Each of these represents potential therapeutic targets in RA (Fig. 1).

AP-1

Introduction

AP-1 is a pivotal transcription factor that regulates T cell activation, cytokine production and generation of matrix metalloproteinases (MMPs) [4]. AP-1 is activated by a number of extracellular signals including cytokines (TNF and IL-1) growth factors, lipopolysaccharide, active oxygen metabolites, stress, a number of tumor promoters and ras oncoprotein. AP-1 regulates a number of genes involved in RA including TNF, IL-1, IL-2, IFN-δ, ICAM-1, GM-CSF, E-selectin and MMPs.

AP-1 is a dimer comprised of members of the Jun and Fos families of transcription factors. Intracellularly, AP-1 is regulated by mitogen activated protein (MAP) kinase signaling cascades leading to activation of three kinases, ERK (extracellular signal regulated kinase), JNK (Jun N-terminal kinase) and p38 family of MAPKs. ERKs are activated by mitogen and growth factors while JNK and p38 kinases are activated by proinflammatory cytokines and cellular stress. All three MAPK pathways regulate the transcription of Fos and Jun family genes. A significant component of the regulation of AP-1 is accomplished through post-translational modification by c-Jun N-terminal kinases JNK-1 and JNK-2, which are terminal members of a MAPK cascade.

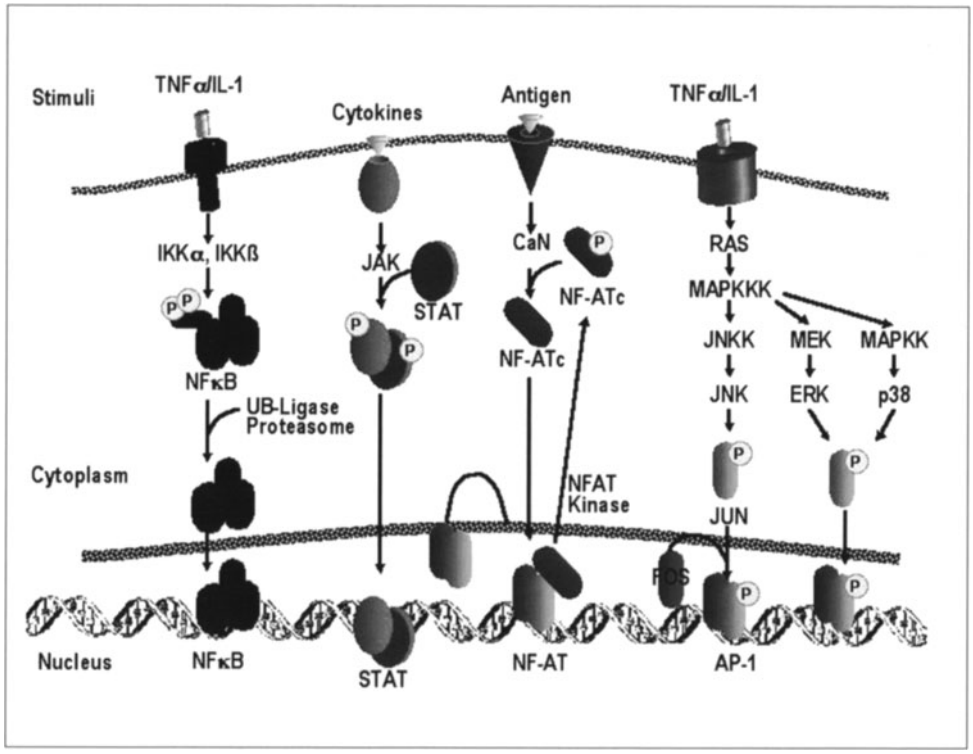

Figure 1
Signal transduction pathways for generation of transcription factors NF-κB, STAT, NF-AT and AP-1.

Role of AP-1 in inflammation

AP-1 proteins play a critical role in RA as evidenced by: (1) AP-1 localization to the cell nuclei in the synovium suggesting cell activation [5]; (2) expression of c-jun and fos proteins in the sublining inflammatory infiltrate; (3) AP-1 expression at the site where AP-1 regulated cytokines and MMP genes are overexpressed [6]; and (4) very high levels of AP-1 activity from nuclear extracts from RA synovium [7].

The precise MAPK pathways activating AP-1 in the synovium are unclear. Recent data has demonstrated that ERK, JNK and p38 MAPK activation were almost exclusively found in RA, but not OA synovial tissue [8]. ERK activation was localized around synovial microvessels, JNK activation was localized around and in mononuclear cell infiltrates and p38 MAPK activation was observed in synovial lining layer and endothelial cells. TNF, IL-1 and IL-6 were major inducers of ERK, JNK and p38 MAP activation in cultured human synovium cells.

AP-1 inhibitors

Recently JNK was shown to be a critical MAPK pathway for IL-1 induced collagenase gene expression in synoviocytes and in adjuvant arthritis [9, 10]. A novel JNK inhibitor SP600125 modestly decreased rat paw swelling but almost completely inhibited radiographic damage associated with reduced AP-1 activity. The data suggests that JNK is an important therapeutic target in RA. More recent data has been generated to demonstrate that complete inhibition of MMP expression and joint destruction will require combined JNK-1 and JNK-2 inhibition.

Mitogen activated protein kinase (MAPK)

Several transcription factor families are involved in the pathogenesis of RA including; (1) mitogen-activated protein kinase (MAPK) and (2) nuclear factor κB (NF-κB) (reviewed in [11]). As noted above, MAPKs are constituents of a signaling cascade leading to the activation of transcription factors. The three major MAPK signaling cascades including ERK, JNK and p38 MAPK are activated by upstream MAPK kinases (MAPKKs), which, in turn, have been activated by MAPK kinase kinases (MAPKKKs). Activation of the MAPK cascade leads to activation of the transcription factor AP-1 which binds DNA resulting in gene transcription of cytokines and matrix metalloproteinases (MMPs). More recent data has also shown that MAPK signal transduction pathways play a critical role in post-transcriptional regulation (control of mRNA stability and translation) of cytokines such as TNF. That MAPK families are involved in the pathogenesis of RA is evidenced by their expression in RA fibroblast-like cells (FLS) and the demonstration of AP-1 in RA synovium. TNF and IL-1 have also been shown to induce ERK, JNK, and p38 MAPK activation in cultured RA synovial tissue cells [8].

The p38 MAPK family

The p38 MAPK family includes 4 subsets: α, β, γ and δ with a different expression in human tissues. Inflammatory cells, including neutrophils, monocytes, macrophages and T cells synthesize preferentially p38α and δ. The synthesis of the p38 MAPK family is dependent on the upstream activation of a series of kinases after cell stimulation by a variety of mediators. In chronic inflammatory conditions such as RA, TNF-α and IL-1β are the main inducers of p38 MAPK expression. The downstream targets of p38 MAPK include other kinases as well as transcription factors involved in the production or the action of several mediators of inflammation (reviewed in [1–3]).

Kinases as substrates of p38 MAPK

Among the several kinases activated by p38 MAPK, the MAPK activated protein

kinase (MAPKAP K) plays a pivotal role in cytokine production, especially TNF-α, IL-1β, IL-6, IFN-γ. Thus p38 MAPK play a dual role for cytokine production as well as the signalling induced by these same cytokines.

Transcription factors as substrates of p38 MAPK

Several transcription factors are induced by p38 MAPK, which can activate the transcription of both c-Jun and c fos-resulting in the generation of AP-1 and thereby influencing host defence responses and inflammation. Other substrates include cytoplasmic phospholipase A_2 (cPL A_2), but p38 MAPK inhibition does not impact on arachidonic acid disposition.

Role of p38 MAPK in inflammation

Recruitment of cells at the inflammatory site requires the activation of adhesion molecules on the surface of leukocytes as well as on endothelial cells. The expression of selectins which regulate rolling of cells is mediated by TNF-α through the activation of three intracellular signalling pathways, p38 MAPK, JNK and nuclear factor κB (NF-κB). p38 MAPK plays also a minor role in the regulation of NF-κB. In a second step, chemokine-activated integrins allow a firmer adhesion of leukocytes to the vessel walls with subsequent diapedesis. Chemokines production is upregulated among others by IL-8 and TNF-α which are p38 MAPK dependent. The cell composition of the inflammatory infiltrate is dependent on the subgroup of generated chemokines, of which several are mediated by the activation of p38 MAPK.

Tissue damage results from the release of toxic metabolites, superoxide anions and degradation enzymes such as metalloproteases. Both p38 MAPK and ERK are involved in the activation of enzymes leading to the oxidative burst and release of superoxide anions.

p38 MAPK inhibitors

Given the pivotal role of p38 MAPK in cytokine production and cytokine-induced cell stimulation, it is logical to consider means of inhibiting p38 MAPK activation as a therapeutic target in diseases with a strong inflammatory component.

The basic mechanism of action of kinases is phosphate transfer from ATP molecules. Despite the high intracellular concentration of ATP, p38 inhibitors competitively bind to the ATP pocket of the p38 MAPK.

There are several chemical classes of p38 inhibitors. The first discovered were the pyridinylimidazoles, which combined immunomodulatory activities as well as inhibitory properties of cyclooxygenase and lipoxygenase. Despite their potent anti-inflammatory activity in animal models, their development was slowed for safety

concerns, including hepatocellular hypertrophy, gastric ulceration and CNS side effects. Structural molecular modifications led to several compounds, which are currently undergoing preclinical and clinical testing.

Since the imidazole moiety by itself does not seem to influence the p38 MAPK inhibitory properties, substitution by pyrrole, pyrazole or pyrazolone groups led to the development of a new chemical family with strong p38 MAPK inhibitory action. Several pharmaceutical companies are pursuing research with such compounds.

Other chemical entities such as indoles and azaindoles have also been developed and are undergoing further development, and more recently new p38 inhibitors structurally different from the pyridinylimidazoles compounds are being tested.

The selective inhibition of p38 MAPK subsets, which have variable cellular expression will lead to specific immunomodulatory and anti-inflammatory activities. The challenges are in developing compound sparing physiologic (as opposed to pathologic) p38 activity and with acceptable toxicity profile.

p38 MAPK may be particularly relevant to the pathogenesis of RA since it may play a central role in regulating the production of, and responsiveness to, proinflammatory cytokines. Five isoforms of p38 MAPK have been identified and p38α is the major isoform activated in most inflammatory cells. p38 MAPK is involved in the activation of proinflammatory cytokines including TNF, IL-1, IL-6, IL-8 as well as Cox-2. In addition, p38 MAPK is involved in TNF induced upregulation of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and E-selectin. Activation of p38 MAPK also leads to an increase in the synthesis of MMPs. Since activated p38 MAPK is localized to synovial endothelial cells as well as the synovial lining layer, it is likely involved in increased angiogenesis and cell recruitment [8].

The rationale for considering p38 MAPK as a therapeutic target also comes from preclinical studies in animal models of RA. Administration of AP-1 decoy oligonucleotides that interfere with binding of AP-1 at the promoter binding region has been shown to suppress IL-1, IL-6 and TNF as well MMPs in synovial tissue with resultant inhibition of murine collagen induced arthritis [12].

The therapeutic potential of inhibiting MAPKs with oral small molecules was recently demonstrated in several preclinical studies. In the collagen-induced arthritis, adjuvant arthritis and *Streptococcus* cell wall models of RA, orally administered selective p38 MAPK inhibitors administered during the established phase of disease caused marked reduction in the clinical severity of the arthritis as well as radiographic damage [13, 14]. A number of oral p38 MAPK inhibitors have been developed for the treatment of RA. The development for a number of these agents has been terminated. One p38 MAPK inhibitor thus far has demonstrated significant clinical benefit in RA. Other agents have been discontinued on the basis of toxicity in long-term animal studies. The side effect profile to date in humans appears good,

but hepatotoxicity remains an issue that may be dose limiting. A number of new p38 MAPK inhibitors are currently being generated which are selective for specific isoforms of p38 MAPK with the hope of reducing toxicity. Although the concept of inhibiting p38 MAPK is inherently sound, more clinical data is needed to conclude that p38 MAPK is a viable therapeutic target in RA.

Nuclear factor-κB

Introduction

The transcription factor NF-κB is one of the most important inducers of inflammation of RA. NF-κB is activated by a large number of extracellular signals that also activate JNK and p38 MAPK pathways including UV light, TNF, IL-1 and lipopolysaccharides. It plays a role in the generation of a substantial number of inflammatory mediators including cytokines (TNF, IL-1, IL-1, IL-6, IL-8), enzymes (Cox-2, cPLA2, iNOS), chemokines (MIP1a, MCP-3, RANTES), adhesion molecules (ICAM-1, VCAM-1, E-selectin) and a variety of anti-apoptotic proteins (TRAF-1 and 2, Bcl-2 homologues, Bcl-X, C-1AP1 and 2). NF-κB activation can prevent apoptosis and therefore has been implicated in the generation of synovial hyperplasia. More recently NF-κB has been implicated in antigen presenting cell function including T cell receptor cell recognition of MHC Class I and Class II, expression of co-stimulatory molecules (CD80/86 and CD40) as well as IL-12 and chemokine production. NF-κB has also been implicated in bone erosion in RA with osteoclast differentiation. Thus, a key soluble mediator driving osteoclast differentiation is receptor activator of NF-κB ligand (RANKL). Binding of RANKL to the cognate receptor (RANK) leads to activation of NF-κB and other transcription factors. The absence of osteoclasts in mice lacking NF-κB (p50) and NF-κB (p52) suggests a critical role for NF-κB in osteoclast differentiation.

NF-κB exists in the cytoplasm in an inactive form associated with inhibitory proteins termed inhibitor of nuclear factor κB (IκB), the most important being IκBα, IκBβ and IκBε. NF-κB is activated through the proteolytic degradation of Ikb upon extracellular signaling. Extracellular stimuli initiate a signaling cascade of MAPKs particularly NF-κB inducing kinase (NIK) and MEKK-1 which cause the activation of two IκB kinases IKK-1 (IKKα) and IKK-2 (IKKβ) that phosphorylate IκB. Once phosphorylated, IκB undergoes a process of ubiquination to generate IκBε3RS, which is subsequently degraded by a 26S proteosome. NF-κB, once freed from IκBs, translocates to the nucleus where it binds to the promoter of its target genes to initiate transcription. Like AP-1, NF-κB is comprised of a number of family members which undergo dimerization to bind DNA. The classic NF-κB dimer (p50/p65) contains REL-A (p65) and NF-κB1 (p50) but other NF-κB containing dimer also exists. The dimeric structure of NF-κB allows for distinct biologic functions to be subserved by individual family members.

NF-κB in inflammation

In rheumatoid synovium, NF-κB, RelA (p65) and p50 are found mainly in the nuclei of synovial macrophages as well as fibroblast-like and endothelial cells [15]. The co-localization of TNF and IL-1 in rheumatoid synovial macrophages suggests that cytokine production is regulated by NF-κB. IL-1 and TNF in turn have been shown to be capable of rapidly activating NF-κB in fibroblast-like synoviocytes.

NF-κB inhibitors

A number of strategies have been developed to inhibit NF-κB activity [16]. Potential points of intervention have included: (1) activation of the IKK; (2) phosphorylation of IκB by IKKβ; (3) ubiquination of IκB; (4) degradation of IκB by the 265 proteosome; and (5) translocation of NF-κB to the nucleus. Inhibition of IKK-2 and not IKK-1 has been demonstrated to prevent TNF mediated cytokine, MMP and adhesion molecule synthesis in fibroblast-like synoviocytes, suggesting IKK-2 is a key target to inhibit NF-κB in RA synovium [17]. Gene therapy to inhibit IKK-2 activity with an IKK-2 dominant negative mutant ameliorated the severity of adjuvant arthritis [18]. Inhibition of NF-κB by inhibiting the proteosome involved in degradation of Ikb revealed profound apoptosis of synovium in rats with streptococcal-cell wall arthritis [19]. Direct inhibition of NF-κB non-specifically using a NF-κB decoy oligonucleotide that binds NF-κB thereby preventing binding to the DNA promoter markedly suppressed collagen induced arthritis [20]. Specific NF-κB inhibition with a novel T cell specific NF-κB inhibitor SP100030 caused improved collagen-induced arthritis clinically and histologically.

NFAT

The nuclear factor of activated T cells (NFAT) family of transcription factors play a critical role in the control of lymphokine gene expression in T cells in a Ca^{2+} dependent manner. NFAT proteins are expressed in several immune related cells, including T cells, B cells, natural killer cells, mast cells, macrophages and endothelial cells.

NFAT is activated by stimulation of receptors coupled to calcium mobilization such as antigen receptors on T and B cells and Fc receptors on macrophages. The NFAT family of factors regulates IL-2, INF-δ, IL-3, IL-4, IL-5, IL-8, IL-13, TNF-α and Gm-CSF as well as CD40 ligand and Fas ligand.

Ligand binding to its receptor leads to activation of phospholipase C, generation of inositol biophosphate and calcium mobilization with subsequent activation of the calcium and calmodulin-dependant phosphatase calcineurin. Calcineurin dephosphorylates NFAT proteins leading to translocation to the nucleus for binding to the appropriate promoter.

NFATc is required for regulation of IL-2 gene transcription. Most of the transcription factors that regulate IL-2 transcription are sensitive to cyclosporin A and FK506. Based on the improvement in RA observed with cyclosporine and FK506, NFAT appears to be an appropriate therapeutic target in RA.

STATs

The molecular components of intracellular signaling pathways in lymphoid cells include the Janus kinase (JAK), signal transducers and activators of transcription (STAT) pathway. The pathway is used by interferons (STAT 4), CSFs, growth factors, IL-2 (STAT 3), and IL-4 (STAT 6). Genes regulated by STATs include E-selectin, C-Fos, C-myc, ICAM-1, FcδR-I.

Following ligand binding and receptor dimerization, JAKs are catalytically activated by phosphorylation and associate with the intracellular domain of the cell surface cytokine receptor. The activated JAKs phosphorylate tyrosine residues on the receptors resulting in recruitment of STATs that are themselves activated (by phosphorylation), leading to release from the receptor docking site to form dimers that translocate to the nucleus to bind to the promoter region of the appropriate gene.

That STATs may be pathogenic in RA is suggested by the finding that STAT activation in rheumatoid SF cells appears to be continuous compared to normal circulating leukocytes [21].

Transcription as a target

The process of transcription involves unwinding the process of DNA encoding the protein. During transcription, a complementary strand of messenger RNA (mRNA) – the sequence sense mRNA transcript – is synthesized from the complementary DNA sequence. The mRNA is modified and sequence elements added to control the translation process. Recently, antisense therapy has been developed to target the mRNA transcript. This involves the use of an antisense oligonucleotide (with a nucleotide sequence complementary and hence antisense) to the mRNA sequence encoding the target protein [22]. When the antisense oligonucleotide binds the mRNA, it prevents the sense mRNA transcript from being translated at the ribosome and hence blocks the unwanted protein synthesis. A human TNF antisense oligonucleotide is currently in Phase II trials in RA. Although recent unpublished data have demonstrated efficacy, additional studies are needed to determine its utility in RA.

TNF-α converting enzyme (TACE) as a target

TNF-α converting enzyme (TACE) is the MMP that processes the 26 kDa membrane bound precursor of TNF-α (pro TNF) to the 17 kDa soluble component. A number of orally bioavailable, selective and potent TACE inhibitors are in development and are currently in Phase II studies in RA [23]. These inhibitors effectively block TACE mediated processing of pro TNF in human monocytes, and are capable of reducing TNF production in normal human subjects. TACE processing of pro TNF has recently been shown to occur intracellularly. One issue raised as a consequence of intracellular processing is the fate of unprocessed pro TNF, since cell surface associated pro TNF could lead to potential biological activity. Recent studies demonstrate that > 80% of unprocessed pro TNF is degraded intracellularly. The rest is transiently expressed on the cell surface.

In animal models of arthritis, oral TACE inhibitors are efficacious therapeutically in established collagen induced arthritis. The efficacy is at least, if not greater than strategies to neutralize soluble TNF, presumably due to greater tissue penetration.

Interleukin converting enzyme (ICE) as a target

Another approach to decreasing cytokine activity is to reduce its production by interfering with its processing and secretion. This approach can be used with IL-1 and IL-18. IL-1β and IL-18 are synthesized in the cytoplasm as an inactive precursor (pro-IL-1β and pro-Il-18). In order for IL-1β and IL-18 to be secreted the proform of the cytokines are processed by interleukin converting enzyme (ICE), a cysteine protease that cleaves pro-IL-β and IL-18 to generate mature forms that can be secreted.

The therapeutic potential for targeting ICE has been demonstrated in animal models of arthritis [24]. ICE knockout mice are not susceptible to collagen-induced arthritis. Treatment with an ICE inhibitor reduced the severity of established collagen induced arthritis. Since inhibition of ICE potentially affects both IL-1 and IL-18, synergistic effects of inhibiting both T cell and non T cell mediated processes may be particularly effective. Recent data has shown IL-18 to have a variety of proinflammatory effects on multiple cells in the synovium. Since inhibition of ICE may prolong the life of cells, the potential for development of malignancies and autoimmune disease may exist. However, ICE knockout mice do not seem to develop these diseases.

Recently, a clinical trial of an ICE inhibitor was discontinued for safety reasons. Whether this technology to inhibit IL-1 will be evaluated further remains unclear.

Conclusion

Advances in the understanding of the intracellular signaling cascades utilized by the new recognized proinflammation molecules in RA have lead to the development of targeted small molecules that have the capability of substantially inhibiting these pathogenic elements. The pace of development of targeted small molecule inhibitors may give rheumatologists the capability of ameliorating the disease process with a generation of therapies that are easily administered and accessible to all patients who require them. Currently, the limiting factor in development appears to be hepatotoxicity. Hopefully this will be resolved. We look forward with great anticipation to further developments in this field.

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Hydroxychloroquine

Beau Bothwell and Daniel E. Furst

University of California at Los Angeles, 1000 Veteran Ave Rm 32-46, Los Angeles, CA 90025-1670, USA

Introduction

Hydroxychloroquine ($C_{18}H_{26}ClN_3O$) and chloroquine, the two 4-aminoquinolones commonly prescribed for treatment in rheumatic diseases, are derived from the bark of the Peruvian cinchocha tree. Along with quinacrine, the two aminoquilones are labeled antimalarials after their long history in the treatment of that disease, highlighted by Pelletier and Caventou's isolation of quinine and cinchonine as active antimalarial agents in 1820. Quinacrine, though not an aminoquinolone, carries within it the imbedded structure of chloroquine.

The first publication using aminoquinolone derivative therapy in rheumatic diseases took place in 1929 with the use of quinine to treat systemic lupus erythematosus [1]. A 1951 article by Page noted the effectiveness of antimalarials in the treatment of both systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) [2], and more recently the use of antimalarials has been extended to a wide range of connective tissue diseases including dermatomyositis [3], palindromic rheumatism [4], juvenile onset SLE [5], eosinophilic fasciitis [6] and osteoarthritis (OA) [7]. While the effectiveness of aminoquinolones in the treatment of connective tissue diseases has been exceeded by other treatments, hydroxychloroquine has remained an important element in the treatment arsenal for two reasons. Firstly for its relative lack of toxicity as compared to other antirheumatic drugs, and secondly because its mechanism of action is different than that of most other DMARDs, hydroxychloroquine can effectively be used in combination therapy.

Mechanism of action

The primary mechanism of action of 4-aminoquinolones is mediated by protonation of these weak bases within the lysosome, thereby increasing the general intra-lysosomal pH. The raised pH of the lysosome disrupts antigen processing and leads to

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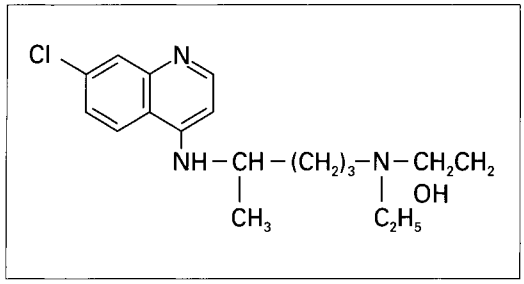

Figure 1
Hydroxychloroquine

decreased stimulation of T cells, decreased granulocyte migration, decrease cytokine production, and downregulation of the autoimmune response [8–10]. Hydroxychloroquine affects platelet activation in SLE associated anti-phospholipid syndrome, a result which may occur through inhibition of the expression of platelet surface markers such as GPIIb/IIIa [11]. In SLE, hydroxychloroquine inhibits *in vivo* apoptosis [12].

Aminoquinolones are racemic mixtures that do not seem to exhibit any chiral inversion, though the racemates are not cleared at the same rate. Hydroxychloroquine is metabolized stereo-selectively and binds to proteins stereo-selectively. The differences in action of the stereoisomers, if they exist, are unknown [13].

Though the antimalarials are very similar in structure, the *in vivo* mechanisms of hydroxychloroquine, chloroquine, and quinacrine may differ significantly. Quinacrine, and to some extent chloroquine, inhibit lipopolysaccharide (LPS)-induced expression of Il-1β and TNF-α. All three antimalarial compounds help limit the synthesis of prostaglandins through inhibition of phospholipase, which in turn inhibits arachidonate acid release and eicosanoid formation. The antimalarial drugs inhibit nuclear events in DNA through the binding of the quinoline ring to the nucleotide bases of DNA [14].

Antimalarials may also interfere with the Golgi complex by blocking the proteolytic conversion of secretary protein precursors such as pro-C3, thereby inhibiting protein secretion and the intracellular processing of proteins, inducing biochemical changes which are associated with morphological changes in the Golgi complex [15].

Clinical pharmacokinetics

Orally administered antimalarials are rapidly and efficiently absorbed into the bloodstream through the intestinal tract, with only small amounts recovered from

stool. This absorption may be enhanced when taken with food [16]. Hydroxychloroquine has an absorption half life of 3 h in the blood, with a distribution half life of 40 h to 5 days in tissues. In the final phase of drug elimination, hydroxychloroquine has a half life of 40–50 days [17].

Once in the body, most hydroxychloroquine is metabolized to desethylhydroxychloroquine, desethylchloroquine, and bisdesethylchloroquine, but up to 25% of ingested hydroxychloroquine is cleared unchanged renally [18]. Munster et al.'s study of 212 patients found a weak but persistent correlation between desethylhydroxychloroquine concentration in the blood and overall efficacy of hydroxychloroquine treatment, as well as an association between high blood levels of hydroxychloroquine (HCQ), the parent compound, and gastrointestinal related adverse events.

There is evidence that chloroquine exhibits strong *in vivo* melanin affinity, which explains the concentration of antimalarial drugs in the pigmented retinal tissues. Bernstein et al. found that following the IV delivery of a 5 mg/kg dose of chloroquine, pigment-normal rabbits accumulated chloroquine in the iris, the choroids, and the pigmented epithelium of the eyes (all melanin-containing tissues), whereas albino rabbits did not accumulate the drug in these tissues [19]. The same study found chloroquine concentrations in the pigmented retina of rats to be 10–20 times greater than in any other tissues.

Oral bioavailability of hydroxychloroquine ranges from 30–100%, resulting in a great deal of kinetic variability between individuals taking the drug [17]. The drug's large volume of distribution (5,500 liters), might be accounted for by its accumulation in cell lysosomes due to its properties as a weak base [13].

There have been relatively few drug interaction studies published regarding hydroxychloroquine. One study published in 2000 by Somer et al. found that hydroxychloroquine increased the bioavailability of metoprolol by 65% [20]. The study authors suggested that hydroxychloroquine inhibits metoprolol metabolism by interfering with its biotransformation through CYP2D6. Another study, published by Carmichael et al. in 2002, detailed the interaction between methotrexate and hydroxychloroquine in 10 healthy subjects [21]. This study found that hydroxychloroquine reduced the maximum concentration of methotrexate in the blood, but also decreased methotrexate's clearance. Overall this was found to increase the area under the concentration curve for methotrexate by 81%. These results may explain the somewhat increased potency of the hydroxychloroquine/methotrexate combination over methotrexate as a single agent, and the extended duration of the effects of methotrexate when combined with hydroxychloroquine. If peak concentrations of methotrexate are related to liver toxicity (a purely speculative notion) the fact that the maximum methotrexate (MTX) concentration is decreased when used in combination with hydroxychloroquine may explain the smaller number of acute liver adverse effects in combination HCQ/MTX therapy as compared to methotrexate in isolation.

Efficacy and effectiveness

The efficacy and effectiveness of hydroxychloroquine for the treatment of RA has been documented by studies beginning in the 1960s. In a 24 week double-blind trial conducted in 1993 in 126 RA patients with disease durations of less than 5 years, the hydroxychloroquine treated group showed improvement in a combined joint swelling and tenderness score, global assessment, and grip strength as compared to the placebo group [22]. A 2000 meta-analysis of four trials (two 24 week trials, one 36 week trial, and one 52 week trial), including 300 patients randomized to hydroxychloroquine and 292 to placebo, found hydroxychloroquine to be statistically better than placebo in most measures, with standardized mean differences ranging from –0.33 to –0.52. This analysis observed no differences in withdrawals due to toxicity compared to placebo [23].

The usual dose of hydroxychloroquine is no more than 6 mg/kg and no higher than 400 mg per day [24]. However a recent trial indicated that the use of 800 or 1,200 mg daily hydroxychloroquine for 6 weeks improved the response rate in patients with RA [25]. Patients in the 6-week double-blinded portion of that study exhibited Paulus response criteria of 47.9% for 400 mg/day, 57.7% for 800 mg/day, and 63.6% for 1,200 mg/day respectively ($p = 0.05$). The hydroxychloroquine dose was reduced to 400 mg for all groups after 6 weeks for the remainder of this 24-week trial. Discontinuations for gastrointestinal (GI) toxicities were increased in a dose response manner, with three, five and six instances, respectively, but no statistical differences were noted [25].

Among 195 patients undergoing treatment with antimalarials for early RA, a delay of therapy by more than 4 months was the only statistically significant predictor of remissions in patients followed for the duration of 2 years [26]. While a cumulative improvement in RA has been shown to be better for methotrexate or intramuscular gold than hydroxychloroquine, all three drugs were more effective in patients with disease duration of less than 1 year [27].

Some patients undergoing hydroxychloroquine therapy have been known to respond for prolonged periods. 30% of patients on hydroxychloroquine in a 541 patient randomized, controlled trial, with a flexible dosing regimen, were in remission after a treatment period of 5 years [28]. Despite this, observational studies indicate that discontinuations for inefficacy were more common for hydroxychloroquine treated patients during long-term follow ups (ranging from 7 months to 13 years) than for patients receiving penicillamine, sulfasalazine, auranofin, intramuscular gold, methotrexate, cyclosporin or azathioprine (50% of patients taking HCQ discontinued treatment during the first 2 years due to inefficacy) [29]. Griffiths et al. documented a median treatment duration of 11 months for hydroxychloroquine when used as initial DMARD therapy, as opposed to 5 months for sulfasalazine and 15 months for methotrexate [30].

A retrospective study published in 1998 by Avina-Zubieta et al. examined a cohort of 940 patients with RA, SLE, palindromic arthritis, or other diagnoses, engaged in aminoquinolone treatment (the study examined data up to a treatment duration of 120 months) [31]. Among this cohort, where 57% used chloroquine and 43% hydroxychloroquine, the hazard ratio for discontinuations because of inefficacy on hydroxychloroquine was significantly higher than on chloroquine (hazard ration equals 1.4, 95% confidence interval: 1.1–1.9), suggesting that hydroxychloroquine is likely to be less effective that chloroquine. On the other hand, 15% of the hydroxychloroquine treated patients experienced adverse events compared to 28% of those taking chloroquine, which along with a lower hazard ratio for discontinuations secondary to toxicity in hydroxychloroquine (hazard ratio = 0.6) suggests that hydroxychloroquine is less toxic than chloroquine.

The literature on antimalarial drugs does not recommend its use in psoriatic arthritis, with several studies documenting their general inefficacy and high level of intolerance in that disease [32–34]. Antimalarial use in osteoarthritis is also controversial with both positive and negative studies [7, 35]. Controlled studies seem not to support its usefulness in this disease.

Toxicity

Though hydroxychloroquine has been shown to be comparatively less effective than other DMARDs, it remains a common recourse in the treatment of rheumatic diseases because of its relatively benign toxicity profile. In 120 patients randomized to hydroxychloroquine from a prospective cohort study of about 400 patients with treatment durations of up to 4 years, 8% of patients receiving hydroxychloroquine eventually discontinued treatment with the drug secondary to an adverse event [36]. In a study of 156 patients from an SLE database where 203 courses of antimalarial treatment were recorded over an average duration of 6.9 years per patient, 97% received hydroxychloroquine [37]. Of these patients, 10% had side effects requiring withdrawal. In a study by Fries et al. of a cohort of 2,747 patients with RA receiving 3,053 courses of 6 DMARDs (hydroxychloroquine, intramuscular gold, D-penicillamine, methotrexate, azathioprine) and 1,309 courses of prednisone over 7,278 patient-years, hydroxychloroquine was found to have the most favorable side effect profile. Hydroxychloroquine patients had a mean toxicity index computed from symptoms, laboratory abnormalities, and hospitalizations attributable to DMARD therapy, of 1.38 ± 0.15 (as opposed to 2.27 ± 0.17 for intramuscular gold, 3.38 ± 0.36 for D-penicillamine, 3.82 ± 0.35 for methotrexate, 3.92 ± 0.39 for azathioprine) [38]. This conclusion was reinforced by Felson et al's meta-analysis [39].

In an overview of clinical trials of early RA by van Jaarsfeld et al., which followed a group of 120 patients who used hydroxychloroquine plus NSAID for up to 4 years, 26% had GI side effects (nausea, diarrhea, colitis, gastric ulceration, and

Table 1 - Possible adverse events

Gastrointestinal	Dermatological	Neuromuscular	Renal	Ocular
nausea	rash	muscle weakness	Proteinuria	corneal
diarrhea	mucocutaneous	vertigo	Elevated serum	deposits
	pigmentation	headache	creatinine	retinopathy
colitis	hair loss	tinnitus		
gastritis	pruritus			
gastric ulceration				
vomiting				

gastritis), 14% mucocutaneous adverse events (AEs) and 12% had renal side effects (increase protein or creatinine, usually associated with NSAID) [36]. Fewer than 5% of patients experienced other side effects. The side effects most commonly associated with hydroxychloroquine are GI in nature, including nausea, vomiting, epigastric pain, cramps, diarrhea, and weight loss [13]. A 1999 HCQ dose-loading study of 212 patients found that groups receiving higher doses of hydroxychloroquine had higher rates of discontinuation for adverse events (3 in the 400 mg/day group, 5 in the 800 mg/day group, 6 in the 1,200 mg/day group), with most AEs being GI-related (64%) [25]. Among other unusual side effects that have occurred are alopecia, pruritus, rashes, and even rarer side effects such as cardiomyopathy and third degree atrioventricular (AV) block [40, 43], blood dyscrasia [41] and precipitation of porphyria [42], which are usually cited as case reports [40–42]. In the previously cited database of 203 courses of antimalarial therapy in SLE patients, 11 of 20 withdrawals (55%) were for GI problems, two patients each withdrew due to headache or dizziness (10%), one withdrew secondary to each hearing loss (5%), one patient secondary to rash (5%), and one developed retinopathy after 6 years at a dose of 6.5 mg/kg/day (corresponding to a rate of retinopathy occurrence of 0.95 cases per 1,000 patient years of hydroxychloroquine) [37]. Two patients developed hydroxychloroquine myopathy (1.9 case per 1,000 patient years) [37]. Most studies of hydroxychloroquine agree that its side effects are infrequent and mild [13, 38, 39].

Despite the rarity of its incidence (see below), retinopathy remains a concern with hydroxychloroquine treatment. A prospective cohort study of 526 Greek patients from 1985–2000 (400 of whom had completed at least 6 years of treatment) examined the occurrence of retinopathy [44]. This study found no occurrence of retinal toxicity in patients during the first 6 years of treatment. One patient developed retinopathy at 6.5 years, and one at 8 years. The incidence of hydroxychloroquine related retinopathy in 400 patients who were treated with recommended doses of the drug for a mean of 8.7 years was 0.5%. As noted above, the

study by Wang et al. calculated 0.95 cases of HCQ related retinopathy per 1,000 patient years of use [37]. A 1999 editorial was able to cite only four cases of retinopathy reported from hydroxychloroquine at doses of less than 6.5 mg/kg/day [45]. If retinopathy does occur, discontinuation of the drug generally gives an excellent prognosis when the patient exhibits normal central and color vision and only relative scotomata. In patients whose vision has degraded to less than 20/20, or have experienced abnormal color vision or absolute scotomata, progressive vision loss may occur even if the drug is discontinued. Most ophthalmologists now consider eye examinations at baseline and every 6–12 months sufficient monitoring for patients with normal vision receiving less that 6.5 mg/kg/day hydroxychloroquine [45].

While defects in accommodation and corneal deposits have also been associated with the use of hydroxychloroquine or chloroquine treatment, the effect on accommodation is easily reversible, and corneal deposits have few, if any, consequences. It is appropriate to separate these latter effects from retinal toxicity in the discussion of the safety of hydroxychloroquine therapy [46, 47].

The effect of hydroxychloroquine on pregnancy has been extensively studied in SLE patients, due largely to the fact that many SLE patients are women of childbearing age [48]. Based on the study of this and other populations, hydroxychloroquine treatment can safely be continued during pregnancy, with the following rationale. Firstly, antimalarial prophylaxis has historically been recommended to pregnant women traveling to malaria-infested areas, and appears to be safe. Secondly, SLE-related flares have been documented when antimalarials have been discontinued during pregnancy. While the 4-aminoquinolone agents cross the placenta and deposit in fetal pigmented tissues, these have not been know to lead to any adverse effects, whereas SLE flares have been know to be detrimental to pregnancy outcomes. Thirdly, as the half life of hydroxychloroquine in the body is 40–50 days, discontinuation of the drug during pregnancy still results in exposure during most of the pregnancy (200–250 days). Finally, the mechanism of action of hydroxychloroquine, such as the lowering of serum lipid levels and the inhibition of platelet aggregation, can potentially actually promote successful pregnancy.

A recent study compared 133 pregnancies among women with connective tissue diseases receiving hydroxychloroquine to a control group of women with similar diseases not on hydroxychloroquine during pregnancy [49]. This study found no statistical difference in pregnancy outcomes between the hydroxychloroquine group and the control group. 88% of pregnancies in the HCQ group (n = 133) and 84% of those in the control group (n = 70) ended successfully with a live birth; three malformations were observed in the HCQ group (one hypospadias, one craniostenosis, and one cardiac malformation) *versus* four in the control group. This study concluded that hydroxychloroquine should be maintained throughout pregnancy for patients with systemic lupus erythematosus.

Place in the rheumatologic armamentarium

Hydroxychloroquine is most frequently used as a single DMARD course of treatment in patients with recent onset, mild RA and is popularly prescribed in this capacity. A survey done in Brittany (France) in 2002 found hydroxychloroquine and injectable gold to be the two most widely used DMARDs in early rheumatoid arthritis [50]. Another survey of 375 rheumatologists found hydroxychloroquine to be the most cited medication for treating patients with mild disease activity/severity [51], a statistic which could be partially due to the relative low cost of hydroxychloroquine therapy in contrast to other single DMARDs. One study showed the RA coded costs of care for patients using hydroxychloroquine therapy to be lower ($227/mo.) than for those on sulphasalazine ($233/mo.) or methotrexate ($340/mo) [30]. In 1995, Prashkar and Meenan modeled the total cost of drug, monitoring, and toxicity for various DMARDs, and found that hydroxychloroquine had the lowest monitoring costs in terms of office visits [52].

A meta-analysis by Felson et al. in 1990 reinforced the impression that hydroxychloroquine is both slightly less effective and less toxic that other generally prescribed DMARDs [39]. A composite measure of outcomes among 66 trials showed antimalarials to be numerically but not statistically better ($p = 0.11$) than auranofin, and similarly numerically but not statistically less effective than other DMARDs. In contrast, antimalarials were found to be generally less toxic than other DMARDs. Most studies contrasting the effectiveness of various DMARDs have not been large enough to avoid the finding of false negatives in terms of the appearance of a lack of differences where differences might in fact exist.

As hydroxychloroquine is less effective than some other DMARDs, its use as a solitary DMARD is recommended primarily for patients with low disease durations and/or mild disease activity/severity. On the other hand, its low toxicity profile, along with the fact that its mechanism of action is different than other DMARDs, makes hydroxychloroquine ideal for use as an element in combination therapy. A survey of Canadian rheumatologists in 2002 found that nearly all (99%) used combination DMARD to treat RA, with the most popular combination being methotrexate and hydroxychloroquine (used by 61% of rheumatologists in the study) [53].

Monitoring

Because hydroxychloroquine is considered a relatively benign DMARD, laboratory monitoring has not been systematized. However, since these patients are also generally on other drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) or methotrexate, the lab monitoring for these drugs is considered adequate for HCQ.

Though the possibility of retinopathy has traditionally been very closely monitored in patients receiving hydroxychloroquine, current ophthalmological opinion indicates that baseline and semi-annual or even annual screening is sufficient. Most ophthalmologists feel that eye examinations every 6–12 months are sufficient monitoring for patients with normal vision, receiving less than HCQ 6.5 mg/kg/day [54].

Two other types of adverse reactions are occasionally associated with hydroxychloroquine, and the patient and physician need to be aware of the possibility that HCQ can be associated with GI and dermatological adverse events. GI disturbances such as nausea, diarrhea, anorexia, abdominal cramps, and vomiting occur not infrequently (see above). These problems usually resolve immediately upon reduction or discontinuation of treatment. Dermatological adverse events can include skin rashes, pigmentous changes in skin and mucous membranes, hair bleaching, and hair loss. These also usually resolve with drug discontinuation [55].

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Sulfasalazine

Cees J. Haagsma

Twenteborg Hospital, P.O. Box 7600, 7600 SZ Almelo, The Netherlands

Introduction

In 1942 Nanna Svartz [1] published an important paper that has since become the benchmark for the present development of drugs: designing antirheumatic drugs based on notions of pathogenesis. She tried to link an antibacterial to an anti-inflammatory agent in order to achieve simultaneous elimination of a putative infectious organism causing rheumatoid arthritis (RA) and suppression of inflammation. A number of compounds were developed and the one containing the antibiotic sulfapyridine and the anti-inflammatory salicylate 5-aminosalicylic acid linked by an azo bond (Structure 1) appeared to be the most promising.

Although initial results in treating arthritis patients were promising, negative results from one study in the UK, and with corticoids entering the pharmacotherapeutic scene, prevented further use in rheumatology. 30 years later McConkey and co-workers reintroduced the drug in rheumatology. It appeared to be effective in an open and extended study [2]. This led to a surge of interest and studies concerning this drug, mainly in RA, but also in spondylarthropathies, e.g., ankylosing spondylitis and psoriatic arthritis. Possible mechanisms of action and other aspects of the (clinical) pharmacology were explored; clinically relevant data on longer-term efficacy, including some data on functional outcome and pharmacoeconomics are now available.

Mechanisms

In their excellent review of the possible mechanisms of action [3] Smedegård and Björk distinguish several mechanisms by which sulfasalazine (SSZ) could exert its actions.

Antibacterial effects of SSZ itself (on which the development of the drug was based) or sulfapyridine on bacterial flora of the gut or systemic infections could

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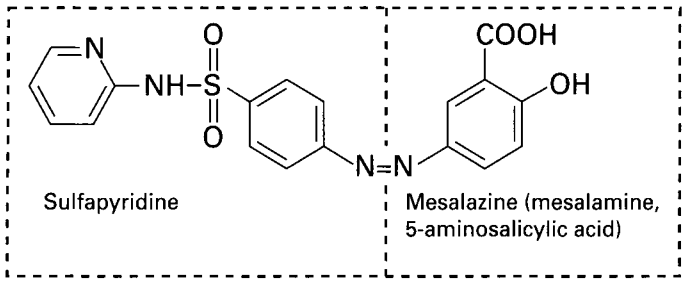

Structure 1
Picture of the chemical structure of SASP, e.g. from Goodman & Gilman's The Pharmacological Basis of Therapeutics, *9th ed., with an indication of the azo-bond*

mediate its efficacy when direct or indirect infectious sequelae are the cause of RA. However, no correlation between change in gut bacteria and clinical response to SSZ was found, and two other sulphonamides (sulphamethoxazole and phthalylsulphathiazole) did not elicit a clinical improvement, although others suggest that cotrimoxazole (a component of which is sulphamethoxazole) has some activity against RA [4].

Anti-inflammatory effects may play a role. SSZ, but not its metabolites, has inhibitory actions *in vitro* on several enzymes involved in the arachidonic acid cascade, e.g., COX-1 and COX-2, 15-PGDH, and also 5- and 15-lipoxygenase and other enzymes involved in the formation of leukotrienes. SSZ has inhibitory effects on various folate-metabolizing enzymes, e.g., on dihydrofolate reductase but also on AICAR-transformylase, thus enhancing the production of adenosine which has several anti-inflammatory properties. SSZ can have effects on granulocytes by altering enzyme and mediator release and inhibiting intracellular activation pathways. Again, this was obvious for SSZ itself, but not for, or much less so, its metabolites, including sulfapyridine. Several of the above-mentioned anti-inflammatory pathways influenced by SSZ are shared by non-steroidal anti-inflammatory drugs. The clinical effects of these drugs and of SSZ are grossly different, both in rapidity of response and disease modifying characteristics, e.g., influence on the rate of radiological deterioration. Interference with anti-inflammatory mechanisms is therefore unlikely to be the primary explanation of the clinical action of SSZ.

Immunomodulatory effects are more likely to account for the way SSZ works in RA. Cellular effects, mainly on lymphocytes, have been studied *in vitro*. Inhibition of T cell proliferation and function, including the release of proinflammatory cytokines was seen. But also inhibition of growth and immunoglobulin synthesis of B cells by SSZ was observed, as well as some influence on monocyte function concerning the release of Il-1, TNF-α and Il-6. In these experiments few or no effects of

the metabolites of SSZ, including 5-ASA and sulfapyridine, were seen, as in the inflammatory models. Influence on angiogenesis could also play a role; inhibitory effects on proliferation of endothelial cells were found, but conflicting results emerged between various cell types. Bovine endothelial cells were inhibited in their proliferation exclusively by SSZ, whereas growth of human umbilical vein cells was inhibited by sulfapyridine only, and not by SSZ itself or 5-ASA. The interpretation of the results of observations in RA patients is complicated by the chicken and egg question: are the observed changes in lymphocyte numbers and function due to direct influence of SSZ and/or its metabolites, or are they merely mirroring the decline in disease activity?

Since the effects on immunological function found *in vitro* were mostly found at concentrations higher than those obtained in serum and joints by the doses used in clinical practice, it poses the question where the SSZ site of action is? Given the possible connection between the gut and inflammatory arthropathy both immunologically and clinically and the much higher concentrations of SSZ in the gut, it is tempting to postulate that the site of action is in the gut or in the gut tissue. Ig A levels and number of circulating Ig A producing cells decline in correlation with the decline in disease activity. Influence on the composition of lymphocytes of the gut mucosa in RA patients and modulation of oral immune response in healthy humans and mice also corroborate the suggestion that the gut is the target of SSZ, but the evidence is thin and circumstantial.

An *in vitro* study found SSZ, but not its metabolites, to be a strong inhibitor of nuclear factor kappa B (NF-κB), thus inhibiting proinflammatory intracellular signalling [5]. Another *in vitro* study showed that the inhibition of TNF-α expression in macrophages by SSZ is mediated by induction of apoptosis, involving the activation of caspase 8. This phenomenon was not observed when using methotrexate, nor by the metabolites of SSZ, sulfapyridine and 5-ASA [6]. Thus, the question of which moiety acts against RA is still matter of debate. The above-mentioned immunological studies point strongly to SSZ itself. In several clinical studies of RA patients however, sulfapyridine did have relevant beneficial effects [7–9]. This clear clinical effect of sulfapyridine questions the relevance of the *in vitro* and *ex vivo* immunological experiments.

Clinical pharmacology

Pharmacokinetics, metabolism and relation to clinical effects

Several authors have reviewed pharmacokinetics comprehensively [10–12]. SSZ is given as enteric-coated tablets. This coating may lead to reduced bioavailability, down to two-thirds of non-coated tablets. As stated in the preceding sections, SSZ consists of two compounds: sulfapyridine and mesalazine (5-aminosalicylic acid)

tied together by an azo-bond (Structure 1), and is split in the colon by bacteria to sulfapyridine and 5-aminosalicylic acid. SSZ itself is variably absorbed, with estimates between 10–30%, as is 5-aminosalicylic acid. SSZ undergoes enterohepatic recirculation. Blood levels of SSZ are about one-third of sulfapyridine and its main metabolite acetyl-sulfapyridine [13, 14] synovial fluid levels are somewhat lower than in plasma. SSZ is highly protein-bound. Its elimination half life in blood is 4–14 h, increasing in elderly people [15]. A small fraction (<10%) of the original dose is excreted unchanged in the urine. Sulfapyridine is absorbed more than 90% and is subject to extensive metabolism: mainly n-acetylation but also hydroxylation, and subsequently glucuronidation. Protein binding is around 50%. In patients with RA significantly higher plasma levels of sulfapyridine were found compared to patients with inflammatory bowel disease. The area under the curve (AUC) was also greater with sulfapyridine, but the AUC of SSZ itself was smaller, probably due to more cleavage of SSZ in RA patients [16]. Acetylatorship is bimodal: fast and slow acetylators. This influences the pharmacokinetics of sulfapyridine; doubling of plasma levels is seen in slow acetylators, the elimination half life being prolonged by 50% or more after a single dose of SSZ, but age has no influence on sulfapyridine levels [15], as can be seen in Figure 1.

Higher sulfapyridine plasma levels and slow acetylators are associated with a greater number of minor gastrointestinal adverse events [17–19], but are probably not related to efficacy [20, 21]. Acetylating capacity is genetically determined, single-nucleotide polymorphisms of n-acetyltransferase-DNA influenced acetylating capacity if both alleles were mutated. These polymorphisms are linked to adverse events in 144 Japanese patients, mainly fever and rash, sometimes leading to hospitalisation [22]. However, such toxicity was not exclusive to the patients with the doubly-mutated enzyme, and the results are contrary to preceding studies in which slow acetylatorship was not associated with more serious adverse events such as those experienced by the Japanese patients. Moreover, the relative lack of gastrointestinal (GI) toxicity in that study (only one patient had GI toxicity without fever or rash), strengthens the impression that it was not a representative patient sample, and generalization to all SSZ users is thus not possible. Routine testing of acetylatorship or determination of mutations in n-acetyltransferase-DNA is therefore not recommended. Maybe genetic testing, possibly only in selected populations, will have a place if it is confirmed that serious adverse events occur excessively with certain mutations.

Dosing

SSZ is given orally. It is initiated at low doses, 1–2 times 500 mg daily, and increased in weekly intervals. Although no extensive studies have been done to establish the optimal dose, 2,000 mg/day is the commonly used dose. Increasing the dose to

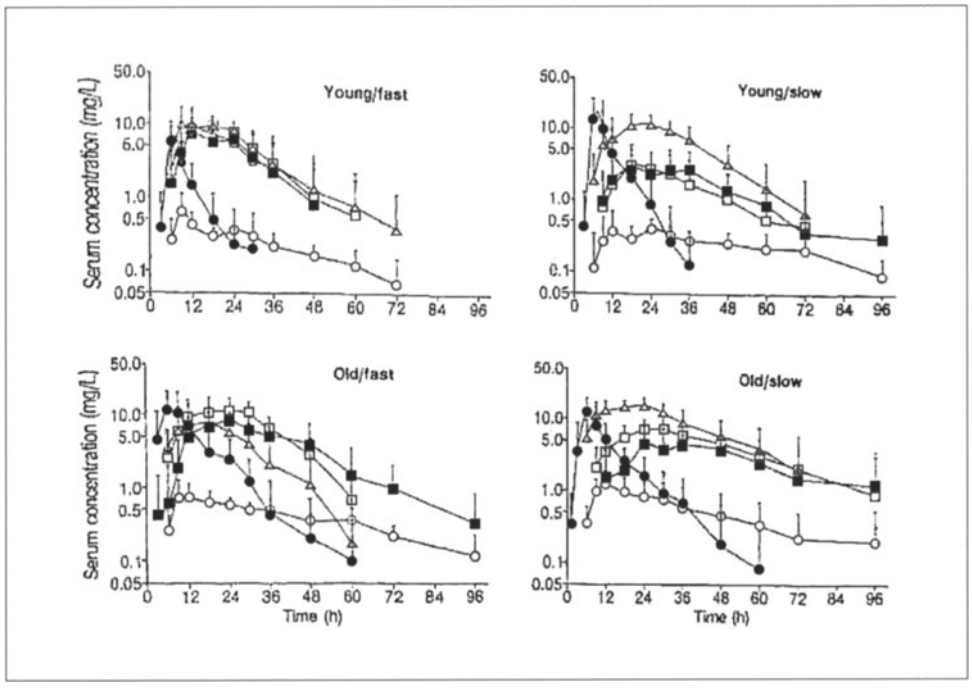

Figure 1
Serum concentration (± SD) of sulfasalazine (SASP, ●), and metabolites, sulfapyridien (SP △), N-acetyl-sulfapyridine (AcSP, □), N-acetyl-sulfapyridine-O-glucuronide (AcSPG, X) and N-acetyl-mesalazine (Ac-ME, ○) after oral administration of sulfasalazine 2 g on day 1 of the treatment schedule in the 4 subgroups of patients: young/fast; young/slow; old/fast; old/slow [15].

≥ 40 mg/kg may correlate with increased efficacy [23]. Indeed, raising the dose from 2,000 to 3,000 mg/day was necessary in 32% of a cohort of RA patients to maintain efficacy, with 50% of those achieving this goal, thus being able to continue the drug. Conversely, 30% of the patients had to lower the dose in order to avoid intolerable side effects [25].

Interactions

Clinically relevant interactions with SSZ are very few. Theoretically, antibiotics and cholestyramine can delay or reduce absorption of sulfapyridine because of antibacterial activity or by binding to SSZ, making it unavailable for bacterial cleavage. The

Table 1 - Clinically significant interactions with SSZ

Drug	Significance	Ref.
Digoxin	Decrease in digoxin blood level of 25%	[30]
Azathioprine	Leucopenia, increased levels of 6-thioguanine nucleotides	[26]

significance of these possible interactions has not been clinically investigated, however, and given the uncertainties concerning the active component of SSZ, it is of doubtful significance. *In vitro* testing of the activity of thiopurine methyltransferase, responsible for the methylation of azathioprine/6-mercaptopurine, revealed that SSZ and its metabolite 5-aminosalicylic acid, but not sulfapyridine nor its metabolites, inhibited the activity of this enzyme [25]. It appeared that this *in vitro* discovered interaction indeed leads to significant increases in blood levels of 6-thioguanine nucleotides and increased occurrence of leucopenia in the SSZ and mesalazine treated patients with Crohn's disease [26]. In RA patients, the combination of azathioprine and SSZ is not often used and hardly studied; only case series exist [27, 28]. In general, the dose of SSZ is lower in treating RA than in treating Crohn's disease, but caution has to be exerted when using the combination of azathioprine and SSZ. Contrary to sulfonamides in general, a case report suggested that SSZ had an inhibiting effect on the anticoagulant effect of warfarin [29]. SSZ can decrease the absorption of digoxin, leading to a decrease of digoxin levels of about 25% [30].

Efficacy

Early experience with SSZ in RA has been discussed in the sections above. More rigorous clinical testing of the drug was undertaken from 1980 onwards. Randomized clinical trials were performed, mostly with limited duration of follow-up. The reported outcomes were often limited to the course of disease activity variables, e.g., joint scores, pain and some measurement of acute phase response. In the following, only results of single drug comparisons will be shown, combinations containing SSZ are discussed in the chapter by Choy and Paulus.

Short-term experience, traditional efficacy variables

Meta-analyses

In 1990 a meta-analysis of comparative efficacy and toxicity of second-line drugs used in RA was published by Felson et al., with an update in 1992 [31, 32]. The

search was limited to the Medline database with an additional bibliographic search of trial reports and reviews. Only randomised trials of a minimum duration of 2 months were evaluated. Nearly all trials lasted less than 2 years with a mean of 37.7 weeks. As measures of efficacy tender joint count, ESR, grip strength, and a "combined effect size", summary measurements were taken. A rather unusual way of standardization was performed: the change of an outcome measurement was divided by the pooled baseline standard deviation for that outcome measure across trials, referred to as the standardized improvement. How dropouts were accounted for is not clear and the authors did not mention which measurement of change of an individual trial was used. No further details about the pooling were given. The "combined effect size" was presented as the mean of standardized improvements of the three variables (tender joint count, ESR, grip strength). Importantly, the authors combined the results for each drug across the treatment groups, weighting each treatment group by size at the end of the trial. A quality assessment was done, assessing 10 characteristics, e.g., patient characteristics as eligibility criteria, randomization, blindness and statistical analysis. In the comparison between drugs, analyses of variance, weighted by treated group size, multiplied by study quality and adjusted for significant co-variants (among them the trial length) were performed, using a fixed effects model. Q statistics were applied to test for heterogeneity, which was not significantly present after adjusting for co-variants. The primary efficacy variable was the composite outcome measure. Toxicity was evaluated by using the proportion of all dropouts and the ones dropping out due to toxicity, again corrected by treatment group size, quality and co-variants. Due to remaining heterogeneity a random-effects model was used in the first analysis, while in the update it was stated that a fixed effects analysis was performed. In the update, toxicity was defined as above and also by weighting a dropout due to toxicity by a modified toxicity index and by the rate of severe toxicities; the toxicity index exceeding a certain value. As in the efficacy studies, results were combined across the trials. There was a considerable overlap in the studies used in the efficacy and toxicity analyses, but this was not complete. Results were plotted as the measure of efficacy *versus* each measure of toxicity.

SSZ ranks with MTX, d-penicillamine and parenteral gold as the most effective, the toxicity being slightly more than MTX but less than gold. The main objection against this meta-analysis is the pooling of results of the individual treatment arms of each drug across the trials, thereby effectively breaking the randomization, which could lead to the situation that the differences found between the drugs mirror differences in study populations rather than true differences between drugs.

Two other meta-analyses specifically addressing SSZ were performed concerning efficacy in RA. A Cochrane Review by Suarez-Almazor et al. was published searching studies published until 1997 [33] summarising six studies [34–39] comparing SSZ with placebo with a follow up of at least 6 months. Only four studies contributed to the full efficacy analysis. Inclusion criteria and application of them (two

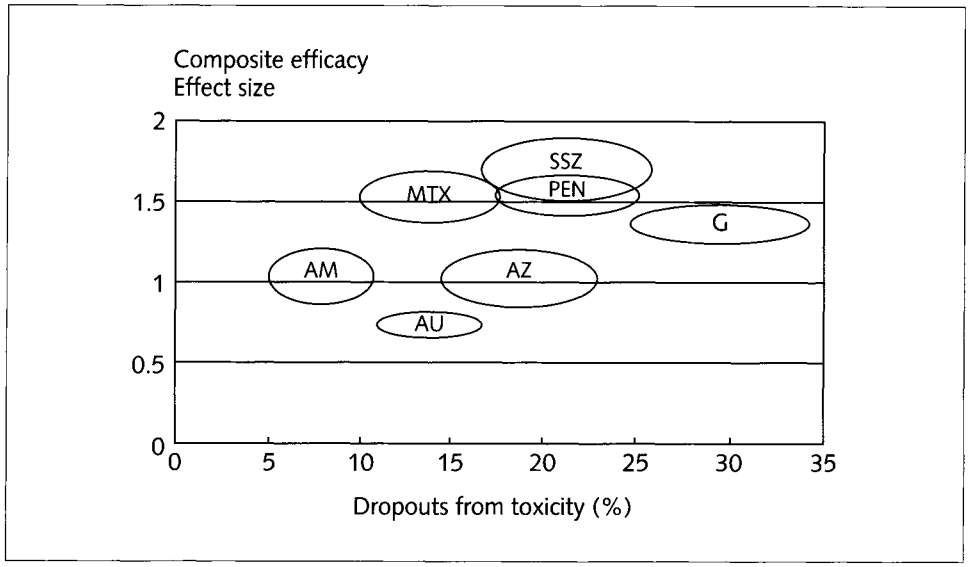

Figure 2
Plot of the corrected composite efficacy versus *the corrected % dropouts is depicted [32].*

reviewers), search strategy (Cochrane Musculoskeletal Group trials register and Medline), data extraction and handling, description of patients, intervention, outcomes and quality of the studies (Jadad score) were adequately described. Differences between studies were tested, using fixed effects models in case of homogeneity and random effects models in case of heterogeneity (ESR). A pooled analysis calculating standardized weighted mean differences of numerical and ordinal outcomes and pooled odds ratios of nominal outcomes (both outcomes at the end of trial) was performed. A clear statistically significant difference favoring SSZ was found for tender and swollen joint scores, pain and ESR. Probably due to a type II error, no statistical differences were found for global assessments of patient and physician. Withdrawals due to inefficacy were higher in the placebo group (OR 0.23) and withdrawals due to adverse reaction higher in the SSZ treated group (OR 3.0). No data on functional parameters were available. More detailed information is given in Table 2. The meta-analysis uses clear inclusion criteria and a reasonable search of the literature, although publication bias is not excluded. The authors excluded non-English studies.

In 1999, a meta-analysis of 15 randomized trials comparing SSZ with either placebo or active control in RA patients [40] was published. Concerning placebo-controlled trials, the same ones as mentioned in the meta-analysis cited above plus a study of shorter (15 weeks) duration [41] and a trial published in Japanese [42] were included. Comparison of the original studies was not possible, since they were

Table 2 - Weighted effect sizes [33]. Sulfasalazine vs. placebo – Efficacy. Copyright Cochrane Library, reproduced with permission.

Outcome title	No. of studies	No. of participants	Statistical method	Effect size
01 Tender joints	6	252	WMD [Fixed] [95% CI]	–2.448 [–4.154, –0.741]
02 Number of swollen joints	6	220	WMD [Fixed] [95% CI]	–2.379 [–3.728, –1.029]
03 Pain	6	173	WMD [Fixed] [95% CI]	–8.711 [–14.801, –2.621]
04 Physician global assessment	6	155	WMD [Fixed] [95% CI]	–0.160 [–0.375, 0.055]
05 Patient global assessment	6	155	WMD [Fixed] [95% CI]	–0.231 [–0.462, 0.000]
06 Functional status	6	0	WMD [Fixed] [95% CI]	Not estimable
07 ESR	6	172	WMD [Fixed] [95% CI]	–17.581 [–21.933, –13.228]
08 Radiological scores	1	73	WMD [Fixed] [95% CI]	–3.600 [–11.130, 3.930]
09 Patients with erosions	1	51	Peto OR [95% CI]	0.59 [0.11, 3.21]

not described in detail. However, the authors do report on and deal with (lack of) homogeneity, and analyze the data with and without the outliers. Not surprisingly, the results of the comparison with placebo were comparable with the analysis of Suarez-Almazor, although the method of reporting the results was different. The authors did not make an individual assessment of the quality of the studies, all were randomized controlled trials, and double blindness was indicated. From the way the authors present the pooled effect estimate, the impression arises that pooling was done across the trials and not a comparison within the individual studies with pooling of the results of each trial. This could affect the randomization. The use of d-statistics however suggests that they did pool the differences between SSZ and placebo of the individual studies and the results of this statistical analysis support the conclusions drawn. The comparison with hydroxychloroquine revealed no important differences between the two drugs, although there was a tendency in some variables in favor of SSZ. The percentage of dropouts due to inefficacy was less in the SSZ *versus* the hydroxychloroquine patients (5% *versus* 15%, $p = 0.055$), the ESR

was lowered more (–43% *versus* –26%, p = 0.10) as was duration of morning stiffness (–59% *versus* –40%, p = 0.09). The course of the various disease activity variables during the study is not analyzed. Only differences between baseline at the end of the study were taken into account. Earlier response to SSZ in one study [43] was thus not noticed, although radiographical analysis of this study revealed less progression in X-ray lesions [44], possibly explained by the earlier effect on synovitis by SSZ. Compared to intramuscular gold, dropouts due to adverse drug reactions were significantly less in SSZ treated patients (12% *versus* 29%, p = < 0.0001) and dropouts due to lack of efficacy were greater (13% *versus* 4%, p = 0.006); no significant differences between other variables were observed. No significant differences or relevant tendencies were found in the pooled results of three trials comparing SSZ and d-penicillamine.

Other studies

Since 1997, several studies comparing SSZ with placebo and other DMARDs have been performed. A comparison between SSZ, leflunomide and placebo showed that SSZ and leflunomide were similar in efficacy and statistically superior to placebo as measured by the primary endpoints: tender and swollen joint counts and patient's overall assessments [45]. It was a 24 weeks study with a possibility for completers to go on in a double-blind extension of 12–24 months, the placebo treated patients switched to SSZ. 358 patients were randomized 2:2:1 to SSZ, leflunomide and placebo respectively. In Table 3 the changes (± SD) in the 6-month results of the primary efficacy variables are given. Although the ESR decreased significantly more in the SSZ group compared to leflunomide, the C-reactive protein (CRP) level decrease did not differ significantly. Radiological and functional measurements are discussed below in the sections on influence on structural damage and on function.

A double-blind, double-dummy comparison of SSZ and MTX and the combination of the two in 115 early RA patients revealed no difference between the two single drugs in tender joints, Ritchie articular index or swollen joints, nor in Disease Activity Score or good response according to EULAR criteria or 20% ACR response [46]. The time to good response tended to be shorter in the SSZ treated patients, but this could be due to the relatively low starting dose of methotrexate (7.5 mg weekly). A study of similar design revealed similar results when comparing the two single drugs, and although the decrease in Disease Activity Score tended to be greater in the SSZ treated group at 52 weeks of follow up (–1.15 and –0.87, SSZ *versus* MTX), there were no differences in percentages of responders at 52 weeks [47].

Recently a double-blind randomized clinical trial comparing SSZ with diclofenac in 117 early RA patients was published [48]. In this rather remarkable comparison the authors found that SSZ had a symptomatic effect (pain score, number of painful

Table 3 - Change of disease activity variables, results after 6 months, means (SD)

Smolen et al. 1999 [45]	**SSZ (n=132)**	**Leflunomide (n=130)**	**Placebo (n=91)**
Tender joint count	–8.1 (7.4)	–9.7 (7.8)	–4.3 (7.5)
Swollen joint count	–6.2 (5.7)	–7.2 (6.6)	–3.4 (6.5)
Patients assessment	–1.1 (1.0)	–1.1 (1.1)	–0.4 (1.1)

joints) as quickly as diclofenac measured at 2 and 4 weeks after start of the medication. Disease activity measured by the Disease Activity Score decreased significantly in both groups at 2 and 4 weeks, at 12 and at 26 weeks this score was lower in the SSZ group ($p < 0.05$). So SSZ had similar early symptomatic effects as the NSAID diclofenac but suppressing clinical disease activity better in the long run. The radiological outcome at 12 months is presented below.

SSZ is superior in efficacy to placebo in the short-term treatment of RA, and probably comparable to various other DMARDs, e.g., methotrexate and leflunomide. Perhaps SSZ is more effective than hydroxychloroquine due to a faster onset of action.

Influence on structural damage

The influence of SSZ on radiographical outcome was assessed in a few studies. The only study comparing SSZ (and leflunomide) with placebo using radiological evaluation has been discussed above; longer-term radiographical analysis was published separately [49]. The X-rays were blinded for treatment and sequence. Radiographs of about two-thirds of the patients were available. Already at 6 months of pharmacological interventions an advantage of active treatment was discernable. The Sharp score at baseline was about 45, about 75% of the patients being erosive, comparable among the three arms of the study. The change during the first 6 months of follow up in the SSZ treated group was 2.32 (SD 10.1), in the leflunomide group 1.23 (2.85), and in placebo treated patients 5.88 (10.0). The differences between active treatment (both leflunomide and SSZ) and placebo were statistically significant. Compared to the estimated baseline annual rate of radiographic progression there was an important slowing of deterioration both in the SSZ and leflunomide treated patients (SSZ from 5.7 to 1.38, leflunomide from 6.1 to 0.97) regarding the 12-month data of active treatment. 61 of the 133 SSZ treated patients and 77 of the 133 leflunomide treated patients remained in the study. The placebo treatment was stopped at 6 months. The same data were analyzed again using the Larsen scoring method with addition of the results of the 24 months extension (from 6–24 months

the study contained only two arms: SSZ and leflunomide) [50]. Not surprisingly the results of the 6 and 12 months analyses were about the same as the evaluation of the patients using the Sharp score. At 24 months of treatment there were no statistically significant or meaningful differences between the treatment groups, notwithstanding suggestions and even straightforward claims of the authors. The results were presented as intention-to-treat, but at 24 months only 27 of the 133 SSZ patients and 28 of the 133 leflunomide patients were still involved in the analysis. Thus, these results can only be regarded as an interesting but premature impression.

Another study examined the difference in radiological outcome [44] between SSZ and hydroxychloroquine; the design and clinical results have been described in the preceding section [43]. At 24 weeks of treatment there was a difference between the two drugs in favor of SSZ in erosion-score although no statistical significance was reached in this relatively small number of patients (median number of erosions 2.5 *versus* 10, SSZ and hydroxychloroquine respectively). The total score (including joint space narrowing) was significantly better in the SSZ treated patients (6.5 *versus* 17, $p < 0.02$). At 48 weeks the difference was more pronounced: the median number of erosions in the SSZ group being 5, in the hydroxychloroquine group 16 ($p < 0.02$), as was the total score: 8 *versus* 33.

The radiographic analysis of a comparison between SSZ and diclofenac48 revealed that early institution of SSZ led to less damage after 12 months of study in an intention-to-treat analysis. The SSZ treated patients had a mean number of new erosions of 2.0 (95% CI 0.9–3.1), compared to diclofenac treated patients (mean 7.5, 95% CI 4.1–10.9), this was significantly better ($p < 0.002$). These results confirm the disease modifying properties of SSZ.

In an older, non-blinded study of 54 RA patients SSZ was compared to d-penicillamine [51]. Radiological deterioration was observed in both groups, with a tendency of greater worsening in the SSZ group. Pullar found in a follow up of 41 patients treated with SSZ that there was deterioration over 2 years, but that this worsening was confined to the first year. The SSZ patients had less progression than a control group, which was of limited value, consisting of only 10 patients who refused second line therapy [52].

In conclusion, SSZ is superior to placebo already at 6 months of treatment concerning retardation of radiographic damage, confirmed by a comparison with diclofenac. The more disease activity is suppressed, the better the radiological outcome is; thus, SSZ seems to prevent X-ray damage better than hydroxychloroquine and appears equivalent to leflunomide in this respect.

Influence on long-term outcome

Information on long-term (i.e., more than 1 year) outcome of SSZ treatment in RA consists of two broad categories. Firstly, long-term clinical trials and long-term

observational studies, of which the traditional outcome measurements are discussed in this section; and secondly drug use survival studies. Few long-term clinical trials and observational studies have been reported.

SSZ (n = 315) was compared to intramuscular gold (n = 203) and d-penicillamine (n = 163) in an observational study for up to 5 years describing the course over time of a clinical score and of two measurements of acute phase: ESR and CRP [53]. The patients included in this study were roughly the same as included in an analysis of treatment termination using life table methods [54], and comprised all patients with definite or classical RA in the unit of the authors receiving one or more of the three drugs. Since the indication to use one or another drug is not clearly stated or known, the treatment order of the drugs was different, and the patient groups were not comparable in various important clinical variables; the three drugs cannot be compared to each other. In all three groups there were significant improvements in clinical score and acute phase response compared to baseline, in SSZ lasting up to 60 months for the patients who continued treatment. Using an arbitrary definition of response, age was lower and disease duration was shorter in the SSZ responders compared to SSZ non-responders.

A comparison of SSZ and d-penicillamine showed that after 5 years only three of the original 28 patients treated with SSZ and 8/26 patients on d-penicillamine were still on their original treatment [51]. In SSZ, lack of efficacy was the main reason of treatment termination (16/28), whereas in d-penicillamine it was toxicity (10/26).

In an open, partly randomized trial SSZ and auranofin were compared [55]. The 200 patients were followed-up for a maximum period of 5 years. Not all patients that were analyzed were allocated to treatment by randomization: if the patient received one of the two study drugs before start of the study the other drug was given in the study, if the patient had received intramuscular gold with ensuing toxicity or lack of efficacy the patient was allocated to SSZ. Among the patients treated with SSZ 31% continued treatment for 5 years, compared to 15% auranofin treated patients; in the latter group patients dropped out mainly because of toxicity, half of these due to diarrhea. Obviously, the percentage of patients pretreated with i.m. gold was particularly low in the auranofin group. In the analysis of patients still on drug treatment, articular index, duration of morning stiffness, pain score, ESR and CRP were lowered during the 5 years of treatment in both groups, with significant changes at 5 years compared to baseline only in the SSZ group. Only the pain score was not significantly different. The between-group comparisons revealed no significant differences. If the patients who continued the full 5 years of treatment were analyzed separately, the same picture emerged. Generally, the patients who continued treatment for 5 years had lower disease activity at baseline. Meaningful comparison between the two drugs is severely hampered by the peculiar way patients were allocated to treatment; the conclusion of the authors that SSZ has a better efficacy/toxicity profile is therefore unjustified.

A study following RA patients for 12 years initially randomized them between SSZ and d-penicillamine [56]. Only seven of the initial 102 patients continued to take SSZ (46% of the patients stopping because of lack or loss of effect, 35% due to toxicity and 19% due to other reasons, including death), 4/98 d-penicillamine. Life table analysis showed no difference between SSZ and d-penicillamine in time on allocated drug (median of 28 and 20 months, SSZ and d-penicillamine respectively). Almost half of the patients died, not related to either SSZ or d-penicillamine according to the authors. Not surprisingly, disease activity did not deteriorate in the patients who were able to survive the 12 years of follow-up.

The comparison between SSZ and leflunomide has been described above in the sections on short-term effects [45] and influence on structural damage [49, 50]. Both the SSZ and the leflunomide group consisted of 133 patients at baseline. The placebo treated patients switched to SSZ after 24 weeks; because of this design this group is excluded from the following discussion. At 6 months 76 SSZ and 80 leflunomide patients entered the next treatment episode lasting from 6–12 months; in the 12–24 month extension 60 patients participated in both groups. Reasons for withdrawal did not differ significantly between SSZ and leflunomide. Concerning the original primary efficacy variables, no differences were seen between SSZ and leflunomide with the exception of patient's global assessment at 24 months. Of the secondary efficacy parameters only the HAQ scores were significantly better in the leflunomide treated patients compared to SSZ at a few time points as will be discussed below in the section on quality of life and function. Interim analyses were not mentioned but have been performed in order to be able to report in the above-mentioned articles. This influences the power of the study. Due to the design of the study the analysis was not intention to treat. Together with the considerable number of withdrawals, the conclusions drawn by the authors can therefore only be tentative [57].

As is clear from above, the problem with determining the efficacy as defined by, e.g., joint scores or other variables in long-term studies, is the substantial withdrawal rate. Another way of analyzing the long-term performance of drugs is to focus on this phenomenon, making a virtue of the need. Studying treatment termination is done with the aid of survival analysis, e.g., life table or Kaplan-Meier survival analysis. Data from treatment as is given in usual care can thus be evaluated.

The obvious disadvantage of this approach is the non-randomized way patients receive their treatment, leading to selection bias and/or confounding by indication. Preceding therapy and the number and attractiveness of pharmacological options beyond the studied drug, and the notion of both the patient and the physician about the drug also influences treatment "survival". These and other influences may vary over time, as was pointed at by Utley et al. [58]. Nevertheless it can provide useful data on the long-term outcome, the more so if the potential biases pointed at above are avoided or compensated for, e.g., by correcting for confounders by Cox proportional hazards analysis.

In 1987 Situnayke and colleagues published the long-term follow up of the treatment of RA with SSZ (317 RA patients), intramuscular gold (201 patients) and d-penicillamine (163 patients) using life table methods [54]. Results of the traditional efficacy variables have been discussed above [53]. Treatment termination rates at 5 years were similar in all three drugs (SSZ 81%, gold 92% and d-penicillamine 83%). Discontinuation rates due to inefficacy were 41%, 30% and 38%, respectively, with a rather evenly distributed cessation during the 5 years. These percentages were 37, 57 and 41 respectively for toxicity-withdrawals, most of the patients stopping the drug in the first year of treatment. Reasons for selecting one or the other drug were not stated, nor were preceding or following therapy, making this study vulnerable to the above-mentioned biases.

86 RA patients were followed for 5 years or until SSZ discontinuation in a study by Jones et al. [59]. No comparator was included in the study. Very few data on patient characteristics were provided. At 5 years 22% of the patients were still on treatment with SSZ. 38 patients (44%) stopped because of inefficacy, 25 (29%) due to adverse effects that occurred almost all in the first 3 months, two patients died from unrelated causes and six patients (7%) discontinued being in remission. In this study the same criticism (no correction for possible confounders) as mentioned with the study of Situnayake is applicable.

A prospective study of patients with recent-onset RA reported the probability of treatment termination of 272 consecutive patients attending two academic hospitals who entered the study by starting second-line antirheumatic treatment [60]. Due to low numbers of other drugs, only the data of SSZ, hydroxychloroquine, aurothioglucose or d-penicillamine were analyzed. All patients had a disease duration of less than 1 year. The mean rank order of prescription of each drug was calculated, which was shortest for hydroxychloroquine (1.10), 1.39 for SSZ and longest for d-penicillamine (2.15) being significantly different. The disease activity as measured by the Disease Activity Score [61] at the start of treatment, was significantly higher in the gold and d-penicillamine group. No other details on the patients were provided. At 2 years significant differences existed between aurothioglucose (43%) and hydroxychloroquine (61%), and between SSZ (46%) and hydroxychloroquine (61%) ($p < 0.01$ and $p < 0.02$, respectively; Cox proportional hazards model with Bonferroni correction) concerning the percentage of drug "survival" with treatment termination due to inefficacy as endpoint . Though the proportion of gold treated patients stopping because of adverse events was larger than in the other groups, this difference did not reach statistical significance. Except for the gold treated patients, most dropouts due to toxicity occurred in the first 6 months. At 3 years the proportions of patients having stopped the treatment were least in d-penicillamine and SSZ (54% and 60%) compared to hydroxychloroquine and i.m. gold (72% and 75%), but the differences were not statistically significant, probably due to the lower number of patients at risk. The influence of rank order of prescription and initial disease activity were studied, with the aid of the Cox pro-

portional hazards model. A higher rank number and higher initial disease activity shortened the length of "survival" on a drug, regardless of which of the four above-mentioned drugs was studied . In this study an attempt was made to avoid selection biases by correction for the rank order of prescription and initial disease activity.

Updated results of one centre of the cohort mentioned above 60, with addition of newly included patients resulted in the follow up of 186 SSZ treated patients [24]. At 48 months of treatment roughly 35% of the patients were still on treatment with SSZ, although 32% of the patients had to increase the dose to 3,000 mg daily to maintain efficacy and in 30% the dose was lowered to avoid unacceptable toxicity. Compared to HCQ when given as a first choice agent, survival in the first 2 years was better for SSZ. When given as a second choice, SSZ-survival was better compared to i.m. gold, mainly due to fewer dropouts for toxicity. Corrections for initial disease activity were not reported.

A Canadian cohort of 128 RA patients was followed retrospectively during 7 years concerning patterns of drug use and long-term effectiveness [62]. Of the original 184 patients, the ones who died, who refused to participate and who were lost to follow up were excluded (30%). SSZ and hydroxychloroquine were often prescribed in combination with other drugs. Prescription patterns between rheumatologists and rank order of use of the various drugs differed significantly. 109 of the 128 patients were given a total of 233 courses with 2nd line drugs. The proportion of patients on treatment at 48 months was highest in MTX (56%) and lowest in SSZ (13%), although the number of patients at risk was not mentioned and presumably low (cumulative number of MTX patients 27, SSZ 33). Therefore, these percentages must have wide confidence intervals. Although detailed information about patients and drug use was available, the authors did not undertake corrections for confounding factors, such as rank number of prescription and combination with another 2nd line drug, except for disease duration, this variable having no influence. For this reason and due to the low numbers studied, the conclusions can only be speculative.

A large cohort of the same region, mostly patients treated in tertiary centers and, probably including the above-mentioned patients, was analyzed using survival methods [63]. Of 1,297 eligible patients, 1,132 patients receiving 2,296 DMARD courses were studied. 1,239 of these were discontinued; in 93 no reason for discontinuation was reported and these cases were treated as missing values and omitted from some of the analyses. In a Cox regression analysis various potential confounders were corrected for. However, disease activity at the start was not included in that analysis. After 3 years 25% of the patients still received SSZ, compared to 50% MTX, 33% antimalarials and i.m. gold and 18% oral gold. Most discontinuations in the SSZ group were due to lack of efficacy. After 6 years of treatment 20% of the patients were still receiving the drugs, with no differences between the individual DMARDs, possibly due to low numbers of patients remaining at risk. Look-

ing at hazard ratios with correction for various confounders, MTX had a significantly lower risk of discontinuation compared to all other drugs, including SSZ (hazard ratio for all causes 2.3, for lack of efficacy 6.7 and for toxicity 1.4, SSZ compared to MTX). From survival curves antimalarials seemed to do better than SSZ, in particular concerning efficacy. The biases and lack of power of the preceding study have been largely overcome in this study.

In a recent meta-analysis of treatment termination rates of DMARDs used in RA patients, data of 110 of 159 studies satisfying the inclusion criteria were used, identified by a search of Medline (1966–1997) and *Excerpta Medica* [64]. The remaining 49 studies did not provide sufficient details to be analyzed. Treatments with SSZ, methotrexate, parenteral gold and hydroxychloroquine were taken in account. Both clinical trials and observational studies were used. Due to limited amount of data, and as the authors state "because it is generally prescribed in mild RA" (probably hinting at selection bias), hydroxychloroquine was omitted from comparison with the other drugs, although survival curves up to 24 months were presented. Study type and year of publication were corrected for by using Cox's proportional hazards model. The combined number of patients at risk at baseline was 2,875, 3,155 and 1,418, respectively. Survival analysis showed that 36% of the patients continued MTX, 23% parenteral gold and 22% SSZ for 60 months. The median survival times were 41, 24 and 18 months, respectively. Censoring other reasons of withdrawal, the rates of continuing with respect to (in)efficacy were 75%, 73% and 53% at 60 months for MTX, parenteral gold and SSZ, respectively. Concerning toxicity, these rates were 65%, 36% and 48%. When correcting for study type and year of publication, looking at all causes of withdrawal, patients treated with SSZ were 1.6 times more likely to fail therapy than MTX; patients on parenteral gold were 1.4 times more likely to fail therapy than those treated with MTX.

A difference with the studies discussed above is the rather evenly distributed withdrawal due to toxicity in SSZ treated patients in this study, contrary to the early dropout due to toxicity with an ensuing plateau in several others. Comparing withdrawal rates of observational and randomized clinical trials, no significant differences were found. The data on the randomized clinical trials have to be interpreted with caution because apparently the data of the treatment arms have been pooled across the different studies rather than comparing the differences within the studies with subsequent pooling of the differences. The authors noted that survival times for MTX and SSZ increased in recent years, the year of publication independently contributing to survival differences. This meta-analysis provides useful data due to its large number of patients. However, it is subject to most of the biases of the individual underlying studies, since no correction of possible confounders other than the year of study was done.

In conclusion, study of long-term results is difficult, mainly due to the substantial withdrawal rate and a number of biases in long-term observational studies, the

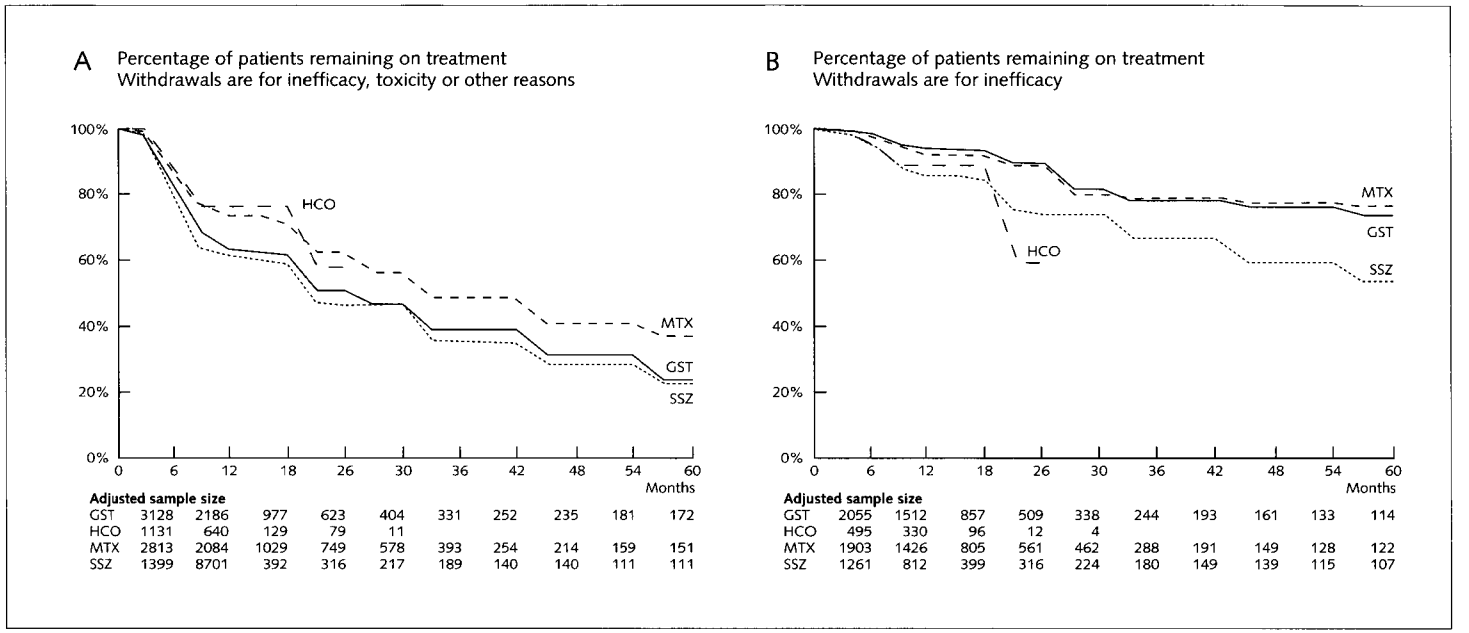

Figure 3

The survival curves are depicted [64].

A. Survival curves representing the percentage of patients withdrawing from each agent because of inefficacy, toxicity or other reasons. Six-month interval data were generated from studies providing withdrawal information for intervals larger than 12 months. The data are from observational studies and randomized controlled trials.

B. Survival curves representing the percentage of patients withdrawing from each agent because of inefficacy. Six-month interval data were generated from studies providing withdrawal information for intervals longer than 12 months. The data are from observational studies and randomized controlled trials.

most important being selection bias. SSZ withdrawal rate is variable between studies. Thus, firm conclusions about long-term use cannot be drawn. With this in mind perhaps SSZ long-term efficacy can be placed between 1) auranofin and hydroxychloroquine, those probably being less effective; and 2) methotrexate, being more effective, although hard data to substantiate this are not available.

Quality of life and function

The impact of SSZ on measurements of quality of life has hardly been studied directly. More information is available on the influence of SSZ on functional outcomes. This was almost invariably assessed with the Health Assessment Questionnaire (HAQ) [65]. The scale of this measurement runs from 0 (best value) to 3 (worst score) with items like dressing and grooming, arising, hygiene, eating and walking. Preliminary information is available on other questionnaires like the AIMS [66], concerning only small, uncontrolled studies.

In the 48-week trial comparing SSZ with hydroxychloroquine discussed above [43], the influence of the treatment on the HAQ was also determined in these early RA patients [67]. Although not all patients could be evaluated, SSZ turned out to be more effective than hydroxychloroquine in terms of the HAQ. In SSZ patients the HAQ score decreased from 0.53 to 0.24, in the hydroxychloroquine treated patients from 0.63 to 0.58 (on a scale of 0–3).

An improvement of long-term functional outcome, as measured by the HAQ, was seen in a cohort of patients, using DMARDs (including SSZ) consistently [68]. The influence of individual DMARDs was not given.

The comparison between SSZ, methotrexate, and the combination of the two in early RA patients [46] showed no significant differences between the three groups in a 1 year treatment period. The HAQ decreased 0.32 (95% CI 0.10–0.53) in the SSZ patients, 0.46 (0.25–0.68) in methotrexate and 0.51 (0.26–0.76) in the combination group.

In the study of Capell [84], 12 years after the start of the study, the HAQ score in the patients still alive who originally had SSZ (40 of 102) was almost the same as at baseline (from 2.13 to 2.25), although only 7/102 were still on treatment with SSZ. In the d-penicillamine group there was a slight increase. However, since so few patients remained on their original treatment no meaningful conclusions can be drawn.

Data on functional measures, the HAQ, and a modification were analyzed in the 6 months study comparing SSZ and leflunomide with an extension to 24 months [45, 57], of which the clinical and radiographical data were discussed in the preceding sections. Data on the HAQ were published in these and various other articles [69–72]. The HAQ scores at baseline and changes at 6, 12 and 24 months are given in Table 4. Not all patients could be evaluated for this analysis; the total num-

Table 4 - Change in HAQ scores, means.

(Smolen 1999 [45], **Scott 2001** [57]	**Change 0–6 months (%, SD, n)**	**Change 0–12 months**	**Change 0–24 months**
SSZ	–0.29 (30, 0.46, 113)	–0.41 (45, 0.49, 62)	–0.36 (45, 0.53, 45)
Leflunomide	–0.50 (44, 0.53, 116?)	–0.58 (50, 0.52, 66)	–0.65 (56, 0.48, 51)
Placebo	–0.04 (4, 0.49, 81)	NA	NA

bers of patients participating are mentioned in the Table – reasons for non-evaluation were not given. In one article [57] two numbers of evaluatable patients in the 6-month leflunomide group are mentioned: 106 and 116. The reason for this difference is not clear. Changes were statistically significantly different between SSZ and placebo at 6 months, leflunomide and placebo at 6 months and between leflunomide and SSZ at 24 months. As early as after 4 and 12 weeks there was a significant difference between SSZ and leflunomide.

Notwithstanding the significant differences at various time points between SSZ and leflunomide, and the large number of publications with strongly-stated conclusions on this single sample of patients, it is important to realize that the HAQ was a secondary efficacy measurement. This implicates that no definite conclusions can be drawn until a study in another group of RA patients, specifically investigating functional measurements, is done and corroborates the findings.

Due to the lack of data, no firm conclusions concerning the comparative effects of SSZ on quality of life or functional measurements can be drawn. Leflunomide is reported to be more effective but, as is pointed out above, this is not certain. However, SSZ seems to be more effective than placebo in this field.

Costs/pharmacoeconomics

RA results in a significant burden of disease, not only in terms of discomfort and disability and even death, but also in terms of substantial resource utilization, direct costs and costs due to loss of productivity, both to the individual and their relatives as well as to society. Very few studies on costs of treatment and financial/economic benefits of treatment with DMARDs have been done. Definitions of costs or the perspective they are looked upon vary. It is difficult to reliably obtain data, and significant differences exist between societies in determinants of costs, making interpretation and comparison of the results particularly difficult. This is true for direct costs, i.e., related to healthcare consumption, but even more so for indirect costs. These are mostly operationalized by loss of wages, ignoring costs, e.g., due to

reduced work performance, or mortality. Direct costs comprise about 15% of all drug related costs including toxicity, according to an estimation of the situation in England in 1992 [73]. The largest portion of these drug related costs is caused by adverse events [74].

Studies concerning SSZ are few. Only one study presented a cost-effectiveness evaluation [75]. It was an analysis of a randomized double-blind 56-week clinical trial comparing SSZ with the combination of prednisolone, methotrexate and SSZ. Further details of the study are given in the chapter on combination therapy (see the chapter by Choi and Paulus). Direct costs were taken in account. Combination therapy was somewhat more expensive in purchase and monitoring costs but resulted in less hospitalization. Overall, there was no significant difference in direct costs between the treatment modalities. Given the greater effectiveness of the combination therapy both in clinical terms and measured in patient utilities, the authors concluded that combination therapy resulted in greater cost-effectiveness in this population of early RA in this healthcare system.

In a systematic review on leflunomide, the authors presented a comparison between leflunomide, methotrexate and SSZ concerning costs of the agents themselves and the monitoring costs based on a British setting in four hospitals [76]. Costs were least for SSZ and most for leflunomide. However these costs probably represent the minor portion of all drug-related costs and depend rather heavily on national prices of the drugs and the monitoring practices.

A study using data of a managed care organization in the USA compared various DMARDs used as initial therapy concerning direct costs, both RA related and non-RA related [77]. It concerned computerized administrative and claims data of individual health plans affiliated with a national managed care organization. No medical records were studied. The number of SSZ treated patients was small (n = 49), most data were available on hydroxychloroquine and methotrexate. Median duration of initial DMARD therapy was especially short in SSZ: a median of 5 months compared to an overall median duration of 10 months. Both RA-related and non-RA related costs were relatively low for SSZ, mainly due to low use of facilities and laboratory costs. However, due to the number of assumptions necessary to estimate costs from this type of data source, the low number of SSZ treated patients, and more importantly the lack of information on the disease status of the patients with ensuing confounding by indication, no solid conclusions can be drawn.

In short, too few studies have been done to reach meaningful conclusions about the relative cost-effectiveness of SSZ, and even data on costs *per se* are scarce and hard to interpret due to lack of clear definitions and difficulty to obtain reliable data. Some indication exists that SSZ is less costly than some other single drug treatments. Further study in this field is urgently needed; firstly to expand and verify the few existing data, and secondly because of the importance of these data in the allocation of healthcare resources to the patients with RA.

Combination therapy

This subject is covered extensively in the chapters on combination therapy and on leflunomide and methotrexate.

Toxicity

Toxicity can be studied, analogous to effectivity, in (short-term) trials. In the long-term toxicity is assessed mostly by observational studies, e.g., analyzing withdrawal rates because of adverse events by survival analysis techniques and also by case reports concerning rare toxicity. As mentioned in the section on clinical pharmacology, the incidence of certain toxicities, GI/central nervous toxicity, is possibly related to the acetylatorship of the patient, which on its turn is genetically determined [22, 78].

Incidence

The incidence of toxicity depends heavily on the definition of toxicity/adverse events (is some degree of causality a prerequisite?) and the way the events are sought for, e.g., voluntary reporting in clinical practice, observational studies or clinical trials. In GCP guided trials any adverse event will be reported possibly leading to an overestimation, whereas voluntary reporting only will result in a lower than expected incidence. With these caveats in mind, the incidence of toxicity of SSZ will be discussed. In the sections about efficacy, data on toxicity have been mentioned for reasons of better appreciation of the balance between efficacy and toxicity. When necessary these data will be presented again, otherwise they will be referred to.

In clinical trials the incidence of all adverse events, regardless of their seriousness or consequences (e.g., treatment termination), is sometimes mentioned. Since no adequate definition of adverse events or toxicity is provided in these trials and causality is often not evaluated, this will not be discussed further. The number of patients withdrawing due to adverse events gives more reliable information and is reported in the majority of the trials. Since withdrawal is a trade-off between efficacy and toxicity, and almost always only one reason of withdrawal is counted, the reported reason of withdrawal does not always reflect the balance between the two. The incidence of toxicity in trials will depend on patient characteristics (e.g., disease duration and comorbidity) but more importantly on study characteristics, with the trial duration as the most important one, since the incidence of toxicity due to SSZ is not evenly distributed in time; it occurs mostly in the first phase of treatment.

Felson [31, 32] reported in his meta-analysis of clinical trials comparing second-line drugs with placebo and with each other, that the rate of dropout due to

Table 5 - Side effect profiles of adverse drug reactions leading to withdrawal [79]

	SSpA	IBD	RA
Gastrointestinal (%)	47	42	49
Cutaneous reactions (%)	20	37	25
Headache (%)	15	8	6
Others (%):	18	13	20
Haematological (%)	9	0	6
Depressive feelings (%)	3	7	1
Elevation of transaminases (%)	6	0	2
"Dizziness" (%)	0	0	3
Others (%)	0	6	8

toxicity of SSZ was about 20% (see Fig. 2). The studies involved in this analysis generally lasted ≤ 1 year. Reasons for withdrawal according to organ system were 4.9% mucocutaneous, 1.6% hematological, 1.1% general effects, i.e., fever, 12.5% nausea and/or vomiting, 1.6% hepatic, 0.5% lung-related and 1.1% other reasons.

In a meta-analysis of SSZ related toxicity Wijnands et al. compared the incidence of adverse drug reactions due to SSZ between different SSZ treated diseases [79]. They include 17 studies on RA, both clinical trials and observational studies. In Table 5 the relative rates of withdrawal are given.

More than one specific adverse event could contribute. No significant differences were observed in these patterns, although there is a tendency for more hematological and hepatic events in the arthritis groups. A possible explanation of these differences is a difference in pharmacokinetics as pointed out in the section on clinical pharmacology [16].

The meta-analysis by Suarez-Almazor comparing SSZ with placebo in six studies [33] revealed that significantly more patients treated with SSZ dropped out due to adverse events, 22 *versus* 8%. The odds-ratios and involved number of patients, totals and divided by organ system are mentioned in Table 6.

In the meta-analysis of Weinblatt et al. [40] the same conclusions were drawn in the comparison of SSZ and placebo, not surprising since the constituting studies were almost the same. The comparison with other DMARDs shows a somewhat higher, but not statistically significant different, withdrawal rate of SSZ treated patients compared to hydroxychloroquine (19% *versus* 13%) based on two studies [43, 80]. There were no significant differences of SSZ compared to d-penicillamine in three studies [40], including analyses by organ system, but as with the study comparing SSZ with hydroxychloroquine this may be due to a type II error. Compared

Table 6 - Sulfasalazine vs. placebo - Withdrawals and dropouts [33]

Outcome title	No. of studies	No. of participants	Statistical method	Effect size
01 Withdrawals and dropouts – Total	6	468	Peto OR [95% CI]	0.70 [0.48, 1.01]
02 Withdrawals due to inefficacy	6	468	Peto OR [95% CI]	0.23 [0.14, 0.37]
03 Withdrawals due to adverse reactions	6	468	Peto OR [95% CI]	3.01 [1.82, 4.99]
04 Withdrawals due to gastro-intestinal adverse reactions	6	390	Peto OR [95% CI]	2.44 [1.12, 5.32]
05 Withdrawals due to skin and mucosal adverse reactions	6	390	Peto OR [95% CI]	3.43 [1.30, 9.09]
06 Withdrawals due to renal adverse reactions	6	390	Peto OR [95% CI]	0.10 [0.00, 5.01]
07 Withdrawals due to liver abnormalities	6	390	Peto OR [95% CI]	3.63 [0.72, 18.23]
08 Withdrawals due to hemaeto-logical adverse reactions	6	390	Peto OR [95% CI]	2.84 [0.48, 16.75]

to parenteral gold (three studies), significantly fewer patients on SSZ withdrew (12% *versus* 29%), mainly due to less mucocutaneous toxicity and proteinuria, although nausea and vomiting occurred significantly more in SSZ40.

Treatment with leflunomide for 6 months resulted in a higher incidence than with SSZ of diarrhea (17% *versus* 9%) but less nausea (10% *versus* 17%); no other significant differences in organ toxicity were observed. The total number of patients withdrawing for reasons of toxicity was 14% and 19%, respectively [45]. Extension of this study showed a decrease in further GI toxicity in both groups, only alopecia was a continuing problem for leflunomide treated patients compared to SSZ. No differences were seen in the incidence of liver enzymes abnormalities or hypertension [57].

Compared to methotrexate there were no significant differences in toxicity between methotrexate and SSZ [46, 47] in early RA patients who received their first course of DMARD treatment. The follow up of these studies was 1 year.

Long-term observations on toxicity have been discussed partly in the section on long-term efficacy. No other pattern of toxicity arises in the long-term, although the incidence of adverse events seems to decline after the first 3–6 months as was found by Amos et al. in their observational study on toxicity of SSZ following 774 patients

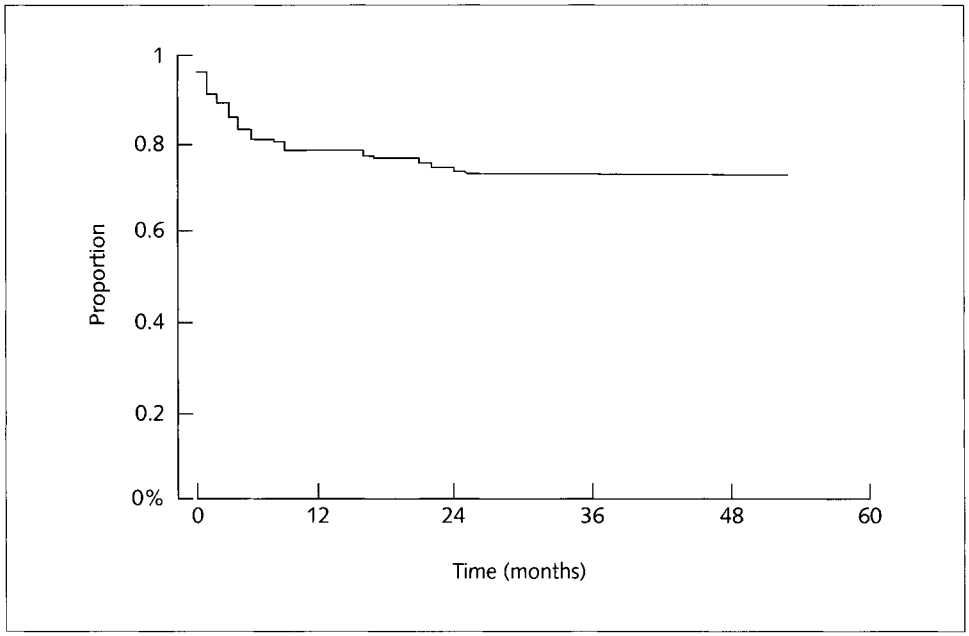

Figure 4
Dropouts due to adverse events in that study occurred mainly in the first 3 months, a plateau being reached after 12 months. In this analysis proper analytic techniques were used [24].

with RA for 1–11 years [81]. Of all patients, 205 (26.5%) had to stop treatment because of an adverse event, 76% of those in the first 3 months. 19.0%, i.e., 147 of the 205 patients had to stop treatment because of central nervous/GI, 4.8% for mucocutaneous, 1.1% for hematological (mainly leucopenia) and 1.6% for other reasons. Notably, 22 of the 147 patients (this is almost 3% of all patients) stopping for central nervous/GI reasons, had to withdraw because of mood disturbances, e.g., depression. The way data were analyzed is not mentioned in the paper, nor are corrections for incomplete follow up (e.g., by survival techniques with censoring), so the numbers may have been influenced by the rather large portion of patients with a relatively short follow up. However, the same picture of early termination because of toxicity emerges in the study by van Riel et al. [24].

In the study of Jones [59] Following 86 RA patients for 5 years or until withdrawal, all withdrawals (n = 25) occurred before 10 months of treatment, 22 patients discontinued treatment for reasons of toxicity in the first 3 months. Most patients withdrew because of GI adverse events (13/25), hematological toxicity accounted for five withdrawals (three patients with mild hemolysis, none because of neutropenia), four patients had dermatological problems and one patients stopped because of elevation of liver enzymes.

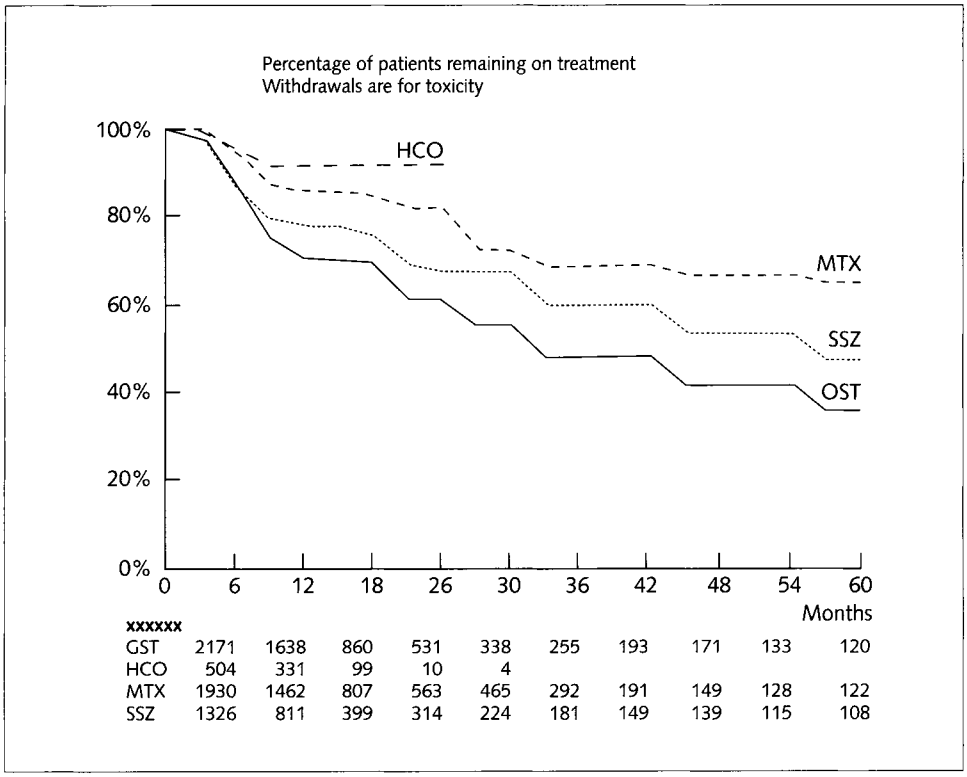

Figure 5
Survival curves representing the percentage of patients withdrawing from each agent because of toxicity. Six-month interval data were generated from studies providing withdrawal information for intervals longer than 12 months. The data are from observational studies and randomized controlled trials [64].

In the meta-analysis of treatment termination rates [64] discussed in the section on long-term efficacy, a slightly different picture emerges. Although many patients withdrawing for reasons of toxicity did so in the first 6 months, no plateau was reached and even after 4 years of treatment with SSZ, the curve was still going downwards as can be appreciated in Figure 5.

In conclusion, overall incidence of SSZ toxicity seems to be comparable with that of methotrexate, both in the short- and in the long-term. In the short-term overall occurrence of adverse events is comparable between SSZ and leflunomide. No long-term evaluations are available yet. SSZ is probably less toxic than intramuscular gold and more toxic than hydroxychloroquine, both on short- and on long-term. The specific patterns of toxicity are different between the various DMARDs, as is the distribution in time. SSZ toxicity occurs mostly early in use, i.e., the first 3–6 months.

Important but rare toxicity

Hematological

As pointed out before, hematological side effects are rare but the incidence is not neglectable (about 1%). The main toxicity is leucopenia. Mostly leucopenia occurs early in the course of treatment and develops gradually, in which case the causality with SSZ is not always clear, or it occurs suddenly with a profound drop in leukocyte numbers, often with a severe neutropenia or agranulocytosis [82–84]. In the latter case patients often acquire an infection, characteristically a throat infection and fever, with or without a candidiasis. But other types of opportunistic infection also occur. On stopping SSZ the leukocyte count returns to normal within 1 or 2 weeks; persistent agranulocytosis is not the usual sequel. In the light of this rapid recovery, hematopoietic growth factor treatment is seldom indicated, although incidentally recovery is not so swift and a serious and even fatal outcome is possible [85]. In such cases granulocyte-macrophage colony-stimulating factor can have a beneficial effect [86–88]. Another hematological toxicity of SSZ is hemolysis. Although rarely mentioned as a reason of withdrawal, it occurs rather frequently in subclinical form in RA patients [89]. Macrocytosis can also occur during SSZ [90], sometimes but not always due to folate deficiency [91, 92]. Significant hypogammaglobulinemia incidentally is seen during SSZ treatment, but infections are rarely a problem [93].

Pulmonary

Although SSZ toxicity of the lungs is rare, there is an increasing number of case reports documenting reversible lung abnormalities during the use of SSZ. The typical picture is cough, dyspnea and fever with sometimes crepitations on auscultation and bilateral infiltrates on chest radiography. Laboratory examination reveals eosinophilia in half of the cases. Microscopic analysis of biopsy specimens shows interstitial inflammation and/or eosinophilic pneumonia. Withdrawal of SSZ is the management of choice; it is unclear whether corticosteroids have a place in management of this drug-induced complication [94, 95].

Gastrointestinal and liver

Elevation of liver enzymes sometimes occurs as is clear from the remarks on general incidence of toxicity. Severe adverse events due to SSZ involving the liver are very rare. A fatal liver failure [96] occurring in the setting of a generalized (hypersensitivity?) reaction was described [97]. Pancreatitis has been reported to occur during

SSZ treatment for inflammatory bowel disease, although the incidence is probably far less than with mesalazine [98].

Renal

While there are some case reports concerning proteinuria and interstitial nephritis as a complication of SSZ use [99], in the UK reporting system on suspected adverse events no reports of interstitial nephritis were received on SSZ contrary to several reports on mesalazine [98]. Contrary to gold, cyclosporine and d-penicillamine, SSZ is judged to have little potential for side effects on the kidney according to a review on renal toxicity of DMARDs [100]. In the meta-analysis of Suarez-Almazor et al. [33], remarkably a lower odds for renal toxicity was seen for SSZ compared to placebo, although, due to the low incidence, the odds ratio had a wide confidence interval including 1.

Central nervous system

Although central nervous system (CNS) adverse events seem to be more frequent, depending whether certain symptoms (e.g., nausea) are thought to be due to either the GI tract or the CNS [101], specific mood changes, i.e., depression, are very rarely described in detail [102]. However, this side effect is mentioned by others to occur in 1–7% [79], being a troublesome side effect, especially when the link to SSZ is not appreciated.

Skin

The incidence of skin reactions was mentioned above. A rare but potentially fatal adverse dermatological event can be a toxic epidermal necrolysis (Lyell's syndrome) [103].

Autoimmune disorders

Several case reports mentioned drug-induced autoimmune phenomena and diseases, e.g., drug-induced lupus erythematosus, with slow acetylatorship as a risk factor [104, 105]. However, in 100 patients treated for 5 years no clinical SLE or similar autoimmune disease was seen, although conversion from ANA negative to positive occurred in 14 patients [106]. Similarly, in a trial comparing SSZ and d-penicillamine, no case of SSZ induced SLE was seen in 102 RA patients followed up for

up to 12 years, although one case was seen in the d-penicillamine treated group [56] and several other studies mentioned in the section on long-term efficacy.

Pregnancy and fertility

SSZ and its metabolites cross the placenta (Hensleigh, 1977 [107], Jarnerot, 1981 [108]). Furthermore, SSZ has some antifolate properties [3]. Nevertheless, SSZ did not seem to cause extra congenital abnormalities or other adverse pregnancy outcomes in more than 2,000 pregnancies during SSZ [109, 110]. SSZ is therefore probably the best choice of DMARD in pregnancy, as was stated in a review by Ostensen [111]. However, some caution is indicated for use near term because of the possibility of kernicterus in the newborn. Adequate supplementation of folic acid seems to be a wise precaution given the antifolate effects of SSZ. The drug can be given relatively safely to lactating mothers, but sulfapyridine levels in mother's milk are about half of the levels obtained in plasma. The effects on male fertility have been described by several authors [112–114], showing a reversible oligospermia and sperm abnormalities, with a return to normal within a few months.

Desensitization

In case of minor toxicity, especially skin reactions and other minor toxicity involving possible hypersensitivity reactions, desensitization using a variety of doses and schedules has been tried, often with success (between 30–90%) if the toxicity already occurred [115–119]. An attempt to avoid side effects of SSZ by pretreatment with a desensitization regimen failed to do so in 422 RA patients randomized to this regimen or placebo [120].

Monitoring

To try to avoid the toxicity described above, various laboratory monitoring practices have been advocated. In gastroenterology often no monitoring at all of SSZ is done if there is no clinical suspicion of side effects [121]. However, in rheumatology regular monitoring is common practice, possibly due to the higher incidence of toxicity in rheumatological patients compared to gastroenterological patients [79]. Determination of acetylatorship does not seem necessary, since only minor side effects as mild hemolysis and gastrointestinal complaints are possibly related to the acetylator status (see section on clinical pharmacology). Most commonly, more frequent monitoring in the first few months is advocated because of the higher incidence of adverse events in that period. Wijnands et al. suggest after baseline deter-

mination of blood count, liver enzymes (AF, gamma GT, AST or ALT and LD), a check of blood count and liver enzymes every 2 weeks in the first 3 months, 4-weekly testing of those parameters in the second 3-months period and every 12 weeks thereafter [122]. In the ACR guidelines of 1996 on monitoring of drug therapy, a schedule for SSZ consisting of a baseline evaluation of complete blood count, and AST or ALT in patients at risk and G6PD-status, with complete blood count every 2–4 weeks in the first 3 months and 3-monthly thereafter is recommended [123]. The British Society for Rheumatology recommends full blood counts (FBC) every 2 weeks and liver function tests (LFTs, including AST or ALT) every 4 weeks for the first 12 weeks and FBCs and LFTs 12-weekly thereafter [124]. Some authors focus on renal side effects and recommend urine testing for proteinuria as with intramuscular gold, based on some case reports and an extrapolation of the incidence [99], notwithstanding the reported low incidence as is described in the section above.

Virtually no research has been done to establish the best way of monitoring; no studies have been done to prospectively assess this issue in a methodologically sound way, e.g., by randomization between various ways of monitoring. An attempt to evaluate monitoring practices was done by Simon et al., finding a lower frequency of laboratory testing than was recommended internationally [125]. What is best also depends on the efforts and costs one is willing to invest in order to avoid toxicity, an estimate of the cost of detecting one adverse reaction of a DMARD was stated to be UK£32.000 (1995 [126]). Furthermore, certainly not all toxicity of SSZ can be avoided by monitoring, especially suddenly occurring serious adverse events like agranulocytosis and acute allergically-mediated toxicity. Careful instruction of the patient is probably at least as important as laboratory monitoring.

Place in the rheumatologic armamentarium

The place of a DMARD in treatment of RA depends on a combination of factors. In the long pharmacotherapeutical career of a RA patient aimed to achieve adequate disease control, most patients, especially the more severely affected ones, will need more than one or two DMARDs, given the (primary of secondary) resistance to the effects of them and the occurrence of toxicity leading to withdrawal of the agent. So the question asked most often is when to employ a certain DMARD rather than if to do so. First of all, the balance between efficacy and toxicity is important in the choice when to use SSZ or another DMARD. This balance can be judged on a group level, and general quantitative data of the preceding paragraphs can be used. In the treatment of the individual patient, individual factors such as comorbidity and issues such as pregnancy and fertility can influence the choice. The patterns of toxicity and the possibility of rare but important specific toxicity or data on teratogenicity can decisively influence the choice of DMARD. Secondly, factors such as

complexity of use, e.g., complicated drug regimens (e.g., when using drug combinations or complicated monitoring) and pharmacoeconomic considerations play a role.

Early in RA, SSZ can be used to treat both patients with mild disease and those with characteristics of bad prognosis (e.g., positive rheumatoid factor, presence of erosions), since from the paragraphs on efficacy and toxicity it is clear that SSZ has a relatively short time to effect and acceptable incidence of adverse events, and has proven beneficial effect on preventing radiological damage. In this phase of the disease SSZ has turned out to be probably equal to methotrexate in direct comparisons. In patients with no features of bad prognosis and mild disease activity, hydroxychloroquine is probably a better choice given its low incidence of toxicity and simpler monitoring.

Later on in the disease, SSZ is comparable to leflunomide although long-term data are not available for leflunomide. Methotrexate is probably somewhat more effective in the long run with a comparable toxicity. Opportunistic infections are no problem with SSZ contrary to methotrexate [127].

SSZ can be used singly or in combination, both early [128–130], or later in the disease [131], the use of combinations is reviewed in the chapter by Choy and Paulus. Its place, relative to the new biologicals and combinations with them, has not yet been determined.

A specific place for SSZ is in case of women with RA wishing to become pregnant. It is probably the only DMARD that can be used relatively safely in pregnancy, although adequate folate supplementation is indicated given the influence of SSZ on folate metabolism.

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Parenteral gold

Rolf Rau

Ev. Fachkrankenhaus Ratingen, Rosenstr. 2, 40882 Ratingen, Germany

Introduction (history of gold treatment)

Following the discovery of the bacteriostatic effect of gold cyanide by Robert Koch in 1890, gold was used for the treatment of tuberculosis and other infections and, from the 1920s, also for rheumatoid arthritis (RA), believed to be caused by streptococcal infection. In 1935 Forestier reported over 550 cases, of whom 70–80% experienced remission or at least transient inactivation of the disease for 2–3 years, and Hartfall reported a clear improvement in 86% and remission in 10% of his 900 patients. In 1960, the Empire Rheumatism Council (ERC) confirmed the efficacy of parenteral gold in RA in a controlled study.

For 5–6 decades, parenteral gold was *the* standard slow-acting antirheumatic drug. In part due to the recent introduction and strong promotion of several "modern" disease-modifying anti-rheumatic drugs (DMARDs), and in part due to (unjustified) fear of toxicity, gold treatment has become less popular. When efficacy data and multiple modes of action are considered, gold could regain an important place in the long-term treatment of RA.

Mechanism of action

With its multiple modes of action, gold remains one of the most fascinating antirheumatic drugs [1, 2]: Gold plays an important role already in uptake and presentation of foreign antigens; it inhibits antigen processing within the lysosomes of macrophages; it suppresses NF-κB binding activity and subsequently proinflammatory cytokine production is reduced, while the production of anti-inflammatory cytokines is upregulated (shift from TH1 to TH2 cells). Gold inhibits proteolytic enzymes and may also inhibit synovial fibroblasts. It is still unclear if there is a common denominator or if the modes of action are independent of each other.

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Humoral immunity

Clinical and biochemical improvement of RA is often associated with a decrease in immunoglobulins, levels of rheumatoid factor complexes, and, simultaneously, a reduction in rheumatoid factor synthesis. B cell IgM production is suppressed by gold sodium thiomalate (GSTM) and auranofin (AF) in a synergistic way [3]. Antibody deficiency is less common [4].

Lymphocytes

Correlating with clinical improvement, the absolute lymphocyte counts decrease under gold treatment, and the number of T and B cells infiltrating the synovial membrane declines. Mitogene- or IL-2 induced T cell proliferation is inhibited. Conversely, the reduced proliferation of lymphocytes in response to stimulation in RA patients (T cell anergy) is normalized after i.m. gold [5]. There is a shift of T-lymphocytes from proinflammatory Th1 to anti-inflammatory Th2 cells [6]. Adjuvant-induced arthritis in the rat can be suppressed. Liposome encapsulated GSTM improved symptoms of collagen-induced arthritis in mice over 50% and prevented cellular infiltration of lymphocytes into the synovial membrane [7]. After passive transfer of adjuvant arthritis, the splenocytes of gold treated rats are able to prevent the development of arthritis in the recipient [8].

Macrophages

Macrophages are of central importance in antigen presentation and cytokine production. Gold salts inhibit antigen processing in the lysosomes, particularly that of peptides rich in amino acids containing sulfur (cysteine, methionine) [9]. The highest levels of gold accumulation are found in the lysosomes of macrophages, so they are termed "aurosomes" here. Low gold concentrations inhibit peptide stimulated chemotactic reactions of monocytes in human blood and the supply of $CD14^+$ monocytes from the bone marrow [10].

Gold reduces both the number of macrophages in the synovial membrane and the expression of proinflammatory cytokines [11], almost as strongly as dexamethasone [12], including the production of IL-1, IL-8 [13] and IL-6 [14] in different cell systems and induces the anti-inflammatory cytokine IL-10. Aurothioglucose also inhibits the activity of NF-κB (and NF-κB binding activity), which is responsible for the gene expression and therefore induction of IL-1, IL-6, and IL-8 [15]. The chemotactic activity of monocytes is reduced [16].

Fibroblasts

Synovial fibroblasts are a crucial component of the infiltrative growth of the pannus. Isolated synovial fibroblasts incubated with GSTM concentrations, which are reached in the synovial membrane during gold treatment show substantial toxic deformation and signs of lysis when examined in a scanning electron microscope [2]. This clear toxic effect may be blocked in vivo by protective mechanisms.

Angiogenesis

Vascular endothelial growth factor (VEGF) is a potent inducer of angiogenesis, which may play an important role in pathogenic vascularization and synovial hyperplasia of RA. A tendency to inhibit the production of VEGF has been shown for GSTM, more so for bucillamine, but not for methotrexate (MTX) or sulfasalazine (SSZ) [17].

Leucocytes

Adhesion molecules "hold" leucocytes on the vessel wall, and thus initiate migration into the synovial membrane. The expression of the adhesion molecule ELAM-1 is inhibited in gold treated patients, resulting in reduced infiltration by granulocytes [18]. Gold also inhibits the phagocytic activity of macrophages and polymorphs in inflamed tissues.

Reactive oxygen species (ROS)

Free oxygen radicals contributing to the inflammatory destruction in RA are deactivated or inhibited by gold salts [19, 20].

Enzymes

Stabilization by gold salts of the lysosomal membrane, and a direct inhibition of these enzymes, has long been known. Following the inhibition of cytokines, smaller quantities of these destructive enzymes are produced. Matrix metalloproteinases (MMPs) are inhibited by binding of gold to the catalytic centers [21]. Moreover, their biological efficacy is lost in part due to the attachment of gold to the disulfide-bridges in the tissue.

Pharmacology of parenteral gold salts

Gold compounds

The gold products currently in use contain water-soluble complexes of univalent Au (gold I) linked to sulfur to form an aurothio-group, which is in turn bound to an organic or inorganic carrier molecule. Sodium aurothiomalate (GST; 46% Au) is an aqueous solution and aurothioglucose (50% Au) is an oil-based suspension. Aurothioglucose seems to have fewer side effects but is no longer on the market in many countries.

Gold distribution

After uptake in the body, the gold atom is released by hydrolytic cleavage and then bound to sulfur-containing amino acids in the serum proteins. The primary distribution space is the intravascular compartment (plasma proteins and blood cells). Extracellular fluids including synovial fluid together with endothelial membranes, glomeruli, and renal tubules represent an intermediate compartment. The deep compartment comprises enzyme proteins and intracellular organelles, particularly in the immunocompetent cells of the synovial membrane, the lymph nodes, and the bone marrow. The highest tissue gold concentrations are found in the kidneys, adrenals, and organs of the reticuloendothelial system [22] (Tab. 1).

Gold is found in synovial lining cells, subsynovial mononuclear cells, and macrophages. At the intracellular level it is found in the mitochondrial and lysosomal fractions, and it is bound to organelle membranes. It passes into lysosomes and alters their structures [23]. During chrysotherapy, gold levels in actively inflamed joints are higher than in inflammation-free joints. The levels in body fluids are lower than in tissue; levels in synovial fluid reach about 50% of the serum concentration.

Pharmacokinetics

About 2–6 h after the first injection of 50 mg GSTM containing 23 mg Au, a mean maximum plasma concentration of 400–700 μg/dl is reached, falling to about 250 μg/dl (approximately 45% of the peak value) after one week [24]. About 40% of the i.m. dose is excreted within a week, 70% of it in the urine and 30% in the faeces. About 300 mg of Au is retained in the body after 20 weeks of treatment with 50 mg per week; at steady-state, which is achieved after about 6–8 weeks, the minimum concentration 1 week after injection is between 300–400 μg/dl. With a 1-month injection interval, the level falls back to a subtherapeutic region of 100 μg/dl after just 2 weeks. 92% of the gold is bound to plasma proteins, 95% of that to

Table 1 - Gold content in organs during parenteral gold treatment [22]

Tissue	Gold content (mg/g tissue)	% of total gold in the body
bone marrow	159	26
liver	148	24
skin	117	19
bone	110	18
muscle	33	5
spleen	19	3
other organs	33	5
Total	619	100

albumin. Gold concentrations below 300 μg/dl are therapeutically inadequate, as the gold is only released from its binding to albumin into the tissue with difficulty [24]. Serum gold concentrations correlate with the dose. There is also a dose effect relationship during gold treatment [24, 25]. Steady state tissue concentrations reached after 20 weeks of weekly injections of 50 mg GSTM cannot be maintained by monthly administration.

Clinical efficacy: Controlled studies

Placebo-controlled studies

Controlled studies were carried out and published as far back as the 1940s, though they did not comply with modern standards with respect to diagnostic criteria, randomization, double-blind evaluation, outcome measures, or patient numbers. In a 9-month trial Ellman [25] treated three groups of 30 patients each with placebo, moderate or high doses of gold and found the highest remission rates (1/9/14 patients) in the high dose group. Further controlled studies all demonstrated superior clinical improvement in gold treated patients over controls. Patients who had to discontinue treatment after a total dose of less than 400 mg responded less often than patients who received higher gold doses (38% *versus* 57%) [26]. The study carried out by the Empire Rheumatism Council [27] is considered proof of the efficacy of parenteral gold treatment: in 100 patients each 50 mg GSTM/week given over 20 weeks turned out to be significantly superior to 0.05 mg GSTM/week after 3, 6 and 18 months (12 months after treatment termination); however, the significant difference had been lost after another year. As a result of this study, the use of gold treatment as repeated short-term courses was given up in favor of long-term treatment.

Comparison of different gold doses

In at least three studies conventional and higher gold doses have been compared and have documented a significant improvement of all disease activity parameters without significant differences between the dose groups; no correlation between gold levels and efficacy or rate of side effects has been established. However, the number of patients (23–38 per group) was too low to demonstrate significant differences in multicenter studies.

Comparison with other DMARDs

Since parenteral gold was the standard DMARD for decades, there are numerous comparative studies with other DMARDs. However, trials conducted following "modern" standards are rare, since sponsors are not available for trials with the "old" drug gold.

Auranofin (AF)

A number of studies have compared parenteral gold (Au) with AF. After 21 weeks of a three-arm, multicenter study an over 50% improvement in pain/tenderness was seen in 9% of placebo- , 34% of AF-, and 48% of Au-treated patients; the respective numbers regarding joint swelling were 12%, 28% and 37% [28]. A study comparing AF with Au over 3 years showed a clinical improvement in both groups, but half the AF patients dropped out because of lack of efficacy; they responded after switching to parenteral gold [29]. In a 1 year study with 120 patients, the dropout rate due to lack of efficacy was twice as high in the AF group, although AF was better tolerated. There was a similar statistically significant improvement in both groups among the 60% of patients who remained in the study [30].

The protocol of another double-blind, double-dummy study comparing 50 mg GSTM per week with 6 mg AF per day in 122 patients allowed to reduce the dose after 24 weeks in case of "clear improvement". This dose reduction was implemented in all patients treated with GSTM, but none of those treated with AF. Following the dose reduction in the GSTM group to 50 mg/month gold serum levels which were five times as high with injectable gold declined to the levels achieved with AF; at the same time all disease activity parameters began to deteriorate again in the parenteral gold group indicating that 50 mg/month is an insufficient dose [31]. The 3-year data were not available, since patients were transferred from GSTM to AF whenever possible at the end of the first and the second year [32].

In a French study with 60 patients, parenteral gold was found to be significantly better than AF in terms of all clinical parameters and the ESR.

Four studies have addressed the question of whether patients previously under good control under parenteral gold could also continue treatment with oral gold. This was possible in a 6 month study without loss of efficacy, but in other studies many patients switching to AF discontinued because of increasing disease activity; they improved again after resuming treatment with GSTM.

Methotrexate (MTX)

Three smaller studies comparing parenteral gold and methotrexate demonstrated significant improvement in both groups, with parenteral gold being slightly superior to MTX.

A two-center, randomized, double-blind study compared 50 mg GSTM and 50 mg MTX per week i.m. in 174 patients with early erosive RA over 1 year. All clinical parameters, the C-reactive protein (CRP) and ESR improved significantly over 50% with no significant difference between the groups. 11.5% of patients in the MTX group and 24.1% in the GSTM group achieved a clinical remission (no swollen joints, ESR <20 mm, no steroids) within 1 year ($p<0.05$). A marked improvement ($>50\%$ reduction in joint count and ESR) was assessed in 68% and 76% of patients treated with MTX or GSTM, respectively. Significantly more patients in the GSTM group were withdrawn due to toxicity. The number of patients taking prednisone was reduced from 21% to 7% with MTX and from 15% to 4% with GSTM [33]. Treatment was continued for an additional 2 years as an open trial with the same MTX dose and a reduced gold dose (50 mg/2 weeks) and revealed a clinical remission in 33%/38% of patients treated with MTX/GSTM. The time to remission was shorter with gold. A greater than 50% improvement was achieved in 78% (MTX) and 87% (GSTM), respectively (ITT analysis). The withdrawal rate for toxicity was significantly greater with GSTM ($p<0.0001$) [34].

128 patients recruited from one center were followed over 6 years demonstrating a significant 40–70% improvement in all parameters compared to baseline. The same improvement was seen in patients withdrawn from gold treatment, while MTX withdrawals experienced a deterioration of their disease [35]. MTX withdrawals improved again after switching to parenteral gold. The comparison between parenteral gold ($n=87$) and MTX ($n=101$) over 1 year did not reveal any differences in respect to efficacy or dropout rate [36].

Treatment with Au or MTX in 141 patients in a randomized but open trial improved all disease activity parameters and pain ($p<0.001$) by 24 and 48 weeks with no inter-group difference (ITT and completer analysis). At 3 months, gold was more effective regarding ESR and CRP. 18% of GST treated patients, compared with 6% of MTX treated patients, achieved a clinical remission. The withdrawal rate for toxicity was higher with GSTM. However, 7% of GSTM withdrawals remained in remission at 48 weeks and 27% continued to show improvement [37].

Sulfasalazine (SSZ) and other DMARDs

Of 128 consecutive patients the first 70 were treated with gold, the following 58 patients started SSZ. Both groups improved significantly within the first 3 months without significant inter-group differences up to 1 year. However, the discontinuation rate for lack of efficacy was 10% in the gold group and 21% in the SSZ group [38].

In a long-term open randomized trial of four DMARDs in 541 patients the proportion of patients who remained on their first DMARD or who were in remission at 5 years was 53% for penicillamine, 34% for GSTM, 31% for AF and 30% for hydroxychloroquine ($p < 0.001$). In patients who stayed on their first DMARD all groups showed a 30–50% improvement in CRP, ESR and Ritchie-index. With gold, CRP had improved from a mean of 43.8 to 20.8 mg/l and ESR from 46.6 to 23.8 mm/h [39].

Meta-analyses

Meta-analyses pooling data from completely different studies may not be very reliable. Placebo-controlled trials may underestimate the potential of drugs because of a tendency to include mild disease with little potential for improvement and therefore only small difference between placebo and active drug.

A meta-analysis from controlled trials revealed a change in favor of gold (adjusted for placebo) as follows: active joint count 30.1% ($p < 0.00001$), functional capacity 13% ($p < 0.0005$), and ESR 19.6% ($p < 0.02$) [40]. Another meta-analysis found auranofin significantly weaker than injectable gold ($p < 0.0001$) and weaker than MTX ($p = 0.006$). The improvement in tender joint count, grip strength, and ESR was greater with gold than with MTX treatment [41].

Another meta-analysis abstracted the numbers of withdrawals for inefficacy or toxicity from 110 randomized controlled trials or observational studies including 2.013 patients on MTX, 2.233 on GSTM, and 1.392 on SSZ. Considering withdrawals for all reasons, GSTM had the highest withdrawal rate; however, significantly fewer patients on GSTM than on MTX discontinued therapy for lack of efficacy [42]. Discontinuing gold treatment cannot be counted as "therapy failures" since many of these patients get into clinical remission [35].

Long-term observational studies

Well conducted long-term observational studies can provide valuable information about effectiveness, remission rate, duration on drug, long-term safety, disability, mortality etc., that cannot be reached in (too short) randomized clinical trials. Only a few long-term follow up studies on gold can be discussed here. Several retrospective studies up to 10 years duration established very good to good therapeutic out-

Table 2 - Clinical results in patients re-investigated after 7 years [46]

	Start	Last visit	p
Joint count (40 joints)	15.4	6.0	<0.001
Grip strength (bar)	0.36	0.45	
ESR/h	57.5	22.8	<0.001
Joints with deformities (40 joints)	6.2	12.2	<0.001
Joints with erosions (hands and feet)	7.2	10.0	<0.001
Larsen score per joint	0.82	1.05	<0.001
Superimposed osteoarthritis per patient	3.13	5.42	<0.001
Operated joints	0.5	1.3	<0.001

comes in over 60%, and a moderate effect in another 20%, of patients treated with i.m. gold. Treatment of 316 consecutive patients over an average of 37 months (with an average cumulative dose of 3,060 mg GSTM) resulted in a significant improvement of disease activity; 38.7% achieving a state of near-remission with only two swollen joints and an ESR <20 mm/h). 33% of patients discontinued treatment because of side effects, 12.7% because of remission; 56% continued to be treated with gold [43]. 102 patients with at least 3 years of continuous gold treatment had a complete follow up over 7 years; groups of patients with different disease duration demonstrated similar improvements: the swollen joint count decreased from about eight at baseline to about three after 1 year and around two after 7 years [44]. 112 gold treated patients of the same cohort had significantly less severe disease with respect to swollen joints, limitation of motion, ESR, radiographic score, hemoglobin than a control group of 138 patients treated with a variety of different DMARDs. Only 11% of the gold group was on corticosteroids compared to 45% of patients in the control group. Radiographic progression correlated with time integrated disease activity [45].

In another single-center study [46] 205 patients, (72% DMARD naive) started parenteral gold treatment in 1981. 80% could be re-investigated after 7 years, 13% were deceased, 7% were lost to follow up. All disease activity parameters had improved significantly (SJC 15.4 to 6.0, ESR 57.5 to 22.8), but the Larsen score, the number of deformities and of osteoarthritic or operated joints had increased (Tab. 2).

In most long-term studies patients in early stages responded better than patients with advanced disease, highly active forms demonstrated greater percentage improvement from baseline but less active cases had better outcomes.

When evaluating the data on 98 patients treated with gold for at least 1 year Wolfe et al. [47] found a >50% improvement in all disease activity parameters, pain and disability index with a simultaneous reduction in the mean prednisone dose

from 2.6 to 0.87 mg/day. Even after correcting for the placebo effect, about half the patients showed an improvement of more than 50%.

The strength of gold in the treatment of RA is also documented in a study by Fries [48]: when analyzing new starts of DMARDs or prednisone in 2,898 patients on the basis of immediately prior therapy MTX reduced disability significantly except after i.m. gold; disability increased with hydroxychloroquine, when this was given after i.m. gold. Improvement was greatest always after NSAID only *versus* after DMARD treatment.

Influence on functional capacity/disability

Several studies have documented an improvement of functional capacity with gold treatment. A meta-analysis of controlled trials published in 1989 using the Steinbrocker functional classification, which is very insensitive to change, revealed an advantage of gold over placebo of 13% ($p < 0.0005$) [40].

With the Health Assessment Questionnaire (HAQ) published in 1980, a score of 0 means normal function, 3 represents maximum disability. An improvement of > 0.22 is regarded to be clinically important.

When comparing gold and MTX over 1 year the HAQ (as calculated from the ADL score Hannover) improved from 1.3 to 0.95 with MTX and from 1.23 to 0.90 with gold treatment [33]. This improvement increased further after 2 years and could nearly be maintained after 3 years [34].

With 2.164 DMARD starts in 3.299 consecutively diagnosed RA patients disability was reduced significantly at 3 months by gold, followed, in this order, by SSZ, MTX, and HCQ. At 9 months, the improvement in disability had increased dramatically for gold (0.13, $p < 0.0003$) and methotrexate (0.08, $p < 0.0001$) [49].

When analyzing the completers of an observational study over 5 years there was an improvement of the HAQ with HCQ and AF but no change with gold [39].

The study from Glasgow performed in a socially deprived area found only a small but non-significant decrease in the HAQ (baseline values 2.0) with MTX or gold over 24 and 48 weeks [37].

A study investigating all 1,160 RA patients attending the Wichita Arthritis Center from 1980–1989 found an average treatment duration of 3.23/2.61/1.96 years for MTX/gold/HCQ. The baseline HAQ for MTX and gold was substantially higher than for HCQ ($p<0.001$). Due to longer treatment duration the area under the curve (AUC) total disability averted was greatest with MTX; when annualized, however, gold was insignificantly better than MTX, both better than HCQ ($p < 0.01$) [50].

Completers of a 5 year follow up study under gold treatment had improved in their disease activity parameters by 66% to 75% irrespective of their disease duration at baseline (0–2, >2–5, >5 years). Functional capacity improved significantly

in all three groups during the first 4 years; however, after 5 years only the group with the shortest disease duration had maintained an improvement of around 30% (1.88 at baseline, ~ 1.00 during years 1–4, 1.25 at 5 years). In early disease disability may be caused to a greater proportion by synovitis and therefore be more reversible. Only 9% of the discontinuations in this study were caused by lack or loss of effect [51].

The HAQ improved from 1.32–1.02/0.98 after 6/12 months ($p < 0.0001$) in patients with active RA treated with i.m. gold, while there was no significant change in a control group treated with NSAID and corticosteroids only [52].

Influence on quality of life and mortality

Studies on the influence of gold on quality of life are rare. At least two previous studies with 150–200 patients each documented that the quality of life in patients still on gold was better than that of withdrawals after 5–10 years and better than in patients on MTX treatment, who very often suffer from some nausea. When the mortality among 573 RA patients hospitalized in Finland in the early 1960s was checked in 1989 gold-treated patients could be shown to have a distinctly higher survival rate [53]. This result was confirmed in an own study following 134 consecutive patients with active early RA over 9 years; highly significantly fewer patients initially treated with gold were deceased than patients treated with other DMARDs, most of them with MTX [54].

Influence on radiographically documented progression

The inhibition of radiologically-documented progression is the most important proof for the disease modifying effect of a drug. Such an effect is well established for gold treatment [55].

In the ERC study the patients were treated only for 6 months which might explain that there was no significant difference between patients treated with gold and controls [27]. Two previous American trials and a Dutch study over 3 years demonstrated the superiority of gold over placebo and the greater efficacy of higher doses in inhibiting radiographic progression.

In a retrospective study evaluating patients 68 months after the initiation of gold treatment 47 patients who discontinued treatment after a total dose of < 500 mg showed distinctly greater progression than 188 patients who continued gold treatment [56].

A 2-year gold treatment of 73 patients resulted in remission in 27, > 50% improvement in 20, and improvement of < 50% in 26. Patients in remission had significantly the lowest increase in destruction and joint space narrowing scores. The

clinical response correlated closely with the development of new erosions and deformities; persistent joint swelling and progression were closely related [57].

Assessment of the radiological course in European comparative studies using GSTM and AF over 1 year revealed a significantly lower progression under parenteral gold. Progression in the second half year was slower than in the first half year [58].

Treatment of patients with early RA with gold or i.m. MTX [33, 34] resulted in a significant slowing down of radiographic progression during the second when compared with the first half year, non-significantly more so with gold [59]; 36 month ITT data demonstrated less progression during the second and third than during the first year. Again, there was a non-significant advantage for gold [60].

Patients who discontinued MTX treatment showed substantial radiographic deterioration after 24 months, whereas gold withdrawals had the same favorable course as completers up to month 48 [35]. After 6 years, only 14% of the total study population had markedly progressed to a score of over 20% of the maximum score, 42% had a moderate progression to between 5–20% and 44% had very low progression of $< 5\%$ of the maximum possible score [61]. This demonstrates that patients treated early and sufficiently dosed with gold or MTX monotherapy have a favorable outcome.

In a 7 year open study [45] approximately 65% of patients had progressed radiographically to at least 10% of the maximum possible Larsen score, and only 10% had progressed to 50% or more of the maximum possible score. Within those patients who could be followed for 20 years (usually a negative selection) 40% of patients had reached 40%, 80% of patients had reached 20% of the maximum possible score.

Another follow up of 205 patients over 7 years [46] demonstrated a deterioration of the mean Larsen score from 0.82 per joint to 1.05 only. A radiographic progression was seen most frequently in the wrist joints. On the other hand, an improvement in the Larsen score was encountered in 7–10% of joint groups (Tab. 3).

In an open randomized study over 18 months in 375 patients with severe early active RA radiographic progression was non-significantly smaller with gold than with cyclosporine [62]. After 36 months (ITT analysis) gold also demonstrated non-significantly less progression [63].

After 10 years of open long-term treatment study with parenteral gold the median Larsen score had deteriorated from 18 to 84 ($p = 0.0001$) after 10 years. However, only 28 of the original 93 patients attended the outpatient clinic after 10 years, and only in seven of these radiographs were taken [64]; this may be a very negative selection of patients. Moreover, all operated joints were given the highest score of 5, which is not justified, since many joints being operated are relatively normal at the time of the operation, for example, if all five MTP-joints are resected because of some luxation.

Table 3 - Mean progression of the Larsen index per joint (32 joints) in 205 patients during 7 years [46]

	Start	Last visit	p
All joints	0.82	1.05	<0.001
MCP joints	0.6	0.9	<0.001
PIP joints	0.5	0.7	<0.001
Wrists	1.1	1.5	<0.004
MTP joints	0.8	1.1	<0.001

Radiologic progression in joint groups: PIP joints 18.5%; MCP joints 22.6%; Wrists 27.6%; MTP joints 25.8%; Improvement (Larsen score) 7–10%

A macroradiographic study by Buckland-Wright answered many questions regarding radiographic change with gold treatment [65]: patients with early active RA were treated with GSTM immediately after presentation, 6 months later or not at all and followed for 18 months. In both gold groups there was a significant increase in the computer-calculated erosion area during the first half year, in the second half year there was no change and in the third half year the erosion area became smaller. No change in the width of the joint space was observed at any time. The "no gold" group continued to deteriorate in the same way as 34 historical controls treated with another compound.

The studies cited are summarized in Table 4. One curious clinical observation was confirmed in a study: the proximal interphalangeal (PIP) joints of the ring finger and the neighboring metacarpophalangeal (MCP) joints had less destruction than the respective joints at the contralateral side [66].

Combination with methotrexate

Patients with long lasting active disease not sufficiently responding to gold treatment switched to MTX monotherapy (n = 97) or the combination gold + MTX (n = 126) in a non-randomized open fashion. Both drugs were given at full dose. Starting from comparable baseline data (ESR 55.1 and 56.7 mm/h, respectively), a significant improvement of equal size was observed with over 50% decrease in swollen joint count in 62/70% after 1 and 3 years with MTX and 55/85% in the combination group. The improvement in the ESR was similar [67]. These data suggest that the combination was not superior. However, subsequent evaluation of the radiological courses demonstrated twice as much progression in the combination

Table 4 - Radiological progression of rheumatoid arthritis under parenteral gold treatment

Author	Total	AU	Co	Ra	Duration of disease (years)	Duration of study (months)	Gold salt dose	Result	P
Ellman et al 1940 [25]	90	60	30	31	7	9	200 mg/week (n=7) 100 mg/week (n=9) 0 mg/week (n=12)	Reduction in swelling and osteoporosis	n.s.
ERC 1960 [27]	185	90	95	185	2.8	18 or 30	1000 mg/20 weeks (n=90) 0.5 µg/week (n=95)		
Luukkainen 1980 [56]	235	188	47	235	< 3	74	2391 mg (n=188) 285 mg (n=47, discontinued early), premature discontinuation	Reduction in destruction, early treatment better	
Sharp 1982 [57]	73	73		73		6,12,18,24	25–50 mg/week (27 remissions 20> 50% better)	Improvement in destruction, joint-space narrowing, and new erosions	s.
Larsen 1984 [58]	232	113	119 (AF)	232	3	6,12	1.3 g i.m. (50 mg/week) 6 mg/day oral	Reduction in progression in the second half year. Au better than AF.	s.

Table 4 (continued)

Author	Total	AU	Co	Ra	Duration of disease (years)	Duration of study (months)	Gold salt dose	Result	P
Rau 1990 [33]	174	87	87 (MTX)	174	11 mths	0,6,12	50 mg/week for 1 year (50 mg/2 weeks after 12 months)	From second half yr progression inhibition. More so with gold (n.s.)	s.
Menninger 1992 [34]	100	43	57 (MTX)	100	11 mths	0,6,12, 24,36	50 mg/week for 1 year (50 mg/2weeks after 12 months)	Less progression in the second and third yr. More inhibition with gold (n.s.)	s. s.
Buckland-Wright 1993 [65]	29	13, 16 after 6 mo.	34	29	< 2	0,6, 12,18	50 mg/week ~ up to 1 g, then 50 mg/month	First half year = increase in erosive area, second half year = no change, third half year = reduction, NSAID = continuous deterioration	

AU, number of patients treated with gold; Co, number of controls; Ra, number of joints examined radiologically; Aur, number of patients treated with auranofin; MTX, number of patients treated with methotrexate; ECR, Empire Rheumatism Council; NSAID, non-steroidal anti-inflammatory drugs; n.s., non-significant

group during the year before baseline. This indicates a greater severity of the disease in the combination group and, therefore, a stronger effect of the combination.

A very interesting Canadian study recently performed clearly demonstrated the effect of parenteral gold when added to MTX. Patients with suboptimal response to MTX (≥ 15 mg/week) and still active disease were randomized to i.m. placebo (n = 30) or i.m. gold (n = 40). One observer monitored the side effects; the second observer conducted the joint examination. The proportion of ACR 20 responders was 56% for gold *versus* 28% for placebo (p = 0.017; logistic regression Odd's ratio = 3.4). Three patients each discontinued treatment for side effects, nine patients of the placebo group and two of the gold group discontinued for lack of efficacy. This study was performed in 11 centers experienced in the use of i.m. gold and allowed dose adjustment in the case of adverse events [68].

Toxicity

Gold is regarded as a considerably toxic drug. This is still, in part, due to experience in times where higher gold doses were used and monitoring was less strict. The side effect rates in newer studies differ greatly, but many also report a high side effect rate. Most adverse events occur within the first 3 months, 2/3 during the first year [33, 34], particularly at high doses [33, 69] and within blinded randomized trials where the doses cannot be adapted [33, 34]. Side effects lead to discontinuation of the drug in 10–30% of patients. The frequency of specified side effects within different trials or meta-analyses [33, 38, 40, 41, 70] are shown in Table 5. Most frequently skin and mucous membranes were affected, followed by proteinuria, while the incidence of other adverse events was very low. Most side effects are harmless and disappear completely without sequels. Moreover, very often they indicate a good response to treatment [35, 71]. When analyzing the ARAMIS data with 2.747 patients, over 7.278 patient years [72] hydroxychloroquine was found to be least toxic (toxicity index 1.38), followed by intramuscular gold (2.27). Methotrexate (3.82) and prednisone (3.83) were more toxic. According to Fries [73] gold has the best efficacy/toxicity relationship of all DMARDs. The drug survival rate analyzed according to Kaplan-Meier was not fundamentally different from that of methotrexate; the discontinuation rate under gold was higher than with MTX only during the first 6 months [73]. An analysis of 1,666 deaths in RA patients performed in Finland at a time when gold was the predominantly used DMARD attributed 10% to the treatment, most of which were caused by non-steroidal anti-inflammatory drugs (NSAIDs), none by gold [74]. The life expectancy of patients with RA can even be increased by gold treatment [53].

Combination with gold does not increase the toxicity of MTX: in a long-term study a total of 20.6% (MTX) and 15.1% (combination MTX/gold) of patients, respectively, were withdrawn for side effects; there was no difference in the type or

Table 5 - Incidence/withdrawal rate due to side effects

	Clark [40] % with-drawals	**Fries** [48] Incidence per 1,000 patient years	**Felson** [41] % with-drawals	**Rau** [33] % incidence	**Peltomaa** [38] % with-drawals	**SmithKline** [70] % incidence
No. of patients		2.747		174		
Drug	Placebo/Au	Au	Placebo/Au	MTX/Au	SSZ/Au	AF/Au
All	12/23		15/30		29/24	–
Skin rash	5.2/19.8	103	1.2/13.0	3/50	7/14	32/41
Mucositis	–	47	0.6/1.8	7/20	0/6	15/18
Proteinuria	2.3/3.0	24	0.5/3.7	0/10	0/4	7/12
Eosinophilia	–	–		7/10	0/2	
Low WBC ↓	–	4	0.1/1.5	2/0	5/4	1.5/2.9
Low platelets ↓	–	4	0.1/1.1	0/2	0/0	1.9/1.9
Elevated liver enzymes	–	0	0.4/0.9	34/9	12/6	4/4
Pulmonal	–	–	0.1/0.3	5/3	12/6	

severity of adverse events; after 5 years, 54% of patients in both groups were still being treated [67].

It has to be stated that the withdrawal rate for lack of efficacy is lower with gold treatment then with other DMARDs [38, 42].

Transient unpleasant increases in joint pain, swelling of the joints, and tiredness which may occur a few hours to a few days after injection often indicate a good response to treatment and are no indication for its discontinuation.

Skin and mucous membranes

Dermatitis and stomatitis represent about 80% of gold side effects. They can be extremely variable and mimic various skin diseases: maculopapular, erythematosquamous, and lichenoid changes are most common. Histologically, a dermatitis-eczema type, a vasculitis type, a lichenoid type, and an urticarial type can be distinguished. Generalized exfoliative dermatitis and Lyell's syndrome are particularly feared, but rare. Skin reactions may be due to nickel impurities in the gold product, and may be induced by intense sunlight and cannot be reliably predicted by intracutaneous tests. They are fully reversible after weeks or months, and treatment can usually be started again after the skin reaction has subsided [75]. Der-

matitis often coincides with a distinct reduction in disease activity or even remission [71]. 15% of patients complain of hair loss, rarely total alopecia. Both are fully reversible.

Mucositis can take the form of erosive or ulcerative stomatitis, gingivitis, or pharyngitis, often preceded by a metallic taste in the mouth.

Chrysiasis, a blue discoloration of the skin as a result of increased deposition of gold, can be observed in patients after many years of gold treatment. Gold deposits in the cornea and lens are harmless, correlate directly with the total cumulative dose, and occur in 75% of patients with total doses above 1,500 mg.

Kidneys

Gold nephropathy is seen in 3–10% of cases (Tab. 5) and manifests itself, mostly during the second quarter of treatment, predominantly in mild or moderate proteinuria. In 0.2–2% a nephrotic syndrome occurs with 24 h protein excretion of over 3.5 g, and development of oedema. Less often, microscopic hematuria or cylinduria is seen. Kidney function is not affected or recovers rapidly. Histologically, in most cases there is a diffuse or segmental membranous glomerulonephritis with deposits of antigen–antibody complexes and simultaneously proliferation of mesangial cells [76], less often mesangial nephritis. The symptoms of gold nephropathy regress fully within weeks or months without residual damage [76]. Gold treatment should be discontinued at proteinuria of 0.3 g/24 h. It can be resumed without side effects in some patients [77]. In most patients the recurrence of proteinuria can be prevented by combination with low doses of methotrexate (7.5–10 mg/wk) [78]. Like dermatitis, nephropathy is also often accompanied by remission of the arthritis.

Bone marrow

Changes in the blood count under gold therapy range from mild eosinophilia through leucopenia and thrombocytopenia up to agranulocytosis, pancytopenia, and aplastic anemia. Eosinophilia, usually transient, occurs in about 5%, and in half of the cases is followed by other side effects.

Thrombocytopenia occurs in 0–3%. It is usually caused by increased peripheral utilization in association with proliferation of megakaryocytes in the bone marrow, less often by bone marrow depression. About 85% of reported cases are HLA-DR3 positives, compared with 30% of all RA patients.

Leucopenia is rare and of variable severity and duration. The most dreaded complication of gold treatment – pancytopenia and bone marrow aplasia – occurs at an incidence of less than 0.5%. Its prognosis has been substantially improved by bone marrow transplantation and the administration of granulocyte-stimulating factors.

Liver

Side effects in the liver are uncommon. "Gold hepatitis" has disappeared since disposable syringes and disposable needles have been introduced. Rare cases of intrahepatic cholestasis or an increase in transaminases have been observed. The liver biopsy may show biliary stasis, ballooning of the hepatocytes, isolated cell necrosis, and periportal infiltrates. One case of fatal liver necrosis has been described. Even after severe intrahepatic cholestasis, gold treatment can be resumed later, starting with small doses.

Liver changes that occur during gold treatment need to be distinguished from the frequent involvement of the liver in RA and reactions to other drugs (e.g., diclofenac, postoperative heparin) [79].

Rare side effects

Enterocolitis is a rare, but serious, complication occurring after low total doses. It results in slimly or bloody diarrhea coupled with stomach pains, retching, and fever.

Acute pulmonary symptoms in the form of coughing, expectoration, chest pain, and patchy infiltrates in the x-ray as well as restrictive reduction in pulmonary function are rare and need to be distinguished from rheumatoid lung (higher rheumatoid-factor titer, rheumatoid nodules) [80]. Bronchoalveolar lavage mainly reveals lymphocytes. Rapid restitution occurs after discontinuation of gold treatment and the administration of systemic glucocorticoids. Single fatal cases have been described, however.

Post-injection reactions

Vasomotor (nitritoid) reactions in the form of reddening in the face, weakness, nausea, retching, profuse sweating, and hypotension as a consequence of dilatation of the arterioles are of unclear etiology and, in our experience, very rare. Hypertensives under treatment with angiotensin converting enzyme (ACE)-inhibitors are at particular risk [81]. Degradation products including malic acid, formed due to exposure to light (darkening of the otherwise pale yellow solution) are a possible cause.

Gold during pregnancy and lactation

Gold salts pass through the placenta and can be deposited in fetal tissue as well [82]. Increased numbers of malformations were induced in studies in gold-treated rats.

No increased malformation rate was found in 102 patients given gold treatment during the first half of pregnancy [82]. Available data do not indicate termination of pregnancy due to current gold treatment. But treatment may be discontinued, since the disease activity declines markedly during pregnancy anyway.

Gold salts pass into the milk, so toxic reactions are possible on breastfeeding. However, the American Academy of Pediatrics does not prohibit breastfeeding.

Place in the rheumatologic armamentarium

In the last two decades, a number of new DMARDs (MTX, SSZ, leflunomide, biologics) have been introduced, making injectable gold less popular in the treatment of RA. For their approval by the authorities the new drugs have been examined in large extremely well funded multicentric studies with the participation of all opinion leaders worldwide who propagated the use of these compounds.

Large multicenter studies performed with modern methodology provide a high ranking within the "evidence based medicine (EBM)" which cannot be achieved with older drugs tested with an "outdated" methodology. For obvious reasons, no company will invest much money in modern studies with old (and cheap) drugs. Moreover, in the face of many new drugs being developed, established centers have no patients left for routine treatment. So, more and more doctors have no chance to get personal experience with parenteral gold.

Another reason for the decline in gold use may be its perception as a toxic drug. This is questionable, as discussed under "Toxicity". In clinical practice, with patients and doctors having experience with the drug, and in studies allowing an adaptation of the dose [68], permanent withdrawal is rare. According to Fries [48] parenteral gold is less toxic than MTX, and it is the DMARD with the best efficacy/toxicity relationship [49]. As patients with rash tend to have the best long-term response, it is misleading if patients who discontinue are classified as treatment failures in clinical trials.

Given these disadvantages, the results of clinical studies with gold are promising: it has been shown to be more effective than placebo [25–27, 40], more effective than auranofin [28–32] and sulfasalazine [38], as effective or more effective than MTX [33–37, 41]. Even when comparing the results of studies performed with biologics gold seems to perform as or nearly as good as biologics: the EULAR moderate response rate, based on the DAS, was 68%, 74% and 75% after 1, 2 and 3 years, the EULAR good response was 28%, 33% and 36%, respectively [34]. Within 3 years clinical remissions were achieved by 38% of patients [34]. This compares very favorably with the results obtained with biologics. Gold treatment is significantly less often discontinued for lack or loss of efficacy than MTX [42]. In controlled clinical trials and long-term observations over 5 years all disease activity parameters had decreased by 50–75% from baseline [33, 34, 44]. Gold is the drug inducing

most long lasting remissions [34, 44, 46, 67, 69, 72]. It improves functional disability and quality of life [33, 34, 40, 49–52].

Gold has been convincingly shown to inhibit structural damage as demonstrated in radiologic scores [45, 55–60, 65]. Inhibition of progression lags behind clinical improvement for 6–12 months. This is why the inhibitory effect can best be demonstrated by comparing the progression rate during the first half year with that during the second half year (or the first and the second year). The inhibition of progression may be faster and more pronounced with TNF blockers. However, methodological differences have to be considered. Older studies were scored knowing the chronological sequence of the films thereby overestimating progression, while studies with biologics were scored with unknown sequence thereby underestimating progression. This is true especially in patients with advanced disease.

Personally, I would classify DMARDs into three groups: strong DMARDs (parenteral gold, MTX, TNF-blockers), moderate DMARDs (D-penicillamine, SSZ, AZA, leflunomide, IL-1 RA), weak DMARDs (hydroxychloroquine, auranofin). In my view gold is the ideal first line DMARD and it is still used as such in our department: there is a high probability that the patient will improve or even reach remission. An improvement can be recognized by the patient and the doctor within 3 months. If side effects occur the chance for remission is even greater. If gold has to be discontinued because of toxicity, the patient has lost no time, since, as a rule, toxicity is coincident with clinical improvement.

Suggested procedure and monitoring in practice (recommendations of the German Society for Rheumatology)

Prerequisites for gold treatment

The patient must have a reliable diagnosis of RA, he/she must be informed about the possibility and the type of side effects and that an improvement can be expected only after 3–4 months. Many rheumatologists prescribe low doses of prednisone (5–7.5 mg/day) to "bridge the gap". 5 mg prednisolone daily for 6–12 months have been shown to inhibit radiologic progression until gold becomes effective [83]. Some authors recommend monthly i.m. injection of 40–80 mg triamcinolone acetonide for a few months. We try to avoid routine treatment with corticosteroids. Early introduction of gold therapy is more effective than delayed treatment.

Contraindications to gold treatment

A contraindication is arthritis associated with collagen vascular disease; bone marrow depression, severe general disease or disease of the liver or kidney, ulcerative

colitis, heavy-metal or gold allergy, and anticoagulation (i.m. injections). The mere presence of antinuclear antibodies is no contraindication.

Monitoring

Before starting treatment, a full history and complete clinical examination including the skin and mucous membranes has to be performed. The following parameters have to be obtained at baseline, every 2 weeks for the first 3 months, thereafter every 4 weeks: CRP, ESR, full blood count including differential and platelet count, alkaline phosphatase, glutamate pyruvate transaminase (GPT), creatinine and urine status. Before every injection, the patient must be asked about side effects, the skin and mucous membranes must be examined.

(Temporary) discontinuation of treatment

(Temporary) discontinuation of treatment is indicated in the case of exanthema or stomatitis, hepatitis or enterocolitis, leucopenia (< 3,000/μl), persistent eosinophilia of more than 12%, granulocytopenia (<2,000/μl), thrombocytopenia (<100,000/μl), aplastic anemia (to be distinguished from inflammatory and hemorrhagic anemia), persistent proteinuria (> 0.3 g/l), cylinduria, hematuria, pulmonary infiltrates, severe infections.

Gold dosing

10 mg GSTM in the first week, 20 or 25 mg in the second and third week, followed by 50 mg weekly for 6 months; thereafter, many physicians reduce the dose to 50 mg monthly for long-term treatment. As this dose is too low for most patients to maintain clinical effect, we recommend 50 mg/week up to a total dose of 2,000 mg, thereafter 50 mg every 2 weeks for long-term treatment or, if the patient is in remission, 50 mg per month.

Parameters of disease activity, radiological progression, functional capacity must be checked on a regular basis to evaluate the efficacy of treatment. In case of increasing disease activity the dose should be increased back up to 50 mg per week for a period of 3 months. If the response continues to be unsatisfactory, gold can be combined with parenteral (on the same day, even in the same syringe) or oral MTX.

After discontinuation for side effects, treatment can be started again with low and slowly increasing doses. Following nephropathy, the recurrence of proteinuria can be prevented in most cases by combination with 7.5–10 (–15) mg MTX per

week. A lower gold dose in patients with side effects can be justified since these patients use to respond to lower doses also.

Duration of treatment

Many authors recommend the permanent continuation of treatment. However, the recurrence of symptoms after months or years can be expected only in about half of the patients and a second course of gold in the event of reactivation of the disease is also effective [84], often even in less than 3 months [85]. The maximum cumulative dose is not known. We have treated patients with total doses of 20–30 g without detectable toxicity.

Treatment of side effects

Most side effects subside on their own over a period of weeks or months. Gold induces dryness of the skin with itching and dermatitis. Daily moisturizing and fattening of the skin can avoid skin problems. Antihistamines and topical corticosteroids can be helpful as well as systemic corticosteroids in generalized exanthema. Stomatitis and gingivitis occasionally require mouth rinsing with lidocaine or lozenges containing corticosteroids. Corticosteroids are also recommended in nephrotic syndrome, thrombocytopenia, enterocolitis and pulmonary infiltration, MTX is helpful in nephrotic syndrome. In severe thrombocytopenia or granulocytopenia platelet transfusions, granulocyte-stimulating factors or bone marrow transplantation, respectively, may be necessary.

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Azathioprine

David Kane[1] *and Barry Bresnihan*[2]

[1]School of Clinical Medical Sciences (Rheumatology), 4th Floor, Cookson Building, University of Newcastle, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK; [2]Department of Rheumatology, St. Vincent's University Hospital, Dublin, Ireland

Mechanisms: *In vivo* and *ex vivo* studies

Ex vivo/in vitro studies

Azathioprine is a synthetic purine analogue – the 1-methyl-4-nitro-5-imidazolyl derivative of thioguanine. The principal metabolite of azathioprine is 6-mercaptopurine and this is metabolised by hypoxanthine guanine phosphoribosyl transferase (HGPRT) to produce the active metabolites thioinosinic and thioguanylic acid. These metabolites inhibit intracellular function by interfering with adenine and guanine ribonucleotide production through suppression of inosinic acid synthesis [1]. These effects result in downregulation of actively proliferating cells with a particular effect on bone marrow.

In vivo studies

The reduction in intracellular purine synthesis by azathioprine is associated with a decrease in numbers of circulating B and T lymphocytes, particularly CD8 cells [2] and with reduced IgG and IgM synthesis [2], diminished interleukin-2 (IL-2) secretion [3] but no effect on serum levels of interleukin-6 or soluble interleukin-2 receptor [4].

Clinical pharmacology

Pharmacokinetics

Absorption/half life/distribution/elimination

Azathioprine is almost completely absorbed from the upper gastrointestinal tract, attaining peak plasma levels within 1–2 h. Azathioprine is rapidly distributed as it

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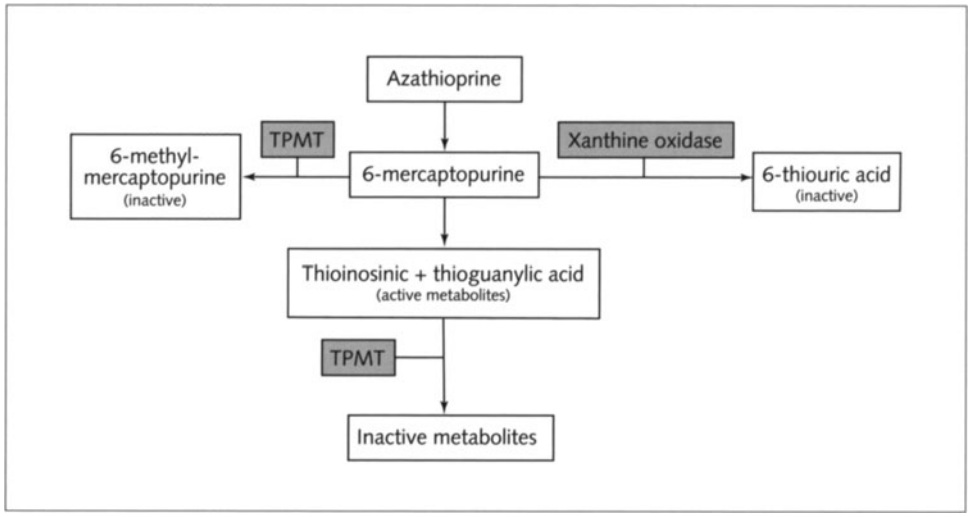

Figure 1
Inactivation of 6-mercaptopurine and its active metabolites is regulated by two key enzymes – thiopurine methyltransferase (TPMT) and xanthine oxidase. Functional polymorphisms of the TPMT gene can be screened for and will cause reduced TPMT enzyme activity with increased azathioprine toxicity. Administration of xanthine oxidase inhibitors causes a predictable increase in azathioprine toxicity and should lead to adjustment of the dose of azathioprine dosage.

is only 30% protein bound. The serum half life of azathioprine is short, being approximately 0.2 to 0.5 h as 20–45% of the drug is excreted in the urine and the remainder is rapidly converted to 6-mercaptopurine by the action of glutathione in red blood cells [1, 5]. Thus, serum azathioprine levels are not used in monitoring for dose related toxicity.

Metabolism (Fig. 1)

The metabolism of azathioprine is important in understanding drug interactions and toxicity. Thiopurine methyltransferase (TPMT) is the key enzyme regulating conversion of 6-mercaptopurine to the inactive metabolite 6-methyl-mercaptopurine, in addition to the inactivation of active thiopurine metabolites of azathioprine. Functional genetic polymorphisms causing reduced TPMT enzymatic activity have been described. 10–15% of patients treated with azathioprine and 1 in 300 individuals in the general population have negligible enzyme activity. This causes preferential accumulation of active thiopurine metabolites which result in increased azathioprine toxicity (mainly haematological cytopaenias) [6, 7].

Analysis of the TPMT gene prior to the administration of azathioprine can identify individuals with homozygous TPMT deficiency who are at risk for early (up to 6 weeks) and severe marrow toxicity resulting in discontinuation of azathioprine [8, 9]. However the more frequent TMPT heterozygotes develop toxicity at a later stage – often due to another triggering event or TMPT inhibiting drug – and only 27% of leucopoenic events were related to TPMT polymorphisms in one series of patients with inflammatory bowel disease [9]. At present TPMT gene testing is very limited in rheumatological practice but experience in oncology suggests that pharmacogenetic screening has the potential to become a useful and cost-effective technique in predicting drug toxicity in rheumatology [10, 11].

Interactions

TPMT activity is also inhibited by sulphasalazine, 5-acetylsalicylic acid preparations as well as by furosemide and thiazide diuretics [12]. Co-prescription of these medications with azathioprine – particularly in TPMT heterozygotes – may further increase toxicity and requires careful consideration and close monitoring for marrow toxicity.

6-Mercaptopurine is oxidised to 6-thiouric acid by xanthine oxidase and inhibition of xanthine oxidase by allopurinol increase the toxicity of azathioprine and fatalities have been reported [13]. Thus, allopurinol should be avoided in patients taking azathioprine, but if they must be used together a 50–75% reduction in the dose of azathioprine is necessary.

Efficacy

Most studies of azathioprine in the management of inflammatory arthritis do not meet the current standards for assessing immunological therapy in rheumatology and there are very limited data on the effect of azathioprine on functional measures, quality of life or long-term disease outcome.

Placebo controlled trials

Since the first placebo controlled trial in 1969, a number of controlled, double-blind studies have demonstrated that azathioprine is more effective than placebo in the treatment of rheumatoid arthritis (RA) [14–19]. Only three studies [14, 15, 19] met current pharmacological study standards or had sufficient standardised data to be included in a meta-analysis [20]. The pooled data on 81 patients – with 40 patients receiving azathioprine 2-2.5 mg/kg/day for 6 months – confirmed a significant reduction of 29–60% in tender joint scores in favour of azathioprine. Other effects

Table 1 - Comparison of efficacy of azathioprine and methotrexate [30]

	Azathioprine (n=32)	Methotrexate (n=30)
Tender joints (max 53)	–6.2 (–10.2, –2.3)	–10.5 (–13.9, –7.1)
Swollen joints (max 46)	–2.8 (–5.7, 0.0)	–5.8 (–8.4, –3.2)
Global assessment by patient		
Pain (max.100 mm)	–18 (–29.5, –6.6)	–26.6 (–35.6, –17.5)
General Health (max. 100 mm)	+0.9 (–8.0, +9.9)	–5.3 (–17.5, +6.9)
ESR (mm/hr)	–23.1 (–32.8, –13.5)	–24.1 (–32.4, –15.8)
CRP (mg/l)	–31.6 (–42.8, –20.3)	–23.7 (–31.5, –15.9)
Disease Activity Score	–0.97 (–1.34, –0.60)	–1.39 (–1.74, –1.04)

Comparison of methotrexate (starting dose 7.5 mg/wk, increased according to clinical response to maximum dose 15 mg/wk) and azathioprine (starting dose 100 mg/day, maximum dose 150 mg/day) in the management of rheumatoid arthritis (duration of study = 48 wks).

in favour of azathioprine included a small (11–14%) but non-significant reduction in erythrocyte sedimentation rate (ESR) in two of the pooled studies [15, 19] and a significant reduction in swollen joint count by 50% in 19 patients in one study [15].

Functional and quality of life measures currently used were not evaluated. Woodland showed a trend to improved Steinbrocker functional score in patients receiving azathioprine but this was not significant. The clinical benefit of azathioprine was observed as early as 6–8 weeks with full effects at 12–16 weeks but 26% of azathioprine treated patients and 7% of placebo treated patients withdrew because of toxicity at 6 months.

The dose of 2.5 mg/kg per day of azathioprine was significantly better than placebo, while 1.25 mg/kg per day had an intermediate effect [19]. Once a therapeutic benefit is obtained, continued long-term improvement can be maintained when the dose is reduced to 1.5 mg/kg per day [16] while withdrawal of azathioprine was followed by a flare of disease activity in 15 of 16 patients [18].

Comparison with other DMARDs

Azathioprine has been compared to other disease-modifying anti-rheumatic drugs (DMARDs) in the treatment of RA and more extensive data on the effects of azathioprine on clinical and laboratory parameters of RA can be obtained from these studies (Tab. 1). An 18 month study of 121 patients compared parenteral gold, cyclophosphamide, and azathioprine [21]. Cyclophosphamide was marginally the most effective treatment and azathioprine produced similar efficacy to gold. Aza-

Table 2 - Comparison of the effects of azathioprine and other disease-modifying antirheumatic drugs on radiological progression as measured by Larsen or Sharp score

Comparator drug	Study population (study interval)	Radiological assessment	Comparison with azathioprine
Penicillamine [24]	Established RA (24 months)	Larsen score of hands and feet	Reduced progression with azathioprine
Methotrexate [28]	Established RA (12 months)	Sharp score of hands and feet	Similar efficacy
Methotrexate [31]	Established RA (48 weeks)	Sharp score of hands	Reduced progression with methotrexate
Methotrexate [30]	Established RA (48 weeks)	Sharp score of hands and feet	Reduced progression of erosion score with methotrexate
Methotrexate [39]	Established RA (24 months)	Sharp score of hands and feet	Reduced progression with methotrexate

thioprine has been demonstrated to have similar efficacy to parenteral gold [21, 22], penicillamine [23, 24] and chloroquine [22].

Two randomised double-blind studies found that azathioprine and cyclosporin A have similar efficacy [25, 26]. The largest (117 patients) reported no difference between azathioprine 1.5–2 mg/kg/day and cyclosporine 5 mg/kg/day after 6 months of treatment [26]. One smaller open study of 24 patients demonstrated greater improvement in patients taking cyclosporin [27]. More renal toxicity was observed in patients taking cyclosporin.

Four trials have compared azathioprine to methotrexate and demonstrate a trend toward superior clinical and radiological (Tab. 2) benefit of methotrexate [28–31]. Thus, azathioprine efficacy appears to be comparable to parenteral gold, penicillamine, cyclosporin A and cyclophosphamide, although it is probably not as effective as methotrexate.

Functional outcome

Most studies of azathioprine do not use current standardised measures of functional outcome though data from comparative studies of azathioprine and methotrexate suggest similar effects on function in patients with established RA. Azathioprine and methotrexate were equally effective in producing a 50–55% improvement in a seven-item score of activities of daily living in patients with an average disease duration of 8.7 years [28] and in producing an improved modified Health Assessment Questionnaire (HAQ) in 25% of 209 patients with RA [31].

Radiological outcome (Tab. 2)

Detailed information on the effects of azathioprine on radiological progression in RA is obtained solely in established RA and through comparison with other DMARDs (Tab. 2). Azathioprine is more effective in retarding radiological progression than penicillamine and appears to be less effective than methotrexate.

Combination therapy

The combination of azathioprine and methotrexate was not superior to either agent alone, though there was no increase in toxicity [31]. Azathioprine has also been used in combination with other DMARDs such as cyclophosphamide and hydroxychloroquine, though there is no controlled data to confirm if this approach is superior to monotherapy and concerns remain about toxicity [32].

Other inflammatory diseases

Azathioprine has also been used for the treatment of psoriatic arthritis in one double-blind crossover study for 12 months, with patients randomly assigned to drug or placebo for the first 6 months [14]. Though a small study, marked improvements in skin and joint indices were observed solely in the active treatment group. Azathioprine is also used in Reiter's syndrome, Behcet's disease, polymyositis, and systemic lupus erythematosus, and to sustain remissions in systemic vasculitis, including Wegener's granulomatosis, polyarteritis nodosa, and Churg-Strauss syndrome.

Adverse effects

The frequency of serious toxicity of azathioprine in patients with RA is similar to that of most other DMARDs, though more patients remain on methotrexate after 1 year of treatment [30, 31]. Gastrointestinal intolerance, bone marrow suppression and infection are the most frequent side effects of azathioprine at doses of 2.5 mg/kg/day.

Particular caution is required in monitoring dose-related marrow suppression [5, 21]. Azathioprine produces leucopoenia in up to 27% of patients and thrombocytopenia in up to 5% of patients [5]. This usually occurs early in the course of treatment, particularly if a TPMT heterozygote. Genetic screening is not widely practised and the initial stages of treatment require careful monitoring and gradual dose adjustment. Mild degrees of leucopoenia are managed by reducing the azathioprine dose while more severe myelosuppression necessitates drug withdrawal in less than 5% of patients. Thrombocytopenia occurs in up to 5% of patients [33]. Macrocytosis is a common dose-related effect and requires investigation for other potential causes.

Anorexia, nausea, and vomiting occurs soon after the initiation of azathioprine therapy in up to 23% of patients [5]. Diarrhoea also occurs early in 1–5% percent of patients. Rarely dramatic gastrointestinal hypersensitivity reactions may occur within the first few weeks of azathioprine therapy. This is characterised by nausea, vomiting, diarrhoea and fever in combination with rash, myalgias, malaise, abnormal liver enzymes, and hypotension. Oral ulcers may occur with azathioprine therapy, possibly as a sign of leucopoenia, and necessitate checking of the white cell count. Mild elevation of liver enzymes occurs in approximately 5% of patients but progression to cirrhosis has not been described [5]. This is usually managed by dose adjustment unless treatment is for autoimmune liver disease.

Infections occur overall in up to 9% of patients, with most patients restarting azathioprine after the acute illness [5]. Leucopoenia predisposes to increased bacterial infections [34] and viral infections, particularly herpes zoster which may occur in up to 6% of treated patients [34].

Significant concerns exist regarding the risk of malignancy in patients taking azathioprine. Renal transplant recipients who received azathioprine are reported to have a 50- to 100-fold increase in the relative risk of malignant disease [35]. However the malignancy risk is considerably smaller in patients with RA treated with azathioprine, ranging from 2.2–8.7% relative risk [36]. In one study this was not significantly different from that observed in RA patients who received no cytotoxic drugs [36]. The most common tumours implicated are squamous cell carcinomas of the skin, non-Hodgkin's lymphoma, Kaposi's sarcoma, *in situ* carcinomas of the uterine cervix, and carcinomas of the vulva and perineum [35, 36]. It has been estimated that the increased risk of lymphoma is equivalent to one case per 1,000 patient years of treatment with azathioprine [36].

There is a wide experience of the effects of azathioprine during pregnancy from patients with renal transplants. There is definite evidence of foetal harm including lower birth weight, prematurity, jaundice, respiratory distress syndrome and transient immunosuppression though azathioprine is not teratogenic. However the benefit to the patient may outweigh these risks, principally in patients with renal transplant and glomerulonephritis. Azathioprine should be avoided during pregnancy in women being treated for RA.

Place in the rheumatological armamentarium

Azathioprine has proven efficacy for the treatment of rheumatic diseases, particularly RA. It is generally reserved for use in the management of progressive RA, either alone or in combination and is rarely used as a DMARD of first choice. In one series of 1,300 consecutive DMARD courses in RA patients no patient received azathioprine as an initial therapy [37]. Azathioprine accounted for only 0.4% of subsequent DMARD courses, usually in combination or in patients with a mean of five

previous DMARDs. Thus azathioprine is principally used when other DMARDs have failed to control disease, either as an alternative or as add-on therapy. Azathioprine may also be an option when intercurrent illness makes other DMARDs less desirable. When selecting azathioprine it may be preferred to cyclosporine in patients with renal impairment.

Drug dosage and monitoring

When used in the treatment of rheumatic diseases, it is generally recommended that therapy is begun at a dose of 25 to 50 mg/day for the first week to test for drug hypersensitivity. The dose is then increased incrementally by 0.5 mg/kg per day every 4–6 weeks until the desired response is seen or a maximal total dose of 3 mg/kg per day is reached. A lower dose is indicated in patients with renal insufficiency.

The American College of Rheumatology recommend a complete blood count (including haemoglobin, white blood cell count, and platelet count) every 2 weeks during dose initiation or increase, and every 4–12 weeks after a stable dose is achieved [38]. The risk of myelosuppression is maximal in the first 6 months of therapy and it has been estimated that monthly monitoring after the first 6 months detects one haematological adverse event for every 133 patient years. Liver enzyme testing is recommended every 4–12 weeks during azathioprine therapy.

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Methotrexate

Kevin Pile

Institute of Medicine, James Cook University, Townsville Hospital, P.O. Box 670, Townsville, Queensland 4814, Australia

Introduction

Methotrexate (MTX) is a folate analogue originally developed in the 1940s as a highly selective inhibitor of dihydrofolate reductase (DHFR). Its use in the reduction of rheumatoid arthritis (RA) synovitis was first reported in a 1951 paper on six RA patients by Gubner and Ginsburg and explained as a cytotoxic effect on proliferating lymphocytes. Subsequently its efficacy in RA was proven in a series of papers in the mid 1980s and it is now the mainstay of RA therapy, both alone and in combination, as recommended in the guidelines of the American College of Rheumatology 2002 [1].

Pharmacology

Low dose MTX is the most widely used treatment employed in rheumatology to modify the clinical features of RA and retard disease progression [2], with commonly used doses ranging from 7.5 to 25 mg/wk. Low dose weekly oral MTX is actively absorbed from the proximal jejunum, and may be taken regardless of meals. Plasma levels peak 1–2 h after administration, and clearance from serum occurs within 24 h. Mean absolute bioavailability is 70–80%, with large inter-individual variation from 30–90% observed, and moderate intra-individual variability. Low dose MTX may be given parenterally to aid compliance, and possibly to ensure more uniform bioavailability, and is also reported to reduce acute gastrointestinal (GI) discomfort. MTX is absorbed more rapidly and reaches higher serum concentrations after intramuscular (IM) or subcutaneous (*s.c.*) administration compared to oral administration. The mean absolute oral or parenteral bioavailability is very similar with low doses of MTX, suggesting that route of administration is interchangeable [3]. Patients who do not respond to oral medication may be switched to IM or *s.c.* administration with the hope that a better response may occur [4]. Low

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dose MTX may also be injected intra-articularly, with mean synovial methotrexate concentration exceeding serum by a factor of 10 during the 24 h post-dose period. Despite this, plasma kinetics is unaltered making it unlikely that intra-articular MTX will yield an advantage over systemic therapy [4].

Approximately 10% of the MTX's administered dose undergoes hydroxylation *via* aldehyde oxidase in the liver to 7-hydroxy-methotrexate (7OH-MTX), a significantly less potent metabolite. MTX and 7OH-MTX are both transported intracellularly by both passive transmembrane diffusion and carrier-mediated active transport. After entering the cell, MTX is polyglutamated with up to six new glutamic acid moieties, *via* folylpolyglutamate synthase. MTX is a monoglutamate, such that polyglutamation maintains a low intracellular concentration of monoglutamate-MTX that never reaches steady state, allowing intracellular accumulation of vast quantities of MTX. Polyglutamate-MTX (poly-MTX) cannot be transported extracellularly unless hydrolysed to the monoglutamate state by polyglutamate hydrolase. The polyglutamation of MTX effectively increases its intracellular life, and also enhances its enzyme inhibitory potency as the number of glutamic acid moieties increases, for example MTX-pentaglutamate is up to 2,500-fold more potent than the native monoglutamate in inhibiting 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (AICAR T'ase).

Methotrexate is primarily eliminated *via* the kidneys, with glomerular filtration and active secretion utilising the transport mechanism for organic acids, and active reabsorption unaffected by acidic compounds from the distal tubule. Tubular reabsorption is saturated prior to secretion, with considerable inter-individual variation in saturation point for both reabsorption and secretion. Thus, non-linear elimination may result from MTX doses of 7.5–30 mg and contribute to the variability in serum methotrexate concentrations. Some authors have found a positive correlation between MTX clearance and creatinine clearance, and its use in renal insufficiency should be viewed as hazardous. A lower dose should be used in patients with chronic renal impairment and temporary cessation of MTX treatment may be required at times of volume depletion (such as perioperatively). Similarly, co-prescription of agents known to impair glomerular filtration rate (GFR), such as aminoglycosides and cyclosporin, should be undertaken with caution. It has also been reported that prolonged MTX usage itself, may reduce renal function and hence its own clearance [5]. A possible mechanism is to increase plasma adenosine levels activating A1 receptors in the renal parenchyma, thereby diminishing renal blood flow, and salt and water excretion [6]. Due to MTX's low protein binding and high tissue distribution neither haemodialysis, nor peritoneal dialysis would be an effective way to clear the drug in the event of overdose or toxicity. While MTX is actively excreted in bile and this is responsible for 10–30% of clearance, an extensive enterohepatic circulation ultimately results in only 1–2% faecal excretion. Interruption of the enterohepatic circulation using cholestyramine or charcoal may be trialled in severe toxicity due to renal insufficiency or after poisoning.

Many patients taking low dose MTX are also treated with non-steroidal anti-inflammatory drugs (NSAIDs) in order to suppress the symptoms of inflammation. While renal blood flow and renal function can be influenced by NSAID use, the co-administration of commonly used agents (aspirin, diclofenac, naproxen, indomethacin and ibuprofen), with the possible exception of very high dose aspirin [7], has no effect on the area under the curve (AUC), systemic clearance or half life of low dose MTX as used in the treatment of RA [8]. Probenecid significantly decreases the renal excretion of MTX and should be avoided [9]. Additionally, bone marrow suppression has been occasionally seen with the combination of MTX and cotrimoxazole, the latter possessing anti-folate activity also.

Method of action

Cellular effects of MTX

MTX was designed as an inhibitor of DHFR, blocking the intracellular production of reduced tetrahydrofolate (THF). THF is the single carbon donor involved in the *de novo* pathways for both purine and pyrimidine synthesis as a prelude to DNA and RNA synthesis and cell proliferation. Poly-MTX inhibits the conversion of dihydrofolate to tetrahydrofolate by DHFR. By affecting the intracellular folate pool, MTX influences the activity of methylenetetrahydrofolate reductase (MTHFR), and the generation of 5-methyl-THF. The latter is the methyl donor for the conversion of homocysteine to methionine, which is converted to S-adenosyl-methionine (SAM), a key methyl donor as part of the synthesis of DNA, proteins, phospholipids and neurotransmitters.

Polyglutamation also increases the potency of MTX inhibition of thymidylate synthase as part of the *de novo* pyrimidine pathway. Similarly the *de novo* purine pathway is inhibited by poly-MTX *via* its effect on glycinamide ribonucleotide transformylase and AICAR T'ase. Poly-MTX has a higher affinity for the enzymes of the purine pathway, suggesting that the inhibition of pyrimidine biosynthesis will be minimal compared to purine biosynthesis. Based on its ability to inhibit DHFR, the original method of action for MTX in RA was postulated as the inhibition of activated lymphocyte proliferation, although there is no convincing evidence that low dose MTX inhibits lymphocyte proliferation in RA patients.

Adenosine induced immunosuppression

Current hypotheses favour low dose MTX having an anti-inflammatory action over an anti-proliferative action. In general, low dose MTX alters the cytokine balance by inhibiting the production of proinflammatory cytokines (TNF-α, IL-6) and

enhancing anti-inflammatory cytokines (IL-1 receptor antagonist). MTX's modulation of the cytokine network increases Th2 cytokines (IL-4, IL-10) and decreases Th1 cytokines (IFN-γ, IL-2).

The major anti-inflammatory effect of low dose MTX appears to be the intracellular accumulation of AMP and its conversion to adenosine in the extracellular space. In animal models, low dose MTX leads to the extracellular accumulation of the potent anti-inflammatory adenosine. Poly-MTX's potent inhibition of AICAR T'ase leads to the accumulation of AICAR, with *in vitro* studies showing this ultimately leads to the release of adenosine from cells. AICAR inhibits the deamination of adenosine monophosphate (AMP), the intracellular accumulation of AMP leads to the production of excess intracellular adenosine that is then released into the extracellular space. AMP that leaves the cell can also be converted to adenosine. The accumulation of AICAR also inhibits the conversion of AICAribonucleoside to AICAR, with AICAribonucleoside inhibiting the conversion of adenosine to inosine. The effect of extracellular adenosine has been reviewed, whereby it can bind to the transmembrane G-protein coupled adenosine surface receptors (A1, A2α, A2β, A3) [6, 10]. Extracellular adenosine acts predominantly *via* ligation of the A2α receptors that are present on neutrophils, macrophage-monocytes, lymphocytes and basophils. Binding increases intracellular cAMP leading to immunosuppression by inhibition of phagocytosis; inhibition of secretion of TNF-α, IFN-γ, IL-2, IL-6, IL-8, and HLA expression; and increased secretion of Il-10. Binding of adenosine to A3 receptors on macrophage-monocytes leads to inhibition of secretion of TNF-α, IL-12, IFN-γ, and IL-1ra.

Inflammatory cell proliferation and apoptosis

There is little evidence that low dose MTX inhibits lymphocyte proliferation as originally proposed as its method of action in treating RA. MTX concentrations achievable in serum with low dose RA therapy (50 μg/ml) can induce significant cell growth inhibition and apoptosis in immature monocytic cell lines, but have little or no effect on synovial macrophage proliferation. It has been suggested that low dose MTX may inhibit the recruitment of immature and inflammatory monocytes into inflammatory sites, and reduce their survival in the inflamed synovium, but have little or no effect on tissue infiltrating monocytes and resident macrophages [11].

Outcomes

MTX has been shown to improve the signs and symptoms of disease, improve function, and reduce disease progression when compared to placebo, disease-modifying anti-rheumatic drugs (DMARDs), and biological modifiers. Similarly in combina-

tion with a number of DMARDs and biological agents it has been shown to improve the signs and symptoms of disease, and function, with less evidence for alteration of disease progression [1].

Dosage

The commencement dose of MTX is 7.5–10 mg/wk as a single dose, which can be split to three doses over 36 h, if acute GI symptoms dictate. The rate of dose escalation is frequently 2.5–5 mg at intervals of 2–4 weeks, leading to a maintenance dose of 7.5–25 mg/wk. The approximate time to initial benefit is 1–2 months, levelling out at 6 months with the initial clinical improvement seen with MTX being maintained for up to 132 months of therapy [12]. A relationship between oral dosage and efficacy has been found in the range 5–20 mg MTX weekly. The plateau of efficacy is attained at approximately 10 mg/m^2/wk in most subjects, with no clear relationship between pharmacokinetic parameters and clinical response. Overall, the dosage must be individualised because of inter-individual variability in the dose-response curve [9]. Oral bioavailability of higher dose MTX (25–40 mg/week) is only two-thirds that of subcutaneous administration, such that to improve MTX efficacy at doses of 25 mg/week or more, parenteral administration should be used [13].

Signs and symptoms

A meta-analysis of the four pivotal DBPCTs of MTX in RA, from the mid 1980s, showed that MTX-treated patients had a 37% greater improvement than placebo in both tender and swollen joint counts, 39% greater improvement in joint pain, and 46% greater improvement in early morning stiffness [14].

The primary endpoint in more recent RA studies is the American College of Rheumatology (ACR) response criteria, which represents a percentage improvement from baseline in a range of clinical variables. It includes improvement in the number of tender joints, the number of swollen joints; and improvement in three of the following five areas: pain assessment on visual analogue scale, global assessment by both patient and physician, acute phase reactants, and functional status as measured by self-administered questionnaire. Originally designed to distinguish active from placebo therapy, the results are expressed as the percentage of the cohort of interest achieving the endpoint, which may be a 20%, 50%, or 70% improvement in the above individual parameters. The literature consensus is that the clinical improvement occurring with MTX, as determined by the number of tender and swollen joints and the composite ACR response, plateaus at about 6 months, but may be maintained for up to 132 months [12, 15]. Improvement in indices of disability may

be slower and has been shown to continue to improve into the fourth year of therapy [16].

In studies undertaken as part of the FDA approval for leflunomide (LEF), MTX was evaluated in RA patients who had not previous received MTX. In a placebo controlled trial of MTX and LEF (US301), 182 subjects received MTX 7.5–15 mg/wk with folate 1–2 mg daily for 52 weeks. MTX was initiated at 7.5 mg/wk, increased to 10 mg at week 4, and at 12 weeks 53% of subjects were increased to 15 mg/wk [15]. In the second active controlled trial (MN302), 498 subjects received the same MTX regime, but only 10% received folate supplementation usually after an adverse event [17]. The ACR responses are shown in the Table 1. In US301, MTX and LEF were statistically superior to placebo, and equivalent to each other after 12 months of therapy. In MN302 MTX was statistically superior to LEF using ACR response rates, although both treatments were statistically equivalent as measured by radiographic progression.

The 2-year blinded ITT results of US301 are also shown, and include 26 Canadian subjects not included in the original report. Not surprisingly, those continuing into the second year of the study had better 52 week ACR responses than the cohort overall, and this pattern continued at week 104 [18]. Of the 387 MTX receiving patients who completed the first year of MN302, 320 continued into a second year of double-blind treatment. During the second year little or no improvement occurred in any of the primary endpoints. There was no further increase in the percentage of ACR 20% responders, which at 2 years was 72%; the apparent increase due to the selection process between years 1 and 2.

MTX has also been compared to sulphasalazine (SSZ) alone, and in combination. Dougados et al. [19] recruited 205 patients with ACR criteria RA of less than 1-year duration, of which 69 received MTX only. The MTX doses used were low, commencing 7.5 mg/wk without folate supplementation, increasing to 15 mg/wk at week 16 if efficacy was inadequate. The ACR 20 response was 59% at 1-year, and was not significantly different from SSZ alone or the combination MTX/SSZ. The authors also reported no significant difference between treatment groups using the European disease activity scores for good and moderate responders.

In a study of 217 MTX naïve subjects with RA of less than 3 years duration, MTX was compared to etanercept, the MTX dose escalated over 8 weeks from 7.5 mg to 20 mg/wk, [20]. The ACR responses to etanercept were significantly greater than MTX for most evaluations in the first 6 months, but were the same over the second 6-month period.

For the treatment of the signs and symptoms of RA, MTX is superior to placebo and comparable to newer agents in trials up to 2 years. Analysis that plots the AUC of the response curves shows a benefit for both leflunomide and etanercept in the initial speed of response onset. Whether this translates into a longer-term benefit is contentious and needs to be balanced against the diarrhoea induced by the leflunomide loading dose and the economic cost of biological therapy.

Table 1 - ACR responses to methotrexate treatment: Intent to treat outcomes

Study	US301 [15]	US301 [18]	MN302 [17]	Dougados [19]	MTX *versus* etanercept [20]
Duration	52 wks	104 wks	52 wks	52 wks	52 weeks
MTX dose	7.5–15 mg/wk	7.5–15 mg/wk	7.5–15 mg/wk	7.5–15 mg/wk	Mean 19 mg/wk
Folate	Yes	Yes	10%	No	Yes
ACR ≥20%	46%	48%	65%	59%	65%
ACR ≥50%	23%	28%	44%		42%
ACR ≥70%	9%	12%	10%		22%

Radiographic progression

Radiographic progression may be evaluated using the Sharp score, which sums the erosion and joint space narrowing subscores measured at articular interfaces of the hands and feet [21]. The Sharp score or its modification evaluates joint erosions on a 0–5 point scale, and joint space narrowing on a 0–4 point scale. The scoring range of 0 (no damage) to 398/422/440 (severe joint destruction) is a highly sensitive and reproducible measure of progression in early disease – the maximum score altered by the number of joints evaluated [21–23]. Even with severe damage scores above 200 are infrequently seen. An estimated progression of radiographic damage can be defined as the Sharp score at baseline divided by disease duration at baseline. This imputed rate assumes linear radiographic progression and no follow on effect from previous treatment or uncontrolled disease – assumptions which may not be valid. Wolfe and Sharp [24] showed that the rate of progression of joint space narrowing increases with time but that the rate of progression for erosions does not change with time.

Studies from the early 1990s on radiological progression conflicted as to the benefit of MTX, although a meta-analysis showed that MTX slowed the appearance of new erosions more effectively than azathioprine and as effectively as IM gold [25]. In trial US301 MTX significantly reduced X-ray progression, and was the first 12-month placebo controlled trial of MTX to do so. The baseline total Sharp score was 22.8 in the MTX group and 25.4 in the placebo group; the MTX group increasing a mean 0.88, compared to the placebo increase of 2.16 and estimated yearly progression 3.5, $p = 0.02$. That the progression in the placebo group was less than predicted may represent a flaw in the calculation, or the influence of 63% of the placebo-treated patients receiving active treatment. In the active comparator MTX *versus* LEF study (MN302), the baseline total Sharp score was 24.6 in the MTX group

and 24.9 in the LEF group; the MTX group increasing a mean 1.62, which was less than the estimated progression of 6.5/year, and non-significantly less than LEF's 2.5 increase. In the trials US301 and MN302 in which LEF and MTX were compared, the 1 year results from the two treatments were not statistically significantly different, and neither erosion nor joint space narrowing scores were significantly different [23].

The 2-year radiographic data for MN302 noted no further increase in joint damage in the subjects treated with LEF and a small improvement in the subjects treated with MTX. The net result was a small but significant reduction in disease progression with MTX [17]. The 2-year data from US301 comparing the mean changes in total Sharp scores from baseline over 12 and 24 months of active treatment, both the LEF and MTX groups demonstrated statistically equivalent retardation of disease progression. The MTX groups change in total Sharp score at 2-years was 1.2 with an imputed yearly progression of 3.75/year. In addition, an evaluation of the erosion and joint space narrowing subscores demonstrated retardation of disease progression [18].

The short duration of disease in the study of Dougados [19] was reflected in the baseline total joint scores of 6–9. During the study the three treatment groups (MTX, SSZ, MTX/SSZ) declined similarly with increases in total joint score of 3.5–4.5. This rate of progression is greater than expected compared to the estimated disease progression within this study and when compared to the imputed progression of the other studies described here.

In a comparative study of MTX *versus* etanercept, the baseline Sharp scores were 11–13 reflecting earlier disease. The mean increase in erosion score in the MTX group was 0.68 at 6 months and 1.03 at 12 months; significantly greater than that observed with etanercept 25 twice weekly, but less than the estimated rate of progression 5 per year. Interestingly, the rate of change in both the total Sharp score and the erosion score was significantly slower in the second 6 months ($p \leq 0.005$), and was similar to etanercept. During the latter period of the study both MTX and etanercept were equivalent clinically, showing no difference in the ACR 20/50/70 response rates. It was postulated that methotrexate halted erosions in 60% of patients over the year of the trial. Decreases in clinical evidence of disease activity were correlated with the absence of radiographic evidence of progression. The strongest correlate of the absence of progression was decreased serum C-reactive protein concentrations in the group treated with etanercept [20].

Despite MTX's long history, it is only recently that its impact on radiological progression has been proven. Its efficacy in halting radiological progression is similar to leflunomide over a 2-year period, and less than etanercept during the first 6-months of treatment. The probability that among the cytokine soup of RA, some cytokines are more important in cartilage and bone degradation than others, may lead to combination therapies aiming at achieving the different endpoints of symptom and sign reduction *versus* retardation of radiological progression. While intu-

itive that alteration of radiological progression should impact on long-term disability, further proof is required. There may be no relation between radiologic damage and Health Assessment Questionnaire (HAQ) score in early RA interventions [26], in accordance with studies showing that HAQ scores at the group level do not increase during the first decade of RA [27]. The hypothesis has been that suppression of clinical and laboratory indices of disease activity would retard radiological progression, with cumulative inflammatory burden measured as area under the inflammatory curve associated with radiological progression. This is supported by the above correlations, with the altered rate of disease progression in the second half of the etanercept/MTX study allowing MTX a slower onset of therapeutic benefit. Sharp et al. [23] in their analysis of radiographic progression found only weak/mild correlations (< 0.4) between radiographic progression and the clinical variables of final ACR 20 response, AUC for ACR 20, average decreases in ESR/CRP, and HAQ scores. Supporting this was the trial adding infliximab to patients who had active RA disease despite at least 12.5 mg of MTX per week (mean 16 mg). In this study the rate of progression of joint damage in those receiving MTX alone and MTX plus varying infliximab regimes, was similar irrespective of whether a clinical response had occurred [28]. Clinical response was defined as $> 20\%$ decrease in the number of tender joints, the number of swollen joints, or the serum C-reactive protein. Previous reports have more closely correlated changes in ESR/CRP with radiographic progression, and while the short observation period may have contributed, the difference remains unexplained. Heterogeneity within the RA population could lead to variation in the individuals TNF-α contribution to the inflammatory process, with TNF-α having a critical role in the progressive bone and cartilage damage. While MTX modulates the cytokine environment and TNF-α levels, it would be expected to do this less than a specific antibody.

Disability

Analysis of function/disability and health-related quality of life utilise the HAQ and Medical Outcomes Study 36 Item Short-Form Health Survey (SF36). The HAQ has been administered to thousands of RA patients, and as a measure of arthritis-related disability found to be sensitive to change and highly correlated with a variety of clinical outcomes. It assesses on a 0 (no difficulty) to 3 (unable to perform) scale functional ability in a variety of areas, i.e., the ability to dress, arise, eat, walk, maintain personal hygiene, reach and grip. A decrease in the HAQ disability index of 0.22 is considered the minimum clinically meaningful difference, one that is apparent to patients [29]. A recently recognised problem is that the HAQ score may not increase during the first decade of RA, and that it may measure disease activity along with disability until late in the disease [27, 30]. With the SF-36, eight aspects of health status are assessed on 0 (worst) to 100 (best) subscales: general and men-

tal health, physical function, social function, physical and emotional health, pain, and vitality. No minimum clinically meaningful difference for the SF36 in RA has yet been reported.

Trial US301 demonstrated a decrease in the HAQ of 0.26 in the MTX group over 1 year compared to no change with placebo therapy. The physical components of the SF36 also significantly improved a mean 4.6 *versus* placebo 1.0, $p < 0.05$. Analyses of physical function in the year-2 cohort demonstrated that the HAQ and SF-36 improvements achieved at 1-year were maintained but not bettered over the next 12 months of therapy with both MTX and LEF [18]. In MN302 there was a statistically significant benefit of MTX over LEF, with a HAQ reduction of 0.46 *versus* 0.39, $p < 0.05$. In those patients who entered and completed the second year of the trial, the HAQ improvement was no longer significantly different between therapies, but remained clinically meaningful, being 0.5 for MTX and 0.45 for LEF [17].

Dougados et al. [19] reported no difference in HAQ improvement in early RA patients treated for 1-year with either MTX, SSZ, or the combination MTX/SSZ. All treatment groups improved a mean 0.7.

Long-term observational studies have also provided information on MTX's impact on disability. The 5-year results from 95 of 123 patients who in 1983 entered a long-term open study of methotrexate showed a significant improvement in the modified HAQ, 0.57 at last visit compared to baseline (0.57 *versus* 0.79, $p < 0.001$) [31]. Ten of the original 29 who entered Kremer's long-term observational study of methotrexate in 1982 were followed a mean 13.3 years, and the functional class (1–4) of the patients who remained in the study was maintained from baseline without deterioration [32]. Ortendahl et al. [16] using the ARAMIS database studied 437 patients commencing MTX during the period 1988–1996. The authors were surprised to find that improvement in disability (using HAQ) continued into the fourth year of therapy, and the plateau did not occur until 30–42 months of therapy. While MTX doses increased during the period of study, this was not a predictor of HAQ disability. Other factors that may have lead to apparent continuing reduction in disability include the ongoing benefits from earlier suppression of inflammation or more recent changes in concomitant medications influencing either bioavailability of the MTX or acting additively/synergistically.

Factors influencing outcomes

An analysis of factors predicting response to RA treatment (particularly for MTX), found disease duration had the strongest effect on the likelihood of response; with 53% of patients responding who had disease duration < 1 year, declining to 38% after 5–10 years of disease, and to 35% for > 10 years disease duration [33]. Other factors decreasing the response rate were any prior use of DMARD, higher disease

functional class, low disease activity (using patient global assessment), and female gender.

Resistance to MTX therapy is well described in the oncology literature and some features may be applicable to rheumatology. Factors influencing resistance are the membrane transport of methotrexate intracellularly, and its subsequent polyglutamation. Reduced levels of folylpolyglutamate synthase lead to reduce intracellular MTX concentrations and hence activity. The efflux of MTX from cells is increased with increased expression of the P glycoprotein, further increasing resistance. Low DHFR levels have been reported that would further increase resistance. There are however few studies of MTX resistance in RA, although one study has shown increased P glycoprotein in RA patients refractory to MTX compared to MTX responsive individuals (reviewed in [34]).

Reiterating that the methylenetetrahydrofolate reductase (MTHFR) enzyme impacts on several crucial cellular processes, and is known to be polymorphic, the C677T alanine to valine substitution leads to a thermolabile variant with decreased enzyme activity, and is able to influence the clinical effects of drugs such as anticonvulsants and oestrogen. The effect of C677T polymorphism on MTX toxicity in patients undergoing bone marrow transplantation showed a higher incidence of oral mucositis in those with the homozygous and heterozygous genotypes, reaching statistical significance in the homozygous genotype [35]. In a prospective study of homocysteine levels among 105 RA patients treated with MTX, SSZ), or MTX/SSZ, the C677T genotype was studied. Homocysteine levels increased more in the MTX/SSZ compared to MTX, compared to SSZ. The MTHFR genotype influenced the rise in homocysteine, with heterozygous patients having higher plasma homocysteine levels after 1 year than patients without the mutation. Baseline values for homocysteine were high for the homozygous C677T mutation, and did not increase further, suggesting a ceiling effect. A higher rise in plasma homocysteine (17%, $p < 0.05$) was found in patients experiencing a GI adverse effect (nausea, abdominal pain) than in patients without an adverse event. The authors concluding that RA patients treated with MTX have increased plasma homocysteine levels, which may be further increased by the C677T polymorphism [36]. In another study of 236 RA patients, 8% were homozygous for the mutation, 40% heterozygous, and 52% were wildtype. The presence of the C677T mutation either heterozygous or homozygous was associated with a two fold relative risk (CI 1.09–3.7) of MTX discontinuation due to GI symptoms, hair loss, and hepatotoxicity, particularly ALT elevation (RR 2.38, CI 1.06–5.3) [37]. No relation was seen between the polymorphism and the efficacy of MTX in this study. In a retrospective study of 106 RA patients treated with MTX, mucocutaneous and hepatic toxicity, as well as fatigue were more common in patients homozygous or heterozygous for the C677T mutation (RR 1.25, CI 1.05–1.49). They also assessed the A1298C glutamine to alanine polymorphism, which reduces MTHFR activity, although not as a thermolabile variant. Patients homozygous or heterozygous for A1298C received lower doses of MTX and had

improvements in CRP and ESR ($p < 0.05$), but not in tender or swollen joints. Thus, the C677T polymorphism made patients with RA more sensitive to MTX toxicity, whereas the A1298C polymorphism made them more responsive to treatment [38]. Hyperhomocysteinemia is an independent risk factor for cardiovascular disease, and the elevation of homocysteine as a result of MTX therapy can be offset by folic acid supplementation.

Combination therapy

The large proportion of RA patients who continue to have clinically evident synovitis despite maximisation of single drug therapy has lead to increasing use of combination therapies. American rheumatologists almost universally use two drug combinations, with two-thirds using a combination of at least three DMARDs to treat a subset of RA patients [39]. Combination therapy for the treatment of RA synovitis is advocated on the basis of (a) modifying or inhibiting the immuno-inflammatory cascade at multiple sites thereby gaining a greater degree of inflammatory suppression and less treatment resistance *via* redundancy of inflammatory pathways, and (b) the selection of combinations that will interact neutrally or beneficially on toxicity profile. Multiple combination studies of traditional DMARDs have been published, many of which include MTX as a core component. The combination of MTX with the anti-TNF-α antibodies or TNF receptor medications is also critical in reducing immune responses to the antibodies and improving the efficacy of treatment classes.

Combination therapy can be delivered in at least three different styles. Multiple medications can be initiated together and maintained, or after a period gradually withdrawn in a "step down" approach. Alternatively the medications can be "stepped up", commencing with one agent and adding rather than swapping if the desired outcome is not achieved. Outcomes in combination trials can be evaluated in terms of both improved signs and symptoms, and reduced progression or toxicity. Whilst these changes may be indicative of a true additive or synergistic effect of the combination, the pharmacokinetic influence of the combination also needs to be evaluated.

The "step up" addition of cyclosporin (2.5–5 mg/kg/day) to the maximally tolerated dose of methotrexate (< 16 mg/wk) has been studied in a DBRPCT of 148 RA patient who continued to have active RA [40]. The treatment group had a significant improvement in tender joint count, swollen joint count, physician and patient global assessment, joint pain, and disability as measured by HAQ. There was no difference in the reported toxicities. Subsequently the study was extended openly for a further 6 months with the combination arm remaining unchanged and maintaining their clinical status over that time. The methotrexate/placebo arm was converted to methotrexate/cyclosporin as per the original active arm, and had significant

improvement in clinical parameters. The basis for this improvement however may be more pharmacokinetic than combination therapy. Fox et al. [41] added cyclosporin 3 mg/kg/day to steady dose methotrexate (7.5–22.5 mg/wk) in RA patients, and noted a 26% increase in mean peak plasma concentration of MTX and an 18% increase in mean plasma AUC MTX, and an 80% reduction in the metabolite 7-OH methotrexate. The latter is less efficacious than MTX in rat adjuvant arthritis, and 4–17 times less cytotoxic in human cell culture. By altering the pharmacokinetic balance in favour of MTX, an increased efficacy may be explained by the combination of MTX and cyclosporin. The combination of MTX and hydroxychloroquine (HQ) is also widely used, with the combination shown to reduce the risk of acute liver damage compared to MTX alone [42]. The authors postulated that HQ stabilised hepatic lysozymes and reduced damage, although altered MTX bioavailability as has been shown for another antimalarial chloroquine may be contributing [43]. Co-administration of MTX and HQ increases the AUC values for MTX by an average 65%, with a lower maximum MTX concentration and a longer time to maximum MTX concentration. The reduced Cmax may reduce the risk of acute hepatotoxicity, with the greater AUC explaining the greater potency of the MTX-HQ combination [44]. These changes in kinetics however raise a caution for this combination in patients with reduced renal function or the elderly, and extra vigilance is indicated.

Based on the biochemical mechanisms underlying the therapeutic efficacy of low dose MTX and LEF in the treatment of RA being quite different, Kremer et al. [45] undertook a 24 week DBRPCT of leflunomide added to MTX in 263 patients with active disease despite MTX therapy. The ACR 20, 50, and 70 results for the MTX/LEF combination were 46%, 26%, and 10% respectively – all significantly different compared to the group continuing on MTX with the addition of placebo. But is the outcome better than what you would expect by simply stopping MTX and commencing LEF? As there was no LEF-only arm in the study, the result is not attainable from that study. Certainly the combination results are no better than results from LEF-only studies, although disease severity factors need to be considered when comparing across trials.

In a groundbreaking study of 102 predominantly MTX naïve patients, O'Dell et al. demonstrated the potential of "triple therapy" commenced together [46]. Patients were randomised to MTX alone, SSZ/HQ, or all three drugs. The main endpoint being the achieving of three of the following: morning stiffness < 30 minutes or decreased by 50%; joint tenderness decreased by 50%; joint swelling decreased by 50%; and an ESR < 30 mm/h for women and < 20 mm/h for men. The doses of SSZ and HQ remained fixed at 500 mg and 200 mg twice daily, respectively. The MTX was started at 7.5 mg/wk and increased at 3 months to 12.5 mg/wk if the 50% response not achieved and if needed to 17.5 mg/wk at 6 months. The results were at least a 50% improvement at the 2-year endpoint in 77% of the triple therapy regime, 40% of the SSZ/HQ group, and 33% of the group receiving MTX

alone. In those patients who had maintained a response at 2 years, this had usually occurred by 9 months, and 50% response was maintained in 73% of the triple therapy cohort at 3.3 years [39]. The improved outcome in the triple therapy may not only be due to HQ altering the bioavailability of the MTX, as the MTX dose could be increased in an effort to reach the main endpoint. At the end of the study the mean doses of MTX were almost identical (16 mg) in both the MTX alone, and triple therapy groups. The results raised the question as to whether a double combination of either MTX/HQ or MTX/SSZ would do just as well, particularly as the MTX/HQ combination was the most frequently prescribed in the US. The same network of investigators then compared triple therapy with MTX/HQ, and MTX/SSZ in 171 RA patients. Over half were already receiving MTX 17.5 mg/wk, and continued to have active disease. The primary endpoint was an ACR 20 response at 2 years, and the design allowed escalation of the SSZ dose from 500 mg bd to 1 g bd, and the MTX dose from 7.5 up to 17.5 mg/wk, with a fixed dose HQ 200 mg bd. The 2-year intent-to-treat analysis showed the ACR 20 response was achieved in 78% of subjects treated with triple therapy, in 60% treated with MTX/HQ ($p = 0.05$), and in 49% treated with MTX/SSZ ($p = 0.002$). A similar trend was seen for the ACR 50% response being 55%, 40% ($p < 0.1$), and 29% ($p = 0.005$) respectively [47]. The triple therapy was well tolerated and superior to MTX/SSZ, but only marginally superior to that of MTX/HQ. Sub-analysis according to prior MTX exposure reduced the cohort sizes, with triple therapy no longer significantly better than MTX/SSZ in the MTX naïve group, and no longer significantly better than MTX/HQ in the MTX prior users. The simple analysis of comparing the endpoints between treatment groups may be overly simplistic for combination trials. At this point in time, we are not reliably able to predict which patient will respond to which medication, and failure to respond to one medication does not invariably mean you will fail the next. Therefore, how to know whether starting multiple therapies at once is better than commencing individual drugs and swapping to the next if an objective outcome is not achieved within a fixed time. AUC analysis may demonstrate a faster onset, hopefully without medication toxicity – but in a chronic disease this faster onset may only be of benefit in those with early disease.

Toxicity

Numerous authors have reported MTX continuation rates of 60% at 5 years, 50% at 7–8 years, and around a third at 11–13 years [12, 48]. Continuation argues against severe toxicity, but does not imply ongoing efficacy – the latter dependent on alternative treatment options, which fortunately have recently increased significantly. Analysis of MTX continuation for therapy started after 1999, has shown a 3-year retention rate of 51%, being significantly better than sulphasalazine or leflunomide [49].

Table 2 - MTX associated toxicities (reviewed in [4, 34])

nodulosis	8%
hypersensitivity pneumonitis	2–7%
CNS disturbance (headache, fatigue, fuzziness)	13–35%
Post-dose reactions	10%
gastrointestinal symptoms (oral ulcers, nausea, vomiting, diarrhoea)	20–60%
elevated hepatic transaminases	20–58%
infections	60%
anaemia/thrombocytopenia	1–2%
leucopenia	2–21%
rash	2–15%
alopecia	5%

Long duration clinical studies of 5+ years, show that 10–30% of RA patients will cease therapy due to toxicities as listed in Table 2.

Much of the toxicity linked to MTX usage is ascribed to the inhibition of DHFR and its antagonism of folate metabolism. The antagonism of folate metabolism has greatest effect in the GI tract, liver, and bone marrow due to the high cell turnover in these areas and demand for purines, thymidine, and methionine. Other proposed mechanisms of MTX toxicity include inhibition of purine metabolism, inhibition of adenosine deaminase with resultant increase in adenosine and deoxyadenosine, decreased polyamine synthesis, and decreased homocysteine metabolism.

Oral ulceration, nausea and fatigue symptoms occur very frequently and are probably related to intracellular depletion of folates, resulting in increased adenosine and hyperhomocysteinemia. Hence the recommendation to supplement with oral folate 1 mg/day, which mitigates particularly the mucosal and GI toxicity, without affecting the therapeutic efficacy of MTX [50–52]. A split dose regimen of three divided doses given at 12 h intervals may reduce the GI complaints, headache and fatigue early after drug ingestion on the basis of prevention of excessive release of adenosine in the central nervous system (CNS).

In a 48-week DBRCT patients with active RA received MTX plus either placebo, folic acid (1 mg/day), or folinic acid (2.5 mg/wk). MTX was increased from 7.5 mg/wk to a maximum of 25 mg/wk according to clinical response, and the folate supplementation doubled for MTX > 15 mg/wk. Toxicity related withdrawals were 38% in the placebo group, 17% in the folic acid group, and 12% in the folinic acid group. The differences were explained by the decreased incidence of elevated liver enzymes in the supplemented group, but GI and mucosal side effects were not altered. There was no difference in disease activity scores between the groups,

although the mean dosages of MTX at the end of the study were lower in the placebo group (14.5 mg/wk) than in the folic and folinic acid groups (18 and 16.4 mg/wk, respectively) [53]. Whittle and Hughes in their 2004 review recommended the pragmatic dosing schedule of 5 mg of oral folic acid on the morning following the day of MTX administration, noting that supplementation did not significantly reduce MTX effectiveness in RA and that it offset the elevation of homocysteine associated with the use of MTX [54]. This may help reduce the risk of cardiovascular disease, which is over-represented in RA patients, and for which hyperhomocysteinemia is an independent risk factor.

Elevation of hepatic transaminases is relatively common with MTX therapy, leading in clinical trials to withdrawal in 5%, and is the prompt for dosage reduction in most studies. While cirrhosis may occur its incidence is low and depends on comorbid factors, alcohol intake and compliance with monitoring. Most cirrhosis figures predate the ACR guidelines on MTX monitoring and range from 1–30 cases per 1,000 after 5 years of use [4]. A 3.5-year prospective study utilising liver biopsies in RA patients receiving MTX up to 35 mg/wk has shown no correlation between liver MTX or Poly-MTX concentrations and clinical response or toxicity, histology, or liver function tests. Liver biopsies at 1, 2, and 3.5 years after baseline did not progress using the Roenigk score. Thus, measurement of serum MTX levels is unlikely to be useful in predicting significant hepatotoxicity [55].

Monitoring and dose modification should restrict most enzyme increases to below two times ULN (upper limit of normal), with liver biopsy only for those patients who need to continue MTX and who continue to have enzyme abnormalities. Weinblatt et al. reporting the 132 month data in his long-term prospective study detailed the serial liver biopsies at 24, 48 and 72 months [12]. The majority were Roenigk class I, with several showing improvement from II to I during the study. No biopsies showed moderate to severe fibrosis or cirrhosis.

On of the more unexpected toxicities from MTX therapy in RA patients is the accelerated formation of rheumatoid nodules, which may occur after a few months or several years. Typically, multiple small, painful nodules develop over the fingers and pulp spaces. It has been reported that MTX induced adenosine release from cultured peripheral blood monocytes, acts *via* A1 receptors to enhance giant cell formation, a surrogate marker for nodule formation [6]. Colchicine *via* its effect on microtubule function, abrogates the effect of adenosine A1 ligation on neutrophil function, and has been shown to interfere with granuloma formation. Merrill et al. in an uncontrolled trial published in abstract, treated 14 patients with MTX induced nodulosis with colchicine 0.6 mg bd and continued MTX. Approximately half the patients noted marked resolution of their nodules in less than 2 weeks, with the benefit occurring in those whose nodules were less than 6 months old [56]. A subsequent case report similarly reported regression of nodules for a year after colchicine treatment [57].

A sustained dry cough without dyspnoea or constitutional symptoms is reported in MTX users, and is attributed to mucosal/airway irritation, with a benign bronchoalveolar lavage [58]. Low dose MTX can also induce interstitial lung injury associated with severe hypoxemia in 2–7% of patients. Patients generally present with shortness of breath, tachypnoea, dry cough and fever. Radiographs show bilateral particularly bibasal "fluffy" interstitial and alveolar infiltrates. Biopsy specimens may show mononuclear cell desquamation into the alveoli with a tendency to form giant cell multinucleated non-caseating granulomas. About 40% of patients have concomitant eosinophilia and a fifth have a rash. Treatment consists of cessation of MTX, supportive measures, and high dose corticosteroids. Recommencement of MTX in proven cases of pneumonitis should be avoided, and while most patients with MTX-induced lung disease have a complete recovery, some have permanent lung damage.

The strongest predictors for lung injury is age >60 years, diabetes mellitus, rheumatoid pulmonary involvement, previous use of DMARDs, and hypoalbuminemia [59]. A baseline chest radiograph is currently recommended prior to commencing MTX [1], with a proposal to include baseline pulmonary function tests (FEV1, VC, DLCO). A HRCT of the lungs should be obtained if the DLCO was <70% of predicted, and MTX should not be prescribed in the presence of interstitial lung disease [60].

Autoimmune diseases including RA have a 2–5 fold risk of developing non-Hodgkin's and Hodgkin's lymphoma, however no additional risk has been noted in patients treated with low dose MTX [61].

Infections, predominantly upper respiratory tract infections, bronchitis, and pneumonia occur at an increased rate compared to placebo (60% *versus* 48%), and need to be interpreted in light of the high background rates.

MTX has been used both as an abortifacient and for the medical management of ectopic pregnancy, with a high risk of teratogenicity [62]. It is therefore contraindicated in women attempting to conceive or not using a reliable form of contraception. Apparently normal children have been born to fathers being treated with MTX, although it has been recommended to wait at least 3 months after MTX cessation, which is longer than one spermatogenic cycle, before attempting conception [63].

Monitoring

Methotrexate is one of the least expensive medications, but has the highest costs associated with monitoring including laboratory and clinic visits [64].

The ACR 2002 guidelines advocate baseline haematology, renal and liver function tests, supplemented with hepatitis B and C serology in high-risk patients. A chest radiograph is recommended if none are available within the previous year. Subsequently CBC, creatinine, and LFT's are repeated monthly for 6 months and 1–2

monthly thereafter. For minor transaminase elevations of less than two times ULN, the testing is repeated within 2–4 weeks. Moderate elevations of up to three times ULN both close monitoring and dosage reductions are initiated. Persistent elevation of greater than two times ULN or elevation greater than three times ULN lead to discontinuation and liver biopsy as necessary [1]. The original 1994 ACR guidelines for monitoring liver toxicity, recommended liver biopsy if half of the 4–6 weekly aspartate aminotransferase (AST) determinations within a 12 month period were abnormal (> ULN), or there was a decrease in serum albumin below the normal range in the setting of well controlled RA [65]. Abstention or restriction to minimal alcohol intake is also recommended.

Placement

Whether a patient is MTX naïve or had a suboptimal response to MTX is currently the major branch point in the ACR 2002 decision map in the management of RA, and all patients should be considered for MTX treatment at the time of RA diagnosis. Individual factors such as family planning, pregnancy, and alcohol intake may impact on that decision but it needs to be considered.

A cost-effectiveness analysis for MTX naïve RA patients evaluated five monotherapy options: etanercept, LEF, MTX (up to 15 mg/wk), SSZ, and no DMARD. The total cost of therapy with each agent was composed of direct costs associated with treating MTX naïve RA patients, combined with indirect costs incurred of lost productivity due to morbidity. MTX was shown to be a cost saving option compared with no second line agent, and indistinguishable from SSZ on cost-effectiveness. LEF was not cost effective relative to MTX in the modelling unless its efficacy was substantially better than MTX or the cost of LEF was reduced by at least 30%. Etanercept was efficacious but at a very high cost. The conclusion being MTX was cost effective in MTX naïve RA patients in achieving a ACR 20 or weighted ACR 70 response [66].

Optimal care and subsequent outcome of RA patients requires early and efficient control of rheumatoid synovitis. Strategies that modify the amplification of the immune and inflammatory pathways at multiple sites appear to have objectively better outcomes in the first years of disease, and MTX currently has a pivotal role in these protocols.

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Leflunomide

Alberta Hoi[1] *and Geoffrey Littlejohn*[2]

[1]Centre for Inflammatory Disease, Monash University, Monash Medical Centre, Melbourne, Australia; [2]Department of Rheumatology, Monash Medical Centre, 246 Clayton Rd, Clayton, Melbourne, Victoria 3168, Australia

Introduction

Leflunomide (HWA486) is a small molecule disease-modifying anti-rheumatic drug (DMARD) developed in the mid 1980s and 90s. It is structurally unrelated to other currently available immunomodulatory agents and was first evaluated in experimental models of autoimmune disease and post-transplant graft *versus* host disease. Its action as an anti-inflammatory and immunomodulatory drug has been applied to the treatment of rheumatoid arthritis (RA) in both *in vitro* and *in vivo* studies. A number of well-conducted randomised controlled studies led to its approval for the treatment of RA in the late 1990s. Leflunomide is effective in improving symptoms of RA and prevention of radiographic erosions. Like methotrexate or sulfasalazine, which are two commonly used first-line DMARDs, leflunomide has been shown to improve function and quality of life in patients with active RA.

Mechanism of action

Although the exact pathogenic mechanisms of RA remain poorly understood, inflammation and subsequent joint destruction are two constant features, both thought to be initiated by the activation and proliferation of specific immune cells that are amenable to modulation. T cell activation, in particular, can stimulate other inflammatory cells such as macrophages and synovial fibroblasts to produce mediators that may perpetuate the inflammatory cascade. Control of T cell proliferation by interfering with their progression through the cell cycle appears to be a logical therapeutic target in RA.

Leflunomide is a low molecular weight (270 kDa) synthetic isoxazole derivative and is also a pro-drug that is rapidly converted to its active metabolite A77 1726 on first-pass metabolism through the liver (Fig. 1). A77 1726 has been shown to regulate lymphocyte proliferation both *in vivo* [1, 2] and *in vitro* [3–7] using a number of cell lines, including murine B and T cells as well as lymphoma cells.

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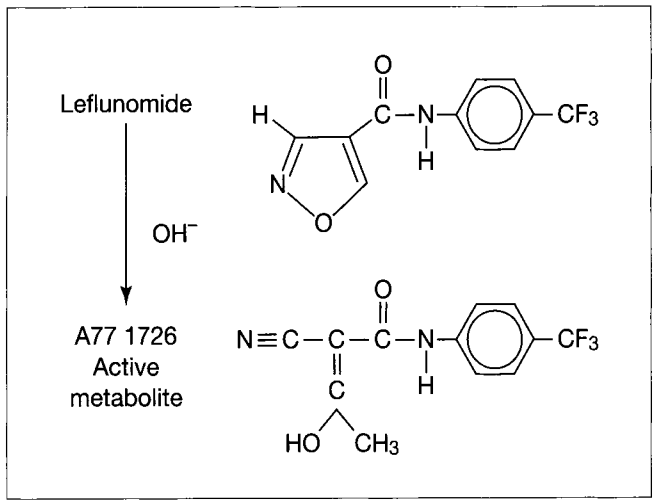

Figure 1
Chemical structure of leflunomide and active metabolite

Activated CD4+ T cells proliferate rapidly during the progression of RA, a process involving the *de novo* synthesis of pyrimidines [8]. The pyrimidine pool, and to a lesser extent the purine pool, within the activated lymphocytes must expand significantly during proliferation. This places a unique and extraordinary demand on the activated lymphocytes to induce and upregulate the *de novo* synthesis pathway in order to keep up with the demand. Insufficient pyrimidines result in a block in DNA synthesis that prevents these lymphocytes from progressing from G1 to S phase.

The anti-proliferative effect of A77 1726 can be reversed by the addition of uridine or cytidine, while the purine nucleotides adenosine and guanosine have no effect [3, 4, 7, 9]. Intracellular nucleotide pools of uridine triphosphate (UTP) and cystine triphosphate (CTP) are also reduced significantly by A77 1726 at doses observed to have anti-proliferative effect, thus suggesting that A77 1726 acts *via* inhibition of the *de novo* pyrimidine synthesis [7, 10, 11]. At this dose, A77 1726 does not appear to affect adenosine triphosphate (ATP) and guanosine triphosphate (GTP) levels.

Reversible inhibition of dihydroorotate dehydrogenase

The primary mode of action of leflunomide is thought to be the selective but reversible inhibition of dihydroorotate dehydrogenase (DHODH) [7, 12]. The *de novo* synthesis of uridine-5'-monophosphate (UMP) from ATP and glutamine is a

multi-step reaction that involves a number of enzymes. DHODH is uniquely situated on the outer face of the inner mitochondrial membrane, which means its precursor (dihydroorotate) and product (orotate) must diffuse across the mitochondrial membrane. This is the rate-limiting step in the *de novo* synthesis of pyrimidines. The inhibitory action of A77 1726 on DHODH is suggested by an accumulation of dihydroorotate in human T lymphoblastoid cells [13], and the reversal of the anti-proliferative effect of A77 1726 by exogenous orotate [14]. DHODH is the only one of the six enzymes catalysing the *de novo* UMP biosynthesis that is susceptible to inhibition by A77 1726 [9, 15]. The *in vivo* inhibition of delayed-type hypersensitivity by the analogues of A77 1726 also correlated well with the *in vitro* potency of DHODH inhibition [16].

Low levels of ribonucleotide uridine monophosphate (rUMP), due to inhibition of DHODH through A77 1726, are detected by the cell and provide a signal resulting in cell cycle arrest in late G_1, and with p53 is thought to be pivotal in regulating the G_1 checkpoint [17]. The low concentration of rUMP triggers p53 to become activated and translocate into the nucleus, where it upregulates another cell cycle regulatory gene, p21, which interacts with the cyclin-dependent kinases, with the end result being cell cycle arrest in late G_1 before the cell is irreversibly committed to the phase of DNA replication.

Interestingly, one of the mechanisms of action of low dose methotrexate appears to be by an inhibition of purine ribonucleotide synthesis and a stimulation of pyrimidine synthesis in mitogen-stimulated T lymphocytes [18]. Combination therapy with methotrexate and leflunomide thus may provide synergistic immunomodulatory effect. Cell dependent B cell formation of autoantibodies is also inhibited by A77 1726 [5]. The significant anti-rheumatic effect in animal models of arthritis by leflunomide is likely based on its anti-proliferative effect on activated lymphocytes and possibly the effect on humoral response from activated B cells arthritis [16, 19].

Tyrosine kinase inhibition – effect on signal transduction

The dose needed to inhibit *de novo* pyrimidine synthesis and lymphocyte proliferation does not normally inhibit T cell mediated signal transduction events [4, 10] and therefore does not interfere with functions of memory T-helper cells. At much higher doses however, A77 1726 is a weak tyrosine kinase inhibitor. Interference with the early signal transduction events may prevent the transition of cells from the resting G_0 phase to the G_1 phase [10, 20]. A77 1726 has been shown to inhibit *Src* family (p56*lck* and p59*fyn*) mediated protein tyrosine phosphorylation. p56*lck* and p59*fyn* are important in the mobilisation of intracellular calcium upon cellular activation and T cell receptor mediated complex signalling respectively [20], and therefore play a crucial role in early T cell activation. Furthermore, IL-2 driven proliferation is also inhibited by A77 1726, *via* inhibition of tyrosine phosphorylation of

the beta-chain of the IL-2R, and Jak1 and 3, which are protein tyrosine kinases initiating signalling by IL-2R [10].

Anti-inflammatory actions of leflunomide

In addition to the anti-proliferative effect and interference with T cell signalling, A77 1726 also has broad anti-inflammatory effects. A77 1726 is a potent inhibitor of nuclear factor κB activation [21] by preventing degradation of its natural inhibitor IκB. IκB normally binds non-covalently to NF-κB and traps it in its inactive state. Degradation of IκB results in nuclear translocation of the p65 subunit. A77 1726 blocks the degradation of IκB that is otherwise an essential step for NF-κB activation. This prevents activation of NF-κB mediated by a range of inflammatory stimuli, including TNF-α [21]. There is also a dose-dependent effect on cytokine production. TNF-α production by activated human cultured macrophages from RA patients is inhibited by A77 1726 [22] while the production of the immunosuppressive cytokine TGF-β is augmented [23]. A77 1726 has similarly been shown to inhibit the activity of COX-2 in macrophages [22, 24].

By way of reducing ATP dependent pools of UTP, the membrane biosynthesis and post-translational glycosylation of adhesion molecules may be inhibited [25]. The expression and upregulation of adhesion molecules are crucial in the process of leucocyte recruitment, and leflunomide may have an inhibitory effect on the recruitment of inflammatory cells to the joints in RA [26, 27]. Leflunomide can also prevent contact activation of monocyte by lymphocytes and favours the inhibition of proinflammatory cytokines, such as IL-1β and MMP-1 [28]. Cellular infiltration to the inflamed synovium is similarly reduced as suggested by a reduction of chemokines such as monocyte-chemotactic protein-1 (MCP-1), thymus- and activation-regulated chemokine (TARC) and macrophage derived chemokine (MDC) [29].

Leflunomide is effective in not only reducing the general inflammation process in RA but also the local production of metalloproteinases in synovial tissue. This suggests a mechanism by which it acts to prevent joint destruction [27].

Applied clinical pharmacology

Leflunomide is rapidly and almost completely converted to its active metabolite A77 1726, by first pass metabolism in the gut wall and liver and the bioavailability is not influenced by the presence of food. A77 1726 has a long half life of between 15–18 days because of its extensive protein binding in plasma and enterohepatic recirculation [30]. About 90% of a single dose of leflunomide is eliminated, about half in urine primarily as glucuronides and an oxanilic acid derivative of A77 1726, and about half in faeces, primarily as A77 1726 itself. Factors such as age, sex and body

size have a small but clinically irrelevant influence on the clearance of A77 1726. The experience with leflunomide in patients with end stage renal failure is limited, but the steady state concentrations of A77 1726 in plasma are within the expected therapeutic range. A77 1726 did not appear to be dialyzable on haemodialysis or chronic ambulatory peritoneal dialysis [31]. Nonetheless, because the kidneys play a role in drug elimination, physicians need to exercise caution when prescribing leflunomide to patients with significant renal impairment. On the other hand, because leflunomide is highly protein bound and relied heavily on enterohepatic recirculation for its clearance, and also given the risk of hepatotoxicity, leflunomide is contraindicated in patients with hepatic impairment.

Because of the long half life it may take up to 20 weeks to reach steady state plasma concentration without a loading dose. The usual loading dose consists of 100 mg daily for 3 days in order to achieve rapid attainment of steady-state levels. Maintenance dose is usually 20 mg daily, although a lower dose of 10 mg daily may be used. The lower dose may be indicated for patients who cannot tolerate the higher dose due to adverse effects or when used in combination with methotrexate. The use of activated charcoal or cholestyramine to facilitate drug elimination reduces the plasma half life of A77 1726 to approximately 1 day. In practice, many clinicians are either not using or varying the loading dose with the expectation of less "nuisance" problems with diarrhoea or nausea, both of which may influence early patient compliance. Anecdotally, clinical efficacy is maintained but delayed by a few weeks.

Weekly dosing of leflunomide (100 mg per week) has been examined in a pilot study of eight refractory RA patients [32]. Although the weekly dose group appeared initially to lag behind the usual daily dose group by 6 months there was no clinical difference between the two groups.

Clinical efficacy in RA

The clinical efficacy of leflunomide in the treatment of RA has been confirmed by a number of Phase III multicentre randomised double-blinded clinical trials. A meta-analysis of this data shows equivalent efficacy of leflunomide to methotrexate and sulfasalazine in these trials [33].

MN301 was the first phase III study of 358 patients in Europe randomised to either leflunomide, sulfasalazine or placebo for 26 weeks [34]. The Leflunomide Rheumatoid Arthritis Investigators Group (US301) was the Northern American counterpart, which investigated 482 patients on either leflunomide, methotrexate or placebo for 52 weeks [35]. The main outcome measure for efficacy in these trials was the ACR responder rate. An ACR20 responder, as defined by the American College of Rheumatology (ACR) criteria, is a person with greater than or equal to 20% improvement in both tender and swollen joint counts, and in three of the following

five criteria: physician's global assessment of disease activity, patient's global assessment of disease activity, function and disability measure, visual analogue pain scale, and either ESR or CRP. In most studies, ACR20 was the primary endpoint, although in some studies, ACR50 and ACR70 responder rates were sought. ACR50 and ACR70 correspond respectively to a greater than or equal to 50% and 70% improvement in the ACR criteria.

In both MN301 and US301, leflunomide has been shown to be no different to its active comparator in the respective studies. The ACR20 responder rate in MN301 was 48% in the leflunomide group compared to 44% in the sulfasalazine group ($p > 0.05$) [34]. In US301, 52% *versus* 46% of patients achieved ACR20 in the leflunomide and methotrexate groups, respectively ($p > 0.05$) [35].

MN302 was a multinational multicentre double-blind trial of 999 patients on either leflunomide or methotrexate for 52-weeks, with the option of continuation for a second year [36]. In this 2-year follow up study, leflunomide continued to be effective in the treatment of RA. However, when compared to methotrexate, the current gold standard first line DMARD, a few interesting observations were made. There was a statistically significant benefit from methotrexate over leflunomide with 64.8% of patients *versus* 50.5% achieving ACR20 by the end of 1 year. However, this difference was not considered clinically meaningful and became less distinct at the end of 2 years [36] (Fig. 2).

In US301, while there was no overall difference in ACR20 responder rates between leflunomide and methotrexate, both ACR50 and ACR70 responder rates were significantly better in the leflunomide group (Fig. 3). The onset of action was observed as early as 4 weeks in the leflunomide group. When compared with sulfasalazine in another multinational randomised double-blind study, leflunomide was found to be superior according to patient and physician global assessment at 2 years [37].

It is difficult to directly compare the results of US301 and MN302, which both compared leflunomide to methotrexate. These studies found seemingly contradictory results that likely relate to three main differences between the two studies. The mean duration of disease in patients from MN302 was shorter than those from US301, even though the percentages of early RA (duration of disease less than 2 years) were about the same. In US301, all patients were methotrexate naïve, whereas in MN302, patients only required to go through a washout period if they have been previously on methotrexate. Also of note, less than 10% of patients were given folate supplementation in MN302, whilst all patients received folate supplementation in US301.

Long-term efficacy of leflunomide was demonstrated by the Utilisation of Leflunomide in the Treatment of Rheumatoid Arthritis (ULTRA) Trial Investigator Group, which was a continuation from US301, to see if the therapeutic efficacy and safety were sustained over 24 months [38]. Leflunomide has shown similar ACR responses when compared to methotrexate at 2 years, regardless of whether one

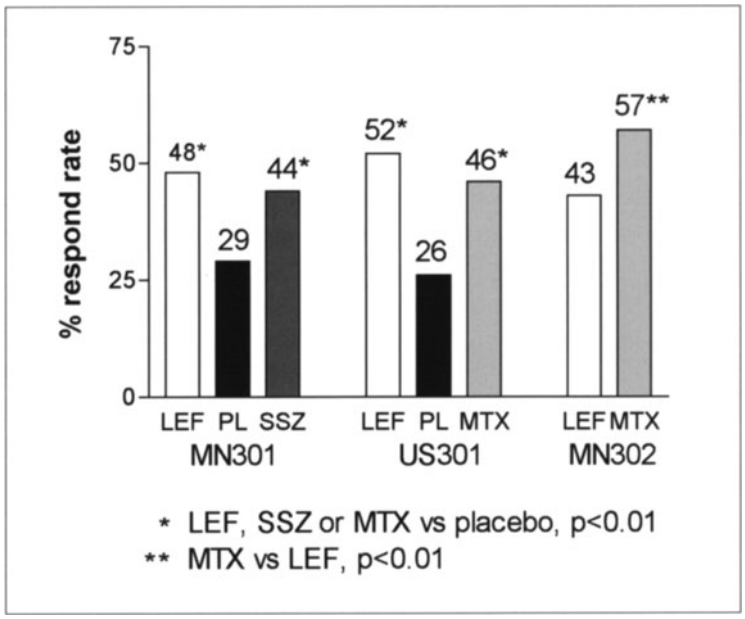

Figure 2
ACR20 responses

uses the intention-to-treat or the year-2 cohort only [38]. In addition, 163 patients from the pooled subjects of the European studies (MN301 and MN302) completed an open-labelled extension study and showed sustained ACR responses over the 5 years on leflunomide [39].

Function and quality of life

In addition to the primary efficacy endpoints, leflunomide has also been shown to significantly improve physical function and health-related quality of life [34–36]. The Stanford Health Assessment Questionnaire (HAQ) disability index can be used not only as a measure of physical function, but can predict morbidity and mortality in patients with RA [40, 41]. Leflunomide has been shown to have a significant reduction in the HAQ score as early as four weeks after commencement of therapy [34] and the improvement is sustained to 5 years of follow up [38, 39, 42]. The mean change in HAQ 2 years from baseline was similar for leflunomide and methotrexate in one study [36] with a slight statistical superiority for leflunomide in another [38], although this difference was less than the minimum clinically important difference (MCID). The minimum clinically important difference (MCID)

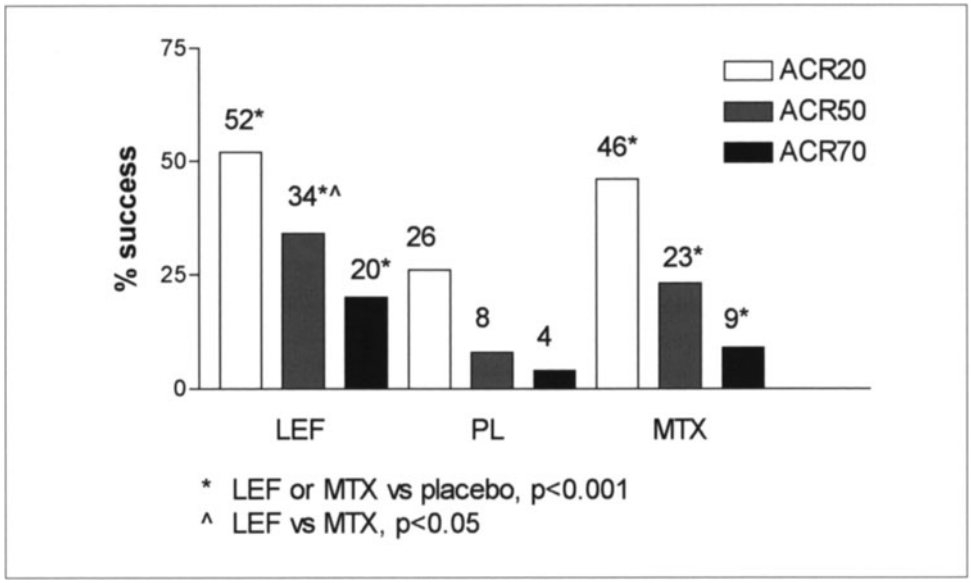

Figure 3
ACR response rates in US301

is a concept widely accepted for the interpretation of functional outcome studies in treatment for RA. It is the degree of improvement that is perceptible to patients [43, 44]. The MCID in the HAQ disability index is an improvement by at least 0.22 [45] (Figs 4 and 5).

The Short Form 36 is a validated generic measure of health-related quality-of-life which includes a physical component and a mental component. The survey consists of eight domains that give a composite score from 0 to 100. Improvements in HAQ disability index is closely reflected by SF-36, particular its physical component [46]. Leflunomide improved health-related quality-of-life significantly more than placebo (mean change of 7.6 *versus* 1.0, $p < 0.001$) and more than methotrexate (7.6 *versus* 4.6, $p < 0.001$) [35]. The improvements in function and quality of life were sustained over 12 and 24 months, and were statistically superior to methotrexate or sulfasalazine at 24 months [38, 42].

Radiological progression

Leflunomide is effective in reducing radiographic progression, as assessed by a number of radiographic scoring systems that take account of the extent of erosions and

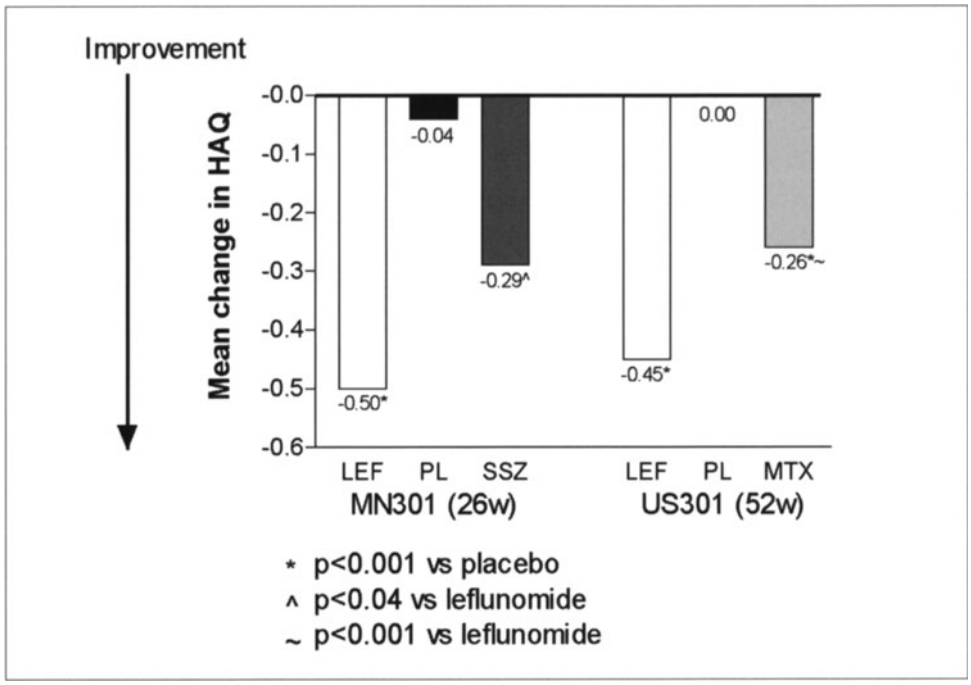

Figure 4
Change in Health Assessment Questionnaire score

joint space narrowing. The Sharp score has been the most widely used. Results from three randomised controlled trials of leflunomide in patients with active RA have shown that leflunomide is equally effective in slowing radiographic progression when compared to methotrexate and sulfasalazine and superior to placebo [47]. In US301, leflunomide and methotrexate both significantly retarded radiographically assessed progression of RA compared to placebo, but there was a statistically significant difference between leflunomide and methotrexate at one year, with mean change in Sharp score of 0.53 *versus* 0.89 ($p = 0.05$) [35] in favour of leflunomide. In MN302 however, there was no statistical difference between leflunomide and methotrexate, with mean change in Sharp score of 2.48 *versus* 1.62 ($p = 0.2940$) [47]. There appears to be no difference in the mean change in Sharp score in leflunomide *versus* sulfasalazine groups in MN301 in the original 6-month analyses, and the 12 months extension (protocol MN303). The retardation of radiographic progression is evident even after 6 months of treatment on leflunomide, as seen in MN301, and is sustained over at least 2 years of observation [38, 48]. Differences between radiological damage outcomes in these various studies probably relate to

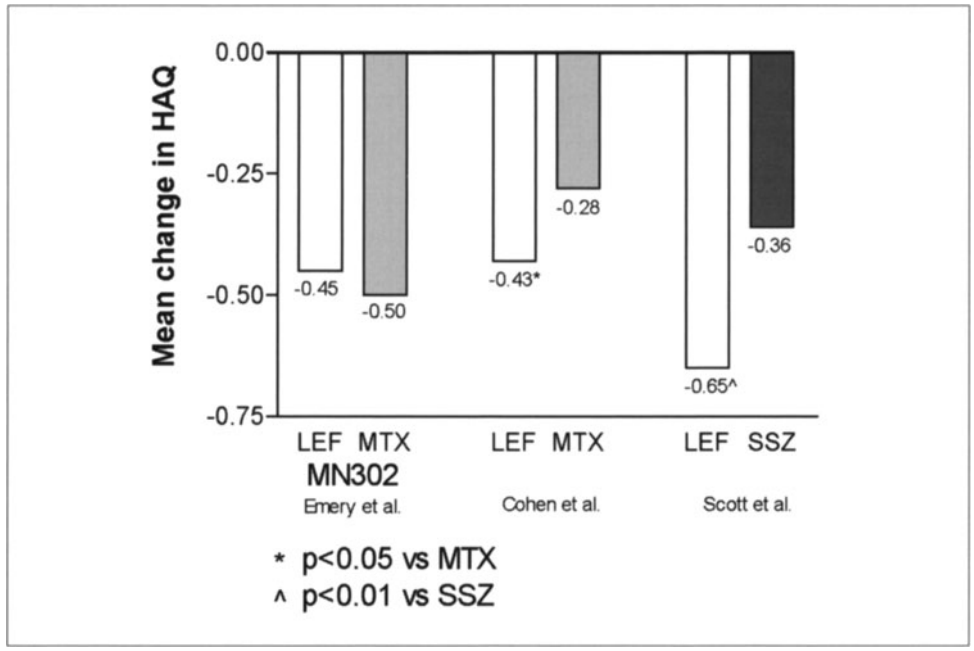

Figure 5
Change in Health Assessment Questionnaire score: Two-year results

differences in characteristics of the inception cohort such as base-line damage associated with disease severity and duration (Fig. 6).

More sensitive techniques for the detection of joint inflammatory activity and damage have been devised with particular emphasis on magnetic resonance imaging (MRI). Not only is MRI more sensitive than plain radiography to detect bony erosion, it also gives valuable information on synovial and marrow inflammation. In support of the known anti-inflammatory effect from basic science studies, leflunomide has been shown to be more effective than methotrexate in reducing synovial inflammation as demonstrated by dynamic gadolinium enhanced MRI, especially with regard to the initial rate of enhancement [49].

Combination therapy in RA with MTX

The notion of "step-up" therapy in patients with persistent active RA despite on adequate dose of a first-line agent is widely accepted. The combination of leflunomide and methotrexate has potential synergistic actions given their different mechanism of action. Combination therapy with leflunomide and methotrexate has been

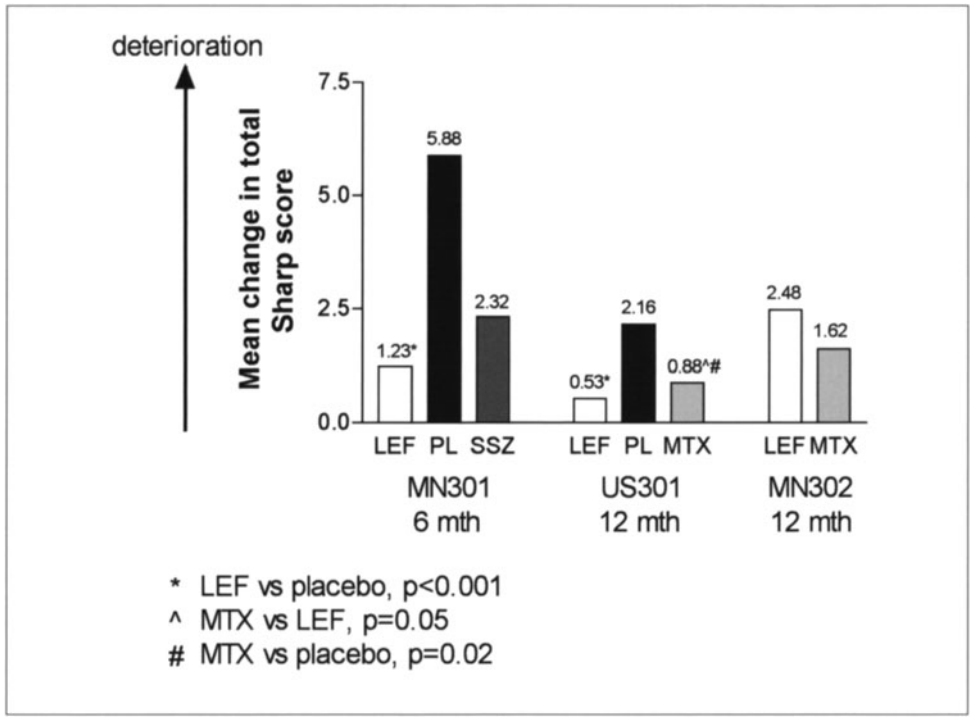

Figure 6
Change in total Sharp score

shown to be effective and well tolerated in patients inadequately responding to methotrexate alone [50]. 46% of patients on leflunomide/methotrexate combination achieved an ACR20 response at the end of the 24-week study, compared to 19.5% of patients on methotrexate alone (mean dose of 16.5 mg/wk). The mean change in HAQ score was -0.42 for the leflunomide group (compared to -0.09 for the placebo group) and was nearly twice the MCID. There was no increase in frequency of adverse events or discontinuation rates.

The combination of infliximab and leflunomide was associated with a significant rate of adverse reactions and is not recommended [51] .

Leflunomide in other diseases

Leflunomide has been used for the treatment of psoriatic arthritis and skin psoriasis anecdotally for some time [52, 53]. In a recent randomised controlled study called Treatment with leflunomide in Psoriatic Arthritis (TOPAS), leflunomide was

shown to be more effective than placebo in the treatment of psoriatic arthritis [54]. In this study, there was also a significant improvement in the psoriatic skin lesions in terms of extent of skin involvement as well as severity of target lesion. Physical function and health-related quality of life also improved significantly on leflunomide.

It is recalled that leflunomide was initially developed in the mid 1980s for experimental models of autoimmune disease [55–58]. The subsequent experience with leflunomide in human systemic lupus erythematosus (SLE) is limited. One published pilot study involved retrospective examination of a small cohort of lupus patients using leflunomide with the drug appearing to be efficacious and safe [59]. As discussed previously, because leflunomide inhibits tyrosine kinase phosphorylation only at higher doses, the dose required to treat SLE may theoretically be higher than that required for the treatment of RA [1, 20].

Another major area of interest is the role of leflunomide in the prevention and treatment of graft rejection having been shown to be effective in this role in various animal models [60, 61] In addition, leflunomide has been used anecdotally for a range of rheumatological conditions, from Felty's syndrome [62], vasculitis [63], and sarcoidosis [64].

Toxicity

The most common adverse events associated with leflunomide treatment are gastrointestinal (diarrhoea, nausea, dyspepsia), rash and reversible alopecia [34, 36]. Effects are usually mild and occur in first 3 months of therapy. Other adverse events occurring in over 5% of patients in trials and which require clinical surveillance include hypertension and upper respiratory tract infection.

The cytostatic effect of leflunomide may explain some of the side effect profile, such as reversible alopecia and conversely the lack of opportunistic infections. Most memory T cells circulate in the G_0 phase, and therefore do not require DHODH for any *de novo* pyrimidine synthesis, and are not susceptible to the antiproliferative effect of leflunomide. In addition, because of the sparing of the salvage pathway, the replicating cells in the gastrointestinal tract and haemopoietic system are relatively unaffected, thus explaining the lack of mucositis or marrow toxicity [17, 65]. Haematological adverse events are rare, but reversible pancytopenia has been reported, particularly when used in combination with other drugs that may cause marrow toxicity [66, 67]. Long-term study of up to 5 years follow up has shown no unexpected late or cumulative effect on adverse events [39].

Gastrointestinal complications, and in particular, diarrhoea, tend to occur early but may improve with time and/or dose reduction. Rats given high doses of leflunomide (35 mg/kg/day) developed diarrhoea and liver abnormalities. On autopsy, the

villi of the small bowel are short and the mature epithelial cells substituted by low columnar cells with or without dysplasia [6].

Weight loss associated with leflunomide is an adverse effect that has been a little controversial. In controlled studies, the incidence of weight loss has not been reported to be different to placebo [34, 68]. However, some reports have associated weight loss with leflunomide therapy [69, 70]. The mechanism by which leflunomide may cause weight loss is likely multifactorial, and may be independent of the diarrhoea or other gastrointestinal side effects. Leflunomide may increase metabolic requirement by interference with the oxidative phosphorylation and ATP generation thus inducing a catabolic state from insufficient ATP in the mitochondria [70].

The other main concern with regard to the use of leflunomide has been the hepatotoxicity. Liver histology in rats on high-dose leflunomide show a number of non-specific toxic changes such as fatty degeneration, atrophy and necrosis of hepatocytes in the central lobular regions [6]. When used as monotherapy in RA clinical trials abnormal transaminase levels, such as ALT and AST, were noted in 5.4–14.8% of patients but these effects were generally mild (less than two-fold elevations) and reversible and usually resolved while continuing treatment [34–36]. Marked elevations (greater than three-fold upper limit of normal [ULN]) were infrequent and generally reversed with dose reduction or cessation of treatment. The risk obviously increases significantly when leflunomide is used in combination with methotrexate [71]. In a community-based observational cohort study of patients taking leflunomide, with or without other DMARDs including methotrexate, the percentage of patients having at least one episode of abnormal ALT, as defined by elevation above the upper limit of normal, was 30% [72]. Patients from this study were managed on usual care, and no stringent exclusion criteria meant that potential problems such as other comorbidities, polypharmacy and poor compliance with monitoring were more likely than in randomised controlled trials [72]. In fact, post-marketing surveillance published by the European Agency for the Evaluation of Medicinal Products (EMEA) and FDA reveal that almost all cases of hepatic adverse event reactions had other confounding factors present. There was no consistent pattern of clinical or biochemical presentations, and because the incidence of serious hepatic dysfunction is so rare that the exact rate cannot be calculated.

Recently, leflunomide has been shown to increase plasma levels of cholesterol and LDL in a progressive manner [73]. The longer the patients remain on leflunomide, the higher the plasma concentrations of cholesterol and LDL as compared to pre-treatment levels. While the magnitude of this increase was around 17% and 27% for total cholesterol and LDL respectively, this may be sufficient to have an impact on increasing cardiovascular risk.

Leflunomide is absolutely contraindicated in women who are or may become pregnant, because of its teratogenic effects in animal studies [74]. Most physicians would recommend termination of pregnancy if patient has been on leflunomide, even though there have been two reported cases of delivery of full-term healthy

infants [75]. Because of its prolonged half life, any woman taking leflunomide who is contemplating pregnancy should accelerate the elimination of the drug by taking cholestyramine (8 g tds orally for 11 days). Leflunomide is also contraindicated in nursing mothers and children.

Any suspected toxicity may be further evaluated by use of a short course (1–2 days) of cholestyramine at lower dose (4 g three times daily). This will often reverse the side effect, be it rash or diarrhoea or other, quite quickly.

Place in the rheumatologic armamentarium

The efficacy and safety profile of leflunomide is congruent with its use in early RA, particularly when assessment indicates that significant persistent joint inflammation and damage are likely to occur. Its cost and shorter duration of clinical observation relative to methotrexate would place it as a second line DMARD in most circumstances. A typical pattern of use is to replace methotrexate, in situations where methotrexate toxicity precludes continuation, or to add it to methotrexate, if effective clinical response is not present with routine doses of say 20–25 mg per week. In clinical practice, leflunomide appears more potent than sulfasalazine in the early aggressive RA patient. In established active RA, leflunomide may be either substituted for or added to other routine agents if clinical response is inadequate or if damage is not modified.

Because the cost of leflunomide is about 8–12 times less than that of the biologics, this drug would generally be used before biologic therapies are initiated. Practice algorithms incorporating cost-efficacy and clinical outcomes, based on adequate patient numbers in different social arenas, are yet to be clarified.

Suggested monitoring

Baseline investigation should include hepatitis B and C serology and any persistent hepatic dysfunction investigated prior to starting leflunomide. Patients should be advised to reduce alcohol consumption. The American College of Rheumatology has published guidelines for monitoring liver function tests (LFTs), based on the findings of EMEA. Most of the putative hepatic adverse events occurred within six months after commencement of the drug [72]. It is therefore reasonable to monitor LFTs every 4–6 weeks during this period. Monitoring at this frequency should be continued for at least 12 months if patients are taking combination therapy with methotrexate or other hepatotoxic drugs. Thereafter, LFTs should be repeated at least every 3 months, provided the test continues to be normal.

As with methotrexate, persistent liver function abnormalities should not be tolerated. For mild to moderate elevation (2–3 times ULN), the dose should be reduced

and washout with cholestyramine should be considered if the patient is symptomatic. For marked elevation (grater than three times ULN), leflunomide should be stopped immediately and washout with cholestyramine given unless there is another obvious cause. If there is no DMARD alternative appropriate for ongoing use then re-challenge with leflunomide once liver function abnormalities settle may necessitate consideration of a liver biopsy to ensure there is no underlying hepatic fibrosis.

In addition to the monitoring required for liver toxicity, all women of childbearing age should be counselled to use effective forms of contraception and have a negative pregnancy test before beginning the drug. It is good practice to review birth control methods at follow up visits with patients taking leflunomide.

Conclusion

Leflunomide, used as monotherapy for RA, is overall at least as effective, if not superior, to other small molecule comparators. It has clinically important effects on disease activity, health-related function, quality of life and radiological indices. It has benefits of once daily dosing and an acceptable side effect profile requiring a relatively simple monitoring and surveillance program. Side effects are usually mild but if more severe they will generally respond to drug washout procedures. Issues regarding pregnancy require careful review. When used in combination, particularly with methotrexate, added clinical benefits are seen but increased surveillance is needed. Leflunomide is effective in established disease but an increased role in early, potentially severe, disease appears likely.

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Cyclosporin

Arno W.R. van Kuijk[1,2] *and Ben A.C. Dijkmans*[2,3]

[1]Department of Clinical Immunology and Rheumatology, Academic Medical Center, P.O. Box 22660, 1100 DD Amsterdam, The Netherlands; [2]Jan van Breemen Institute, Dr Jan van Breemenstraat 2, 1056 AB Amsterdam, The Netherlands; [3]Department of Rheumatology, Vrije Universiteit Medical Centre, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

Introduction

Cyclosporin A (CsA) was discovered and isolated in 1971 as a fungal metabolite produced by *Tylopocladium inflatum* with only weak antimicrobial properties. After the initial deception the immunosuppressive effect of CsA was discovered and explored in the Sandoz laboratories in the years thereafter [1, 2]. Since its first use in humans in 1978 CsA has led to a dramatic improvement in the field of organ transplantation [3]. Based on its mode of action it has also been used as an immunosuppressive agent in many other diseases, mainly autoimmune or rheumatologic conditions. The success of CsA in the clinical treatment of organ transplant patients was never equalled in rheumatology, but CsA certainly has a place as immunosuppressive agent in the treatment of many autoimmune diseases, especially in rheumatoid arthritis (RA).

The first trials of CsA in RA were initiated in the early 1980s based upon information derived from studies in transplant patients [4–8]. Therefore, patients started with high doses of CsA (i.e., 5–10 mg/kg/day), which were decreased during the trial. Clinical improvement of RA was noted, but there were considerable side effects, especially renal dysfunction and hypertension were a major problem. Later the current strategy in rheumatic diseases ("go low, go slow") was introduced with less adverse events [9]. This chapter will focus on the mechanism of action, pharmacological aspects and clinical use of CsA in RA.

Mechanism

The immunosuppressive properties of CsA have been extensively investigated, including the intracellular signalling pathway it blocks. As a result the mode of action of CsA is largely revealed. Most of the effect on the immune response is caused by a relatively selective inhibition of T cell activation [10, 11].

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CsA is a lipophilic cyclic peptide that binds with high affinity to cyclophilin, a cytoplasmic binding protein that is part of a group of so-called immunophilins. *In vitro* the cyclophilin-CsA complex competitively binds to and inhibits calcineurin, a serine/threonine phosphatase [3, 10, 11]. The inhibition of calcineurin blocks the translocation of a nuclear transcription factor (NF-AT) and nuclear factor κB (NF-κB), leading to inhibition of transcription of several T cell cytokine genes. The inhibition of interleukin-2 (IL-2) has been studied most intensively, but there is also inhibition of IL3, IL4, CD40-ligand, granulocyte-macrophage colony stimulating factor (GM-CSF), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) [10–14]. The impaired production of these cytokines leads to inhibition of T cell activation and T cell dependent immune responses.

Moreover, there is supposed to be a direct effect of CsA on cytokine production by fibroblast-like synoviocytes. The production of proinflammatory cytokines IL-6, IL-15 and TNF-α and of vascular endothelial growth factor (VEGF) by these synoviocytes is found to be downregulated and production of anti-inflammatory IL-10 upregulated [15, 16].

Despite the large number of *in vitro* studies, only a few have investigated immune function in patients treated with CsA. A differential effect on T helper cells in patients with active RA has been reported, shifting the cytokine expression from a Th1- towards a more Th2-pattern [17]. CsA therapy has not been shown to significantly lower the titre of gamma globulines or rheumatoid factor, products of B cell derived plasma cells, in RA [18, 19]. An important difference with most of the other immunosuppressive agents is the absence of myelosuppressive activity.

Clinical pharmacology

Two formulations of CsA are available for oral use, the older liquid or oil-based Sandimmune® and the newer micro-emulsion Neoral®. The active drug is similar in both formulations. Much of the research in this area has arisen from the field of organ transplantation, but is also relevant to rheumatology.

Absorption

Oral CsA is only partly and highly variably absorbed by the small intestine and this is dependent upon bile salts. As a result the absorption is increased when CsA is ingested after a fatty meal [20, 21]. The average bioavailability is approximately 30%, but varies from 5–80% [22]. The absorption is higher and there is substantially less variability with the newer micro-emulsion formulation Neoral® than with the older Sandimmune® [20, 23, 24].

Much of the poor absorption and bioavailability can be explained by P-glycoprotein activity and the cytochrome P450-3A enzyme system. P-glycoprotein, a drug efflux pump and the product of the multi-drug resistance gene, pumps drugs (CsA among others) out of the cell. P-glycoprotein is expressed not only on liver cells, but also on the surface of intestinal epithelial cells, that are responsible for the absorption of CsA [25]. Moreover there is metabolism of CsA by cytochrome P450-3A, both in liver and intestinal epithelium [26].

Distribution

The time to the peak serum concentration is highly variable, from 1 to 8 h after ingestion, as is the elimination half time. Because of the lipophilic nature of CsA, it is widely distributed outside the blood volume. In blood most of the CsA can be found inside the erythrocytes and in plasma the drug is largely bound to lipoproteins [27]. Because of its distribution whole blood essays should be used, when CsA concentration is measured [20, 28]. Since whole blood cyclosporine concentration is not a useful predictor for clinical response or toxicity in rheumatic diseases, these concentrations are not routinely measured in the treatment of patients with RA.

Metabolism and elimination

CsA is mainly metabolised by the cytochrome P450-3A enzyme system in the liver. CsA is extensively metabolised to more than 20 metabolites. The immunosuppressive abilities of most metabolites are negligible [29].

Drug interactions

Many other drugs can influence the bioavailability of CsA (Tab. 1) by interference with the two main determinants of CsA absorption and elimination (P-glycoprotein and cytochrome P450). Drugs metabolized by the cytochrome P450-3A enzyme system may compete with CsA leading to impaired clearance of CsA and an increased concentration of the drug. Examples are erythromycine, diltiazem and ketoconazole. Because some of these drugs also interact with P-glycoprotein, the resulting interaction may be a combination of metabolic inhibition and decreased drug absorption [25]. On the other hand there are drugs that induce the cytochrome P450 enzyme system (examples phenytoin, rifampicin) leading to an enhanced metabolism of CsA and a decreased concentration of the drug.

Caution is advised if CsA is combined with other potential nephrotoxic medication such as aminoglycosides, vancomycin, co-trimoxazole, aciclovir and ampho-

Table 1 - Clinically important drug interactions with CsA

Increased CsA concentration	
Calcium-channel blockers:	diltiazem, verapamil, amlodipine, nicardipine
Antifungals:	ketoconazole, fluconazole, itraconazole
Antibiotics:	erythromycin, clarithromycin
Other drugs:	allopurinol, amiodarone, danazol, bromocriptine, metoclopramide, (high dose) methylprednisolone
Food/drinks:	grapefruit
Decreased CsA concentration	
Antibiotics:	nafcillin, rifabutin, rifampicin, isoniazide
Anticonvulsants:	phenobarbital, phenytoin, carbamazepine
Other drugs:	octreotide, ticlopidine

tericin B. It is controversial whether non-steroidal anti-inflammatory drugs (NSAIDs) increase CsA nephrotoxicity. NSAIDs and CsA have been co-administered in many patients both in daily practice as in clinical studies without serious complications [30]. Nevertheless, co-administration of CsA with an NSAID should always be accompanied with careful monitoring of renal function. When a 30% rise of baseline serum creatinine level occurs, that does not respond to a reduction of CsA dose according to the international guidelines [9], most authors advise to discontinue the NSAID as well. CsA can increase the toxicity of other drugs, such as inducing myopathy and rhabdomyolysis with lovastatin (or other statine), and increased toxicity of colchicine and digoxin.

In conclusion, oral CsA as Neoral® has reasonably stable pharmacokinetics, but knowledge of possible drug interactions is essential for use in daily practice.

Efficacy

During the last years insight has been gained into the pathogenetic mechanisms of inflammation in RA via immunohistochemical studies of synovium. The interaction between antigen presenting cells, lymphocytes, macrophages and other cells involved has been examined and many pro- and anti-inflammatory cytokines have been recognized [31]. The pathway to chronic autoimmune inflammation is still far from fully understood, but it is likely that T lymphocytes are involved in the early phase of the disease, and that a subset of T cells is probably necessary for the persistence of inflammation. The supposed importance of T cells in chronic inflammation represents a rationale for a T cell-directed therapy, such as CsA.

CsA as monotherapy in RA

As mentioned previously, the first intervention trials in RA in the 1980s were based upon data generated by studies in transplant patients. Patients usually had a long-standing disease duration and started with a high dose of CsA (10 mg/kg/day). A clear beneficial effect on clinical disease activity was reported by most investigators, but there were quite severe side effects, mainly hypertension and renal dysfunction, causing a high withdrawal rate [4, 6, 32]. Then studies with a lower starting dose of CsA were performed in order to reduce toxicity with preservation of the beneficial therapeutic effect [5, 8, 33], but despite good effect on disease activity with average doses between 5–10 mg/kg/day adverse effects remained a major drawback.

The first controlled studies in which a CsA dose between 2.5–5 mg/kg/day was used, were published in the early 1990s. The largest study with a clear positive result was performed by Tugwell et al. [34]. They examined the efficacy and safety of CsA 2.5 mg/kg/day in a placebo-controlled trial in 144 patients (72 in each arm) over 6 months. Significant improvement was observed in joint score (average improvement 31% versus 8%), swollen joint count (SWJ improvement 23% *versus* 7%) and tender joint count (TJC improvement 23% *versus* 3%). At 6 months 31% *versus* 7% had a 50% reduction in joint score in favour of the CsA group. At 6 months in the CsA group three patients were lost due to lack of efficacy and three because of adverse events, *versus* 21 and one, respectively, in the placebo group. In contrast with the clinical findings, there was no decrease in erythrocyte sedimentation rate (ESR).

The other controlled studies in both long-standing and early RA are listed in Table 2 [35–44]. The overall conclusion is that CsA, used as monotherapy in a dose between 2.5–4.5 mg/kg/day, is an effective disease modifying antirheumatic drug (DMARD). As a matter of fact none of the "classic" DMARDs proved to be more effective than CsA in any of these trials; however, only a few trials with a large number of patients have been performed and a head-to-head comparison with sulfasalazine was never performed. During the last decade emphasis is laid on the property of a DMARD to slow the radiographic progression; this progression is proved to be slowed down by CsA [38, 41–44]. Because no large controlled studies of more than 2 years have been published, long-term efficacy cannot be judged easily.

CsA in combination therapy in RA

Combination therapy of CsA with other DMARDs was first reported in small series and open uncontrolled studies. Bensen et al. [45] added low-dose CsA 2.5 mg/ kg/day for 6 months in patients partially responding to methotrexate (MTX) (n = 20) and parenteral gold (n = 20). When compared with baseline SJC improved with 57% in the CsA plus gold group and 70% in the MTX and CsA group at 6 months.

A few controlled trials have convincingly demonstrated that combination with CsA gives an additive therapeutic effect. In the study by Tugwell et al. [46] CsA or

Table 2 - Monotherapy CsA in RA: Overview of controlled trials

First author	Year	Ref	Patients n=	Duration months	Mean dose mg/kg/day	CsA *versus* drug	Clinic score	Radiol score
Long-standing RA								
Tugwell	1990	34	144	6	3.8	Placebo	Better	n.a.
Van Rijthoven	1991	35	92	6	4.4	d-penicillamine	No diff	n.a.
Ahern	1991	36	52	6	3.4	Azathioprine	No diff	n.a.
Kruger	1994	37	117	6	4.2	Azathioprine	No diff	n.a.
Forre	1994	38	122	11	3.9	Placebo	Better	better
Early RA								
Landewe	1994	39	44	6	3.6	Chloroquine	no diff	n.a.
Van den Borne	1996	40	44	24*	2.7	Chloroquine	better at 1 y	n.a.
Pasero	1996	41	340	12	2.9	Several DMARDs	no diff	better
Pasero	1996	42	253	24	3.0	Several DMARDs	n.a.	better
Zeidler	1998	43	375	18	3.2	Parent gold	no diff	no diff
Drosos	1998	44	103	24	3.0	Methotrexate	no diff	no diff

Clinical and Radiological scores are expressed from the viewpoint of CsA:
"better" means that CsA performed significantly better than the comparison drug
"n.a." means no data available
"no diff" means no difference detected in study
**The study performed by Van den Borne [40] is an 18 months extension of the study by Landewe [39]*

placebo was added in a dose of 2.5–5 mg/kg/day for 6 months to 48 patients with a non-complete response to MTX 15 mg/week. In the MTX plus CsA group 48% met the ACR-20% response criteria as compared with 16% in the group treated with MTX plus placebo. The combination MTX and CsA was well tolerated and there was no substantial difference in side effects. In an open label extension of this study up to 1 year this improvement was sustained [47]. In a recent study by Marchesoni et al [48] in early RA the combination MTX and CsA was compared to MTX monotherapy for 12 months (30 patients in both groups) to determine radiologic progression using the Sharp-van der Heijde method. There was an increase in damage score in both groups, but there was a significant difference between the two groups in favour of the combination MTX and CsA. Interestingly, another recent report by Fox et al [49] studied the pharmacokinetics of the combination MTX plus CsA in RA patients, showing that the combination with CsA leads to higher concentrations of MTX. This may in part explain the additive effect of combining MTX and CsA, but could also increae the risk of adverse events due to MTX.

The combination of CsA with other DMARDs than MTX has been studied less extensive. In 40 RA patients with a non-complete response to parenteral gold therapy, CsA or placebo was added in a randomized controlled trial [50]. The 6 months results were not decisive: the overall health and efficacy scores were better in the gold plus CsA group, but no differences were detected by the Health Assessment Questionnaire (HAQ) or arthritis impact measurement scale (AIMS). Van den Borne et al. [51] tried to confirm an additive effect of combining low-dose CsA (1.25 or 2.5 mg/kg/day) with chloroquine in 88 RA patients with a non-complete response to chloroquine alone. The differences between the placebo and CsA groups were not significant, but there was a trend towards an additive effect. This study was hampered by a very high response in the placebo-group (ACR-20% improvement in placebo group 28% *versus* 50% in the higher CsA group).

No controlled trial of combination therapy with one of the new biologicals has been published so far. Temekonidos et al. [52] treated 18 patients with refractory RA, who could not tolerate MTX, with the combination of CsA and infliximab-infusions for 12 months. There were two adverse events (one tuberculosis, one hypersensitivity reaction), but overall treatment was well tolerated and most people responded well (80% were ACR-20% responders, 39% were also ACR-50% responders). So CsA can be a good alternative in combination with infliximab for patients that do not tolerate MTX.

Toxicity

In most studies with a duration of 6–12 months CsA has been well tolerated by most of the patients with limited serious toxicity, despite the high percentage of minor side effects. The main concern of administrating CsA is renal dysfunction.

Renal dysfunction

Renal dysfunction is the most common and most serious adverse effect of CsA. The exact mechanism by which CsA causes renal dysfunction is not known. There is an increased renal afferent vasoconstriction by a higher production of vasoconstrictive agents (endothelin and thromboxane) and a decreased production of vasodilator prostaglandins [53]. This results in a decreased renal perfusion and glomerular filtration rate, and hypertension.

Almost all patients treated with CsA have a measurable rise in serum creatinine level. In most studies with a duration of 6–12 months an average 20% rise in serum creatinine concentration was noted. This serum creatinine rise usually occurs in the first 2–3 months of treatment, is relatively stable in the first year and reversible after dose reduction or discontinuation of the drug [54, 55].

It is unclear whether there is a risk of more structural renal impairment when CsA is used for a longer period of time. Van den Borne et al. [56] found a small but irreversible loss of renal function in patients treated with combination CsA and chloroquine, but renal function was not affected if patients were treated according to the international guidelines [9]. In a considerable number of patients with a stable increase of serum creatinine during the first year, accepted according to the guidelines, Yocum et al. [57] reported that a rise of creatinine over 30% unresponsive to dose reduction occurred after the first year of treatment. On the other hand in a study by Pasero et al. [58] a surprisingly low percentage of side effects was reported after 3 years of CsA use in early RA. Moreover 80% of patients in this study were still on CsA medication after 3 years.

CsA-induced pathologic changes are characterized by striped interstitial fibrosis, tubular atrophy and arteriolar abnormalities [59], but these are rare when CsA is used in a low dose. In a study by Rodriguez et al. [60] renal biopsies were performed on 60 RA patients with longterm CsA-use (average 87 months). Hardly any pathologic changes attributable to CsA-induced nephropathy were found, and not at all in patients with a starting dose below 4 mg/kg/day.

Probably a patient with longstanding RA is more prone to develop renal dysfunction [61]. This may be explained by subclinical organ damage that is already present in longstanding RA as a result of either the disease itself or medication used in the years before, leading to an over-estimation of toxicity caused by CsA. In order to minimize renal damage, the importance of treating a patient according to the international guidelines should be emphasized.

Hypertension

Approximately 10–20% of RA patients receiving CsA develop hypertension. The hypertension is usually mild and can be controlled by either reducing the CsA dose or adding an antihypertensive drug. The exception to this is the patient with pre-

existing hypertension, that may have a more serious increase in blood pressure which is more difficult to treat.

Hypertension can be treated with calcium channel blockers such as nifedipine or isradipine, without interference with CsA metabolism. Calcium channel blockers that do interfere with CsA metabolism are not recommended (e.g., verapamil, diltiazem), nor are diuretics, ACE-inhibitors and angiotensin II receptor antagonists. Betablockers may be a good alternative treatment for hypertension.

Malignancy

In organ transplant patients an increased risk of malignancy is reported with the use of CsA, especially skin cancer and lymphoma [62, 63]. A retrospective controlled cohort trial in 208 RA patients did not reveal such a correlation [64], suggesting no increased risk of malignancy in RA patients treated with CsA. On the contrary, this study suggested a protective rather than a tumor-promoting effect [64, 65]. There are some rare case-reports however about Epstein-Barr virus related B cell lymphomas, which are reversible after discontinuation of immuno-suppressive therapy [66, 67].

Infection

No increased risk of infection with the use of CsA has arisen from the placebo-controlled trials in rheumatic disorders. In transplant patients CsA-containing combination immunosuppressive therapy shows a marginally decreased risk of infection compared with other combination immunosuppressive regimens [68].

Other (less serious) adverse effects

Many minor adverse effects of CsA have been reported in clinical trials. Gastrointestinal complaints are the most common (15–25%) but are usually mild and seldom give rise to discontinuation of the drug. Other frequent complaints (10–25% of patients) are hypertrichosis and hirsutism, gingival hyperplasia, headache and tremor. Less common (< 10%) are paraesthesia, dizziness, breast tenderness (women), gynaecomastia (men), oedema and many dermatologic and neurological complaints. Serum tests may show hyperkalaemia, hypomagnesemia, increased triglyceride level, increased serum concentration of uric acid and alkaline phosphatase.

CsA-induced tremor is usually mild and can disappear despite continued therapy. Although hypertrichosis is usually mild and well tolerated by most patients, excessive hair growth may be a problem in some patients.

Table 3 - Clinical use of cyclosporine in rheumatic disease

1. Select appropriate patient
   Contra-indications: current or past malignancy (except basal cell carcinoma of skin), renal impairment, uncontrolled hypertension, hepatic dysfunction
   Caution: high age (> 65 years), controlled hypertension, drug-interactions with CsA, pregnancy, breastfeeding, obesity
2. Measure serum creatinine level at least twice before starting CsA therapy and average these to obtain a reliable baseline creatinine level
3. Start low: initial CsA dose 2.5 mg/kg/day in two separate doses
4. Stay low: maximum CsA dose 4 mg/kg/day
5. Monitor blood pressure and serum creatinine level initially every 2 weeks for 3 months and then monthly if stable
6. If serum creatinine level rises > 30% above baseline level, reduce the CsA dose by 1 mg/kg/day. Check serum creatinine level within 1–2 weeks after dose reduction and temporarily discontinue CsA if creatinine level remains > 30% above baseline level
7. When creatinine level returns to within 15% above baseline, CsA can be restarted at a lower dose

From: [9]

Place in the rheumatologic armentarium

Cyclosporine has been used in rheumatic diseases for 20 years. It has proved to be effective as monotherapy in RA [34–44], but it hardly has any advantages over other established DMARDs. In addition, the careful selection and monitoring of patients necessary for safe use make it not the number one drug in daily practice.

The combination of MTX and CsA is more effective than MTX alone [46–48] and usually well tolerated. So, CsA can be a useful alternative both as monotherapy or in combination with MTX, the combination being more effective than CsA alone [69], especially in those patients who responded poorly to, or did not tolerate, other DMARDs. Furthermore it can be used in combination with infliximab for patients that do not tolerate MTX [52].

Suggested monitoring

Safe and effective use of CsA for RA requires appropriate patient selection and careful monitoring of therapy. International guidelines for the use of CsA in rheumatic disease have been published and are shown in Table 3 [9]. CsA is started at a dose of 2.5 mg/kg/day divided in 2 doses per day. Like most other classic DMARDs the

response to CsA is slow, usually noticeable after 8–12 weeks of treatment. To improve clinical response the daily dose can be increased by 0.5 mg/kg/day in 4 week intervals to the maximum dose of 4 mg/kg/day. If there is no clinical improvement in 6 months of treatment, CsA should be stopped because of lack of efficacy. In case of remission, CsA dose can be reduced by 0.5 mg/kg/day at 4–8 week intervals to determine the lowest effective dose for the individual patient (and diminish toxicity).

To minimize toxicity blood pressure and serum creatinine should be measured every 2 weeks for the first 3 months of treatment, and afterwards every month if the patient is stable. It is very important to determine the baseline serum creatinine level before start of therapy, at least two measurements should be obtained for this. An increase of the serum creatinine level within 30% above baseline level is considered acceptable. If the serum creatinine concentration rises above this level, the CsA dose should be reduced according to the guidelines. If the serum creatinine level does not return within acceptable limits, CsA should be stopped.

Other labarotory tests that should be obtained before, and regularly after starting treatment are serum potassium, magnesium, liver enzymes and uric acid, because they can be influenced by CsA.

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Tetracyclines

Kristin Bird[1] *and James R. O'Dell*[2]

[1]Division of Rheumatology, 96 Jonathan Lucas St, Suite 912, P.O. Box 250623, Charleston, SC 29425, USA; [2]Section of Rheumatology and Immunology, Department of Internal Medicine, Internal Medicine Residency Training Program, 983025 University of Nebraska Medical Center, Omaha, NE 68198-3025, USA

Introduction

The tetracyclines are a group of antibiotics discovered in the 1940s that were quickly recognized for their effectiveness against a variety of microorganisms. These drugs remain important for treatment of infections caused by mycoplasma, rickettsiae, Chlamydia, and some spirochetes. Tetracyclines were initially proposed as a treatment for rheumatoid arthritis (RA) based on the belief that the disease may be caused or triggered by an infectious etiology [1, 2]. It is clear there is a link between infection and certain cases of polyarthritis related to hepatitis C, parvovirus, and Lyme disease. Although an infectious cause of RA has not been demonstrated, the role of the tetracycline family in treating RA has been re-examined due to the discovery of the anti-inflammatory and immunomodulatory effects of these agents.

Mechanisms of action/clinical pharmacology

The antimicrobial action of the tetracyclines results from their binding to the 30S ribosomal subunit found in bacterial RNA. This binding inhibits bacterial protein synthesis. The most commonly used tetracycline derivatives include tetracycline, doxycycline, and minocycline. Doxycycline and minocycline are considered second-generation tetracyclines because they have enhanced antibacterial activity due to their better absorption, tissue penetration, and longer half life. The primary route of tetracycline elimination is via the kidney and dosages should be adjusted in patients with renal insufficiency.

In addition to their antimicrobial effects, ongoing studies have demonstrated that these drugs exhibit a variety of non-antibiotic effects. Perhaps one of the best-described actions of the tetracyclines is their inhibitory effect on matrix metalloproteinases (MMPs). MMPs are a diverse group of endogenously produced enzymes with substantial effects on extracellular matrix. MMPs thus play an important role

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in tissue remodeling, wound healing, embryogenesis, and angiogenesis. MMPs are made by a variety of cells, such as endothelial cells, fibroblasts, chondrocytes, osteoclasts, and osteoblasts that are involved in the pathogenesis and severity of many types of arthritis. MMPs including collagenases and gelatinases are elaborated in degenerative and inflammatory articular disorders where they may cause progressive destruction of articular cartilage and periarticular bone, thereby contributing to pain, disability, and deformity.

The inhibitory properties of tetracyclines on MMPs were initially characterized in studies of periodontal disease which shares many pathogenic features with RA [3, 4]. Tetracyclines were first shown to inhibit gingival collagenase activity in rats. Additionally, these studies demonstrated that these inhibitory effects could not be attributed to antimicrobial actions because chemically-modified tetracyclines that lacked antibiotic activity were used. Tetracyclines also have been shown to inhibit collagenases in models of experimentally induced arthritis and in samples of synovial tissue or fluid from RA patients [5–12]. These inhibitory effects on MMPs have been most potent with synthetic tetracyclines such as doxycycline and in chemically-modified tetracyclines that lack antimicrobial activity.

A number of other non-antibiotic properties of tetracyclines may contribute to the beneficial actions of these drugs in inflammatory conditions such as arthritis. Many such effects may be due to interactions with mediators of inflammation. Tetracyclines have been shown to inhibit nitric oxide production, to interfere with production of prostaglandins and leukotrienes, and to have antioxidant effects [13–21]. Tetracyclines also influence polymorphonuclear leukocytes and have been noted to inhibit neutrophil chemotaxis and phagocytosis [22–25]. Additional immunoregulatory effects include actions on B and T lymphocytes. Minocycline has been reported to inhibit tumor necrosis factor α (TNF-α) production by T lymphocytes [26–28]. This effect may occur partially due to an induction of interleukin-10 by minocycline which has been demonstrated in synovial fibroblasts [29].

Efficacy

Early reports of the benefits of tetracyclines in treating patients with RA were largely anecdotal and uncontrolled [30, 31]. T. McPherson Brown was an advocate of tetracyclines and other antibiotics in RA. He reported an independent analysis of 98 patients he treated and although most showed clinical benefit, the varying dosing regimens and classes of antibiotics made the results difficult to interpret.

In 1971, a small, controlled trial of 27 patients with RA treated with tetracycline failed to show a statistically significant benefit of tetracycline (250 mg/day) compared with placebo [32]. Although this trial was small and the dose of tetracycline used was low, for a time this was accepted as evidence that tetracyclines were ineffective in RA and these agents fell out of favor.

The use of tetracyclines for treatment of connective tissue disease was revisited when studies such as those discussed previously revealed the non-antibiotic effects of these agents in inflamed gingival and synovial tissue. The improved bioavailability of the newer tetracyclines, minocycline and doxycycline also made them more attractive agents.

In the early 1990s, two open trials evaluated the use of minocycline in patients with RA [33, 34]. Both found minocycline of benefit in reducing disease activity. Doses of up to 400 mg of minocycline were used in the first 16-week trial; however the majority of patients experienced side effects, particularly vestibular, at this dose. A 48-week open trial of lower dose minocycline (200 mg daily) in 18 patients with RA showed benefit in the 12 patients completing the trial.

These open trials were followed by two randomized, controlled, double-blind trials investigating the use of tetracyclines in RA. Each of these studies used minocycline administered at 100 mg twice daily. The first study involved 80 Dutch patients with severe RA [35]. More than 90% of patients were seropositive and had erosive disease with an average disease duration of 13 years. Patients were treated with minocycline or placebo for 26 weeks but were allowed to continue other disease-modifying antirheumatic drugs (DMARDs). The minocycline group showed modest improvement over the placebo group with 38% showing at least 25% improvement in signs and symptoms of arthritis whereas 18% in the placebo group had a similar response ($P = 0.05$).

The minocycline in active rheumatoid arthritis (MIRA) trial was a multicenter trial conducted in the United States lasting 48 weeks [36, 37, 40]. 219 adult patients with active RA were randomized to receive minocycline 100 mg twice daily or placebo. These patients had slightly less severe disease than those in the Dutch trial with about two-thirds having erosive changes. Average disease duration was 8.6 years. Patients were continued on NSAIDs or low-dose corticosteroids (prednisone 10 mg daily or less) but concomitant use of other DMARDs was not allowed. At 48 weeks, more patients in the minocycline group than in the placebo group showed improvements in joint swelling (54% and 39%) and joint tenderness (56% and 41%). ($P < 0.023$ for both).

These randomized, controlled trials provided evidence that tetracyclines had a modest benefit over placebo in patients with well-established RA. A third randomized, placebo-controlled trial investigated use of minocycline in patients with early RA [38]. This 6-month study enrolled 46 patients all of whom were rheumatoid factor positive and had RA for less than 1 year. Patients were continued on stable doses of NSAIDs but oral prednisone, intraarticular corticosteroids, or other DMARDs were not allowed. 65% of patients in the minocycline group showed at least a 50% improvement in composite symptoms of arthritis while 13% of patients in the placebo group had a similar improvement ($P < 0.001$). At the conclusion of the blinded portion of this trial both groups of patients were treated with conventional therapy. A 4-year follow up found that remissions were more frequent and the need for

DMARD therapy was less in the patients treated originally with minocycline compared with the placebo group [39]. This was the first study to address the long-term benefit of minocycline in RA.

After the positive results seen with minocycline *versus* placebo in RA, this drug's benefit when compared to another DMARD was investigated. In 2001, the first randomized, controlled trial comparing minocycline to a conventional DMARD in RA was published [41]. This study randomized 60 patients with active, seropositive RA of less than 1 year's duration to treatment with minocycline 100 mg twice daily or hydroxychloroquine 200 mg twice daily. All patients also received low-dose prednisone. After 2 years, patients treated with minocycline were more likely to achieve an ACR50 response than hydroxychloroquine-treated patients (60% compared with 33%; $P = 0.04$). Minocycline-treated patients were also receiving less prednisone at 2 years than those receiving hydroxychloroquine (mean dosage 0.81 mg/d and 3.21 mg/d respectively; $P < 0.01$).

Four double-blind clinical trials have established the efficacy of minocycline in RA. It appears minocycline may have a more dramatic effect when used early in the course of RA. Whether other tetracyclines such as doxycycline or different classes of antibiotics would give comparable results to minocycline was unknown. Two randomized, double-blind, placebo-controlled trials were designed to investigate this question. The first used oral doxycycline and failed to show any clinical improvement over placebo [42]. In a second trial, 31 patients were randomized to receive intravenous doxycycline 200 mg daily, azithromycin 250 mg orally, or placebo. This trial was stopped prematurely, but in the analysis that was completed there were no significant differences observed across treatment groups in disease activity after 4 weeks [43]. These results suggest that doxycycline and minocycline may differ in their effects on joint inflammation. Because these agents share a similar spectrum of antibacterial activity, the difference may be in the ability of minocycline to more dramatically upregulate IL-10 production as compared to doxycycline [29].

Adverse effects/toxicity

Severe toxicity is unusual with the tetracyclines and most adverse effects are reversible with a dosage reduction or discontinuation of these drugs. In the open trials of minocycline in RA the most common adverse effects were dizziness, vertigo, and nausea [33, 34]. However, in some patients these effects occurred when higher doses of minocycline (up to 400 mg daily) were used. The subsequent trials of minocycline in RA used doses of 200 mg daily and the incidence of vestibular and gastrointestinal symptoms varied from none to more than 50% depending on the trial. However, these side effects lead to drug discontinuation in no more than 7% of patients.

All tetracyclines may cause photosensitive reactions but minocycline, especially with long-term use such as for acne or RA, can result in gray, black, or blue pigmentation of the skin, nails, and mucosal membranes. This has been reported to occur in 4–15% of patients taking cumulative doses of > 100 grams of minocycline for acne and other dermatoses [44, 45]. In RA, some investigators suggest that with continued minocycline therapy lasting longer than 2 years, hyperpigmentation may be seen in 20% of patients [41]. The discontinuation of the drug usually results in resolution of pigmentary side effects but this may take months to years.

Minocycline has been reported to rarely cause interstitial nephritis, hepatotoxicity, severe exfoliative dermatitis, and cytopenias. All tetracyclines should be avoided in pregnant and nursing women because they inhibit skeletal growth in the fetus and newborn. When used in childhood, they may also cause discoloration of the teeth [46].

A lupus-like syndrome associated with polyarthritis, skin rash, pneumonitis, hepatitis, and positive antinuclear antibodies (ANA) or antineutrophil cytoplasmic antibodies (ANCA) has been described in patients taking minocycline for acne and more recently for RA [47–49]. These symptoms typically resolve promptly with drug discontinuation. It has been suggested that recognition of this possible side effect of minocycline may be difficult in RA patients where the symptoms may be thought to represent a flare of their systemic disease.

There are no current guidelines on laboratory monitoring of patients taking long-term tetracyclines for treatment of RA. A conservative approach may be to obtain a complete blood count as well as liver and renal function studies with initiation of therapy and every 3 months thereafter [2].

Conclusions

Although studies examining tetracycline and doxycycline have not shown benefit, four double-blind clinical trials have now clearly established the efficacy of minocycline in RA. It appears minocycline may have a more dramatic effect when used early in the course of RA suggesting that there may be a period of opportunity when disease course and outcome may be significantly and favorably impacted. When compared to hydroxychloroquine, minocycline is superior at least in patients with early disease. How minocycline compares with other DMARDs or its role in combination therapy in RA is still unknown [50–55].

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Systemic glucocorticoids in rheumatoid arthritis

John R. Kirwan and Mark G. Perry

University of Bristol, Academic Rheumatology, Bristol Royal Infirmary, Lower Maudlin Street, Bristol BS2 8HW, UK

Introduction

Glucocorticoids have been therapeutic agents in rheumatology since 1948, when Philip S Hench first used compound E (later renamed cortisone) to successfully treat a patient with rheumatoid arthritis (RA) [1, 2]. Over the intervening years, opinions about glucocorticoids have varied from excitement to complete rejection. Most of the worries concerning their use have been about adverse effects. Despite this historical variability in rheumatological opinion, many patients in practice are prescribed glucocorticoids. Here we review new evidence on the mechanisms of action of glucocorticoids, outcomes of glucocorticoid treatment and adverse effects, and propose an evidence based approach to their effective use in the management of rheumatoid arthritis.

Mechanisms

Glucocorticoids are thought to exert their effect via two mechanisms – genomic and non-genomic. These influence physiological cell mechanisms differently at different doses [3]. Genomic actions have been known for many years, are mediated by cytosolic glucocorticoid receptors, occur at all doses, and are only seen more than 30 minutes after receptor binding (and more usually after many hours). Non-genomic effects are mediated via membrane bound glucocorticoid receptors or biological membranes, occur at higher doses and happen within minutes or even seconds.

Glucocorticoids are lipophilic and therefore pass easily through cell membranes. Once inside the cytoplasm, the glucocorticoid (G) binds to the ubiquitously expressed glucocorticoid receptor (GR), causing a conformational change and allowing translocation of the G-GR complex into the cell nucleus. The G-GR complex has three categories of actions that account for the clinical effects seen in practice: increasing or reducing certain enzyme transcription rates; and post-transcriptional effects. Transcription rate increases are seen with many proteins, but the most important for the glucocorticoid anti-inflammatory effect is lipocortin-1.

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Lipocortin-1 antagonises the enzyme phospholipase A2, and thus inhibits the arachidonic acid cascade and production of the ensuing inflammatory mediators [4]. Transcription inhibition is also an important mechanism of glucocorticoid action, with the most relevant example being inhibition of the synthesis of cytokines such as tumor necrosis factor (TNF), interleukin-2 and interleukin-6. Post-transcriptional effects have been shown to affect messenger ribonucleic acid (mRNA) stability, translation and secretion [5–7]. An example of this effect is seen in the IL-1 induced expression of cyclooxygenase 2 (COX-2) mRNA, where glucocorticoids destabilise the COX-2 transcript [5].

Glucocorticoid effects mediated by mechanisms different to the genomic effects have been apparent from the earliest research into its physiology and pharmacology, although until the 1990s these effects had been generally overlooked and not studied. These actions, now known as non-genomic effects, can be non-specific or specific. In the laboratory the non-specific non-genomic effects occur within seconds, at very high glucocorticoid doses, and appear to result from direct interactions with biological membranes rather than with a specific receptor. The specific non-genomic effects occur within a few minutes and are probably mediated by cell surface membrane-bound glucocorticoid receptors. (It is possible that mitochondrial membranes also have such receptors.) Laboratory examples include rapid aldosterone effects in lymphocytes, vitamin D effects in non-epithelial cells, and glucocorticoid effects on neuronal function [3]. The rationale for specific non-genomic effects is three-fold: firstly many rapid glucocorticoid effects are very selective; secondly they occur at lower concentrations of glucocorticoid than non-specific non-genomic actions (although still equivalent to > 200–300 mg prednisolone daily); and finally it is difficult to explain these effects on the basis of non-specific interactions with membranes. This theory provides a possible explanation for the clinical observation that only high doses of glucocorticoid are generally successful in treating exacerbations of certain diseases such as rheumatoid vasculitis. Thus current understanding suggests that at low doses (prednisolone ≤ 7.5 mg daily) glucocorticoid effects are mediated almost entirely by genomic actions; at medium doses (< 30 mg) greater genomic actions are accompanied by some specific non-genomic actions; at high doses (30–100 mg) specific non-genomic actions increase; and at very high doses (e.g., > 250 mg by IV injection) non-specific non-genomic actions make a major contribution to treatment effects [8].

Applicable pharmacology

All glucocorticoids bind to the same glucocorticoid receptor and their relative potencies relate to their structure and plasma half life [8]. Prednisone (used commonly in clinical practice in North America) is immediately hydroxylated to prednisolone (used widely in Europe). Both are rapidly absorbed from the gastrointesti-

nal tract and bind reversibly to plasma proteins. Prednisolone is rapidly metabolised in the liver, conjugated and excreted in the urine. It has a half life of about 1 hour [9], however its action at the tissue level lasts considerably longer.

Drug interactions with glucocorticoids may occur. Enhancement of the rate of metabolism of some glucocorticoids has been noted with concurrent administration of phenobarbital, phenytoin and rifampicin, probably by inducing hepatic microsomal drug-metabolising enzymes [10]. Glucocorticoids at higher doses may induce new diabetes or make control of diabetes mellitus with insulin or oral hypoglycemic drugs more difficult. When glucocorticoids and thiazides or related diuretics are prescribed together, potassium loss may be increased. This is of particular relevance when cardiac glycosides are co-prescribed, as their toxicity is increased with hypokalaemia. Giving aspirin and glucocorticoids concurrently decreases salicylate levels owing to an increased rate of salicylate metabolism. 40 years ago, when high dose aspirin was a common treatment for RA, a reduction in glucocorticoid dosage in such patients could result in increased plasma salicylate levels with symptoms of salicylate overdosage [11].

Efficacious treatment outcomes in rheumatoid arthritis

There can be little doubt that glucocorticoids have beneficial effects in patients with RA. Traditionally these have been primarily measured in terms of process measures (e.g., inflamed joints and blood tests for inflammatory markers), but a series of studies have now explored the effects of glucocorticoids on radiographic progression (e.g., the development of erosions) and there has been a move to include patient-centred outcomes (e.g., function, or physical and mental wellbeing). Measurement of patient-centred qualitative outcomes has thrown up its own difficulties, but they offer the opportunity to improve our understanding of patients' experiences of RA. In addition, it has become clear that patients and the healthcare professionals looking after them have different views of the importance of different symptoms. For example, patients seem to place the personal impact of a disability above the disability itself [12, 13].

In this review we would like to show that glucocorticoids have three main actions in the context of RA: inhibition of inflammation; retardation of radiographic changes; and modifying (currently poorly defined) outcomes such as a sense of wellbeing. Further, the effects on these outcomes may differ, and this may relate to underlying differences in their pathophysiology.

Effects on inflammation

The dramatic effect of glucocorticoids on the signs and symptoms of inflammation has been clearly apparent since 1948 when cortisone was first used to treat RA, and

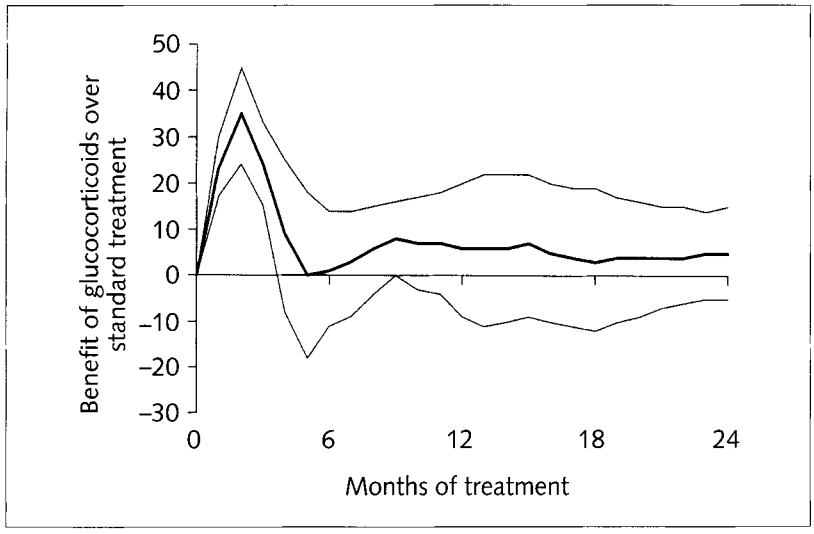

Figure 1
Schematic illustration of symptomatic benefit from trials of glucocorticoid therapy in early rheumatoid arthritis
Five studies are included [16–20]. All patients had disease duration of less than 2 years. The primary clinical outcome is used from each study. The additional benefit for the glucocorticoid treated group is calculated as a percentage of the comparator group (standard treatment) for each time point in the trial, and monthly values interpolated where necessary (only up to 12 months for reference 18). The un-weighted mean value of the trials and 95% confidence intervals were calculated for each month. Because some trials did not make measurements until month 3, it is likely that the initial rate and size of increase in benefit is underestimated. The benefits of glucocorticoids are lost by about 6 months of treatment.

a rapid improvement in tender and swollen joints is equally visible in patients today. The evidence summarised in a Cochrane Library review of efficacy over 1 week, mainly from studies published in the 1950s and 1960s, confirms that there is a large, immediate benefit in relieving symptoms [14]. A three-month randomized controlled trial showed clearly evident benefit at two weeks, and maximal benefit at eight weeks [43]. A second Cochrane Library review of efficacy concentrating on outcomes measured close to 6 months of treatment, concluded that glucocorticoids were significantly more effective than placebo in four of six outcomes measured (tender joints, swollen joints, pain and functional status) [15]. However, contrary to popular belief, this effect is not sustained unless doses are steadily increased. Six randomised controlled trials [16–20, 42] have all indicated this, and can be summarised in Figure 1. A similar phenomenon was observed with intramuscular glucocorticoids [44]. This evidence about longer term, continuing anti-inflammatory

effects suggests that the period of benefit after initiation of treatment is about 6 months. It is worth noting that the small, non-significant benefit thereafter could represent a definite continuing benefit in a few patients. This would be consistent with the clinical experience of finding a small proportion of patients with RA in whom symptom control is impossible without continuing glucocorticoid use. In this respect, it is interesting that in the ARC Low Dose Glucocorticoid Study, about 8% of patients were being treated with glucocorticoids at the end of the post study 1-year follow up [21].

Effects on radiographs

Glucocorticoids slow the radiographic changes seen in the early years in many patients with RA, although it is not known whether this effect persists beyond 3 or 4 years. This slowing of changes was suggested in early studies [22–25], but in the last decade five carefully conducted randomised controlled trials have clarified the issue [17–20, 42]. These have generally used low doses of prednisolone (5–10 mg daily) over 6 months to 2 years in addition to specific anti-rheumatoid ('disease modifying') drugs. Four of these trials have shown marked reduction in the progression of erosive disease (Fig. 2) or in the onset of erosions (Fig. 3). Conversely the WOSERACT data showed no significant difference in erosive disease between the active and placebo groups [42], although concerns have been raised about the accuracy of these data [46]. In contrast to the effect of glucocorticoids on symptoms of inflammation, this effect has been maintained throughout the period of treatment. A comparable trial of intramuscular glucocorticoids in established RA did not show any reduction in progression of erosions [44]. Two of the oral glucocorticoid trials have gone on to show erosive progression resuming in the 6–12 months following discontinuation of glucocorticoids [18, 21], but the onset of erosions and even possibly the rate of erosive progression may remain less even after the glucocorticoids have been withdrawn. If this could be confirmed by specific studies it would have profound consequences for our understanding of both the disease and its treatment. Glucocorticoids have a much smaller effect (if any) on the generalised loss of cartilage visible on radiographs as joint space narrowing. Figure 4 summarises the results from three studies, and is in marked contrast to Figures 2 and 3.

Effects on performance and wellbeing

Glucocorticoids in moderate or high doses can have marked effects on a patient's psychological state, and a range of psychiatric disturbance can be precipitated by glucocorticoids including psychosis and depression. But, even in lower doses, patients report clinically that glucocorticoids affect subjective experiences such as wellbeing

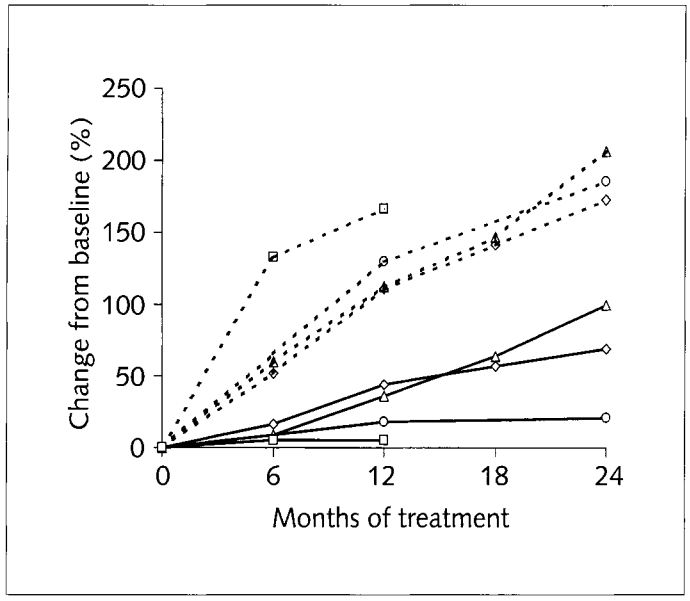

Figure 2
Changes in erosion scores in four trials of prednisolone in early rheumatoid arthritis
Four studies are included. (Square: Boers [18], triangle: van Everdingen [20], circle: Kirwan [17], diamond: Rau [19].) All patients had disease duration of less than 2 years, and had low erosion scores at the start of treatment. The units of measurement differed between the studies, so the proportionate (%) change from baseline during the treatment period is shown for the treated and placebo group from each trial. Glucocorticoids suppress erosion progression substantially, and do so throughout the treatment period.

and fatigue. We have therefore looked at these broader effects that glucocorticoids may have in the anti-inflammatory and anti-erosive studies reported above.

In the 1957 ERC trial [24], aspirin or cortisone acetate (at an equivalent dose to prednisolone 12–15 mg) was given for 3 years. The conclusion of this trial was that the effect of cortisone and aspirin were similar in almost all respects, and this included clinical measures, employment status and ESR. Although "subjective wellbeing" improved similarly in both groups over the first 6 months, by 2 years only the cortisone group still showed a statistically significant difference from their baseline. This difference continued until the end of the third year. Unfortunately, the details of the definition of "subjective wellbeing" are not recorded, but it seems that glucocorticoids influenced these subjective experiences on a different time scale to the clinical signs of inflammation.

In the ARC study [17] disability levels showed a statistically significant improvement for longer than measures of joint inflammation, lasting for 15 months of treat-

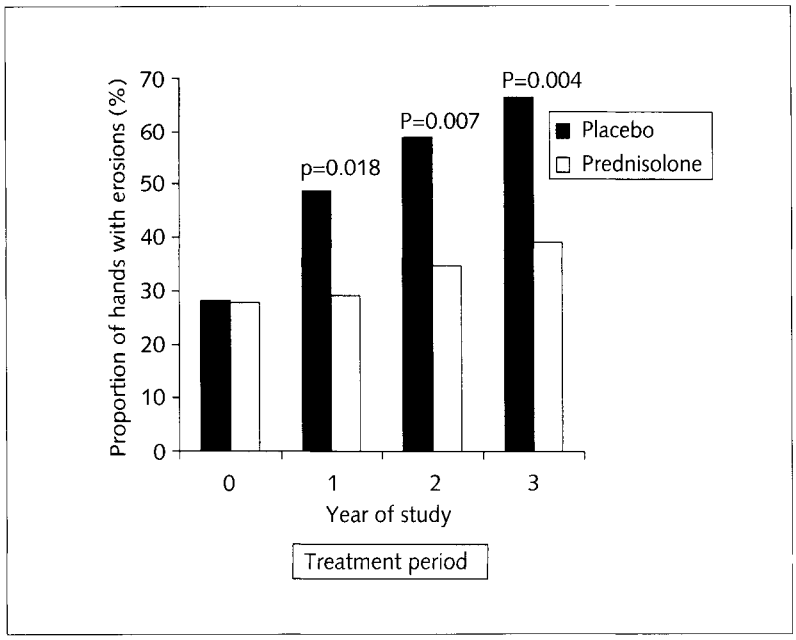

Figure 3
Proportion of hands with erosions in the ARC low dose glucocorticoids in rheumatoid arthritis study [17, 21]
Very few non-erosive hands developed erosions, either during prednisolone therapy or in the year after stopping treatment.

ment. It is possible that something of what patients interpret as 'wellbeing' is reflected in the measurement of disability. Patients involved in discussions about assessing the outcome of RA consider wellbeing, fatigue and sleep disturbance to be important aspects of RA and to be related to the disease process in some way (not just a non-specific consequence of having a chronic disease) [26, 27]. Further work is required to determine how adequate measures of these outcomes can be created.

High dose, short term (pulsed) glucocorticoids

Pulse therapy involves the i.v. infusion of a large dose of glucocorticoid (usually 1 g of methylprednisolone) over a short time – perhaps 60–90 minutes. Many current regimens entail a course of three pulses on alternate days, followed by a resting phase of around 6 weeks. This form of treatment was first used in renal transplants, but

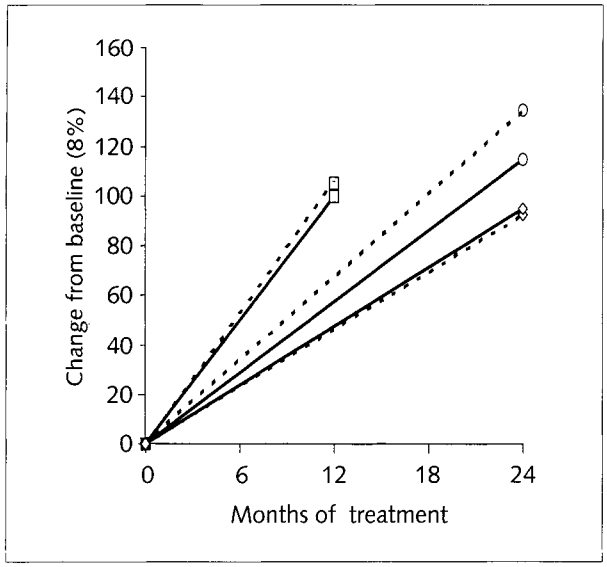

Figure 4
Changes in joint space narrowing in three trials of prednisolone in early rheumatoid arthritis
Three studies are included. (Square: Boers [18], circle: Kirwan [26], diamond: Rau [19].) In each study, the proportionate (%) change from baseline is the same for prednisolone and placebo treated patients.

gradually spread to the treatment of other renal disorders, most notably lupus nephritis where synovial inflammation responded rapidly and for prolonged periods.

Control of arthritis

Methylprednisolone has been used in a number of studies to produce an early initial response in RA patients commencing second-line agents, thus attempting to bridge the gap between initiation and response with these agents. A review [28] of the use of pulsed methylprednisolone stresses the impressive favourable risk:benefit ratio of this therapy with few or minor adverse effects. Most of the serious adverse effects – cardiovascular collapse, myocardial infarction, severe infection – have occurred in patients with compromised cardiovascular or immune systems as a result of their disease, or due to concomitant drug treatment. The minimal effective dose of methylprednisolone is uncertain at present. It has been reported that doses as low as 320 mg may be as effective as 1 g [29] although others [30] have concluded that using only 500 mg results in substantial loss of efficacy. If equivalent oral doses are as effective as i.v. treatment [31], this will allow pulsed treatment to

become an outpatient procedure with reduced costs and less patient discomfort, although Choy [32] found that intramuscular methylprednisolone was superior to equivalent oral doses in their study of glucocorticoids and gold therapy.

Control of severe systemic rheumatoid arthritis or rheumatoid vasculitis

Glucocorticoids are widely used to treat systemic vasculitis in RA, systemic lupus erythematosus (SLE) and other autoimmune diseases and are clearly powerful inhibitors of the inflammatory processes. However, glucocorticoids may not inhibit subsequent organisation of the platelet and fibrin thrombus and induction of endothelial cell and smooth muscle cell proliferation. Thus, in spite of clinical and laboratory improvement after glucocorticoid treatment, patients may go on to develop evidence of progressive ischemia, such as blue digits. Conn [33] suggest that where there is no clinical evidence of active inflammation indicated by fever, myalgias, arthralgias or active skin lesions, and no laboratory evidence of inflammation such as elevation of the erythrocyte sedimentation rate, a more plausible management strategy would be the use of vasodilators and inhibitors of platelet activation. They postulate that glucocorticoids fail to control the generation of platelet-derived thromboxane (possibly because platelets do not possess a nucleus and cannot form lipocortin), but it may be that this deficiency is often offset by the concomitant, widespread use of (platelet suppressing) non steroidal anti-inflammatory drugs (NSAIDSs).

Adverse effects of glucocorticoids in rheumatoid arthritis

Most commentators agree that taking prolonged high doses of glucocorticoid risks serious adverse effects. However, there is much debate on the safety of low dose glucocorticoid and intravenous pulse therapy, particularly when treating RA. When considering the occurrence of adverse effects of glucocorticoids in RA, the dose used is a critical issue, disease duration may be important, and evidence is scarce.

Low dose glucocorticoids

There are four main areas of concern: the development of "glucocorticoid adverse effects" such as obesity, glycosuria and hypertension (as occurs in Cushing's syndrome); the possibility of an exaggerated flare or worsening of symptoms when treatment is stopped; the development of osteoporosis; and adrenal suppression. The literature provides little evidence on which to base judgments in the particular circumstances of RA, and even less regarding low dose glucocorticoids in RA. Randomised controlled trials (RCT) provide some evidence, although the trials reviewed below were not powered to analyse adverse effects.

There are six RCTs that have investigated an equivalent dose of prednisolone ≤ 10 mg daily [17, 19, 20, 35, 42, 43], and one gave an average of prednisolone 12.5 mg daily [18]. In total 774 patients were followed (N = 34 to 167) and the trials lasted from 3 months to 10 years.

General metabolic effects attributable to glucocorticoids include hypertension, obesity and hyperglycaemia. None of the seven RCTs found differences in the rates of developing hypertension. Van Everdingen [20] found a significant increase in weight in the prednisolone treated group, whereas the remaining six trials found no significant change in weight between the two groups. Two of these [18, 42] found that both groups gained weight, and this is likely to be a reflection of better disease control. Million [36] found one patient treated with prednisolone who developed glycosuria, compared with no controls. Van Everdingen [20] found a significant increase in serum glucose levels in the prednisolone treated group, but only two patients developed diabetes compared to one in the placebo group. In the remaining trials, four found no significant difference in the rate of developing diabetes [17, 35, 42, 43] and two did not comment [18, 19]. It is interesting to note that none of the seven trials reported an increase in peptic ulcer disease in the glucocorticoid treated group. This concurs with the usual absence of peptic ulceration in endogenous Cushing's disease [37]. It is possible that low dose glucocorticoids predispose to diabetes, but there is no clear evidence for this. Low dose glucocorticoids may predispose to weight gain when used in active RA but this is not in excess of that seen with better disease control. They do not seem to cause hypertension.

With respect to osteoporosis the evidence is not clear cut. While the occurrence of osteoporosis in patients treated with glucocorticoids for a wide variety of medical conditions is well recognised, the situation in active RA is complicated by the possibility that glucocorticoid treatment may counteract the disease process which is itself related to reduced bone mineral density [47]. In a small trial by Harris [35] there were two vertebral fractures in the prednisolone group and none in the placebo group and van Everdingen [20] found a slight increase in vertebral fractures in patients treated with prednisolone, but these differences were not statistically significant. Indeed, in the four randomised controlled trials of oral glucocorticoids that (at least in some patients) included measurement of bone mineral density (BMD) there were no significant differences between the groups (Tabs 1 and 2). A single RCT has suggested that oral glucocorticoids reduce loss of hand bone mass in RA [45]. In a trial of intramuscular glucocorticoid there was a significant fall in hip BMD but not the lumbar spine [44]. Broader epidemiological and physiological data mean that it is likely that low dose glucocorticoids do have at least some effect on bone metabolism, but it may be that this is effect is negligible in many patients.

Worsening or flaring of symptoms is a commonly voiced concern when attempting to reduce or discontinue treatment. Two randomised controlled trials [21, 35] were designed to allow double-blind analysis of flares after prednisolone was withdrawn, and a third comments on symptoms un-blinded after cessation of pred-

Table 1 - Bone mineral density (BMD) and fractures in RA patients treated with glucocorticoids in randomised controlled trials

Reference	Prednisolone dose	Number of patients	Result
Boers [18]	60 to 7.5 mg	126	Trend for lower BMD on prednisolone at 6 months but no significant difference at 12 months.
Zeidler [41]	5 mg	192	No significant difference in BMD at 24 months.
Van Everdingen [20]	10 mg	81	No significant difference in fractures at 24 months.
Kirwan [34]	7.5 mg	21	No significant difference in BMD at 12. Prednisolone group had higher BMD in hips at 24 months.
Capell [42]	7 mg	167	No significant difference in BMD at 24 months. Anti-resorbtive treatment more frequently used in prednisolone group.

Table 2 - Mean (sd) Changes (%) in bone mineral density in a subset of patients[a] in the ARC low dose glucocorticoid study [34]

Years of treatment	Prednisolone 7.5 mg daily (n = 11)		Placebo (n = 10)	
	Spine	Hip	Spine	Hip
1	–1.6 (5.0)	–2.2 (7.1)	–2.3 (6.5)	–0.6 (5.6)
2	–3.0 (5.6)	–1.2 (3.1)	–1.3 (4.6)	–4.0 (2.5)[b]

[a]Patients were chosen for bone mineral density measurement because they were attending study centres where measurement facilities were readily available at the time of the study. Only those patients for whom measurements at the spine and hip after year 1 and year 2 are included.

[b]P = 0.04 for difference from the prednisolone group (T-test).

nisolone [18]. Hickling [21] (n = 75) stopped prednisolone 7.5 mg daily over 4 weeks and found no significant difference in clinical variables apart from the articular index. Closer inspection of their data shows that the difference in articular index between the prednisolone and placebo group may have been due to a (possi-

bly chance) reduction in placebo scores at that time. Kirwan [43] (n = 143) abruptly stopped prednisolone 7.5, and budesonide 3 or 9 mg overnight and found a significant worsening of joint pain and count over 4 weeks. However, patients became no worse than those being treated with placebo. Harris [35] (n = 34) abruptly stopped prednisolone 5 mg daily overnight, and found a significant increase in joint pain and tenderness. It is important to note that Boers' data [18] is un-blind, that both methotrexate and prednisolone were discontinued within 3 months, and that the most recently discontinued drug was restarted for disease flare. That aside, six of 76 patients needed to restart prednisolone, compared to 13 of 79 restarting methotrexate. Overall the evidence suggests that the chance of disease flare is low if discontinuation of low dose glucocorticoid occurs over a few weeks.

Pulsed IV glucocorticoids

Reports of complications following pulse glucocorticoid therapy have usually arisen in renal transplant patients [37, 38]. Most important of these is sudden death, most probably as a result of ventricular dysrhythmia and consequent myocardial infarction. In three such cases, the IV bolus was administered rapidly (in one case over only 20 seconds) and all were taking frusemide, which may have induced hypokalaemia. It has been suggested that increasing the infusion time to at least 30 minutes might prevent such events. Nevertheless, the incidence of such sudden death appears to be extremely low, given that well over 10,000 renal transplant patients are likely to have been treated with pulse glucocorticoids.

Severe fatal infections have also been reported. However, these are rare, and have occurred in transplant patients on daily doses of azathioprine, often following continued, long-term use of 1 g pulses. *In vitro* studies indicate, however, that methylprednisolone pulses fail to reduce bacterial phagocytosis or killing by human neutrophils

Evidence-based treatment strategy

Based on the evidence reviewed here, it is possible to derive a strategy for the use of low dose glucocorticoid therapy in RA.

Treating symptoms

In the absence of evidence for long term symptomatic control, the use of relatively short-term therapy to help control symptoms for specific reasons is justifiable. One example might be the use of "bridging" therapy between the introduction of slow

Table 3 - Evidence based policy for treating with prednisolone to reduce the progression of erosions in rheumatoid arthritis

Radiographic findings at time of decision	Disease duration			
	Less than 2 years	2–3 years	3–4 years	More than 4 years
Erosions	Treat with prednisolone 7.5 mg daily for approximately 4 years.	Treat with prednisolone 7.5 mg daily for approximately 2 years.	Treat with prednisolone 7.5 mg daily for approximately 2 years.	Do not treat until more evidence is available.
No erosions	Treat with prednisolone 7.5 mg daily for approximately 4 years.	Treat with prednisolone 7.5 mg daily fo approximately 2 years.	Do not treat.	Do not treat.

acting anti-rheumatoid drugs and the time when they begin to reduce disease activity. A second example is to help patients through a particularly difficult or demanding time, such as undertaking a journey or a wedding. Controlling disease during pregnancy might also be achieved with glucocorticoids. In the light of the evidence for accumulation of adverse effects, it is difficult to justify long-term, low dose "background" therapy for symptoms control, a treatment strategy that is currently used in a substantial proportion of RA patients.

Controlling joint destruction

Taking all the studies so far published in full or in abstract form that have used low dose treatment in patients with early disease, the mean disease duration has been about 1 year, and all patients have had their disease for less than 2 years. However, there has not been any indication that the therapeutic response has been related to disease duration within these narrow limits. Taking into account the evidence that most patients who are to develop erosions do so during the first 3 years of their disease, it is not necessary to treat non-erosive patients with a disease duration over 3 years. Thus a treatment strategy can be summarised as shown in Table 3.

Suggested monitoring

When considering what monitoring for adverse effects should be implemented with low dose glucocorticoid therapy, it is clear that evidence is scarce. Following the data presented above, we will consider whether it is worth monitoring patients for osteoporosis or hyperglycaemia.

Guidelines for management of glucocorticoid induced osteoporosis have been published by the Royal College of Physicians (RCP) and the American College of Rheumatology (ACR) for use in patients who are on long-term glucocorticoid treatment, or are starting a course that is expected to last ≥3 months. The RCP [39] suggests that patients in the above group aged ≤65 years with a previous fragility fracture, and all patients aged ≥65 years are at high risk of glucocorticoid induced osteoporosis. These patients should have both general measures to help reduce osteoporosis and start a bisphosphonate without the need to measure BMD. Patients aged ≤65 years without a previous fragility fracture should have BMD measured and, if significant bone loss is present they should start osteoporosis treatment. If BMD is acceptable, they should have repeat BMD measurement at between 1 and 3 years. The ACR [40] recommend patients receiving long-term glucocorticoid therapy ≥5 mg prednisolone equivalent should have BMD measured. Bisphosphonate treatment should be started if the BMD is low or, if the initial BMD is normal, sequential measurements taken after between 1 and 2 years.

Neither of these guidelines is based nor comments on the specific circumstance of low dose glucocorticoids (≤7.5 mg prednisolone equivalent) in RA. Instead they summarise the available evidence, which is primarily RCTs where patients are taking significantly higher doses (from 10 – > 120 mg prednisolone equivalent), and retrospective case-control data (evidence level III) in which the majority of patients were taking glucocorticoids for non-rheumatological conditions, and very few had RA. Further research is needed to clarify whether monitoring for osteoporosis is needed in the specific situation of low dose glucocorticoids in RA. Currently we would not advocate routine testing or treatment for osteoporosis in patients with early RA being treated with low dose glucocorticoid therapy.

It is unclear whether treatment with low dose glucocorticoids predisposes to hyperglycaemia. As patient or laboratory tests for glucose are cheap and readily available, it is reasonable to keep a check on glucosuria.

In summary, glucocorticoids have an important role to play in the management of patients with RA. At low doses, they are effective in treating symptoms of joint inflammation, slowing joint destruction, and also appear to affect performance and wellbeing. In high intravenous doses they can quickly bring rampant disease under control. Concerns regarding adverse effects are important to consider, but careful scrutiny of the available evidence suggests that at low oral doses these are minimal.

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TNF-α inhibitors

Zuhre Tutuncu and Arthur Kavanaugh

University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0943, USA

Introduction

The development of anti-TNF-α therapy has resulted in the potential to radically alter the course of inflammatory diseases such as rheumatoid arthritis (RA). Currently there are three anti-TNF-α agents available for clinical use: infliximab, a chimeric anti-TNF-α mAb; etanercept, a soluble TNF-receptor construct; and adalimumab (formerly know as D2E7), a human anti-TNF-α mAb. All three agents block the ability of TNF to bind to the cell and prevent TNF from stimulating the cell to produce the biochemical agents that are ultimately responsible for the clinical markers of RA: inflammation, tissue damage, pain, stiffness, swelling, tenderness, and eventual deformation of the joint and patient disability.

Tumor necrosis factor α inhibitors: Mechanism of action

Although all three agents are TNF-α inhibitors, there are differences among them. Infliximab and adalimumab are specific for TNF-α; etanercept binds both TNF-α as well as LT-α. While all bind with high affinity, the avidity and hence duration of binding may be greater for the mAb. Effector functions such as induction of cell lysis and apoptosis can be demonstrated by *in vitro* studies with the mAb but not the soluble receptor, although the *in vivo* relevance of this is uncertain. Infliximab and adalimumab neutralize the biological activity of TNF-α by binding with high affinity to the soluble and transmembrane forms of TNF-α and inhibits binding of TNF-α with its receptors.

The TNF-α receptor domains in etanercept bind to two of the three receptor binding sites on the TNF-α trimer, thus blocking the ability of TNF-α to interact with two or more cell-bound TNF-α receptors, a prerequisite for signal transduction. The dimeric structure of etanercept results in a high binding ability for TNF-α, which acts as a competitive inhibitor to the binding of TNF-α to cell surface TNF receptors and thereby inhibits TNF-α-induced proinflammatory activity [1].

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It has been verified by the analysis of many RA patient samples, that there is a cytokine network that is thought to be regulated by TNF-α. Once the TNF-α activity has been blocked, it has been proposed that clinical benefit in patients with RA are brought by various mechanisms, including downregulation of local and systemic proinflammatory cytokine production, reduction of lymphocyte migration into the joint, and reduction of angiogenesis in the joints [2, 3]. The relevance of these has been demonstrated with post-treatment synovial biopsies that showed reduced cellular infiltrates, with fewer numbers of T cells and macrophages present [4]. Also, changes in soluble E-selectin, soluble ICAM-1, VEGF, and circulating lymphocytes with anti-TNF-α therapy correlated with clinical outcome [5].

Applicable clinical pharmacology

Despite potential differences, all agents are effective in controlling the signs and symptoms of RA. TNF-α blockers behave in a consistent manner across different demographic groups (including pediatric *versus* adult patients) and among patients with different diseases of varied severity. Given intravenously, infliximab has a high peak concentration, whereas etanercept and adalimumab, because they are given subcutaneously, have more "flat" pharmacokinetic profiles. Whether these differences in characteristics between the agents will result in differences in other outcomes in RA patients (e.g., effect on radiographic changes), efficacy in other diseases, and toxicities; remains to be shown. Based on small numbers of patients, trying a different TNF-α blocker can be recommended in patients who failed one agent. In one analysis from a Swedish registry of RA patients, patients with insufficient efficacy from etanercept, treatment with infliximab provided better results, and in patients who discontinued infliximab owing to adverse events treatment with etanercept gave at least similar clinical efficacy [6].

Clinical pharmacology studies demonstrate that infliximab has a dose-dependent PK profile following infusions of 1 to 20 mg/kg. In combination therapy with methotrexate (MTX) (7.5 mg once a week), serum infliximab concentrations tended to be slightly higher than when administered alone [7]. It has been estimated that the half life of infliximab is around 8–9.5 days at the 3 mg/kg dose, although longer values have been reported for higher doses [8]. The volume of distribution (Vd) of infliximab at steady state is independent of dose, and there is predominantly intravascular distribution [9, 10]. Median Vd ranges from 3–5 L. The clearance of infliximab is approximately 0.01 L/h. The initial recommended dose of infliximab is 3 mg/kg given as an IV infusion followed by doses at 2 and 6 weeks after the first infusion then every 8 weeks thereafter. Infliximab is approved for use in combination with MTX, although patients have received infliximab with other DMARDs or as monotherapy. At the recommended initial dose, about 25% of patients will have trough concentrations below 1 mg/mL; this is associated with

lesser clinical response [10]. In that case higher doses and/or shorter intervals may be used.

Etanercept is given by self-administered subcutaneous injection at 25 mg twice weekly, or 50 mg once a week. Etanercept is approved for use either alone or in conjunction with methotrexate. When administered subcutaneously, etanercept is absorbed slowly, reaching a mean peak concentration at approximately 50 h after a single 25 mg dose. The immunoglobulin structure affords a half life of 3–4.8 days. The volume of distribution of etanercept suggests predominantly intravascular distribution [11]. The route of clearance from the circulation is unclear although it is presumed that it is mediated through Fc binding by the reticuloendothelial system.

The peak serum adalimumab concentration and area under the curve (AUC) increases linearly with dose over the range of 0.5–10 mg/kg. Adalimumab appears to have a low clearance and distributes mainly in the vascular compartment. Its elimination half life is 10–13.6 days. Adalimumab has been studied with different doses at different intervals. The recommended dose is 40 mg every other week administered subcutaneously, with the possibility of changing dose frequency. Adalimumab is recommended to be used alone and in combination with methotrexate; however methotrexate is shown to reduce the adalimumab clearance after single and multiple dosing 29% and 44%, respectively.

Efficacy

In recent years, true disease modification has become a realistic goal in the clinical care of patients with RA. With early and aggressive treatment involving new drugs and drug combinations, it may be possible to substantially ameliorate the physical, social, and economic consequences of RA. Anti-TNF-αs are very effective, as demonstrated by a number to treat (NNT) of 2 to produce a 20% improvement. For all agents, the NNT for ACR50 is 4 and for ACR70 it is 8 [12]. The clinical benefits of TNF-α blockers are associated with an improvement in various serological parameters, including C-reactive protein, serum amyloid-A, ESR, MMP-1 and MMP-3 levels [13]. Double-blind, placebo-controlled, randomized clinical trials (DBPCRCT) have demonstrated clinical benefit associated with significant improvement in patients with severe disease, often when conventional treatments are unsuccessful. Initial clinical studies based on the use of anti-TNF therapy have suggested a potential beneficial effect in inducing reduction of inflammatory parameters in patients with longstanding active RA. In the earliest controlled trials, the efficacy and tolerability of the TNF-α-blockers in refractory RA patients were demonstrated [9, 14–16]. This, along with the growing safety of experience gained with therapy, provided the rationale for studies with longer duration of therapy.

Multicenter DBPCRCT with infliximab have evaluated the effects of multiple doses over longer time periods. In the ATTRACT trial, addition of infliximab to patients with active disease despite concurrent methotrexate was significantly superior to treatment with methotrexate alone. The initial promising results from 6 months of treatment, have been shown to be sustained through 54 weeks of follow up [8, 17]. In addition to achieving substantial efficacy as measured by ACR 20 clinical response criteria, the use of infliximab was associated with significant improvement in functional status and quality of life [17]. Patients receiving infliximab had an arrest of the progression of joint damage as assessed by x-ray change scores. [10, 17].

Initial studies have demonstrated the efficacy and tolerability of etanercept in both early and refractory disease and also established the optimal dose of 25 mg twice weekly [15, 16]. In a 6-month DBPCRCT with etanercept (10 or 25 mg twice weekly), patients with active and longstanding RA, etanercept was shown to be effective in rapidly reducing disease activity [18]. The efficacy and safety of etanercept together with methotrexate was demonstrated in another trial, where addition of etanercept resulted in rapid and sustained improvement [19]. In the open-label extension part of this study, the patients were able to sustain the improvement and a majority of them were able to decrease their use of methotrexate and/or corticosteroid dose. In one trial two doses of etanercept (10 or 25 mg twice weekly) was compared with an accelerated dosing of methotrexate in methotrexate naïve RA patients with less that 3 years of disease [20]. Radiographic assessments at 0, 6, and 12, 24 months showed that the rate of x-ray progression appeared to be slowed by both agents; the effect of 25 mg etanercept being greater than that of methotrexate.

In a DBPCRCT phase II trial, 284 patients were treated with placebo or one of three doses of adalimumab (20, 40 or 80 mg) by weekly subcutaneous injection for 12 weeks [21]. Clinical results demonstrated the efficacy of adalimumab in comparison to placebo. In a subsequent DBPCRCT study, adalimumab was shown to be safe and effective with concurrent methotrexate therapy [22]. In a multicenter DBPCRCT, 619 patients with active RA with inadequate response to methotrexate were randomized to receive adalimumab 40 mg every other week, adalimumab 20 mg weekly or placebo [23]. Both adalimumab regimens were found to be significantly more effective at reducing signs and symptoms measured with ACR 20 response, and also improving physical function in comparison to placebo. In addition, modified total Sharp scores showed significantly smaller changes in patients treated with adalimumab, and significantly fewer patients had new erosions compared with those taking placebo.

Facing increasing health-related costs and limited healthcare resources, the assessment of cost effectiveness of medical procedures is also gaining considerable importance in the field of rheumatology. The costs associated with RA are substantial. The total cost (sum of direct and indirect costs) of RA was estimated to be $8.74 billion in US (in 1994 dollars) [24]. Indirect costs, primarily attributed to loss

of income, were estimated to be $4,300 to $5,700 per patient per year; however these costs were highly skewed and exceeded $31,000 per year in 10% of patients, presumably those with the most severe disease. Although 12% of patients were hospitalized per year, one-half of direct expenditures for RA in one US survey related to costs of hospitalization. Of note, almost half (43%) of medical admissions for RA were concerned with managing adverse effects of drug therapy [25].

Biologic agents are expensive, but annual costs must be weighed against the personal and social expense of joint arthroplasty, hospitalizations, disability, and diminished quality of life that accompanies poorly controlled RA. There have been several cost analysis studies with TNF-α blockers. The main limitations of these studies relate to the data available, since the costs of RA accrue over the course of many years, whereas the inhibitors have been brought to the clinic relatively recently.

The total costs associated with the administration of etanercept alone and infliximab + methotrexate in combination used in the treatment of RA were compared. Overall treatment with infliximab was more expensive than etanercept alone due to the additional costs associated with administration procedures and the use of methotrexate, when the efficacy of etanercept is assumed to be equivalent to the efficacy of infliximab [26].

In a study that compared etanercept, leflunomide, methotrexate and sulphasalazine and no therapy, incremental cost effectiveness ratios were calculated from weighted average of proportions achieving ACR70, 50 and 20. It was concluded that the most efficacious option, etanercept, incurs much higher incremental costs per ACR20 or ACR70 weighted response than other options analyzed [27].

54 week results from a DBPCRCT were projected into lifetime economic and clinical outcomes in a study by Wong et al. Direct and indirect costs, quality of life, and disability estimates were based on trial results, ARAMIS data base outcomes and published data. Infliximab plus methotrexate was found to be cost-effective with its clinical benefit providing good value for the drug cost, especially when including productivity losses. The marginal lifetime cost effectiveness ratio was $30,500 per discounted quality-adjusted life-year (QALY) gained, considering only direct medical costs. When applying a societal perspective and including indirect or productivity costs, the marginal cost-effectiveness ratios for infliximab was $9,100 per discounted QALY gained. Because most well-accepted medical therapies have cost-effectiveness ratios below $50,000 to $100,000 per QALY gained, results below this range are considered to be cost-effective [28].

In another cost-effectiveness analysis done by Kobelt et al, the cost per QALY of infliximab was estimated on the basis of a clinical trial comparing infliximab plus methotrexate with methotrexate alone in patients in ATTRACT trial. The effect of infliximab was estimated using disease progression models based on data from Sweden and England. It was concluded that although 1 to 2 years of infliximab treat-

ment reduced direct and indirect resource consumption in both Sweden and England, these do not offset the drug cost. However, the cost-effectiveness ratios remain within the usual range for treatments to be recommended for use [29].

In a British study to test the cost-effectiveness of etanercept based on the etanercept monotherapy trial [30] 6-monthly trend in Health Assessment Questionnaire (HAQ) disability score was simulated for 10,000 patients' lifetimes. HAQ scores were converted to QALYs. Primary analysis included drug costs, monitoring and hospitalizations. It was concluded that etanercept is cost-effective when compared with non-biologic agents and recommended for use in patients who failed at least two DMARDs.

The ultimate value of TNF-α blocking therapy will be determined by long-term data on safety, efficacy, and radiologic regression information. Additional long-term observational data on the incidence of joint arthroplasty and disability will help to place the issue of societal costs in a better prospective [31].

Toxicity

Based on the data from clinical trials and practical experience etanercept, infliximab, and adalimumab have generally been well tolerated [8, 12, 17–20]. TNF-α plays a key role not only in the pathogenesis of autoimmune diseases, but also in normal immune homeostasis. Therefore, some concern exists regarding the occurrence of infections and malignancy in patients treated with these agents. Post-marketing experience and pharmacovigilance programs are required to determine the overall safety profile of TNF-α blockers.

Infusion reactions/Injection site reactions

Infliximab has been associated with infusion reactions, the most common of which are headache (20%) and nausea (15%). These are usually transient, rarely severe, can typically be controlled by slowing the rate of infusion or by treatment with acetaminophen or antihistamines [8, 18]. The infusion reactions tend not to increase over time and seldom require discontinuation of therapy [8]. With etanercept and adalimumab, cutaneous reactions at injection sites represent the most frequent side effect, although they rarely cause discontinuation of therapy [19, 20, 23]. Injection site reactions, which occur in approximately 49% of patients treated with etanercept and 24% of those treated with adalimumab, typically consist of erythematous or urticarial lesions [19, 23]. Although they can arise at sites of previous injections, these reactions seem to be limited to the skin and are not associated with other features of immediate hypersensitivity. Reactions typically occur close to initiation of treatment and abate over time, even with continued dosing.

Antigenicity

The development of antibodies to a therapeutic agent could diminish its half life and consequently decrease its efficacy. They might also lead to adverse effects including immune-complex formation or hypersensitivity reactions. RA trials of infliximab with or without concomitant methotrexate treatment revealed that immunogenicity was decreased by concomitant MTX, due perhaps in part to the increase in the half life of infliximab associated with MTX use [7]. The frequency of antibody to infliximab and adalimumab formation may be inversely related to the drug dosage. Population pharmacokinetic analyses revealed that there was a trend toward higher clearance of adalimumab in the presence of anti-adalimumab antibodies. Routine testing for antibodies to TNF-α inhibitors is not currently recommended.

Infection

Given that TNF is a key mediator of inflammation, a major concern surrounding the use of TNF inhibitors is the potential to increase the risk for infection. Of note, while inhibition of TNF in animals does not appear to increase their risks for infection with most pathogens, it does interfere with the ability to mount an inflammatory response against intracellular organisms. In experimental models, TNF blockade impairs the resistance to infection with mycobacterium [32, 33], *Pneumoocystis carinii* [34], fungi [35], *Listeria monocytogenes* [36], and *Legionella* [37]. Comorbidities, such as diabetes mellitus, heart disease, disability, and concurrent immunosuppressive medication all contribute to the risk of infection. It is already well-documented that infections occur more frequently and are important contributors to the accelerated morbidity of RA patients when compared to normal population. How much of this susceptibility relates to disease itself and how much is caused instead by effects of immunomodulatory drugs (e.g., steroid, cytotoxic drugs) is difficult to define [38, 39].

In RA trials with anti-TNF therapies, the number of reports for infections tended to be somewhat higher among patients receiving TNF-α inhibitors. In all studies the most frequent infection was upper respiratory infection. Other frequent infections included sinusitis, urinary tract infection, bronchitis, and pharyngitis. In some studies, a slightly greater propensity to develop upper respiratory infections has been seen in patients receiving TNF-α inhibitors, particularly at higher doses. However, the incidence of severe infections has typically been comparable to that seen in the placebo groups, and significant sequelae occurring as a consequence of infection also appears similar to that in placebo groups. Incidence of serious infection rates were 0.04 in both etanercept and placebo groups; 0.03 in both infliximab and placebo groups; 0.04 and 0.02 in adalimumab and placebo groups respectively [8, 19, 20, 40]. Although these results are reassuring, clinicians need to monitor the patients

closely for signs and symptoms of infections. Anti-TNF-α therapy can mask the initial infections. When using TNF inhibitors, holding treatment should be a consideration when there is a potential for serious infection.

Opportunistic infections, particularly disseminated mycobacterium tuberculosis (TB), are a major area of concern with the use of TNF-α inhibitors [8]. To date, a greater number of cases have been seen among patients receiving infliximab than the other TNF-α inhibitors, but this may in part relate to issues such as particular patient population exposed. Approximately three-quarters of TB cases associated with infliximab were diagnosed within the first three infusions of infliximab, implying reactivation of latent infection [41]. Of note, more patients treated with all the currently available TNF-α inhibitors have disseminated TB and unusual other presentations compared with the overall population, highlighting the need for clinical suspicion and close follow up. Current US guidelines recommend purified protein derivative (PPD) skin testing prior to infliximab and adalimumab therapy. If the PPD test is positive without evidence of active infection, then treatment for latent TB should commence before or with infliximab therapy.

Malignancy

Anti-TNF drugs can theoretically affect the host defense against malignancy. To date, the occurrence of solid tumors seen in clinical trials and long-term follow up of patients from clinical trials of the various TNF-α inhibitors in RA patients does not appear to exceed the rate that would be expected in this population [8]. As is the case for infections, the incidence of certain malignancies is higher than expected in rheumatoid patients with severe disease and receiving other immunosuppressant drugs. Lymphoma rates in RA patients who take TNF-α inhibitors are elevated, but it is not known if this exceeds the risk that would be expected from RA alone [40]. Longer term follow up of larger numbers of patients will provide clinicians a better view about safety of these agents in this regard.

Autoimmune disorders

Approximately 10% of the patients treated with any TNF-α inhibitor develop antibodies to double-stranded DNA (ds-DNA). However, few (0.2–0.4%) treated patients develop symptoms consistent with drug-induced lupus [8, 19, 42]. The mechanism and the significance of the development of antibodies are uncertain. Of note, most patients did not develop life-threatening lupus involvement (e.g., nephritis, CNS lupus) and rarely developed the panolopy of other autoantibodies characteristic of idiopathic systemic lupus erythematosus (SLE) (e.g., anti-Sm/RNP, anti-Ro/La, anti-Scl70). A few patients have been reported to develop anti-cardiolipin antibodies, mostly asymptomatic. Most of the patients who developed lupus-like symptoms improved on discontinuation of anti-TNF-α therapy. While the rare

occurrence of autoimmune disorders has not dissuaded most clinicians from using TNF-α inhibitors in RA, some remain cautious about using them in patients with a history of SLE.

Demyelinating syndromes

Several cases of multiple sclerosis (MS)/demyelinating disease have been reported with anti-TNF-α therapy in patients with RA, PsA and Crohn's disease [43]. In addition, two studies of TNF-α inhibitors in MS patients showed worsening of MS related symptoms and exacerbations in the treated group [44, 45]. Although there is supporting evidence that the incidence of MS may be increased in patients with RA, the association between anti-TNF-α therapy and MS remains unclear. The risk of developing a demyelinating disease is very small; however anti-TNF therapy should be withheld in patients with demyelinating diseases or showing neurologic signs and symptoms during anti-TNF-α therapy.

Congestive heart failure (CHF)

There is data suggesting that TNF-α may play a role in the pathogenesis of CHF. In a pilot trial designed to evaluate the effect of infliximab on clinical status in patients with stable class III or IV congestive heart failure (CHF), patients were randomized to receive either placebo or infliximab (5 mg/kg or 10 mg/kg) at weeks 0, 2, and 6 weeks. No clinical benefit was observed; in fact higher incidences of mortality and hospitalization for worsening of CHF were seen in patients treated with infliximab, especially those treated with a higher dose. Trials of etanercept for CHF similarly failed to show clinical benefit. Therefore, pending further studies, patients with CHF should probably not be treated with TNF-α inhibitors.

Pancytopenia

Rare reports of pancytopenia including aplastic anemia have been reported in patients treated with etanercept. The causal relationship to therapy remains unclear, but caution should be exercised in patients who have a previous history of hematologic abnormalities.

Place in rheumatologic armamentarium

In long-term open label follow up studies of patients from the clinical trials, responses appear to have been sustained over the course of several years [46]. Based on the promising results of a clinical trial of 69 patients, etanercept has been approved for the therapy of juvenile rheumatoid arthritis (JRA) and further safety

was demonstrated [47, 48]. With the growing evidence that suggests TNF-α's role in psoriatic arthritis (PsA), and ankylosing spondylitis (AS), TNF-α blockers have been studied in these conditions. The safety and efficacy of anti-TNF-α therapy were demonstrated in DBPCRCTs in PsA and AS patients [49–51].

Monitoring

No specific laboratory monitoring is currently required by regulatory agencies during therapy with TNF inhibitors, even though continued vigilance is recommended for the use of these agents. Everyone who is under consideration for such treatment should be carefully evaluated for the presence of infection, and prophylactic antituberculous treatment should be started if latent tuberculosis is discovered. TNF-α blockers should not be started, or should be discontinued, when serious infections occur. Clinicians may obtain intermittent assessment of the complete blood count (CBC), because of the rare occurrence of myelosuppression and concern about the risk of infections. Assiduous monitoring of patients for any sign and symptom of infection, demyelinating disease and malignancy is requisite during treatment with TNF-α inhibitors.

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Anakinra in rheumatoid arthritis

Barry Bresnihan

Department of Rheumatology, St Vincent's University Hospital, Elm Park, Dublin 4, Ireland

Introduction

Interleukin-1 (IL-1) plays a central role in the pathophysiology of rheumatoid arthritis (RA) [1]. The IL-1 gene family includes IL-1α, IL-1β and IL-1 receptor antagonist (IL-1Ra) [2]. Extracellular IL-1α, which is membrane-associated, and IL-1β, which is the soluble form, are agonist molecules that can influence the functions of most cell types. Activated monocytes and macrophages are the principal source of IL-1α and IL-1β. There are two distinct IL-1 receptors, designated type I (IL-1RI) and type II (IL-1RII) [3, 4]. IL-1 binding to IL-1RI results in signal transduction and cell activation. IL-1RII is a "decoy" receptor that functions by scavenging IL-1α and IL-1β, but does not have a role in cell signaling [5]. Soluble IL-1RII (sIL-1RII) is important in regulating IL-1-mediated functions. Binding of IL-1 to IL-1RI produces many effects that are central to the pathogenesis of RA [3, 4, 6–8]. The pivotal role of IL-1 in the pathophysiology of RA was highlighted by inducing the pathologic features of RA in rabbits following transfer of the human IL-1β gene, resulting in the constitutive expression of IL-1β by synovial cells [9].

IL-1Ra is the third member of the IL-1 gene family [2–4, 10]. It is also produced primarily by activated monocytes and tissue macrophages. The agonistic effects of IL-1 are partially blocked by the interaction between IL-1Ra and IL-1RI. When IL-1Ra binds to IL-1RI, it blocks the binding of IL-1α and IL-1β and inhibits signal transduction. The agonistic effects of IL-1 and the antagonist effects of IL-1Ra are also tightly regulated by sIL-1RII [11]. The role of IL-1Ra in downregulating IL-1-mediated pathophysiological pathways was demonstrated in IL-1Ra knockout mice that developed a form of chronic arthritis closely resembling human RA [12]. IL-1β, TNF-α and IL-6 were overexpressed in the joint tissues, highlighting the importance of IL-1Ra in regulating local proinflammatory and tissue damaging cytokine networks.

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Clinical efficacy

Conventional response criteria

Five randomised, placebo-controlled clinical trials of anakinra in RA have been completed (Tab. 1). Clinical trials of anakinra in other rheumatic diseases have not been reported. Almost 3,000 patients with RA were recruited. In four studies, the primary endpoints were related to clinical efficacy. The primary outcome measures in the fifth were related to safety. Both the European monotherapy and the methotrexate (MTX) combination therapy studies have been published [13, 14]. A treatment effect was not observed in the low dose monotherapy study. Radiographic analyses will be completed in the confirmatory efficacy study.

In the European monotherapy study, the onset of action was early in the three treatment groups, and a clinical effect was seen as early as 2 weeks (Fig. 1). The clinical effect continued to increase throughout the study, and an American College of Rheumatology (ACR) 20% response [15] was observed in 43% of the patients who were randomised to receive 150 mg/day anakinra, compared to 27% of the patients who received placebo ($p=0.014$). Significant improvements were observed in each of the individual components of the ACR response in the patients who received 150 mg/day anakinra [13].

After completing the 24-week placebo-controlled phase of the study, all patients were offered the option of continuing therapy in a double-blind, 24-week extension study [16]. Patients receiving placebo were randomised to one of the three anakinra dosages, and patients receiving anakinra continued to receive the same dosage. Among the patients who received anakinra and continued into the extension phase, the level of improvement was maintained for 48 weeks. The ACR20 response was 51% at week 24 and 48% at week 48. This effect was consistent across all dose groups. The durability of the response to anakinra was further demonstrated in an evaluation of the sustained ACR20 response, which was similar during the first and second 24-week periods (36% and 42%, respectively).

The rapid onset of action was also observed in the MTX combination therapy study [14]. At 24 weeks, 42% of the patients receiving 1 mg/kg/day anakinra achieved an ACR20 response, 24% an ACR50 response, and 10% an ACR70 response. The improvements in the individual components of the ACR response were most clearly seen in the patient-centered outcomes, such as the patient pain score, the Health Assessment Questionnaire (HAQ) [16], and the patient assessment of disease. Thus, in each of these three outcome measures, the improvements in patients who received 2 mg/kg/day anakinra were highly significant, compared to placebo ($p>0.001$). In the physician-centered outcomes, such as the tender and swollen joint counts and the physician assessment of disease, the placebo responses were greater and the separation between the placebo and the optimal therapeutic responses were less. The improvement in the tender joint count in patients receiving

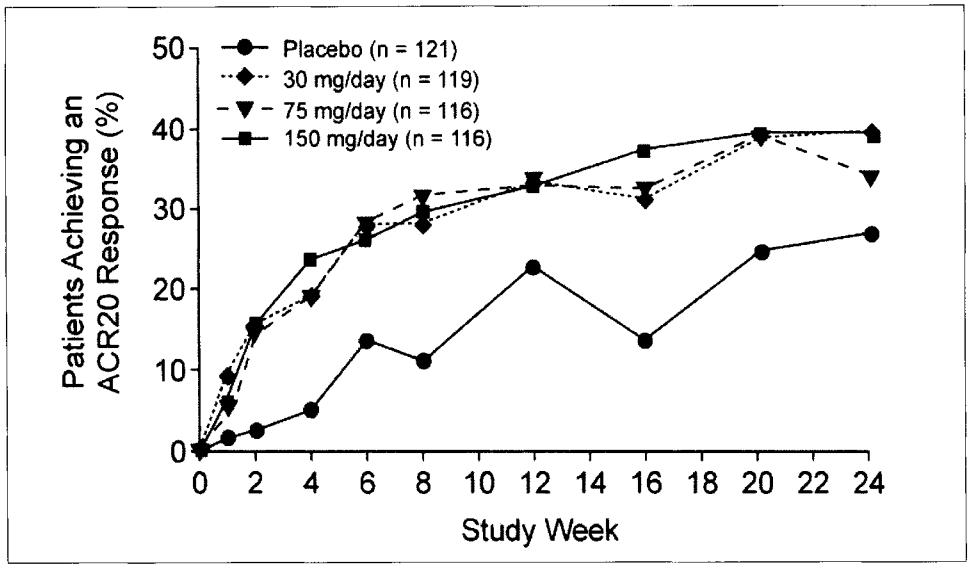

Figure 1
Efficacy of anakinra in the European monotherapy study. The American College of Rheumatology 20% (ACR20) responses over 24 weeks to 30, 75, and 150 mg/day anakinra and placebo are demonstrated.

Table 1 - Randomised, placebo-controlled trials of anakinra

Study	Daily dosages of anakinra	n
European Monotherapy Study [10]	0, 30, 75, 150 mg	472
Low-Dose Monotherapy Study [11]	0, 2.5, 10, 30 mg	141
Methotrexate Combination Therapy Study [12]	0, 0.04, 0.1, 0.4, 1.0, 2.0 mg/kg	419
Confirmatory Efficacy Study [13]	0, 100 mg	501
Safety Study [14]	0, 100 mg	1399
TOTAL		2932

n, number of patients who received at least one dose of the study drug.

anakinra failed to reach statistical significance, compared to placebo, although the improvements in the swollen joint count and physician assessment of disease were significant in the patients who received 2 mg/kg/day anakinra ($p > 0.05$).

The confirmatory efficacy study evaluated 501 patients who demonstrated an inadequate clinical response to therapeutic doses of MTX and were randomised to

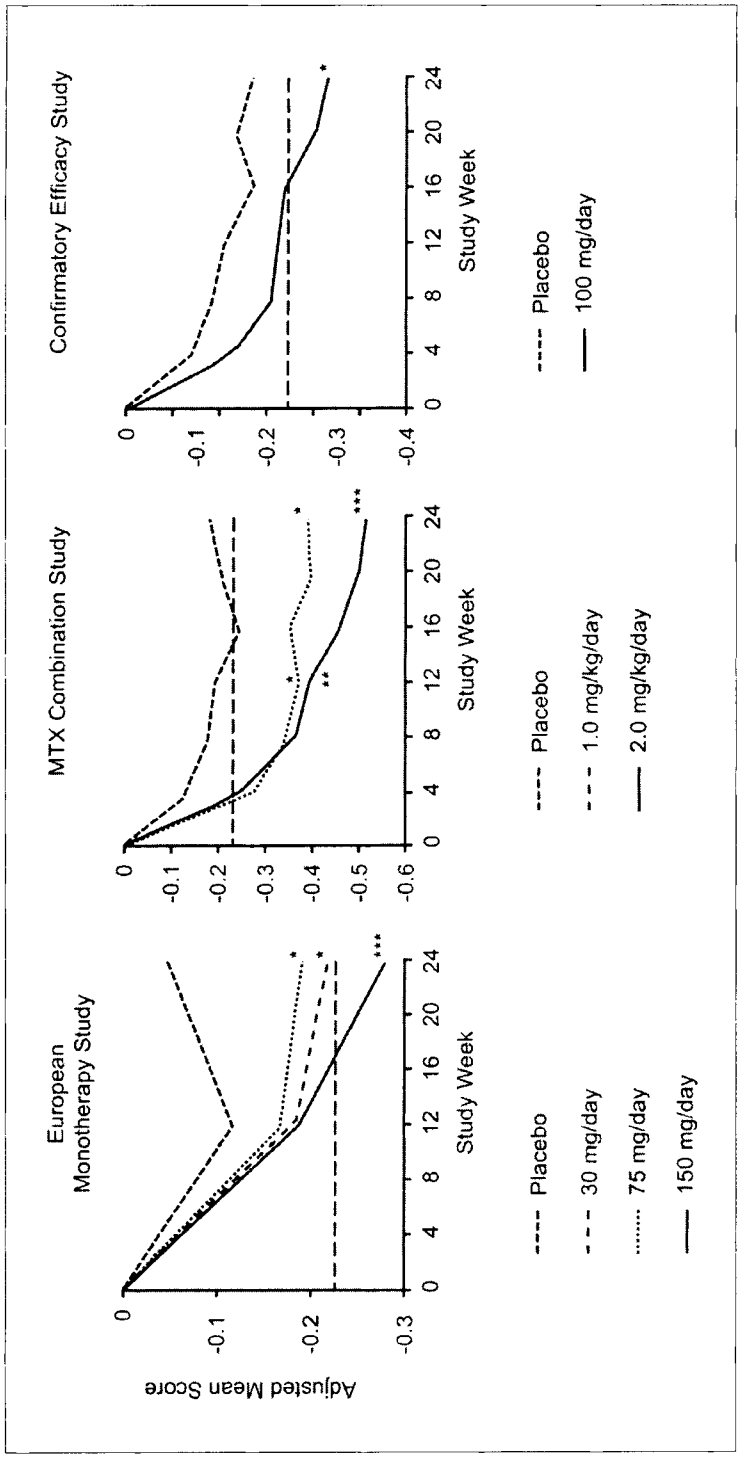

Figure 2
Improvements in the Health Assessment Questionnaire (HAQ) during three randomized clinical trials of anakinra.

receive either placebo or a fixed dose 100 mg/day anakinra in combination with maintenance MTX. At 4 weeks, significantly more patients receiving anakinra had achieved an ACR20 response ($p < 0.01$). At 24 weeks, 38% of the treatment group achieved an ACR20 response, compared to 22% of the placebo group ($p < 0.001$). Consistent with the previous MTX combination therapy study, the improvements in the individual components of the ACR response were most evident in the patient-centered outcomes.

Improvements in function

In each of the three anakinra studies that evaluated therapeutically effective dosages, clinically meaningful improvements in the HAQ scores (a reduction of greater than 0.22) were observed (Fig. 2). In the European monotherapy study, patients receiving each of the anakinra dosages demonstrated reductions in the HAQ scores at 24 weeks that were significantly better than placebo, and the improvement observed in the patients who received 150 mg/day anakinra was clinically meaningful [13]. Similarly, in the MTX combination and the confirmatory efficacy studies, patients receiving anakinra dosages 1 or 2 mg/kg/day, or the fixed dose of 100 mg/day, demonstrated reductions in HAQ scores that were clinically meaningful and significantly better than placebo [14].

A second validated measure of function, the Economic Resource Survey, was employed in the European monotherapy study to evaluate patient and caregiver days of missed work or domestic activity in successive 4-week periods [18]. There were rapid gains in the number of days at work or domestic activity in the treated patients (Fig. 3). The increases in productivity were dose-related with a total of 15.7 days gained over 24 weeks in patients receiving 150 mg/day anakinra, compared to 3.6 in the placebo group ($p = 0.026$). Moreover, the percentage of patients receiving 150 mg/day anakinra with at least one missed day of work or domestic activity decreased by 20%, from 48% at baseline to 28% at 24 weeks. In the placebo group, the decrease was only 6%. At 48 weeks, patients who received anakinra for the entire duration of the study demonstrated greater benefit during the second 24-week treatment period than the first [18]. For example, the patients who received 150 mg/day anakinra for 48 weeks demonstrated a mean gain of 22.4 days productivity during the second 24-week period, compared to 14.0 during the first. Patients who received any dose of anakinra for 48 weeks demonstrated a mean gain of 17.0 days productivity during the second 24 weeks, compared to 12.2 during the first.

Finally, the Nottingham Health Profile is a validated instrument that provides indications of patients' perceived health problems. The scale contains 38 items that can be grouped into six sections: mobility (eight items), pain (eight items), sleep (five items), social isolation (five items), emotional reactions (nine items), and energy (three items). In the patients who received anakinra in the European monotherapy

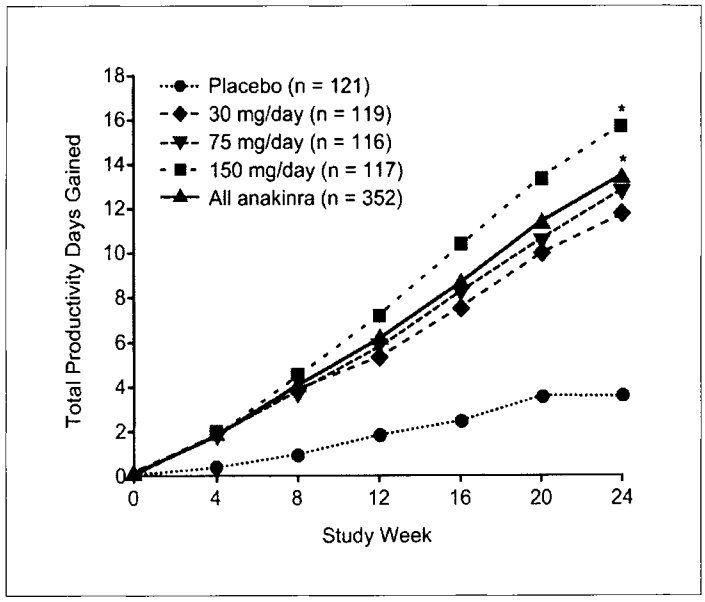

Figure 3
Improvements in total productivity during the European monotherapy study. The number of work days gained over 24 weeks are demonstrated.

study, there were significant improvements in four of the six sections after 24 weeks, compared to the placebo group [19].

A small cohort of patients entering the European monotherapy study underwent synovial biopsy before and after treatment [20]. Anakinra resulted in reduced mononuclear cell infiltration of the synovial membrane, which may represent the *in vivo* inhibition of biologically relevant IL-1β-mediated pathophysiological effects.

Prevention of structural damage

Radiographs of the hands and wrists were obtained at weeks 0, 24 and 48 and scored according to Genant's modification of Sharp's method [21]. The mean change in the total modified Sharp score of patients who completed 48 weeks treatment with anakinra was 2.12, significantly less than 3.81 observed in patients who received placebo for 24 weeks and anakinra for 24 weeks ($p = 0.015$) (Fig. 4). The mean change in the erosion score of patients who received anakinra treatment was 1.15, which was significantly less than 2.03 observed in the patients who received placebo for 24 weeks and anakinra treatment for 24 weeks ($p = 0.006$). A significant reduction in the erosion score was observed with each of the three dosages. The

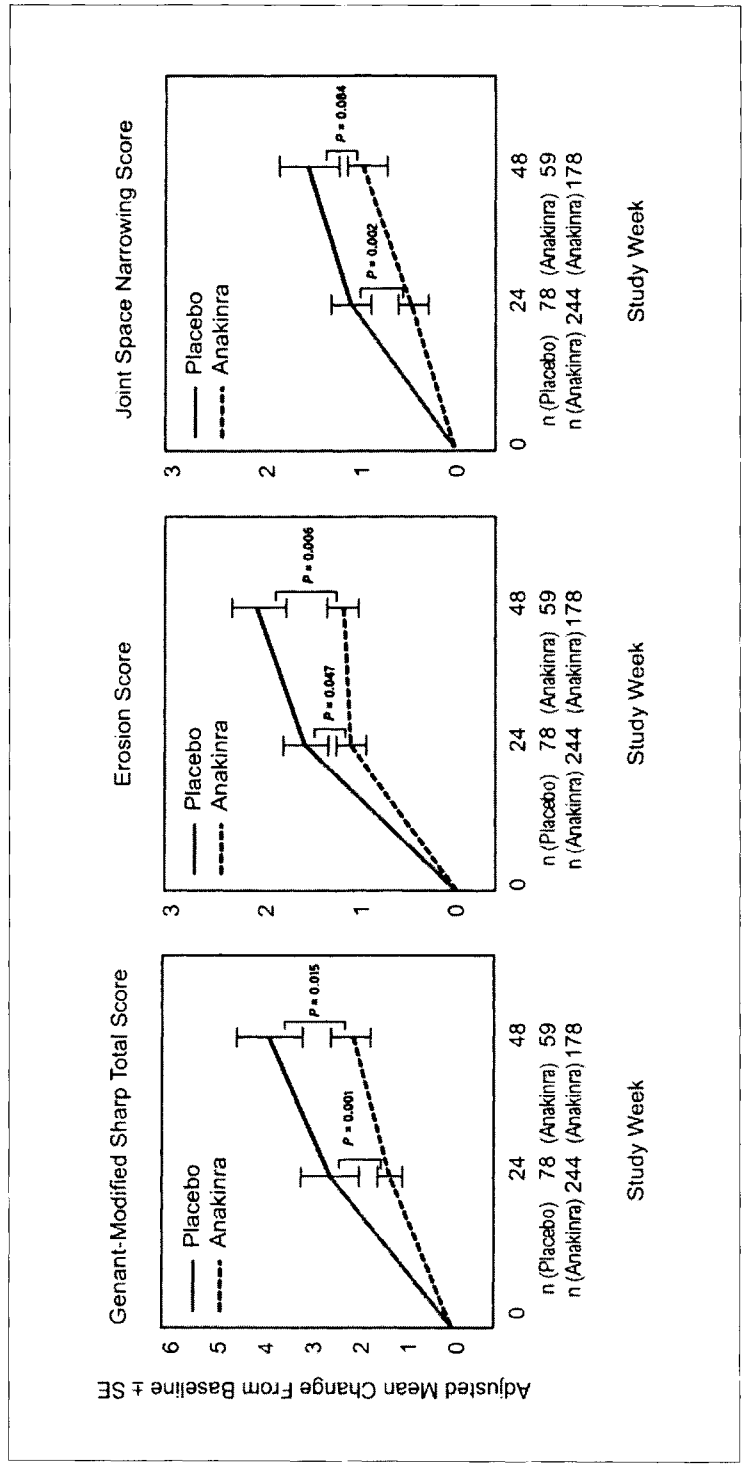

Figure 4
Changes in total modified Sharp scores in the extended European monotherapy study.

mean change in the joint space narrowing score was 1.53 in placebo-treated patients, compared to 0.89 in anakinra-treated patients ($p = 0.084$).

Changes in the rate of joint damage during the two 24-week treatment periods were compared. In the patients who received placebo during the first 24 weeks, a significant reduction of the median change in the total modified Sharp score from 1.95 to 0 after randomisation to anakinra treatment was demonstrated ($p < 0.001$). The patients with a complete set of radiographs who completed 48 weeks anakinra treatment demonstrated a significant reduction in the median total modified Sharp score from 0.51 after the first treatment phase and 0 following the second.

Safety

At the time of licencing in the US, November 2001, the majority of treated patients ($n = 1,812$) had received anakinra for more than 6 months [22]. Of these, 1,379 had received doses of 100 mg/day or more. A total of 570 patients had received anakinra for more than 1 year, and 167 for more than 3 years. An injection site reaction was the most frequent adverse event, observed in 64.4% of patients who received all doses of anakinra, compared to 26.9% of patients who received placebo. Most reactions occurred during the first 4 weeks of treatment, and were mild and transient. An injection site reaction was the most frequent reason for withdrawal, observed in 5.6% of anakinra-treated patients, and in 1.3% of those who received placebo. Serious adverse events were reported in 8.4% of patients receiving < 100 mg/day anakinra, 7.1% of those receiving 100 mg/day, and 12.2% of those receiving > 100 mg/day, compared to 6.5% of patients receiving placebo. Withdrawal due to any adverse event, which included worsening of RA, occurred in 12.9% of patients receiving anakinra, compared to 11.6% of patients receiving placebo.

The crude incidence of malignancy among all patients who received anakinra was 0.9%, compared to 0.8% among the patients who received placebo [22]. The exposure-adjusted malignancy rate per 100 patient-years of study drug was 1.2 for anakinra-treated patients and 2.0 for patients who received placebo. The most frequently observed malignancy was breast cancer (six cases in patients who received anakinra). Histologic details, available in four, confirmed ductal carcinoma in all.

Infection and anakinra

Patients with RA are at increased risk of developing infections compared to people who do not have RA [23]. This may be due to the immunomodulatory effects of RA, or to therapeutic agents with immunosuppressive effects that are used in treatment. Infections were observed infrequently during the placebo-controlled randomised

clinical trials of anakinra [13, 14, 16]. In the 24-week European monotherapy study, an infection resulting in antibiotic therapy occurred in 12% of the placebo group, and in 15–17% of the three anakinra treatment groups [13]. About half of the infections were respiratory infections, which were usually mild. Six patients were hospitalized for infections: one patient each in the placebo and 75 mg/day anakinra groups for respiratory infections, and four patients in the 150 mg/day group (respiratory infection, bursitis, infected bunion, and herpes zoster). An infection resulted in premature withdrawal from the study of one patient who received placebo (< 1%), one who received 30 mg/day anakinra, none who received 75 mg/day, and two (< 2%) who received 150 mg/day.

In the 24-week MTX combination therapy study, upper respiratory tract infections and sinusitis were reported in 22% and 15%, respectively, of placebo-treated patients, and by 14–24% and 5–14%, respectively, of anakinra-treated patients [14]. No serious infections were noted during this study, and infection did not result in premature withdrawal of any patient.

Long-term follow up

The FDA Arthritis Advisory Committee reviewed a database consisting of 2,978 patients who received anakinra treatment [22]. Of these, 2,184 were treated for 6 months or longer, and 2,233 received dosages of 100 mg/day or more. The incidence of serious adverse events in patients who received any dose of anakinra (7.9%) was similar to the incidence in 759 patients who received placebo (6.5%). Moreover, the incidence of any infectious episode was similar in patients who received anakinra (39.3%) and in patients who received placebo (36.2%). Put in context, these data are encouraging. In a large cohort of patients with RA followed over a mean 12.7 years/patient, 64% had at least one infection, and 48% had at least one serious infectious episode [24]. The reported incidence of an infectious episode in patients who received <100 mg/day anakinra was 37.2%, 39.8% in those who received 100 mg/day, and 42.9% in those who received > 100 mg/day. The incidence of serious infectious episodes in patients who received placebo was 0.7%, and 1.1%, 1.2%, and 2.0% in patients who received < 100, 100 and > 100 mg/day anakinra, respectively. The incidence of withdrawal of treatment due to an infectious episode was 0.5%, 1.2%, and 1.0% in patients who received < 100, 100, and > 100 mg/day anakinra, respectively, compared to 0.8% in patients who received placebo. There have been no reports of opportunistic infections, including tuberculosis, in patients who received anakinra treatment.

Pneumonia was the most frequently reported infectious episode, occuring in 14 patients (0.6%) who received anakinra (two in the < 100 mg/day anakinra group and 12 in the 100 mg/day group), and in none of the patients who received placebo. None of the episodes was fatal. In patients with a history of pneumonia ran-

domised to receive anakinra, the incidence of serious infection was 2.7%, compared with 0.0% in those with a history of pneumonia who were randomised to receive placebo. Nine of the 14 patients who developed pneumonia had significant comorbidity, including chronic obstructive pulmonary disease (COPD), coronary artery disease, congestive heart failure (CHF), coronary artery bypass graft, pulmonary fibrosis, and asthma. In patients with a history of asthma who were randomised to receive anakinra treatment, the incidence of serious infections was 4.5%, compared to 0.0% in those with a history of asthma who were randomised to receive placebo. 11 of the 14 patients who developed pneumonia were receiving a concomitant DMARD, and 11 were receiving corticosteroid therapy. Anakinra treatment was discontinued in five of the patients developing pneumonia, and was continued in nine.

The contribution of immunosuppressive agents, such as corticosteroids, to the risk of infection was examined in 1,399 patients recruited to the large Safety Study [25]. This cohort of patients had a range of comorbidities and received a variety of concomitant medications. The percentage of patients receiving corticosteroids at baseline was somewhat higher among the 23 who were randomised to receive anakinra and developed a serious infection (82.6%) than the remaining patients who received anakinra (56.5%) or placebo (60.6%) and did not develop a serious infection. This analysis suggests that concomitant use of corticosteroid therapy could increase the risk of serious infection in patients receiving anakinra. Finally, an analysis of exposure-adjusted event rates of serious infection across all the anakinra studies suggested that the risk of serious infection did not increase over time.

The safety of anakinra treatment in patients with RA and a history of lymphoma, lymphoproliferative disease and other malignancies has not yet been established. Similarly, the safety of anakinra during pregnancy and lactation is unknown.

Indications

Anakinra, like the TNF-α-targeted therapies, is recommended for the treatment of active RA after an adequate trial of at least one DMARD, usually MTX [26]. In the US, anakinra may be prescribed either as monotherapy or in combination with MTX. In Europe, anakinra may be prescribed only in combination with MTX. How should clinicians select which targeted therapy to prescribe first? Three clinical trials that evaluated different cytokine-targeted therapies in combination with effective therapeutic doses of MTX have been published [4, 27, 28]. Each of the studies employed different designs, patient selection criteria, and outcome measures. The anakinra and etanercept studies were conducted over 24 weeks; the infliximab study continued for 54 weeks. There were demographic and clinical differences between the study populations. The numbers of patients in the placebo groups that achieved an ACR20 response ranged between 17% in the infliximab study and 27% in the

etanercept study. The numbers of patients in the relevant treatment groups (anakinra 1 mg/kg/day; infliximab 3 mg/kg every 8 weeks; etanercept 25 mg twice weekly) that achieved ACR20 responses on completion ranged between 42% in both the anakinra and infliximab studies and 71% in the etanercept study, increases of approximately 2 to 2.5-fold more than the respective placebo groups. ACR50 responses were achieved by 24% in the anakinra study, 21% in the infliximab study, and 39% in the etanercept study. ACR70 responses were observed in 10–15% in the three studies. The magnitude of the ACR responses to each of the three different cytokine-targeted therapies, when administered in combination with effective therapeutic doses of MTX, were similar to the responses reported in other studies that evaluated anakinra and etanercept in monotherapeutic regimes [3, 29], and infliximab in combination with low-dose MTX [30].

Among the challenges that face rheumatologists in the new era of targeted therapies is how to choose the optimal regime for each patient. At present, there are no reliable predictors of response to one or other cytokine-targeted approach. There are no data to suggest that patients who are unresponsive to TNF-α-targeted therapy might respond to IL-1 inhibition, or that patients who respond inadequately to anakinra might be candidates for TNF-α-blockade. Targeted biologic therapies modulate specific pathophysiological pathways and are likely to impact differently on individual clinical aspects of disease. This issue represents a potentially very rewarding area for future clinical research.

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Combination therapy

Ernest Choy[1] *and Harold E. Paulus*[2]

[1]Sir Alfred Baring Garrod Clinical Trials Unit, Academic Department of Rheumatology, King's College, London, UK; [2]UCLA School of Medicine, Division of Rheumatology, Los Angeles, California, USA

Introduction

Clinicians who treat rheumatoid arthritis (RA) always have utilized all of the therapeutic modalities available to improve the outcomes of patients with a chronic disabling disease that only rarely exhibits a sustained treatment-induced remission. As new treatments have been developed, they have been tried and added to existing therapies [1]. Narcotic analgesics were supplemented with salicylates, and later with corticosteroids. Early disease modifying anti-rheumatic drugs (DMARDs) such as anti malarial drugs and parenteral gold compounds were added to these "background" therapies, and in controlled clinical trials, their addition proved more beneficial than the addition of placebo. However, because the benefit of these treatments is associated with varying degrees of suppression of (rheumatoid) inflammation, adverse effects are frequently associated with concomitant suppression of normal protective immune and inflammatory mechanisms. Patients recognize that increased benefit is frequently associated with increased side effects. Consequently, they are anxious to try complementary and alternative medications that promise fewer side effects, and are willing to try new drugs and combinations of drugs that may have a more favorable benefit/risk ratio.

History of combination DMARDs (Tab. 1)

The earliest report of DMARD combination therapy for RA was published in 1959 by Michotte and Vanstype [2] who found that 3 months of intravenous gold injections followed by 12–15 months of chloroquine in seropositive patients was associated with clinical benefit and loss of rheumatoid factor positivity in 80% of patients with less than 1 year of RA, but in only 33% of those with a greater disease duration. In 1963 Sievers and Hurri [3] compared 240 patients treated with an antimalarial drug alone (either chloroquine or hydroxychloroquine) with 248 patients

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Table 1 - Some early reports combining disease-modifying anti-rheumatic drugs (DMARDs)

Author	Trial design	DMARD tested
Michotte, Vanstype 1959 [2]	Open, sequential	IV gold followed by chloroquine
Sievers and Hurri 1963 [3]	Open, observational	Antimalarial, or IM gold + corticosteroid
McCarty et al. 1982 [5, 6]	Open, observational	HC + CTX + Aza
Bunch, et al. 1984 [7]	Double-blind, parallel	HC; d-pen; HC + d-pen
McKenna et al. 1985 [9]	Double-blind, parallel	IM gold; d-pen; IM gold + d-pen
Gibson et al. 1987 [8]	Double-blind, parallel	d-pen + chloroquine; d-pen; chloroquine
Scott, et al. 1989 [10]	Double-blind, step-up	IM gold + placebo; IM gold + HC

HC, hydroxychloroquine; CTX, cyclophosphamide; Aza, azathioprine; d-pen, d-penicillamine

treated with the combination of gold injections plus an antimalarial drug during 3–6 month hospitalizations. This observational study was not randomized or double-blind and the treatment groups were not enrolled concurrently, but the ages, sex ratios and disease durations of the two groups were comparable. Among the patients treated with an antimalarial drug alone during hospitalizations in 1957, 1958 or 1959, 36% had remission or major improvement, compared to 43% of those treated with the combination during similar hospitalizations in 1960 or 1961. The results were better for patients with Stage I or II RA; 49% and 66% respectively of antimalarial or combination therapy had remission or major improvement. However, these early findings with a combination of DMARDs were not pursued, following advice [4] in the 1966 edition of *Arthritis and Allied Conditions* that toxicity was increased by concomitant use of gold and chloroquine.

In 1982, McCarty [5] first reported his experience using additive DMARD therapy in 17 patients with erosive seropositive RA who had failed to benefit from hydroxychloroquine. Low doses of azathioprine and cyclophosphamide were added, and after an average of 27 months follow up five patients were in complete remission and in nine patients some radiographic erosions showed recortication. In a follow up report [6] 16 of 31 patients had achieved complete remission, but toxicity was a major problem as four patients developed malignancies (colon, lung, endometrium and erythroleukemia), five early macular degeneration, five herpes zoster, four pneumonia and five cystitis.

The first balanced, double-blind, randomized study of a DMARD combination was published in 1984 by Bunch et al. [7]. 56 patients with 6.1 years average dura-

tion of RA were randomized to receive hydroxychloroquine (2.2 mg/kg daily) plus placebo, or d-penicillamine (7 mg/kg daily) plus placebo or the combination of d-penicillamine and hydroxychloroquine. There was less benefit and less toxicity with the combination than with d-penicillamine or hydroxychloroquine alone in this relatively small study. Additional small double-blind clinical trials failed to show benefit when d-penicillamine was combined with chloroquine [8] or with aurothiomalate injections [9], and one unbalanced, prospective, double-blind trial reported increased benefit and increased toxicity with the combination of aurothiomalate and hydroxychloroquine, compared to aurothiomalate plus placebo [10]. Beginning in the 1990s, large prospective double-blind trials of combination DMARD therapy were published and are summarized below.

Rationale for combination DMARDs

Various rationales have been proposed to justify combination DMARD therapy. Combining drugs with different sites of action may increase efficacy e.g., methotrexate plus cyclosporine. The precise mechanisms of action of biologic DMARDs make this an attractive hypothesis for combining agents with complementary mechanisms of actions, e.g., a tumor necrosis factor alpha (TNF-α) inhibitor with an interleukin-1 (IL-1) inhibitor. If one believes that there is a "therapeutic window of opportunity" for some discrete time after the onset of RA, the proper combination of DMARDs may be able to "cure" the disease, perhaps by preventing the transition to a self-sustaining autoimmune process. Combining drugs with different toxicities may decrease risk, particularly if lower doses can be used to achieve the same benefit with the combination. High doses of one or more toxic interventions may eradicate a "pathogenic clone" of autoimmune cells, e.g., stem cell transplantation after marrow ablation. Perhaps addition of a second drug may prevent the development of resistance to a drug; for example, methotrexate decreases the development of neutralizing antibodies to infliximab [11], thus prolonging and increasing the benefit of chronic infliximab therapy. When there has been an insufficient partial response to one DMARD, a second DMARD may be added to the first, rather than substituted for it, in order to retain the partial benefit of the first DMARD while waiting for the possible benefit of the second drug, thus avoiding an RA flare if the partial benefit of the first drug is lost before that of the (slowly-acting) second drug occurs.

Issues in combination therapy

In 1991, Boers and Ramsden [12] attempted to review publications of combination therapy for RA using explicit formal methodological review criteria [13], and found

numerous problems. Six randomized trials and one prospective cohort study met their inclusion criteria, but only one study was judged to provide strong enough evidence to support its conclusions [10]. Many of the problems apply to all clinical trials, e.g., studies must be randomized and double-blind with a limited number of prespecified outcome measures, ideally including a composite index of arthritis activity; bias due to contamination (when a combination patient stops one of the drugs, thus becoming a single drug subject), co-intervention (as with systemic or intra-articular corticosteroids), or poor compliance with dosage regime; inadequate statistical power; failure to account for withdrawals thus biasing the analyses of the remaining subjects. An additional problem encountered by meta-analysts is incomplete reporting of trial data, often because of editorial space constraints.

Although *ad hoc* DMARD combinations are often used by practitioners, pharmaceutical companies have been reluctant to sponsor such clinical trials because they usually include a competitor's DMARD. NIH has been reluctant to sponsor them because combination DMARD clinical trials are large and expensive, are frequently negative, and rarely lead to major scientific advances. In addition, the number of possible combinations of drugs, doses, dosage regimens and patient cohorts (early RA, later RA, erosive, seropositive or seronegative, etc.) and patient demographics (young, elderly, educational attainment, work status, etc.) are almost infinite, making it difficult to compare the results of one trial with those of another. Consequently, there has not been a systematic approach to the clinical evaluation of combination therapies, and relatively few of the needed studies have been done.

Study design (Fig. 1)

Studies of combination DMARD therapy present additional problems. One would like to know whether the combination of standard doses of A and B is more or less effective and/or toxic than A or B alone. Alternatively, one may hypothesize that combining less than standard doses of A and B is as effective but less toxic than standard doses of A or B alone. Ideally, such a study would randomize patients who had never taken A or B, to standard doses of A, B or both A and B; lower doses of A, B, or both A and B, or placebo A and B. The study would allow one to evaluate whether the efficacy and/or toxicity of A and B are additive, synergistic or antagonistic. However, the anticipated relatively small differences between the effects of the various active treatment groups imply that demonstrating statistically significant differences would require larger sample sizes than practicably feasible, and no such studies have been done with DMARDs. A few studies used a balanced (*parallel*) design [7, 14, 15], comparing standard doses of each drug with standard doses of the combination in *de novo* patients who had no previous use of either drug, but no significant differences were found. One study [16] compared standard doses of azathioprine or methotrexate with lower doses of each in combination. Another study

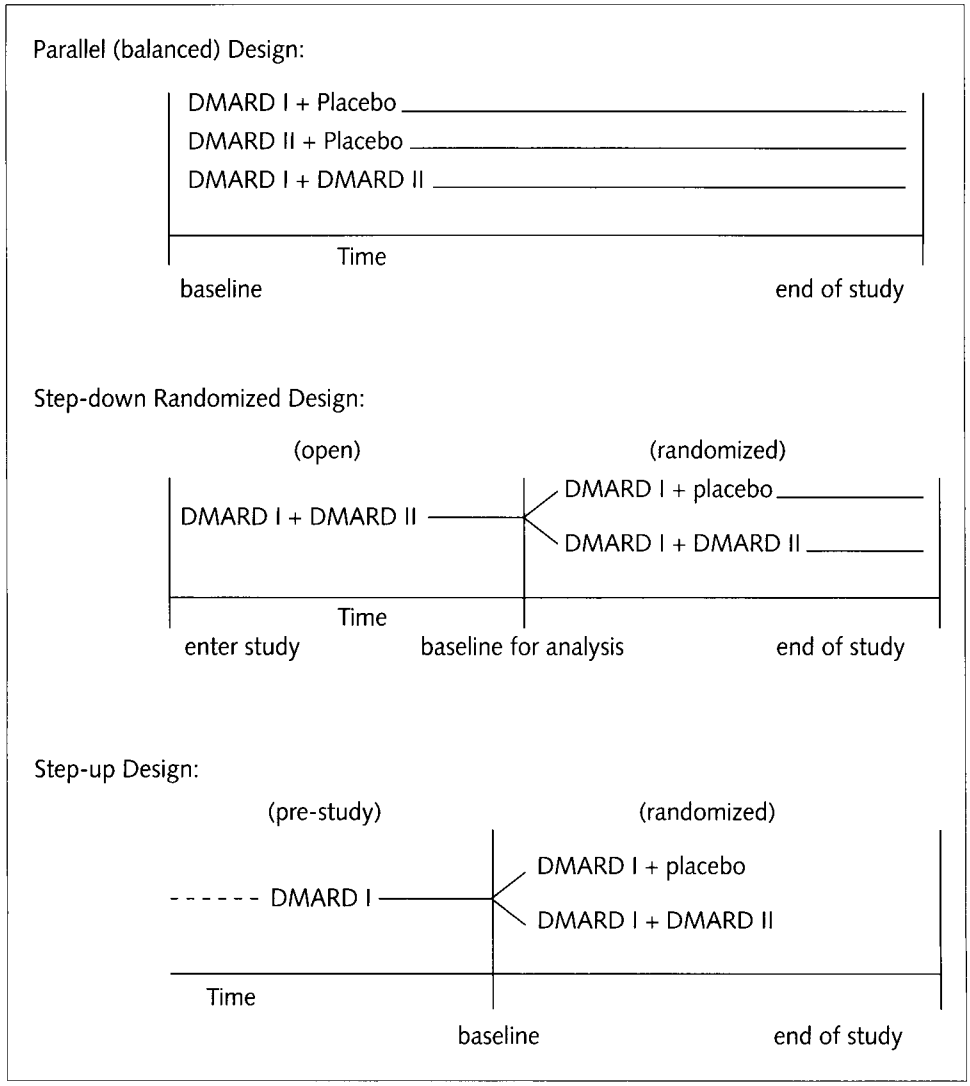

Figure 1
Study designs for randomized clinical trials of DMARD combinations

[17] used a step-down design. It treated all patients with the combination of hydroxychloroquine and methotrexate for 24 weeks; patients who completed this un-blinded phase were then randomized to hydroxychloroquine or placebo, adding methotrexate (or not) if their RA flared. Step-down designs often involve the withdrawal of corticosteroids from a combination that includes a corticosteroid [18].

Many recent studies have used an unbalanced (step-up) design in which patients who have tolerated, but had an incomplete response to drug A have been randomized to add either drug B or placebo to continuing treatment with the first DMARD. These trials can be interpreted as a comparison of drug B with placebo (with drug A part of continued background therapy) or as a comparison of drug B with the combination of A and B. Step-up designs resemble usual clinical practice where an inadequate response to a DMARD often prompts the addition of another DMARD. In this situation, it is relatively easy to show that the added DMARD is more beneficial than added placebo, and this design has been used in patients who have continued active disease despite a reasonable course of methotrexate therapy, e.g., adding cyclosporine A or a biological therapy.

Early *versus* established RA

Randomized control trials (RCTs) of DMARD combinations can be categorized by the patient population studied into two groups: early or established RA. The rationales for using combination therapy in these two patient populations are different. Initially, combination DMARD therapy has focused on patients with established or "refractory" RA but interest in treating patients with early RA by combination DMARDs to improve long-term disease outcome has increased recently. Although it is tempting to pool results from studies in early and established RA together, there are potential pitfalls. Response to DMARD treatment in clinical trials is influenced by disease duration as demonstrated by Anderson et al. who analyzed individual data of 1,435 patients from 14 randomized clinical trials. They found that, in both placebo and active treatment groups, response rates were strongly affected by disease duration [19]. Response rate was 53% in patients with 1 year of disease or less, 43-44% for 1–5 years' disease duration, 38% for 5–10 years, and 35% for > 10 years. It is therefore best to consider trials in early and established RA separately initially and then determine whether the results are concordant.

Early RA

Over the last two decades, rheumatologists have rejected the traditional treatment pyramid for RA and opted for early introduction of DMARD and a sawtooth approach [20]. This strategy is supported by studies showing that conventional monotherapy based on the pyramid approach did not improve the prognosis of RA [21]. Moreover, longitudinal cohort studies showed that erosive disease in most patients started within the first 2 years of disease [22]. This epidemiological evidence is further supported by many RCTs in early RA showing that early introduction of DMARD monotherapy in early RA is superior to delayed DMARD therapy

Table 2 - Randomized controlled trials of disease modifying anti-rheumatic drug combinations in early rheumatoid arthritis

Author	Trial design	Duration of RA (months)	DMARD tested
Van Den Borne et al. [72]	step-up	Early RA	HC *versus* HC+CyA
Kirwan et al. [24]	Parallel	Early RA	DMARD *versus* DMARD + P
Haagsma et al. [73]	Parallel	Early RA	SSZ *versus* MTX *versus* MTX+SSZ
Dougados et al. [31]	Parallel	<12	MTX *versus* SSZ *versus* MTX+SSZ+HC
Gough et al. [74]	step-down	Early RA	SSZ *versus* SSZ+MP
Boers et al. [18]	step-down	Early RA	SSZ *versus* SSZ+MTX+P
Ferraccioli et al. [34]	Step-up	Early RA	MTX (+CyA) *versus* CyA (+MTX) *versus* SSZ
Marchesoni et al. [33]	Step-up	Early RA	MTX+CyA *versus* MTX
van Everdingen et al. [25]	Step-up	Early RA	P+SSZ *versus* SSZ
Möttenen et al. [29]	Parallel	Early RA	MTX + SSZ + HC + P *versus* single DMARD + P
Proudman [32]	Step-up	Early RA	SSZ *versus* MTX+CyA+ intra-articular steroids

P, prednisolone; MTX, methotrexate; SSZ, sulfasalazine; CyA, cyclosporin A; HC, hydroxychloroquine; MP, methylprednisolone

in improving symptoms and signs although only a few studies demonstrated significant reduction in radiological joint damage [23]. Since most DMARDs have a slow onset of action and the efficacy of monotherapy is limited but joint damage occurs early in the disease, the rationale for more aggressive therapy in patients with early RA to improve long-term outcome is appealing. Hence, many researchers have examined combinations of DMARD in early RA often by step-down or parallel approaches.

Eleven RCTs studies have examined the effect of combination DMARDs in early RA (Tab. 2). Three were step-up, four were parallel and four step-down in design. Two were open-label trials but change in radiological joint damage was included as a secondary endpoint. Two studies combined corticosteroids with DMARD monotherapy. Kirwan added prednisolone (7.5 mg daily) to any DMARD [24] while van Everdingen et al. randomized early RA patients to 10 mg daily of prednisolone or placebo and then sulfasalazine was added to those patients with inadequate response [25]. In both studies, initial clinical improvement was better in the prednisolone group than in the placebo group for a limited period but differences

were insignificant at the end of the trials. Interestingly, radiological damage was statistically significantly less in the prednisolone group in both studies. However, follow up data of patients who took part in the study by Kirwan, demonstrated that radiological damage increased once prednisolone was withdrawn [26]. In the study by van Everdingen et al., patients in the prednisone group had more osteoporotic fractures [25].

Three large RCTs, COBRA, FIN-RACo and an European study, examined methotrexate in combination with sulfasalazine and corticosteroids with/without hydroxychloroquine in early RA. In the COBRA study [18], 155 early RA patients were randomized to either sulfasalazine monotherapy or to the following combination therapy: sulfasalazine (SSZ), methotrexate (stopped at 40 weeks) and step-down prednisolone (initially 60 mg/day, tapered in 6 weekly steps to 7.5 mg/day and stopped after 28 weeks). At week 28, 72% and 49% respectively of patients in the combination and monotherapy groups met ACR20 response criteria. Radiographic damage was statistically significantly less in the combination therapy group. The difference remained significant at the end of the trial, at which point both groups were on sulfasalazine monotherapy. The data suggested that combined therapy immediately suppressed damage progression, whereas sulfasalazine did so less effectively. A follow up study for the subsequent 4–5 years of SSZ monotherapy, showed that radiological progression rates in the initial combination therapy group (5.6 points per year) remained less than in the initial sulfasalazine monotherapy group (8.6 points per year) [27]. Using generalized estimating equations to adjust for differences in treatment and disease activity during follow up, the between-group difference in the rate of radiological progression was 3.7 points per year. These data suggested that intensive combination treatment in early RA might lead to sustained suppression of the rate of radiological progression independent of subsequent antirheumatic therapy. Moreover, economic analysis of direct and indirect costs by means of cost diaries and interviews, showed that over the total trial period the mean total costs per patient were $10,300 and $12,800, for combination and sulfasalazine therapy, respectively. Interestingly, for the first 28 weeks, total costs were $5,900 and $7,900 ($p = 0.04$) although the differences in total costs were no longer significant at the end of the trial [28]. This suggests that combination therapy in early RA is cost effective, and supports the hypothesis of an early "window of opportunity."

In the FIN-RACo study, 199 patients with early RA were randomly assigned to a combination of methotrexate, sulfasalazine, hydroxychloroquine, and prednisolone or a single DMARD with or without prednisolone for 2 years [29]. Disease remission, the primary outcome measure, was achieved in 25% of patients treated with combination therapy, and 11% of those treated by monotherapy after 1 year. Radiological joint damage score was statistically significantly less in the combination group. The difference remained significant after 5 years. The data from this study supported the findings in the COBRA study and argued strongly for more

aggressive intervention with combination DMARDs at the onset of RA. Interestingly, analysis by logistic regression showed that remission in the monotherapy group but not the combination therapy group was dependent on the duration of symptomatic period prior to DMARD therapy [30]. The duration of symptomatic period was less than 4 months in 33% of patients in the combination therapy group and 27% in the single DMARD group. Logistic regression included disease duration, the presence of shared epitope allele, sex, age, seropositivity, number of positive ACR 1987 classification criteria present at baseline, and the duration of symptomatic period as covariates. The duration of symptomatic period was the only significant predictor for remission for patients in the single DMARD group, but not in the combination therapy arm. This implies that in patients who had symptoms for more than 4 months before the diagnosis of RA was made, combination DMARD should be used instead of monotherapy.

Similarly, a multicenter European study assessed the effect of sulfasalazine, methotrexate or both in 205 patients with RA for 1 year or less in a RCT [31]. The mean change in the DAS disease activity score was -1.15, -0.87, -1.26 in the sulfasalazine, methotrexate, and combination groups respectively ($p = 0.019$). Radiological progression was slightly less in the combination therapy group: total damage score was +4.6, +4.5, +3.5, in the sulfasalazine, methotrexate, and combination groups, respectively. However, adverse events were more frequent in the combination group.

Two randomized controlled trials compared methotrexate plus cyclosporin with/without intra-articular steroids with monotherapy. In an open-label study, Proudman et al. compared methotrexate plus cyclosporin and intra-articular steroids with sulfasalazine in 82 early RA patients [32]. After 48 weeks, ACR20 improvement was seen in 58% in the combination therapy group compared with 45% in the sulfasalazine group. The difference was not statistically significant. Radiographic damage score increased by a median of 1 in the combination group and 1.25 in the sulfasalazine group. However, the number of patients withdrawn due to lack of efficacy in the combination group (one of 40) was statistically significantly less than the sulfasalazine group (10 of 42). Another RCT evaluated the effect of adding cyclosporin (3 mg/kg/day) to methotrexate (10–15 mg/week) partial responders versus methotrexate monotherapy in 42 patients with early RA [33]. After 12 months, ACR20 response was seen in 75% of patients in the cyclosporin plus methotrexate group compared with 59% in the methotrexate monotherapy group. The difference was not statistically significant. However, radiological damage, measured by van der Heijde modified Sharp method, was significantly less in the combination therapy group (0.87) compared with methotrexate alone (7.3; $p < 0.02$). The sample sizes of both studies are relatively small and lack statistical power to detect small but clinically relevant improvement.

Recently, Ferraccioli et al. assessed step-up combination therapy with methotrexate, and cyclosporin A in early RA [34]. In an open-label trial, 126 consecutive

patients with early active RA were enrolled. Patients were started on monotherapy with either methotrexate, cyclosporin A or sulfasalazine. After 6 months, patients on methotrexate were given cyclosporin and *vice versa*, while patients taking sulfasalazine remained on sulfasalazine. After 18 months, almost 90% of patients on combination therapy achieved ACR50 response criteria compared with 24% in the control group.

Overall, there is some evidence to suggest DMARD combinations, primarily, methotrexate plus sulfasalazine plus steroids with/without antimalarials may be superior to monotherapy (principally sulfasalazine) in early RA. However, it is important to assess whether combination therapy is superior to methotrexate monotherapy which is the commonest used DMARD in early RA. Furthermore, identifying prognostic factors that can be used to predict patients with good prognosis may avoid the need to treat all patients with aggressive therapy.

Established RA

In established RA, the step-down approach is uncommon except when corticosteroids are used as bridge therapy when a DMARD is started. The step-up approach is increasing popular although the deficiency of the step-up design has been discussed previously. Initial trials of DMARD combinations were negative but recent trials produced more positive results (Tab. 3).

Initially, antimalarials were often combined with other DMARDs including intramuscular gold [35, 10], D-penicillamine [8] and sulfasalazine [36]. All had negative results except one study [10] that compared intramuscular gold plus hydroxychloroquine with intramuscular gold alone. Although in this study combination therapy was more effective, adverse events were also more frequent. Subsequently, two large balanced RCTs compared methotrexate plus auranofin with either methotrexate or auranofin [14], and compared methotrexate plus azathioprine with either methotrexate or azathioprine [37]. These studies recruited 335 and 209 patients, respectively. In both studies, monotherapy and combination therapy had similar efficacy.

In the 1990s, a number of RCTs evaluated methotrexate plus antimalarials with/without sulfasalazine [38–40]. All these studies suggested that combination therapy was more effective than monotherapy. Among these, the largest was by O'Dell et al. who enrolled 102 patients in a 2-year parallel-designed study comparing methotrexate alone (7.5 increasing to 17.5 mg per week), with sulfasalazine (500 mg twice daily) plus hydroxychloroquine (200 mg twice daily), and with all three DMARDs [40]. 77%, 33% and 14% of patients treated with all three drugs, methotrexate alone and sulfasalazine plus hydroxychloroquine, respectively, achieved at least 50% improvement. Subsequently, the same research group conducted a 2-year, double-blind, placebo-controlled trial comparing the triple combi-

Table 3 - Randomized controlled trials of DMARD combinations in established rheumatoid arthritis

Author	Trial design	DMARD tested
Tugwell et al. [42]	Step-up	MTX *versus* MTX+CyA
Porter et al. [35]	Step-up	Gold *versus* Gold+HC
Yasuda et al. [75]	Step-up	Gold *versus* Gold+Buc
Bendix et al. [76]	Step-up	Gold *versus* Gold+CyA
O'Dell et al. [40]	Parallel	MTX *versus* SSZ+HC *versus* MTX+SSZ+HC
Willkens et al. [37]	Parallel	Aza *versus* MTX *versus* MTX+Aza
Faarvang et al. [36]	Parallel	HC *versus* SSZ *versus* HC+SSZ
Ferraz et al. [39]	Parallel	MTX *versus* MTX+Chloro
Scott et al. [10]	Parallel	Gold *versus* Gold+HC
Gibson et al. [8]	Parallel	D-Pen *versus* HC *versus* D-Pen+HC
Calguneri et al. [77]	Parallel	MTX/SSZ/HC *versus* MTX+SSZ/MTX+HC *versus* MTX+SSZ+HC
Williams et al. [14]	Parallel	Auranofin *versus* MTX *versus* MTX+auranofin
Trnavsky et al. [38]	Parallel	HC *versus* MTX+HC
Ciconelli et al. [78]	Step-down	SSZ *versus* SSZ+MP
Corkill et al. [79]	Step-down	Gold *versus* Gold+MP
Van Gestel et al. [80]	Step-down	Gold *versus* Gold+MP
Van der Veen et al. [81]	Step-down	MTX *versus* MTX+P *versus* MTX+MP
Wong et al. [82]	Step-down	Gold *versus* Gold+MP

P, prednisolone; MTX, methotrexate; SSZ, sulfasalazine; CyA, cyclosporin A; HC, hydroxychloroquine; MP, methylprednisolone; Aza, azathioprine; Gold, intramuscular gold; Buc, bucillamine; Pen, penicillamine; Chloro, chloroquine

nation with methotrexate plus hydroxychloroquine (200 mg twice per day) and with methotrexate plus sulfasalazine 1 gm (twice per day) [41]. 171 patients were randomized. The ACR20 response criteria was achieved in 78%, 60% and 49% of patients receiving the triple combination, methotrexate plus hydroxychloroquine and methotrexate plus sulfasalazine, respectively, at the end of the study. The differences between triple therapy and both dual therapies were statistically significant.

Tugwell et al. added cyclosporin A or placebo to RA patients with a partial response to methotrexate in a 6-month RCT [42]. 48% of patients in the cyclosporin group and 16% in the placebo group met ACR20 response criteria. Effect on radiological joint damage was not assessed. Subsequently, a number of observational studies have reported that methotrexate plus cyclosporin has a favor-

able long-term tolerability profile [43–45]. This was one of the first studies to use the step-up design which has become popular in established RA. Subsequently leflunomide, intramuscular gold and tacrolimus have all been tested using step-up designs in methotrexate partial responders.

Leflunomide is an immunosuppressant and a new DMARD. It suppresses cellular proliferation by inhibiting the pyridinoline pathway. In a 6 month RCT, leflunomide or placebo was added to 263 RA patients with partial response to methotrexate [46], 46.2% of the patients in the leflunomide group met ACR20 response criteria compared with only 19.5% in the placebo groups. The difference was statistically significant. An open-label study found no significant pharmacokinetic interaction between leflunomide and methotrexate [47]. Since both leflunomide and methotrexate are associated with hepatotoxicity and pancytopenia, careful monitoring of liver enzymes and full blood count are essential. The European Agency for the Evaluation of Medicinal Products has adopted a very cautious approach and advised against using this combination in patients with RA.

Lehman et al. examined the effect of adding intramuscular gold, one of the oldest DMARDs in partial responders to MTX. 70 patients were randomized to receive either intramuscular gold or placebo for 48 weeks. ACR20 response rate was 56% in the combination group and 28% in the placebo group ($p = 0.017$) [48].

Tacrolimus is an immunosuppressant used commonly in preventing transplant rejection. Its mode of action is similar to cyclosporin. In a RCT of patients with established RA, it suppressed disease activity when given as monotherapy [49]. In a 6 month open-label trial in 80 RA patients, 3 mg/day of tacrolimus was added to background methotrexate [50]. At the end of the trial 52.5% of patients achieved ACR20 responses. There was a slight increase in mean creatinine level from 0.74 ± 0.16 mg/dl to 0.81 ± 0.22 mg/dl.

In summary, there is strong evidence to suggest combination therapy is more effective than monotherapy in established RA, although many studies are step-up in design and may overestimate the magnitude of benefit. Moreover, the sample sizes of these studies are too small to assess whether combination therapy is associated with a higher risk of side effects. Currently, all the effective DMARD combinations include methotrexate. Since there are many patients who are intolerant of methotrexate, there is a need to assess the effect of DMARD combinations that do not include methotrexate.

Combinations including biologics (Tab. 4)

Methotrexate plus biologics

Cytokine antagonists, etanercept [51], infliximab [11, 52], adalimumab [53] and anakinra [54] have been added to methotrexate, in patients who had a partial

Table 4 - Randomized controlled trials of DMARD combinations including biologics

Author	Trial design	Combination tested
Maini et al. [11]	Step-up	MTX *versus* infliximab *versus* MTX + infliximab
Weinblatt et al. [51]	Step-up	MTX *versus* MTX + etanercept
Lipsky et al. [52]	Step-up	MTX *versus* MTX + infliximab
Cohen et al. [54]	Step-up	MTX *versus* MTX + anakinra
Weinblatt et al. [53]	Step-up	MTX *versus* MTX + adalimumab
Smolen et al. [55]	Parallel	MTX *versus* infliximab + MTX
Klareskog et al. [56]	Parallel	MTX *versus* etanercept *versus* MTX + etanercept
Combe et al. [57]	Step-up	SSZ *versus* etanercept *versus* SSZ + etanercept
Edwards et al. [61]	Step-up	MTX *versus* rituximab *versus* rituximab + CP versus MTX + rituximab
Weinblatt et al. [65]	Step-up	Etanercept *versus* etanercept + CTLA4-Ig

MTX, methotrexate; SSZ, sulfasalazine; CP, cyclophosphamide

response to methotrexate, in "step-up" randomized placebo controlled trials. In all these studies, combination treatment was superior to methotrexate monotherapy in improving symptoms and signs. In parallel design trials, combinations of infliximab with methotrexate were more effective than methotrexate alone in early aggressive RA [55], and the combination of etanercept and methotrexate was significantly better than either alone in established RA [56].

Etanercept (10 mg or 25 mg twice per week) or placebo was administered to 89 patients who were partial responders to methotrexate for 24 weeks. At the end of trial, 71% and 27% of the patients receiving etanercept and placebo respectively met the ACR20 criteria [51]. Cohen et al. assessed the effect of anakinra in a similar randomized controlled trial. 419 patients were randomized to placebo or 0.04, 0.1, 0.4, 1, or 2 mg/kg daily of anakinra for 6 months [54]. ACR20 responses in the 1 mg/kg (46%) and 2 mg/kg (38%) groups were statistically significantly greater than the placebo group (19%). In the ATTRACT trial, 428 patients who had active RA despite methotrexate therapy were randomized to either placebo or infliximab (3 or 10 mg/kg every 4 or 8 weeks) for 54 weeks [52]. ACR20 response was achieved by 51.8% of infliximab treated patients compared with 17% in the placebo group. In addition, radiological progression in the infliximab treated group was significantly less than in the placebo treated patients. Interestingly, methotrexate reduced the number of patients who developed human anti-mouse antibody responses to infliximab, which may be a biological basis for the benefit of this combination [11].

In the ASPIRE trial [55], 1,049 patients with less than 3 years duration (mean 0.6 year) of active RA and no more than three prior doses of methotrexate were randomized to methotrexate (7.5 mg increasing to 20 mg per week by week 8), methotrexate plus infliximab 3 mg/kg or 6 mg/kg (week 0, 2, 6 and then every 8 weeks) for 54 weeks. Both combination regimens were more effective than methotrexate alone. With the highest dose, 50% of patients achieved ACR 50 and 37% ACR 70 responses, compared with 32% and 21%, respectively, for methotrexate alone. Radiographic progression was arrested in both infliximab groups.

The TEMPO trial [56] used a parallel (balanced) design to evaluate etanercept, methotrexate or the combination in 682 randomly assigned patients with active RA in a 12 month study. All had had an inadequate response to prior DMARDs; 42% had prior exposure to methotrexate, but had washed out of methotrexate for at least 24 weeks and other DMARDs for at least 1 month. Mean RA duration was about 6.5 years. The clinical response to the combination was significantly better than to either methotrexate (17 mg/week) or etanercept (25 mg biw); 69% had ACR 50 and 43% ACR 70 responses respectively, compared to 43% and 19% for methotrexate, and 48% and 24% for etanercept alone. "Remissions" (DAS<1.6) occurred in 14%, 18% and 37% with methotrexate, etanercept and the combination, respectively. The patients had substantial radiographic damage at baseline, but there was no progression with the combination therapy which was significantly better than with etanercept or methotrexate alone.

Other DMARD plus biologics

While adding biologics to methotrexate partial responders has been shown to be effective, many RA patients are partial responders to other DMARDs but the value of adding biologics in these patients has not been studied in detail. Recently, a double-blind RCT compared adding etanercept or placebo to sulfasalazine partial responders [57]. 254 RA patients who were partially responsive to sulfasalazine (2–3 g/day) were randomized to receive etanercept 25 mg twice weekly (with sulfasalazine discontinued at trial baseline), etanercept 25 mg twice weekly plus continued sulfasalazine, or continued with sulfasalazine alone. At the end of the 24 week trial, ACR20 response criteria were met by 74%, 74% and 28% of etanercept, etanercept plus sulfasalazine and sulfasalazine patients, respectively. Adverse events (headache, nausea, asthenia, pruritus) were less common in the etanercept only group compared with combination therapy group. Hence in sulfasalazine non-responders, substituting etanercept for sulfasalazine rather than adding etanercept to sulfasalazine is the most appropriate treatment. This study also highlighted a major deficiency of the step-up designs. It does not address whether the addition of a DMARD or biologic is superior to switching to a new DMARD or biologic in patients who have suboptimal response to DMARD monotherapy.

In an open-label study, patients intolerant of methotrexate were treated with low dose cyclosporin (2 mg/kg/day) and prednisone (5 mg/day). Infliximab (3 mg/kg) was added for 12 months [58]. 80% of patients achieved the ACR 20 response criteria while 39% satisfied the ACR50 response criteria. However, one patient developed pulmonary tuberculosis.

Two studies examined the addition of infliximab to leflunomide partial responders. In an open label study of 20 patients [59], although disease activity improved, adverse events were common. 11 of the 20 patients had to be withdrawn due to side effects. The commonest adverse event was eczematous skin rash. Serious adverse reactions occurred in five patients including one case of Stevens-Johnson syndrome. In a similar study of 40 patients [60], a high drop-out rate due to adverse events was also noted. Interestingly, the proportion of patients with a positive anti-nuclear antibody titer rose from 11% at week 0 to 38% at week 24, 61% at week 36, and 100% at week 60. The mean anti-dsDNA titer was over 100 by week 24 although clinical lupus was not seen. Therefore, leflunomide plus infliximab has been associated with a high incidence of side effects and probably should be avoided in RA.

An aggressive combination pulse therapy combines methotrexate or cyclophosphamide with high dose steroids and rituximab, a depleting anti-B cell monoclonal antibody. A recent randomized, double-blind, placebo controlled trial examined these combinations in 161 RA patients who were methotrexate partial responders [61]. Patients were randomized to one of four treatment groups: Group A: continuing methotrexate alone; Group B: rituximab alone (2 × 1 g intravenous infusions); Group C: rituximab (2 × 1 g intravenous infusions) plus pulse cyclophosphamide and Group D: rituximab plus methotrexate. All groups also received an intensive 17-day course of corticosteroids (total dose of 960 mg). An interim analysis of the study with the first 122 patients showed that 33%, 58%, 84% and 80% of patients in Group A, B, C and D, respectively, met the ACR20 response criteria. Benefit seems to be sustained for 6 months.

Combinations of biologics

Both TNF-α and IL-1 are potent proinflammatory cytokines. Their roles in the pathogenesis of synovitis and joint damage are confirmed in animal models, *in vitro* experiments, clinical studies and cytokine blockade therapies. Although both TNF-α and IL-1 antagonists are efficacious in RA, complete disease remission is uncommon. Therefore, there is a rationale to combine TNF-α and IL-1 antagonists. A small open label study assessed combining anakinra with etanercept [62]. 58 RA patients, on 25 mg twice per week of etanercept, added anakinra 1 mg/kg/day. Among 21 subjects who discontinued early, 11 had adverse events and seven reported lack of benefit. 28 subjects experienced 48 infectious episodes. Four had serious episodes (two cellulitis and two community acquired pneumonias) where

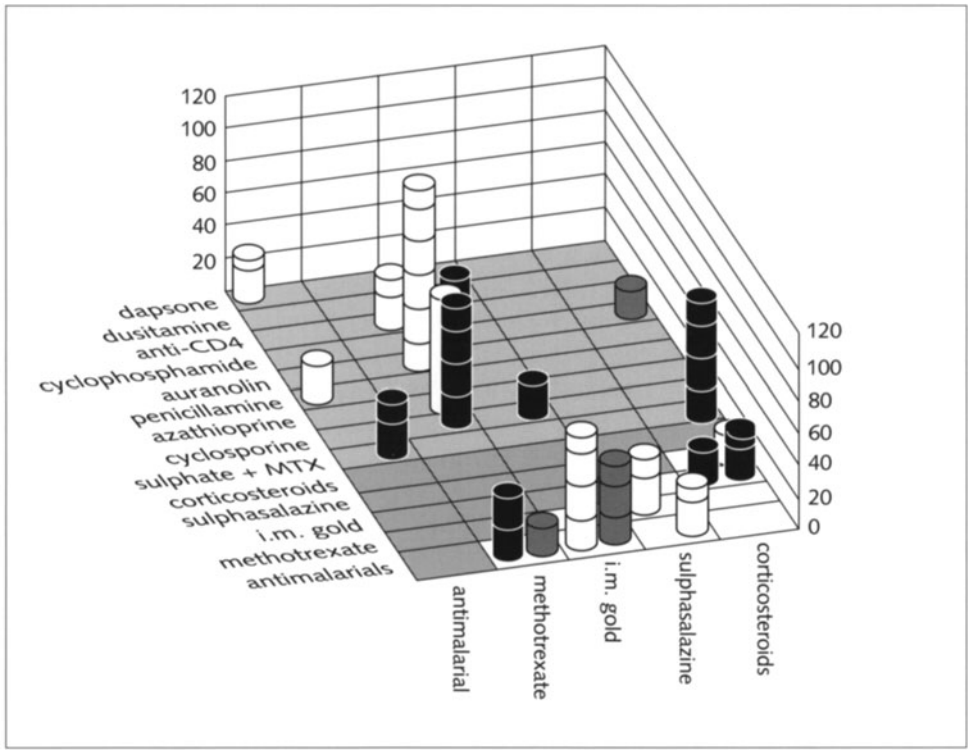

Figure 2
Epidemiological approach to DMARD combinations (Reproduced with permission from article by Verhoeven et al. British J Rheumatology (1998) 37: 612–619).
Three-dimensional summary of the efficacy of combination DMARD therapy. From the perspective of the five most frequently used drugs, the matrix describes the combinations of all single drugs reviewed and one two-drug combination. Each bar describes a specific combination trial; its length reflects the sample size of the combined treatment group, its shade reflects the evidence that combined treatment was significantly better than the single drug(s). Dark gray, strong evidence that the combination is better or much better; light gray, evidence that the combination is better (any evidence or trend); white, no evidence that the combination is better. Because the five primary drugs are repeated on the long axis, a dark area in the matrix indicates overlap.

hospitalization was required. Although there was no control group, some subjects exhibited improvements over baseline. However, more recently a large randomized placebo control trial of etanercept plus anakinra involving 44 centers failed to show any significant therapeutic advantage in combination therapy over monotherapy [83].

In animal models of RA, anti-TNF and anti-CD4 monoclonal antibodies were synergistic in the suppression of synovitis [63]. CTLA4-Ig (BMS-188667) inhibits T cell activation by binding to the co-stimulatory molecules, CD-80 and CD-86 receptors on antigen presenting cells thereby preventing their interaction with the CD28 molecule on T cells. It is an effective treatment for active RA at a dose of 10 mg/kg monthly by intravenous infusion [64]. Recently, a randomized, double-blind, placebo controlled study of CTLA4-Ig (2 mg/kg) was given in combination with etanercept. In a 6-month study to assess the safety and efficacy of CTLA4-Ig in subjects with active RA while on etanercept therapy [65], a total of 121 subjects were randomized with 85 and 36 subjects in the 2 mg/kg, and control groups, respectively. After 6 monthly infusions, the percentages of subjects achieving a modified ACR20 response criterion (which excluded ESR/CRP) were 48% and 28% in the CTLA4-Ig plus etanercept and placebo plus etanercept groups respectively. These differences were statistically significant. The addition of CTLA4-Ig in RA patients with an inadequate response to etanercept appeared well tolerated. Since CTLA4-Ig and etanercept inhibit different immune pathways, a higher dose of 10 mg/kg of CTLA4-Ig may be more effective than 2 mg/kg.

Strategy in choosing DMARD combinations

Epidemiological comprehensive model

In a systematic review of combination therapy in 1998 [66], Verhoeven et al. suggested that each combination of DMARD needs careful study of its therapeutic potential in several trials, which can subsequently be pooled by meta-analysis (Fig. 2). This is the most thorough and comprehensive approach. However, as more DMARDs become available, the number of possible combinations increases exponentially. Besides, the difference between combinations and monotherapy is often small; therefore a large sample size is needed to provide the necessary statistical power. The resources necessary to undertake all these studies may be insurmountable.

Animal models

Animal models of RA, such as collagen-induced arthritis, are used extensively in testing potential biological treatments for RA. Typical examples are cytokine inhibitors and anti-lymphocyte monoclonal antibodies [63]. However, their value in assessing DMARD for RA has not been examined thoroughly. Some studies have examined combining DMARD with biologics. Using collagen-induced arthritis as a model of RA, Williams et al. examined the combined therapeutic effect of

cyclosporin with anti-TNF monoclonal antibody [67]. Cyclosporin at a dose of 20 mg/kg was effective in reducing the severity of established collagen induced arthritis. The effects of cyclosporin and anti-TNF monoclonal antibody were found to be additive. However, in this study of collagen-induced arthritis the dose of cyclosporin used was five-fold higher than that used in the routine treatment of RA. Nonetheless, cyclosporin (2 mg/kg/day) has been reported to be effective in combination with anti-TNF monoclonal antibody in an open-labeled study in patients who cannot tolerate methotrexate [58].

Combining cyclosporin and methotrexate has also been shown to be effective in collagen-induced arthritis [68]. Rats with collagen-induced arthritis were given either 0.3 mg/kg/week or 0.8 mg/kg/week of methotrexate, 4 mg/kg/day or 10 mg/kg/day cyclosporin, or combinations of both. Incidence and severity of arthritis were less in the animals treated by combination therapy, compared with controls.

Overall, the usefulness of animals model in assessing DMARD and combinations of DMARD needs to be assessed more thoroughly. If an ideal animal model could be identified, it could provide a useful screening tool prior to testing DMARD combinations in patients.

Extrapolation based on mechanism of action and drug interactions

Münster et al attempted to provide a rational model for using DMARD combinations in RA by tabulating their known mechanisms of action, kinetics, and toxicity [69]. From these matrices potential positive or negative interfaces among combinations were hypothesized. They found that the model has only limited value since knowledge in many areas of kinetics and mechanisms of action is lacking. Evidence for the mode of action of the drugs is fragmentary and weakened by methodological problems not least of which is extrapolating from *in vitro* effects to an *in vivo* mode of action.

Review of efficacy and toxicity of combination therapy

A recent meta-analysis of combination DMARD therapy from 28 selected studies in RA showed that overall combination therapy is superior to monotherapy (RR = 0.43, 95%CI 0.28–0.65, p = 0.00005), however, there is also a slightly higher risk of toxicity (RR = 1.21, 95%CI 1.0, 1.46, p = 0.05) [70]. Nevertheless the benefit/risk ratio, in general, is in favor of combination therapy. Sensitivity analyses suggested that efficacy and safety depended on the specific DMARD combination. Combinations that have positive benefit/risk ratios include methotrexate plus sulfasalazine or antimalarials, or all three, or sulfasalazine plus antimalarials. Methotrexate plus biologics is superior to methotrexate monotherapy. Most of these

trials were carried out in established RA and the evidence supporting combination therapy is strongest in established disease. Although accumulating evidence suggests a strong trend in favor of combination therapy, one cannot be absolutely confident that combining DMARDs is superior to methotrexate monotherapy, the current gold standard for treating patients with early RA.

Recommendations for clinical practice

The ultimate goals in managing RA are to prevent or control joint damage, prevent loss of function, and decrease pain. Randomized controlled trials have shown that some combinations of DMARDs are more effective than DMARD monotherapy in improving symptoms and signs in RA. Some combinations are also more potent than DMARD monotherapy in reducing the progression of joint damage. These combinations of DMARDs are now being used in routine clinical practice. In the UK and Canada, methotrexate plus antimalarials and triple therapy are the most commonly used combinations. In most cases, DMARD combinations are used in a step-up manner in patients whose diseases are sub-optimally controlled by monotherapy which is in line with clinical practice as recommended in the American College of Rheumatology 2002 guidelines on the treatment of RA [71]. There are good theoretical reasons to believe that the step-down approach should be the logical approach to take in earlier RA especially recent evidence suggested combination therapy in early RA could reduce structurally damage for at least 5 years after the initial treatment. However, combination DMARDs need to be shown to be superior to methotrexate, the current gold standard, in early RA. With the emergence of cytokine antagonists, there are further options in combining them with DMARDs. The value of these combinations needs to be assessed in early RA.

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Hematopoietic stem cell transplantation for the treatment of severe autoimmune diseases

Alan Tyndall[1] *and Jacob M. van Laar*[2]

[1]Department of Rheumatology, University of Basle, Felix-Platter Spital, 4012 Basel, Switzerland; [2]Department of Rheumatology, Leiden University Medical Center, The Netherlands

Introduction

In humans, autoimmune diseases (ADs) represent a heterogeneous group of disorders with diverse genetic and environmental etiological factors.

Despite the mostly successful use of glucocorticosteroids and immunosuppressive agents there are still patients who either do not respond or require more toxic drugs to achieve or maintain clinical remission, and this subgroup poses a serious treatment dilemma.

The dose of cytotoxic drugs such as cyclophosphamide (CY) has been limited by bone marrow toxicity, but improving hematopoietic stem cell transplantation (HSCT) techniques allows one to exceed these limits, then "rescue" the patient with autologous HSCs. The observation in some patients receiving HSCT for conventional indications that a coexisting AD also improved suggested that HSCT could be a viable option for selected AD patients. The concept was also supported by animal model data. This led to an international collaboration, and currently worldwide around 600 patients have received an HSCT as treatment of an AD.

This Chapter summarises the theoretical and practical background of such a treatment strategy, the results of the Phase I and II studies so far and how this experience has been exploited in designing the running Phase III randomised comparative trials and parallel science programs such as immune reconstitution.

The first consensus statement concerning the use of HSCT in the treatment of severe AD was published in April 1995 [1] and the first case report in October 1996 [2]. Results of the autologous HSCT programs have suggested that in favourable outcomes, a resetting of a dysregulated autoaggressive immune system may be occurring, rather than total ablation of autoimmune inducing cells.

Antirheumatic Therapy: Actions and Outcomes, edited by Richard O. Day, Daniel E. Furst, Piet L.C.M. van Riel and Barry Bresnihan

Autoimmune disease mechanism

Despite the heterogeneous clinical expression of AD in humans, it seems clear that most AD share several or all of the following features. They are polyclonal, with rarely a defined inciting single antigenic epitope and by the time of clinical disease expression, there has been extensive epitope spreading and effector cell recruitment [3]. The innate immune system and tissue environment probably play a vital role in determining whether an antigen will evoke an immune reaction or anergy/tolerance [4] and a genetic component is present, but not sufficient. This genetic factor is mostly encoded within the major histocompatibility complex (MHC), but multiple other genes on different chromosomes play a role. In insulin dependent diabetes mellitus for example, at least 19 such regions are proposed [5], disease initiation and perpetuation probably involves activation and disturbance of specific subsets of regulatory T cells. The recent re-evaluation of a subset of $CD4^+$ $CD25^+$ T cells which have suppressor activity [6] supports this concept of dysregulation, rather than "all or nothing" events, as in malignant clonal disease. The complexity of these diseases is further illustrated by the observation that clinical expression is often dependent on a mixture of inflammatory and scarring processes.

Presentation of self antigens probably occurs continuously, but under normal circumstances produces either apoptosis, anergy or tolerance if presented without co-stimulatory molecules such as by non-professional APC lacking B7 (CD80) [7]. Which T cells are needed for this autoaggressive reaction? It is known that autoreactive T cells escape thymic deletion and remain in the periphery, but with low affinity. Under the circumstances described above, these lymphocytes may be activated and induce an autoimmune process. This reaction is probably in turn controlled by regulatory T cell subsets, especially early in the process.

Breakdown of this regulatory network over time allows clinical expression and the development of chronic AD. Reversal of this vicious circle and reinstitution of the normal regulatory network but not eradication of the last single autoreactive cell is one of the postulated mechanisms behind the concept of HSCT for treatment of AD.

Coincidental AD in patients receiving HSCT for another indication

A number of case reports have been published over the past 20 years describing patients receiving HSCT for a conventional indication (e.g., aplastic anaemia or malignancy) in which a coincidental AD improved or even fully remitted. In the majority of initial reports allogeneic HSCT was employed (Tab. 1). Many of these patients remained free of both the haematological and the autoimmune disease. In some patients relapse occurred and in one such patient full engraftment with donor-type lymphocytes [8] were observed. More recent reports have included response

Table 1 - Coincidental autoimmune disease and allogeneic HSC transplantation

Disease for which transplant performed	AD present	Outcome of AD	Patient outcome	Reference
SAA	RA	Remission	Died	Baldwin et al. 1977 [28]
SAA	RA	Remission	Died	Baldwin et al. 1977 [28]
SAA	RA	Remission	Died	Baldwin et al. 1977 [28]
SAA	RA	Remission	Well	Baldwin et al. 1977 [28]
SAA	RA	Partial	Well	Jacobs et al. 1986 [29]
SAA	RA	Remission	Well	Lowenthal et al. 1993 [30]
SAA	RA	Remission	Well	Lowenthal et al. 1993 [30]
AML	Psoriasis	Remission	Well	Eedy et al. 1990 [31]
CML	Psoriasis	Remission	Well	Yin and Jowitt, 1992 [32]
AML	Ulcerative colitis	Remission	Well	Liu Yin and Jowitt, 1992 [32]
ALL	Autoimmune hepatitis	Remission	Well	Vento et al. 1996 [33]
CML	Multiple sclerosis	Remission	Well	McAllister et al. 1997 [34]
Various	Hyperthyroidism	No recurrence		Nelson et al. 1997 [35]
	IDDM	No recurrence		
	SLE, RA	No recurrence		
	Crohn's disease	No recurrence		
	Vasculitis	No recurrence		
	Dermatitis herpetiformis	No recurrence		
MALT lymphoma	Sjoegrens syndrome	No effect	Alive	Ferraccioli et al. 2001 [36]

SAA, severe aplastic anemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; RA, rheumatoid arthritis; IDDM, insulin dependent diabetes mellitus; SLE, systemic lupus erythematosus.

following autologous HSCT (Tab. 2) emphasising the fact that genetic predisposition alone is not sufficient for AD expression [3].

There are also case reports of transfer of AD through allogeneic HSCT, including myasthenia gravis, thyroid disease, insulin dependent diabetes mellitus, celiac disease and psoriasis with arthritis [9]. In one patient, production of autoantibodies (anti Clq) were detected in a recipient following HSCT from a donor with known systemic lupus erythematosus (SLE), but clinical disease did not develop [10].

Table 2 - Coincidental autoimmune disease and autologous HSC transplantation

Disease for which transplant performed	AD present	Outcome of AD	Patient outcome	Reference
NHL	Myasthenia gravis	Remission	Well	Salzmann et al.1994 [37]
Ovarian cancer	Thyroiditis	Relapse	Alive	Euler et al. 1996 [38]
NHL	Myasthenia	Relapse	Died	Euler et al. 1996 [38]
NHL	SLE	Relapse	Alive	Euler et al. 1996 [38]
NHL	Atopic dermatitis	Relapse	Alive	Euler et al. 1996 [38]
NHL	RA	Relapse	Alive	Snowden et al. 1997 [39]
CML	SLE	Remission	Alive	Meloni et al. 1997 [40]
NHL	SLE	Remission	Alive	Jondeau et al. 1997 [41]
NHL	RA	Relapse	Alive	Cooley et al. 1997 [42]
NHL	RA	Relapse	Alive	Cooley et al. 1997 [42]
AML	Psoriasis	Relapse	Alive	Cooley et al. 1997 [42]
Plasma cell leukaemia	Psoriasis	Relapse	Alive	Cooley et al. 1997 [42]
NHL	Crohn's disease	Remission	Alive	Kashyap et al. 1998 [43]
Hodgkin's	Crohn's disease	Remission	Alive	Musso et al. 2000 [44]

NHL, non-Hodgkin's lymphoma. Others see Table 1.

In interpreting these case reports, it is important to remember the following facts: there is selection bias and details of AD severity or extent are often lacking, making it difficult to determine the clinical relevance of the outcome.

There is sufficient evidence in these reports to assume some modification of the AD process following HSCT, justifying further clinical trials.

Animal models

Support for the concept of HSCT in the treatment of AD is also found in animal models. Since the original observation by Denman et al. in 1969 that SLE could be transferred from a susceptible to a non-susceptible strain through allogeneic bone marrow transplantation [11], and later by Morton et al. [12], many proofs of concept observations have been published. This has recently been extensively reviewed

[13, 14]. In interpreting these data it is important to distinguish models in which AD is genetically and inevitably programmed, e.g., the MLR/lpr mouse and those in which a genetic component plus a trigger are required, e.g., the buffalo rat and adjuvant arthritis. The latter is more like human AD, as reflected in concordance rates between identical twins i.e., 15% in SLE, 18% in rheumatoid arthritis (RA) and 25% in multiple sclerosis (MS) and 50% in insulin dependent diabetes mellitus (IDDM). In addition, it is important to distinguish between HSCT performed to prevent AD occurring, or HSCT to treat established AD.

As in human AD, the autoimmune process has been active at a cellular level often long before the clinical features become manifest. These data have been summarized by Van Bekkum [15] whose work in adjuvant arthritis, and later experimental allergic encephalomyelitis (EAE) demonstrated that not just allogeneic but also autologous HSCT [16] could prevent and treat AD. In addition, a significant peripheral immunological tolerance was induced, especially in the arthritis model. It is hoped that such immunomodulation will also occur in humans, and that HSCT will induce more than just profound immunosuppression.

Treatment of human autoimmune disease with hematopoietic stem cell transplantation

Currently around 600 patients worldwide have received a BMT as treatment of an AD alone, 468 of whom are registered in the European Group for Blood and Marrow Transplants (EBMT) and the European League Against Rheumatism (EULAR) database (Tab. 3). The majority of patients have had either severe multiple sclerosis (MS) or systemic sclerosis (SSc), also called scleroderma. This reflects the fact that there is no reliably effective alternative treatment option in these disorders. However, as the experience grew, other ADs were transplanted, mostly in the context of combined Phase I and II trials and following the consensus guidelines developed at international meetings [17–18] early in the program.

The quintessence of these guidelines was:

1. HSCT regimes
   A limited number of protocols only should be employed (Tab. 4). This was mostly followed and allowed some comparison of intensity *versus* toxicity/benefit to be drawn (see below under "Outcome").
2. Patient selection
   Patients should have had failed conventional therapy and have a poor prognosis concerning life or vital organ function. There should be enough reversible or maintainable vital organ function to ensure a decent quality of life if the immunological/inflammatory process were arrested or reversed. The patient should have sufficient capacity to withstand the HSCT procedure.

Table 3 - EBMT/EULAR autoimmune disease autologous HSCT database

Disease and disease category		N
Neurological disorders	Multiple sclerosis	135
	Myasthenia gravis	2
	Polyneuropathy	2
	Amyotrophic lateral sclerosis	2
Rheumatological disorders	Systemic sclerosis	72
	Rheumatoid arthritis	72
	Juvenile idiopathic arthritis	51
	Systemic lupus erythematodes	55
	Dermatomyositis	7
	MCTD	4
	Morbus Behçet	3
	Psoriatic arthritis	2
	Ank. Spondylitis	2
	Sjoegren	1
Vasculitides	Wegener's	3
	Cryoglobulinemia	4
	Not classified	2
Hematological immuncytopenias	Immune thrombopenia	12
	Pure red cell aplasia	4
	Autoimmune hemolytic anemia	4
	Thrombotic thrombocytopenic anemia	3
	Evans syndrome	2
Gastrointestinal	Enteropathy	2
	Inflammatory bowel disease	1
Other		3
Total		453

Status at August , 2002

As the program proceeded, certain clinical parameters and treatment related factors emerged as being associated with an unacceptable risk, such as a mean pulmonary artery pressure > 50 mm Hg in SSc, high disability scores in MS and total body irradiation (TBI) without lung shielding in SSc. This experience was then exploited in the design of the Phase III randomised studies.

Following the initial consensus meetings, single case reports and small series of patients transplanted for the treatment of severe AD have been published (Tab. 5). These reports demonstrate a heterogeneity of patient selection, target AD and outcome, and have in part formed the basis for further prospective trials.

Table 4 - Conditioning regimens used with HSCT in autoimmune diseases

Conditioning regimen	Total
Cyclophosphamide	115
Cyclophosphamide ± ATG ± other drugs	110
Cyclophosphamide + radiation ± other drugs or ATG	43
Busulfan ± cyclophosphamide ± ATG ± other drugs	25
BEAM ± ATG	80
Other/missing	66
Total	439

**TBI, Total body irradiation (includes some patients with total lymphoid irradiation); ATG, anti thymocyte globulin; BEAM, BCNU, VP16, ara-C, melphalan*

In the EBMT/EULAR database the most commonly transplanted diseases are MS, SSc, RA, JIA and SLE, the data coming from over 100 transplant centres in more than 20 countries. There were long-lasting responses in all disease categories, but they were achieved at a price, the overall actuarially adjusted transplant-related mortality (TRM) being 7% (5–9) [19]. This was higher than the predicted 3% for autologous HSCT overall and reflects the general overall level of illness and multi-organ involvement of many AD patients compared with, for instance, breast cancer patients undergoing high dose chemotherapy and HSCT. In fact, there is a marked difference between AD groups with a TRM of 11% in SLE and only one patient (1.4%) with RA. There are also different response rates and types. In RA, JIA and SLE more patients responded early but later relapsed than for MS and SSc.

Systemic sclerosis (SSc)

SSc is a multi-organ AD with immunological, vascular and collagen overproduction components.

In the first 45 patients, an improvement of 25% or more in the skin score (measured by the modified Rodnan method) was seen in 70% of the patients, with a TRM of 17% [20]. Several protocols were used, mostly either Cy based (4 g /m^2 Cy mobilisation and Cy 200 mg/kg body weight conditioning or radiation 8 Gy/Cy 120 mg/kg body weight. With further patient recruitment and longer term follow up, the TRM of the EBMT registered patients fell to 8.5% , considered to be related to more careful patient selection. Lung function tended to stabilise and some factors were identified as potentially hazardous for HSCT, e.g., pulmonary hypertension

Table 5 - Case reports and small series of autologous HSC transplantation for autoimmune diseases

Disease (number of patients)	Disease outcome	Patient outcome	Reference	
RA (1)	remission	alive	Durez 1998	[45]
RA (1; syngeneic donor)	remission	alive	McColl 1999	[46]
RA (6)	5 PR	alive	Pavletic 2001	[47]
RA (6)	6 PR	alive	Bingham 2001	[48]
RA (14, 12 transplanted)	8 PR	alive	Verburg 2001	[49]
RA (1)	PR	alive	Kim 2002	[50]
RA (33)	23 PR	alive	Moore 2002	[51]
AOSD (1)	remission	alive	Lanza 2000	[52]
JIA (4)	4/4 remission	alive	Wulffraat 1999	[53]
JIA (1)	N/A	died	Quartier 1999	[54]
JIA (1)	remission	alive	Nakagawa 2001	[55]
SSC (1)	remission	alive	Tyndall 1997	[56]
SSC (1)	remission	alive	Martini 1999	[57]
SSC (19)	13/15 PR	4 died	McSweeney 2002	[21]
SSC (12)	8/11 PR	5 died	Farge 2002	[58]
SLE (1)	remission	alive	Marmont 1997	[59]
SLE (1)	remission	alive	Musso 1998	[60]
SLE (1)	remission	alive	Fouillard 1999	[61]
SLE (1)	remission	alive	Trysberg 2000	[62]
SLE (1)	N/A	died	Shaughnessy 2001	[63]
SLE (1)	relapse	alive	Rosen 2001	[64]
SLE (2)	2/2 remission	alive	Wulffraat 2001	[65]
SLE (1)	remission	alive	Brunner 2002	[66]
SLE (15)	12/15 remision	alive	Traynor 2002	[67]
MCTD (1)	PR	alive	Myllykangas 2000	[68]
PM (1)	remission	alive	Baron 2000	[69]
PM (1)	PR	alive	Bingham 2001	[70]
MS (3)	3/3 PR	alive	Burt 1998	[71]
MS (24)	18/23 PR	1 died	Fassas 2000	[72]
MS (5)	2/3 PR	2 died	Openshaw 2000	[73]
MS (11, 8 transplanted)	7/8 PR	alive	Kozak 2000	[74]
Crohn's disease (2)	2/2 remission	alive	Burt 2003	[75]
Mixed (7)	4/7 remission	1 died	Rosen 2000	[76]
Mixed (19)	17/19 PR	alive	Rabusin 2000	[77]
Mixed (7)	4/7 remission	alive	Musso 2001	[78]

> 50 mmHg mean pulmonary arterial pressure, severe cardiac involvement, severe pulmonary fibrosis and uncontrolled systemic hypertension.

A long-term follow up of this cohort showed no further transplant related deaths and trend to durable remissions (EBMT database).

A multicentre US study of 19 SSc patients utilising a regimen of Cy 120 mg/kg, TBI 8 Gy and equine ATG 90 mg/kg body weight and a CD 34 selected graft product showed a sustained benefit in 12 patients at median follow up of 14.7 months [21]. Four patients died, three from treatment-related causes and one from disease progression. In two cases a fatal regimen-related pulmonary toxicity occurred, which was not seen in the subsequent 11 cases in whom lung shielding was employed.

Twelve patients had a sustained and significant improvement of skin score and functional status to a degree not previously seen with other treatment modalities.

Rheumatoid arthritis (RA)

A retrospective analysis of the first 78 registered patients showed significant improvement, with 67% achieving an ACR-50 response at some time post-transplant [79]. Most of the patients had failed a median of 5 (range 2–9) conventional disease modifying anti-rheumatic drugs (DMARDs) before the transplant. Some degree of relapse was seen in 73% of patients post-transplant, but was in most cases relatively easy to control with drugs which had proven ineffective pre-transplant. At 12 months post-transplant, more than half of the patients had achieved an ACR-50 or more, and of these, just over 50% had not restarted DMARDs. The median follow up was 18 (6–40) months, and at this time the majority of patients received a conditioning regimen of Cy 200 mg/m^2 alone and received peripherally harvested stem cells after either G-CSF or Cy/G-CSF (equal numbers) mobilisation.

Only one TRM was reported, a patient who 5 months post transplant (Busulphan/CY) died of sepsis, with a coincidental non small-cell lung carcinoma being discovered at autopsy. In the opinion of the investigators, this was not considered to be a transplant induced tumor.

A multicentre trial in Australia failed to show any advantage of CD 34 selection of the graft after non myeloablative conditioning with Cy [22].

Juvenile idiopathic arthritis

A total of 51 children with idiopathic juvenile arthritis, mostly the systemic form called Still's disease, have been registered. Most of these cases were treated in two Dutch centres using a bone marrow obtained stem cell source and a conditioning protocol of Cy 200 mg/kg body weight, TBI 4Gy and ATG [23], (N. Wulffraat, personal communication).

In the whole group there were 15 complete remissions and three partial remissions reported. In those attaining remission, the corticosteroid dose could be reduced and some patients experienced puberty and catch up growth. Three patients died from macrophage activation syndrome, thought to be related to intercurrent infection or uncontrolled systemic activity of the disease at the time of transplantation. Protocols were modified accordingly, such that systemic activity is controlled before the transplant with methyl prednisolone intravenously. Since this modification, no further such deaths have occurred.

Systemic lupus erythematosus (SLE)

Of the 55 registrations in the EBMT/EULAR database, most had either renal and/or central nervous system (CNS) involvement, and 21 had failed conventional Cy treatment. A peripheral stem cell source after mobilisation with Cy and G-CSF was used in the majority. 23 patients received a conditioning with Cy and ATG, 11 Cy plus TBI and four other regimens were employed. An unselected graft was used in 29, with CD34 selection in 19. There were five deaths due to treatment and one from progressive disease, resulting in an actuarially adjusted TRM of 10% (2–20).

In those 53 patients with sufficient data for analysis, 66% achieved a "remission", defined as a SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) of ≤ 3 and steroid reduction to < 10 mg/day. Of those achieving remission, 32% subsequently relapsed to some degree and were mostly easily controlled on standard agents which had previously been ineffective.

There were 12 deaths after 1.5 (0–48) months of which seven (12%) were related to the procedure.

Traynor and colleagues [24] reported on nine patients with severe SLE who were mobilised in a transplant protocol. One died as a result of infection following mobilisation and another 3 months later from active CNS lupus, having not proceeded to transplant. The seven remaining were free of signs of active lupus at a median follow up of 25 months post-transplant. The high-dose chemotherapy consisted of cyclophosphamide 200 mg/kg, methylprednisolone 1 gm and equine ATG 90 mg/kg.

The numbers of cases with vasculitis, Behçets disease, relapsing polychondritis and other ADs are too small to draw meaningful conclusions, with further Phase I and II standardised protocol pilot studies proceeding.

Prospective randomised controlled clinical trials

Criteria for moving to Phase III randomised controlled trials (RCTs) are: enough information is available from Phase I/II trials; inherent mortality of the disease justifies the risk of the procedure; prognostic factors of the disease are known to define

patients at high risk for disease progression; HSCT morbidity and mortality is acceptably low; risk of disease progression after HSCT is low, and little or no alternative conventional therapy is available.

Such criteria are currently sufficiently met for systemic sclerosis, multiple sclerosis and rheumatoid arthritis.

In the ASTIS (Autologous Stem cell Transplantation International Scleroderma) Trial patients are selected who have less than 4 years of diffuse skin involvement and evidence of progressive and organ or life threatening disease. The primary endpoint on which the trial is powered is event-free survival at 2 years, events being arbitrarily but precisely defined to capture irrever-sible and severe end-organ failure or death.

Exclusion criteria are based on the Phase I and II data to avoid an unacceptably high TRM risk together with a minimal chance of clinically significant improvement.

The treatment arm is mobilisation with Cy 4 g/m^2 and G-CSF, followed by CY 200 mg/kg body weight conditioning plus ATG and a CD 34 selected graft. The control arm is monthly IVI pulse CY 750 mg/m^2 for 12 months.

The ASTIS trial is running, and further details are available on the website: www.astistrial.com. So far, 26 patients have been randomised and there has been no transplant related mortality.

A similar study is being planned by a US consortium (P. McSweeney, personal communication).

The ASTIRA (Autologous Stem cell Transplantation International Rheumatoid Arthritis) Trial

Active RA patients who have failed at least four DMARDs including methotrexate and TNF alpha blocking agents with a disease duration between 2–15 years will all receive stem cell mobilisation with Cy 4 g/m^2 and G-CSF. Randomisation will then occur to either continued conventional therapy with either methotrexate or leflunomide or conditioning with Cy 200 mg/m^2 and ATG. The graft will not be manipulated, and maintenance with methotrexate or leflunomide will be given. The primary endpoint is the number of patients reaching a good or moderate EULAR response and/or an ACR 20 at 6 months. 16 patients in each arm are required, calculated on a > 50% difference in the two groups and the trial is running.

In SLE, a Phase II study is being planned to assess the role, if any, of post-transplant maintenance (e.g., mycophenelate mofetil) therapy to retain remission.

The results of Phase I/II trials in JIA using Cy alone *versus* Cy and TBI suggested no advantage of the TBI (Wulffraat, personal communication). Further Phase II studies will be performed to assess the optimal regimen for a Phase III study.

Open issues

Allogeneic HSCT

The international guidelines stipulated that autologous HSCT should be the preferred approach. So far, this has been mostly adhered to with only a few allogeneic HSCT for AD alone having been performed in refractory cytopaenias.

Arguments not to use allogeneic HSCT remain the same. Treatment related toxicity is high, graft-*versus*-host-disease (GVHD) cannot yet be avoided and might interfere with the pre-existing disease without the potential benefits of added "graft-*versus*-autoimmunity". Unlike in malignancy, there is no definable clone of autoaggressive cells to be eradicated. Furthermore, incomplete or slowed immune reconstitution after allogeneic HSCT might lead to late development of a donor-type AD, even more so in predisposed patients.

It remains open whether reduced intensity conditioning regimens might alter the perspectives. They have been shown to reduce early mortality. So far, they have not reduced risk of GVHD and long-term follow up is required.

Still, there is consensus that it might be appropriate under carefully selected conditions to begin the planning of Phase I/II studies to evaluate the role of allogeneic HSCT. Conditioning with Cy ± ATG as used for aplastic anaemia for many years might be the most appropriate choice.

Immune reconstitution

So far, anecdotal data has not produced an immune cell phenotypic pattern which reliably predicts either remission or relapse. As already known, the CD-8 RO pos "memory cell" compartment expands post-transplant, with later appearance of CD8 and CD4 RA pos "naive" T cells. CD 19 and 20 B cells and NK cells reconstitute within weeks to months, but CD4 cells may take months to years, depending on the severity of the conditioning and T cell depletion and probably also the underlying disease.

Early data give hints of potential laboratory markers of response and relapse of the RA synovium [25], but further work is required and ongoing. The finding of T cell receptor excision circles in T cells recently exiting the thymus [26] has allowed a more detailed analysis of normal and autoaggressive T cell reactions following HSCT for AD.

Ablative therapy without HSCT

Hematopoietic stem cells resist the cytotoxic effects of cyclophosphamide, and therefore theoretically, a HSCT is not needed following aplasia induction and G-CSF sup-

ported reconstitution. Such a strategy has been successfully employed in aplastic anaemia and applied to SLE [27]. Early results are encouraging, but a significant number of patients had not had conventional pulse cyclophosphamide therapy and the reconstitution times, especially for platelets, were prolonged compared to rescue with HSCT. Both procedures remain research based rather than standard therapy.

Summary

The role of stem cell transplantation in the treatment of severe, therapy refractory autoimmune disease remains experimental, with data on around 600 patients being sufficiently encouraging to proceed to randomised prospective trials in the major diseases: SSc, RA, MS and soon JIA and SLE. An impressive international collaboration has, and is, reducing duplication of effort with shared data bases, protocols, patient selection and endpoints.

The concept of resetting a dysbalance in the complex immune network, rather than total eradication of clonal autoimmunity is emerging.

Further clinical trials are required to establish the place, if any, HSCT has in such treatment, and a parallel science program continues to explain the pathophysiological mechanisms of these immune modulating strategies.

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