Michael W. Stewart

Diabetic Retinopathy

Current Pharmacologic Treatment and Emerging Strategies

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ISBN 978-981-10-3508-1 ISBN 978-981-10-3509-8 (eBook)
DOI 10.1007/978-981-10-3509-8

Library of Congress Control Number: 2017933287

Printed on acid-free paper

This Adis imprint is published by Springer Nature
The registered company is Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Introduction

Diabetes mellitus (DM) currently affects more than 8% of the US population and ranks seventh as a cause of death. Approximately 90% of affected patients have type 2 DM and because of the growing obesity epidemic, which predisposes patients to the development of type 2 DM, the number of affected patients is increasing rapidly. Despite the large number of patients in industrialized nations, the majority of diabetics live in developing countries with the greatest number found in India. The abandonment of traditional foods in favor of westernized diets that are rich in processed carbohydrates and saturated fats has increased the incidence of metabolic syndrome in many of these countries. The number of patients with DM challenges healthcare systems throughout the world with both industrialized and developing countries struggling to manage the global diabetes epidemic.

Diabetic retinopathy (DR) is the most common neurodegenerative and microvascular complication affecting patients with DM. Comorbidities such as systemic arterial hypertension and hyperlipidemia predispose to the development of DR, but the most important risk factors are average serum glucose levels and duration of disease. Internists and endocrinologists have an increasing number of drugs at their disposal to treat DM, but several factors including national and regional drug availability, drug costs, healthcare insurance coverage, access to medical care, patient education, and patient compliance (among others) limit the effectiveness of treatment and influence the development of retinopathy. Chapter 1 of this book discusses the incidence and prevalence of DM and DR from both global and regional perspectives.

The pathophysiology of DR is extraordinarily complex and remains incompletely understood. Because 75% of diabetes-related vision loss results from diabetic macular edema (DME) and most of the remaining cases stem from complications of proliferative diabetic retinopathy, research and drug development has focused on the retinal vascular endothelial cell. Several chemokines and inflammatory cytokines, including vascular endothelial growth factor (VEGF), have been associated with the development of DR. Unfortunately, the pertinent biochemical pathways are rarely linear, with numerous crosstalk associations and feedback loops that make it difficult to fully understand the differences between causative molecules

and disease markers. Chapter 2 delves into the complex biochemical mix found in eyes with DR.

Only during the past 3 decades have proven treatments for DR (laser photocoagulation and vitrectomy surgery) emerged. Chapter 3 discusses these alternative treatments and highlights their effectiveness in the treatment of DR.

The relatively recent (1989) discovery of VEGF has fueled most of the recent advances in the treatment of DR. VEGF inhibitory drugs have been available to ophthalmologists since 2004, but their use in patients with DME has advanced more slowly than in patients with neovascular age-related macular degeneration. Nevertheless, anti-VEGF treatment has become the preferred treatment for DME, and their use in PDR is increasing. Chapter 4 describes the available anti-VEGF agents and details the important clinical studies that have shaped our current approaches to the treatment of DR.

Inflammation plays a key role in the development of DR, and its discovery quickly led to the use of intravitreal triamcinolone acetonide injections, and, more recently, dexamethasone and fluocinolone sustained release inserts. Chapter 5 discusses both the off-label use of corticosteroids and the pivotal phase III trials that have led to regulatory approval of sustained release inserts.

With data from prospective trials that have evaluated several drugs, treatment choices for DME and DR abound. Chapter 6 constitutes the author's attempt to present evidence-based treatment guidelines after a synthesis of the literature. Several important questions regarding treatment choices remain unanswered, and these will be addressed in accordance with the best available data.

Multiple lines of evidence implicate the vitreoretinal interface in the development of DR. Several drugs and preparations that induce posterior vitreous detachment are being evaluated in patients with DR. Chapter 7 discusses the off-label use of both approved and investigational drugs that promote vitreous detachments.

Despite our available treatments, many patients still respond poorly to current therapies. Development of new drugs, novel formulations of existing medications, and extended duration drug-release devices are proceeding on several fronts. Chapter 8 discusses off-label use of approved medications as well as the current status of investigational products.

Drug safety is an important outcome during the development and approval of all pharmaceuticals, and it becomes particularly important in diabetic patients, many of whom have advanced vascular disease. The pivotal trials found acceptable safety profiles for each of the approved drugs, but important ocular and systemic risks must be considered when treating patients. Chapter 9 discusses the risks associated with pharmacotherapy.

Pharmacotherapy for DME comes at a significant cost to patients, medical care insurance companies, and national healthcare programs. The cost-effectiveness of available treatment strategies has been calculated in several economic analyses. Chapter 10 discusses some of the economic impacts of pharmacotherapy.

The diabetic retinopathy body of literature has become vast, and published reports vary from case discussions through multicenter, randomized, phase III trials. With tens of thousands of manuscripts published in hundreds of journals, this book

is not and cannot be a complete review of the literature. Contradictions in the literature are frequent and recommendations for nearly every contingency have been advanced. The best data come from carefully planned, multicenter, randomized, prospective, double-blind trials. This book attempts to discuss these studies in the greatest detail as they provide the most reliable information. Other studies that lack the same rigorous methodology have been included, but these are fewer in number, and their conclusions must be viewed with greater skepticism. Despite the focus on the pivotal trials, some important studies have undoubtedly been omitted.

This book comes after years of writing about drug pharmacokinetics, pharmacodynamics, and clinical pharmacotherapy. My interest in academic writing has been nurtured and supported by several excellent physicians including Mayo Clinic colleagues James Bolling, MD; Tom Liesegang, MD (past editor-in-chief of the *American Journal of Ophthalmology*); and George Bartley, MD (editor-in-chief of *Ophthalmology*). Others who have significantly supported my academic development include Phil Rosenfeld, MD; David Browning, MD; Kurt Gitter, MD; son-in-law Gregory P. Forlenza, MD; and son Michael Llort Stewart. I am forever indebted to Maurice B. Landers, III, MD, who has served as my mentor and valued colleague for 30 years.

I dedicate this work to the ladies in my life: my daughter-in-law Gwen Hochman Stewart, JD; daughter Tania Llort Stewart, MBA; and granddaughters Olympia Stewart Forlenza, Julia Claire Stewart, and Genevieve Stewart Forlenza. Most importantly, I thank my wife Enid Llort, MBA, from whom I have received never-ending support and encouragement. She is the smartest, most intuitive woman I have ever met, and without her pushing and pulling me for the past 37 years, I would never have been able to compose this volume.

Contents

Chapter 1
Diabetes and Diabetic Retinopathy: Overview of a Worldwide Epidemic

1.1 Introduction

By 2013, the world's population reached 7.2 billion with 138.8 million annual births and 54.9 million annual deaths [145]. The global population is projected to continue to increase, reaching 8.3 billion people by 2013, with 13% of these people falling into the fastest-growing age group – those over the age of 65 [119]. The world's changing demographic profile results from a remarkable transition in the causes of death. No longer are people dying primarily from communicable diseases. Rather, most deaths today are the result of noncommunicable diseases, a shift that has dominated the statistical patterns of industrialized countries for decades and is now also the trend in low- and middle-income countries.

Between 1970 and 2010, global life expectancy increased from 56.4 to 67.5 years for males and from 61.2 to 73.3 years for females [162]. During this same period, age-specific death rates were declining. Longer life expectancy prolongs exposure to various environmental and age-related risk factors and increases the incidence of deaths from noncommunicable diseases. In 2013, more than two-thirds of global deaths were due to noncommunicable diseases, with 17.3 million deaths due to cardiovascular and circulatory diseases, 8 million deaths due to cancer, and anywhere from 1.5 to 5.1 million deaths due to diabetes mellitus, making diabetes the eighth leading cause of death worldwide [30, 154]. Among older adults, diabetes is most heavily concentrated in Oceania, Latin America, North Africa, and the Middle East where a high body mass index is an important risk factor. High blood pressure is an important risk factor in Central and Eastern Europe, whereas high body mass index is important in Latin America, Oceania, North Africa, and the Middle East [98]. Of the 671 million obese individuals worldwide, 62% live in developing countries [122, 123]. The incidence rates of diabetes are likely to continue rising in association with the rapidly increasing obesity epidemic.

Diabetes mellitus (DM) represents a leading cause of morbidity and mortality throughout the world. Approximately 347 million persons worldwide (approximately 8.3% of the adult population) have diabetes mellitus [30, 154], and this has

M.W. Stewart, *Diabetic Retinopathy*, DOI 10.1007/978-981-10-3509-8_1

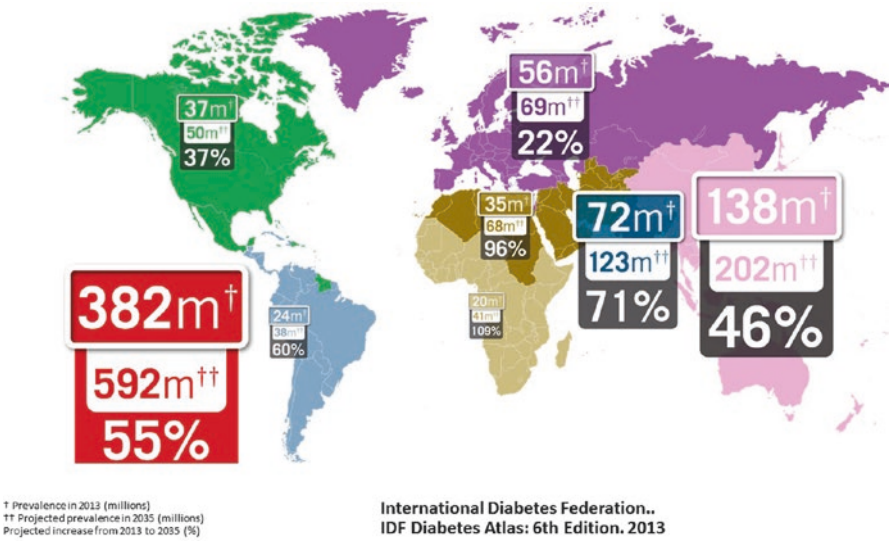

Fig. 1.1 This drawing shows projected increases in the prevalence of diabetes mellitus by continent from 2013 to 2035. The total number of diabetic patients throughout the world is projected to increase by 55% to 592 million. Increases are particularly significant in developing areas of Africa, the Middle East, and India

been projected to grow to 592 million by 2035 (Fig. 1.1) [12], numbers that have been revised upward several times in recent years. Diabetes ranks among the top ten causes of disability, primarily due to loss of ambulation from lower limb amputations and blindness due to diabetic retinopathy (DR). Because of its effects on pregnancy, DM adversely affects more than 21 million live births annually.

Diabetes disproportionately affects poor societies, as low- and middle-income countries account for 80% of diabetes-related mortality [72]. Diabetes-attributed mortality outstrips the combined annual deaths from malaria (600,000), tuberculosis (1.5 million), and AIDS (1.6 million) [169–171]. When combined with cardiovascular disease, cancer, and chronic respiratory diseases, diabetes accounts for an annual global death toll of 35 million – over 60% of the world's mortality.

Worldwide concern over the increasing importance of DM even led to the passage of a 2006 United Nations resolution designating November 14 as "World Diabetes Day." It was not the mortality rate, however, but rather the macroeconomic impact that prompted global action on diabetes and other noncommunicable diseases (NCDs). It has been estimated that for every 10% rise in the incidence of NCDs, a country loses 0.5% of its gross domestic product [152]. Noncommunicable diseases ranked among the top global risks to business due to the trillions of dollars incurred annually through lost productivity [168]. Diabetes incurs an annual healthcare cost of US$548 billion for 20–79-year-olds [30]. In low- and middle-income countries, blindness due to diabetes often disables breadwinners, burdens caregivers, and perpetuates a cycle of poverty.

Type 1 diabetes mellitus (T1DM) results from unopposed autoimmune destruction of the islet β-cells of the pancreas, which leads to insufficient production of insulin. Only 5–10% of worldwide cases of diabetes are classified as type 1 with the balance being type 2. Genetic factors appear to play important roles in the development of T1DM [112, 129, 153] but efforts to identify the responsible genes and their variants have met with limited success. More than half of the significant association signals for T1DM have been identified within chromosome 6p21 [15, 125, 129, 147], which maps to the human leukocyte antigen (HLA) region. This constitutes the most important region in the vertebrate genome regarding infection and autoimmunity and is crucial in regulating adaptive and innate immunity. A recent study of 4075 patients with T1DM identified 452 associated genes. Seven genes including four nonhuman leukocyte antigen (HLA) genes (RASIP1, STRN4, BCAR1, and MYL2) were replicated in at least one independent population and were differentially expressed in peripheral blood mononuclear cells or monocytes [136]. This emphasizes the fact that the genetic susceptibility pattern for T1DM appears complex [73] and may involve both susceptible and protective haplotypes. Despite our improved understanding of genetic associations, the triggering mechanism for the development of T1DM remains unknown, and in genetically susceptible individuals, it may result from exposure to as yet unidentified environmental factors.

The relative contributions of these haplotypes and their interactions with environmental factors and other genetic loci might partially explain the ethnic and racial variations in the frequency of T1DM [71]. Type 1 diabetes predominantly affects individuals of European ancestry, with the highest rates in Finland and Sardinia [11]. Asian and sub-Saharan African countries generally report low frequencies of T1DM, but Kuwait and China recently have reported higher rates [113, 180]. The SEARCH Study for Diabetes in Youth (2009) determined that T1DM remains a Caucasian-dominated disease with prevalence rates among 0–19-year-olds of 2.00 per thousand in non-Hispanic white patients, 1.31 for African-Americans, 0.99 for Hispanics, 0.94 for Navajos, and 0.52 for Asians and Pacific Islanders [11, 103].

Remarkable economic growth in Asia during the past 30 years has greatly improved the region's standard of living and extended life expectancy. Growth is transforming the region from a predominantly low-technology, agrarian society toward an industrialized society with greater urbanization. This has increased food availability and caloric consumption and created a more sedentary lifestyle with a declining rate of communicable diseases. Obesity rates have increased as have the prevalences of T2DM, further straining the already challenged healthcare systems in the developing countries of the region.

Type 2 diabetes is characterized by peripheral insulin resistance, impaired regulation of hepatic glucose production, and declining ß-cell function that eventually leads to ß-cell failure and dependence on exogenous insulin [154]. With rapid growth in the prevalence of T2DM in the last two decades, there has been a surge in the reports of T2DM-related diabetic retinopathy (DR), especially from Asia. In 2030, the largest numbers of patients with diabetes will be in India and China. About 150 million people in China currently show early symptoms of diabetes [27].

By the year 2033, 80 million people in India will suffer from T2D [154] and 1 million people with diabetes will die every year [37, 56].

1.2 Incidence of Diabetes

Substantial data showing the prevalence rates of DM among specific ethnic and racial populations and within several countries has been published. There appears to be uniform agreement that the prevalence rates among most of these groups will significantly increase in the foreseeable future. Some authors have characterized the rate of increase in the global prevalence of T2DM, particularly in some areas of the globe, as alarming [157, 164]. It is estimated that the prevalence of diabetes among people over the age of 16 years will rise by 28.3% between 2010 and 2030, with 54.5% of this increase being attributed to increased obesity [2]. The incidence of T1DM is also rising but not to the same degree [11].

In 2008, there were an estimated 2.4 million Canadians (out of a population of approximately 30 million) with DM, and this is expected to increase to 3.7 million by 2018 [66]. Five and one-half percent of the adult population of France suffers from T2DM. Among affected patients, the average age is 65.9 years, 55% are men, the prevalence of obesity is 43%, and 18% take insulin [46]. The number of people with DM in the Middle East is expected to triple from the year 2000 to 2030 to approximately 60 million [148].

The prevalence of T2DM in South African adults rose from 5.5% in 2000 to 9.0% in 2009. Two million South Africans currently live with T2DM with 115,000 new cases estimated to occur each year [12]. Eye screening programs have been ineffective because an estimated 55% of adults with diabetes remain undiagnosed [65].

According to the International Diabetes Federation, Brazil's population of patients with DM is the fourth largest in the world (11.9 million in 2013) with an estimated prevalence of 10.3% [8].

The annual age-adjusted prevalence and incidence rates of diabetes in the United States did not change significantly during the 1980s, but each rate increased yearly from 1990 through 2008 before leveling off until 2012. The prevalence per 100 population was 3.5 in 1990, 7.9 in 2008, and 8.3 in 2012 (Fig. 1.2). The incidence per 1000 persons was 3.2 in 1990, 8.8 in 2008, and 7.1 in 2012. Trends in many subpopulations were similar to these overall trends, but the incidence rates among non-Hispanic blacks ($P = .03$) and Hispanic adults ($P = .01$) continued to increase at rates significantly greater than those for non-Hispanic white adults. The prevalence rate increased faster for adults with a high school education or less, than for those with more than a high school education [57]. Based upon population and obesity trends, it was recently predicted that for a child born in the United States in 2000, the lifetime probability of being diagnosed with diabetes is 33% for males and 39% for females [117].

For predisposed individuals who do not yet meet criteria for the diagnosis of DM, early changes in lifestyle can prevent the onset of T2DM. The Diabetes Prevention

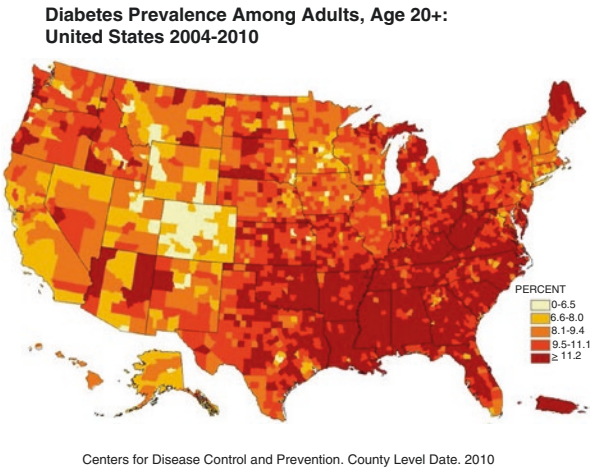

Fig. 1.2 This figure shows the county-by-county 2010 prevalence of diabetes mellitus in the United States. Note the very high prevalence rates in the southeastern United States, Puerto Rico, and scattered counties throughout western states in which there are high populations of Native Americans

Program was a multicenter, randomized, controlled clinical trial that enrolled overweight individuals with elevated blood glucose levels but without definitive criteria for the diagnosis of diabetes. The consistent adoption of lifestyle changes including a low-fat diet, weight loss, and increased physical activity reduced the development of T2DM by 58% compared with placebo. Metformin (850 mg twice daily) lowered the incidence of diabetes by 31% compared with placebo. The study also demonstrated that approximately 8% of the patients who were prediabetic by commonly used clinical criteria already had diabetic retinopathy [7]. This suggests that the current criteria necessary for the diagnosis of DM exclude some patients who already show evidence of diabetes-related damage. Therefore, an argument can be made for changing the criteria required to diagnose T2DM.

Good control of blood glucose concentrations is the best way to prevent the development and progression of diabetes-related complications, but many patients with DM fail to achieve or maintain adequate metabolic control. Unfortunately, patients who manage to keep their HbA1c low have an increased risk of symptomatic hypoglycemia. The incidence of severe hypoglycemia in the Diabetes Control and Complications Trial (DCCT) was three times higher in the intensive treatment group compared with the conventional treatment group. Therefore, it appears unrealistic for patients with T1DM to pursue perfect glucose control because of the elevated risk of hypoglycemic episodes. Intensive glycemic control in the DCCT was also associated with a weight gain of 4.6 kg more than those in the conventional treatment group [51, 52]. It is difficult for patients with T2DM to achieve optimal metabolic control as patients in the intensive treatment group in the UKPDS were also plagued by increased hypoglycemic episodes and weight gain [51]. Metabolic control deteriorated over time in the UKPDS, possibly because of progressive loss of islet beta cell function [156]. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study was stopped because of increased all-cause mortality in people whose glucose was extremely tightly controlled with insulin and multiple oral agents [1].

Obesity is a major risk factor for the development of T2DM [44, 55, 68, 97, 160], and increased prevalences of both diabetes and obesity are closely correlated [110]. The increases in obesity prevalence rates may have recently plateaued [50, 128] because of declines in overall food purchases and caloric intake, which could mean that the growth rate of T2DM may stabilize [53, 122]. Bariatric (weight loss) surgery is an effective and increasingly commonly used treatment for obese patients with T2DM [179].

Physical inactivity as part of a sedentary lifestyle is considered to be an important modifiable risk factor for T2DM and cardiovascular disease. Increased physical activity may slow the onset and progression of insulin resistance and improve glycemic control, blood pressure, and the lipid profile [69, 76].

1.3 Incidence of Diabetic Retinopathy

Visual impairment is a major public health problem that significantly diminishes the quality of life of affected individuals. Visually impaired patients report having difficulty reading, driving a car, and preparing meals [58, 105, 106, 161]. Vision impairment leads to higher incidences of social isolation, poor overall health, and falls. Not only does vision impairment impose a major burden on the affected individuals but also on their families, caregivers, and society.

Diabetes is the leading cause of vision loss in patients between the ages of 20 and 74 years in industrialized nations [33, 140]. Diabetes affects all parts of the eye, ocular adnexae, neurosensory pathway, and ocular motility system, but most DM-related vision loss stems from retinopathy due to microvascular complications. Early diagnosis and treatment of DR is important because substantial and permanent vision loss may occur if DR is left untreated for 1 year or longer [3, 47]. An estimated 50,000 new cases of retinal neovascularization and diabetic macular edema (DME) occur yearly [120, 130], and as many as half of the patients who would benefit from treatment remain untreated [87].

Preventable causes of blindness such as cataracts, glaucoma, and infectious diseases are viewed as the most important ophthalmic public health problems in many parts of the world, making screening for diabetic retinopathy and subsequent treatment lesser priorities (Fig. 1.3). Fortunately, the incidence rate of nonproliferative diabetic retinopathy appears to be declining in the United States, thus supporting the contention that more aggressive management of DM and its comorbidities limits the development of DR-related vision loss. In response to the results of randomized, controlled trials, the initiation of the VISION 2020 program in 1999 [54, 172] and DR surveillance programs [141] has intensified control of risk factors [38, 143, 156], and continuing improvements in healthcare systems have contributed to the decreasing rates of DR [81]. Unfortunately, studies from less developed areas of the world have been more limited in scope and do not show similar trends [148].

The number of people worldwide with DR will increase from 126.6 million in 2010 to 191.0 million by 2030, and the number with vision-threatening diabetic retinopathy (VTDR) is estimated to increase from 37.3 million to 56.3 million

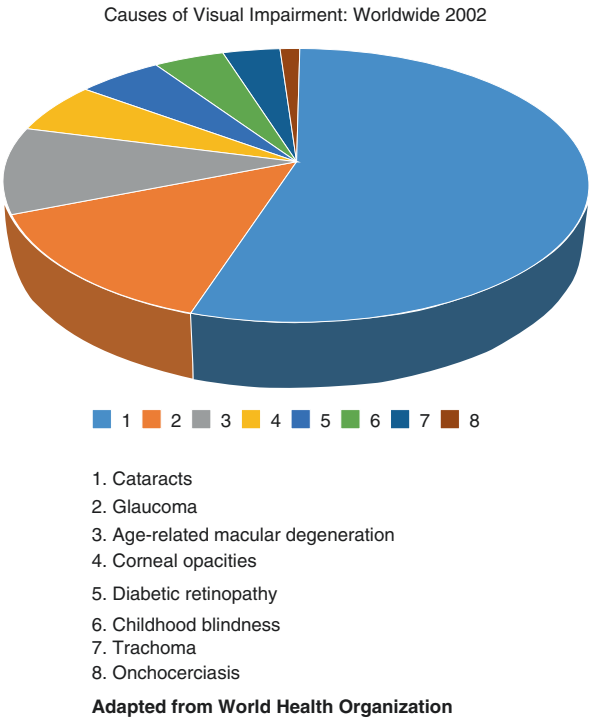

Fig. 1.3 This pie chart shows the most common causes of blindness throughout the world in 2002. Cataracts account for most cases of blindness, though these are almost exclusively from developing countries and reflect an inadequate supply of the healthcare services that are abundant in industrialized countries. Diabetic retinopathy accounts for about 5% of worldwide blindness (fifth on the list) and is a major cause of blindness in industrialized countries

[183]. Among patients aged 20–79 years with diabetes, the worldwide prevalence of any grade of DR has been estimated to be 35% and that of PDR to be 7% [80]. Among patients with DM for at least 25 years, 50% of those with T1DM and 15% of those with T2DM will have proliferative retinopathy [6].

A pooled analysis using individual participant data (22,896 individuals) from 35 population-based studies (1980–2008) from around the world was performed to determine the prevalence of DR [177]. The overall prevalence of DR was 34.6% (95% CI 34.5–34.8%) for any DR, 6.96% (CI 6.87–7.04%) for proliferative DR, 6.81% (CI 6.74–6.89%) for diabetic macular edema, and 10.2% (10.1–10.3%) for VTDR. All DR prevalence endpoints increased with diabetes duration, hemoglobin A1c levels, and blood pressure measurements, and were higher in people with T1DM compared with T2DM. The prevalence estimates of any DR and VTDR were similar in men and women and were highest in African-Americans and lowest in Asians [146, 176]. Higher total serum cholesterol was associated with a higher prevalence of DME, bringing clarity to previously conflicting reports about this risk factor [167]. The authors concluded that there are approximately 93 million people with DR, 17 million with proliferative DR, 21 million with diabetic macular edema, and 28 million with VTDR worldwide.

An analysis of pooled data from several population-based studies estimated that approximately 40% of patients with diabetes who are over the age of 40 years have some retinopathy and 8.2% have vision-threatening retinopathy [81].

Many have attempted to define the contribution of hereditary factors to the development of DR [144], but no firm data suggests that DR has a genetic component. This differs from diabetic nephropathy where important genetic associations have been described recently [43]. Candidate gene studies and GWAS (genome-wide association studies) may ultimately find genetic linkage to retinopathy phenotypes.

The WHO (World Health Organization) has estimated that diabetic retinopathy accounts for approximately 15–17% of total blindness in Europe and the United States [134]. The widespread use of drugs that inhibit the actions of vascular endothelial growth factor (VEGF) has significantly decreased the incidence of blindness due to age-related macular degeneration (AMD), prompting some authors to speculate that diabetes has become the overall leading cause of blindness [28, 45]. Further complicating the care of many of these patients is the fact that diabetes is also a leading cause of renal failure through its effects on the microvasculature [29, 77, 126]. Since prevalence rates of systemic arterial hypertension – a well-established risk factor for DR – are also increasing, the importance of DR to vision loss in industrialized nations is unlikely to diminish [70, 131].

Diabetic retinopathy is responsible for up to 17% of all blindness in parts of the Americas, Europe, and the Western Pacific [163]. The numbers of patients with both DM and DR appear to be increasing, and by 2050 as many as 50 million or more individuals in the United States will have DM with half having some form of retinopathy [10, 18, 19, 75, 81, 116]. In 2004, the Eye Diseases Prevalence Research Group estimated the prevalence of diabetic retinopathy from the compilation of eight separate population-based studies from the United States and elsewhere that had been conducted in the late 1980s or early 1990s [81]. Their report recommended that more recent estimates of diabetic retinopathy prevalence be obtained from the nationally representative sample of the National Health and Nutrition Examination Survey (NHANES) [181].

The 2005–2008 National Health and Nutrition Examination Survey evaluated 5222 Americans aged 40 years and over for general visual impairment (distance visual acuity worse than 20/40 in the better-seeing eye) and visual impairment not due to refractive error (distance visual acuity worse than 20/40 after refraction). The overall prevalences of visual impairment and of visual impairment not due to refractive error were 7.5% (95% CI, 6.9%, 8.1%) and 2.0% (95% CI, 1.7%, 2.3%), respectively. Not surprisingly, the prevalence of visual impairment not due to refractive error was significantly higher among people with DR (3.5%) compared to those without DR (1.2%) [28]. The prevalences of DR and vision-threatening DR were found to be 28.5% (95% CI, 24.9–32.5%) and 4.4% (95% CI, 3.5–5.7%). A slightly greater prevalence of DR was found among men (31.6%, 95% CI, 26.8–36.8%) than women (25.7%, 95% CI, 21.7–30.1%, $P = 0.04$). Non-Hispanic blacks, compared to non-Hispanic whites, had higher incidences of DR (38.8%, 95% CI, 31.9–46.1% vs. 26.4%, 95% CI, 21.4–32.2%, $P = 0.01$) and vision-threatening retinopathy (9.3%, 95% CI, 5.9–14.4% vs. 3.2%, 95% CI, 2.0–5.1%, $P = 0.01$). Male gender was independently associated with the presence of DR (OR 2.07, 95% CI 1.39–3.10), higher HbA1c (OR, 1.45, 95% CI, 1.20–1.75), longer duration of diabetes (OR, 1.06 per year duration, 95% CI, 1.03–1.10), insulin use (OR, 3.23, 95% CI, 1.03–1.10), and higher systolic blood pressure (OR, 1.03 per mmHg, 95% CI, 1.02–1.03).

A previous analysis of NHANES III data suggests that the prevalence of diabetic retinopathy is 46% higher in non-Hispanic black individuals and 84% higher in Mexican Americans than in non-Hispanic whites [61, 63]. The prevalence of vision-threatening diabetic retinopathy was 190% higher in non-Hispanic blacks and 130% higher in Mexican Americans than in non-Hispanic whites, probably because non-Hispanic blacks and Mexican Americans have poorer diabetes control and are less likely to be screened and treated in a timely manner [127]. Non-Hispanic black individuals and Hispanics are less likely to use eye care services [182]. These findings highlight the need to reduce disparities in care among racial, ethnic, and socioeconomic groups [182].

Latino Americans have one of the highest rates of visual impairment but the prevalence and risk of undetected eye disease cannot be accurately quantified. In one study, the 4-year incidences of best corrected visual impairment and blindness from DR in Latinos were 1.2 and 0.3%, respectively [159]. Access to healthcare by this population is inconsistent and unequal compared to other populations, and this may influence disease statistics. In contrast, no association was found between socioeconomic status and DR in cohorts of Mexican Americans and Caucasian patients with T2DM in Texas [60]. Approximately 13% of African-Americans have T2D, with the prevalence and incidence of T2DM being at least twice as high as that among white Americans [18, 62].

There are more than two million Native Americans on the North American continent, comprising more than 500 tribal organizations, and a comprehensive review of complications of T2DM in this indigenous population reveals high prevalence rates of DR for all populations studied. High prevalence rates of DR have been observed among the Alberta First Nations of Canada (40%) and the Pima Indians in Arizona (37.8%). In the Southern Alberta Study of Diabetic Retinopathy, DR in nonnatives tended to be more advanced, but the prevalences of DR in native and nonnative Canadians were identical (40%).

The Eye Disease Prevalence Research Group estimated that 4.1 million Americans had diabetic retinopathy in 2010 and projected that this would rise to 7.2 million by 2020 [118]. It has been estimated that one in every 12 Americans with diabetes over the age of 39 has vision-threatening retinopathy. A recently published study of the prevalence of DR and vision-threatening DR in a nationally representative sample of US adults aged 40 years or older showed that approximately 1.5% (95% CI, 1.1–2.2%) of adults with diabetes had proliferative DR and 2.7% (95% CI, 1.8–4.0%) had CSME [181]. Over a 10-year period, nonclinically significant DME and CSME will develop in 14% and 10%, respectively, of Americans with known diabetes [89]. Approximately half of all patients with DME will lose two lines or more of vision within 2 years [47, 48].

A cross-sectional, subgroup analysis of fundus photographs from 1038 participants with diabetes aged 40 years or older showed that 55 had DME, for an overall weighted prevalence of 3.8% (95% CI, 2.7–4.9%); this translates to 746,000 persons with DME in the US 2010 population. There were no differences in prevalence rates due to age or gender, but the chances of having DME were higher for non-Hispanic blacks than for non-Hispanic whites (odds ratio [OR], 2.64). Elevated

hemoglobin A1c levels (OR, 1.47; $P < .001$) and longer duration of diabetes (OR, 8.51; $P < .001$) were also associated with DME [13].

Diabetic retinopathy appears to be strongly associated with all-cause mortality. An analysis of 20 studies with data from 19,234 patients showed that any degree of DR increased the risk of all-cause mortality by 2.34-fold (95% CI, 1.96–2.80) among type 2 diabetics and 2.41-fold (95% CI, 1.87–3.10) among type 1 diabetics [95]. Fortunately, however, rates of non-ocular diabetic complications have declined during recent decades. The hospitalization rate for lower extremity amputations among individuals with diabetes began decreasing in 1997 [57], and the prevalence of diabetes-related end-stage renal disease decreased between 1996 and 2006 [16]. It is reasonable to assume from these favorable trends in incidence data that improved diabetes care, such as effective management of blood glucose levels, blood pressure, and serum lipid levels, may also be reducing the incidence of diabetic retinopathy [16, 57].

Population-based studies have shown that nearly all patients with T1DM and more than 60% of those with T2DM develop DR during the first two decades of the disease [51]. Recent population-based studies have reported decreases in the prevalence and incidence of severe DR [67, 92, 106]. These findings, however, were limited to regional populations, and broad application of this data to national populations cannot be done with certainty [181]. The prevalence of DR has been changing because of improvements in management of blood glucose, serum lipids, and blood pressure [4]. In the 8 years between the beginning of the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) and the beginning of the Beaver Dam Eye Study (BDES), the prevalence of any DR in persons with T2DM fell by 30% (from 50% in the WESDR in 1980–1982 to 35% in the BDES in 1988–1990), and the prevalence of vision-threatening DR fell by 70% (from 10% in the WESDR in 1980–1982 to 3% in the BDES in 1988–1990) [85, 86, 88].

The Wisconsin Epidemiologic Study of Diabetic Retinopathy reported that the 10-year incidence of DME in the United States was 20.1% among type 1 diabetics, 25.4% among insulin-dependent type 2 diabetics, and 13.9% for non-insulin-dependent type 2 diabetics. The prevalence of DME was 3% (diabetes duration, 0–14 years) or 6% (>15 years) in mild NPDR, 37% (0–14 years) or 63% (>15 years) in moderate/severe NPDR, and 73% (0–14 years) or 74% (>15 years) in PDR [82]. Nearly half of the patients with DME will lose two or more lines of visual acuity within 2 years.

National DR screening programs have been introduced to all four countries of the United Kingdom during the last decade resulting in significant reorganization of care for these patients [17, 124, 135, 141]. Analysis of a large UK database found that 15.8–18.1% of diabetics had center-involving macula edema [80]. Of these, 14% of eyes had mild NPDR, and 24% of eyes with moderate NPDR, 31% of eyes with severe NPDR, and 22% of eyes with PDR also had CSME (whether or not center-involving). Prevalence figures for DR are approximately three times higher in the hospital databases than whole population estimates, reflecting the tendency that patients with more advanced grades of DR are managed in the hospital systems. The prevalence of any grade of DR was around 80% in this study, compared with

20–40% in most population studies of patients with diabetes. PDR prevalence was around 20%, and CSME prevalence was around 18% in the hospital database, compared with 1–8% and 6%, respectively, in the general populations [14, 107, 111, 133, 150, 178].

From the English National Health System database, the number of people with diabetes in England in 2010 was estimated at 2,342,951 of which 2,334,550 were over the age of 12 years. An estimated 166,325 (7.12%) had DME in one or both eyes, and of these, 64,725 individuals had DME that reduced the visual acuity to poorer than 6/6 in at least one eye. The overall health and social care costs in 2010, from screening through treatment, rehabilitation, and care in the home, were estimated at £116,296,038.

The community-based National Diabetic Retinopathy Screening Service for Wales performed a cross-sectional analysis of 91,393 persons with diabetes (5003 with T1DM and 86,390 with T2DM) at their first screening from 2005 to 2009. The prevalence of any DR and sight-threatening DR in patients with T1DM was 56.0% and 11.2%, respectively, and in patients with T2DM was 30.3% and 2.9%, respectively. The presence of DR was strongly associated with increasing duration of diabetes for patients with either T1DM or T2DM and was also associated with insulin therapy in patients with T2DM.

A UK database study evaluated the incidence of DR in newly diagnosed diabetics [100]. By 9 years after the diagnosis of diabetes, 28% of T2DM and 24% of T1DM patients had developed DR (7899 incident DR cases). During the first 2 years with diabetes, the incidence rate was almost two times higher in patients diagnosed with diabetes in 2006–2007 than among those diagnosed in 2000–2001. Among patients with retinopathy at baseline, the study found a cumulative incidence of DME in 12.1% of T2DM patients and 18.8% of T1DM patients within 9 years.

The World Health Organization estimates that over 40 million Chinese will have diabetes by 2030 [164]. Similar to the studies in South Asia, the Beijing Eye Study reported that DR is a relatively minor cause of blindness (7.7%) in the Chinese population [78]. Nonetheless, with continued economic progress, this is becoming a threat to public health in many areas. In China, the prevalence of diabetic retinopathy among people with diabetes is predicted to reach 43% [177], with up to 9.2 million people in rural areas having diabetic retinopathy and 1.3 million having sight-threatening diabetic retinopathy. Reflecting the fact that DM and DR are related to lifestyle, DR is more prevalent in South Asians living outside the Indian subcontinent [138] and in urban cities [137] compared to those who reside in rural areas within India [115]. Projections suggest that for India alone, 0.7 million people will have proliferative diabetic retinopathy (PDR) in 2030, and 1.8 million will have clinically significant macular edema [139].

A Japanese study found a significant correlation between the severity of DME and the DR grade [175]. In this report, 28% of patients with mild/moderate NPDR, 67% with severe NPDR, and 51% with PDR also had DME.

Studies from Australia that date back more than three decades support the impact of health education and better glycemic control on the prevalence and incidence of DR [35, 104, 114]. In the past three decades, the prevalence and incidence of

DR among patients with T1DM have declined in the United States, Australia, and other developed countries [183]. The earliest clinic-based study of DR in Australia, the Newcastle Diabetic Retinopathy Study (1977–1988), reported a 35% prevalence of DR, but subsequent population-based studies have reported lower rates [109] with the Australian Diabetes, Obesity, and Lifestyle study (AusDiab) reporting a DR prevalence of only 21.9%.

Comparing the prevalence rates for DR among studies is difficult because of the changing classification of diabetes over time, the different grading protocols employed, and differences in the characteristics of "similar" populations [177, 178, 181]. Findings from these studies, however, can provide policy makers with important information to plan eye care services, with the understanding that the prevalence of sight-threatening DR may be underestimated. The strong association between duration of diabetes and risk of retinopathy underscores the importance of early detection via office-based examinations or screening programs. An effectively structured screening program may reduce the incidence of blindness by 40% within 4 years [121].

Improving glycemic control by lowering the level of glycosylated hemoglobin (HbA1c) is the most effective way to slow the progression of DR. Keys to the discovery that optimal metabolic control could reduce the incidence and progression of DR were the DCCT and the UKPDS trials. Not only did intensive glycemic control inhibit the development of DR, these effects persisted well beyond the course of treatment [39, 40].

The Diabetes Control and Complications Trial (DCCT) (1982–1993) was a multicenter, controlled clinical trial that compared the effects of intensive blood glucose control (INT) with standard control (CON) on the onset and progression of DR. The Epidemiology of Diabetes Interventions and Complications (EDIC) study (1994–present) is an observational follow-up of the DCCT cohort. Of the 1441 DCCT subjects, 726 had no DR (primary prevention cohort) and 715 had mild DR (secondary intervention cohort) at baseline. Subjects were followed for a mean of 6.5 years. The median HbA1c was 7% in the INT group compared with a median of 9% in the CON group. INT reduced the adjusted mean risk for the development of DR by 76% and slowed progression of DR by 54% compared with CON.

Following DCCT, the HbA1c levels in the original INT and CON groups converged (year 8, INT 7.98%, CON 8.07%), but the INT group continued to enjoy a durable effect with significantly lower incidence of further DR progression (hazard reduction, 53–56%). Serious retinal outcomes and the need for procedures to treat them were reduced by 50% in the original INT group.

DCCT demonstrated that intensive glucose-lowering therapy for a mean of 6.5 years reduces the risk of development and progression of retinopathy by as much as 76% compared with conventional therapy. Much of the original effect persisted for over 18 years of follow-up in EDIC – the so-called metabolic memory – as the cumulative incidence of each retinal outcome continued to be lower in the former INT group. Interestingly, the year-to-year incidence of these outcomes is now similar in the two groups because of a reduction in risk in the former CON group [40].

1.4 Comorbidities for Diabetes and Diabetic Retinopathy

Higher levels of hemoglobin A1c, longer duration of diabetes, insulin use, and higher systolic blood pressure have been independently found to be associated with diabetic retinopathy [85, 86, 151, 165, 166, 182]. The early randomized controlled clinical trials showed that modifying risk factors such as hyperglycemia and blood pressure could reduce the burden of diabetic retinopathy and prevent vision loss [38, 155, 156] (Table 1.1). Efforts to prevent the development and progression of

Table 1.1 This table lists some of the important trials that have studied the effects of the major systemic contributing factors (hyperglycemia, systemic arterial hypertension, hyperlipidemia) on the development and progression of diabetic retinopathy

Important trials associating diabetic retinopathy with major systemic risk factors	
Hyperglycemia	
Diabetes Complications and Control Trials (DCCT) Type 1 diabetes mellitus	Intensive glucose control: 1. Reduced the adjusted mean risk for the development of DR by 76% 2. Slowed progression of DR by 54%
UK Prospective Diabetes Study (UKPDS) Type 2 diabetes mellitus	Intensive glucose control: 1. Reduced progression by 35% per A1C point 2. Reduced moderate vision loss by 47%
Systemic arterial hypertension	
Wisconsin Epidemiologic Study of Diabetic Retinopathy	Progression of retinopathy was associated with: 1. Higher diastolic BP at baseline 2. Increase in diastolic BP over a 4-year period
UK Prospective Diabetes Study (UKPDS) Type 2 diabetes mellitus	Found that systolic BP <150 mmHg: 1. Decreased the progression of DR 2. Decreased the need for macular laser photocoagulation for DME
ACCORD-Eye trial	Found that intensive BP control did not decrease the progression of DR
EUCLID study	Found that lisinopril decreased the progression of DR in normotensive type 1 diabetics
Diabetic Retinopathy Candesartan Trials (DIRECT)	In type 1 diabetics, 5 years of treatment: 1. Decreased the incidence of DR 2. Had no effect on progression of established DR In type 2 diabetics, there was a 34% regression of DR ($P = 0.009$) Less severe retinopathy in types 1 and 2 ($P = 0.03$)
RASS trial	Evaluated 285 normotensive patients treated with enalapril, losartan, or placebo for 5 yrs Progression of retinopathy by two steps in: Placebo (38%) Enalapril (25%, $P = 0.02$) Losartan (21%, $P = 0.008$) Enalapril and losartan increased the likelihood of less DR progression by 65% and 70% independent of blood pressure lowering

(continued)

Table 1.1 (continued)

Important trials associating diabetic retinopathy with major systemic risk factors	
Hyperlipidemia	
Fenofibrate Intervention and Event Lowering in Diabetes Study (FIELD)	Found that fenofibrate: 1. Decreased the requirement for the first laser and the development of DME 2. Decreased the need for laser treatment compared to the control group (3.4% vs. 4.9%, $P = 0.0002$) 3. Appeared to have protective effects independent of blood glucose, blood pressure, and baseline lipid values
ACCORD-Eye study	The addition of fenofibrate to basal statin therapy resulted in: 1. A decrease in the progression of DR, in a similar manner to that observed with intensifying blood glucose control, but with a good safety profile without increasing the risk of hypoglycemia 2. Questions regarding fenofibrate's mechanism of action and the pathogenesis of DR/DME

DR diabetic retinopathy, *BP* blood pressure, *DME* diabetic macular edema

DR should target individuals with the highest risk of developing retinopathy, those with longer duration of diabetes, and those using insulin. Improved availability of care for vulnerable sections of the population should be able to reduce the risk of blindness in diabetes. Identifying and protecting individuals at risk of T2DM, by reducing body weight and increasing physical activity, may also help delay the onset of T2DM and reduce complications of diabetic retinopathy.

Diabetic retinopathy is associated with increased cardiovascular (CV) and all-cause mortality in patients with T2DM and T1DM [41, 84, 149, 158]. Diabetic retinopathy also predicts all-cause mortality, more than just CV events. Several mortality studies showed that CV disease was the cause of death in fewer than 35% of diabetic patients [61, 84], suggesting that an alternative mechanism increases the death rate in many DR patients. An autonomic neuropathy could link DR and CV events since it was recently demonstrated that autonomic deregulation can lead to alterations in blood pressure and cardiac rhythm and the development of DR [5, 94].

In a recent systematic review of 17 studies of 14,896 people with T2DM (mean age 58 years and mean follow-up 9 years), people with any retinopathy were more than twice as likely to die or suffer a fatal or nonfatal CV event than people without retinopathy, and a fourfold higher risk was noted for people with advanced retinopathy. The same review also included four studies of 4438 people with T1DM (mean age 33 years and mean follow-up 12 years) and reported a 3.5–4-fold higher risk of death as well as CV events in the presence of any retinopathy and a sevenfold higher risk with advanced retinopathy.

Several explanations may account for the relationship between retinopathy and CV outcomes. First, both retinopathy and incident CV outcomes are recognized consequences of diabetes. Second, the degree of retinopathy is progressively related to the degree of several independent risk factors of CV outcomes, including hyperglycemia, elevated blood pressure, albuminuria, renal insufficiency, hyperlipidemia,

and other abnormalities. People with these risk factors would therefore have both more severe retinopathy and a higher incidence of CV outcomes. Third, the microvascular abnormalities present in the retina may also be occurring in many other vascular beds, and CV outcomes may be due in part to accumulated microvascular abnormalities in the myocardial microcirculation, arterial wall, and elsewhere. If this is true, changes in retinal pathology may closely reflect changes in microvascular pathology in these other vascular beds, and people with the most rapid progression in retinal pathology may be the ones most likely to suffer adverse CV events.

Recognizing and classifying diabetic retinopathy can improve the accuracy of a diabetic patient's CV risk stratification. A dilated fundus examination is inexpensive and should be routinely performed annually to screen for retinopathy, making this examination highly cost-effective. Previously mentioned findings support the view that changes in the retina may reflect changes in an individual's CV risk and may therefore identify those individuals whose CV risk is rising and who may benefit from particularly aggressive CV risk reduction therapies. Such a possibility should be tested in future clinical trials. Moreover, a retinal benefit of some therapy after only a few years (despite no CV effect during that period) may predict a CV benefit over a much longer period of time. Indeed, the findings of both the Diabetes Control and Complications Trial (in patients with T1DM) and the UK Prospective Diabetes Study (in patients with T2DM) showed that therapies that reduce the progression of retinal disease in the short-term subsequently reduce CV outcomes after long-term follow-up. If subsequent studies, including ongoing follow-up of the ACCORD participants, support this finding, retinal assessments may become integral to the regulatory assessment of the CV risk of drugs to treat diabetes. Furthermore, whether retinopathy progresses or not could be a gauge of whether a particular patient is reducing his or her risk of CV outcomes in response to the cardioprotective therapies that have been prescribed and provide an empirical basis to change therapies that are not working.

Hypertension is a major risk factor for the development and progression of both DR and DME. The WESDR found that progression of retinopathy was associated with higher diastolic blood pressure at baseline and an increase in diastolic blood pressure over a 4-year follow-up period [90]. The UKPDS demonstrated that control of blood pressure (systolic blood pressure <150 mmHg) led to a reduction in the progression of diabetic retinopathy and reduced need for laser treatment in the tight blood pressure control group compared with the less tight control group [156]. In the United Kingdom, this finding has contributed to the establishment of a target blood pressure measurement in diabetic patients of 140/80 mmHg. The American Diabetes Association and the National Institutes of Health recommend a target blood pressure of less than 130/80 mmHg for diabetics. The ACCORD study, however, produced contrasting findings to those of UKPDS in terms of the effects of intensive blood pressure control on the progression of DR [74]. In ACCORD, intensive blood pressure control did not reduce progression of DR.

The SisHyperDia national registry for diabetes and hypertension found that 4.3% of diabetic patients had foot disorders, 2.2% had a previous amputation, 7.8% had renal disease, 7.8% had a previous myocardial infarction, and 8% had a previous

stroke [142]. Registry data also suggested that age- and gender-adjusted mortality figures showed a 57% higher risk of death among diabetes.

A meta-analysis that included 40 trials (100,354 participants) evaluated the effects of HTN on morbidity and mortality in diabetics. It concluded that each 10 mmHg lowering of systolic blood pressure reduced mortality rates (relative risk [RR], cardiovascular events (RR, 0.89 [95% CI, 0.83–0.95]), coronary artery disease (RR, 0.88 [95% CI, 0.80–0.98]), stroke (RR, 0.73 [95% CI, 0.64–0.83]), albuminuria (RR, 0.83 [95% CI, 0.79–0.87]), and retinopathy (RR, 0.87 [95% CI, 0.76–0.99]) [45].

Attempts have been made to determine whether blocking the renin-angiotensin system (RAS) can reduce the incidence or progression of DR. The EUCLID study group investigated the effect of lisinopril, an angiotensin-converting enzyme (ACE) inhibitor, on progression of retinopathy in normotensive subjects with T1DM [21, 22]. The investigators found that lisinopril can decrease retinopathy progression in non-hypertensive patients with T1DM and little or no nephropathy. It is not known if this effect is due to RAS blockade or incremental lowering of blood pressure in normotensive subjects. Other studies, however, have not shown that ACE inhibitors decrease retinopathy development in normotensive patients with T1DM [20, 96].

The Diabetic Retinopathy Candesartan Trials (DIRECT) were a group of large, randomized trials designed to determine if blockade of the RAS with candesartan reduces the incidence or progression of DR in normotensive patients without albuminuria [22]. Almost 5 years of candesartan treatment in patients with T1DM reduced the incidence of retinopathy by two or more steps (ETDRS) in severity by 18% ($P = 0.0508$) and, in a post hoc analysis, reduced the incidence of retinopathy by three-step progression by 35% ($P = 0.034$). In patients with T1DM, there was no effect on progression of established retinopathy, but 5 years of candesartan treatment to patients with T2DM resulted in a 34% regression of retinopathy ($P = 0.009$). A significant overall trend toward less severe retinopathy was noted in patients with both T1DM and T2DM ($P = 0.03$). Though the analysis was corrected for blood pressure values obtained throughout the trial, it could not be proven conclusively that the effects on retinopathy were due to RAS blockade and not to blood pressure lowering [173]. The favorable effect of blocking the RAS was confirmed by the RASS study [102], a multicenter, randomized, double-blind, placebo-controlled, investigator-initiated trial conducted on 285 normotensive patients treated with enalapril 20 mg/day, losartan 100 mg/day, or placebo for 5 years. The primary functional endpoint was a two-step progression of retinopathy severity, which was seen in 38% of patients receiving placebo, but only 25% of those receiving enalapril ($P = 0.02$) and 21% of those receiving losartan ($P = 0.008$). Enalapril and losartan increased the likelihood of slowing the progression of retinopathy by 65% and 70%, respectively, independent of changes in blood pressure.

In 1991, Gordon et al. [59] found that lipid-lowering therapy reduced hard exudates and microaneurysms in DR and may decrease the risk of vision loss in patients with DR [26]. Several large studies have subsequently studied this question by randomizing patients to statin and or fenofibrate treatment.

The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial was a large, randomized, multicenter, controlled trial that evaluated the effects of

intensive versus standard glucose lowering, intensive versus standard systolic blood pressure (SBP) lowering, and the addition of a fibrate versus placebo to a statin on the 5-year incidence of serious CV outcomes in people with type 2 diabetes and other CV risk factors. After a mean of 3.5 years, patients receiving intensive glucose control were switched to standard glucose control because they had a higher risk of death from all causes (5% vs. 4%) [1]. Tighter glucose control lowered the risk of retinopathy progression, but among patients without DR at baseline, no differences in the incidence of developing retinopathy were seen. By 4 years, the risk of having a CV event increased to 38% (hazard ratio [HR] 1.38; 95% confidence interval [CI], 1.10, 1.74) for each category change in retinopathy severity. Compared with no retinopathy, the adjusted hazard ratios (95% CI) for the development of cardiovascular outcomes rose from 1.49 (1.12–1.97) for mild retinopathy to 2.35 (1.47–3.76) for severe retinopathy. Although the relationship was insignificant after adjusting for baseline and follow-up A1C levels, systolic blood pressure, and lipids, the authors concluded that the retina may reflect the effect of metabolic and hemodynamic factors on future CV outcomes and that these two complications of diabetes may have shared pathophysiology. ACCORD found that fenofibrate combined with simvastatin reduced the progression of DR at 2 years, but the ACCORDION extension trial showed no effect at 8 years [25].

Both the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) and ACCORD trials suggested an important role of fenofibrate for the treatment of DR, and results were aggregated for analysis. These trials included 11,388 patients with T2DM, of which 5701 were treated with fenofibrate (+/−statin) for up to 5 years. In the FIELD study, retinopathy progression was defined as laser treatment for PDR or macular edema or an increase by ≥2 steps on the Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale. Disease progression in the ACCORD-Eye study was defined as an increase of ≥3 steps on the ETDRS severity scale or proliferative disease requiring laser or vitrectomy treatment. In FIELD, fenofibrate (200 mg/day) reduced the requirements for laser therapy and was shown to arrest disease progression in patients with preexisting diabetic retinopathy. In ACCORD-Eye, fenofibrate (160 mg/day) taken with simvastatin yielded a 40% reduction in the odds of retinopathy progression when compared with simvastatin alone over 4 years. Fenofibrate reduced the need for first laser treatment by 31% ($P = 0.0002$) and progression of diabetic retinopathy with absolute reductions of 5.0% over 5 years ($P = 0.022$, FIELD) and 3.7% over 4 years ($P = 0.006$, ACCORD-Eye) [24, 79, 167, 174].

A meta-analysis of 35 articles from 1980–2008 found that higher total serum cholesterol was associated with a higher prevalence of DME, thereby clarifying previously conflicting reports regarding this risk factor [167, 177]. This was particularly relevant to trials suggesting that fenofibrate, a lipid-altering agent, may slow the development and progression of DR [79]. Fenofibrate acts mostly on triglycerides, and its effects on retinopathy in those trials were independent of lipid levels achieved. Statins, however, did not affect DR severity in the few studies in which cholesterol was evaluated, although this was not a primary outcome [31, 32].

High caloric and sodium intakes are significant and independent risk factors for the progression of DR in African-Americans with T1DM [140]. Other contributing factors in all ethnic groups include high body mass index (BMI) [36, 93], lack of physical activity, dyslipidemia in CSME [108], microalbuminuria [23], smoking, and socioeconomic factors [132]. Elevated hemoglobin levels predict the incidence of PDR in T1DM [34], whereas moderate alcohol consumption reduces the risk of PDR [9]. Advancing age at onset significantly decreases the long-term risk of PDR with the highest risk in the 5–14 years group and the lowest risk in the 15–40 years group [64]. Hormonal changes induced by puberty, including increased growth hormone and insulin-like growth factor, and the effect of the duration of prepubertal diabetes on the development of DR remain controversial [42, 49].

Increased ocular axial length, as is seen in high myopia, may be protective against the development of DR [99]. The relationship between smoking and the development of DR is unclear as some studies have discovered a link [91], whereas others have not [101].

1.5 Conclusions

The rapidly increasing number of patients with diabetes mellitus throughout the world threatens to stress healthcare systems. Epidemiological studies show that there have been remarkable improvements in the care and management of diabetes over the last 30 years that have resulted in significant decreases in the prevalence and incidence of DR in patients with T1DM [83]. Unfortunately, only limited long-term epidemiological data are available to determine whether similar trends exist for patients with T2DM [83]. Since optimal treatment of the key risk factors for DR and DME requires coordinated medical treatment, ophthalmologists caring for patients with diabetic eye disease should discuss management options with patients and primary care physicians.

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Chapter 2
The Diabetic Retina: Anatomy and Pathophysiology

2.1 Introduction

Throughout the world diabetic retinopathy (DR) has emerged as a major cause of permanent loss of vision among people over the age of 20 years. Retinopathy has generally been considered a vasculopathy that results from breakdown of the blood-retinal barrier and closure of retinal capillaries. Recent evidence, however, suggests that DR begins as a neuro-retinopathy with vascular changes occurring later during the disease.

Knowledge of retinal anatomy and pathophysiology are important to surgeons so that diagnoses and disease characterizations can be made accurately. Additionally, a detailed understanding of vitreoretinal anatomy enables surgeons to correctly deliver intravitreal depot injections of pharmaceutical agents and perform complex vitreoretinal surgery.

The biochemical abnormalities responsible for the development of diabetic retinopathy may not be important during the day-to-day practice of ophthalmology, but these abnormalities form the base of knowledge from which drugs have been developed to treat DR. Biochemical pathways that are pertinent to the development of diabetic retinopathy are complex and highly interrelated. Recognizing pivotal molecules such as vascular endothelial growth factor (VEGF) from within the biochemical milieu is an extraordinarily difficult process but one that is critical to drug development.

This chapter will cover many of the important highlights of retinal biochemistry in the setting diabetes, though a deep analysis of all affected pathways is beyond the scope of a single book chapter. The chapter begins with a discussion of retinal anatomy, then transitions to the microscopic composition of the blood-retinal barrier, and the biochemical abnormalities found in eyes affected by diabetes mellitus (DM). Finally, a brief discussion of the funduscopic characteristics of diabetic retinopathy will be provided.

M.W. Stewart, *Diabetic Retinopathy*, DOI 10.1007/978-981-10-3509-8_2

2.2 The Retina

2.2.1 History

The retina was first described by Herophilus of Chalcedon (c. 300 B.C.) and it was eventually named by Rufus of Ephesus (c. 110 A.D.). To early anatomists the retina appeared as a net that surrounded and supported the vitreous. Galen noted structural similarities between the retina and the brain, but he did not understand the retina's function. Kepler was the first to suggest that the retina served as the eye's primary photoreceptor tissue. Treviranus (1835) performed the first detailed microscopic analysis of retinal structure by employing alcohol fixation. Only with the use of electron microscopy, trypsin digest, clinical fluorescein angiography, and optical coherence tomography were scientists able to understand the retina's cellular connections, ultrastructure, and retinal vasculature, and ultimately correlate anatomic and clinical findings [169].

2.2.2 Anatomy

The retina is a translucent tissue that lines the posterior two-thirds of the eye from the macula posteriorly to the ora serrata anteriorly [87] (Fig. 2.1). The retina is contiguous with the optic disc since axons of the retinal nerve fiber form the optic nerve as they leave the eye. Anteriorly, the retina attaches to the nonpigmented epithelium of the pars plana at the ora serrata. Interdigitations between the photoreceptor outer segments and the cells of the retina pigment epithelium weakly attach the outer retina to the wall of the eye. The retina is internally attached to the vitreous at the optic nerve, macula, retinal vessels, and vitreous base.

The central 5–6 mm of the retina, referred to as the retina centralis, has more than one layer of ganglion cells (Fig. 2.2). The central 1.5 mm of the retina is bounded by the termination of the retinal capillary circulation and is called the macula lutea because of the high concentration of xanthophyll in the ganglion and bipolar cell layers [218]. The 0.35 mm depression within the central macula, called the fovea by clinicians and the foveola by anatomists, has only cones in the photoreceptor layer. The center of the foveal depression, the clivus, is situated 3.4 mm temporal and 0.8 mm inferior to the center of the optic disc.

Beyond the macula, the retina extends past the vortex veins until terminating at the ora serrata. The equatorial diameter of the human eye averages 24.06–24.08 mm, from which the retinal area is calculated to be 1206 mm^2 [169, 218]. The ora serrata lies between 5.73 mm (nasally) and 6.52 mm (temporally) posterior to Schwalbe's line and is characterized by protruding extensions of the retina (teeth) that are separated by indentations of the ciliary epithelium (ora bays) from the pars plana. The strong adhesion between the vitreous and the peripheral retina and pars plana, the

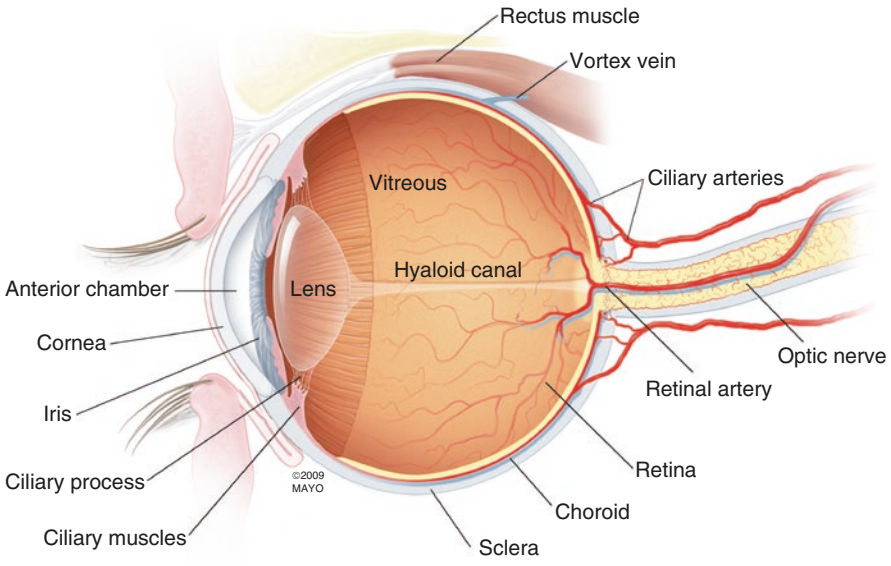

Fig. 2.1 This drawing details the important anatomic and circulatory structures of the eye and supporting tissues. The retina lines the inner, posterior two-thirds of the globe from the optic nerve to the ora serrata (just beyond the termination of the retinal arterioles). The vitreous is the most voluminous (4 ml) tissue within the eye

so-called vitreous base, extends from 1.8 to 3 mm posterior to the ora bays to 1–2 mm anterior to the ora serrata [150].

A cross-sectional view through the retina just outside the retina centralis shows nine layers (from internal to external): nerve fiber layer, ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, outer nuclear layer, external limiting membrane (ELM), rod and cone inner and outer segments, and retinal pigment epithelium (RPE) (Figs. 2.3 and 2.4). The retina is thickest around the optic disc because of the densely packed nerve fiber and ganglion cell layers, but thickness tapers to 0.18 mm at the equator and 0.11 mm at the ora serrata, since the density of all neural elements decreases in the peripheral retina.

The layered pattern of cells and connecting neural fibers within the retina enables photic energy to be converted into neuronal signals. Rhodopsin within the outer segments of rods and cones detects incoming photons and converts their energy into an electrical potential by depolarizing adjacent cellular membranes. Photoreceptor cells synapse with dendrites of bipolar cells (first-order neurons) and the processes of horizontal cells (integrating neuronal cells) in the outer plexiform layer. In the inner plexiform layer, bipolar cells synapse with dendrites of ganglion cells (second-order neurons) and amacrine cells (which provide cross wiring). Ganglion cell axons comprise the nerve fiber layer, which aggregate to form the optic nerves until they synapse with third-order neurons in the lateral geniculate body.

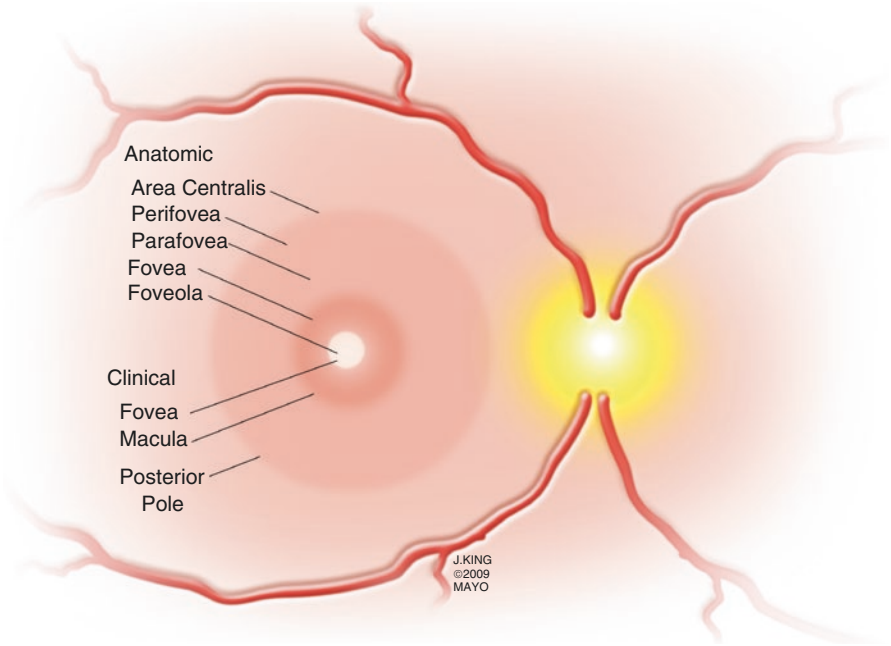

Fig. 2.2 This drawing shows the anatomic and clinical areas of the macula. Despite being referenced more frequently by anatomists, the terms parafovea and perifovea are often used in clinical practice to describe the locations of macular pathology

The central 0.4 mm of the macula is capillary-free with its cells obtaining their nutrients from the choriocapillaris [54]. The fovea contains long and slender red and green (but not blue) cones that are aligned perpendicular to the RPE, which maximally sensitizes them to incoming photons. Because the fovea lacks the inner nuclear layer, inner plexiform layer, ganglion cell layer, and nerve fiber layer, light scatter is minimized. The external plexiform layer has an unusual configuration as its axons make a right angle turn to assume a course parallel with the retina surface. After coursing a short distance parallel to the retinal surface (while forming Henle's layer), the axons turn perpendicular to the RPE to synapse with dendrites of overlying bipolar cells outside the central 0.2 mm diameter of the fovea.

Xanthophyll pigments within bipolar and ganglion cells give the retina its characteristic yellow color and function to decrease chromatic aberration, absorb potentially toxic blue light, and scavenge free radicals. Lutein is scattered throughout the posterior pole, whereas zeaxanthin is concentrated in the foveal region [81].

The parafovea and perifovea are frequently referenced regions in clinical practice. The parafovea is a 0.5 mm ring of retina that surrounds the fovea and is characterized by an accumulation of ganglion and inner nuclear cells with a thickened Henle's layer. The density of cones in the parafovea is lower than within the fovea and rods are beginning to be found. The perifovea is the outermost ring of the retina centralis, between 1.25 and 2.75 mm from the foveal center. The perifovea

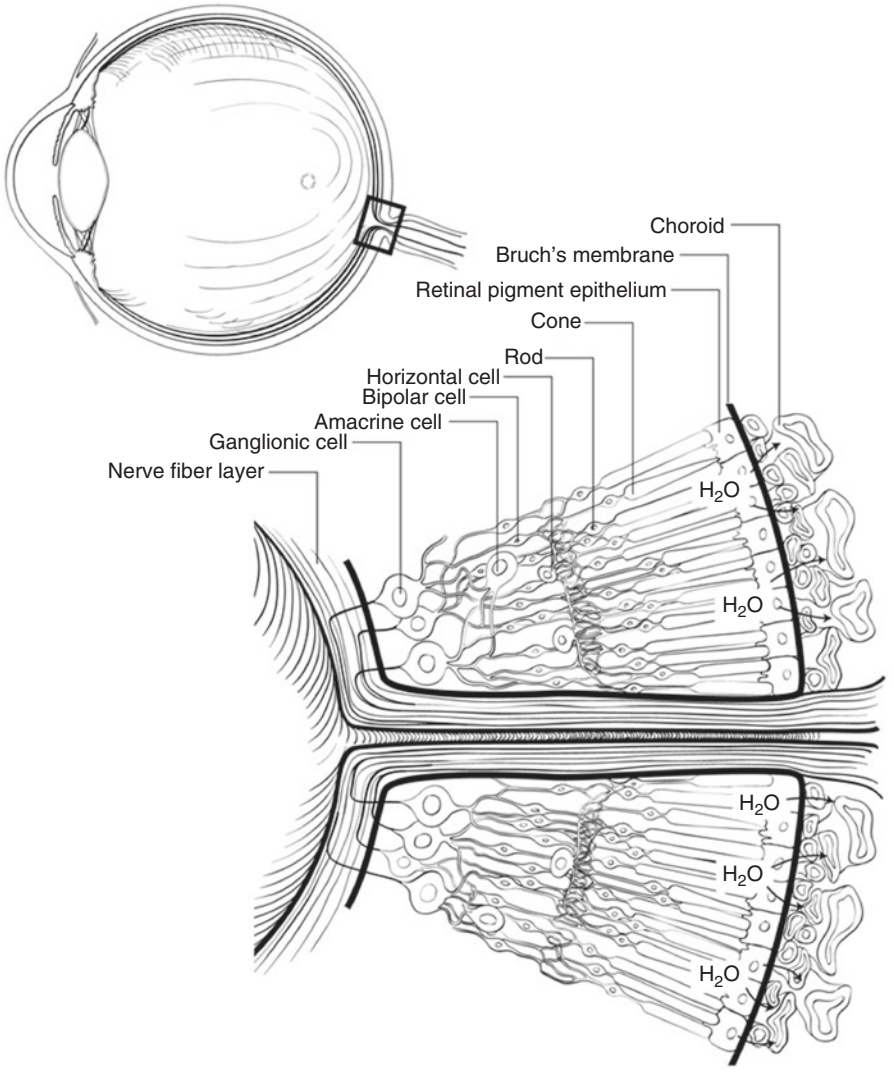

Fig. 2.3 This cross-sectional drawing of the retina near the optic nerve shows the layers of the retina and the locations of important cell types. The pumping mechanism of the retinal pigment epithelium (RPE), which removes water from the subretinal space to keep the photoreceptors in contact with the RPE, is illustrated

begins where the ganglion cell layer is four nuclei thick and ends where it thins to a single layer.

The ora serrata is the anterior boundary of the retina where it forms the junction between the multilayered neurosensory retina and the monolayered, nonpigmented epithelium of the ciliary body. The tissue in this region is thin and avascular and enjoys a close relationship with the vitreous base and zonular fibers. Collagen fibrils

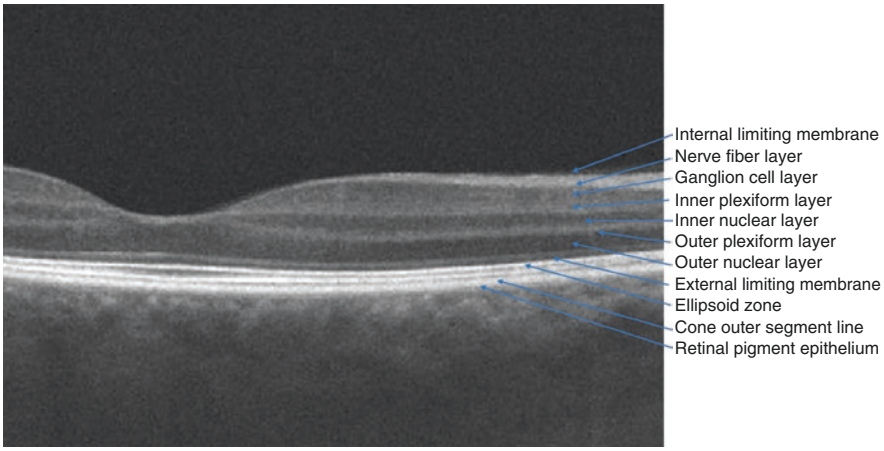

Fig. 2.4 The labeling on this spectral domain optical coherence tomography (OCT) scan through the papillomacular bundle highlights the tissue layers of the retina and important barrier structures. Structures that are commonly identified on the OCT that are often not referred to by anatomists as retinal layers – internal limiting membrane and external limiting membrane – have been included

insert into the internal limiting membrane (ILM) of the retina at the vitreous base, which normally extends from 2 to 4 mm posterior to the ora. The vitreoretinal adhesion is particularly strong along the posterior margin of the vitreous base, which makes this a common site for the development of retinal tears. A gradual loss of the nerve fiber layer, ganglion cell layer, and plexiform layers occurs near the ora as these layers are replaced with neuroglia and Müller cells. Both the ILM, into which vitreous base inserts, and the external limiting membrane (ELM), which continues anteriorly between the pigmented and nonpigmented layers of pars plana, are thickened in this region.

2.2.3 Microanatomy of the Retina Neurons

Cells within the retina fall into one of the three histologic groups: neuronal, glial, and vascular. The intricate interactions between the three types of neural cells, photoreceptors, interneurons, and ganglion cells, give the retina its primary function – converting light energy into electrical impulses. The densely packed rod and cone photoreceptor cells act as the primary neurons in the visual pathway by detecting individual photons to accurately construct visual images. Any change in the axial arrangement of the photoreceptors alters visual perception by producing micropsia if the cells are abnormally separated or metamorphopsia if the alignment is irregular.

Cone photoreceptor cells are comprised of four distinct anatomic portions: inner segments, outer segments that contain the visual pigment, a perikaryal region that contains the cell nucleus, and a synaptic terminal. The three different types of cones each contain only one light-sensitive pigment, which results in three different

spectral sensitivities. The blue, green, and yellow cone pigments maximally absorb incident light with wavelengths of 450 nm, 530 nm, and 565 nm, respectively. The visual pigment in rods, rhodopsin, is composed of the light-sensitive chromophore retinol attached to the protein opsin [197] and is most sensitive to light with a wavelength of 500 nm.

The photoreceptor outer segments have two important anatomic connections, to the inner segments (cell bodies) of the photoreceptor cells and to the extracellular matrix that separate the outer segments from the RPE. The acid mucopolysaccharides of the matrix are probably synthesized by the photoreceptor inner segments and form the "glue" that keeps the outer segments attached to the RPE [5]. The nuclei of the cones are situated 3–4 μm internal to the ELM and comprise the outer nuclear layer. The photoreceptors form synaptic junctions with both interneurons and Müller cells. The plasma membrane of each photoreceptor and Müller cell is differentiated into a dense band known as the external limiting membrane. Rods and cones do not come into direct contact as they are insulated from each other by the Müller cells. The Müller cells contact each other in zonula adherens, which are thought to form a relative diffusion barrier between the intercellular space of the inner retina and the extracellular matrix between the photoreceptor outer segments and the RPE.

The outer plexiform layer lies between the inner and outer nuclear layers. The synaptic zone is composed of numerous intercellular junctions and synapses between neural and glial processes and creates the middle limiting membrane. This resembles the ELM and may also act as a partial barrier to diffusion of fluid and larger molecules as it may prevent exudates, hemorrhages, and cysts from spreading throughout the entire retina.

The inner plexiform layer is located between the ganglion and inner nuclear cell layers and contains Müller cell branches, retinal blood vessels, and synaptic processes of the bipolar, ganglion, and amacrine cells. Synaptic units called dyads each consist of a bipolar cell contacting two processes, one from a ganglion cell. Dyad density is approximately 2.9 million/mm^2 [47].

The ganglion cell bodies comprise a distinct layer between the inner plexiform and nerve fiber layers. Throughout much of the retina, there is one ganglion cell for every 100 rods and every 4–6 cones, but the ganglion cell to photoreceptor ratio is higher in the macula. This creates a smaller receptor field for each ganglion cell and greater image resolution. There are no ganglion cells at the center of fovea, but the ganglion cells are so densely packed within the extrafoveal macula that there may be two or more for each cone [42]. Ganglion cells consist of two types: midget and diffuse. Midget ganglion cells cover small areas (<10 μm^2) and synapse with only one midget bipolar cell, though each midget bipolar cell may synapse with several ganglion cells. The large, polysynaptic, diffuse ganglion cells synapse with retinal bipolar and amacrine cells via dendrites and cells in the lateral geniculate body via axons.

Ganglion cell axons course through the nerve fiber layer of the inner retina until entering the optic nerve. The axons remain unmyelinated until they reach the lamina cribrosa at which point they acquire myelin sheaths. Axons directly contact each

other without interposed glial cells, except for interdigitating Muller cell processes. Most axons assume a generally radial course toward the optic nerve except for those immediately temporal to the disc, which form the papillomacular bundle. Since these axons are the first to develop, they form the center of the optic nerve. The nerve fiber layer thickens as axons converge at the optic nerves.

Axons cannot survive if they are detached from their respective cell bodies [152] as both proximal and distal axonal degeneration are seen after acute retinal or optic nerve ischemia. Cotton wool spots or optic disc edema are usually seen on funduscopic examination. Though cotton wool spots were long believed to represent focal infarctions of the retinal nerve fiber layer, they may actually be boundary sentinels of inner retinal ischemia [135]. After axonal degeneration, residual defects in the nerve fiber layer can often be seen on optical coherence tomography (OCT) scanning or funduscopic examination.

Müller cells form tight junctions with both other Müller cells and with neural cells. A continuous row of Müller cell zonula adherens forms the external limiting membrane, which acts as a relative barrier to metabolite movement into and out of the retina [142]. Müller cells comprise the majority of retinal glial cells but astrocytes are more widely distributed between blood vessels and neurons.

2.2.4 Intercellular Spaces

Neural cells are 10–20 μm apart within the retina, with spacing similar to that found in the brain. Intercellular spaces are filled with low density matrix material that permits diffusion of even large proteins [173] until they reach the relatively impervious external limiting membrane. The potential subretinal space is filled with an inter-photoreceptor matrix that is comprised of glycosaminoglycans, glycoproteins, and filamentous structures [85]. The most common inter-receptor matrix protein is interstitial retinal binding protein (IRBP), which is synthesized and secreted by rod photoreceptor cells and binds all-trans retinal and 11-cis retinal. Little is known about other matrix proteins.

2.2.5 Internal Limiting Membrane

The ILM is composed of laminin, basement membrane (BM) proteoglycans, fibronectin, and collagen [96] and represents the retina's only true basement membrane. The outer portion consists of the basement membrane of the Müller cells, whereas the inner portion is formed by vitreous fibrils and mucopolysaccharides. The ILM is 2000 nm thick over the macula but thins to only 20 nm over the fovea where the density of Müller cells decreases [209]. Müller cell processes form a continuous but uneven border of attachment with the ILM, though the exact nature of the vitreous attachment to the ILM is not known (see Chap. 7).

2.2.6 Circulation

The retina's demand for oxygen is higher than that of any tissue in the body, and to meet this high metabolic demand, the retina relies on two distinct circulations: the inner two-thirds of the retina relies on the retinal vasculature, whereas the outer one-third relies on the choroidal circulation. Flow through the choroidal circulation is high but variable with easy molecular transfer with the surrounding tissues. Flow through the retinal circulation is low but constant with a high rate of oxygen extraction [11].

2.2.7 Arteries

The central retinal artery (CRA) penetrates the optic nerve about 10 mm posterior to the globe, leaves the nerve at the optic disc, and rapidly branches into progressively smaller arterioles. The central retinal artery supplies the entire inner two-thirds of the retina, except in the 20% of eyes with cilioretinal arteries. The histologic structure of the CRA resembles that of other comparable-sized arteries with a luminal diameter of 200 μm, a wall thickness of 35 μm, a single layer of endothelial cells, a subendothelial elastica, an internal elastic lamina, a medium of smooth muscle, and an external elastic lamina that merges with the adventitia. When the artery enters the eye, the elastic lamina is lost but the muscularis is unusually prominent. Degenerative diseases that affect muscular arteries, such as atherosclerosis and giant cell arteritis, also affect the intraneural retinal artery. Arteries within the retina are spared from giant cell arteritis because they lack an internal elastic lamina, whereas atherosclerosis, with its subendothelial plaque formation and hyperplasia of the intimal and endothelial layers, can affect any portion of the retinal artery.

The retinal artery divides into superior and inferior branches immediately upon entering the eye, then to smaller branches according to either dichotomous (equal-sized bifurcation) or side-arm branching. The arteries are situated in the nerve fiber layer or ganglion cell layers, and only the smaller arterioles descend into the inner plexiform layer to supply capillaries [88]. Strong connections exist between the arteries and cortical collagen in the ILM. Vitreous traction on the ILM can elevate the retinal arteries without affecting deeper retinal layers. The arteries are usually superficial to the veins but may dive to the inner nuclear layer at arteriovenous crossing sites.

Within smaller branches of the artery, the muscular layer thins from seven cell layers at the disc to two layers at the equator, and the luminal diameter thins from 120 μm at the disc to 8–15 μm at the equator. The vascular endothelial cells contain tight junctions that prevent the passage of large molecules into or out of the vascular lumens [167], which limits transfer of materials to diffusion and endothelial pinocytosis. Müller and astrocyte cell processes surround the vessels and insulate them from surrounding retinal neural tissue.

Tissue oxygen tensions, metabolic by-products, and intraocular and systemic blood pressures autoregulate the retinal circulation [52]. It is not known if sympathetic or parasympathetic nerves innervate the retinal arteries, but studies suggest that adrenergic binding sites are present on cells and that retinal blood flow can be altered by adrenergic agonists and antagonists [58, 168].

2.2.8 Veins

The central retinal vein consists of a layer of endothelial cells, subendothelial connective tissue, a medium of mostly elastic fibers, a few smooth muscle cells, and a thin connective tissue adventitia. The vein is separated from the surrounding neural tissue by Müller cells and astrocyte processes. The diameter of the vascular lumen decreases from 150 μm at the disc to 20 μm at the equator. In the peripheral retina, smooth muscle cells in the medium are lost and pericytes take their place. Muscle cells enable the venous diameter to change according to the pressure differential across the lumen. In patients with diabetes or carotid artery disease, sluggish flow relaxes the smooth muscles and the veins become sausage-shaped. The central retinal vein is the only outlet for the retinal circulation, but potential anastomoses between the retinal and choroidal circulations at the disc may become manifest in cases of central retinal vein occlusion or compressive lesions of the optic nerve.

2.2.9 Capillaries

Capillaries are found throughout the retina except in the foveal avascular zone, the retina adjacent to major arteries and veins, and the far periphery. The capillary network originates from the arterioles in the ganglion cell layer and spreads through the inner nuclear cell layer. No vessels are found in the outer plexiform and outer nuclear layers. Capillaries are distributed in two layers with a superficial network in the ganglion and nerve fiber cell layers and a deeper network in the inner nuclear layer. The diameters of these vessels range from 5 to 10 μm. The volume of the outer vascular network is relatively constant throughout the retina, whereas the volume of the inner network varies with the thickness of the nerve fiber layer. Though only one capillary layer is found in the perifoveal region, up to four different capillary layers are found in the peripapillary region.

Peripapillary capillaries drain directly into venules on the optic nerve [84]. Within 2 disc diameters of the nerve, these capillaries have long, straight, or slightly curved paths with minimal anastomoses. This unique anatomy makes the capillaries susceptible to elevated IOP and changes in retinal perfusion pressure, which has been used to explain the development of arcuate scotomas in glaucoma, peripapillary flame-shaped hemorrhages in papilledema and hypertension, and cotton wool spots in disorders causing retinal ischemia.

The capillary wall consists of endothelial cells, pericytes, and a basement membrane. The narrow vascular lumen (3.5–6 μm) and thin endothelial cell bodies cause nuclei to bulge inward, which requires erythrocytes to distort and mold when passing.

The capillary endothelial cells are connected by tight junctions that comprise the inner blood-retinal barrier (BRB) [167]. Pinocytotic vesicles transfer metabolites from the circulation to the retina. Diseases such as diabetes that damage the endothelium and disrupt the BRB allow protein and lipid to leak into the retina. The leakage is reversible through endothelial cell mitosis and the formation of new tight junctions [206].

Pericytes are situated within the endothelial cell basement membranes. Contraction of mammalian pericytes has not been demonstrated in vivo, but pericytes contain contractile proteins and contract in vitro when exposed to endothelin [29], thromboxane A_2 [46], and angiotensin II [132]. Loss of pericytes, due to ischemic retinopathies such as diabetes mellitus, weakens capillary walls and results in the formation of microaneurysms [35].

2.3 Hemodynamics, Macular Edema, and Starling's Law

Movement of fluid both into and out of capillaries depends upon hydrostatic and oncotic pressures. Formation and resorption of macular edema can be described by Starling's law (Fig. 2.5).

The four primary Starling's forces are as follows:

1. Hydrostatic pressure within the capillary lumen (P_c)
2. Hydrostatic pressure within the retinal interstitium (P_i)

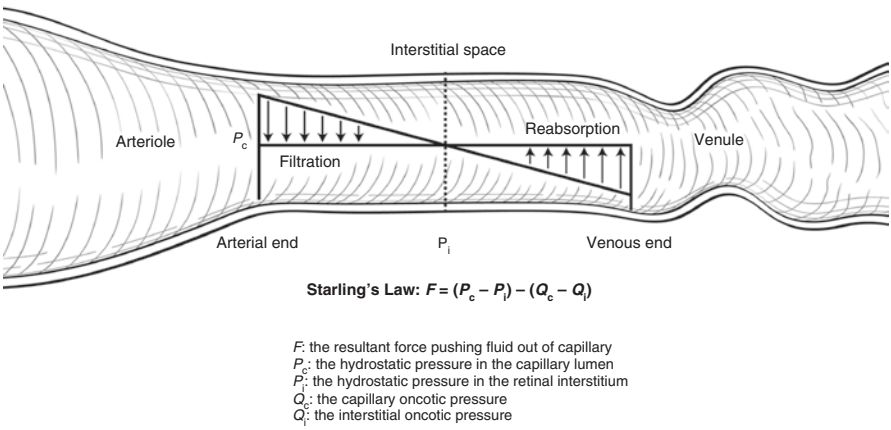

Fig. 2.5 Starling's law states that movement of fluid across capillary walls is determined by the net hydrostatic pressure less the net oncotic pressure. In eyes with diabetic retinopathy, greater blood flow increases the net oncotic pressure, and breakdown of the blood-retinal barrier enables protein to accumulate in the interstitium and increase the net oncotic pressure. The end result is net fluid movement out of the capillaries into the interstitium leading to macular edema

3. Capillary oncotic pressure (Q_c)
4. Interstitial oncotic pressure (Q_i)

Capillary hydrostatic pressure is primarily determined by systemic blood pressure, whereas tissue hydrostatic pressure is approximately the same as intraocular pressure. Most of the capillary oncotic pressure is created by albumin, whereas, with healthy vascular endothelium, tissue oncotic pressure is determined by interstitial proteins. The net force pushing fluid out of capillaries is the difference between hydrostatic pressures and oncotic pressures and can be represented by the following equation:

$$F = (P_c - P_i) - (Q_c - Q_i)$$

where F is the resultant force that determines fluid movement. If F is positive, fluid moves out of the capillary into the interstitium and forms tissue edema. If F is negative, then the net movement of fluid is out of the tissue and into the capillary. At equilibrium:

$$F = 0 = \Delta P - \Delta Q$$

where there is no net movement of fluid across the capillary walls.

Edema can be defined as the abnormal swelling of soft tissues, which in the case of diabetic retinopathy represents the retinal interstitium. Edema can be cytotoxic, where the fluid accumulates within cells, or vasogenic, where fluid accumulates within the interstitial spaces. Cytotoxic edema occurs with severe ischemic conditions such as central retinal artery occlusions. Starling's law applies to the formation of vasogenic edema, which is the most common form of edema in retinal vasculopathies such as diabetic macular edema and retinal vein occlusions.

Retinal edema occurs when the net hydrostatic force (forcing fluid into the interstitium) exceeds the net oncotic force (drawing fluid into the capillary lumen) across capillary walls. This usually occurs because of an increase in transluminal hydrostatic pressure, as with systemic hypertension or ocular hypotony, or because of a decrease in transluminal oncotic pressure, as occurs with increased interstitial proteins due to breakdown of the BRB or with a decrease in plasma proteins as with liver disease or protein wasting nephropathies.

Intraluminal pressure falls through the length of the arteriole system, but hydrostatic pressure in the capillaries and venules still depends upon the arterial blood pressure. Systemic arterial hypertension increases capillary hydrostatic pressure and aggravates the severity of diabetic macular edema. Patients with diabetic macular edema should undergo a thorough blood pressure assessment, and if systemic hypertension is discovered, appropriate pressure lowering treatment should be initiated. Reduction of systemic blood pressure may be sufficient to improve diabetic macular edema in some cases [134, 186].

Poiseuille's law describes flow within a tube, where the resistance to flow is inversely related to the radius of the lumen raised to the fourth power. Retinal arterioles dilate under hypoxic conditions such as diabetic retinopathy, which decreases resistance and increases hydrostatic pressure [178–180]. Retinal blood vessels have been observed to progressively dilate as diabetic macular edema forms [118].

Macular edema also forms in eyes with ocular hypotony. Since tissue hydrostatic pressure is equal to intraocular pressure, hypotonous eyes develop macular edema

due to a widened hydrostatic pressure gradient. Edema may improve in these eyes if intraocular pressure rises [115]. When the retina is subjected to vitreous traction, interstitial hydrostatic pressure decreases and macular edema or subretinal fluid accumulates [122].

Capillary oncotic pressure decreases in patients with hypoalbuminemia due to the nephrotic syndrome, protein-deficiency malnutrition, or severe liver disease. On the other hand, breakdown of the BRB allows albumin to leak into the interstitial spaces and increase tissue oncotic pressure. Endothelial damage, like that seen with diabetic retinopathy and vein occlusions, compromises the intercellular tight junctions and breaks down the blood-retinal barrier. Increased vascular porosity results in oncotic pressure shifts and the development of interstitial macular edema. There is a strong correlation between increased vascular endothelial growth factor (VEGF) levels, breakdown of the BRB, and the formation of macular edema [148].

2.4 Biochemical Basis for Diabetic Retinopathy

Patients with DM develop similar microvascular abnormalities in the retinal vasculature, renal glomeruli, and vasa vasorum of the peripheral nerves. Alterations in retinal blood flow and increases in vascular permeability are seen soon after the development of DM. Decreased activity of vasodilators such as nitric oxide together with increased activity of vasoconstrictors such as angiotensin II and endothelin-1 can be measured. The resultant qualitative and quantitative abnormalities in the extracellular matrix contribute to increases in vascular permeability. Microvascular cell loss results from programmed cell death. Cell loss together with the overproduction and deposition of extracellular matrix proteins and periodic acid-Schiff-positive proteins induced by growth factors such as TGF-β leads to progressive capillary occlusion. Chronic hyperglycemia decreases the production of endothelial and neuronal cell trophic factors, which leads to edema, ischemia, and hypoxia-driven neovascularization [25]. In nondiabetic patients, atherosclerosis begins with endothelial dysfunction [131], whereas in diabetic patients initiation seems to involve insulin resistance [90].

Four hypotheses have been advanced to explain the mechanism of hyperglycemia-induced microvascular damage (Fig. 2.6). These are:

1. Increased polyol pathway flux
2. Accumulation of advanced glycation end products (AGEs)
3. Activation of protein kinase C (PKC)
4. Increased hexosamine pathway flux

Inhibitors specific to aldose reductase, AGE formation, PKC activation, and the hexosamine pathway each prevent various diabetes-induced abnormalities. No common element to these pathways had been noted until the recent discovery that each causes overproduction of superoxide by the mitochondrial electron transport chain (Fig. 2.7) [25]. Consistent with this observation is that both diabetes and hyperglycemia increase oxidative stress [79].

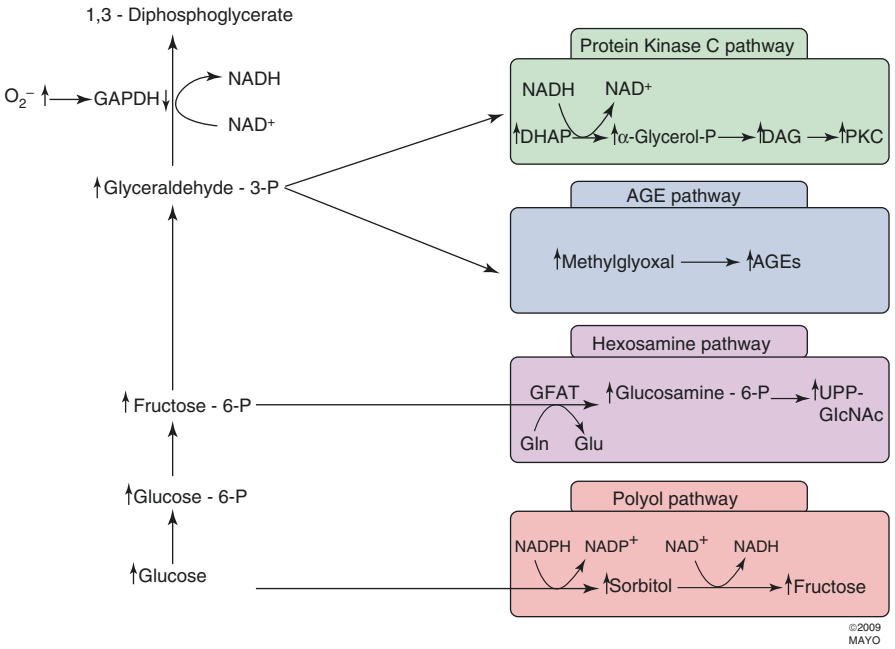

Fig. 2.6 Hyperglycemia dysregulates four biochemical pathways: protein kinase C pathway, advanced glycation end product (AGE) pathway, hexosamine pathway, and polyol pathway. Each of the pathways has been associated with the development of diabetic retinopathy

To understand how hyperglycemia increases reactive oxygen species (ROS), one must examine changes in the electron transport within the mitochondria. Hyperglycemia causes overproduction of the electron donors (NADH and $FADH_2$) by the tricarboxylic acid (TCA) cycle [49], which increases the proton gradient across the inner mitochondrial membrane. This sufficiently prolongs the lifespan of electron transport intermediates, such as ubisemiquinone, to generate superoxides. Experimental evidence shows that two regulatory enzymes can be exploited to uncouple hyperglycemia-induced production of ROS. Upregulation of manganese superoxide dismutase (MnSOD) eliminates the production of reactive oxygen species, and an excess of uncoupling protein-1 (UCP-1) eliminates the protein electrochemical gradient [73]. Furthermore, overexpression of either MnSOD or UCP-1 prevents PKC activation of the hexosamine pathway, AGE formation, and an increase in polyol pathway flux. This evidence strongly supports the contention that excessive superoxide is pivotal to the unified theory of diabetic retinopathy.

Other experimental evidence links hyperglycemia, ROS, and the four previously mentioned biochemical pathways. A hyperglycemia-induced increase in ROS decreases glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activity, which increases the concentrations of upstream glycolytic metabolites. These increase flux through the polyol pathway. Elevated triose phosphate levels increase the formation of methylglyoxal-derived AGE, the most common AGE that results from hyperglycemia.

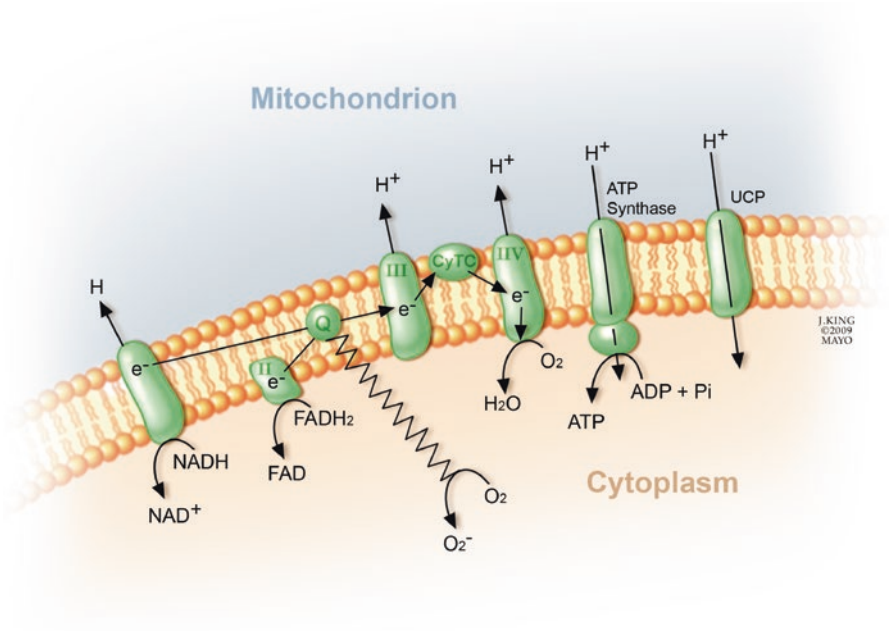

Fig. 2.7 In the presence of O_2, electron transfer through the mitochondrial cytochromes regenerates adenosine triphosphate (ATP) from adenosine diphosphate. Dysregulation of each of the previously mentioned biochemical pathways disrupts electron transfer and shunts the electrons toward the synthesis of superoxide (O_2^-)

Triose phosphate levels rise due to GAPDH inhibition by ROS [50]. ROS-induced decreases in GAPDH activity increase the concentration of fructose-6-phosphate, the primary substrate for the hexosamine pathway. Inhibition of GAPDH increases concentrations of dihydroxyacetone phosphate, which increases DAG concentrations and activates PKC.

Several experimental models have shown that elevated MnSOD or UCP-1 activity prevents hyperglycemia-induced complications. Overexpression of either protein prevents monocyte adhesion to aortic endothelial cells [25], hyperglycemia-induced decrease in eNOS activity [49], and collagen-induced platelet aggregation and activation [210]. Increased MnSOD activity prevents increase collagen synthesis [37] and decreases programmed cell death induced by hyperglycemia.

Continued oxidative stress leads to chronic inflammation, which is a strong mediator of most chronic diseases including diabetes. Oxidative stress upregulates several transcription factors including AP-1, HIF-1α, β-catenin/Wnt, NF-κB, PPAR-γ, p53, and Nrf2. Activation of these factors leads to the expression of over 500 genes, including those for growth factors, inflammatory cytokines, chemokines, cell regulatory molecules, and anti-inflammatory molecules [156].

Inflammatory cells also produce soluble mediators, such as metabolites of arachidonic acid, cytokines, and chemokines that recruit additional inflammatory cells to the site of damage and produce more reactive species. These key mediators

activate signal transduction cascades and induce changes in transcription factors that mediate immediate cellular stress responses. Induction of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), aberrant expression of inflammatory cytokines (tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6) and chemokines (IL-8, CXC chemokine receptor 4 (CXCR4)), and alterations in the expression of specific microRNAs have also been reported to play a role in oxidative stress-induced inflammation [92].

Our current understanding of retinal biochemistry identifies a series of pathways, all of which share substrates and enzymes and are regulated by positive and negative feedback loops. This complicated biochemical mosaic allows for considerable and complicated crossover reactions among pathways, which suggests that successful inhibition of any single pathway will probably produce an incomplete clinical response. If the unified theory of diabetic retinopathy – hyperglycemic production of reactive oxygen species – is correct, then limiting the formation of ROS may ultimately yield the most effective therapies.

A considerable amount of clinical research continues to focus on decreasing diabetic complications by minimizing changes in the four hyperglycemia-driven pathways. Further discussion of these pathways, therefore, appears warranted.

2.4.1 Increased Polyol Pathway Flux

Aldose reductase (the first enzyme in the polyol pathway) has a low affinity for glucose at normal concentrations, but during hyperglycemia glucose is increasingly converted to sorbitol with associated decreases in NADH. Sorbitol is subsequently oxidized to fructose with reconstitution of NADH. Sorbitol oxygenation increases the NADH/NAD+ ratio in the cytosol, which inhibits glyceraldehyde-3-aldehyde dehydrogenase (GAPDH) activity. This increases concentrations of triose phosphate [205], which increases formation of methylglyoxal (a precursor of AGEs) and diacylglycerol (DAG) (which activates PKC). Reduction of glucose to sorbitol consumes NADPH, and since NADPH is also required to reduce glutathione, this could worsen oxidative stress. Unfortunately, attempts to inhibit the polyol pathway *in vivo* have yielded only mixed results. A 5-year study of polyol pathway inhibition in diabetic dogs prevented diabetic neuropathy but failed to prevent retinopathy [51]. Zenarestat, an aldose reductase inhibitor, had a positive effect on diabetic neuropathy in humans [77].

2.4.2 Advanced Glycation End Products (AGEs)

Intracellular hyperglycemia promotes the formation of AGEs, which are found in elevated concentrations in diabetic retinal blood vessels [182, 184, 185] and glomeruli [89]. The intracellular auto-oxidation of glucose to glyoxal, the decomposition

of the Amadori product (glucose-derived 1 amino-1-deoxyfructose lysine adducts) to 3-deoxyglucosone, and the fragmentation of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate to methylglyoxal, each reacts with amino groups of intracellular and extracellular proteins to form AGEs [44, 192, 202]. The AGE inhibitor aminoguanidine partially prevents microvascular damage in animal models [176] and lowers urinary protein concentrations and may slow progression of retinopathy in humans [23].

The intracellular production of AGE precursors damages target cells by modifying critical proteins and altering their functions. These change extracellular matrix components and integrins and modify plasma proteins that bind to AGE receptors. The end result is receptor-mediated production of reactive oxygen species.

The formation of AGEs alters the properties of several extracellular matrix proteins. Advanced glycation end product-induced cross-linking expands the molecular packing of type I collagen and changes the composition of type IV collagen in basement membranes, thereby altering the function of blood vessels [91]. Advanced glycation end product formation on laminin decreases the self-assembly of polymers, decreases binding to type IV collagen, and decreases binding to heparin sulfate proteoglycan [30]. The formation of AGEs on the extracellular matrix interferes with matrix-cell interactions. Modification of type IV collagen-binding domains in the matrix decreases endothelial cell adhesion.

Several cell-associated proteins that bind AGEs have been identified. These include OST-48, 80 K-H, galectin-3, macrophage scavenger receptor type II, and RAGE. These proteins mediate the long-term effects of AGEs on macrophages, glomerular mesangial cells, and vascular endothelial cells through the expression of cytokines and growth factors (interleukin-1, insulin-like growth factor-I, tumor necrosis factor-α, TGF-β, macrophage colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and platelet-derived growth factor) by macrophages and mesangial cells and the expression of pro-coagulatory and pro-inflammatory molecules (thrombomodulin, tissue factor, and VCAM-1) by endothelial cells. Many of these ligands bind to endothelial AGE receptors and mediate VEGF-induced capillary wall hyperpermeability [129].

2.4.3 Activation of Protein Kinase C (PKC)

The protein kinase C family is made up of at least 11 isoforms, 9 of which are activated by diacylglycerol (DAG). Intracellular hyperglycemia increases DAG synthesis from dihydroxyacetone phosphate in both the retina and renal glomeruli [117]. Hyperglycemia also activates PKC isoforms indirectly by ligating AGE receptors [153] and increasing activity of the polyol pathway [103]. Activation of PKC-β isoforms depresses nitric oxide production and increases endothelin-1 activity to mediate blood flow abnormalities in the retina and kidney [93].

Activated PKC increases the accumulation of matrix protein by upregulating the expression of TGF-β1, fibronectin, and type IV collagen, which is mediated by

downregulating nitric oxide [38]. Protein kinase C overexpresses the fibrinolytic inhibitor PAI-1 and activates NF-kB in endothelial and vascular smooth muscle cells [151, 211] and regulates and activates various membrane-associated NADP(H)-dependent oxidases.

A PKC-β-specific inhibitor reduces activity in the retina and renal glomeruli of diabetic animals, reverses diabetes-induced increases in retinal mean circulation time, normalizes glomerular filtration rate, and corrects urinary albumin excretion [116].

2.4.4 Increased Hexosamine Pathway Flux

Excess flux through the hexosamine pathway activates genes that lead to vascular endothelial dysfunction and other changes that are seen in diabetic retinopathy. Excess intracellular glucose in the form of fructose-6-phosphate is diverted from glycolysis in order to provide substrates for reactions requiring UDP-N-acetylglucosamine. End products of these reactions include proteoglycans and O-linked glycoproteins. The mechanism by which increased hexosamine pathway flux mediates hyperglycemia-induced increases in gene transcription is not known, but covalent modification of the transcription factor Sp1 by N-acetylglucosamine (G1cNAc) might explain the link between the hexosamine pathway and hyperglycemia-induced changes in transcription of the gene for PAI-1. The glycosylated form of Sp1 upregulates transcription more than the deglycosylated form. Glycosylated Sp1 is inversely associated with phosphorylated Sp1, which suggests that the two molecules compete for the same binding site and may represent a more generalized mechanism for regulating glucose-responsive gene transcription [82].

2.5 Early Pathophysiology of Diabetic Retinopathy

One of the earliest histopathological changes observed in diabetic patients and diabetic models is thickening of the capillary BM (basement membrane) [66, 67]. Thickening is thought to result from increased synthesis of collagen IV, fibronectin, and laminin and/or reduced degradation of the BM by catabolic enzymes [128, 158, 182, 183]. It remains uncertain whether BM thickening is of primary or secondary importance in the development of DR, but it has been speculated that these matrix modifications may contribute to impaired endothelial-pericyte communication, defects in capillary autoregulation, or inappropriate cell interaction with constituent BM proteins [20, 146, 159, 184].

Mitochondria of retinal pericytes exhibit fragmentation, metabolic dysfunction, and reduced extracellular acidification when exposed to high glucose environments. This may be responsible for the early apoptotic death of both pericytes and smooth muscle cells during DM [67, 138]. The ratio of pericytes to endothelial cells in

patients with diabetes decreases from 1:1 to 1:4 [19, 80]. Vascular endothelial cells become dysfunctional at the same time, but over the short term, they can sufficiently compensate for the developing cellular deficit. Because of prolonged exposure to the diabetes-induced biochemical milieu, the replicative ability of the endothelial cells ultimately becomes critically compromised [83, 126].

It has been proposed that the primary cellular defect in diabetic retinopathy lies with the vascular endothelium and that retinopathy could be considered an endotheliopathy [104]. The endothelium is critical to normal function of other cells in the capillary complex [66], and though the precise basis of endothelial, pericyte, and smooth muscle dysfunction in the diabetic retinal microvasculature remains obscure, it is most likely related to an array of cumulative biochemical insults coupled with impaired ability of the cells to repair and renew themselves. Retinal pericytes and smooth muscle cells rely on key growth/survival factors, such as PDGF (platelet-derived growth factor)-B [61, 86], which is selectively depleted during diabetes [36] and is closely related to the formation of acellular capillary casts [80].

2.6 Macular Edema

Retinal nutrition is supplied by two distinct circulations: the choriocapillaris nourishes the outer third of the retina and the two vascular layers (superficial plexus in the axon and ganglion cell layers, deep plexus in the inner nuclear layer) of the retinal capillary circulation. Two distinct, though often coexisting, types of diabetic maculopathy are seen: ischemic maculopathy results from capillary dropout, and macular edema results from breakdown of the blood-retinal barrier in the retinal capillaries and/or RPE.

The exact location of macular edema – extracellular or intracellular – has not been well established as previous studies have implicated both locations. Histologic studies of enucleated eyes with diabetic retinopathy have identified swelling and degeneration of Müller, bipolar, ganglion, and photoreceptor cells [59] and extracellular cystic spaces [69]. Though angiographic ischemia and macular edema may coexist, their effects on visual acuity appear to be independent. Arend [12] found no correlation between blood flow and macular edema, thereby concluding that inner retinal ischemia does not cause macular edema. Retinal thickening appears to correlate with blood-retinal barrier breakdown, but ischemia was not correlated with macular thickening, thereby suggesting that the major cause of retinal thickening was extracellular expansion [174]. These studies conclude that ischemia may lead to a small amount of intracellular swelling, but extracellular fluid accumulation is the primary contributor to macular edema [8].

Even before the development of obvious retinopathy, diabetes can either increase or decrease retinal blood flow. Once retinopathy becomes established, however, retinal blood flow usually increases [171]. The autoregulatory function of retinal blood vessels is lost, and both arterioles and venules are noted to dilate and elongate. This leads to decreases in arteriole resistance and loss of pressure across the length of the

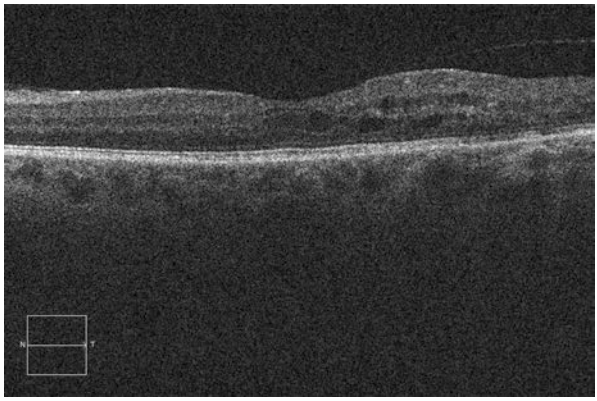

Fig. 2.8 This optical coherence tomography scan demonstrates cystoid macular edema, most which can be seen in the inner and outer nuclear layers

arteriole. Effective intraluminal pressure in the retinal capillaries increases, which, according to Starling's law, increases fluid movement into the extracellular space. The concomitant release of vasoactive cytokines (such as VEGF) from endothelial cells, neuroglia, and activated leukocytes breaks down the blood-retinal barrier via several mechanisms and leads to albumin movement into the interstitium.

Total flow through the choroidal circulation decreases in patients with diabetes [120]. Bulk flow to the choroid is relatively unchanged in diabetics, but indocyanine green angiography shows focal areas of choroidal non-perfusion in eyes with diabetic pigment epitheliopathy. Significant leakage across the pigment epithelium sometimes occurs in eyes that have only minimal retinopathy [200]. Both neovascularization within the choroidal and occlusion of the choriocapillaris occur more commonly in diabetics than in individuals without diabetes. These processes probably result from the same inciting events – basement membrane degeneration and angiogenesis – that lead to the development of diabetic retinopathy [63].

Breakdown of the blood-retinal barrier can be detected by vitreous fluorophotometry before edema accumulates within the retina. This probably results from early protein loss from the gap junctions that allows molecules smaller than albumin to pass out of the capillary lumen. The first manifestation of diabetic retinopathy in other patients, however, may be a retinal pigment epitheliopathy [200]. Retinal pigment epithelial dysfunction is probably underappreciated in patients with DR since overlying retinal vascular disease masks RPE dysfunction on fluorescein angiography.

Histopathologic studies have shown that cystoid macular edema develops in the inner nuclear layer and the outer plexiform layer (Fig. 2.8) [194], but large volumes of fluid can "spill over" to the outer nuclear layer (Fig. 2.9). Both histologic studies and OCT scans suggest the presence of multiple cystoid spaces, but further analysis shows that fluid exists in large cavities that are often spanned by Müller cells.

Fluid accumulation due to leaking capillaries of the inner retina is usually limited by the inner and outer plexiform layers, causing fluid to accumulate within the inner nuclear layer. This fluid displaces tissue in the plexiform layers, increases tissue density, and forms a relative barrier to further spread of the edema. Breakdown of the outer blood-retinal barrier often causes fluid to accumulate between the outer

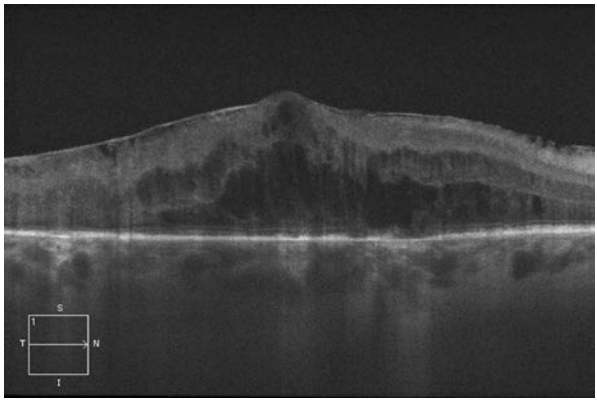

Fig. 2.9 This optical coherence tomography scan through the macula shows significant edema in the outer nuclear layer. Note the prominent epiretinal membrane and the intact external limiting membrane and ellipsoid zone

plexiform layer and the external limiting membrane. As demonstrated in horseradish peroxidase studies in diabetic rats [193], eyes with intact external limiting membranes will develop limited exudative retinal detachments with fluid accumulation between the retinal pigment epithelium and photoreceptor outer segments.

2.6.1 Blood-Retinal Barrier

The retina is protected by two distinct blood-retinal barriers: the inner BRB consists of the retinal capillary endothelial cells and their tight junctions, and the outer BRB consists of the retinal pigment epithelium and their tight junctions. The cell membranes of the epithelial and endothelial cells are bilayers with hydrophilic glycerols bounding hydrophobic long-chain fatty acid moieties. Under physiologic conditions, the cell membranes allow small hydrophobic molecules, water, and small uncharged polar molecules to pass, but form an impenetrable barrier to ions and larger uncharged polar molecules.

Adjacent retinal vascular endothelial cells and retinal pigment epithelial cells are connected by tight junctions that are composed of several intercellular proteins including occludin, claudin (23 isoforms), 7H6, cingulin, zonula occludens (ZO)-1,2,3, junction adhesion molecule (JAM), membrane-associated guanylate kinases with inverted domain structures (MAGI)-1,2,3, partition defective genes (PAR)3/6, and multi-pdz protein-1 (MUPP1) (Fig. 2.10) [133]. Some of the proteins have been extensively studied and well characterized, with occludin and the claudins controlling much of the barrier function [106]. Loss of occludin, which is specific to vascular endothelial cells, [10] in diabetic rats correlates with increased blood-retinal permeability to albumin (molecular weight of 66 kD) but not rhodamine-dextran (molecular weight of 10 kD), which may be due to changes in hydrophobicity but not pore size. Claudin-5 prevents passage of small (<0.8 kD) molecules across the blood-brain barrier [141] and probably serves the same purpose in the retina [55]. In retinal capillary endothelial cell tight junctions, ZO-1 is believed to coordinate the intracellular assembly of the junctional complex [53] and is a reliable indicator of

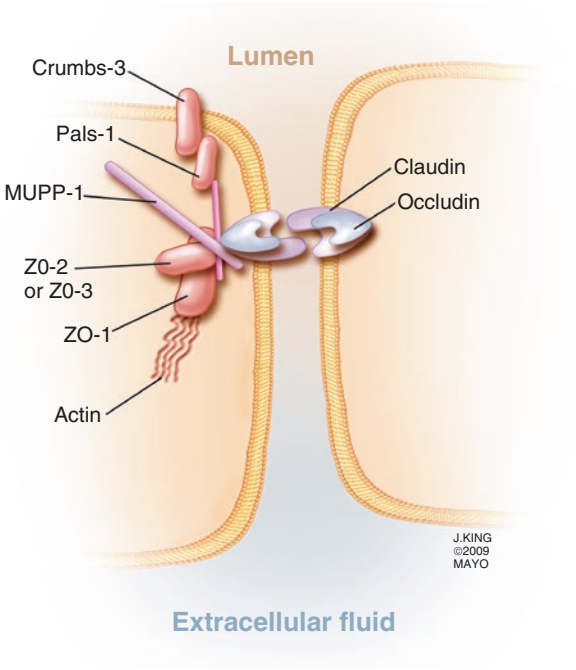

Fig. 2.10 This drawing shows several of the important proteins forming a tight junction between vascular endothelial cells. Note that occludin and the claudins span the intercellular space, while several intracellular proteins, including the multi-pdz protein-1 (MUPP1), support the complex

overall tight junction function [68]. The junctional adhesion molecules (JAM) facilitate interactions between endothelial cells and leukocytes and assist with tight junction assembly. Junctional proteins may assist with cellular structure since intracellular, circumferential actin bundles aggregate at sites of occludin-positive cell contact, but not at sites that lack occludin.

Transport of ions and molecules across the BRB may be either transcellular or paracellular (Fig. 2.11) [181]. Transcellular passage occurs because of changes in the barrier state or pumping capacity of endothelial cells, whereas paracellular passage occurs because of loss of integrity of the tight junctions. In rat and monkey models of diabetes and in humans, increased fluorescein levels in the vitreous can be detected with vitreous fluorophotometry before fluorescein angiograms show evidence of diabetic retinopathy [41, 97]. These observations suggest that early breakdown of the BRB allows passage of small molecules such as fluorescein but not larger proteins such as albumin, which are necessary for the formation of macular edema.

2.6.2 Biochemical Abnormalities Responsible for Diabetic Retinopathy

During recent years there has been considerable focus on the role of inflammation in the pathophysiology of diabetic retinopathy (Table 2.1) [191]. Increased expression of pro-inflammatory cytokines within the neural retina and upregulation of

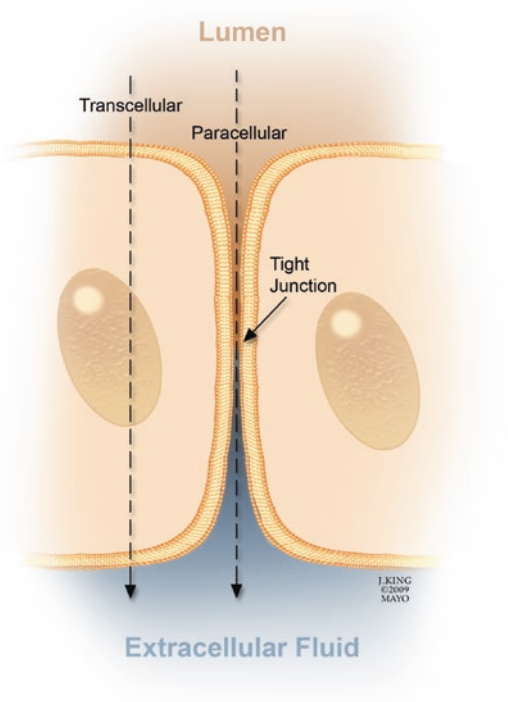

Fig. 2.11 Fluid movement across the blood-retinal barrier (BRB) may be paracellular (through the intercellular tight junctions) or transcellular (across the cell). Vascular endothelial growth factor-mediated breakdown of the BRB promotes fluid movement via both pathways

Table 2.1 This table lists several of the chemokines (pro-inflammatory molecules) and cytokines (growth factors) that have been associated with the development of diabetic retinopathy

Chemokines
Chemokine (C-C) motif ligand 2 (CCL2)
Endothelin-1
Intercellular adhesion molecule-1 (ICAM-1)
Interleukin 1α (IL-1α)
Interleukin 1β (IL-1β)
Interleukin 6 (IL-6)
Interleukin 8 (IL-8)
CXCL 10/Interferon induced protein 10 (IP-10)
Monocyte chemotactic protein 1 (MCP-1)
P-selectin
Vascular cell adhesion molecule-1 (VCAM-1)
Cytokines
Angiopoietin – 2 (Ang 2)
Platelet-derived growth factor AA (PDGF-AA)
Transforming growth factor β (TGF-β)
Tumor necrosis factor-α (TNF-α)
Vascular endothelial growth factor (VEGF)

vascular adhesion molecules that promote leukostasis have been linked to both neurovascular dysfunction and formation of acellular capillaries [98]. Evidence suggests that leukocytes may actively damage the retinal vascular endothelium [123, 189]. Global mRNA expression profiling has detected altered expression of pro-inflammatory cytokines not only in the retinal vessels [72] but also in the neuroglia [26]. Within the diabetic retina, a complex milieu of dysregulated pro-inflammatory factors, including interleukin (IL)-1α, IL-1β, IL-6, and tumor necrosis factor (TNF)-α, develops [191]. Though microglia and infiltrating monocytes are central to inflammatory-mediated central nervous system pathology, the role of these cells in diabetic retinopathy is considerably less well understood. A number of in vitro studies and in vivo investigations of animal models and human postmortem specimens indicate that activation of retinal microglia could play an important regulatory role in diabetes-mediated retinal inflammation [213] by modulating cytokine expression [27] and other pathologic responses [119]. Monocytes and microglia play important roles in retinal homoeostasis, but they are also central to neuroinflammation, and these have been shown to increase in diabetes, in both humans and animal models [15, 160, 214].

Very soon after the onset of experimental diabetes in animals, a variety of molecules are upregulated in the retinas and physiological changes are observed. Retinal oxygen tensions decrease before the loss of retinal capillaries in rodent models of diabetic retinopathy [39, 125]. Experimental diabetes in animals results in an increase in retinal VEGF mRNA within the first week, and some authors have concluded that VEGF upregulation is caused by oxidative stress [48, 147]. Upregulation of VEGF correlates with increased expression of intercellular adhesion molecules (ICAM-1), leukostasis, and breakdown of the blood-retinal barrier (BRB) [3]. Pro-inflammatory cytokines such as TNF-α [99] and complement factors [215] are upregulated, and activation of the kallikrein-kinin [121] and renin-angiotensin systems [32] occurs. Each of these changes has been implicated in the pathogenesis of DR, and since some of these pathways are not VEGF dependent, they provide further evidence that inflammation and hypoxia may be independently responsible for DR [4, 43, 45]. Vitreous concentrations of pro-inflammatory cytokines (TNF-α, IL-8, IL-6, VEGF), chemokines (monocyte chemotactic protein-1 (MCP-1)), and other proteins (endothelin-1, sE-selectin, ICAM-1, CXCL10/IP-10) are higher in patients with proliferative diabetic retinopathy or diabetic macular edema than in controls [109].

Evidence that links leukostasis to oxidative stress and other downstream mediators comes from the observation that alpha-lipoic acid decreases leukocyte adhesion. Mechanisms linked to PKC pathways are responsible for hemodynamic alterations that occur concomitantly with leukostasis [1].

Advanced glycation end products bind to specific membrane receptors to upregulate vascular cell adhesion molecule-1 (VCAM-1), which promotes leukostasis and may accelerate diabetic vasculopathy [2, 164].

A few hours of hypoxia increases passage of retinal RNA into the general circulation in humans, and when oxygen tensions are normalized, the levels return to normal within 24 h [100, 207]. Hypoxia stimulates the release of hypoxia-regulated

vasoproliferative factors, such as VEGF, but VEGF has been found to be increased in retinas of diabetic animals before capillary closure, indicating that other factors upregulate it early during the course of diabetes. VEGF has been shown to break down the BRB by both promoting molecular movement across cells and by damaging tight junctions. VEGF injections induce membrane fenestrations in rat and frog endothelial cells but not monkey cells. Transcellular gaps occur with increased frequency and lead to increased hydraulic conductivity. Within 24 h after vascular endothelial cells are perfused with VEGF, vesiculovacuolar organelles form a continuous, transcellular chain, separated only by fenestrations [16–18].

When exogenous VEGF is administered to eyes affected by diabetes, three changes in the tight junctions have been observed: (1) tight junction proteins are phosphorylated; (2) existing junctions are reorganized; (3) junctional protein levels are decreased [55].

2.6.3 Mechanism of Blood-Retinal Barrier Breakdown

The exact mechanism by which breakdown of the blood-retinal barrier occurs is not fully understood. Current evidence suggests that it may be due to either damage of the junctional complexes between capillary endothelial cells and RPE cells or changes in the cells' membrane state or pumping capacity. Proposed mechanisms that cause leakage have included the development of fenestrations across the endothelial cell cytoplasm, increased transcellular transport via vesicles, and increased infolding of the RPE that promotes choroidal to subretinal space transudation [195]. High glucose concentrations have been shown to reduce electrical resistance of cultured capillary endothelial cells and induce insulin-mediated breakdown of the blood-retinal barrier [76]. The discovery of vascular endothelial growth factor (VEGF) and other inflammatory cytokines, which exert considerable effects on junctional and interstitial proteins, has focused attention on the tight junctions. Cultured astrocytes simultaneously increase both intercellular barrier function and ZO-1 synthesis, suggesting a strong link between the two processes [68].

Diabetes changes the concentrations of junctional and matrix proteins. Occludin is decreased in the retinas of diabetic rats, and occludin and ZO-1 levels are decreased in cultured brain endothelial cells treated with VEGF [199]. Levels of MMP-2, MMP-9, and MMP-14 mRNA are increased in the retinas of diabetic animals. Cells treated with MMP-2 and MMP-9 develop altered gap junctions through increased transepithelial electrical resistance and degradation of occludin [74].

Though the precise biochemical pathway that leads to breakdown of the blood-retinal barrier is unknown, a large volume of research has focused on vascular endothelial growth factor. Much has been learned about VEGF since its discovery in 1989, and understanding its effects has been critical to developing therapy for clinically significant diabetic retinopathy. Detailed VEGF chemistry will be covered in Chap. 4, but general actions of VEGF on the blood-retinal barrier and development of neovascularization will be discussed in this chapter.

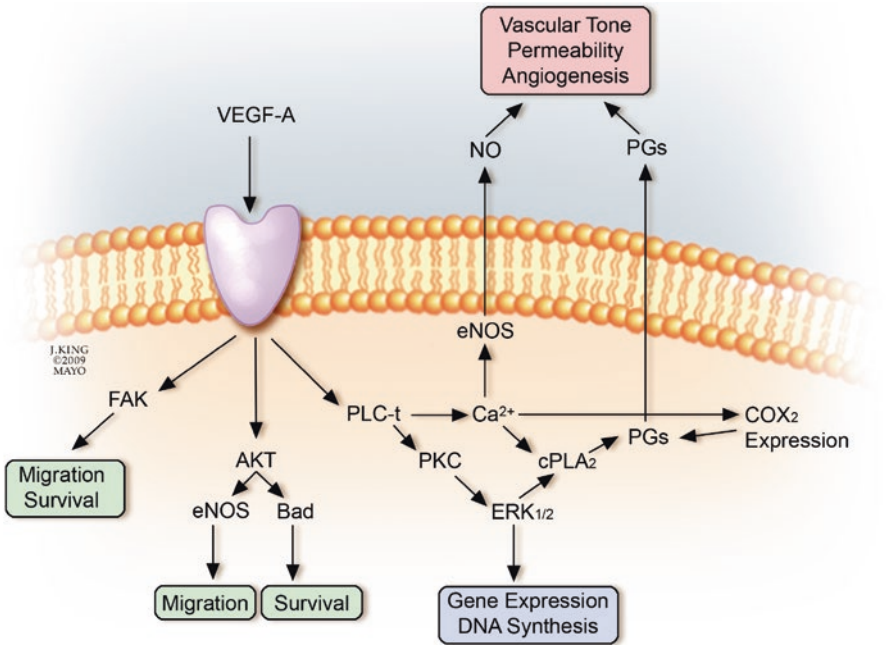

Fig. 2.12 Vascular endothelial growth factor-A binds to transmembrane receptors and activates several downstream pathways. These promote cellular survival, proliferation, and migration, and they increase blood flow through vasodilation and angiogenesis

Most ocular actions of VEGF result from the binding of VEGF-A isoforms to the transmembrane receptor VEGFR2. Vascular endothelial growth factor-mediated dimerization and phosphorylation of VEGFR-2 increase vascular permeability and stimulate angiogenic and mitogenic changes in vascular endothelial cells [56, 57].

Vascular endothelial growth factor increases vascular permeability through several mechanisms (Fig. 2.12). First, it stimulates inositol triphosphate (IP_3), which releases intracellular calcium. High calcium concentrations increase nitrous oxide and cyclic GMP levels and relax vascular smooth muscle. Vascular permeability, angiogenesis, vessel diameter, and blood flow are proportional to nitrous oxide synthetase levels [62]. Secondly, VEGF stimulates DAG production, which increases cellular permeability directly through DAG-sensitive Ca^{2+} channels. Thirdly, increased synthesis of DAG activates PKC.

In mouse models of experimental neovascularization, exogenous administration of a VEGF-trap decreases neovascularization by 66% [162], and transfer of the VEGFR-1 genes decreases neovascularization by 53–86% [13, 71]. These data suggest that VEGF is critically important to the development of neovascularization. VEGF is sufficient to cause leakage from normal blood vessels as shown in animal studies following implantation of sustained VEGF-release devices [145] or intravitreal injections [162]. Though hundreds of papers have been published regarding the

effect of VEGF on vascular permeability, there have been very few *in vitro* studies. Notable findings include the ability of VEGF to increase hydraulic conductivity [17] and diffusive permeability to albumin [208], but VEGF has no effect on the oncotic reflection coefficient (the probability that a molecule will bounce off a pore rather than go through it) to albumin [16]. In fact, hydraulic conductivity (a measure of the ease by which fluid moves through a lumen) and compliance (the inverse of stiffness) may be stimulated separately, suggesting that permeability and angiogenesis may be stimulated or inhibited separately.

In addition to its effects on the retinal vasculature, VEGF protects against apoptosis by acting as a survival factor for endothelial cells both *in vitro* and *in vivo*. Vascular endothelial growth factor prevents starvation-induced endothelial apoptosis by blocking the phosphatidylinositol 3-kinase/V-akt murine thymoma viral oncogene homolog (PI3 kinase/Akt) pathway, possibly by inducing the anti-apoptotic proteins Bcl-2, A1, XIAP, and survivin.

Vascular endothelial growth factor induces inflammation by upregulating ICAM-1 (intercellular adhesion molecule) synthesis, which causes leukocyte adherence to vascular walls [130]. These activated leukocytes synthesize VEGF, thereby amplifying VEGF production [70]. Adherent leukocytes narrow capillary lumens and decrease downstream perfusion, which exacerbates localized retinal ischemia, further amplifies VEGF production, and compromises the blood-retinal barrier [22].

Intravitreal VEGF levels correlate with the severity of diabetic retinopathy as higher concentrations are generally seen in eyes with PDR and lower concentrations in eyes with BDR [24]. Even within the subset of patients with DME, aqueous VEGF levels closely correlate with the severity of DME [65].

Vascular endothelial growth factor is upregulated by several growth factors including TNF-α, TGF-β, epidermal growth factor, insulin-like growth factor-1, fibroblastic growth factor, platelet-derived growth factor, and also by inflammatory cytokines including IL-1α and IL-8. One of the major stimulants of VEGF mRNA expression is low tissue O_2 tension. Similarities exist between the hypoxic regulation of VEGF and erythropoietin. A 28-base sequence in the promoter of the human VEGF gene, which has similar binding characteristics to hypoxia-inducible factor (HIF)-1α, a key mediator of hypoxic responses, has been identified [166]. Hypoxia-inducible factor has been termed the "cell's oxygen sensor" since it serves as the key transcriptional regulator of the hypoxic response.

In the presence of oxygen, free HIF-1α is hydroxylated by prolyl hydroxylase. Hydroxylated HIF-1α binds to the von Hippel-Lindau factor (pVHL), and the pVHL/HIF-1α complex is subsequently degraded by intracellular proteasomes [95]. Low tissue oxygen tension prevents hydroxylation of HIF-1α and allows it to dimerize with HIF-1β [161]. The stabilized dimer moves to the nucleus where it binds to the promoter region of the VEGF gene and stimulates transcription. Hypoxia increases levels of HIF-1α subunits both by stabilizing the protein [198] and allowing HIF-1α mRNA accumulation [201]. Stabilization of HIF-1α during hypoxia requires an intact mitochondrial electron transport chain to generate reactive oxygen species (ROS), and it is the concentration of ROS that may constitute the cell's oxygen sensor [165]. Low intracellular oxygen tension is

indirectly represented by high levels of ROS, which limits a cell's ability to hydroxylate HIF-1α.

In addition to ROS, several other molecules including IGF-1 and 2 and AGEs affect the stability of HIF-1α. Insulin-induced stabilization of HIF-1α with subsequent upregulation of VEGF may explain why very tight glucose control worsens retinopathy [154]. Several biochemical opportunities exist for HIF-1α modulation, including phosphatidylinositol 3-kinase inhibitors, mitogen-activated protein kinase inhibitors (MAPK), prolyl-4-hydroxylase domain activators, microtubule disrupting agents, cyclooxygenase-2 (COX-2) inhibitors, heat shock protein inhibitors, and antisense therapy. Breakthroughs in treating ischemic retinal disease may follow new cancer therapies such as topotecan and camptothecin analogs [149].

The protein kinase C pathway is upregulated by the presence of diacylglycerol. Activated PKC increases vascular permeability via two mechanisms: endothelial cell contraction through phosphorylation of the cytoskeletal proteins caldesmon and vimentin [177] and the activation of serine/threonine phosphatases or the inactivation of kinases, both of which dephosphorylate the tight junction proteins occludin and the claudins [34]. Protein kinase C increases matrix protein deposition – a hallmark of diabetic retinopathy – by inhibiting NO synthesis, which leads to increased TGF-β1, fibronectin, and type IV collagen [187].

Several studies have shown how activated PKC may contribute to BRB breakdown. Protein kinase C inhibitors block VEGF-mediated BRB breakdown in nondiabetic rats [7]. The intravitreal injection of PKC can induce BRB breakdown in nondiabetics. Protein kinase C inhibitors prevent hyperglycemia-induced VEGF production [204], and in advanced diabetic retinopathy, PKC can mediate the action of VEGF, thereby creating a reinforcing cycle. Hyperglycemia may worsen existing retinopathy by activating PKC and also by upregulating p42/p44 MAPK. Both of these pathways depend on HIF-1α stabilization [154].

Maintenance of the normal retinal vasculature requires a precise balance of angiogenic factors (such as VEGF) with inhibitors (such as angiostatin and pigment epithelium-derived factor (PEDF)) [136]. Pigment epithelium-derived factor, which is secreted by Müller and endothelial cells, has anti-inflammatory and anti-permeability properties [127]. Vitreous levels of PEDF are low in eyes with BDR, and levels rise to normal after pan-retinal photocoagulation for proliferative diabetic retinopathy. Pigment epithelium-derived factor modulates the vasculopathic effects of VEGF by decreasing HIF-1α levels via MAPK-mediated activation, which stimulates endogenous PEDF production [217], and by competitive binding with VEGF for VEGFR-2.

Patients with rheumatoid arthritis appear to have less severe diabetic retinopathy, which has prompted speculation that the anti-inflammatory medications they take antagonize the effects of vasoactive cytokines. Though these drugs may antagonize the effects of VEGF, they do not decrease VEGF levels. Aspirin and TNF-α inhibitors decrease ICAM-1 levels and leukocyte adhesion by decreasing nitrous oxide synthetase expression. Aspirin decreases the expression of integrins that bind to ICAM-1 [LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18)].

2.6.4 Renin/Angiotensin System

The renin/angiotensin system (RAS) modulates a diverse group of biological functions [105]. The system's major active product, angiotensin II, is converted from angiotensin I by angiotensin-converting enzyme (ACE). Angiotensin II modulates vasoconstriction, electrolyte homeostasis, drinking behavior, and pituitary hormone release [6, 40, 94]. As a growth factor, angiotensin II promotes cellular differentiation, apoptosis, and the deposition of extracellular matrix [101, 143, 144, 188]. Angiotensin receptors are found in endothelial cells, glia, and neurons [139, 140], which suggests that angiotensin II may regulate functions within these cells.

Angiotensin exerts significant effects on vascular smooth muscle cells including growth, proliferation, and the deposition of extracellular matrix proteins [190]. These are mediated by factors such as TGF-β1, PDGF, VEGF, insulin-like growth factor, and connective tissue growth factor [203]. Though the effects of angiotensin II on pericytes are less well studied, they appear to include pericyte uncoupling and migration [102].

The pro-angiogenic effect of angiotensin II on mammalian retinas with oxygen-induced retinopathy is mediated by VEGF [56]. Pharmacologic blockade of the RAS decreases angiogenesis by downregulating VEGF and VEGFR2.

The ACE inhibitor captopril prevents degeneration of retinal capillaries, and the angiotensin receptor antagonist losartan inhibits leukostasis in streptozotocin-induced rats and vascular cell adhesion molecule-1 (VCAM) expression in human vascular endothelial cells [216]. Inhibition of angiotensin-converting enzyme prevents the retinal overexpression of VEGF in experimental diabetes [75] and the progression of diabetic retinopathy in normotensive patients with type I diabetes [31].

Elevated angiotensin II levels correlate with vitreous VEGF concentrations in patients with diabetic macular edema [64]. Experimental evidence suggests that this may be mediated through the AT1-R/NF-κB pathway [139], which creates new target sites for the prevention of diabetic retinopathy.

2.7 Development of Proliferative Diabetic Retinopathy

Apoptosis of retinal vascular endothelial cells together with the formation of pericyte ghosts results in acellular capillaries, hypoxia due to retinal non-perfusion, and with sufficient upregulation of cellular growth factors, proliferative retinopathy [21, 138]. Death of the vascular cells results from the upregulation of several cytotoxic factors including TNF-α. The forkhead transcription factor FOXO1 regulates cell death after being upregulated by TNF-α. Inhibition of FOXO1 by RNAi reduces cell apoptosis and microvascular cell death both *in vitro* and *in vivo* in type 1 and type 2 diabetic rats.

These factors significantly increase the activities of genes that modulate endothelial cell behavior, including angiogenesis and vascular remodeling. Occlusion

of retinal capillaries leads to patchy non-perfusion, with the inner two-thirds of the retina suffering from hypoxia. Hypoxia-induced stabilization of HIF-1α upregulates VEGF production, which is temporally related to the development of neovascularization [4].

In addition to VEGF, other growth factors such as insulin-like growth factor-1, hepatocyte growth factor (HGF), basic fibroblast growth factor (b-FGF), platelet-derived growth factor, and pro-inflammatory cytokines and angiopoietins are involved in the pathogenesis of PDR. Also found within the retina and vitreous are anti-angiogenic factors such as pigment epithelium-derived factor (PEDF), TGF-β, thrombospondin, (TSP), and somatostatin. It is widely believed that neovascularization results from an imbalance of angiogenic and anti-angiogenic factors [170].

2.8 Clinical Findings of Diabetic Retinopathy

The clinicopathology of the diabetic retina has been extensively studied, but the precise cellular and molecular defects that lead to retinal vascular, neural, and glial cell dysfunction remain somewhat elusive. Though vascular dysfunction and decreased perfusion remain the hallmarks of diabetic retinopathy, a growing body of evidence suggests that neuroretinal function is compromised, probably before overt vascular changes are seen [9]. Electrophysiological studies of patients with diabetes suggest that alterations in the neural retina, including loss of color vision [157] and contrast sensitivity [175], and abnormalities in the electroretinogram (ERG) occur soon after the onset of diabetes [212]. Glial abnormalities develop during hyperglycemia, with Müller cells overexpressing glial fibrillary acidic protein [137], with concomitant synthesis of glutamate as a function of disruption of the glutamate transporter [155]. This may contribute to excitotoxicity and eventual depletion of retinal neurons, thus making them an integral part of diabetic retinopathy [124]. Taken together, the retinopathy literature suggests that neurodegeneration constitutes a significant pathophysiological defect in diabetes.

The earliest functional changes in nonproliferative diabetic retinopathy (NPDR) cannot be visualized with photography, but they include changes in retinal blood flow and loss of autoregulatory mechanisms that adjust retinal capillary perfusion to local metabolic demand [114]. As early as the 1930s, it was suggested that retinal blood flow was markedly altered in diabetic patients [196], and as technology evolved there were indications that retinal vessel caliber consistently increased during diabetes [14, 172]. Since the 1970s it has been recognized that hemodynamic changes in the retinal vasculature play a role in the pathophysiology of diabetic retinopathy and possibly serve as an early indicator of diabetes-related retinal dysfunction [110, 112]. Since then various patient-based studies have shown that retinal hemodynamic abnormalities occur before the onset of clinically observed diabetic retinopathy [28]. In diabetic patients without retinopathy, retinal arteriolar vasoconstriction and decreased total retinal blood flow have been reported [28, 33, 107]. Decreased retinal blood flow early in the course of diabetes may reduce oxygen and

nutrient delivery to the neuro-retina and contribute to the initial neuroglial abnormalities that have been observed in the diabetic retina. As the diabetes progresses, retinal arterioles begin to dilate and bulk retinal blood flow increases in proportion to the severity of retinopathy [163]. Enhanced blood flow may hasten the progression of diabetic retinopathy by causing shear stress-induced endothelial cell damage and death [114]. A recent trial has suggested that these changes could be regarded as robust risk factors for the subsequent development of retinopathy [108]. Patients with increased retinal blood flow who fail to demonstrate improved hemodynamic indices after normalization of blood glucose experience a more rapid progression of the disease [78].

The earliest ophthalmoscopically visible signs of diabetic retinopathy are usually retinal capillary microaneurysms. These represent saccular dilations of the capillaries and appear as dark red or white fundus spots. Fluorescein angiography reveals perfused microaneurysms as discrete hyperfluorescent spots with early pooling of dye followed by late leakage. Many microaneurysms are fully or partially perfused during fluorescein angiography, whereas others are sclerosed and non-perfused [111, 113].

Retinal hemorrhages can be either "flame-shaped," which generally occur within the nerve fiber layer and arise from the superficial capillary plexus, or "dot and blot," which occur within the spaces between the vertical-oriented axons of the inner plexiform layer and arise from the deep capillary plexus. Hemorrhages usually indicate a more serious form of DR than do microaneurysms.

Exudation from microaneurysms and leaking areas of the capillary beds lead to diabetic macular edema (DME) (Fig. 2.13). After resorption of interstitial fluid, lipids frequently precipitate and appear as yellow spots in the inner retina [Fig. 2.14]. These are often found at the junction of edematous and non-edematous retina, surrounding areas of microaneurysms.

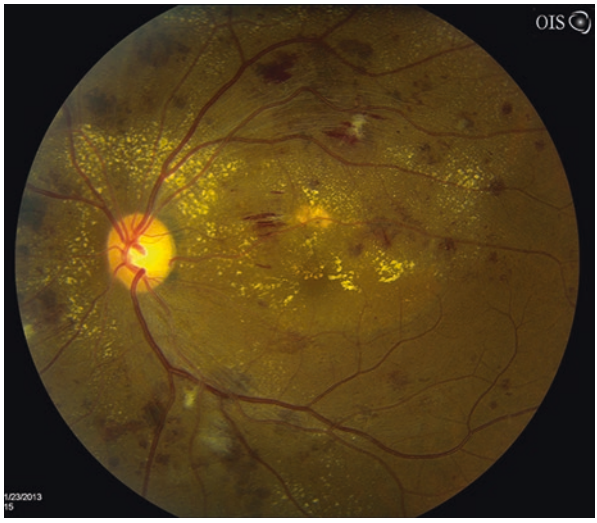

Fig. 2.13 This macular photograph shows significant diabetic retinopathy with flame-shaped and dot-and-blot hemorrhages. Scattered hard exudates are seen primarily in the superior macula, and macular edema obscures the underlying choroidal detail

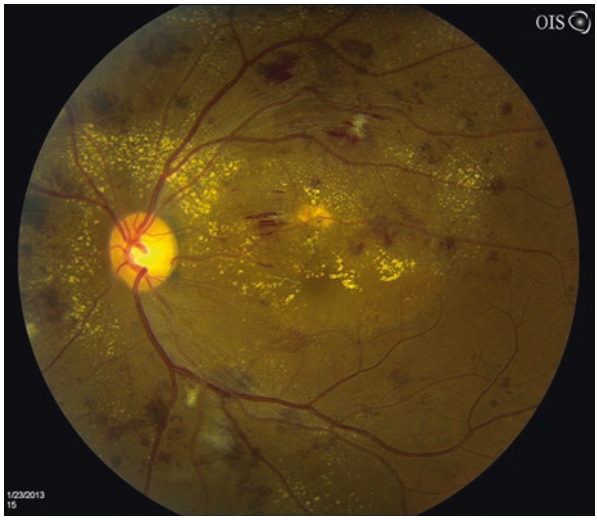

Fig. 2.14 This posterior pole photograph shows nonproliferative diabetic retinopathy with diabetic macular edema and significant hard exudates

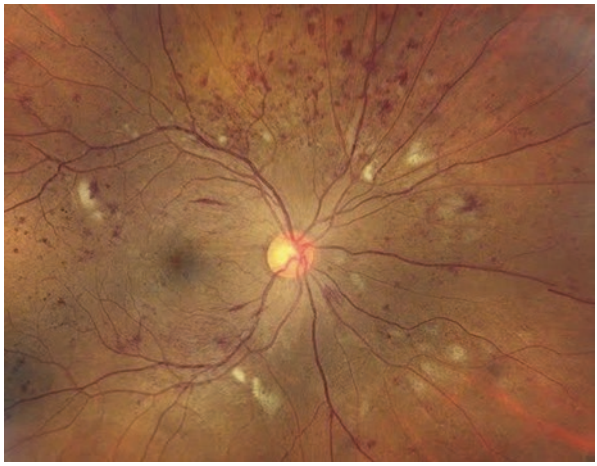

Fig. 2.15 This photograph of the posterior pole shows prominent cotton wool spots (soft exudates) in each quadrant. This indicates advancing retinal non-profusion and progressive ischemia

Cotton wool spots (CWS) and intraretinal microvascular abnormalities (IRMA) are usually associated with early retinal ischemia (Fig. 2.15). Cotton wool spots represent stasis of flow within the axons, whereas IRMA are vessels that shunt blood past occluded capillary beds.

Advanced retinal ischemia leads to the development of retinal neovascularization. New vessels sprout from pre-existing retinal (neovascularization elsewhere or NVE) or disc (neovascularization of the disc or NVD) vessels and grow along the scaffold provided by the posterior hyaloid surface (Fig. 2.16). The amount of blood vessel growth generally correlates with intraocular VEGF levels, with vessels growing toward higher VEGF concentrations. New vessel growth defines proliferative diabetic retinopathy (PDR) though patients may also display all the characteristics of nonproliferative diabetic retinopathy, including diabetic macular edema.

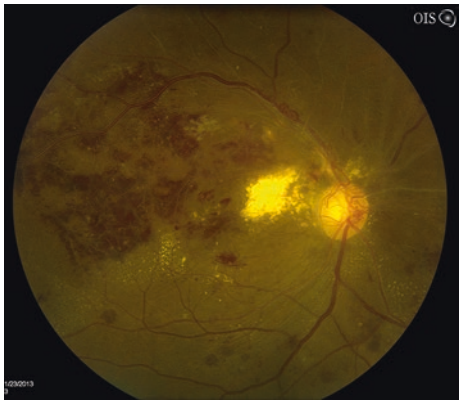

Fig. 2.16 This photograph of the posterior pole shows diabetic retinopathy with a coexisting retinal vein occlusion. Prominent neovascularization of the disc (NVD) due to retinal ischemia can be seen

Most diabetes-related vision loss results from DME due to nonproliferative diabetic retinopathy. Severe vision loss (worse than 20/200) more commonly results from complications of PDR: vitreous hemorrhage, traction and traction/rhegmatogenous retinal detachments, neovascular glaucoma, and widespread retinal non-perfusion [60].

Though diabetic retinopathy is broadly categorized as nonproliferative and proliferative based on the presence of neovascularization, the severity of each condition varies from patient to patient. Nonproliferative DR can be subdivided into mild, moderate, severe, and very severe, depending on the extent of hemorrhages, retinal venous beading, and intraretinal microvascular abnormalities (IRMA) (Table 2.2). Though each of these sub-diagnoses carries a different prognosis, and the frequency of follow-up examinations is usually based on the sub-diagnosis, they are not usually used to make treatment decisions.

Over the past 30 years, macular edema has been classified according to two definitions:

1. Clinically significant macular edema (CSME). This was first used in the Early Treatment of Diabetic Retinopathy Study and is defined as any of the following:
   (a) Thickening of the retina within 500 μm of the fovea
   (b) Hard exudates within 500 μm of the fovea with adjacent retinal thickening
   (c) Thickening of the retina at least 1 disc area in size that extends to within 1 disc diameter (DD) of the fovea

Thickening is determined by contact lens examination of the macula.

2. Diabetic macular edema (DME). This definition, rather than CSME, is used in the era of optical coherence tomography (OCT) and retinal pharmacotherapy. This is defined as a 2 DD area of macular thickening, any part of which is within 1 DD of the fovea. Thickening is determined by OCT scanning.

Historically, color fundus photography has been the "gold standard" for detecting diabetic retinopathy and assessing its severity. In clinical practice, treatment decisions were usually based on the results of biomicroscopic evaluation of the

Table 2.2 This table classifies diabetic retinopathy according to severity

Classification of diabetic retinopathy	
Category	Characteristics
Nonproliferative diabetic retinopathy (NPDR)	
Mild NPDR	Microaneurysms only
Moderate NPDR	More than just microaneurysms (hemorrhages and exudates) but less severe than severe NPDR
Severe NPDR "4:2:1 rule"	Any of the following but with no signs of proliferative retinopathy: Severe intraretinal hemorrhages and microaneurysms in each of the four retinal quadrants Venous beading in two or more quadrants Moderate intraretinal microvascular abnormalities (IRMA) in one or more quadrant
Very severe NPDR	Two or more criteria for severe NPDR but without neovascularization
Proliferative diabetic retinopathy (PDR)	
PDR	Neovascularization of disc or retina (elsewhere) Vitreous or pre-retinal hemorrhage
Severe PDR	At least three of the following characteristics: Neovascularization Neovascularization of the disc Neovascularization at least one-half disc diameter in size Vitreous or pre-retinal hemorrhage

This popular classification system is used by many prospective and retrospective studies

macula, indirect ophthalmoscopic evaluation of the retina, or 30°–50° photographs of the fundus. Fluorescein angiography was usually used to identify areas of leaking in order to guide laser photocoagulation of the macula. Diabetic treatment trials usually required 7-field photographs, which provided views of the retina that extended to the mid-periphery.

Recent advances in technology enable physicians to take widefield and ultra-widefield laser-generated photographs. The Heidelberg Spectralis system with the Ocular Staurenghi 230 SLO Retina Lens (Ocular Instruments, Bellevue, Washington) enables photographers to take color and angiographic images that span 120°. The Optos C200MA noncontact SLO (Optos PLC, Dunfermline, UK) enables the photographer to take ultra-widefield photographs and fluorescein angiography (Fig. 2.17) images that span 200°. The system enables physicians to identify retinal non-perfusion anterior to the equator and may be superior to indirect ophthalmoscopy for the detection of peripheral neovascularization. The extent of peripheral retinal ischemia has been correlated with the severity of treatment-naïve DME, and peripheral ischemia may be identified in eyes where the DME responds poorly to pharmacotherapy. A retrospective study of eyes that responded poorly to anti-VEGF therapy showed that 80% had untreated peripheral non-perfusion and the poorest responders had the largest areas of non-perfusion. Many physicians perform targeted peripheral laser photocoagulation to eyes responding poorly to therapy, but its ability to resolve DME has not been established.

Fig. 2.17 This fluorescein angiogram image taken with an ultra-widefield system shows areas of peripheral capillary non-perfusion and retinal neovascularization in all quadrants. Pre-retinal hemorrhage is noted just beneath the inferotemporal arcade, and the macula is partially obscured by vitreous hemorrhage

2.9 Conclusions

Diabetic retinopathy has historically been considered a retinal vasculopathy, but recent data suggests that it affects the entire neurovascular unit. Diabetes results in dysfunction and eventual death of several key cells that maintain the BRB: pericytes, vascular endothelial cells, Müller cells, and neurons. Complex biochemical pathways upregulate inflammatory chemokines and cytokines that lead to the development of diabetic retinopathy, diabetic macular edema, and retinal neovascularization. Recent advances in our understanding of several important biochemical pathways have helped us identify potential therapeutic targets.

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Chapter 3
Treatment of Diabetic Retinopathy: A Historical Perspective

3.1 Introduction

The lower prevalence of metabolic syndrome throughout the early part of the twentieth century meant that a greater proportion of patients suffered from type 1 diabetes and the total number of patients affected with type 2 diabetes was much lower than it is today. The introduction of insulin in 1922 extended survival for patients with type 1 diabetes mellitus (DM), but life expectancy remained poor because many died of disease-related complications. Since few patients lived long with DM, the incidence of diabetic retinopathy (DR) was low and early diagnosis and treatment were a low priority. In addition, only a small number of ophthalmologists served the population and their ability to accurately diagnose retinopathy was far more limited than it is today.

The development of DR was frequently accompanied by a steady decline in vision often with progression to legal blindness. Most physicians believed that tight glucose control decreased the incidence and progression of retinopathy but conclusive evidence did not emerge until completion of the Diabetes Complications and Control Trial and the United Kingdom Prospective Diabetes Study in the 1990s. Despite best attempts to control glucose, success was limited by inadequate dietary guidelines, lack of home glucose monitoring systems and hemoglobin A1c testing, and a limited choice of medications.

Several treatments for vision-threatening DR were tried, but efficacy was never demonstrated for some of them and significant side effects plagued others. This chapter begins by briefly discussing a couple of DR treatments that were tried and abandoned during the latter half of the twentieth century [46, 61, 131]. Most of the chapter focuses on the use of laser photocoagulation and vitrectomy for the treatment of diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR). Laser and vitrectomy were introduced in the 1960s and 1970s, respectively, and still play an important role in the treatment of DR. Recent technological advances in the forms of micropulsed and navigated laser and small gauge vitrectomy have made these treatments easier to apply with potentially less damage to

M.W. Stewart, *Diabetic Retinopathy*, DOI 10.1007/978-981-10-3509-8_3

surrounding tissues. Current research with these modalities attempts to determine how they fit into today's pharmacology-based treatment regimens.

3.2 Pituitary Ablation

Evidence supporting the notion that pituitary ablation alters the course of DM surfaced in the 1930s after pituitary glands had been removed from dogs that had undergone pancreatectomies [56] and cats that had undergone hypophysectomies and adrenalectomies [81]. Some observers believed that a "fundamental neural mechanism" was responsible for stabilization of retinopathy [156], while others suggested that pituitary ablation decreases or eliminates growth hormone, adrenocorticotrophin, and thyroid-stimulating hormone, which directly or indirectly through their downstream effects worsen DR [51]. Additionally, vision-threatening DR was rarely observed in growth hormone-deficient diabetic dwarfs. Support for pituitary ablation as a treatment for DR originally came from a case report (1953) of a woman whose DR regressed after she developed postpartum pituitary necrosis.

Few studies have compared the results of pituitary ablation for DR with control patients. In one report, 4 of 19 patients that underwent radioactive yttrium ablation of the pituitary experienced improved vision after 1 year, compared to 0 of 22 controls. Neovascularization of the retina decreased in 30% of ablated patients but in none of the controls [60]. In another report, 9 of 33 patients that underwent surgical resection of the pituitary stalk experienced decreased vision, compared to 19 of 33 controls. Retinopathy improved in 16 ablated patients but in only 9 controls [125]. In a review of 385 ablated patients, retinopathy was stabilized or improved in one-half [9]. Data from one series of patients suggested that improvement in retinopathy does not start until 12 months after ablation, so the authors recommended that ablation be performed early in the proliferative phase of retinopathy [5]. Pooled results from a large symposium reported that 75% of 708 patients had stabilized vision during the first 6 months after ablation [137]. Long-term visual results after ablation have not been well established, but up to one-third of patients with 5-year follow-ups experienced progression of retinopathy after an initial response to surgery [33].

Pituitary ablation was performed via surgical removal or resection, irradiation with yttrium or proton beam, or cryohypophysectomy (Fig. 3.1) [77, 122]. Surgeons believed that a more rapid and complete hypophysectomy had a greater effect on retinopathy [61]. Complications of pituitary ablation included cranial nerve palsies, cerebrospinal fluid rhinorrhea, meningitis, and herpes simplex encephalitis [133]. Following ablation, patients required lifelong hormonal replacement with corticosteroids and possibly thyroid and sex hormones.

Predicting which patients with DR would respond favorably to ablation was often difficult. Vascular lesions associated with both background and proliferative retinopathy were often observed to regress, but fluorescein angiography demonstrated that ischemia improved slowly [69]. Contraindications to pituitary ablation included retinal detachment, preretinal fibrosis, advanced age with cardiovascular

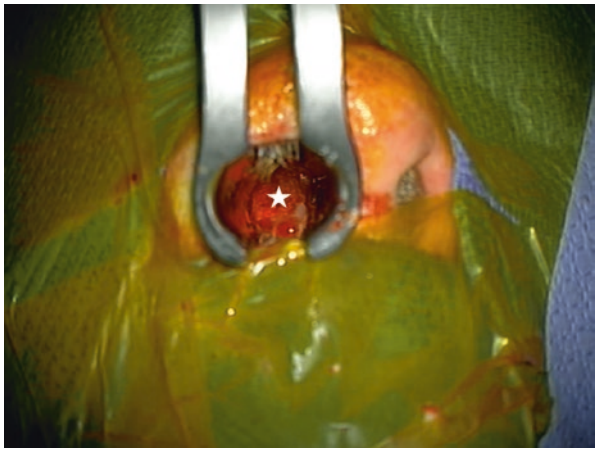

Fig. 3.1 This intraoperative photograph shows exposure of the pituitary gland (*beneath the white star*) via a trans-nasal approach. Trans-nasal hypophysectomies are no longer performed for diabetic retinopathy but are frequently employed for large pituitary tumors

disease, and the inability to tolerate long-term, complex hormonal replacement. The ideal patient was below the age of 40 years with good renal function, but 75% of patients failed to meet the surgical criteria.

Pituitary ablation was performed on hundreds of patients, but firm evidence supporting its efficacy was never obtained. Due to its lack of established efficacy and unacceptable complication rates, pituitary ablation had been discontinued at some medical centers by the mid-1960s. After the introduction and widespread use of laser photocoagulation, nearly all centers had discontinued pituitary ablation by the mid-1970s.

3.3 Interferon

Interferon is a pluripotent endogenous protein with antiviral, antiproliferative, and immunogenic activity that has been shown to limit endothelial cell motility and proliferation in vitro [38, 147]. Recombinant α-interferon has been used to treat pulmonary hemangiomatosis and human immunodeficiency-related Kaposi sarcoma [134, 153]. Since interferon only mildly interferes with glucose tolerance, it would be unlikely to interfere with metabolic control in patients with DM [151].

In a 4-month prospective study, 3 patients with proliferative diabetic retinopathy were treated with systemic interferon [131]. Subjects experienced mild fatigue, nausea, myalgias, leukopenia, thrombocytopenia, and transient ataxia. Fasting hyperglycemia and postprandial hyperglycemia increased from 2 to 6% despite a 17 to 68% increase in insulin dosage. During treatment with interferon, DR was stable without new hemorrhages, but all patients had new retinal bleeding after interferon was discontinued.

Interferon has also been evaluated for the treatment of neovascular age-related macular degeneration [48], but since these studies failed to show a therapeutic effect, no further testing of interferon in patients with DR was performed.

3.4 Laser Photocoagulation

Damage to the retina by excessive light has been recognized for centuries. Socrates warned against directly viewing solar eclipses and advised his pupils to indirectly view the reflected image of the eclipse off of a pool of water. Solar maculopathy was first described in the seventeenth century [50], and it has been suggested that the artist Degas suffered solar damage to the macula when standing watch on a sunny day. Hundreds of solar burns occurred in Europe during a solar eclipse in 1912.

These events represent the results of a photochemical effect on the retina and not the thermal or photocoagulative effect that occurs with photocoagulation. These observations, however, inspired researchers to develop light-based devices to photocoagulate the retina. Experimental photocoagulation of rabbit eyes with a concave mirror and convex lens was first performed in 1867 [18] and duplicated in 1882 [21]. A carbon arc lamp produced the same effect in1893 [154].

The first experimental photocoagulation of the human retina (1927) was performed in an eye scheduled for enucleation because of a choroidal melanoma. Sunlight focused on the retina for 10 min caused hyperemia and retinal edema [88]. The first instrument specifically designed for therapeutic photocoagulation of the retina (1949) focused the sun's rays with a Galilean telescope and mirror [93]. This instrument was relatively unreliable because it depended on weather conditions. A next-generation device used a high-intensity carbon arc [94]. Though much more reliable, the carbon arc released soot and undesirable particles. The next light source was the xenon lamp, which produced a bright light with long-duration burns of moderate intensity over proliferating blood vessels [95, 96]. The xenon lamp was an effective photocoagulator for years, but it was eventually replaced by the ruby laser, the argon blue-green and green lasers, the krypton laser, the tunable dye laser, and most recently the infrared diode and frequency-doubled diode laser (Table 3.1). These lasers produce monochromatic light with small spot sizes and better control of power and pulse duration. They enable physicians to treat not only proliferative retinopathy with broad areas of photocoagulation but also macular edema with precise placement of small spots. The diode lasers are highly efficient as they derive power from standard electrical outlets without the need for specially installed, high voltage outlets.

Each of these instruments produces a collimated beam of coherent light that is transmitted through the ocular media to the retina where it is absorbed by retinal chromophores – xanthophyll, hemoglobin, and melanin. Light absorption produces a photothermal effect that heats the surrounding tissues and induces inflammation and edema that eventually causes localized tissue necrosis. High-intensity laser pulses produce burns that affect all levels of the retina, retinal pigment epithelium (RPE), and choroid, whereas low-energy pulses affect primarily the photoreceptors and RPE but spare the inner retina [17, 43]. Longer wavelengths penetrate deeper into the choroid because they are absorbed poorly by xanthophyll chromophores. Histopathologic studies confirm that the resultant scars vary according to wavelength, but the clinical importance of this has not been proven.

Table 3.1 Commonly used laser systems are listed

Common types of laser used for retinal photocoagulation	
Long-duration laser systems	
Argon	Argon gas in replaceable tube Wavelengths in blue (488 nm) and blue-green (514 nm) part of spectrum Use has diminished because of high-power requirements and need to periodically replace tube
Krypton	Krypton gas in replaceable tube Wavelengths in red (627 nm) part of spectrum Use has diminished because of high-power requirements and need to periodically replace tube
Tunable dye	Dye allows modification of wavelength including 577 nm, highest uptake of hemoglobin
Frequency-doubled YAG (solid state)	Solid-state laser uses 120 volt power output Spots at wavelength of 532 nm Device has long lifespan
NAVILAS	Computer-guided system delivers spots directly to spots identified on fluorescein angiography Some surgeons believe that NAVILAS delivery may be better than surgeon directed photocoagulation
Micropulse laser systems	
Visible and infrared	Subthreshold laser delivery. Repeated delivery may be performed. Some surgeons suggest that subfoveal delivery is safe Has not been subjected to randomized, multicenter, masked trials

Several advantages and disadvantages are discussed

While physicians were evaluating laser photocoagulation, retinal photography was also being developed. High-quality, stereoscopic color fundus photographs enabled physicians to identify microaneurysms and areas of diffuse macular thickening. Stereoscopic photos were critical for enrolling patients in prospective studies for the treatment of DME. Fluorescein angiography showed that DME develops from both leakage of microaneurysms and diffuse leakage from incompetent capillary beds.

3.4.1 Techniques for Performing Laser Photocoagulation

When choosing to perform laser photocoagulation of the retina, ophthalmologists can choose among different machines and techniques. Several manufacturers produce user-friendly, reliable lasers that produce excellent retinal burns. Most current laser systems have abandoned the use of Nobel gases (argon and krypton) and tunable dyes and have migrated to diode technology. Diodes are reliable, durable, and portable and require minimal servicing, and unlike the old gas-generated lasers, diodes work with 120 volt electrical outlets, thereby making installation easier and use in the clinic and operating rooms safer. They produce a 532 nm beam that is

moderately absorbed by oxyhemoglobin and minimally absorbed by retinal xanthophylls.

The most common laser delivery methods include the slit-lamp biomicroscope, indirect laser ophthalmoscope, and intraocular probe. In the outpatient or clinic setting, slit-lamp delivery systems are most commonly used, particularly for the treatment of DME (Fig. 3.2). The retina is visualized through a high-diopter condensing lens or a contact lens which may be flat (for the macula), may invert images (such as the panfundus or Mainster lenses that are generally used for panretinal or scatter photocoagulation), or may be mirrored (for treatment of the peripheral retina). Panfundus and Mainster lenses magnify the spot size by factors of 1.75 and 2.2, respectively. Topical anesthesia is usually sufficient for most treatments though retrobulbar anesthesia improves pain control and limits ocular motility.

Macular edema is usually treated with the slit-lamp delivery system through a flat lens. Microaneurysms are focally treated with 50–100 μm spots and sufficient power to blanch them. A complete or modified grid pattern of spots is used to treat broader areas of capillary leakage (Fig. 3.3). A typical laser treatment includes 50–100 μm spot sizes, power to produce light burns with only mild blanching of the

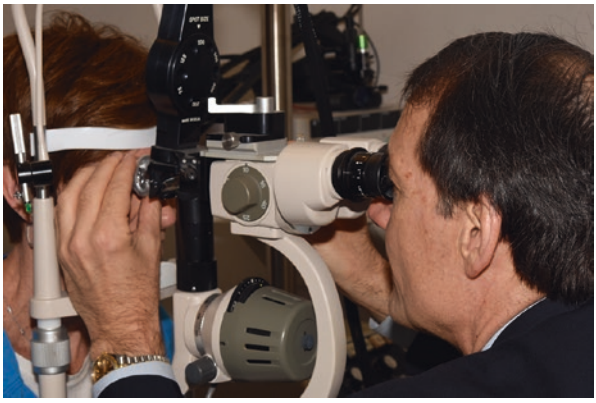

Fig. 3.2 Laser treatment of diabetic macular edema and proliferative diabetic retinopathy is most commonly performed with the slit-lamp delivery system. The surgeon directly visualizes the retina through a contact lens and focuses the laser beam on microaneurysms, areas of capillary leakage, and areas of peripheral retina

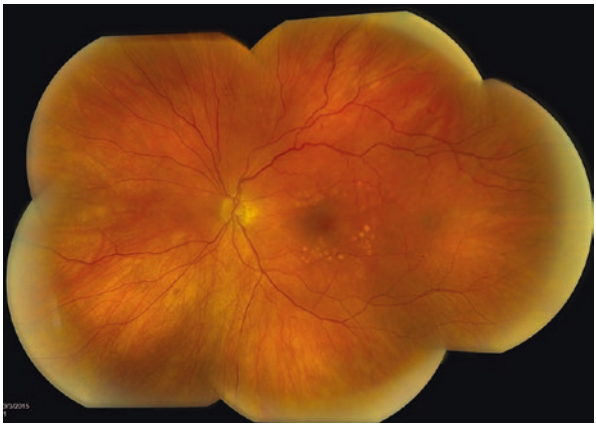

Fig. 3.3 This composite photograph shows scarring from a previous modified grid laser pattern in the macula. Note that the spots are not confluent and they do not completely encircle the macula

retina and pigment epithelium, and separation of burns by 2 spot diameters. Spots are not placed within 500 μm of the fovea during the initial treatment, but if edema persists and visual acuity remains decreased, subsequent spots can be placed to within 300 μm of the fovea but outside of the foveal avascular zone. If the foveal avascular zone is larger than normal because of capillary non-perfusion, spots should be kept further from the fovea to avoid areas of perifoveal non-perfusion.

Treatment recommendations have changed since the older ETDRS guidelines when spots were larger, closer together, and more powerful (Fig. 3.4). The new guidelines produce a subtler treatment pattern that is believed to cause fewer permanent scotomas. Since the spacing between spots is increased, there remains sufficient space for repeat laser treatments, if needed.

Treatment of non-center-involving edema, even in the anti-VEGF drug era, usually includes focal treatment of microaneurysms and a modified grid pattern to areas of edema. Some studies suggest that focal treatment of microaneurysms may not be required as treatment of the RPE may produce the desired effect [115]. Extrafoveal edema often resorbs after laser and the effect may be durable, thereby preventing the subsequent need for a series of anti-VEGF injections. If necessary, laser treatments can be repeated at 3–4-month interval. When a retreatment is planned, fluorescein angiography may help identify areas of persistent leakage.

Complications of focal/grid laser are unusual but can be serious. Laser photocoagulation permanently damages photoreceptors and retinal pigment epithelium, and large, heavily pigmented laser scars may cause paracentral scotomas. Densely applied treatment patterns decrease color vision and contrast sensitivity. Laser spots can enlarge and encroach on the fovea – referred to as "laser creep." In one series of patients with DME, enlarging laser scars reached the fovea in 11 of 203

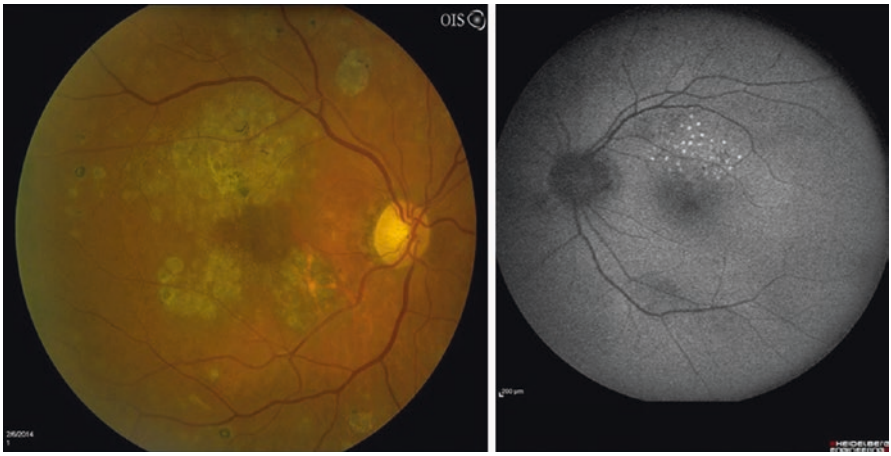

Fig. 3.4 The figure on the left shows confluent laser spots resulting from large, intense laser spots that may have been placed too close together. These spots had been placed according to the original ETDRS guidelines. The figure on the right shows a treatment pattern that was done in accordance with the new modified treatment guidelines. The spots are smaller, less intense, and spaced farther apart. This decreases the likelihood of spot enlargement or "laser creep"

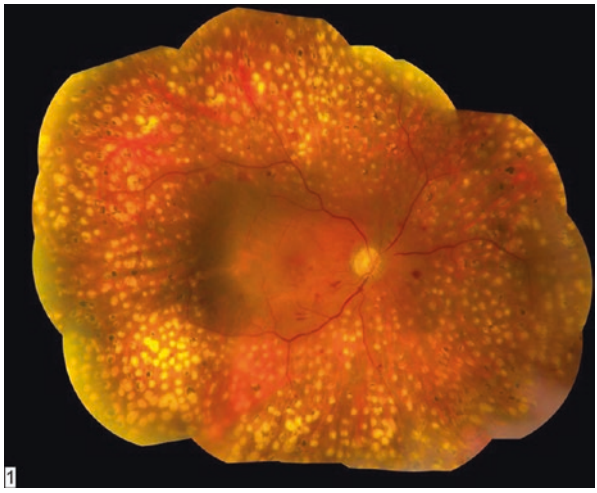

Fig. 3.5 This composite fundus photograph shows mature laser scars following a completed panretinal photocoagulation treatment. The treatment has been extended posteriorly to the vascular arcades to achieve maximum effect

eyes [127]. Laser scars in the posterior pole expand more than those in the periphery possibly because the greater density of photoreceptors facilitates heat transfer [87]. Surgeons must be careful not to use too much energy when using small spot sizes because of the risk of an RPE rupture. Rupture can be followed by choroidal neovascularization with accompanying subretinal hemorrhage, exudation, and disciform scarring [80, 82].

Several pathophysiologic mechanisms have been proposed to explain why macular laser improves DME. Laser is believed to increase local oxygen tension [135], decrease autoregulatory vasoconstriction [45], decrease the entire area of abnormal leakage [155], restore the outer blood-retinal barrier [42, 89], decrease the production of vasoactive cytokines (primarily VEGF), and increase phagocytosis by the RPE and retinal glial cells. RPE cells at the margins of burns may modulate the production of cytokines through their interactions with photoreceptors [28, 100].

Proliferative diabetic retinopathy has been treated with scatter or panretinal photocoagulation (PRP) for the past 40 years. Moderate-intensity spots of 350–500 μm in diameter and 0.075–0.2 s. in duration are delivered through an inverting, panfundus contact lens or 3-mirror contact lens to the mid-peripheral retina. Spots are spaced 1 diameter apart and are applied from just outside the major vascular arcades to the far peripheral retina (Fig. 3.5). Complete treatment usually consists of 1200–3000 spots, but more may be delivered either primarily or for resistant cases. In the Diabetic Retinopathy Study (DRS), photocoagulation was applied directly to patches of new blood vessels, but since this does not adequately address the underlying cause of neovascularization and fails to promote regression, this strategy has been abandoned. Panretinal photocoagulation can also be performed with an indirect ophthalmoscopic delivery system, which enables physicians to more completely treat the far peripheral retina (Fig. 3.6). Treatments are often divided into 2–3 sessions to improve patient comfort. Many physicians choose to photocoagulate the inferior retina first so that if the post-laser course is complicated by a

Fig. 3.6 Panretinal photocoagulation can be delivered through the indirect ophthalmoscope with the patient in the supine position. Since ultra-widefield angiography has shown that broad areas of peripheral retinal non-perfusion may contribute to the development of neovascularization, many surgeons advocate the routine use of indirect ophthalmoscopic laser delivery in all cases of proliferative diabetic retinopathy

vitreous hemorrhage, the view of the superior retina is less likely to be obstructed and the course of treatment can be completed. Smaller spot sizes and longer wavelengths (such as 810 nm) penetrate vitreous hemorrhages better.

Preretinal neovascularization usually regresses within 3 weeks of adequate PRP (Fig. 3.7) [24], and patients who have a favorable early response usually have a better visual outcome than those who do not [149]. Persistent or progressive neovascularization despite the performance of a standard course of PRP may respond to additional laser. Indirect laser-assisted treatment with up to 8000 spots [150] has been described. Ultra-widefield fluorescein angiography can visualize persistent neovascularization and identify untreated areas of peripheral retinal ischemia. These images can be used to guide the placement of supplemental photocoagulation.

Panretinal photocoagulation that spares the posterior retina and preserves the paracentral visual field is sufficient to cause neovascular regression in many cases [7]. Ultra-widefield fluorescein angiography has subsequently shown that most retinal non-perfusion is found in the mid- and far periphery, thereby validating the logic of this approach.

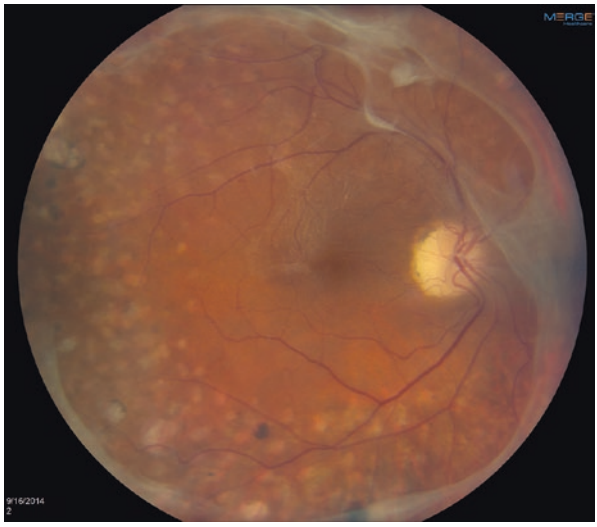

Fig. 3.7 This photograph of the posterior pole shows panretinal photocoagulation scars that have been extended to the vascular arcades. A near-complete ring of preretinal fibrosis extends around the macula, and an epiretinal membrane is causing mild macular wrinkling. This represents successful post-laser regression of what were previously active neovascular fronds

Exudative choroidal detachments may complicate intensive, widespread PRP, but this rarely occurs after split-session treatments [57]. High-intensity burns can rupture Bruch's membrane and lead to retinal and vitreous hemorrhages and the ingrowth of choroidal neovascular membranes [11]. Peripheral visual field loss and night blindness can follow high-intensity PRP. Worsening of preexisting vitreoretinal traction can occur after intense photocoagulation [92], but treatment with divided sessions usually avoids this complication.

Broad areas of mid-peripheral and peripheral retina must usually be ablated to successfully involute neovascularization. Physicians have long appreciated that the severity of neovascularization correlates with the amount of retinal ischemia [98], but the biochemical processes responsible for angiogenesis were not understood. The recent discovery of vascular endothelial growth factor (VEGF) [16, 32], the understanding of its pivotal role in angiogenesis [10, 31, 47, 138], and its detection in the vitreous in eyes with PDR suggest that it plays an important role in eyes with PDR.

Several lines of evidence associate VEGF upregulation with retinal neovascularization. Tissue hypoxia is the most important upregulator of VEGF synthesis [26], and advanced DR is characterized by broad areas of capillary closure with retinal ischemia. The cell's oxygen sensor, hypoxia-inducible factor (HIF)-1α, is stabilized when oxygen tension is low, enabling it to upregulate the synthesis of VEGF (Fig. 3.8) [44, 148]. Administration of exogenous VEGF into monkey eyes induces retinal neovascularization and other findings typical of DR [144]. Panretinal photocoagulation is thought to improve overall retinal oxygen tension by either destroying large areas of ischemic retina or increasing oxygen diffusion from the choroid into the remaining retina. Improved retinal oxygenation downregulates VEGF and involutes immature neovascular vessels.

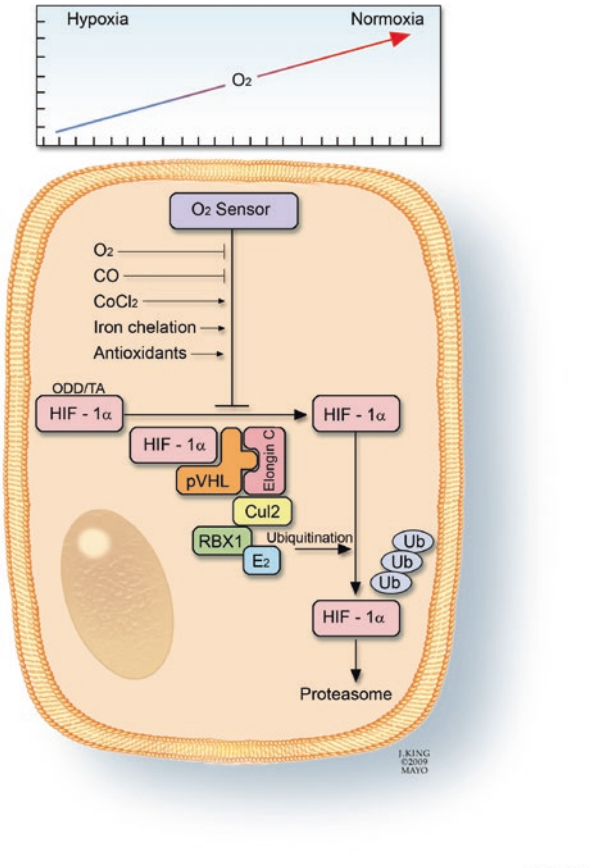

Fig. 3.8 This drawing shows the mechanism by which hypoxia-inducible factor (HIF)-1α acts as the cell's oxygen sensor. In the presence of sufficient oxygen, HIF-1α binds to the von Hippel-Lindau complex, undergoes ubiquination, and is eventually degraded in the proteosomes. This process can be affected by factors such as carbon monoxide, iron chelators, and antioxidants

3.5 Treatment of Background Diabetic Retinopathy

The Early Treatment of Diabetic Retinopathy Study (ETDRS) produced level I evidence supporting the use of laser photocoagulation for patients with clinically significant macular edema (CSME) due to diabetic retinopathy (Table 3.2). The ETDRS was designed to test the theory that laser photocoagulation is superior to observation for patients with CSME [29]. Diabetic patients were eligible for enrollment if they had biomicroscopic evidence of CSME, which was defined as follows:

1. Retinal thickening within 500 μm of the fovea
2. Hard exudates within 500 μm of the fovea that were accompanied by adjacent thickening
3. Retinal thickening of 1 disc diameter in area part of which was within 1 disc diameter of the fovea

Table 3.2 The definitions of clinically significant macular edema (CSME) and diabetic macular edema (DME) are listed

Clinically significant macular edema is diagnosed if ANY of the following is met:
1. Thickening at or within 500 μm of the fovea
2. Hard exudates within 500 μm of the fovea with adjacent retinal thickening
3. An area of macular thickening at least 1 disc area in size, any part of which is within 1 disc diameter of the fovea
**To make the diagnosis of CSME, macular thickening is diagnosed by slit-lamp biomicroscopy
Diabetic macular edema is diagnosed if the following is met:
1. Thickening within 2 disc diameters of the fovea
For clinical purposes, DME is classified as:
1. Center-involving *or* non-center-involving
2. Diffuse *or* focal

The presence of clinically significant macular edema was used to guide laser photocoagulation, but this definition has become less important since the introduction of retinal pharmacotherapy

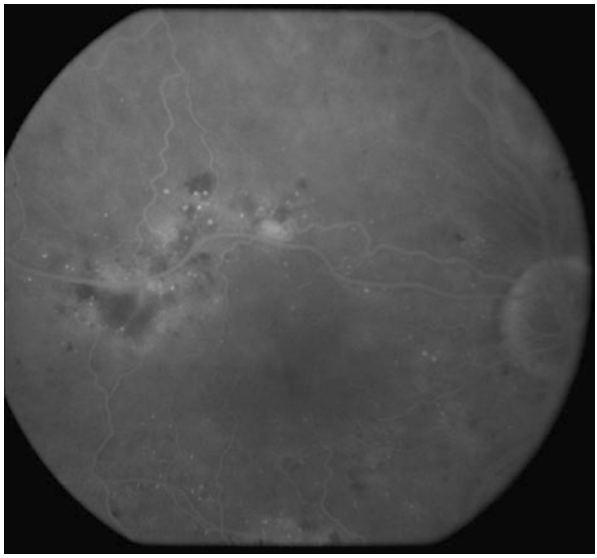

Fig. 3.9 This venous phase frame from a fluorescein angiogram shows areas of dye leakage in the superior macula from microaneurysms and capillary beds. Macular edema is usually found in areas that display these findings

Eligible eyes had baseline visual acuities that ranged from 20/20 to 20/200. Fluorescein angiography (FA) was performed at baseline, but angiographic findings were not used to define CSME (Fig. 3.9). Angiography served two main purposes in patients with diabetic macular edema. First, it identified microaneurysms and areas of diffuse capillary leakage, information that helped physicians accurately place laser spots. Second, the FA identified areas of capillary non-perfusion, information that helped physicians set appropriate visual expectations with the patients and helped them avoid treating areas of non-perfusion.

Enrolled eyes were randomized to observation or fluorescein angiography-guided laser photocoagulation. Treatment was applied focally to microaneurysms and in a grid pattern to areas of diffuse capillary leakage. Microaneurysms were treated directly with spot sizes of 50–100 μm and durations of 0.05–0.1 s until either darkening

or whitening of the microaneurysms was observed; areas of diffuse capillary leakage received a grid pattern of laser with moderate-intensity burns, spot sizes of 100 μm separated by 100 μm, and duration of 0.05–0.5 s. Spots were not applied within 500 μm of the foveal center, and treatment could be extended out past 3000 μm from the fovea. Eyes were eligible for retreatment on a quarterly basis to areas of persistent or new leakage that were untreated or judged to be incompletely treated. Argon laser was used during the ETDRS, but other wavelengths are likely to be as effective [116]

Overall in the ETDRS, 30% of laser-treated patients experienced some improvement in BCVA. Patients receiving laser had a 50% decrease in their risk of moderate vision loss (15 letters) over the course of 3 years. Unfortunately, only 3% of laser-treated patients improved by +15 letters (three lines) at 3 years and 40% of laser-treated eyes had persistent CSME at 12 months [30]. Nonetheless, the trial results suggested that all eyes with CSME should be treated with laser, including those with 20/20 visual acuity.

Persistent DME following laser photocoagulation is not uncommon as the closure rate of microaneurysms with focal laser is only 70% [78], and the need for repeat treatment to areas of diffuse capillary leakage is common. Unless the underlying disease process is halted, new areas of macular thickening frequently develop.

3.6 Treatment of Proliferative Diabetic Retinopathy (PDR)

Randomized clinical trials have validated the efficacy of panretinal photocoagulation for proliferative diabetic retinopathy, and many of these were summarized in a 2007 systematic review [102]. An early British trial used the xenon arc photocoagulator [12] to treat PDR, and this was soon followed by the National Eye Institute-sponsored Diabetic Retinopathy Study (DRS; ClinicalTrials.gov number, NCT00000160). The DRS was one of the first multicenter, randomized studies in medicine and served as a model for subsequent collaborative trials [139]. The DRS enrolled 1758 patients with visual acuities of 20/100 or better in each eye and with either proliferative diabetic retinopathy in at least one eye or severe nonproliferative diabetic retinopathy in both eyes. Each patient was randomly assigned to undergo panretinal photocoagulation with either the xenon arc photocoagulator or argon laser in one eye, with the other eye serving as the untreated control. Treatment consisted of 800–1600 burns of 0.1 s duration and new blood vessels were treated directly. Panretinal photocoagulation reduced the risk of severe vision loss (defined as visual acuity of 20/800 or worse at two consecutive 4-month visits) from 14.0 to 6.2% over a 2-year period [139] and from 33.0 to 13.9% over a 5-year period [142].

There were no significant differences between the xenon arc and argon groups regarding the primary outcome, but eyes treated with argon maintained better visual acuities and fewer visual field and night vision defects. Panretinal photocoagulation reduced the overall incidence of severe vision loss by at least 50% [140]. Eyes at high risk of vision loss included those with moderate neovascularization of the disc (covering at least ¼ of the disc area) and those with any neovascularization of the disc (NVD) or elsewhere (NVE) that was accompanied by preretinal or vitreous hemorrhage [141].

The ETDRS (NCT00000151) enrolled 3711 patients with mild-to-severe nonproliferative or early proliferative diabetic retinopathy in both eyes. Each patient was assigned to undergo panretinal photocoagulation in one eye, while treatment in the fellow eye was deferred until the development of high-risk PDR. Rates of severe vision loss at 5 years were 2.6% following early treatment and 3.7% following deferred treatment [30]. The ETDRS showed that performing PRP in eyes with advanced pre-proliferative diabetic retinopathy generally does not change the visual outcome. The Diabetic Retinopathy Clinical Research Network published guidelines for the treatment of proliferative diabetic retinopathy [23]. Treatment should be initiated promptly when an eye develops high-risk PDR as delaying treatment increases the short-term risk of vision loss because vitreous hemorrhage may develop.

Treatment of coexistent diabetic macular edema and high-risk PDR is particularly challenging. Following PRP, 25–43% of eyes may develop DME or experience an increase in macular leakage with resulting loss of visual acuity [54, 90, 91]. The mechanism responsible for the development of DME after PRP is not well understood, but the accumulation of leukocytes in the posterior pole and the increased release of permeability promoting chemokines and cytokines (including VEGF) due to laser damage may play a role [40, 113, 146]. The ETDRS previously recommended macular laser photocoagulation for DME either before or at the time of PRP [1, 30]. Macular laser photocoagulation performed at these times decreased the risk of moderate visual acuity loss by 50%, but patients experienced minimal visual improvement and only 3% experienced three-line improvements in VA at 3 years. Unfortunately, 12% of eyes lost three lines of VA at 3 years, and 40% of eyes with baseline macular edema had persistent edema at 12 months.

These treatment guidelines were formulated before the introduction of retinal pharmacotherapy, and recent studies have demonstrated that the intravitreal injection of triamcinolone acetonide or anti-VEGF agents prior to PRP minimizes the exacerbation of DME and in many cases with pre-existing edema actually improves VA. Furthermore, pharmacotherapy is synergistic with panretinal photocoagulation in promoting the regression of retinal neovascularization [4, 14, 145, 163]. In a randomized, comparative study, intravitreal triamcinolone was superior to bevacizumab at resolving macular edema and preventing PRP-induced VA loss in eyes with preexisting macular edema, while both drugs were effective at preventing the development of edema in eyes with dry maculas [15]. Recent findings suggest that ranibizumab monotherapy effectively treats PDR through 2 years [157], so anti-VEGF injections may be increasingly used to treat both DME and PDR over extended periods of time, thereby delaying or even avoiding the need for macular laser or PRP.

3.7 New Laser Technologies

The ETDRS provided physicians with a proven strategy to treat CSME, but concerns regarding treatment intensity and subsequent complications lingered. Large spot size, insufficient burn separation, and excessive treatment intensity may all

limit visual acuity gains and induce post-treatment scotomas. Clinicians have modified treatment strategies, and laser manufacturers have developed new technologies in attempts to improve efficacy and safety.

3.7.1 Micropulse Laser

A new laser delivery method featuring short bursts or "micropulses" rather than a continuous wave was introduced in 1990 [118]. Compared to standard pulse thermal laser, micropulse spots heat the target tissues less, spare the overlying photoreceptors, and theoretically reduce iatrogenic side effects such as scarring and fibrosis [19]. Micropulse does not adversely affect the neurosensory retina and leaves it undamaged. The mechanism by which micropulse may exert a treatment effect is unclear though "normalizing RPE function" and decreasing cytokine production have been suggested [20, 41].

The primary determinant of tissue heating is the duty cycle or frequency of micropulses. Longer periods between pulses – lower duty cycle – heat the tissue less. A lower duty cycle limits heat diffusion, produces smaller spot sizes, and creates less thermal tissue damage. Since longer wavelengths (647 and 810 nm) are absorbed less by the retinal chromophores, more energy reaches the RPE without affecting the overlying retina. By micropulsing an infrared laser, energy is delivered directly to the RPE [27, 118].

Early micropulse treatment of DME produced faint scars because physicians sought to mimic the more familiar results that they achieved with thermal laser [39, 103, 109]. They subsequently learned to decrease the duty cycle to achieve a clinical effect without the formation of permanently visible RPE scarring. By not creating a tissue scar, micropulse preserves the outer retina and avoids the adverse effects that characterize thermal laser [99]. Over the years, there has been a slow increase in the popularity of micropulse laser for DME as new delivery devices have become more refined.

Treatment of eyes with macular edema is said to follow a "low-intensity/high-density" pattern. Near confluent spots are delivered throughout the macula with some authors treating to within 200 μm of the fovea. Typical treatment parameters include the following: spot size of 75–125 μm, pulse duration of 300 ms with a 15% duty cycle, and power of 1000 mW. If a tissue reaction is seen, the power is reduced by 200 mW increments until no further reaction is noted [117].

A 2005 pilot study with the subthreshold diode micropulsar (SDM) laser, which uses an 810 nm diode laser at suboptimum intensity, suggested that SDM laser photocoagulation produces results similar to those achieved with thermal laser photocoagulation without causing iatrogenic retinal damage [84]. Small, retrospective studies have shown improvements in macular edema and visual acuity [73, 112].

In a large prospective study with 220 patients, mean CMT improved from 353 to 215 μm at 1 year and mean visual acuity was stable (0.21 LogMAR to 0.18 LogMAR) [117]. A 2009 prospective, randomized controlled trial compared the

efficacy of SDM laser photocoagulation with conventional argon green laser delivered according to the modified ETDRS protocol. The BCVA remained stable in both arms at 12 months but laser scars were identified in only 13.9% of eyes treated with SDM compared to 59% of eyes treated with argon laser [34]. In a study of 24 eyes that had undergone SDM laser, fundus autofluorescence at 1 year failed to detect damage to the RPE [152].

In a long-term (median follow-up of 47 months) study of 252 eyes treated with micropulse, none of the eyes treated with low irradiance (<350 W/cm^2) had detectable damage on fundus photography, fluorescein angiography, or SD-OCT [85]. Unfortunately, visual acuity results from this study were not reported.

The absence of observable damage to the photoreceptors and RPE paved the way for high-density SDM treatment of the macula. By delivering approximately 900 spots to the macular in a confluent pattern that spares the fovea, lateral spread of thermally stimulated cells is promoted in an effort to improve visual outcomes. A 2011 study suggested that high-density SDM produces superior improvements in macular edema and BCVA compared to both low-density SDM and conventional laser therapy [76]. The 577 nm micropulse may also be superior to the 810 nm laser at closing microaneurysms [58].

Micropulse laser treatment of DME appears promising, but current data does not suggest that its results are superior to those of standard thermal photocoagulation. Large, randomized, controlled trials against standard laser photocoagulation and in combination with anti-VEGF therapy are needed to better understand the role of micropulse in the management of DME.

3.7.2 Navigated Laser

Another newly developed method of delivering subthermal laser to the macula involves placing spots in a horseshoe-shaped grid pattern with a 577 nm semiautomated patterned scanning laser (PASCAL, Topcon, Capelle aan den IJessel, the Netherlands). This system allows surgeons to deliver multiple spots simultaneously to the macula or peripheral retina in a preprogrammed pattern. The PASCAL uses burns of 10 or 20 ms that localize to the outer retina, thereby avoiding damage to the RPE and inner retina [105, 106]. Since the PASCAL uses visible wavelength, burns can be seen with SD-OCT 1 h after treatment. Treatment effects are localized to the junction of the photoreceptor inner and outer segments and RPE apices. At 12 months, SD-OCT shows treatment effects at the RPE apices but without overlying changes in the photoreceptors [68].

Panretinal photocoagulation treatment technique has not changed since the DRS, but the PASCAL laser shortens treatment duration and decreases pain while producing the same outcomes [2, 8]. The PETER PAN study showed that the PASCAL laser produced results similar to standard PRP but with lower power settings and less vision loss [107]. Single-session, complete PRP with the PASCAL appears as effective as multi-session single-spot PRP treatments with no difference in the incidence

of macular edema [108]. This technology has the potential to improve patient compliance and decrease the overall cost of care by requiring fewer treatment visits.

The navigated laser photocoagulator (NAVILAS®, NAVILAS Laser System, Irvine, CA, USA) tracks the eye and simultaneously integrates digital fluorescein angiographic information. This stabilizes the real-time retinal image and overlays the acquired fundus photograph to improve the accuracy of laser delivery [70]. In a small retrospective study of seven patients with DME treated with the NAVILAS system, median central foveal thickness decreased (248–220 μm) and LogMAR VA improved (0.695–0.477) [62]. In the 12-month CAVNAV study for treatment of DME, patients receiving anti-VEGF therapy combined with navigated laser required fewer anti-VEGF injections than did patients treated with anti-VEGF injections and standard thermal laser photocoagulation [6].

3.8 Pars Plana Vitrectomy

Approximately 60% of patients with PDR achieve satisfactory regression of neovascularization within 3 months of PRP [149], but many patients require additional laser and 4.5% ultimately undergo pars plana vitrectomy because of vitreous hemorrhage and/or traction retinal detachments [37]. Vitreous hemorrhage is a cause of significant vision loss in patients with PDR, and over the course of 3–10 years, vitreous hemorrhage persists or worsens in two-thirds of patients [164]. For recurrent or non-clearing vitreous hemorrhage, vitrectomy is indicated to clear the visual axis, allow adequate laser photocoagulation to be applied to prevent further bleeding, and address fibrovascular traction [160].

Pars plana vitrectomy has been used to treat DME for more than two decades. In the first vitrectomy for DME series, nine of ten patients with biomicroscopically evident vitreomacular traction experienced improved vision [79]. Encouraged by these results, surgeons began operating on eyes with DME, without concern for the condition of the posterior hyaloid. (Fig. 3.10). Many early studies reported improved postoperative VA, but these were often retrospective, with poor VA measurement protocols and without OCT images. These served as encouraging pilot studies but provided insufficient data for surgeons to make firm treatment recommendations.

Subsequent studies enrolled patients prospectively and measured macular thickness with time-domain optical coherence tomography (OCT), and some included control arms. Dozens of studies have been published with visual acuity results that vary considerably, but most show objective evidence of postoperative macular thinning by OCT. Few of the studies included spectral domain OCT analysis of outer retinal integrity, and none has produced level I evidence supporting the use of vitrectomy for the treatment of DME.

Landers et al. performed a literature review and composite analysis of vitrectomy for DME [74, 75] that included 37 manuscripts published between 2002 and 2012 [3, 13, 25, 35, 36, 49, 52, 53, 55, 63–67, 71, 72, 83, 86, 101, 110, 111, 119–121, 123, 124, 126, 128–130, 132, 136, 143, 158, 159, 161, 162]. These studies reported

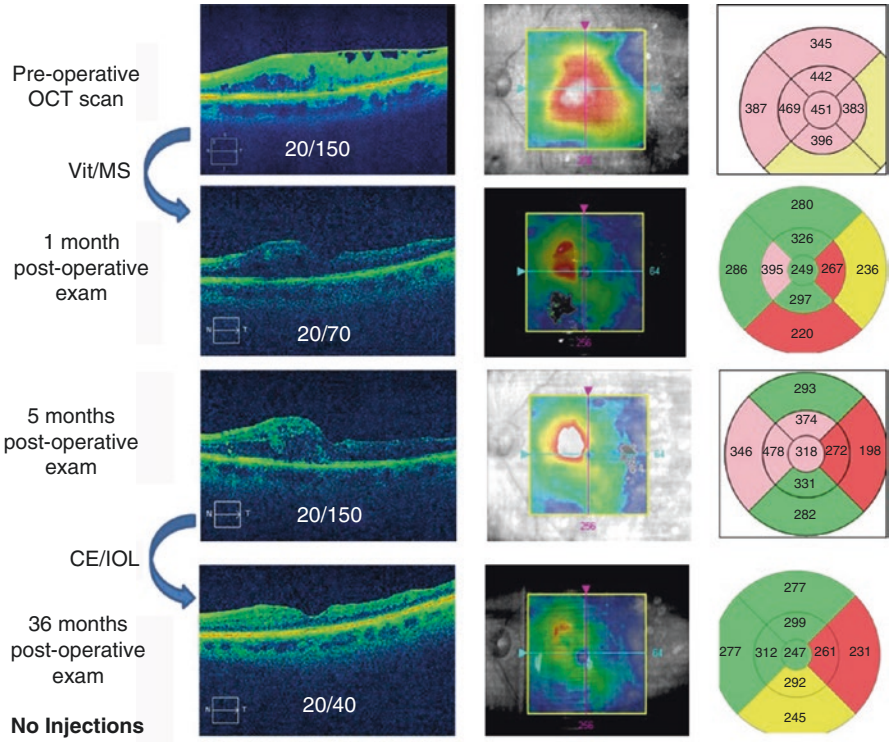

Fig. 3.10 This figure shows an eye with diabetic macular edema that underwent pars plana vitrectomy. Prior to vitrectomy (*top row*), the eye had a prominent epiretinal membrane and thickened macula. At 1 month (*second row*) and 3 months (*third row*) postoperatively, the macular edema had improved but had not completely resolved. The visual acuity at 5 months had dropped to 20/150 because of a nuclear sclerotic cataract. Following cataract removal, the visual acuity improved to 20/40. By 36 months postoperatively the macular edema had resolved. This eye did not require pharmacotherapy at any time. *CE/IOL* cataract extraction with posterior chamber lens insertion

excellent mean thinning of the macula (−187 μm), which compares favorably with anti-VEGF therapy [22, 97, 99, 114]. Improvements in VA were generally favorable but highly variable.

The DRCR.net performed a prospective, single-arm vitrectomy study that enrolled 241 patients who, in the opinion of the primary investigator, would not benefit from additional standard therapy [36]. The mean subfield thickness decreased from 412 to 278 μm at 6 months with more thinning in eyes that had greater preoperative thickness, the presence of an epiretinal membrane, and the presence of vitreomacular traction. Although the average VA (20/80) of the entire cohort did not change, significant visual acuity improvements were seen in eyes with worse baseline vision ($P < 0.001$) and in those requiring the removal of epiretinal membranes ($P = 0.006$). The authors concluded that vitrectomy may have a role in eyes with vitreomacular interface abnormalities but that vitrectomy for eyes without traction could not be recommended.

Most eyes that were enrolled in the DRCR.net study had advanced DME that had been previously treated with laser and/or corticosteroids. Many of these eyes may have already suffered irreversible macular damage that would not have improved with vitrectomy or any other treatment. Chhablani et al. correlated visual acuity improvement after vitrectomy with the preoperative integrity of the external limiting membrane (ELM) and inner segment/outer segment (IS/OS) [13]. They found that ELM integrity on SD-OCT correlated with better final visual acuity and IS/OS integrity correlated with the greatest improvement in vision. Spectral domain OCT was not available to the DRCR.net study, so detailed evaluation of outer segment health was not possible.

In one novel study of 20 patients with chronic DME and subfoveal fluid, saline was injected beneath the fovea, after which an air-fluid exchange was performed followed by facedown positioning [104]. Eighty-five percent of patients had complete resolution of edema, and the VA improved by three lines in 65% of patients.

3.9 Conclusion

Laser photocoagulation still plays an important role in the management of PDR though anti-VEGF therapy has been recently shown to be equally effective. Laser photocoagulation remains the preferred treatment for most eyes with macula-sparing DME, but its role in the management of center-involving DME is unclear. Vitrectomy for DME is usually reserved for eyes that fail other therapies, but its role as an early treatment for DME continues to be evaluated [59].

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Chapter 4
Targeting Vascular Endothelial Growth Factor

4.1 Introduction

Laser photocoagulation was the preferred treatment for the complications of diabetic retinopathy – diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) – for several decades with level I evidence emerging from the Early Treatment of Diabetic Retinopathy Study [45] and the Diabetic Retinopathy Study (DRS) [166–168]. Laser reduced the risk of severe vision loss (<5/200) in eyes with PDR and moderate vision loss (>15 letters) in eyes with DME by one half, but significant improvements in visual acuity (>15 letters) were unusual.

In an attempt to improve the treatment of macular edema, physicians began injecting triamcinolone acetate into the vitreous of the eyes with macular edema due to diabetic retinopathy and retinal vein occlusions [77, 91, 114] early in the twenty-first century. Corticosteroids improved macular edema dramatically but the high incidences of posterior subcapsular cataracts and elevated intraocular pressures limited their widespread adoption. Surgeons frequently performed pars plana vitrectomy for advanced complications of PDR (vitreous hemorrhage, traction retinal detachment, traction/rhegmatogenous retinal detachment) [169] and even for DME that was accompanied by vitreomacular traction [106]. Surgical outcomes for PDR were superior to the natural history of the disease [169], but vitrectomy only benefited a small number of patients with vision loss due to DME [60].

Effective pharmacotherapy for DME eventually came from a surprising source – tumor biology [24, 56]. The discovery of vascular endothelial growth factor (VEGF) and the early understanding of its role in neovascular age-related macular degeneration, macular edema due to retinal vein occlusions, DME, and PDR [5] set in motion a pharmacologic revolution in ophthalmology. The development of potent ocular pharmaceuticals created a paradigm shift in the treatment of center-involving diabetic macular edema and more recently in the treatment of PDR (Fig. 4.1).

Many manuscripts describing the treatment of DME with anti-VEGF drugs have been published, but most are flawed with small numbers of patients, short durations of treatment and follow-up, lack of control arms and randomization, and inadequate

M.W. Stewart, *Diabetic Retinopathy*, DOI 10.1007/978-981-10-3509-8_4

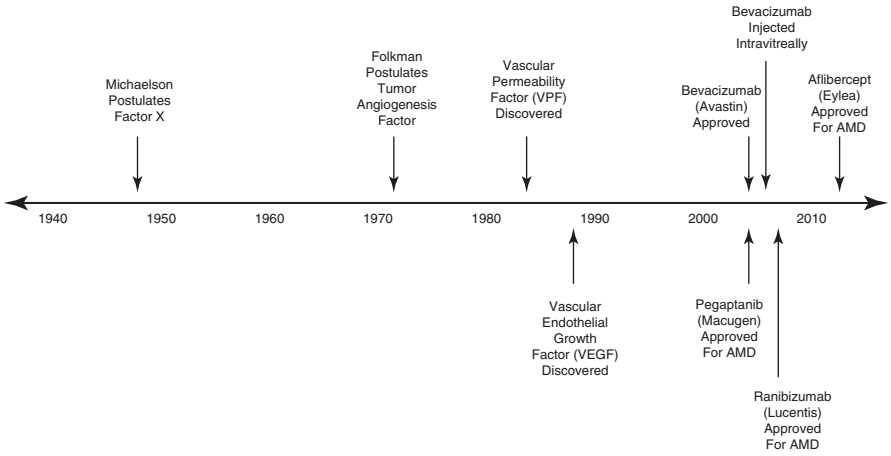

Fig. 4.1 Important milestones in the discovery of vascular endothelial growth factor and the development of VEGF-inhibitory drugs. Each of the VEGF-inhibitory drugs was initially approved by the US Food and Drug Administration for the treatment of neovascular age-related macular degeneration (nAMD) with approvals for diabetic macular edema lagging by 6 years (ranibizumab) and 3 years (aflibercept)

masking. Fortunately, several high-quality, randomized, controlled, double-masked, multicenter trials have been published, and these constitute the focus of this chapter.

4.2 Vascular Endothelial Growth Factor

Pathologic ocular neovascularization has long been recognized as the sequela to severe vascular injury. Michaelson (1948) postulated the existence of a soluble factor X that he believed was responsible for new blood vessel growth in conditions such as neovascular glaucoma (Fig. 4.2) [119]. Folkman (1971) noted that both primary and metastatic solid tumors in children were accompanied by leashes of blood vessels through which the tumor obtained oxygen and nutrients [61]. He postulated that solid tumors synthesized a pro-angiogenic substance that promoted blood vessel growth necessary to sustain tumors.

Vascular permeability factor (VPF) was discovered in 1983 [155], and Ferrara and Connolly independently discovered vascular endothelial growth factor (VEGF) [25, 56] 6 years later. Sequencing analysis showed that both investigators had identified the same molecule, which turned out to be the previously discovered VPF. This set in motion a multi-specialty, international research initiative that characterized VEGF, its receptors, and its downstream pathways.

VEGF is actually a group of closely related molecules that segregate into seven families: VEGF-A, VEGF-B, VEGF-C, VEGF-D, the orf virus encoded VEGF-E,

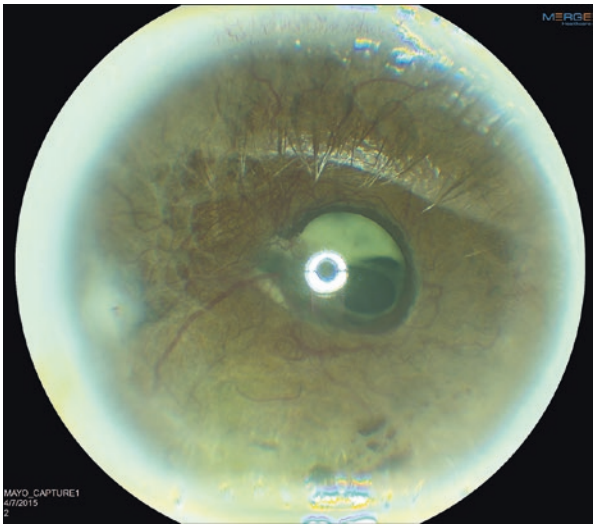

Fig. 4.2 Slit lamp photograph showing severe neovascularization of the iris. Contraction of the neovascular membrane on the anterior surface of the iris everts the pupillary margin and exposes the posterior pigmented epithelium of the iris (ectropion uvea)

VEGF-F, and placental growth factor (PlGF) (Table 4.1). Most of these families consist of several isoforms with similar binding sequences.

VEGF-A isoforms are the most important contributors to ocular angiogenesis. The 14 kb VEGF-A gene resides on chromosome 6p21.3 [174]. Splicing of eight exons and seven introns creates at least six major ($VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$, and $VEGF_{206}$) and several minor isoforms within the VEGF-A family [84, 170]. In addition, the split product $VEGF_{110}$ is produced when tissue-sequestered isoforms with 165 or more amino acids are cleaved by plasmin and matrix metalloproteinase-3. The shorter non-heparin-binding isoforms are biologically active but with only 10–20% the biological activity of $VEGF_{165.}$ VEGF naturally exists as a homodimer, so it presents two receptor-binding sites to its environment [95, 104].

Each of the VEGF families controls a different type of angiogenesis (Table 4.1). VEGF-B stimulates coronary blood vessel growth, and VEGF-C and VEGF-D promote lymphangiogenesis by activating VEGF receptor (VEGFR) 3 [94]. The exact role of PlGF in angiogenesis is unclear, but its binding to VEGFR1 may force VEGF-A to preferentially bind to VEGFR2 [137], the receptor primarily responsible for ocular angiogenesis. PlGF may play an active role in the development of DR as it has been found in the retinas of diabetic rats [89, 99].

The common receptor-binding sequence of all VEGF-A isoforms resides in the amino acid region 82–93, whereas the heparin-binding sequence is found in the 111–165 amino acid region of longer isoforms. Molecular charge determines isoform diffusibility with the acidic $VEGF_{121}$ isoform being freely diffusible whereas the basic $VEGF_{206}$ isoform is nearly totally bound to interstitial matrix. Fifty to

Table 4.1 The various VEGF families, the most important isoforms, and their major physiologic effects

Vascular endothelial growth factors and their major functions	
VEGF-A ($VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$)	Responsible for most angiogenesis in adults Responsible for most ocular neovascularization and breakdown of the blood retinal barrier
VEGF-B	Responsible for coronary angiogenesis Promotes tumor metastasis Found in choroidal neovascular membranes
VEGF-C	Main function is lymphangiogenesis Found to participate in angiogenesis, perhaps including choroidal neovascular membranes Participates in neural development and blood pressure regulation
VEGF-D	Main function is lymphangiogenesis surrounding pulmonary bronchioles
VEGF-E	Encoded by orf viruses
VEGF-F	Expressed in the venom of the habu snake
Placental growth factor 1 and 2	Found in choroidal neovascular membranes Important for vasculogenesis and angiogenesis during ischemia, inflammation, wound healing, and cancer

The isoforms of VEGF-A are most important for ocular angiogenesis. Placental growth factor has been found in experimental models of diabetic retinopathy and in the aqueous of eyes with diabetic retinopathy, but its contribution to the development of diabetic retinopathy in humans is unknown

seventy percent of the electrically neutral $VEGF_{165}$ is bound to the interstitial matrix, and heparin-binding sequences enable isoforms of 165 amino acids and longer to bind to interstitial matrix heparin moieties. Sequestration of these longer molecules provides a readily available reservoir of VEGF when injury or inflammation induces a protease-mediated breakdown of the interstitium. The heparin-binding domain also enables $VEGF_{165}$ to bind to the transmembrane neuropilin-1 co-receptor.

Diffusible VEGF binds to the extracellular binding domains of three transmembrane receptors: VEGFR1 (flt-1), VEGFR2 (flk-1), and VEGFR3 (flt-4). Each of these receptors is composed of three major parts: the seven extracellular immunoglobulin-like VEGF-binding domains, the single transmembrane region, and the intracellular tyrosine kinase moieties [28, 165]. VEGFR1 binds VEGF-A, VEGF-B, and placental growth factor; VEGFR2 binds VEGF-A and VEGF-C; and VEGFR3 binds VEGF-C, VEGF-D, VEGF-E, and VEGF-F. VEGFR-1 binds VEGF-A with a much higher affinity than does VEGFR2. Binding of VEGF by VEGFR1 and VEGFR2 occurs at the second and third extracellular domains. Alternative splicing of the RNA transcript for the binding domains of the VEGF receptors creates soluble VEGFR2 (sVEGFR2) and VEGFR1 (sVEGFR1), which inhibit vascular development by promoting vascular maturation and maintaining corneal avascularity [137].

The role of VEGFR1 in angiogenesis is unclear because its downstream activity varies with both the age of the individual and the cell type. Activation of VEGFR1 during embryological development stimulates angiogenesis, but during adulthood it dampens VEGF activity by acting as a decoy receptor.

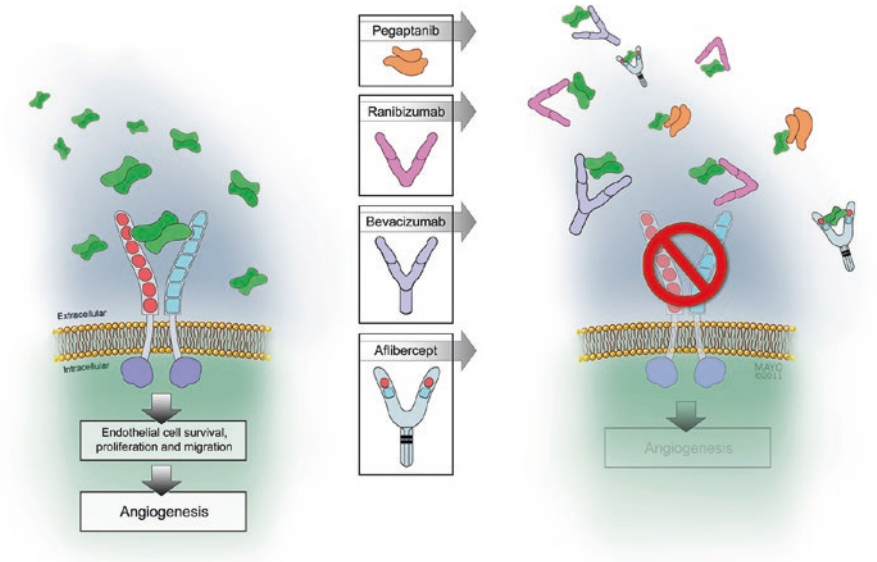

Fig. 4.3 Diffusible dimers of vascular endothelial growth factor (VEGF) bind to transmembrane receptors causing them to dimerize. Receptor dimerization leads to conformational changes and activation of the cytoplasmic tyrosine kinase moieties. These phosphorylate several cytoplasmic proteins, which upregulate downstream biochemical pathways. Each of the four available VEGF-inhibitory drugs binds to diffusible VEGF to prevent receptor binding

VEGF-A activity occurs when either naturally occurring $VEGF_{121}$ or $VEGF_{165,}$ or the fibrin-split product $VEGF_{110}$, binds to VEGFR2 (Fig. 4.3). Each of two VEGFR2 receptors binds to the VEGF dimer causing conformational changes in the 3-dimensional structures of the receptor molecules and activating their intracytoplasmic tyrosine kinase moieties (Fig. 4.2). This phosphorylates several intracellular enzymes that activate downstream pathways, which lead to VEGF's potent effects (Fig. 4.3) [21, 48, 79, 164].

Hypoxic environments created by processes such as tumor growth and occlusive vasculopathies upregulate VEGF [42]. Tissue hypoxia reduces hydroxylation of the cell's "oxygen sensor" HIF-1α, preventing it from binding to the von-Hippel Lindau factor, and undergoing ubiquination and subsequent destruction within proteosomes. Stable HIF-1α dimerizes with HIF-1β to form a complex that enters the cell nucleus where it activates the promoter region of the VEGF gene and also upregulates both VEGFR1 and VEGFR2 [71, 172] synthesis (Fig. 4.4).

VEGF is upregulated during starvation and by several metabolic factors, chemokines, and cytokines. A variety of metabolic regulators including ROS (reactive oxygen species such as superoxide (O_2^-)) increase the synthesis of both VEGF and its receptors [173]. Several growth factors including epidermal growth factor, tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β, basic fibroblast growth factor, interleukin-6, insulin-like growth factor-1, keratinocyte growth

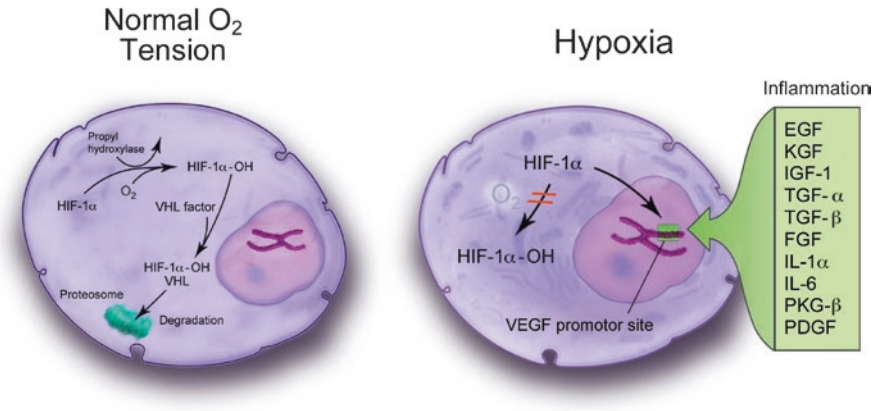

Fig. 4.4 Hypoxia inducible factor (HIF)-1α is normal tissue oxygen conditions and is subsequently metabolized in the proteosomes. Under hypoxic conditions and in the presence of various chemokines and cytokines, HIF-1α becomes stabilized by dimerizing with HIF-1β, enters the cell nucleus, and binds to the promoter region of the vascular endothelial growth factor gene

factor, and platelet-derived growth factor upregulate VEGF and may work in conjunction with tissue hypoxia [128]. Oncologic mutations that stimulate RAS, a family of ubiquitous intracellular GTP-ases, upregulate VEGF [78]. Mechanical forces such as sheer stress and stretch that occur with vitreomacular traction promote VEGF production [46].

VEGF has been detected in both the neuroretina and pigment epithelium [97], and it can be produced by several cell types including pericytes, glia, vascular endothelial cells, invading leukocytes, and retinal pigment epithelium [2, 134]. Some VEGF isoforms modulate the synthesis of others by affecting HIF-1α stability [183], and VEGF inhibitors probably function via a similar mechanism.

PlGF and VEGF-B binding to VEGFR1 activates signaling pathways implicated in monocyte chemotaxis [36] and inflammatory-related angiogenesis. Though PlGF appears to be a critical mediator of inflammatory angiogenesis, it does not appear to directly influence embryologic or physiologic adult angiogenesis [59]. Tyrosine kinase activity of VEGFR1 is relatively weak but several downstream signaling molecules including phospholipase C, the p85 subunit of PI-3 kinase, growth factor receptor bound protein, SHP-2, and NcK are associated with VEGFR1 phosphorylation sites. These pathways result in eNOS activation and calcium influx in vascular endothelial cells.

VEGFR2 activation is responsible for the full spectrum of VEGF-A-mediated responses within human endothelial cells (survival, proliferation, migration, and formation of a vascular tube) by stimulating Raf-MEP-ERK and MAPK pathways [164]. These pathways phosphorylate several endothelial cell proteins including phospholipase-3, PI-3 kinase, RAS GTP-ase activating protein and the Src family [48, 79]. They stimulate cell migration via FAK and paxillin, PI-3 kinase, and MAPK and prolong survival via PI-3 kinase and Akt/PKB [21].

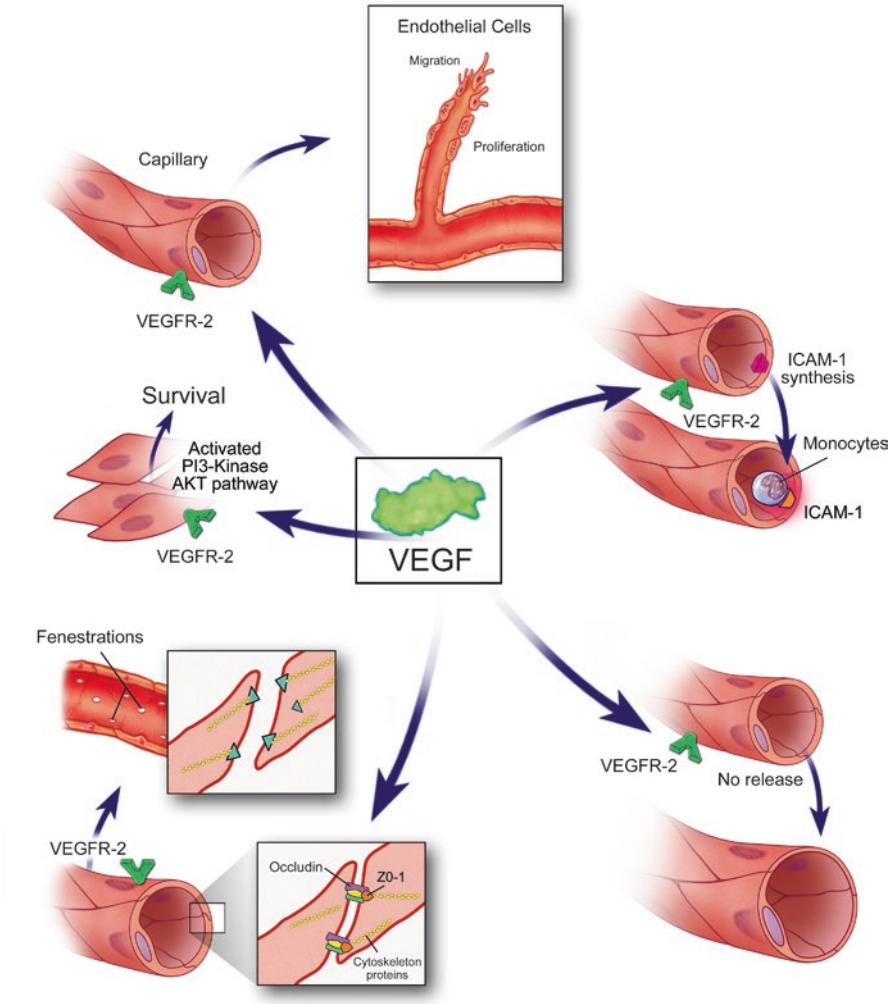

Fig. 4.5 The many functions of vascular endothelial growth factor A

VEGF-A isoforms also bind to the heparin-binding domains of neuropilin-1 and neuropilin-2, transmembrane proteins with small, non-catalytic tails that present $VEGF_{165}$ favorably to VEGFR2. The neuropilin-modulated binding of $VEGF_{165}$ increases its biological activity by tenfold over that of $VEGF_{121}$, which cannot interact with neuropilin [63].

VEGF-A upregulates gene expression that promotes the division, migration, and survival of vascular endothelial cells, regulates vascular dilation and permeability, and stabilizes immature blood vessels (Fig. 4.5) [55]. VEGF-A enhances vascular permeability 50,000 times more than does histamine, increases nitric oxide production, and stimulates the synthesis of several molecules that are important to angiogenesis, such

as matrix metalloproteinases, which break down the interstitial matrix to allow advancement of proliferating endothelial cells [175]. $VEGF_{165}$ induces endothelial cell growth by activating the Raf-MEK-Erk pathway [52]. VEGF degrades the blood retinal barrier (BRB) in venules but not capillaries [126] by causing a calcium-mediated increase in hydraulic conductivity [14], increase in endothelial cell fenestrations [147], and opening of intercellular tight junctions by phosphorylating several junctional proteins [67]. Increased permeability allows plasma to extravasate and form an interstitial fibrin gel into which endothelial cells can proliferate and migrate [44]. VEGF-A increases hexose transport to meet the metabolic demands of neovascularization [138]. VEGF-A produces a dose-dependent vasodilation in vitro via nitric oxide synthesis [102] and retinal vasodilation in animal models and human retinal vein occlusions [51, 171].

VEGF-A promotes the survival of rat endothelial cells in vitro and in vivo, while VEGF blockade causes extensive apoptotic changes in the vasculature of neonatal but not adult mice [73]. Newly formed but not mature vessels are VEGF dependent [16, 181], and a complete vestment of pericytes is key to establishing VEGF independence [16]. Nonetheless, VEGF-A plays a role in maintaining the choriocapillaris in adults [53]. VEGF-A administration to hypoxic vascular endothelial cells upregulates phosphatidylinositol (PI)-3 kinase-Akt pathways and the expression of antiapoptotic proteins Bl-2 and A1 and prevents apoptosis and regression of the vasculature [6, 72]. The effects of VEGF on the retinal pigment epithelium remain controversial as some investigators have noted breakdown of the outer blood-retinal barrier whereas others have not [1, 74, 85, 122].

VEGF-A promotes monocyte chemotaxis and colony formation by granulocyte-macrophage progenitor cells. VEGF-A upregulates the synthesis of intercellular adhesion molecule (ICAM)-1 and vascular cellular adhesion molecule (VCAM)-1, which allows natural killer (NT) cells to adhere to vascular endothelial cells.

Exogenous administration of VEGF into primate eyes induces changes that resemble diabetic retinopathy [171]. VEGF has been successfully blocked in animals with soluble decoy receptors [93], tyrosine kinase inhibitors, and antibodies directed against the VEGF dimer or its receptors [101]. In animal models, blocking VEGF pathways appears to be more effective than blocking other angiogenic pathways [52]. Drugs that block VEGF work primarily by reestablishing the blood-retinal barrier, "normalizing" the circulation, and preventing further new blood vessel growth.

Healthy human eyes have vitreous VEGF concentrations of 8.8 +/− 9.9 ng/ml [5], whereas patients with DME and other retinal vascular conditions have elevated intraocular VEGF concentrations [5, 80]. Following pars plana vitrectomy, the intravitreal half-life of VEGF in rabbit eyes decreases by 75%.

4.3 Anti-VEGF Drugs

Currently available drugs that inhibit the actions of vascular endothelial growth factor are listed in Table 4.2. Included are the major binding and pharmacokinetic properties of each drug.

Table 4.2 Drugs that bind to and inhibit the actions of vascular endothelial growth factor that have been used to treat ocular angiogenesis

Important characteristics of drugs that bind vascular endothelial growth factor				
Characteristic	Aflibercept	Bevacizumab	Pegaptanib	Ranibizumab
Description	Fusion protein with receptor sequences bound to Fc fragment of IgG	Recombinant, humanized, murine antibody to VEGF-A	Pegylated aptamer	Recombinant, humanized, murine antibody fragment to VEGF-A
Molecular weight (kDa)	115	149	50	48
Isoforms and families bound	VEGF-A, VEGF-B, placental growth factor	VEGF-A	$VEGF_{165}$	VEGF-A
Binding affinity for $VEGF_{165}$ (pmol)	0.5	58–1000	46–192	50
Intravitreal half-life (days)	9 (estimated)	9.8	7 (estimated)	7.1
Serum half-life (days)	6	21	0.2	2–4

The major binding and pharmacokinetic properties of each drug are detailed

4.3.1 Pegaptanib

Pegaptanib is a 28-base oligonucleotide – a modified RNA fragment or aptamer – that attaches to the heparin-binding domain of $VEGF_{165}$. It has a molecular weight of 50 kDa, a negative charge of 28, and a hairpin structure. The addition of a 40 kDa polyethylene glycol chain (pegylation) increased the drug's binding affinity (K_D = 50 pM for $VEGF_{165}$) and stability by protecting it against the action of endonucleases [152]. After injection into monkey eyes, the intravitreal concentration of pegaptanib decreases according to a first-order kinetics model with an intraocular half-life of 94 h. Pegaptanib accumulation in the serum is limited by both metabolism (endo- and exonuclease actions) and urinary excretion.

Drug developers had been concerned that pan-VEGF suppression might increase the risk of serious systemic adverse events so they developed pegaptanib to inhibit the actions of diffusible $VEGF_{165}$ but not the shorter natural $VEGF_{121}$ and fibrin split product $VEGF_{110}$. Pegaptanib's exact mechanism of action is not fully known, but it may prevent VEGF binding to neuropilin-1 (by obstructing the heparin-binding site), or it may stereoscopically interfere with VEGF's receptor-binding site. Pegaptanib reduces endothelial cell proliferation [15], completely inhibits VEGF-mediated vascular permeability in a guinea pig model, and reduces experimental neovascularization, leukostasis, and BRB breakdown in diabetic rats [86]. In vitro studies suggest that pegaptanib inhibits VEGF-mediated processes as effectively as monoclonal antibodies [50]. A preclinical study in rhesus monkeys determined that

several doses of pegaptanib were safe, and intraocular samples obtained 7 and 28 days after intravitreal injections demonstrated no molecular instability [43].

The important pegaptanib drug trials and their key findings are listed in Table 4.3.

The VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group (VISION) trials led to United States Food and Drug Administration (US FDA) approval of pegaptanib for the treatment of neovascular age-related macular degeneration (AMD). Compared to observation, patients receiving injections of 0.3 mg pegaptanib every 6 weeks experienced only half as much vision loss at 1 year (−14 vs. −7 letters) [76]. In the post-approval Evaluation of Efficacy and Safety in Maintaining Visual Acuity with Sequential Treatment of Neovascular AMD (LEVEL) trial, maintenance therapy with pegaptanib sustained most of the gains in vision previously acquired with the more potent, nonselective VEGF inhibitors bevacizumab and ranibizumab [62].

Following the nAMD trials, pegaptanib underwent clinical testing for the treatment of DME. In a phase II trial, 172 patients were randomized to receive one of three pegaptanib doses (0.3 mg, 1 mg, or 3 mg) or sham injections every 6 weeks for 36 weeks. A greater proportion of patients receiving 0.3 mg pegaptanib than sham improved by at least 10 letters (34% vs. 10%; $P = 0.003$) and at least 15 letters (18% vs. 7%; $P = 0.12$). Patients receiving 0.3 mg pegaptanib as compared to sham

Table 4.3 Important trials with pegaptanib and details their key findings

Important diabetic macular edema trials with pegaptanib		
Trial and phase	Cohorts	Key findings
Macugen DME trial Phase II 172 patients	Pegaptanib 0.3 mg 1.0 mg 3.0 mg Sham	At 36 weeks, patients treated with 0.3 mg pegaptanib compared to sham/laser: 1. Greater likelihood of 10-letter gain (34% vs. 10%, $P = 0.003$) 2. Trend toward 15-letter gain (18% vs. 7%, $P = 0.12$) 3. Greater improvement in mean BCVA (+4.7 vs. −0.4 letters, $P = 0.02$) 4. Greater improvement in macular thickness (−68 μm vs. +4 μm, $P = 0.02$)
Querques et al. Retrospective 63 patients	Single cohort	At 36 weeks compared to baseline, patients had significant improvements in: 1. BCVA ($P < 0.019$) 2. Macular thickness ($P < 0.001$)
Macugen study group Phase II/III 260 patients		At 102 weeks, patients treated with 0.3 mg pegaptanib compared to laser: 1. Greater likelihood of 10-letter gain (36.8% vs. 19.7%, $P = 0.0047$) 2. Trend toward 15-letter gain (16.5% vs. 10.2%, $P = 0.2466$) 3. Greater improvement in mean BCVA (+6.1 vs. +1.3 letters, $P < 0.01$) 4. Fewer patients required laser for persistent edema (25.2% vs. 45.5%, $P = 0.003$)

experienced a greater mean improvement in BCVA (+4.7 vs. −0.4 letters; $P = 0.04$) and greater mean decrease in macular thickness (−68 μm vs. +4 μm; $P = 0.02$). One case of endophthalmitis occurred after 652 injections [113].

In a 6-month retrospective study of 63 eyes with DME, patients receiving pegaptanib experienced significant improvements in BCVA ($P = 0.019$) and macular thickness ($P < 0.001$) compared to baseline. Patients were treated every 6 weeks PRN for recurrent edema, and most eyes required at least three injections. Compared to the phase II trial, patients in this retrospective study experienced similar rates of 1-line improvement in vision (55.6% vs. 59%) and reduction in CRT (42% vs. 29.6%) [143].

A phase II/III trial randomized 260 patients to receive pegaptanib or sham injections every 6 weeks [163]. Significantly more patients receiving pegaptanib than sham injections gained at least 10 letters (36.8% vs. 19.7%; $P = 0.0047$) though the proportions gaining at least 15 letters were not significantly different (16.5% vs. 10.2%; $P = 0.2466$). At week 102, patients receiving pegaptanib had greater mean gains in VA (+6.1 vs. +1.3 letters; $P < 0.01$), and fewer pegaptanib-treated patients required grid laser photocoagulation by week 54 (23.3% vs. 41.7%; $P = 0.002$) and week 102 (25.2% vs. 45.0%; $P = 0.003$) for persistent edema. A slightly higher proportion of pegaptanib-treated patients experienced a 25% reduction in CRT. Pegaptanib appeared safe since only two patients suffered cerebrovascular accidents, compared to one patient in the sham group.

Self-reported quality of life (QoL) was assessed with the 25-item National Eye Institute Visual Function Questionnaire (NEI VFQ 25) and the EQ-5D [109]. The NEI VFQ 25 domains of near vision, distance vision, and social functioning (week 54) and distance vision, social functioning, mental health, and composite score (week 102) demonstrated clinically meaningful (>5-point between-group difference) and statistically significant ($P < 0.05$) benefits favoring pegaptanib. No significant difference in the mean change in generic EQ-5D-weighted utility scores was seen. The authors concluded that the VA improvement from pegaptanib treatment versus sham is accompanied by improved vision-related QoL as reported by the DME patient.

Pegaptanib causes marked regression of new vessels in eyes with proliferative diabetic retinopathy (PDR) [3, 13, 101, 116]. Pegaptanib was evaluated as an adjunct to vitrectomy in eyes with recurrent vitreous hemorrhage due to PDR. Fifteen eyes of 14 patients each received one to three pegaptanib injections prior to undergoing vitrectomy for persistent vitreous hemorrhage (VH) or progressive traction retinal detachment. In the majority of patients with VH, pegaptanib enabled surgeons to place additional laser prior to vitrectomy. Surgery after pegaptanib injections was felt to be faster and less challenging compared with conventional vitrectomy for recurrent VH due to PDR [83].

Pegaptanib showed promise in the early DME trials but further development was halted because it was inferior to the pan-VEGF-A blockers bevacizumab and ranibizumab for the treatment of nAMD. The drug's developer appeared unwilling to devote the resources necessary to run large, multicenter, phase III DME trials. Some authors have argued that $VEGF_{165}$-specific binding may have doomed pegaptanib

for the treatment of nAMD because it was unable to prevent the pro-angiogenic effects of $VEGF_{121}$ and $VEGF_{110}$. Since these isoforms appear to be less important in the development of DME, pegaptanib may have been relatively more effective in this condition. The theoretical safety advantages of $VEGF_{165}$ specific binding over pan-VEGF-A binding in a diabetic population that is at risk of Antiplatelet Trialists' Collaborative (APTC)-defined events may also have favored pegaptanib use in patients with DME. Unfortunately, pegaptanib never received US FDA approval for DME, and plans for registration trials are unlikely to be resurrected. Its frequency of use in patients with nAMD and DME remains insignificant.

4.3.2 Bevacizumab

Bevacizumab is a humanized (93% of protein sequences are human), recombinant, murine-derived, monoclonal antibody that binds all isoforms of VEGF-A. Bevacizumab has an impressive dissociation constant for $VEGF_{165}$ (K_D = 56–1100 pM) though its binding affinity is less than that of the approved anti-VEGF drugs [136, 140]. Bevacizumab maximally inhibits proliferation of human umbilical vascular cells and improves their survival when the bevacizumab/VEGF ratio exceeds 2.6:1 [176].

The intravitreal half-life of bevacizumab in human eyes varies between 6.7 and 9.82 days, with an average of 8.25 days [117, 161]. Very poor intraocular penetration occurs after topical administration as only small quantities of drug reach the iris, ciliary body, and retina. Subconjunctival and intravitreal injections in rabbits produce similar serum concentrations, suggesting that the drug passes into the systemic circulation without being altered within the eye. Bevacizumab possesses a long serum half-life (21 days) because of its Fc fragment, and it significantly lowers serum VEGF concentrations [10].

Bevacizumab reaches the subretinal space in rabbit eyes within 2 h after intravitreal injections and reaches the inner retina, choroid, and serum in monkeys within the first day [35, 81, 156]. The peak concentrations in the iris and retina are twice those in the vitreous, suggesting that the drug undergoes active transport and binding within the tissues. Bevacizumab can be internalized by RPE cells though toxicity has not been noted [81].

Bevacizumab was developed for the systemic treatment of solid tumors [57] and is currently approved for advanced colorectal carcinoma, small cell lung cancer, renal cell carcinoma, cervical carcinoma, ovarian carcinoma, and glioblastoma. In a small pilot study, bevacizumab was administered intravenously to patients with nAMD [120]. Patients experienced anatomic and functional improvements, but the incidence of systemic side effects precluded further use. In 2005, bevacizumab was injected intravitreally into patients with macular edema due to central retinal vein occlusion and nAMD [150, 151].

The important bevacizumab diabetic retinopathy trials and their key findings are listed in Table 4.4.

Table 4.4 The important diabetic retinopathy trials with bevacizumab and their key findings

Important diabetic macular edema trials with bevacizumab		
Trial and phase	Cohorts	Key findings
DRCR.net Phase II	Bevacizumab 1.25 mg 2.5 mg Laser	Q6wk injections, 12-week endpoint Bevacizumab-treated patients averaged one-line BCVA gain better than those receiving laser Bevacizumab patients had 11% improvement in macular thickness at week 3, but stable through week 12
Pan-American Collaborative Retina Group 139 eyes	Bevacizumab 1.25 mg 2.5 mg	24 month, retrospective study Patients receiving 1.25 mg and 2.5 mg had improved 1. BCVA (20/150 to 20/75, $P < 0.0001$; 20/168 to 20/114, $P = 0.02$) 2. Central macular thickness (466.5 μm to 286.6 μm, $P < 0.0001$; 423.4 μm to 271.8 μm, $P = 0.001$)
Soheilian et al. (2009) 150 eyes Prospective	3 treatment arms: Bevacizumab Bevacizumab + IVT Laser	At 36 weeks 1. 2 lines improvement in BCVA in 37, 25, and 14.8% of eyes. 2. Significant improvements in central retinal thickness only at 6 weeks
BOLT study Phase II 80 eyes Prospective	2 treatment arms: Bevacizumab q6wk Laser q4month	At 1 year 1. More eyes receiving bevacizumab improved by 15 letters (11.9% vs. 5.3%) 2. More eyes receiving bevacizumab improved by ten letters (31% vs. 7.9%) 3. Bevacizumab eyes had greater improvements in mean BCVA (+8.0 vs. −0.5 letters, $P = 0.0002$) At 2 years 1. More eyes receiving bevacizumab improved by 15 letters (32% vs. 4%) 2. More eyes receiving bevacizumab improved by ten letters (49% vs. 7%) 3. Bevacizumab eyes had greater improvements in mean BCVA (+8.6 vs. −0.5 letters)

The DRCR.net evaluated the short-term (12-week primary endpoint) efficacy of q6week bevacizumab in a phase II DME trial. One hundred twenty-one patients were randomized to receive laser photocoagulation or intravitreal injections of 1.25 mg or 2.5 mg bevacizumab, with or without laser. Patients receiving bevacizumab experienced a one-line improvement in visual acuity compared to those treated with laser. Approximately one-half of the bevacizumab-treated patients experienced a greater than 11% decrease in macular thickness at week 3, but additional improvements through week 12 were not seen. The authors concluded that 6-week injection intervals may be too long and that combining bevacizumab with laser photocoagulation provides no short-term advantage over bevacizumab monotherapy [30].

The Pan-American Collaborative Retina Study (PACORES) reported the results of a 24-month retrospective study of 139 eyes (115 patients) with DME [8]. Patients who received 1.25 mg or 2.5 mg bevacizumab experienced significant improvements in mean VA (20/150 to 20/75, $P < 0.0001$; 20/168 to 20/114, $P = 0.02$) and central macular thickness (466.5 μm to 286.6 μm, $P < 0.0001$; 423.4 μm to 271.8 μm, $P = 0.001$).

In a 36-week prospective study, 150 eyes with DME were randomized to receive bevacizumab monotherapy (q12week injections), bevacizumab in combination with intravitreal triamcinolone, or macular laser photocoagulation [158]. Two Snellen lines of visual acuity improvement were achieved by 37% (bevacizumab monotherapy), 25% (bevacizumab + triamcinolone), and 14.8% (laser) of eyes. Compared to baseline, VA improved significantly at all visits in the bevacizumab monotherapy group, only at weeks 6 and 12 in the combination therapy group, but at no visits in the laser group. Significant improvements in central retinal thickness were seen only at 6 weeks. The authors concluded that quarterly bevacizumab injections are superior to laser photocoagulation but that triamcinolone provides no added benefit.

The Bevacizumab Or Laser Therapy (BOLT) in the management of diabetic macular edema trial best demonstrated the superiority of bevacizumab over laser for the treatment of DME [118, 157]. This prospective, single-center, 2-year trial randomized 80 eyes to receive q6week PRN bevacizumab or q4week PRN laser. At 1 year, more patients receiving bevacizumab than laser gained >15 letters (11.9% vs. 5.3%) and >10 letters (31% vs. 7.9%), and fewer lost >15 (2.4% vs. 26.3%) and >30 (0% vs. 5.3%) letters. Patients receiving bevacizumab experienced greater mean VA improvements compared to laser at 1 year (+8.0 vs. −0.5 letters, $P = 0.0002$) and 2 years (+8.6 vs. −0.5 letters). At 2 years, 49% of bevacizumab-treated patients improved by at least +10 letters and 32% by at least +15 letters, compared to only 7 and 4% of laser-treated eyes. Fewer bevacizumab than laser patients lost 15 letters (0% vs. 14%, $P = 0.03$). Eyes receiving bevacizumab had greater mean decreases in macular thickness compared to laser (−146 μm vs. −118 μm). The median number of treatments through 2 years was 13 bevacizumab injections and 4 laser treatments.

A post hoc analysis of the BOLT data showed that eyes with subretinal fluid at baseline were most likely to have persistent edema at 2 months [157], and the authors noted that resolution of edema by 4 months is a strong predictor of a favorable long-term response. They found that 20% of eyes with persistent edema at 12 months achieved dry retinas at 24 months with VA improvements of at least 15 letters. They stated that although the 4-month response may be predictive of long-term outcome, it should not lead to withholding of therapy.

Intravitreal injections of bevacizumab cause regression of optic disk neovascularization due to PDR [9, 159], but the effect is transient as neovascularization recurs by 12 weeks after single intravitreal injections [92]. Several studies have reported resolution of chronic vitreous hemorrhage after intravitreal injections of bevacizumab [4, 29, 47, 123], but these results must be viewed with caution since bevacizumab likely does not promote the clearing of vitreous hemorrhage, but rather it allows hemorrhage to clear while it prevents further bleeding.

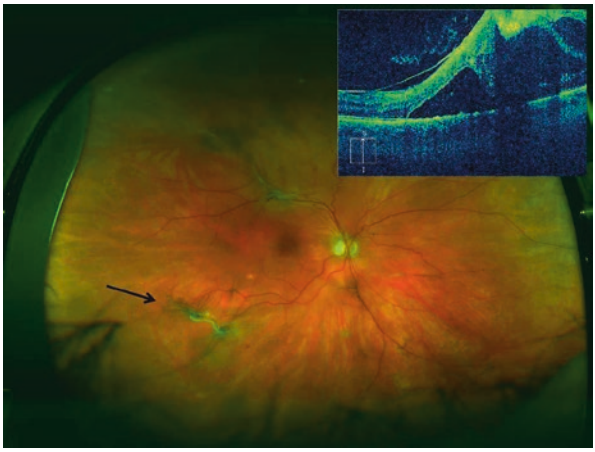

Fig. 4.6 Ultra-widefield photograph showing an eye with proliferative diabetic retinopathy. Fibrovascular proliferation can be seen along the superotemporal arcade causing a traction retinal detachment that threatens the fovea. The vertical optical coherence tomography scan (*upper left corner of image*) was displaced superiorly to better demonstrate the traction retinal detachment in the superior macula that threatens the fovea. Fibrovascular proliferation just outside the inferotemporal arcade features active neovascularization (*black arrow*). An intravitreal injection of bevacizumab was given 3 days prior to pars plana vitrectomy to reduce intraoperative bleeding

Intravitreal injections of bevacizumab have been used as adjuvants to improve surgical outcomes by decreasing intraoperative hemorrhage, facilitating fibrovascular membrane dissection (Fig. 4.6) [22, 27, 29, 87, 108, 123, 135, 146, 148, 180] and reducing the incidence of postoperative vitreous hemorrhage [4, 29, 148, 179]. These studies, however, were limited by relatively small numbers of patients, heterogeneous retinal pathology (TRD and VH were studied together), and varying surgical techniques (multiple surgeons and different gauge vitrectomies). Many surgeons remain concerned that the preoperative administration of bevacizumab may worsen fibrovascular traction [7, 90, 124, 135] and may cause enlargement of the foveal vascular zone [23, 104, 105]. Some surgeons recommend that bevacizumab should be administered only within a few days of planned surgery so that prompt action can be taken if a traction retinal detachment worsens or a traction-rhegmatogenous detachment develops.

In a much larger study, 99 eyes of 90 patients scheduled for diabetic vitrectomy were randomized to receive intravitreal bevacizumab preoperatively or no injection [139]. Thirty-four patients received IVB on an average of 11.5 (range, 3–30) days before vitrectomy. Visual acuity improved significantly after surgery in both groups: from 20/617 to 20/62 in the IVB group, and from 20/443 to 20/86 in the non-IVB group ($P = 0.11$ between groups). Surgery time and postoperative complications (glaucoma, RD, and repeat vitrectomy rate) were similar in both groups. In patients under the age of 40 years, operating time was shorter in the bevacizumab group ($P = 0.02$) with a trend toward better VA. The authors concluded that preoperative

bevacizumab may be a useful adjunct to vitrectomy for severe PDR complicated by TRD, particularly in younger patients.

A meta-analysis of randomized controlled trials compared the safety and functional outcomes of vitrectomy with or without preoperative intravitreal bevacizumab for PDR [182]. Eight trials with 414 eyes of 394 patients were included. The authors found that vitrectomy with preoperative bevacizumab shortened overall surgical time (mean difference of 26.89 min, 95% confidence interval (CI) 31.38–22.39; $P < 0.00001$) and reduced the number of required endodiathermy applications (mean difference of 3.46, $P = 0.02$) compared to vitrectomy alone. The bevacizumab group also experienced less intraoperative bleeding (odds ratio [OR] 0.10; 95% CI 0.02–0.46; $P = 0.003$) and recurrent vitreous hemorrhage within the first month (OR 0.35; 95% CI 0.21–0.58; $P < .0001$), but the incidence of recurrent vitreous hemorrhage after the first month was comparable between the two groups. There were no significant differences in other complication rates between the two groups, except for iatrogenic retinal breaks, which were more likely to occur in the vitrectomy-alone group (OR 0.27, 95% CI 0.12–0.63; $P = 0.003$).

4.3.3 Ranibizumab

Scientists at Genentech recognized that VEGF suppression could be used to treat nAMD, DME, and macular edema due to retinal vein occlusions, but they worried that a full-length antibody such as bevacizumab might have limited efficacy and an unfavorable safety profile. Monkey studies showed that the full-length HER2 antibody (molecular weight 149 kDa) was unable to cross the inner retina after intravitreal injection [125], an observation consistent with the proposed inner retina exclusion limit of 76 kDa [88].They also worried that the antibody's Fc fragment might incite intraocular inflammation or cause systemic adverse events because of a prolonged serum half-life. For these reasons, ranibizumab, an affinity enhanced, humanized, single binding site antibody fragment (Fab), was created in 1996 [54] from a murine antibody to human VEGF. Ranibizumab (MW, 48 kDa) binds all isoforms of VEGF-A, but with a molar affinity for $VEGF_{165}$ that is 5–20 times that of bevacizumab. Its dissociation constant for the binding of $VEGF_{165}$ is 44–192 pM [111, 136]. After successfully completing phase III trials [17, 149], ranibizumab was approved by the US FDA in 2006 for the treatment of neovascular AMD.

Ranibizumab concentrations in the retina are 2.2- to 3-fold lower than in the vitreous [70] but since they are 3000-fold greater than VEGF concentrations, maximum binding of VEGF probably occurs within the retina. The terminal intraocular half-life of ranibizumab in monkey eyes appears to be concentration dependent, ranging from 2.63 days (0.5 mg dose) to 3.95 days (2.0 mg dose) [69]. The intravitreal half-life of ranibizumab in rabbits is slightly shorter at 2.1–3 days [70]. In human eyes, the intravitreal half-life ranges from 7.1 days in a study that sampled aqueous concentrations to 9 days in a population kinetics study [96, 178]. Ranibizumab appears to pass unaltered through the trabecular meshwork and cho-

roid into the systemic circulation where it has a half-life of only 2 h because of rapid ultrafiltration by the kidneys. Serum concentrations are 10,000-fold lower than those in the vitreous [68], and ranibizumab does not appear to suppress serum concentrations of VEGF [10].

Ranibizumab reduces the VEGF-mediated in vitro proliferation of human vascular endothelial cells (HUVAC) in a dose-dependent manner [110] and inhibits VEGF-mediated vascular permeability in guinea pigs. Both ranibizumab and bevacizumab increase vascular endothelial cell apoptosis and decrease their proliferation, migration, and assembly into vascular structures [26]. Both drugs decrease VEGF expression, VEGFR2 phosphorylation, and Akt expression [26]. Ranibizumab causes a greater reduction in endothelial cell proliferation whereas bevacizumab has a greater effect on migration, tube formation, and VEGFR2 phosphorylation [26]. In vitro proliferation of pig choroidal endothelial cells is decreased by ranibizumab (44.1%), bevacizumab (38.2%), and pegaptanib (35.1%) [160].

Pharmacokinetic studies suggested that clinically achievable ranibizumab concentrations within the retina after intravitreal injections were capable of suppressing angiogenic activity for 1 month [68, 100]. This was confirmed by the nAMD pilot studies so monthly injections were used in both the nAMD and DME trials [17, 149]. Excellent results were achieved with monthly dosing of patients with nAMD, but attempts to extend the treatment interval to 3 months resulted in progressive loss of vision [144]. Eyes that were treated with 3 monthly injections and observed for 2 months also showed vision loss of three to four letters [153], suggesting that monthly dosing of patients with nAMD was required to achieve the best results. Therefore, the monthly treatment strategy was carried over to the treatment of DME.

Evidence supporting the use of anti-VEGF therapy for DME emerged from several sources. Elevated concentrations of both VEGF and VEGFR2 were found in animal models of diabetic retinopathy [75]. Exogenous administration of VEGF into monkey eyes produces retinal changes similar to those of diabetic retinopathy, with retinal hemorrhages, increased capillary permeability, and neovascularization [147, 171]. The discovery that these changes could be prevented by the coadministration of VEGF-Trap A40 [142] was important to drug development.

Aqueous VEGF concentrations are higher in patients with DME than in those with nAMD and vein occlusions [64], and VEGF concentrations are higher in patients with severe as opposed to mild DME [65]. In eyes with PDR, vitreous VEGF concentrations fall after successful panretinal photocoagulation [5]. Retinal hypoxia may be the most potent stimulus for VEGF production in diabetes, and treating patients with continuous oxygen supplementation for 3 months significantly reduces DME [132].

4.3.3.1 Ranibizumab Clinical Trials

The important ranibizumab diabetic retinopathy trials and their key findings are listed in Table 4.5.

Table 4.5 Important diabetic retinopathy trials with ranibizumab and details their key findings

Important diabetic macular edema trials with ranibizumab (ran)		
Trial and phase	Cohorts	Key findings
READ-2 Phase II 126 patients 6 months	Treatment arms: RAN RAN + laser Laser	RAN injections at baseline, 1, 3, 5 months At 6 months 1. Mean ΔBCVA were +7, +4, 0 letters 2. Mean changes in central retinal thickness were −95 μm, −82 μm, and −117 μm At 24 months 1. Mean ΔBCVA were +8, +7, +5 letters (laser group eligible for q3month RAN at 6 months)
RISE and RIDE Phase III 759 patients Registration trials	Treatment arms: Monthly RAN 0.3 Monthly RAN 0.5 Sham/laser	At 24 months: 1. More RAN patients had three-line improvement in BCVA (34% – 46% vs. 12% – 18%) 2. RAN patients had greater improvements in mean BCVA (+11 to +13 vs. +0.5 to +3 letters) 3. RAN patients had greater improvements in macular thickness (−250 μm to −270 μm vs. −125 μm to −133 μm) 4. RAN patients had significant improvements in diabetic retinopathy severity scores. At 36 months: 1. No significant changes in BCVA or macular thickness in RAN arms. 2. Sham/laser crossed over to RAN and improved by +2 letters
DRCR.net Protocol I Phase III 854 patients	Treatment arms: RAN + laser RAN + def laser IVT + laser Laser	At 12 months: 1. Improvements in mean BCVA were +9, +9, +4, and +3 letters. 2. BCVA improvements in IVT + laser group that were pseudophakic at baseline were similar to RAN arms. At 36 months: 1. Patients treated with RAN + def laser improved by 2.9 letters more than RAN + laser 2. Compared to patients in RAN + laser, patients in RAN + def laser received three more injections but three fewer lasers
RESOLVE Phase II 152 patients	Treatment arms: RAN 0.3 RAN 0.5 Sham/laser RAN groups were eligible for dose doubling	At 12 months: 1. Mean BCVA improved by +10.3 letters in pooled RAN groups vs. −1 in sham/laser group 2. 2-line and 3-line improvements in 60.8 and 33% of RAN patients and 18.4 and 5% of sham patients 3. 68% of RAN patients required higher drug dose at some point
RESTORE Phase III 345 patients	Treatment arms: RAN RAN + laser Laser	At 12 months: 1. Mean BCVA improvements were +6.1, +5.9, and +0.8 letters. 2. Mean improvements in macular thickness were −118.7 μm, −128.3 μm, and −61.3 μm At 36 months: 1. BCVA in laser arm caught up to RAN + laser after crossover to RAN at 12 months

RAN ranibizumab

Human nAMD trials were evaluating ranibizumab well before pilot studies tested its use in patients with DME. The efficacy and safety of ranibizumab in DME proceeded along three distinct clinical lines: Diabetic Retinopathy Clinical Research Network (DRCR.net), READ/RISE/RIDE, and RESOLVE/RESTORE. The US National Eye Institute, which funded DRCR.net group, was the first to demonstrate the superiority of ranibizumab with immediate or deferred laser over standard of care (laser photocoagulation). The READ trials followed by RISE and RIDE were North American registration trials that led to the approval of monthly 0.3 mg ranibizumab by the US FDA. The RESOLVE/RESTORE trials were performed in the eastern hemisphere and led to the approval of 0.5 mg ranibizumab by the European Medicines Agency (EMA).

Two small pilot studies produced early evidence that intravitreal ranibizumab reduces macular thickening and improves VA in patients with DME. In the READ-1 study, ten eyes were injected at baseline and 1, 2, 4, and 6 months [133]. At the 7-month examination, the mean excess in foveal thickness had decreased by 85% from 503 μm to 257 μm, macular volume had decreased from 9.22 mm^2 to 7.47 mm^2 (a 77% reduction in excess volume), and the mean VA improved by +12.3 letters. Several patients experienced a rapid reduction in macular edema (median of −88 μm, mean of −130 μm) by day 7, and a strong correlation between macular thinning and visual improvement was noted ($r^2 = 0.78$). No safety signals emerged though patients experienced slight increases in blood pressure.

In a second pilot study, ten patients were treated with 0.3 mg or 0.5 mg of ranibizumab at baseline and at months 1 and 2 [24]. The primary endpoints were the incidence and severity of adverse events at 3 months, with secondary endpoints being changes in VA and appearance of the retina (based on fundus photography, fluorescein angiography, and OCT). Two patients experienced mild, sterile inflammation, which was insufficient to alter the performance of subsequent trials. Improvements in visual acuity and macular thickness in the 0.3 mg and 0.5 mg groups were +12 and +7.8 letters and −45.3 μm and −197.8 μm, respectively. Improvements in VA diminished somewhat between 3 and 6 months but were better sustained in the group receiving 0.3 mg.

4.3.3.2 READ-2 and READ-3 Trials

The phase II READ-2, the first prospective, randomized, double-masked, multicenter ranibizumab DME trial, followed on the heels of the phase I READ-1 trial [130]. One hundred twenty-six patients were randomized to three treatment arms: intravitreal injections of 0.5 mg ranibizumab at baseline and months 1, 3, and 5, focal/grid laser photocoagulation of the macula at baseline and at month 3 if needed, and intravitreal 0.5 mg ranibizumab at baseline followed by laser 1 week later. At the 6-month primary temporal endpoint, mean visual acuity improvements were +7, 0, and +4 letters, and mean changes in central macular thickness (CMT) were −95 μm, −82 μm, and −117 μm.

A protocol modification at 6 months allowed patients in the first two groups to receive ranibizumab every 2 months PRN, while patients in the third group became eligible for laser and ranibizumab every 3 months for macular thickness >250 μm. At 24 months, the mean VA improvements in the three arms were +8, +5, and +7 letters, and the mean CMTs were 340, 286, and 258 μm. Visual acuity improvements in the laser group approached that of the ranibizumab group, but the thick maculas in the ranibizumab monotherapy group indicated that these patients were undertreated [131].

A second protocol modification at 24 months allowed patients to receive monthly ranibizumab PRN during year 3. Between the 24- and 36-month visits, patients achieved mean gains of +3, −2, and +2 letters, with each group receiving an average of 5, 2, and 3 ranibizumab injections during the third year [39].

During the exploratory dosing trials for nAMD, a quadruple dose of ranibizumab (2.0 mg) was tested but quickly abandoned because of its high viscosity. Years later, the 2.0 mg dose was reintroduced in the HARBOR trial [20] for nAMD and the READ-3 trial [41] for DME. Patients with DME received either the 2.0 mg or the standard 0.5 mg dose monthly for 6 months then PRN through 12 months. The 0.5 mg dose produced greater improvement in vision than the 2.0 mg dose (+10.88 letters vs. +7.39 letters), and patients receiving the 2.0 mg dose experienced more deaths due to myocardial infarction (3% vs. 0%). Based upon the results from the READ-3 and HARBOR (in which the 0.5 mg and 2.0 mg doses performed comparably), further development of the 2.0 mg dose was halted.

4.3.3.3 RISE and RIDE

READ-2 demonstrated that intravitreal ranibizumab, either with or without accompanying macular laser photocoagulation, was superior to laser for the treatment of DME and had an acceptable safety profile. The disappointing VA results from years 2 and 3 suggested that fixed treatment intervals of 2–3 months were probably too long. Lessons from READ-2 were incorporated into the randomized, multicenter, double-masked, phase III RISE and RIDE registration trials that compared the efficacy of 0.3 mg and 0.5 mg ranibizumab against sham/laser for patients with center-involving DME [129]. The 3-year trials randomized 759 patients to three treatment arms: monthly 0.3 mg ranibizumab, monthly 0.5 mg ranibizumab, or sham. Patients in each group became eligible for rescue laser photocoagulation at 3 months if CRT was >250 μm and if the change in CRT following the previous injection was <50 μm. The primary functional endpoint was the proportion of eyes improving by at least 15 letters. Secondary endpoints included improvement in BCVA, improvement in macular thickness, safety measures, and improvement in VFQ-25 quality of life scores.

At 24 months, significant proportions of patients receiving 0.3 mg ranibizumab, 0.5 mg ranibizumab, and sham injections improved by at least 15 letters in RISE (45%, 39%, 18%) and RIDE (34%, 46%, 12%). Mean visual acuity improvements were particularly impressive in the ranibizumab treated groups in RISE (+13, +12,

+3) and RIDE (+12, +11, +0.5). The corresponding mean improvements in central foveal thickness were −250, −253, and −133 μm (RISE) and −259, −270, and −125 μm (RIDE). Patients receiving ranibizumab required fewer lasers (mean of 0.3–0.8) than those randomized to sham injections (means of 1.8 and 1.6).

The median diabetic retinopathy severity scores (DRSS) in the sham/laser group remained at moderately severe NPDR throughout the study, whereas in patients receiving ranibizumab they improved from moderately severe NPDR to mild NPDR. Fewer patients receiving ranibizumab compared to sham/laser experienced a two-step worsening in the DRSS score (1.7% to 2.1% vs. 9.6%), and fewer ranibizumab eyes developed vitreous hemorrhage. Since visual acuity changes were the same for patients receiving the 0.3 and 0.5 mg doses of ranibizumab, and there appeared to be a dose-dependent risk of stroke, the US FDA approved the 0.3 mg ranibizumab dose for the treatment of center-involving DME in 2012. In 2015, the label was expanded to include the treatment of diabetic retinopathy in eyes with DME.

Patients originally randomized to ranibizumab continued receiving monthly injections during year 3, but those randomized to sham were eligible to cross over to monthly PRN 0.5 mg ranibizumab [18]. Patients in the ranibizumab arms had stable BCVA during year 3 whereas those in the sham arms improved to +4 (RISE) and +5 (RIDE) letters from baseline.

After the 36-month visit, 582 patients from RISE and RIDE were followed in the extension study. All patients were eligible to receive 0.5 mg ranibizumab every 4 weeks if DME was identified by the investigator or BCVA worsened by at least five letters compared to month 36. A mean of 4.5 injections/patient (annualized 3.8) was given during a mean follow-up of 14.1 months. Twenty-five percent of patients did not require any injections during the extension. Best corrected visual acuity in all groups remained stable throughout the extension period and mean CFT increased slightly. Few patients developed PDR during the extension, and those originally randomized to ranibizumab had a lower overall rate of progression to PDR than those originally randomized to sham/laser.

Patients with macular non-perfusion at baseline had lower VA scores than those with good perfusion. But by the end of the trial, patients with non-perfusion had greater improvements in VA and many actually caught up to those with good perfusion. Areas of non-perfusion did not increase in size when exposed to anti-VEGF therapy. These patients also experienced similar two-step improvements in DRSS compared to patients with good macular perfusion.

4.3.3.4 RESOLVE

The phase II RESOLVE trial and phase III RESTORE trials were performed in Europe, Asia, and Australia. In the 12-month RESOLVE trial, 152 patients were randomized to receive monthly 0.3 mg ranibizumab, 0.5 mg ranibizumab, or sham injections [115]. After 1 month, the dose of the injected drug could be doubled if the CRT was greater than 300 μm or greater than 225 μm if the CRT reduction was less than 50 μm following the previous injection. At 3 months, patients were eligible for

rescue laser and additional monthly PRN injections or sham. At the 12-month primary temporal endpoint, BCVA improved by +10.3 letters in the pooled ranibizumab groups but declined by −1 letter in the sham group. Vision gains of +2 lines and +3 lines were achieved by 60.8 and 33% of ranibizumab-treated eyes but only 18.4 and 5% of sham-treated eyes. Mean improvements in CST were −194 μm for the pooled ranibizumab groups and −48 μm for the sham group. Eighty-six percent of all eyes and 68% of those receiving ranibizumab (70–78% of these at the 1-month exam) required the higher drug dose at some point during the trial. The mean number of administered injections was 10.2, and only 4.9% of ranibizumab-treated eyes (compared to 34.7% of sham eyes) required rescue laser.

4.3.3.5 RESTORE

RESTORE was the multicenter (75 sites) phase III ranibizumab registration trial in the eastern hemisphere. Three hundred forty-five patients were randomized to receive ranibizumab + sham laser, ranibizumab + laser, or sham injections + laser. Ranibizumab injections were given monthly × 3 then PRN; laser was performed at baseline then every 3 months PRN [121]. The primary objective of the trial was to demonstrate the superiority of ranibizumab monotherapy or ranibizumab + laser over laser monotherapy as measured by mean improvements in BCVA over 12 months. Secondary objectives included the proportion of patients achieving BCVA of 73 letters (20/40), the time course of the mean change in BCVA and CRT, patient-reported outcomes relative to laser photocoagulation, and safety measures.

At the 12-month primary endpoint, patients in the ranibizumab monotherapy, ranibizumab + laser, and sham/laser groups had improvements in mean BCVA (+6.1, +5.9, and +0.8 letters), BCVA score >73 letters (53, 44.9, and 23.6%), and CRT (−118.7 μm, −128.3 μm, and −61.3 μm). Health-related quality of life (measured by the NEI VFQ-25 questionnaire) improved more in the ranibizumab monotherapy and ranibizumab + laser groups compared to sham/laser ($P < 0.05$ for each). Subgroup analyses showed that patients with baseline BCVA of >73 ETDRS letters or CRT <400 μm saw as well after laser as after ranibizumab. Overall, patients received a mean of seven ranibizumab/sham injections. No cases of endophthalmitis occurred, and ranibizumab therapy was not associated with an increased incidence of cardiovascular or cerebrovascular events.

After the 12-month primary endpoint, 240 patients rolled into the 24-month extension trial. All patients were eligible to receive 0.5 mg ranibizumab according to BCVA and disease progression criteria and at the investigators' discretion. Additional laser photocoagulation was allowed according to ETDRS guidelines. At the preplanned 24-month interim analysis, patients originally receiving ranibizumab monotherapy and ranibizumab + laser maintained improvements in mean best corrected visual acuity (+7.9 letters, +6.7 letters), CRT (−140.6 μm, −133.0 μm), and NEI VFQ-25 composite scores (5.6, 5.8) [103]. Between months 12 and 24, significant improvements in these measures were seen in patients originally treated with sham/laser (+5.4 letters, −126 μm, 4.3). Similar numbers of injections were

performed in each group (3.9, 3.5, and 4.1). No cases of endophthalmitis were reported and the incidences of non-ocular SAEs were low.
Two hundred eight (86.7%) patients completed the 24-month extension study. As-needed ranibizumab treatment led to an overall maintenance of VA and CRT between months 12 and 36 [154]. Best corrected visual acuities at the completion of the study according to initial treatments were +8.0 letters (ranibizumab only), +6.7 letters (ranibizumab + previous laser), and +6.0 letters (laser). Patients in the three treatment arms required between 4.0 and 6.8 (mean for each group) injections over the final 2 years.

4.3.3.6 DRCR.net Protocol I

The DRCR.net Protocol I trial provided the first level I evidence that supported the use of ranibizumab in eyes with DME. Protocol I was a 5-year, multicenter study that randomized 854 eyes with center-involving DME to receive 0.5 mg ranibizumab with prompt macular laser photocoagulation, 0.5 mg ranibizumab with deferred laser (for at least 6 months), intravitreal triamcinolone with prompt laser, or sham injections with prompt laser [31]. During the first year, patients received ranibizumab injections according to the 4:2:7 rule – 4 monthly injections, followed by two injections if fluid persisted, followed by seven visits during which the drug could be administered at the investigator's discretion if there was insufficient improvement. Laser photocoagulation and intravitreal triamcinolone (4 mg) could be repeated quarterly as needed. Patients randomized to the deferred laser group were not obligated to receive laser if the macula was dry at and beyond 6 months.

At 1 year, the median improvements in VA in the ranibizumab + prompt laser, ranibizumab + deferred laser, triamcinolone + laser, and sham + laser groups were +9, +9, +4, and +3 letters, respectively, with most of the gains in BCVA occurring by the 8-week visit. During the first 3 months, patients receiving triamcinolone experienced improvements in VA similar to those receiving ranibizumab, but VA then worsened through 12 months because of the development of steroid-induced cataracts. In the triamcinolone group that was pseudophakic at baseline, 1-year improvements in VA were comparable to those in the ranibizumab groups. There were no significant differences in visual outcomes when the following subgroup analyses were performed: prior treatment for DME, baseline VA, baseline CST, and baseline severity of DR. Improvements in macular thickness were comparable in the groups receiving ranibizumab and triamcinolone, all of which were superior to the group receiving sham/laser. Eyes treated with ranibizumab were less likely to experience progression of diabetic retinopathy. Three patients receiving ranibizumab (0.8%) developed endophthalmitis, and cataracts and elevated intraocular pressure were most commonly seen in patients receiving triamcinolone.

During year 2, the visit interval could be extended to 8 weeks if treatment was deferred at three consecutive visits and to 16 weeks if treatment was not given at the 8-week visit. Patients in the triamcinolone + laser and laser/sham groups were eligible to receive ranibizumab as early as week 74 for persistent edema without

improved vision. The 2-year outcomes were similar to those at 1 year in which 50% of ranibizumab-treated eyes improved by at least 10 letters and 33% improved by at least 15 letters [32]. Compared to the sham/laser group, the mean changes in BCVA in patients receiving ranibizumab + prompt laser, ranibizumab + deferred laser, and triamcinolone + prompt laser were +3.7, +5.8, and −1.5 letters. Forty-three eyes in the sham/prompt laser group were switched to alternative therapy (ranibizumab) within the first 2 years because of "failure," whereas none of the patients randomized to ranibizumab required switching. Elevated intraocular pressures were more common in the triamcinolone/laser group.

At the 3-year visit, the median numbers of injections in the ranibizumab + prompt laser and ranibizumab + deferred laser groups were 12 and 15, respectively [33], and the median numbers of lasers were 3 and 0, respectively. By the 3-year visit, 46% of patients in the ranibizumab + deferred laser group had been treated with laser. Patients treated with ranibizumab + deferred laser improved by +2.9 letters more than those receiving ranibizumab + prompt laser ($P = 0.02$). In the two ranibizumab groups, the percentage of eyes with CST <250 μm was 36% in both groups.

At the 5-year visit, the mean BCVA improvements from baseline were +7.2 letters in the ranibizumab + prompt laser group and +9.8 letters in the ranibizumab + deferred laser group ($P = 0.09$) [49]. In the two groups, there was vision loss of >10 letters in 9 and 8%, improvement of vision by >10 letters in 46 and 58%, and improvement in vision of >15 letters in 27 and 38%. From baseline through 5 years, 56% of patients in the deferred group did not require laser. The mean numbers of ranibizumab injections during the trial were 13 and 17, 54 and 45% did not receive ranibizumab during year four, and 62 and 52% did not receive injections during year 5.

4.3.3.7 Retain

The RETAIN trial explored the viability of a treat-and-extend strategy for DME [141]. Three hundred seventy-two patients were randomized to receive treat-and-extend 0.5 ranibizumab plus laser (G1), treat-and-extend 0.5 mg ranibizumab (G2), or monthly PRN 0.5 mg ranibizumab (G3). All patients received monthly injections until vision stabilized, after which patients in G1 and G2 could be extended at 1 month intervals up to a maximum of 3 months. At 24 months, median BCVA changes in the G1, G2, and G3 groups were +8, +7, and +8 letters. Compared to PRN treatment, patients receiving treat-and-extend required 40% fewer clinic visits, and 70% were extended to a treatment interval of at least 2 months.

4.3.3.8 Ranibizumab for PDR

Ranibizumab not only restores the blood-retinal barrier, thereby allowing the resorption of macular edema, but it also possesses potent anti-angiogenesis activity. It has been used to treat PDR, both as monotherapy and in conjunction with panretinal

photocoagulation. Ranibizumab regresses retinal neovascularization in patients with PDR but since its effects appear to be transient, PRP may still be necessary in some cases to permanently close new vessels.

In a prospective study, ranibizumab combined with PRP was compared to PRP alone for the treatment of high-risk PDR in 40 patients that had not received previous laser photocoagulation [58]. Split-session PRP was administered to all patients at weeks 0 and 2, and the "PRP Plus" group received 0.5 mg ranibizumab at the completion of the first laser session and again at weeks 16 and 32 if needed. Patients were evaluated with ETDRS visual acuity measurements, fluorescein angiography (FA), and OCT. Eyes in both groups experienced significant reductions in FA leakage at all visits though 48 weeks, but this was significantly greater in the PRP Plus group. Best corrected visual acuity worsened in the laser monotherapy group at 16, 32, and 48 weeks but was unchanged in the PRP Plus group. The CMT increased at all visits in the laser group but decreased at week 16 and stabilized through week 48 in the PRP Plus group. The authors concluded that ranibizumab after PRP results in superior reduction in PDR-related FA leakage and improved VA and CMT compared to laser alone.

The DRCR.net performed a randomized clinical trial (Protocol S) with 305 patients at 55 sites to compare panretinal photocoagulation with intravitreal 0.5 mg ranibizumab for PDR [177]. PRP was performed at baseline and ranibizumab was given at baseline followed by q4wk PRN for the PDR, and eyes in both groups with DME were eligible to receive ranibizumab. The primary outcome was change in BCVA and the secondary outcomes included VA area under the curve, peripheral visual field loss (as measured on Humphrey automated visual field testing), incidence of vitrectomy, development of DME, and persistent or new neovascularization. Improvements in BCVA for the ranibizumab and PRP groups were +2.2 and +0.2 letters, respectively (95% CI, −0.5 to +5.0). The group receiving ranibizumab experienced less peripheral visual field sensitivity loss (−23 dB vs. -422 dB; 95% CI, 213–531 dB; $P < 0.001$), fewer vitrectomies (4% vs. 15%; 95% CI 4%–15%; $P < 0.001$), and less development of DME (9% vs. 28%). A median of seven ranibizumab injections was administered through year 1 and ten injections through year 2. Forty-five percent of eyes required additional PRP after the initial treatment, and 53% of eyes in the PRP group required ranibizumab for DME. Only one eye developed endophthalmitis after a ranibizumab injection. The authors concluded that ranibizumab may be a reasonable alternative to PRP over the course of 2 years.

4.3.4 Aflibercept

Aflibercept, previously referred to as the VEGF Trap-eye, is a 115 kD, recombinant, high-affinity, soluble, decoy-receptor molecule. Aflibercept is composed of the second extracellular binding domain from VEGFR1, and the third binding domain from VEGFR2 fused to the constant region (Fc) of a human IgG1 molecule [82].

As a result, aflibercept binds all isoforms of VEGF-A, VEGF-B, and PlGF. Unlike ranibizumab and bevacizumab, aflibercept is composed entirely of human amino acid sequences. It possesses a high binding affinity for $VEGF_{165}$ ($K_D = 0.45$ pM) and PlGF2 ($K_D = 45$ pM) [136] because of its "two-fisted" grasp of the VEGF dimer that is 100-fold stronger than bevacizumab and ranibizumab and exceeds even that of the native VEGFR1 and VEGFR2 receptors. In luciferase-binding assays, aflibercept is 45–92 times as potent as bevacizumab and ranibizumab at preventing VEGF-A isoforms from binding to VEGFR1 and 33–51 times as potent at preventing binding to VEGFR2 [136]. Aflibercept blocked the VEGF-A-induced activation of VEGFR1 and VEGFR2 in human endothelial cells with greater potency than bevacizumab or ranibizumab and also blocked VEGF-A- and PlGF-induced migration of endothelial cells [136].

Aflibercept has an intravitreal half-life of 4.7 days in rabbits [66], considerably longer than either ranibizumab (2.88 days) [11] or bevacizumab (4.32 days) [12], but its half-life in human eyes has not been determined. Mathematical modeling based on binding affinities and estimated intravitreal half-lives predicted that aflibercept would have a longer duration of action than bevacizumab and ranibizumab [162]. After intravitreal injection, aflibercept passes unaltered into the systemic circulation where its half-life is approximately 6 days. Aflibercept binds plasma VEGF and lowers serum concentrations to below 10 pg/ml (the lower detectable limit of some assays) for at least 7 days [10]. Clearance of aflibercept from the serum via renal clearance of bound complexes and Fc-mediated pinocytotic pathways resembles that of full-length antibodies [37].

The important aflibercept diabetic retinopathy trials and their key findings are listed in Table 4.6.

Table 4.6 Important trials with aflibercept and details their key findings

Important diabetic macular edema trials with aflibercept		
Trial and phase	Cohorts	Key findings
DA VINCI Phase II 221 patients	Treatment arms: IAI 0.5 mg q4wk IAI 2.0 mg q4wk IAI 2.0 mg q8wk IAI 2.0 mg PRN Laser	At 1 year 1. Improvements in mean BCVA were +11.0, +13.1, +9.7, +12.0, and −1.3 letters 2. Improvements in mean CST were −165.4 μm, −227.4 μm, −187.8 μm, −180.3 μm, and −58.4 μm 3. Fewer laser in AFL groups (0.5–0.8) compared to laser group (2.5)
VIVID and VISTA Phase III 872 patients	Treatment arms: IAI 2 mg q4wk IAI 2 mg q8wk Laser/sham	At 1 year 1. Improvements in mean BCVA were +10.5 and +12.5, +10.7 and 10.7, and +0.2 and +1.2 letters At 2 years 1. Improvements in mean BCVA were +11.5 and +11.4, +9.4 and +11.1, and +0.9 and +0.7 letters

IAI aflibercept

4.3.4.1 Pilot Study

In a small pilot study, five patients with DME received single intravitreal injections of 4 mg aflibercept. At 4 weeks, the mean excess macular thickness decreased from 108 μm to 59 μm, and the BCVA improved by an average of +9 letters. At 6 weeks, four of the five eyes still had improved excess thickness (median improved from 108 μm to 74 μm; two were normal), and the mean BCVA had improved by +3 letters [40].

4.3.4.2 DA VINCI

The prospective, multicenter, phase II DA VINCI trial randomized 221 patients with center-involving DME to five treatment arms: intravitreal 0.5 mg aflibercept every 4 weeks (0.5q4), 2 mg every 4 weeks (2q4), 2 mg every 8 weeks after 3 monthly loading injections (2q8), 2 mg PRN after 3 monthly loading doses (2PRN), and quarterly laser PRN/sham [38]. Patients receiving aflibercept were not offered rescue laser until 6 months. At 1 year, the mean visual acuity improvements were +11.0, +13.1, +9.7, +12.0, and −1.3 letters for each arm; the proportions improving by at least 15 letters were 40.9, 45.5, 23.8, 42.2, and 11.4%; and the mean improvements in CST were −165.4, −227.4, −187.8, −180.3, and −58.4 μm. Patients in the 2PRN and 2q8 groups received an average of 7.4 and 7.2 injections. The laser/sham group received a mean of 2.5 laser procedures compared to 0.5–0.8 in the aflibercept arms. Improvements in DR severity scores were seen in 31–64% of aflibercept patients but only 12% of laser patients, whereas worsening in DRSS was seen in 0–14% of aflibercept patients compared to 24% of laser-treated patients.

4.3.4.3 VIVID and VISTA

The Study of Intravitreal Administration of VEGF Trap-Eye in Patients with DME (VISTA; NCT01363440) and the VEGF Trap-Eye in Vision Impairment due to DME (VIVID; NCT01331681) [98] were similarly designed, double-blind, randomized, phase III trials that enrolled 872 patients (eyes) (VISTA, 466; VIVID, 406) with center-involving DME. VISTA-DME was performed in the United States and Canada whereas VIVID-DME was run in Australia, Europe, and Japan. Eligible patients had type 1 or 2 diabetes mellitus with BCVA from 24 to 73 letters (20/40 to 20/320) and central macular thickening on OCT. Eyes were randomized 1:1:1 to receive intravitreal aflibercept injections (IAI) 2 mg q4wk, IAI 2 mg q8wk after 5 monthly loading doses, or laser photocoagulation/sham injection. Patients were eligible for laser retreatment every 12 weeks if ETDRS-defined edema was present. All study eyes were eligible for rescue treatment beginning at 24 weeks if they lost >10 letters of BCVA on two consecutive visits or >15 letters at any visit from the

previous best measurement, and the BCVA was worse than baseline. For laser-treated eyes, additional treatment consisted of 5 monthly doses of 2 mg IAI followed by injections every 8 weeks, and for IAI treated eyes, active laser was performed.

The primary temporal endpoint was at 52 weeks, but patients receiving IAI will continue to receive therapy through 148 weeks, and patients randomized to laser/sham will be eligible to cross over to IAI during year 3. The primary efficacy endpoint was the mean improvement in ETDRS BCVA at 52 weeks. Secondary efficacy endpoints included the proportions of patients gaining ≥15 letters, the proportions of patients gaining ≥10 letters, the proportions of eyes experiencing a two-step improvement in the ETDRS Diabetic Retinopathy Severity Scale (DRSS) score, the mean changes in central retinal thickness (CRT) as measured by OCT, the change from baseline in the National Eye Institute Visual Function Questionnaire-25 (NEI VFQ-25) near activities subscale score, and the change from baseline in the NEI VFQ-25 distance activities subscale score. VISTA enrolled a greater proportion of Black patients and VIVID enrolled a greater proportion of Asian patients. More eyes in VISTA, compared to VIVID, had previously received anti-VEGF injections (42.9% vs. 8.9%).

Mean BCVA changes from baseline to 52 weeks for the groups receiving IAI 2 mg q4wk, IAI 2 mg q8wk, and laser/sham were +12.5, +10.7, and +0.2 letters ($P < 0.0001$) in VISTA and +10.5, +10.7, and +1.2 letters ($P < 0.0001$) in VIVID. When eyes receiving additional rescue therapy were included in the analysis, those in the IAI groups changed by +10.7 to +12.4 letters from baseline whereas those in the laser groups changed by +4.2 and +3.5 letters. Visual acuity gains were significantly greater in the IAI groups in both patients who had and had not received prior anti-VEGF therapy. The corresponding proportions improving by ≥10 letters were 64.9, 58.3, and 19.5% ($P < 0.0001$) in VISTA and 54.4, 53.3, and 25.8% ($P < 0.0001$) in VIVID. The proportions improving by ≥15 letters were 41.6, 31.1, and 7.8% ($P < 0.0001$) in VISTA and 32.4, 33.3, and 9.2% ($P < 0.0001$) in VIVID. The proportions that lost ≥15 letters were 0.6, 0.7, and 9.1% ($P < 0.0001$) in VISTA and 0.7, 0, and 10.6% ($P < 0.0001$) in VIVID. Compared to laser, most patients receiving IAI did not lose any letters from baseline: 94.2, 92.7, and 57.1% in VISTA and 94.1, 91.9, and 62.9% ($P < 0.0001$) in VIVID. Significantly more patients treated with IAI q4wk and q8wk than laser experienced a two-step improvement in DRSS in both VISTA (33.8% and 29.1% vs.14.3%) and VIVID (33.3% and 27.7% vs. 7.5%). Mean changes in CRT were −185.9 μm, −183.1 μm, and −73.3 μm in VISTA and −195.0 μm, −192.4 μm, and −66.2 μm in VIVID. The mean +/− SD in NEI VFQ-25 scores for the IAI q4k groups were significantly different from the laser groups only for the near activities subscale scores in VISTA (9.0 +/− 20.6 vs. 5.4 +/− 20.4; $P = 0.0168$). For patients treated with laser/sham, the mean numbers of lasers were 2.7 and 2.1 in VISTA and VIVID, respectively. More patients in the laser group than the IAI groups received additional (rescue) therapy (VISTA, 31.2% vs. 0.7% and 2.6%; VIVID, 24.1% vs. 4.4% and 8.1%).

Incidences of ocular and non-ocular adverse events and serious adverse events including Antiplatelet Trialists' Collaborative-defined vascular events and deaths were similar among all groups. The incidences of ocular and non-ocular adverse events were similar across all treatment groups. Serious non-ocular adverse events were uncommon (hypertension: 9.7%; cerebrovascular accidents: 1.1%; and myocardial infarction: 1.1%). Incidences of intraocular inflammation were 0.2% (4/1832 injections), 0.1% (1/1284 injections), and 0.5% (1/212 injections) in VISTA and 0.2% (4/1566 injections), 0.4% (5/1186 injections), and 0.7% (1/135 injections) in VIVID. Both laser patients developed inflammation before receiving rescue aflibercept. There were no cases of endophthalmitis. The incidences of congestive heart failure and anemia were higher in the aflibercept groups, and the incidences of myocardial infarction and osteoarthritis were higher in the laser groups. The total numbers of vascular deaths were 2, 2, and 2, and the total numbers of deaths were 2, 4, and 2 due to additional deaths from B-cell lymphoma and lung carcinoma in the 2 mg q8wk group.

The mean improvements in BCVA from baseline to week 100 in the 2q4, 2q8, and laser arms in VISTA (+11.5, +11.1, and +0.9 letters) and VIVID (+11.4, +9.4, and +0.7 letters) resembled those at the 52-week primary endpoint [19]. The proportions of eyes that gained >15 letters were 38.3, 33.1, and 13.0% ($P < 0.001$) in VISTA and 38.2, 31.1, and 12.1% ($P < 0.001$) in VIVID. Significantly more eyes receiving aflibercept than laser achieved >two-step improvements in DRSS in both VISTA (37.0, 37.1, and 15.6%) and VIVID (29.3, 32.6, and 8.2%).

Eyes in the aflibercept arms of both VISTA and VIVID had sustained improvements in VA and CRT through the 148-week visit. After week 100, eyes in the laser groups crossed over to receive monthly aflibercept but experienced mean VA improvements of only 1 letter. Rescue aflibercept had been allowed for laser treated eyes that initially lost at least 10 letters. In the VIVID trial, 82% of eyes required either rescue or as-needed aflibercept as did 87% of eyes in VISTA. Eyes in the laser groups that required rescue therapy achieved better final visual acuities than those that did not require rescue therapy [Justus Ehlers, Macula Society, Miami Beach, FL, February 26, 2016]. Only 12% of aflibercept treated eyes required laser at some point during the trials. Eyes with limited responses at 12 weeks (<10% improvement in CRT) ultimately went on to mean visual acuity improvements of +7.8 letters [Rishi Singh, Macula Society, Miami Beach, FL, February 25, 2016].

At week 100, eyes in the laser groups averaged −84 μm of macular thinning and after crossover to aflibercept this increased to −110 μm by week 148. In contrast, the IAI 2q4wk and 2q8wk groups achieved significantly better thinning of the macula (−200 and −190 μm).

In 2014, aflibercept received US FDA approval for the treatment of center-involving DME and soon thereafter for the treatment of diabetic retinopathy in patients with DME. It has been approved by the European Medicines Agency and with the recent approval in Egypt is now available for the treatment of DME in 31 countries.

The ability of intravitreal aflibercept to prevent DR progression in eyes without DME and PDR is being evaluated in the PANORAMA trial. Patients with moderately severe BDR are randomized to receive sham injections or aflibercept every 8 or 16 weeks. This trial resembles the DRCR.net Protocol W trial in which ranibizumab is being evaluated.

4.4 Other Studies

In a retrospective analysis of eyes receiving anti-VEGF therapy, Wycoff noted that two-line improvements in DRSS occurred most frequently in eyes with moderately severe DR, as opposed to those with milder NPDR or with PDR [Charles Wycoff, Retina Society, Paris, FR, October 8, 2015]. The reason for this finding is not known, but it may indicate that the reversibility of DR is related to disease severity and may be influenced by the contribution of chemokines or cytokines other than VEGF.

The best results from anti-VEGF therapy appear to occur when monthly injections are continued for at least 5 months. Unfortunately, a small proportion of eyes (15%) will fail to respond well to monthly therapy and post hoc analyses suggest that these can be reliably identified by poor resolution in macular thickness (<10%) or improvement in BCVA (< 5 letters) at 3 months [David Boyer, Retina Society, Paris, FR. October 8, 2015]. A small retrospective series found that the 1-month response to bevacizumab injections could be reliably determined by the 1 h response (542.3 ± 127.7 μm at baseline to 516.9 ± 123.4 μm 1 h after injection, $P < 0.001$, and to 345.5 ± 110.0 μm at 1 month after injection, $P < 0.001$; $r = 0.515$) [112].

None of the registration trials allowed switching of anti-VEGF drugs in eyes that respond poorly to initial therapy and reliable data regarding this strategy is limited. A retrospective study of 21 eyes evaluated the efficacy of switching from bevacizumab or ranibizumab to aflibercept. Patients had received a median of 6 injections before the switch and 3 aflibercept after the switch. The mean CFT decreased from 453.52 ± 143.39 μm immediately prior to the switch to 362.57 ± 92.82 μm (Wilcoxon signed-rank test, $P < 0.001$) after the first injection and to 324.17 ± 98.76 mm ($P < 0.001$) at the study's conclusion. The mean visual acuity was 0.42 ± 0.23 logMAR just prior to the switch, 0.39 ± 0.31 logMAR after one aflibercept injection, and 0.37 ± 0.22 logMAR at the end of follow-up. The final visual acuity was significantly better than visual acuity before the switch ($P = 0.04$). The authors concluded that eyes with DME that are unresponsive to multiple ranibizumab or bevacizumab injections can demonstrate anatomic and visual improvement after switching to aflibercept [107].

A post hoc analysis of the DRCR.net trials showed that eyes with poor responses at 3 or 6 months eventually achieved 5–7 letters of improvement in BCVA simply by continuing intensive intravitreal therapy [Daniel Martin, Macula Society, Miami Beach, FL, February 26, 2016]. The authors stated that the perceived improvement

with switching anti-VEGF drugs may actually be the result of continued, intensive anti-VEGF therapy.

4.5 Comparison Trials

Prior to the publication of DRCR.net Protocol I, a Bayesian network meta-analysis compared the efficacies of ranibizumab and aflibercept for the treatment of DME [145]. For 10-letter gains, the results slightly favored ranibizumab (RR 1.59, 95% credible interval 0.61–5.37).

A 48-week, randomized, prospective trial of 45 patients compared intravitreal bevacizumab with ranibizumab [127]. Patients in both groups experienced significant improvements in BCVA at all visits ($P < 0.05$), but those receiving ranibizumab had greater improvements at weeks 8 ($P = 0.032$) and 32 ($P = 0.042$). Mean CST significantly improved in both arms at all visits with no difference noted between the drugs. Patients receiving bevacizumab required more injections than those receiving ranibizumab (means 9.84 vs. 7.67).

The only trial to directly compare aflibercept, bevacizumab, and ranibizumab treatment of DME was the prospective, randomized, multicenter DRCR.net Protocol T trial [34]. Six hundred sixty patients at 89 sites received 1.25 mg bevacizumab, 0.3 mg ranibizumab, or 2 mg aflibercept. Entry criteria included BCVA from 20/32 to 20/320 with center-involving DME by clinical exam and OCT. Patients were treated every 4 weeks unless the BCVA reached 20/20 or better with a CST below the eligibility threshold, or over the past two injections there was no BCVA change of 5 letters or more or a 10 % change in CST. Beginning at week 24, injections were withheld if the BCVA change was < 5 letters and the CRT change was < 10 % over two injections irrespective of BCVA. Patients were eligible for laser photocoagulation at 24 weeks for persistent edema.

At 52 weeks, mean numbers of injections were 9 (aflibercept), 10 (bevacizumab), and 10 (ranibizumab) ($P = 0.045$). Laser photocoagulation was performed in 37, 56, and 46% of eyes ($P < 0.001$). Mean changes in BCVA were +13.3 letters (aflibercept), +9.7 letters (bevacizumab), and +11.2 letters (ranibizumab) ($P < 0.001$, aflibercept vs. bevacizumab; $P = 0.03$, aflibercept vs. ranibizumab). A preplanned subgroup analysis was critical to uncovering significant differences in efficacy among the drugs. For eyes with baseline BCVA of 20/32 to 20/40, mean changes were +8.0 (aflibercept), +7.5 (bevacizumab), and +8.3 letters (ranibizumab). When baseline VA was ≤20/50, mean changes in BCVA were +18.9 (aflibercept), +11.8 (bevacizumab), and +14.2 letters (ranibizumab). The average changes in CST were −169 μm, −101 μm, and −147 μm. Only two eyes developed endophthalmitis, and there were no significant differences in the rates of serious adverse events ($P = 0.40$), hospitalization ($P = 0.51$), death ($P = 0.72$), or major cardiovascular events.

Visual acuity gains were sustained in all groups at 2 years, but the differences in BCVA gains between the drugs narrowed. Aflibercept produced significantly better

vision gains than bevacizumab but was not statistically better than ranibizumab [Wells, Mac Society].

4.6 Conclusions and Outstanding Questions

The phase III registration trials showed conclusively that treatment with bevacizumab, ranibizumab, or aflibercept is superior to sham/laser for the treatment of DME. Treated patients are also more likely to experience significant improvements in DRSS.

Important questions regarding treatment of DR persist. It is not known how effective anti-VEGF therapy will be on DRSS progression and regression if therapy is administered less frequently. For example, patients in BOLT and Protocol I were treated less frequently than in RISE/RIDE and VIVID/VISTA, and their improvement in DRSS were also less.

The recent DRCR.net Protocol S provided the first evidence that anti-VEGF therapy may be as good as or better than panretinal photocoagulation for PDR. Both longer duration and confirmatory studies with other anti-VEGFs need to be performed.

The best treatment strategy for poor responders has not been determined. Many options are now available to treating physicians, but randomized controlled trials are difficult to structure and have not yet been performed. Management strategies for these challenging eyes will be discussed in detail in Chap. 6.

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Chapter 5
Corticosteroids: Targeting Multiple Cytokines and Chemokines

5.1 Introduction

Drugs that inhibit vascular endothelial growth factor (VEGF) are effective for center-involving diabetic macular edema (DME) since most eyes respond well to regimens that include repeated intravitreal injections. A significant minority of patients (approximately 40%), however, exhibit an inadequate response and require additional or alternate therapy. In the phase III anti-VEGF registration trials, laser photocoagulation served as secondary therapy for patients with inadequate responses after 3–6 months of monthly anti-VEGF injections [52, 66]. Supplemental laser usually resolves persistent edema slowly, but it has not been shown to incrementally improve VA in most cases [26, 62].

Glucocorticoids were the first drug class shown to improve DME in randomized clinical trials [34–36]. Triamcinolone acetonide, the first drug to be tested (Kenalog®; Bristol-Myers Squibb, New York, NY), was not originally formulated for intraocular use, and it was associated with a high risk of cataract and elevated intraocular pressure. Topical and periocular corticosteroids [24] do not significantly improve DME because they do not penetrate the sclera sufficiently to produce therapeutic concentrations within the retina. Intravitreally injected corticosteroids resolve macular edema and improve visual acuity in both treatment-naïve eyes and those previously treated with laser and/or anti-VEGF therapy. As a result, significant interest in treating DME with intravitreal corticosteroids has developed, and this chapter reviews the current state-of-the art corticosteroid use in the management of patients with DME.

5.2 Characteristics of Corticosteroids

The primary structure of VEGF – a long amino acid sequence – prevents it from crossing the cell membrane's lipid bilayer, whereas the lipophilic ring structures of corticosteroids enable them to pass easily. Corticosteroids bind to heat shock

M.W. Stewart, *Diabetic Retinopathy*, DOI 10.1007/978-981-10-3509-8_5

proteins and several soluble steroid receptors within the cytoplasm [20] and the resultant complex migrates to the cell nucleus (Fig. 5.1). The heat shock protein dissociates, leaving the corticosteroid-receptor complex to bind to the promoter or repressor regions of several genes [11, 29]. The corticosteroid-receptor complex can

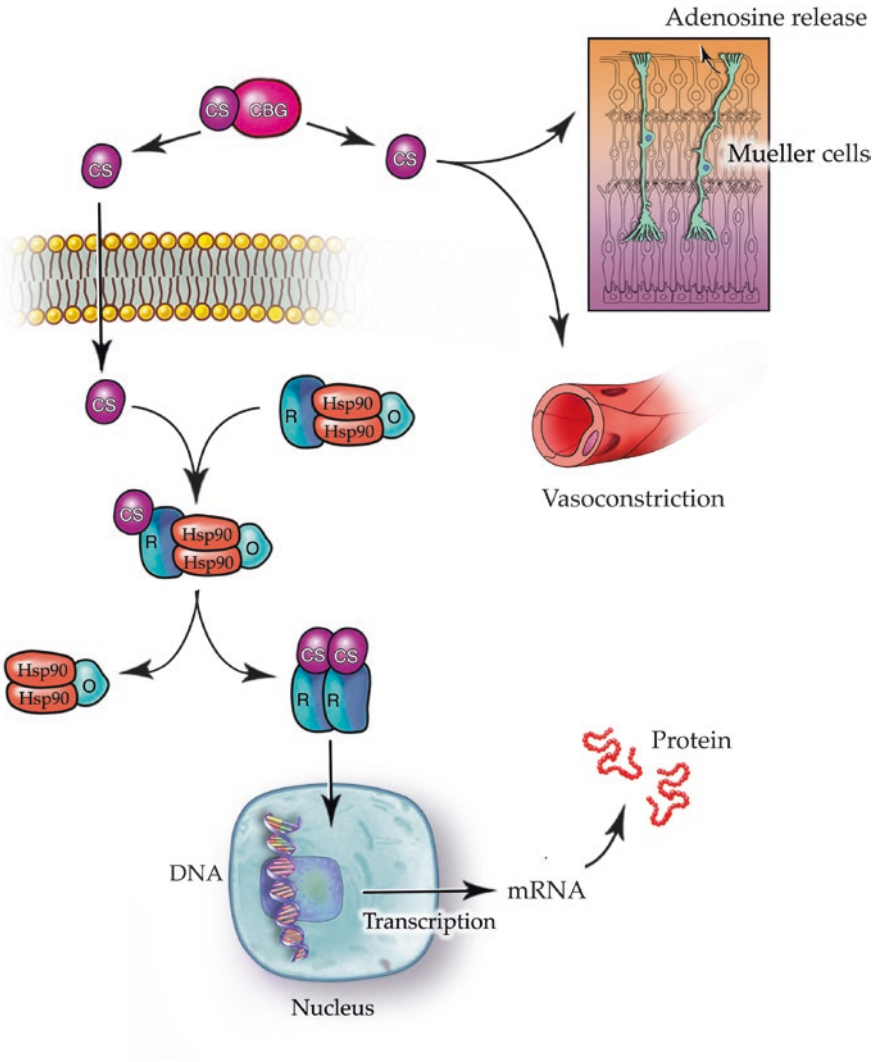

Fig. 5.1 This drawing shows the mechanisms through which corticosteroids exert their effects. Corticosteroids dissociate from serum transport proteins, enter target cells by crossing cell membranes, and bind to cytoplasmic receptors that include heat shock proteins (hsp90). The corticosteroid-receptor complexes enter cell nuclei, interact with DNA receptor sites, and suppress nuclear factor (NF)-κB. This increases the expression of many species of mRNA and suppresses others; both mechanisms affect the synthesis of thousands of proteins. Corticosteroids also act as survival factors for neuroglia and directly promote vasoconstriction

also produce signal transduction within the cytoplasm, stimulating the release of molecules such as annexin-1 [77], a modulator of leukocyte migration [23].

Corticosteroids improve macular edema via several mechanisms. They bind to the promoter region of the VEGF gene and downregulate VEGF synthesis [33, 74]. The resultant free-VEGF levels drop significantly, though they remain 100-fold higher than after the intravitreal injection of anti-VEGF drugs. Steroids decrease exudation by favorably altering the Starling's law equilibrium through vasoconstriction. Steroids decrease average capillary lumen pressure, decrease the mean transcapillary exudative pressure, and reduce the hydrostatic pressure gradient.

Inflammation contributes significantly to the pathogenesis of diabetic retinopathy, and corticosteroids are potent anti-inflammatory molecules. Corticosteroids repress several key pro-inflammatory transcription markers such as nuclear factor-kappa B (NFκB) and activator protein 1, thereby disrupting the pro-inflammatory feedback loop that is critical for the development of DME [80, 89]. Corticosteroids inhibit phospholipase A2 [79], which is upregulated in the retinas of streptozotocin-induced diabetic rates [40, 57] and has been associated with increased ICAM-1, VEGF, and TNF-α expression, and the formation of DME [57, 67]. Corticosteroids are known to reduce tissue edema and downregulate the release of prostaglandins and histamines [87]. Corticosteroids inhibit the synthesis of endothelial nitric oxide synthase (eNOS), a potent vasodilator [56]. Each of these factors causes vasodilation and/or edema and leads to choroidal thickening. Corticosteroids decrease the synthesis of several chemokines, intercellular adhesion molecules, and growth factors that inhibit the migration and margination of polymorphonuclear leukocytes [55]. These are capable of disrupting the VEGF feedback loop and downregulating several VEGF-mediated processes. Corticosteroids also upregulate the expression of anti-inflammatory proteins that downregulate the inflammatory response: interleukin (IL)-10, adenosine, and IκBα (the natural inhibitor of NFκB).

Steroids maintain and restore the blood-retinal barrier by preventing phosphorylation of tight junction proteins [39, 86]. Corticosteroids improve tight junction integrity by upregulating junctional protein expression and facilitating their translocation to the cellular borders [3] and by protecting against oxidative stress-induced phosphorylation of tight junction proteins in RPE cells [63]. Corticosteroids increase fluid movement through the retina by stabilizing Müller cells and improving aquaporin-4 (AQP-4) and potassium channels [70, 90].

The neuroprotective capabilities of a constant infusion of low-dose fluocinolone were demonstrated in transgenic S334ter-4 and Royal College of Surgeons rats. Fluocinolone preserves outer nuclear layer cell morphology, stabilizes a- and b-wave electroretinography amplitudes, and reduces neuroinflammation [37, 38].

Triamcinolone, dexamethasone, and fluocinolone are all fluorinated glucocorticoids that lack mineralocorticoid activity. They differ according to their glucocorticoid-receptor binding affinities (dexamethasone > triamcinolone > fluocinolone) and their lipophilicity (triamcinolone > fluocinolone > dexamethasone). These characteristics may be partially responsible for their relative potencies (triamcinolone = 5, dexamethasone = 25, fluocinolone = 25, compared to cortisol = 1) (Table 5.1).

Table 5.1 This table lists the important characteristics of the corticosteroids used for the treatment of diabetic macular edema

Characteristics of corticosteroids that are used to treat diabetic retinopathy			
Characteristic	Dexamethasone	Fluocinolone	Triamcinolone
Molecular weight (kDa)	0.392	0.452	0.394
Binding affinity (nmol)	5.4	2.0	1.5
Intravitreal half-life	5.5 h	Unknown	18 days (crystalline)
Relative potencies(cortisol = 1)	25	25	5

Corticosteroids differ according to the proteins that they regulate. Triamcinolone, dexamethasone, and fluocinolone each upregulate over 6000 proteins in each of two types of retinal pigment epithelial cells, but only 15–25% of these proteins are upregulated by all three corticosteroids. Within trabecular meshwork (TM 86) cells, dexamethasone upregulates transcripts associated with RNA posttranscriptional modifications, fluocinolone affects lipid metabolism, and triamcinolone affects cell morphology; within TM 93 cells dexamethasone affects histone methylation, fluocinolone affects the cell cycle, and triamcinolone affects cell adhesion [65].

Solubilized fractions of the 3 corticosteroids have brief intravitreal half-lives of only 2–5 h [29]. Crystalline deposits of triamcinolone acetonide are minimally soluble in aqueous, and they slowly release steroid with a half-life of approximately 18 days [75]. Since triamcinolone and dexamethasone are less lipophilic than fluocinolone, some authors speculate that fluocinolone may accumulate less in the anterior chamber and cause fewer steroid-related intraocular pressure rises [69].

Corticosteroid administration by several routes (topical, periocular, intraocular, systemic) frequently increases intraocular pressure and causes posterior subcapsular cataracts [5, 6, 43]. Cataract formation and IOP elevation may be due to activation of a corticosteroid receptor found in both the lens and trabecular meshwork [34].

5.3 Corticosteroid Delivery to the Eye

The timeline in Fig. 5.2 shows important milestones in the use of intraocular corticosteroids for the treatment of DME.

Since corticosteroids are small and lipophilic, they easily cross cell membranes and the blood-retinal barrier to reach glucocorticoid receptors in target tissues. Parenterally administered corticosteroids have large volumes of distribution, so high doses would need to be given to improve retinal vascular conditions and significant side effects would be frequent. Corticosteroid drops and periocular depot injections would be unlikely to cause systemic adverse events, but they must penetrate several tissue layers to reach the retina in therapeutic concentrations [44].

The composition of tears together with their rapid elimination through the lacrimal drainage system via the blink mechanism constitutes the major barriers to topically administered drug penetration into the eye. Drug that manages to penetrate the

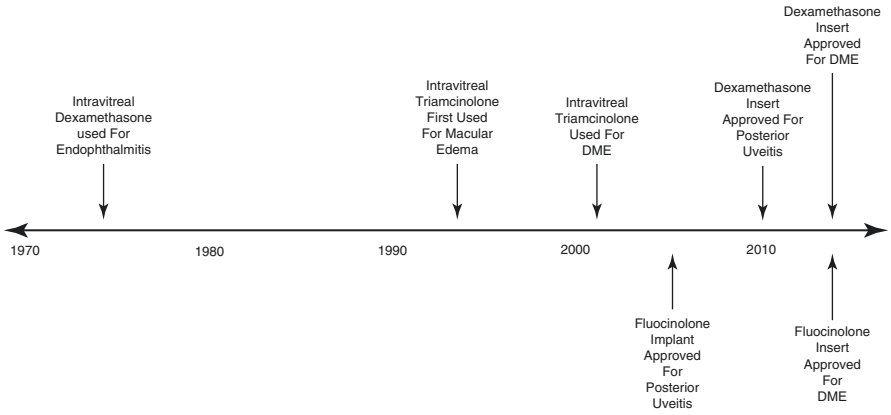

Fig. 5.2 This figure details the timeline for the intraocular use of corticosteroids. Many of the most important milestones in the history of corticosteroid use and delivery system creation are listed

corneal and conjunctival epithelium may be rapidly eliminated by conjunctival lymphatics and uveal blood flow. Unfortunately, topically administered drugs generally have intravitreal bioavailability of less than 0.001% [60].

Topical corticosteroids are effective against several chorioretinal vascular conditions in rats because the diffusion distance from the cornea to the retina is very short. The larger anteroposterior diameter of the human eye (approximately 24 mm), however, makes it nearly impossible to achieve clinically active concentrations within the retina. Corticosteroid formulations that may enhance ocular penetration and facilitate diffusion to the macula will be discussed in Chap. 8.

Sub-Tenon's injections of triamcinolone acetonide treat uveitis and postoperative cystoid macular edema but are ineffective against DME [24]. Drug must penetrate several anatomic barriers (episclera, sclera, choroid, and retinal pigment epithelium) to reach the retinal vasculature. Furthermore, flow within the conjunctival lymphatics and vitreous moves drug away from the retina [50]. Together, these diffusion barriers decrease intravitreal bioavailability after a sub-Tenon's injection to 0.01–0.1% [30]. Drug injections directly into the vitreous bypass the outer blood-retinal barrier and produce maximum bioavailability [30].

Several sustained-release reservoirs and extended duration strategies have been developed to prolong the retention of steroids within the vitreous. Triamcinolone sustained-release systems (Kenalog®; Trivaris®, Allergan, Irvine, CA, USA; Triesence®, Alcon, Fort Worth, TX, USA) are currently available for periocular and intraocular use (Fig. 5.3). Attempts to create sustained-release triamcinolone devices have thus far been unsuccessful, and further development has been halted. Biodegradable nanocarrier systems (liposomes, nanoparticles, nanocrystals, and nanosystems) are in various stages of development and may ultimately be used to treat posterior-segment disease [48]. Three sustained-release corticosteroid devices have been successfully developed and approved by the US Food and Drug Administration (US FDA) – the dexamethasone phosphate insert (DEX, Ozurdex®,

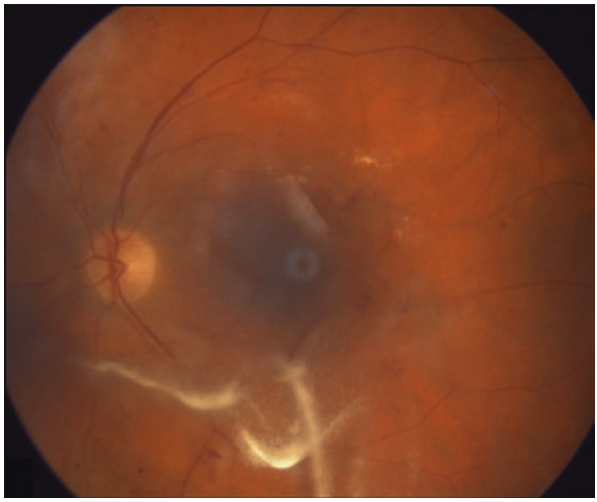

Fig. 5.3 This fundus photograph was taken 1 day after intravitreal injection of triamcinolone acetonide. Drug suspended in the posterior vitreous can be seen over the macula

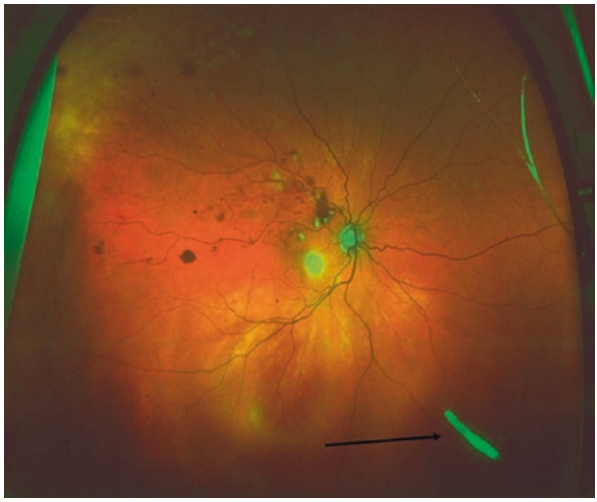

Fig. 5.4 This ultra-widefield fundus photograph shows a dexamethasone insert in the inferior vitreous (*black arrow*)

Allergan, Irvine, CA, USA) (Fig. 5.4), the fluocinolone acetonide insert (Iluvien®, Alimera Sciences, Alpharetta, GA, USA), and the fluocinolone acetonide implant (Retisert®, Bausch & Lomb, Rochester, NY, USA). These will be discussed later in this chapter.

5.4 Triamcinolone

Elevated aqueous levels of interleukin (IL)-8, interferon-induced protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), and VEGF were found in 22 eyes of 11 patients with DME compared to 6 control eyes [76]. In the 11 patients

Table 5.2 This table lists the important diabetic macular edema trials with triamcinolone acetonide and details their key findings

Important diabetic macular edema trials with triamcinolone		
Trial and phase	Cohorts	Key findings
DRCR.net Protocol B	Treatment arms IVT 1 mg IVT 4 mg Laser	At 2 years 1. Improvements in mean BCVA were −3, −2, and +1 letters 2. Elevated intraocular pressures seen in 16%, 33%, and 13% 3. Cataracts developed in 23%, 61%, and 13%
Gillies et al. (2006)	Treatment arms IVT Laser	At 2 years 1. Eyes treated with IVTA were twice as likely to achieve 10 letter improvement in BCVA 2. 44% of IVT eyes required intraocular pressure lowering medications
Kriechbaum et al. (2014)	Treatment arms IVT (8 mg) Bevacizumab (2.5 mg)	Comparable improvements in BCVA and macular thickness at 3 months At 12 months, VA stable in bevacizumab arm but decreased in IVT arm due to cataracts
DRCR.net Protocol I 854 patients	Treatment arms RAN + laser RAN + def laser IVT + laser Laser	At 12 months 1. Eyes receiving IVT + laser achieved 5 fewer letters of visual improvement compared to RAN groups 2. Pseudophakic eyes receiving IVT + laser had comparable BCVA improvements as RAN groups

IVT intravitreal triamcinolone

with DME, one eye was injected with 4 mg of triamcinolone acetonide (TA), and the other was injected with 1.25 mg of bevacizumab. Four weeks later, steroid-injected eyes showed a reduction in IP-10, MCP-1, VEGF, IL-6, and platelet-derived growth factor-AA (PDGF-AA), whereas only VEGF was reduced in bevacizumab-injected eyes. Significant clinical trials with intravitreal triamcinolone are detailed in Table 5.2.

Pilot studies showed that intravitreal injections of triamcinolone acetonide (IVTA) effectively reduce DME [4, 59]. In the prospective, randomized DRCR.net Protocol B trial, 1 and 4 mg intravitreal TA every 4 months were compared to laser photocoagulation. At 4 months, patients receiving 4 mg of TA were more likely to achieve +10-letter improvements in BCVA compared to laser (27% vs. 17%) and had greater mean decreases in central retinal thickness (CRT) (−98 μm vs. −39 μm). At the 2-year primary endpoint, however, the mean BCVA improvement in the laser group was +1 ± 17 letters compared to −3 ± 22 letters and −2 ± 18 letters in the 1 and 4 mg TA groups, respectively. Elevated IOP was seen in 33% of eyes receiving 4 mg triamcinolone, 16% of those receiving 1% triamcinolone, and 13% of those treated with laser. At 2 years, more patients receiving triamcinolone required glaucoma medications (4 mg, 13%; 1 mg, 6%; laser, 3%) and more developed cataracts (4 mg, 61%; 1 mg, 23%, laser, 13%) [25].

In a 2-year randomized clinical trial, IVTA was administered as often as every 6 months [36]. Eyes receiving IVTA were twice as likely as those treated with laser monotherapy to achieve +10-letter improvements in BCVA. The majority of eyes

developed IOP problems: >5 mmHg rise in IOP (68%), need for glaucoma medication (44%), and need for cataract surgery (54%).

The effect of triamcinolone and bevacizumab on subfoveal choroidal thickness (SFCT) was investigated in a prospective, randomized study of 51 eyes with DME [78]. Compared to baseline, the mean SFCT in eyes receiving IVTA was significantly reduced at 24 h (−5.2%) and 12 weeks (−8.2%) ($P < 0.01$ for both). Eyes receiving single injections of bevacizumab experienced significant decreases in SFCT from 24 h to 4 weeks, but not at 8 or 12 weeks. The authors suggest that the presence of inflammatory factors other than VEGF may be responsible for the differences between IVTA and bevacizumab observed after 4 weeks. A more likely explanation is that the shorter intraocular half-life of bevacizumab produces a duration of action of less than 8 weeks.

A 12-month randomized, prospective trial compared 3 monthly injections of bevacizumab (2.5 mg) with a single injection of IVTA (8 mg) followed by monthly and q4month PRN injections, respectively [53]. Eyes in both the bevacizumab and triamcinolone arms experienced improved BCVA (0.30–0.23 logMAR for bevacizumab vs. 0.32–0.26 logMAR for triamcinolone) and reduced macular thickness (505–358 μm for bevacizumab vs. 490–308 μm for triamcinolone) at 3 months. Visual acuity continued to improve through 12 months in the bevacizumab group (0.18 LogMAR) but had decreased in the triamcinolone group (0.36 LogMAR) because of cataracts.

The efficacy of IVTA was evaluated in 20 eyes that had been unresponsive to at least 3 monthly intravitreal bevacizumab injections [46]. At least 2 months after the last intravitreal bevacizumab, an aqueous humor sample was obtained, and vascular endothelial growth factor, IL-2, IL-6, IL-8, tumor necrosis factor-α, and transforming growth factor-β2 concentrations were measured with a multiplex cytokine array. Triamcinolone was injected intravitreally, and BCVA and central subfield thickness were evaluated through 3 months. The mean BCVA was 47.1 ± 18.9 letters at baseline, 53.3 ± 19.7 letters at 1 month ($P = 0.002$), and 52.4 ± 19.1 letters at 2 months ($P = 0.041$), but the acuity gains were not sustained at 3 months (50.9 ± 18.6 letters; $P = 0.204$). A mean decrease in central subfield thickness (−11%) was seen in 12 eyes at 1 month. Multivariate analysis showed that the intraocular concentration of IL-8 was an independent factor for anatomic response at 1 month ($P = 0.006$). The authors concluded that intravitreal triamcinolone may be an attractive treatment option for patients who have poor short-term responses to bevacizumab.

Diabetic macular edema may develop or worsen after panretinal photocoagulation (PRP) for PDR. In a prospective study of eyes with severe diabetic retinopathy, 91 eyes (46 eyes with DME; 45 eyes without DME) of 76 patients underwent PRP with IVTA (30 eyes), PRP with intravitreous bevacizumab (31 eyes), or PRP alone (30 eyes) [19]. The primary outcome measures included changes in BCVA and central macular thickness (CMT) at 1 and 3 months. Secondary outcome measures were the proportions of eyes with BCVA gain or loss, and decreased or increased CMT. In eyes with DME, there was significant worsening in BCVA ($P = 0.031$) in the PRP group but significant improvement in BCVA ($P = 0.012$) in the IVTA group. In eyes without CSME, those receiving PRP alone experienced

significant worsening in BCVA from 0.18 to 0.26 at 1 month ($P = 0.008$) and to 0.27 at 3 months ($P = 0.005$). In eyes without DME, there was significant increase in CMT from 209.75 to 259.00 μm at 1 month ($P = 0.023$) and to 276.14 μm at 3 months ($P = 0.011$) in the PRP group; in eyes with DME, the proportion of eyes with improved BCVA and decreased CMT was significantly higher in the IVTA group (75% and 100%, respectively) than in the IVB group (37.5% and 62.5%, respectively). The authors concluded that IVTA and bevacizumab may be effective adjunctive treatments to PRP by minimizing the risk of PRP-induced macular edema and visual loss.

The DRCR.Net Protocol I trial randomized 854 patients to receive ranibizumab + prompt laser, ranibizumab + deferred laser, IVT + prompt laser or prompt laser + sham. At 1 year, the median improvements in BCVA in each of the four treatment arms were +9, +9, +4, and +3 letters, respectively [26]. Patients receiving triamcinolone experienced BCVA improvements during the first 3 months that were similar to patients receiving ranibizumab, but BCVA then worsened through 12 months because steroid-related cataracts developed. One-year BCVA improvements in the triamcinolone subgroup that was pseudophakic at baseline were comparable to those in the ranibizumab groups.

Eyes randomized to prompt laser + triamcinolone were eligible to receive ranibizumab as early as week 74 for persistent DME with no improvement in BCVA. At the 5-year concluding visit, mean improvements in BCVA in the four arms were +10, +8, +7, and +5 letters [31]. During the first 2 years of the trial, pseudophakic eyes that were randomized to IVT + laser had better improvements in BCVA compared to the entire group receiving triamcinolone. By the 5-year visit, however, the BCVA gains in the pseudophakic eyes receiving triamcinolone resembled those in the entire triamcinolone arm.

5.5 Dexamethasone

The dexamethasone posterior-segment drug delivery system (DEX, Ozurdex®, Allergan Inc., Irvine, CA, USA) is a biodegradable, sustained-release reservoir. The DEX contains 0.7 mg of micronized, preservative-free dexamethasone in a biodegradable cylinder (6 mm long by 0.46 mm diameter) made of a copolymer of poly (lactic-co-glycolic) acid that slowly breaks down into glycolic acid and water while simultaneously releasing dexamethasone over the course of 3 months. The cylinder is preloaded into a single-use applicator and then injected across the pars plana into the anterior vitreous through a 22-gauge needle. The needle is advanced through the sclera in a tunneled fashion, and the tract self-seals after removal. The procedure is performed in the clinic under aseptic conditions after the instillation of topical and subconjunctival anesthesia.

A prospective, comparative study showed that pain after dexamethasone injection under topical anesthesia was comparable to that after anti-VEGF injections that were delivered with much smaller needles [64]. The insert should not be injected

into aphakic and pseudophakic eyes that lack an intact barrier between the vitreous and anterior chamber because it can migrate into the anterior chamber and damage the corneal endothelium [49, 51, 58]. After injection, the insert usually settles into the inferior vitreous base without being noticed by patients. After approximately 3 months, the insert dissolves completely, and intraocular dexamethasone concentrations fall to near zero. In addition to rare cases of endophthalmitis and vitreous hemorrhage, injections have been complicated by inadvertent insert placement into the crystalline lens [7, 21, 32] or fragmentation of the insert [1, 27, 71, 72].

Drug release from the insert follows a biphasic pattern with intraocular concentrations peaking at 2–6 weeks (vitreous, 100–1000 μg/mL; retina, 100–1000 μg/gm), followed by a sharp drop during the third month, a lower plateau for 3–4 months (vitreous, 0.1–1 ng/mL; retina, 0.1–1 ng/gm), and non-detectable levels by months 7–8 [17]. The intraocular dexamethasone concentrations are higher and steadier during the initial plateau than can be achieved with topical [83], subconjunctival [81], periocular [84], and oral administration [82].

Previous vitrectomy accelerates clearance of drugs from the eye, but rabbit studies show that time-related intravitreal dexamethasone concentrations after the injection of the insert were the same in vitrectomized and non-vitrectomized eyes [18]. The CHAMPLAIN study was a prospective, open-label, 26-week trial of DEX in 55 eyes with DME that had previously undergone vitrectomy. At 8 weeks after injection, the mean BCVA improved by +6.0 letters, with 30.4% improving by >10 letters, and mean CRT changed by −156 μm [9]. These improvements were similar to those achieved in non-vitrectomized eyes in the phase III MEAD trial [10], suggesting that DEX functions well following vitrectomy.

Plasma dexamethasone concentrations after intravitreal injection of the DEX are below the lower limit of quantification (50 pg/mL), so the likelihood of patients developing systemic corticosteroid-related effects is very low [28].

In the ORVO study [14], protein arrays were performed on aqueous samples taken before and 4 weeks after a dexamethasone insert. In many patients, aqueous hepatocyte growth factor and EG-VEGF reductions at 4 weeks mirrored the reduction in macular edema.

Important trials that featured dexamethasone inserts are detailed in Table 5.3.

In an initial 6-month trial, single applications of 0.35 and 0.7 mg dexamethasone inserts were performed in eyes with macular edema due to diabetic retinopathy, retinal vein occlusions, posterior uveitis, and the Irvine-Gass syndrome [54]. A subset analysis showed that patients with uveitis and Irvine-Gass responded equally well to the insert compared to eyes with the other diagnoses [85].

Patients in this trial with DME (n = 171) were fully reported in a separate analysis [42]. Enrollment criteria for this subgroup included patients with DME and baseline BCVA measurements from 20/40 to 20/200. Key exclusion criteria included a history of vitrectomy, moderate or severe glaucoma, poorly controlled systemic arterial hypertension (systolic blood pressure >160 mmHg and/or diastolic blood pressure >90 mmHg), and poorly controlled diabetes mellitus (HbA1c >13%). Patients were randomized 1:1:1 to receive the high-dose insert (700 μg), the low-dose insert (350 μg), or observation. The insert (20-gauge) was inserted through the

Table 5.3 This table lists the important diabetic macular edema trials with the dexamethasone insert and details their key findings

Important diabetic macular edema trials with the dexamethasone insert		
Trial and phase	Cohorts	Key findings
Macular edema trial 171 patients	Treatment arms DEX 700 μg DEX 350 μg Observation	At 90 days 1. Proportions of patients improving by 10 letters were 33%, 21%, and 12% ($P = 0.007$) 2. Greater improvements in central macular thickness in DEX 700 μg compared to laser ($P = 0.03$) At 6 months 1. Proportions of patients improving by 10 letters were 30%, 19%, and 23%
PLACID trial Phase II	Treatment arms DEX + laser Laser	More patients receiving DEX + laser improved by 10 letters at 1 month ($P < 0.001$) and 9 months (31.7% vs. 17.3%; $P = 0.0076$) but not at 12 months Area under the curve analysis showed greater BCVA improvements in DEX + laser group
CHAMPLAIN trial open-label 55 patients	Single-arm DEX in patients with previous vitrectomy	Mean change in central retinal thickness was −156 μm at 8 weeks ($P < 0.001$) but only −39 μm at 26 weeks ($P = 0.004$) Mean change in BCVA was +6 letters at 8 weeks ($P < 0.001$) but only +3 letters at 26 weeks ($P = 0.046$)
MEAD trials Phase III 253 patients	Treatment arms DEX 700 μg DEX 350 μg Sham	At 3 years 1. More patients receiving the DEX improved by at least 15 letters (22.2%, 18.4%, 12.0%; $P < 0.018$) 2. Mean improvement in BCVA in pseudophakic eyes receiving DEX was +7 letters 3. 29.7% of DEX group had IOP >25 mmHg, but only one patient required incisional glaucoma surgery 4. 66% of DEX group developed cataracts and 61% underwent surgery

DEX dexamethasone insert, *BCVA* best corrected visual acuity, *IOP* intraocular pressure

pars plana with suturing of the incision sites. Eyes that lost 15 letters of BCVA were eligible for whatever treatment was proposed by the investigator. The primary outcome measure for these groups was a +10-letter improvement in BCVA at day 90. Additional outcome measures included the proportion of eyes improving by at least +15 letters, changes in dye leakage on fluorescein angiography, reduction in CRT, and safety measures.

At day 90, more patients in the group receiving the 700 μg insert than the 350 μg insert or observation groups experienced 10-letter improvements in BCVA (33.3% vs. 21.1% vs. 12.3%) ($P = 0.007$, 700 μg group vs. laser). At day 180, the corresponding +10-letter BCVA improvements were 30%, 19%, and 23% ($P > 0.4$ for treated vs. observed eyes). At day 60, more patients receiving the 700 μg insert than observation achieved +15-letter improvements ($P = 0.01$). There were significantly greater improvements in CRT for treated vs. observed eyes ($P = 0.03$ at day 90). A dose-response trend was noted at all time points, but there were no significant differences between the 700 and 350 μg DEX arms.

Compared to the observation group at day 90, patients receiving the 700 μg DEX had significantly greater improvements in CRT (+30.2 μm vs. −132.3 μm) and fluorescein dye leakage. Four patients in the observation group and two in the 350 μg DEX group were treated with rescue laser or triamcinolone, but none in the 700 μg DEX group.

A subset analysis explored the relationship between macular thickness and VA improvements at sites that used OCT during the trial [8]. The macular thickness at baseline inversely correlated with BCVA (r = 20.406, $P < 0.001$), and patients treated with 350 or 700 μg DDS experienced significant decreases in macular thickness at day 90 ($P = 0.002$). There was a modest inverse correlation between changes in macular thickness from baseline to day 90 and improvement in BCVA in the 700 μg DEX group (r = 20.530, $P = 0.009$), but the correlation was weaker and not statistically significant in the 350 μg DEX group (r = 20.206, P = 0.304). The authors concluded that the correlation between baseline BCVA and macular thickness in patients with persistent macular edema was modest and that improvement in BCVA after treatment with the 700 μg DEX was consistent with changes in macular thickness measured using OCT.

The DEX was tolerated well, and there were no cases of endophthalmitis and no differences in cataract progression among the three groups. By the 90-day visit, an IOP of >25 mmHg had been measured in 12.7% of patients in the 350 μg DEX group, 7.5% in the 700 μg group, but none in the observation group. By day 180, the comparable proportions were 16.4%, 13.2%, and 0%. Most of the IOP increases were seen in the first week of the study, and many were single occurrences. No eyes required laser trabeculoplasty, and none required incisional glaucoma surgery.

Compared to patients enrolled in the DRCR.net triamcinolone trial, patients receiving DEX had worse baseline VA and longer durations of edema at enrollment. These differences make direct comparisons of these corticosteroid trials problematic. Inserts had to be surgically implanted in this study, but for the subsequent phase III trials, a single-use 22-gauge applicator was used [41].

A randomized, double-masked, multicenter, phase II trial (PLACID) compared the intravitreal injection of the dexamethasone insert + laser photocoagulation against laser monotherapy in 253 patients with DME [12]. Dexamethasone/sham was injected at baseline, and laser was performed 1 month later. During the 12-month trial, patients could receive up to three additional lasers and one additional insert. The primary efficacy variable was the percentage of patients who achieved a +10-letter improvement in BCVA. Other key efficacy measures included the change in BCVA from baseline and the change in vessel leakage as determined by fluorescein angiography. Safety outcomes included adverse events and elevations in intraocular pressure. Significantly more patients in the DEX + laser group than in the laser monotherapy group improved by +10 letters at month 1 ($P < 0.001$) and month 9 (31.7% vs. 17.3%, $P = 0.0076$), but not at month 12 (27.8% vs. 23.6%). The mean improvement in BCVA in the combination group exceeded that in the laser group (up to +7.9 vs. +2.3 letters) at all time points through month 9 ($P = 0.013$). Area under the curve analysis showed significantly more improvement in BCVA in eyes receiving DEX over both the 0–6-month and

0–12-month periods ($P < 0.001$). The central retinal thickness improved significantly more in the DEX combination group at 4 months, and the vascular leakage decreased more in the combination group at 12 months. Angiographically measured decreases in the area of diffuse capillary leakage were significantly greater in the DEX group ($P = 0.041$).

Elevations in IOP were modest, and all were managed with topical pressure-lowering medications. Among eyes treated with DEX plus laser, 15.2% (19/125) had IOP increases of at least 10 mmHg from baseline, 16.8% (21/125) had an IOP of at least 25 mmHg, and 4.0% (5/125) had an IOP of at least 35 mmHg at some point during the study. By month 12, the IOP in all eyes had returned to less than 25 mmHg. In all cases, the elevated IOP was managed with IOP-lowering medication or observation as none required surgery. Cataract development occurred more commonly in the DEX group than in the laser group (95% vs. 22%, $P = 0.017$). Cataract surgery was performed in 4 eyes in the DEX group and 5 eyes in the laser group. The authors concluded that DEX plus laser effectively reduces edema and improves vision better than laser monotherapy. The 12-month results, however, suggest that 6-month treatment intervals may be too long to produce optimal results [12].

The CHAMPLAIN trial was a prospective, multicenter, open-label, 26-week study in eyes that had undergone previous vitrectomy [9]. Enrolled patients had undergone PPV an average of 31 months before study entry, most commonly for vitreous hemorrhage, proliferative DR, epiretinal membrane, DME, or vitreomacular traction syndrome. Fifty-five patients with treatment-resistant DME (average duration of 43 months) received a single intravitreal injection of 0.7 mg DEX. The primary efficacy outcome measure was the change in CRT from baseline to week 26. The mean change from baseline central retinal thickness (403 μm) was −156 μm at week 8 ($P < 0.001$) and −39 μm at week 26 ($P = 0.004$). The mean increase in BCVA from baseline was +6.0 letters at week 8 ($P < 0.001$) and +3.0 letters at week 26 ($P = 0.046$). At week 8, 30.4% of patients had gained +10 letters in BCVA. Conjunctival hemorrhage, conjunctival hyperemia, eye pain, and increased IOP were the most common adverse events. The authors concluded that treatment with the dexamethasone intravitreal insert led to statistically and clinically significant improvements in both vision and vascular leakage from DME in previously vitrectomized eyes. Comparable efficacy in vitrectomized eyes was found in a recent, retrospective study of 58 patients [61].

The long-term safety and efficacy of the dexamethasone insert was compared to sham injections in two randomized, double-blind, multicenter, phase III registration trials (MEAD) [10]. From baseline to 3 years, more patients receiving the 0.7 mg and 0.35 mg inserts compared to sham experienced >15-letter improvements in VA (22.2% vs. 18.4% vs. 12.0%; $P < 0.018$). The mean improvement in VA among patients receiving the 0.7 mg insert was +7 letters. Patients that were pseudophakic at baseline had relatively stable improvements in VA throughout the trial, whereas those who were phakic at baseline experienced a rapid improvement in VA, a decline beginning at week 24 due to the formation of cataracts, and finally an improvement in VA after week 52 as cataracts were removed.

More patients receiving the insert experienced IOP readings of at least 25 mmHg (29.7% vs. 4.3% sham). In most cases, the increase in IOP was transient as the incidence peaked at 6 weeks following DEX insertion and returned to baseline in 6 months. The incidence and severity of IOP elevation after each DEX injection was consistent throughout the 3-year study. In most cases, IOP elevation was managed with topical medications or by observation, and only three patients (0.4%) required incisional glaucoma surgery. IOP elevations tended to occur early, as 75% of spikes were diagnosed after the first 2 insertions and 85% after the first 3.

Among phakic patients, 66.0% of patients treated with DEX experienced development or progression of cataracts (cortical, nuclear, or subcapsular) compared with 20.4% of sham-treated patients. Nearly 56% of dexamethasone insert-treated patients compared with 7.2% of sham-treated patients required cataract surgery during the study. The incidence of cataract-related adverse effects increased throughout the duration of the study with most cataract surgeries performed during the second and third years.

In addition to cataract progression and IOP elevation, the most frequent adverse events were conjunctival hemorrhage (23.5%), vitreous hemorrhage (10.0%), macular fibrosis (8.3%), conjunctival hyperemia (7.2%), eye pain (6.1%), vitreous detachment (5.8%), and dry eye (5.8%). Retinal tear, retinal detachment, vitreous loss, and endophthalmitis occurred in approximately 2% of patients.

Use of the dexamethasone insert is contraindicated in patients with active or suspected ocular or periocular infections, including viral, mycobacterial, and fungal diseases, and glaucoma patients with cup-to-disk ratios of at least 0.8. The use of the dexamethasone insert may increase the risk of secondary infections.

The UK National Institute for Health and Care Excellence has recommended the use of the dexamethasone intravitreal implant for pseudophakic patients with diabetic macular edema (DME) who are unresponsive to or unsuitable for non-corticosteroid therapy. In 2014, the US FDA approved the DEX for the treatment of DME in eyes that were pseudophakic or were scheduled to undergo cataract surgery. Shortly thereafter, the indications were expanded to include phakic eyes.

A 12-month head-to-head comparison of bevacizumab and the dexamethasone insert included 88 eyes of 61 patients with center-involving DME. Eyes were randomized to receive intravitreal injections of bevacizumab q4weeks PRN or the dexamethasone insert q16weeks PRN. Improvement of at least +10 letters was seen in 40% of eyes receiving bevacizumab and 41% of eyes receiving the dexamethasone insert ($P = 0.83$). None of the bevacizumab-treated eyes lost 10 letters, compared to 11% of the dexamethasone-treated eyes, primarily because of cataracts. Dexamethasone-treated eyes had greater decreases in CMT (−187 μm vs. −122 μm, $P = 0.015$). Not surprisingly, bevacizumab-treated eyes required more injections (8.6 vs. 2.7). An elevation in IOP of at least 5 mmHg was seen at some point in 46% of dexamethasone-treated eyes but only 19% of bevacizumab-treated eyes. The authors concluded that the dexamethasone insert and bevacizumab lead to comparable improvements in VA, but dexamethasone produces superior drying of the macula with fewer injections.

5.6 Fluocinolone

Two sustained-release fluocinolone devices have been approved for the treatment of posterior-segment conditions. The 0.59 mg fluocinolone acetonide implant (Retisert®, Bausch & Lomb, Rochester, NY, USA) is surgically implanted through a 4 mm pars plana incision and sutured to the sclera for fixation. This device was originally based on the design of the sustained-release ganciclovir reservoir (Vitrasert®) but was modified in 2011 to include a silicone elastomer strut (as opposed to polyvinyl alcohol) because of spontaneous detachment of the reservoir after several years within the eye. This implant was developed, tested, and US FDA approved for the treatment of chronic noninfectious posterior uveitis [45]. The device elutes fluocinolone at a rate of 0.6 μg/day during the first month, which drops to 0.3–0.4 μg/day for the next 35 months. The implant precludes the need for regularly administered immunosuppressive therapy and thereby avoids the side effects that often accompany systemic drug use. Pricing of the implant reflects this trade-off as the implant wholesales for $17,000 US.

The fluocinolone implant was studied in 80 eyes with DME [69]. The results from this feasibility study served as the basis for a subsequent 4-year, 23-center trial that randomized (2:1) 159 patients with center-involving DME to receive the 0.59 mg fluocinolone implant or standard-of-care (SOC) treatment (laser or observation at the discretion of the investigating physician) [68]. Eligible patients had macular thickening at least 1 disk area in diameter that involved the fovea. Baseline visual acuities varied from 20 letters to 68 letters (20/50 to 20/400). All eyes had previously received laser photocoagulation >12 weeks prior to enrollment. The primary outcome was the proportion of patients improving by at least 15 ETDRS letters at 6 months, and the secondary outcomes included the improvement in macular thickness, the mean change in BCVA, the change in leakage on fluorescein angiography, and the diabetic retinopathy severity scores (DRSS). Safety evaluation included the incidences of adverse events. The implant was surgically placed at day 1, but no subsequent surgery (including cataract extraction) was allowed for 6 months. Laser photocoagulation for diffuse macular edema could not be performed within the first 6 months, but focal treatment of microaneurysms was allowed earlier.

Visual acuity improved by >3 lines in more patients receiving the implant than SOC at 6 months (16.8% vs. 1.4%; $P = 0.0012$), 1 year (16.4% vs. 8.1%; $P = 0.0012$), and 2 years (31.8% vs. 9.3%; $P = 0.0016$). The lack of a significant difference in VA at 3 years (31.1% vs. 20.0%; $P = 0.1566$) probably reflects depletion of the implants. The number of eyes with the FA implant with no evidence of foveal thickening was lower than those receiving SOC at 6 months ($P < 0.0001$), 1 year ($P < 0.0001$), 2 years ($P = 0.016$), but not 3 years ($P = 0.861$). More eyes receiving the implant experienced improvements in DRSS from 6 months through 3 years. At 1 year, 20% of implanted eyes experienced a two-step improvement in DRSS, but this had fallen to 12% by 3 years. By 3 years, 20 implanted eyes required 32 laser treatments, whereas 28 SOC eyes required 52 laser treatments. Intraocular pressure >30 mmHg was noted in 61.4% of implanted eyes compared to only 5.8% of SOC

Table 5.4 This table lists the important diabetic macular edema trials with the fluocinolone insert and details their key findings

Important diabetic macular edema trials with the fluocinolone insert		
Trial and phase	Cohorts	Key findings
FAMOUS trial Phase I 35 patients	Treatment arms FA 0.2 μg/day FA 0.5 μg/day	At 12 months 1. Excellent sustained-release of fluocinolone 2. Reduction of macular edema 3. Improvement in visual acuity
FAME trials Phase III 956 patients	Treatment arms FA 0.2 μg/day FA 0.5 μg/day Sham	At 24 months (primary endpoint) 1. The proportions of eyes improving by 15 letters were 28.7%, 28.6%, and 16.2% ($P = 0.002$) 2. Improvements in mean BCVA of +4.4, +5.4, and +1.7 letters 3. 25% of patients required a second insert before 36 months 4. Eyes with chronic edema (>3 years) had greater chance of three-line improvement

FA fluocinolone acetonide insert, *BCVA* best corrected visual acuity

eyes, and by 4 years, 33.8% of implanted eyes required incisional glaucoma surgery to lower the pressure. Cataract removal was performed in 91.4% of phakic eyes but only 20% of SOC eyes by 4 years. Because of the high rate of incisional glaucoma surgeries, further development of the fluocinolone implant for DME was halted.

The fluocinolone insert (Iluvien) is a nonbiodegradable cylindrical tube (3.5 × 0.37 mm) that is injected with a 25-gauge preloaded inserter. The inserts are made of polyvinyl alcohol, similar to the original design of the fluocinolone implant. The drug release characteristics of the fluocinolone acetonide insert (FA) were originally studied in rabbits. After injection of the 0.2 μg insert, drug concentrations in ocular tissues peaked between days 2 and 8, reached a steady-state level by 3 months, and slowly decreased through the remainder of the study. Fluocinolone was found in most ocular tissues through 2 years, but it was not detected in the anterior chamber at most time points [47].

Important trials that featured the fluocinolone insert are detailed in Table 5.4.

The insert was originally studied in a 1-year pharmacokinetic study (FAMOUS). Inserts that eluted either 0.2 μg/day or 0.5 μg/day provided consistent sustained drug delivery for 1 year with associated reduction in DME [15].

The insert underwent testing for DME in two parallel, 101 center, randomized, phase III registration trials (FAME) [13]. A total of 956 patients were randomized to receive sham (185), a 0.2 mg (375), or a 0.5 mg insert (393). At entry, eyes had BCVA between 20/50 and 20/400 despite having received at least one previous macular laser treatment. Patients with preexisting glaucoma were excluded from the trial. Six weeks after randomization, subjects were eligible for rescue laser. One year after randomization, additional inserts or sham injections could be given if necessary. The primary outcome was an improvement in BCVA of >15 ETDRS letters at month 24. Secondary outcomes included other measures of visual function and foveal thickness.

The mean duration of DME at baseline was 3.5–3.9 years. Significant visual acuity improvements were noted in both FA treatment groups at 3 weeks and at every

time point thereafter. The proportions of patients reaching the primary endpoint were 28.7% (low dose), 28.6% (high dose), and 16.2% (sham; $P = 0.002$ for each). The mean improvements in BCVA at month 24 were +4.4, +5.4, and +1.7 letters ($P = 0.02$ and $P = 0.016$ compared to sham). A final VA of 20/40 or better was achieved in 33%, 31%, and 16% of eyes ($P = 0.0185$ and $P = 0.0064$ compared to sham), whereas a final acuity of <20/200 was achieved in 14% of insert eyes and 12% of sham eyes. For eyes with at least 3 years of DME prior to the study, 34% in the low-dose group improved by at least 15 letters (versus 13.4% of sham; $P < 0.001$). The treatment benefit in these eyes did not appear to depend on baseline anatomic characteristics [Cunha-Vaz]. Eyes receiving the insert had significantly greater improvements in foveal thickness at all time points. Final CST of <250 μm was achieved in 40%, 47%, and 51% of eyes. Because of recurrent or persistent edema, 23.5% (low dose) and 26.4% (high dose) of eyes required at least 2 insert injections. Fewer insert than sham patients required laser photocoagulation treatments (36.7%, 35.2%, and 58.9%). Significantly more phakic patients receiving the insert (74.9% and 84.5% vs. 23.1% sham) required cataract surgery and their final BCVA improvements were similar to eyes that were already pseudophakic at baseline. Eyes that underwent cataract surgery by the conclusion of the trial had better average visual acuities than when the trials began [88]. Incisional surgery to control glaucoma was required in 3.7%, 7.6%, and 0.5% of eyes. A secondary analysis showed that eyes receiving the 0.2 mg insert experienced less progression of PDR compared to controls (17% vs. 31%; $P < 0.0001$) [2].

A preplanned subgroup analysis of the FAME trials compared VA improvements in eyes with chronic (>3 years) versus non-chronic (<3 years) DME. At the 36-month endpoint, more eyes with chronic edema treated with inserts improved by 3 lines (34% vs. sham (13.4%); $P < 0.001$) compared to eyes with non-chronic edema (22.3% vs. sham (27.8%); $P = 0.275$). The authors speculated that chronic edema is more sensitive to corticosteroids because it is chemokine-driven, whereas non-chronic edema is less responsive to corticosteroids because it is VEGF-driven [22].

A pharmacokinetic analysis of aqueous fluocinolone concentrations that had been obtained during the previously discussed fluocinolone implant and insert trials was subsequently performed [16]. At 1 month after insertion, the anterior chamber fluocinolone concentrations were 2.17 ng/ml (0.2 μg/day insert), 3.03 ng/ml (0.5 μg/day insert), and 6.12 ng/ml (0.6 μg/day implant). At 3 months, mean FAc levels were 1.76, 2.15, and 6.12 ng/ml, respectively. The low-dose and high-dose inserts produced relatively stable aqueous drug concentrations (0.45–1.18 ng/ml and 0.84–1.50 ng/ml) between 6 and 30 months after injection that were far below the concentrations produced by the implant (>6 ng/ml) through 15 months. The authors concluded that the inserts provide high fluocinolone concentrations for the first 6 months that reach a lower steady state through 3 years. The higher aqueous fluocinolone concentrations from the implant may result from its relatively anterior scleral fixation point immediately behind the lens, which probably explains the considerably higher incidence of glaucoma.

Because the insert has an extended duration of action and has only recently been approved, very little post-approval data is available. A small retrospective study of 15 eyes with chronic edema unresponsive to other therapies showed improved VA

in 11, stable VA in 2, and decreased VA in 2 after injection of the low-dose insert. Elevated IOP occurred in 2 eyes with one requiring cyclocryodestruction to control the intraocular pressure [73].

The 0.19 mg insert has been approved for the treatment of diffuse DME in 17 countries through the European Repeat-Use application procedure (Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Ireland, Italy, Luxembourg, the Netherlands, Norway, Poland, Portugal, Spain, Sweden, and the United Kingdom) as well as the USA (2014). The package insert specifies that Iluvien is indicated in eyes that do not have steroid-responsive intraocular pressures, but the required method of steroid challenge is not specified. Some investigators propose that a sufficient challenge includes the administration of topical corticosteroids, the peri- or intraocular administration of triamcinolone, or the intravitreal use of the dexamethasone insert.

5.7 Conclusions

Intraocular corticosteroids successfully reduce excess macular thickness in eyes with DME and improve BCVA in most cases. The currently available drugs and devices possess longer mean durations of action than the anti-VEGF drugs, but their use is complicated by higher incidences of cataracts and glaucoma. Because of this less favorable safety profile, corticosteroids are regarded as second-line therapy by most physicians. Considerable work needs to be done to better define the role of steroids as primary, secondary, or combination therapy for DME. The current role of corticosteroids in the management of DME will be discussed further in Chap. 6.

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Chapter 6
Current Treatment Recommendations

6.1 Introduction

The use of the intraocular pharmaceutical agents that were discussed in Chapters 4 and 5 has transformed the treatment of diabetic retinopathy (DR). Before the introduction of potent pharmacotherapy for DR, early diagnosis of DR followed by prompt laser photocoagulation to eyes at risk of vision loss was the most effective way to stabilize vision [23]. Significant improvements in visual acuity (VA) were rarely achieved in patients with established diabetic macular edema (DME), so the primary goal of laser photocoagulation was to prevent further loss. Pars plana vitrectomy for persistent vitreous hemorrhage in eyes with healthy maculae sometimes produced dramatic improvements in VA, but, unfortunately, these were a small minority of patients with diabetes-related vision loss [52].

Administration of intravitreal pharmacotherapy (Fig. 6.1) now gives patients with vision loss due to DR an excellent chance of achieving a clinically meaningful (>5 ETDRS letters) improvement in VA. Effective treatment regimens usually require years of frequent assessments and several administrations of one or more drugs, often in conjunction with laser photocoagulation and surgery [8, 23]. These demanding regimens can be expensive and they challenge even the most compliant patients, but for patients and physicians who adhere to a sensible treatment strategy, the results can be gratifying and life-changing.

The goal of this chapter is to synthesize the results produced by the randomized trials that were presented in previous chapters to propose data-driven – whenever possible – treatment guidelines that can be used in most clinical situations. Some new data will be introduced in this chapter, but most will be summarized from previously referenced trials. Despite the plethora of published manuscripts from the past 10 years that have provided level I and II evidence supporting the treatment of DR, conflicting results challenge certain recommendations, and significant gaps in our knowledge remain. Fortunately, however, our present knowledge enables us to make sound, evidence-based, scientific decisions when treating most patients.

M.W. Stewart, *Diabetic Retinopathy*, DOI 10.1007/978-981-10-3509-8_6

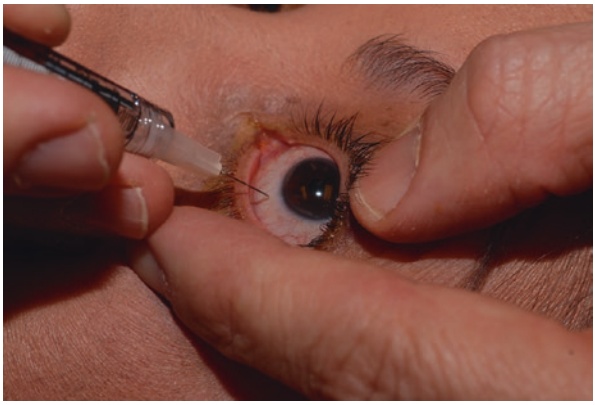

Fig. 6.1 This photograph demonstrates the technique used by the author for an intravitreal injection of a vascular endothelial growth factor inhibitory drug. An eyelid speculum is not used as the surgeon manually retracts the eyelids. Injecting the drug into the vitreous offers the following advantages over systemic administration: 1. Drug is deposited close to the target tissue. 2. The vitreous acts as a slow-release depot that naturally enables extended duration therapy. 3. Systemic exposure to the drug is limited because of the low total dose

6.2 General Medical Care

The first two chapters of this volume detailed the pathophysiology of DR, its important risk factors, and exacerbating conditions. To help patients with their ophthalmic and systemic health, ophthalmologists must remember that DR represents the ocular manifestation of a multisystem disease that includes both microvascular (nephropathy and neuropathy) and macrovascular (cardiovascular and cerebrovascular) conditions. Ophthalmologists have the opportunity to not only maintain and improve visual function but, through counseling of patients and communication with primary care physicians, to improve glucose control, minimize the progression of systemic vascular diseases, reduce morbidity, and prolong life expectancy. Ophthalmologists should develop and maintain close relationships with primary care physicians, internists, and endocrinologists. Important systemic interventions that may prevent or control DR are listed in Table 6.1.

6.2.1 Diabetes Mellitus

The duration over which the patients have had diabetes mellitus (DM) and the average degree of glucose control are the major determinants of both the development and progression of DR [17]. The Diabetes Control and Complications Trial (DCCT) showed that the benefits of "tight" glycemic control on the progression of DR took at least 2 years to become manifest, but once established, these benefits persisted for years [16]. Patients with type 1 DM in the DCCT that received standard diabetes care had average hemoglobin (Hb) A1c concentrations of 9 mg/100 ml, whereas those receiving intensive care had average HbA1c concentrations of 7 mg/100 ml.

Table 6.1 Systemic medical conditions that affect the development and progression of diabetic retinopathy

Systemic medical conditions and diabetic retinopathy	
Study	Key findings
Diabetes mellitus	
UK Prospective Diabetes Study (UKPDS)	Type 1 diabetes mellitus 4209 patients (1977–1999) Intensive versus conventional therapy: 35% reduced progression per A1C point 47% reduction in moderate vision loss
Diabetes Control and Complications Trial (DCCT)	Type 1 diabetes 1441 patients (1983–1993) Intensive versus conventional therapy (HbA1C 7.0 vs. 9.0) 50% reduction in advanced retinopathy Intensive control can temporarily worsen retinopathy in the short term
Systemic arterial hypertension	
Wisconsin Epidemiological Study of Diabetic Retinopathy	Progression of retinopathy was associated with: Higher diastolic blood pressure at baseline Increase in diastolic blood pressure over a 4-year period
UK Prospective Diabetes Study (UKPDS)	Systolic blood pressure <150 mmHg: Decreased the progression of DR Decreased the need for macular laser photocoagulation for DME
EUCLID study	Lisinopril decreased the progression of DR in normotensive type 1 diabetics
DIabetic REtinopathy Candesartan Trials (DIRECT)	In type 1 diabetics, 5 years of treatment: Decreased the incidence of DR Had no effect on progression of established DR In type 2 diabetics: 34% regression of DR ($P = 0.009$) Less severe retinopathy in types 1 and 2 ($P = 0.03$)
RASS trial	Evaluated 285 normotensive patients treated with enalapril, losartan, or placebo for 5 years Progression of retinopathy by 2 steps: Placebo (38%) Enalapril (25%; $P = 0.02$) Losartan (21%; $P = 0.008$) Enalapril and losartan increased the likelihood of less DR progression by 65 and 70% independent of blood pressure lowering
Hyperlipidemia	
Fenofibrate Intervention and Event Lowering in Diabetes Study (FIELD)	Found that fenofibrate: • Decreased the requirement for the first laser and the development of diabetic macular edema • Decreased the need for laser treatment compared to the control group (3.4% vs 4.9%; $P = 0.0002$) • Appeared to have protective effects independent of blood glucose, blood pressure, and baseline lipid values
ACCORD-Eye Study	The addition of fenofibrate to basal statin therapy resulted in: • A decrease in the progression of DR, in a similar manner to that observed with intensifying blood glucose control, but with a good safety profile without increasing the risk of hypoglycemia • Questions regarding fenofibrate's mechanism of action and the pathogenesis of DR/DME

Important studies that demonstrated the effectiveness of key interventions are listed
DR diabetic retinopathy

The UK Prospective Diabetes Study (UKPDS) demonstrated that improved glucose control in patients with type 2 DM significantly decreases the progression of DR [74]. By routinely asking patients about their HbA1c levels, ophthalmologists and their staffs reinforce the notion that glucose control is the most important modifiable risk factor for DR. Though lower HbA1c concentrations can further reduce the risk of DR development and progression, levels below 7 mg/100 ml can be difficult to achieve in patients with type 2 diabetes, are associated with higher frequencies of symptomatic hypoglycemia in patients receiving exogenous insulin, and may be associated with an increased risk of cardiovascular events and mortality [27].

Eyes with vision-threatening DME may benefit from improved glucose control since lowered HbA1c levels can delay or even prevent the need for ophthalmic interventions. The DCCT showed, however, that DME can suddenly and dramatically worsen in some patients when glucose control is improved, so these patients should be monitored carefully when tighter glucose control is being implemented. Patients should be warned that tighter glucose control can worsen DME but that the ocular benefits of tighter control accrue after 2 years.

Recent data suggest that improved control of modifiable systemic risk factors may reverse DME. In the RISE/RIDE trials, 18% of patients in the sham group had visual improvement of at least +15 letters, and 30% of patients in the sham arm never received "rescue" laser [8]. For patients with DME treated with anti-VEGF therapy, the composition of the anti-glycemic regimen (insulin vs. oral hypoglycemic) does not appear to influence the efficacy of the ocular therapy [46].

Prior to the introduction of pharmacotherapy, patients with center-threatening DME and poor glucose control would routinely receive prompt macular photocoagulation [23] because laser could not be counted on to improve VA if the edema spread to the fovea and adversely affected vision. These same eyes can now be observed while glucose control is improving, because expanding edema with worsening VA can probably be reversed by initiating pharmacotherapy.

Diabetic macular edema has been attributed to pioglitazone (Actos) and rosiglitazone (Avandia) [64], but the ACCORD trial failed to find an increase in VA loss in patients receiving thiazolidinediones [70]. Some investigators speculate that DME development due to these drugs is idiosyncratic and that ophthalmologists should consider asking that they be discontinued if a patient develops suspicious DME.

6.2.2 Systemic Arterial Hypertension

Systemic arterial hypertension (SAH) potentiates hyperglycemia-induced damage to capillary endothelial cells and results in blood-retinal barrier breakdown. Adequate blood pressure control (systolic pressure between 130 mmHg and 150 mmHg) decreases the adverse effects of SAH on DR [34, 37, 74], but tighter control may not provide additional advantage [27]. Angiotensin-converting enzyme (ACE) inhibitors should be considered to delay both the onset and progression of DR [24, 39]. Some evidence suggests that ACE inhibitors reduce the likelihood of

developing DR in diabetic patients who are not hypertensive [39]. Losartan and candesartan reduce the progression of DR in diabetic patients with SAH [39, 66].

6.2.3 Hyperlipidemia

Elevated blood lipid concentrations potentiate blood-retinal barrier breakdown and sometimes leads to the accumulation of lipid precipitates in the retina (lipemia retinalis). Though the incidence of lipemia retinalis may be dropping, large lipid deposits within the fovea can damage photoreceptors and irreversibly decrease vision. Lowering serum triglyceride levels with statin drugs decreases vision loss in patients with DR. Some investigators also recommend adding fenofibrate [70] because it further lowers serum lipid concentrations and may decrease vision loss by another unidentified mechanism [33].

6.2.4 Sleep Apnea

Obstructive sleep apnea (OSA) has been associated with DME, but treatment of OSA has not been shown to alter the course of DME. Individual cases of DME resolution after successful treatment of OSA have been reported [29].

6.3 Screening Guidelines

Diabetic retinopathy screening guidelines have been published by the American Academy of Pediatrics (AAP) [2, 44], the American Academy of Ophthalmology (AAO) [1], the American Diabetes Association (ADA) [3], and the Canadian Ophthalmological Society (COS) [10]. Guidelines vary slightly among the organizations, but the overall recommendations are remarkably similar for patients with type 1 DM. The AAP recommends that ophthalmologic examinations begin "3–5 years after DM is diagnosed if the patient is >9 years of age" followed by annual examinations [2]. The AAO Preferred Practice Pattern recommends that ophthalmologic exams begin "3–5 years after DM is diagnosed" followed by annual examinations [4]. The ADA Position Statement recommends the first eye exam "within 3–5 years after diagnosis of diabetes once the patient is 10 years of age or older" followed by yearly examinations [3]. The Canadian Ophthalmological Society recommends that screening for DR should begin "5 years following the diagnosis of diabetes" or at puberty followed by yearly examinations [10]. For patients with type 2 DM, the initial ophthalmic examination should be performed shortly after diagnosis, and this should be followed by yearly exams.

These guidelines are based on data accumulated from several studies, most of which found that DR develops between 8 and 10 years after DM is diagnosed [38, 45, 75]. Some patients, however, develop proliferative diabetic retinopathy (PDR) and peripheral neuropathy within 1–2 years of diagnosis [22, 30] despite excellent glucose control. No cases of PDR have been discovered in the first decade of life, but hormonal changes associated with puberty may increase the risk of developing DR during the second decade [22, 30, 37, 44]. For these reasons, the guidelines generally recommend that screening for patients with type 1 DM begin 3–5 years after diagnosis, once the patient is either 10 years old or has reached puberty – whichever occurs first.

Adherence to DR screening guidelines, unfortunately, is generally very poor. A study of 902 patients with type 1 DM reported that 28% had never received an eye examination and 11% of them were at high risk of vision loss [77]. A study from a tertiary care pediatric clinic reported that only 35% of diabetic children between the ages of 15 and 20 years had been referred for an eye exam [63]. During the first year after screening guidelines for DR were implemented in Australia, no change in practice patterns were noted as only 60% of at risk patients received an eye examination in 2010 [48]. A database claims study pegged the annual ophthalmic examination rate at 34%, with only 16% of patients receiving examinations during consecutive years [51].

Diabetic retinopathy can progress rapidly during pregnancy, presumably due to elevated estrogen levels and the presence of an insulin-like growth factor [36]. Patients who develop gestational diabetes are not at risk of developing DR, but if a patient with diabetic retinopathy becomes pregnant, monthly examinations should be performed until delivery.

6.3.1 Screening Methods

Color fundus photographs remain the gold standard for detecting DR, but routine fundus photos have not been proven necessary if patients receive dilated fundus examinations. Photographic screening programs have become particularly important in developing countries where an insufficient supply of physicians and long travel distances preclude the performance of regular dilated fundus examinations [55]. Telemedicine DR screening programs can be created with modestly priced standard or non-mydriatic fundus cameras, and office personnel can be trained to take high-quality fundus photographs. These programs can generate high-quality photographs that enable readers to rule out vision-threatening retinopathy with a high degree of certainty. Adequate fundus images can be obtained from 85% of patients in rural settings even with the use of non-mydriatic cameras [12]. The value of these screening programs stems from the high DR detection rates – 12 to 29% in some programs.

Recently developed adapters can transform nearly any cellular telephone into a high-quality fundus camera [61]. The low cost of the adapters together with the widespread availability of cellular telephones allows the placement of inexpensive

fundus cameras into nearly any office. With this technology, patients may receive high-quality retinopathy screening evaluations in their primary physicians' offices, after which photos can be electronically transmitted to ophthalmologists' offices or reading centers for evaluation. Patients with high-risk retinopathy can be referred for complete ophthalmology examinations or scheduled for future photographs. Issues regarding Health Insurance Portability and Accountability Act (HIPAA) compliance and insurance billing can be challenging, but systems that address these concerns have been developed.

Some reading centers employ specially trained technicians to grade clinical photographs, whereas others use ophthalmologists and retina specialists. As screening programs expand and include more patients, the number of transmitted photographs may overwhelm the ability of reading centers to properly evaluate them. Computer programs are being developed to efficiently identify abnormal digital photos, make accurate diagnoses, determine the risk of vision loss, and either recommend referral to an ophthalmologist or defer for future screening [32, 76]. Developing and validating such software is a complicated task, and powerful hardware is needed to read thousands of photographs. Software developers in several countries are developing programs that may be commercially available within 5 years.

6.4 Imaging

High-resolution, stereoscopic fundus photography remains the most accurate way to assess the presence and severity of diabetic retinopathy. Diabetic seven-field photographic surveys remain the standard for assessing DR severity in both the macula and mid-periphery (Fig. 6.2), but newly available ultra-widefield photography is easy to perform, can be done through undilated pupils, and often provides excellent views of both the macula and peripheral retina. Stereoscopic macular photographs can identify the degree and extent of macular thickening, but spectral domain

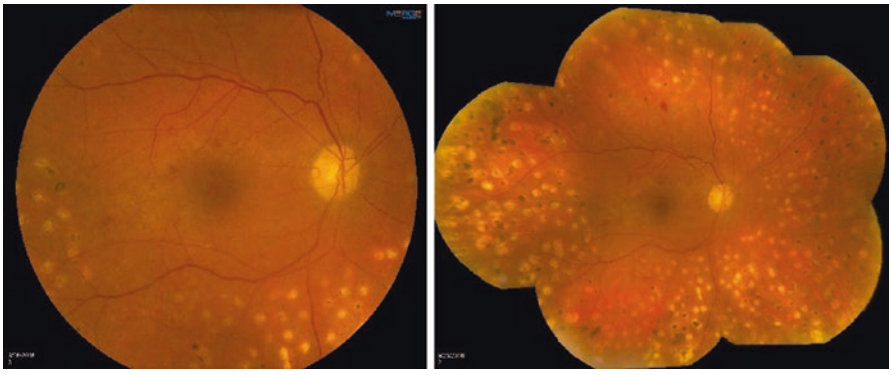

Fig. 6.2 This composite photograph compares a single 50° photograph (*left*) to a seven-field composite (*right*). Note the better view of the mid-peripheral retina with the seven-field composite

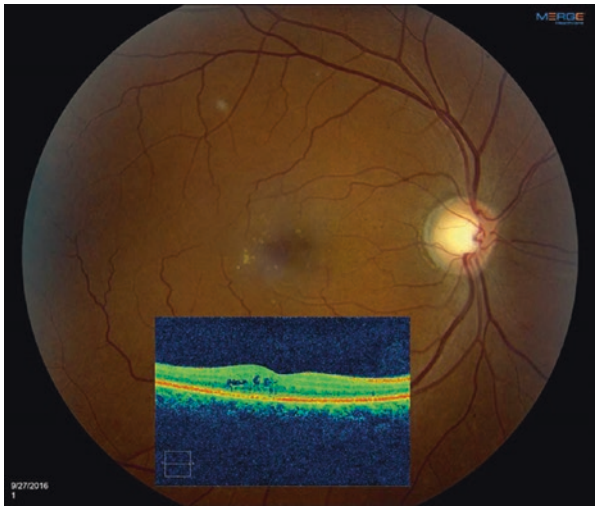

Fig. 6.3 This composite figure shows a color fundus photograph of an eye with diabetic macular edema with a superimposed horizontal optical coherence tomography (OCT) scan through the macula. The photograph shows scattered microaneurysms and hard exudates in the temporal macula, but retinal thickening can only be appreciated with the OCT

optical coherence tomography (SD-OCT) is widely available, is easier to perform, and provides quantitative measurements for serial comparisons.

Fluorescein angiography identifies perfusion defects in the retinal vasculature and helps surgeons plan laser photocoagulation sessions. Laser treatments can be directed at leaking microaneurysms and capillary beds [56], and angiography is necessary if treatment with the NAVILAS system is being planned [41]. Angiography also identifies areas of perifoveal capillary non-perfusion, which should be avoided during macular laser photocoagulation. Identifying macular non-perfusion helps set reasonable expectations for visual recovery. Capillary non-perfusion of the macula is not a contraindication to pharmacotherapy, but visual outcomes in these eyes are usually poorer despite satisfactory resolution of edema.

Persistent retinal neovascularization can cause repeated vitreous hemorrhages even after the placement of what appears to be adequate PRP. Persistent peripheral NV can best be detected with ultra-widefield angiography [35], which can guide the placement of additional PRP.

Spectral domain optical coherence tomography (OCT) imaging of the macula has become indispensable for evaluating DME (Fig. 6.3). OCT produces detailed cross-sectional images with accurate thickness and volume measurements [18]. The pivotal phase III drug trials used OCT imaging to include and exclude patients. Furthermore, OCT measurements are the best way to monitor the effectiveness of treatment and determine the need for retreatment or switching therapy.

OCT angiography (OCTA) is a recently introduced technology that visualizes patent retinal and choroidal blood vessels without the use of an injected dye. OCTA detects perfusion defects in both the superficial and deep retinal capillary plexuses and may ultimately replace fluorescein angiography for evaluating retinal vascular disease.

6.5 Treatment of Diabetic Macular Edema

6.5.1 Non-Center-Involving Diabetic Macular Edema

A decision tree for the treatment of DME is shown in Fig. 6.4.

Eyes with non-center-involving DME usually have excellent visual acuity unless the foveal avascular zone is affected by capillary dropout. When evaluating these eyes, physicians must decide if treatment is indicated or if the eye can be carefully followed. None of the pivotal pharmacotherapy registration trials enrolled patients with non-center-involving DME, so evidence-based treatment guidelines from the pharmacotherapy era are lacking. Before ocular pharmacotherapy became available, preventing foveal thickening and its associated loss of VA was critically important, but now that pharmacotherapy can resolve center-involving edema and reverse mild visual deficits in most cases, many of these eyes can be followed until edema affects the fovea.

Since publication of the ETDRS results, macular laser photocoagulation was performed when eyes developed clinically significant macular edema (CSME) [23]. Clinically significant macular edema no longer dictates the need for treatment in the pharmacotherapy era, but it helps identify eyes with non-center-involving macular edema that threatens the fovea. Eyes with hard exudates within 500 μm of the fovea and adjacent macular thickening, or with an area of macular thickening 1 disc diameter (DD) in size any part of which is within 1 DD of the fovea, are at increased risk

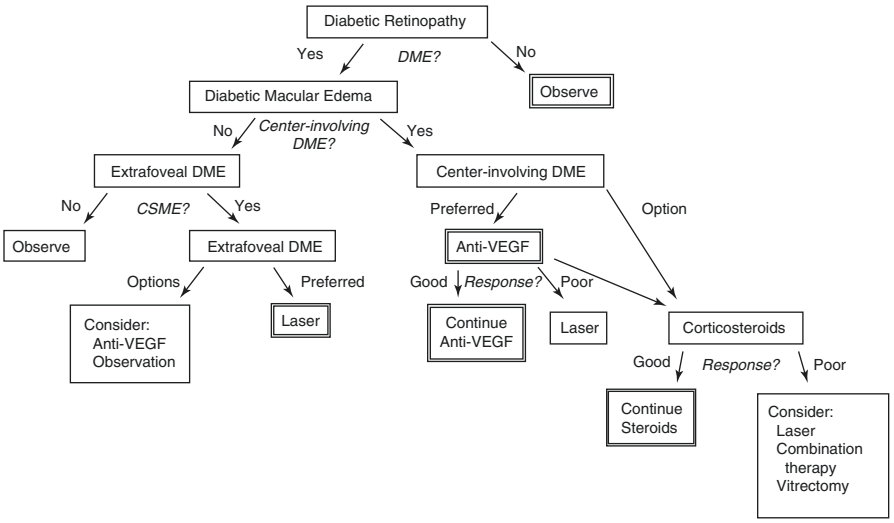

Fig. 6.4 This figure shows a paradigm that can be used to guide treatment for most eyes with diabetic macular edema. Due to the complexity of the disease and the variability of responses, direct application of this paradigm may not be possible in all cases. *DME* diabetic macular edema, *VEGF* vascular endothelial growth factor, *CSME* clinically significant macular edema

of vision loss over the next 3 years and should be watched carefully. When considering treatment of eyes with CSME, physicians should remember that this diagnosis was based on contact lens biomicroscopy and not SD-OCT scanning.

Many physicians perform laser photocoagulation to eyes with non-center-involving CSME to avoid beginning a series of anti-VEGF injections. Low-intensity laser photocoagulation of microaneurysms and areas of capillary leakage [54] (as discussed in Chap. 3) frequently resolves edema and prevents or delays progression to center-involving edema.

6.5.2 Center-Involving Diabetic Macular Edema

The pivotal phase III drug trials demonstrated that anti-VEGF therapy with ranibizumab or aflibercept improves VA and resolves center-involving edema better than laser photocoagulation. Patients enrolled in these trials had best corrected visual acuity (BCVA) measurements that ranged from 20/40 to 20/320 [40, 53], but eyes with center-involving DME and BCVA better than 20/40 have not been rigorously compared to laser in pharmacotherapeutic trials. Therefore, firm treatment guidelines for eyes with center-involving DME but good VA are not yet available. The ongoing DRCR.net Protocol V is comparing laser photocoagulation with intravitreal aflibercept for eyes with BCVA better than 20/32 [73]. Treating these eyes with anti-VEGF injections is a reasonable strategy since this rapidly resolves edema, improves vision, and avoids complications resulting from laser photocoagulation. If we extrapolate from DRCR.net Protocol T, each of the 3 anti-VEGF drugs should work equally well for this set of patients [21]. Focal or grid-pattern laser photocoagulation also remains a reasonable alternative for these eyes, particularly if patients are still asymptomatic. Since the visual acuity in these eyes is already good, laser is being performed primarily to prevent loss of vision.

The RESTORE trial showed that for eyes with CRT <400 μm, visual improvements are the same whether treated with laser or ranibizumab [50]. These results convinced the National Institute for Health and Care Excellence (NICE) of the UK Department of Health to have physicians use ranibizumab for eyes with CST >400 μm but not for those with CST <400 μm [62]. Post hoc analyses of other phase III trials failed to uncover similar results, and surgeons in other countries generally use anti-VEGF drugs as first-line therapy in eyes with CMT between 300 and 400 μm. When caring for a patient with good VA, however, one must remember that treatment should be based on the patient's complaints and wishes, and not solely on the appearance of the OCT.

Eyes with center-involving DME and BCVA of 20/32 or worse most closely resemble those enrolled in the phase III registration trials for ranibizumab and aflibercept [40, 53]. Trials with off-label bevacizumab have provided level II data with which to base clinical decisions [49, 65]. The National Eye Institute-sponsored DRCR.net Protocol T trial showed that for eyes with baseline BCVA of 20/32 to 20/40, each of the anti-VEGF drugs produces VA gains of approximately +8 letters [21]. But for eyes with baseline BCVA of 20/50 or worse, aflibercept (+18.9 letters)

produces greater gains in VA than ranibizumab (+14.2 letters) and bevacizumab (+11.8 letters) at 1 year. Aflibercept also produces greater macular thinning (−169 μm) than either ranibizumab (−147 μm) or bevacizumab (−101 μm). On average, patients received fewer aflibercept (9) than ranibizumab (10) or bevacizumab (10) injections. Unfortunately, Protocol T used a complicated retreatment algorithm that is difficult for most practices to follow. The simplified version of the Protocol T algorithm says that patients should receive 5 monthly injections followed by continued injections until stable. The 2-year data has been presented but not yet published in peer-review journals. Gains in BCVA were maintained for all groups, but for patients with baseline BCVA of 20/50 or worse, the difference between the aflibercept and ranibizumab groups decreased to 2 letters and was no longer statistically significant.

The pivotal phase III anti-VEGF trials evaluated the efficacy of monthly (ranibizumab and aflibercept) or bimonthly (aflibercept) injections on center-involving DME and patients were switched to PRN ranibizumab after 12 months in RESTORE and after 36 months in RISE/RIDE. Aggressive treatment with monthly injections probably produces the best possible visual results (Fig. 6.5), but these regimens are expensive and compliance is difficult to maintain. In contrast, DRCR.net Protocols I and T featured monthly injections for 4 months or until dry, before switching to

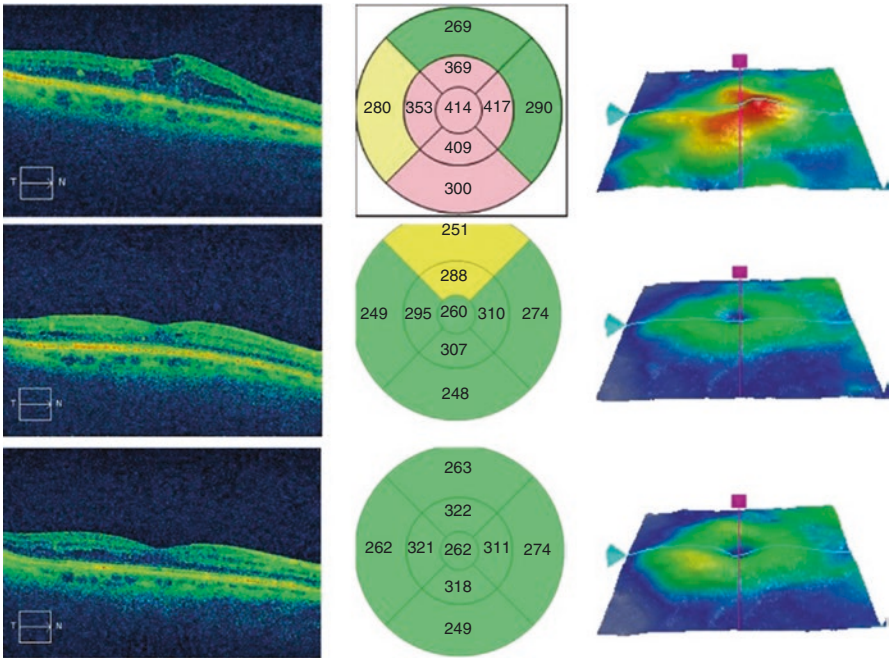

Fig. 6.5 This figure shows the optical coherence tomography scans of an eye with a visual acuity of 20/40 and center-involving DME (*top row*). After 10 monthly injections of ranibizumab, the DME resolved and the visual acuity improved to 20/30 (*middle row*). Examinations were then performed every 4–8 weeks with additional injections given as needed for recurrent edema. During the next 3 years, the patient required only 7 injections to maintain a dry macula and stable visual acuity (*bottom row*)

monthly PRN injections that are based on retreatment criteria that many physicians believe are too complex to use in most clinical settings. Compared to monthly injections, as-needed treatment regimens reduce the number of injections but not the number of clinic visits.

Most surgeons treat DME with a treat-and-extend (T&E) regimen to decrease the number of injections and minimize the number of office visits. The 24-month, single-masked RETAIN trial compared T&E + laser, T&E, and PRN ranibizumab regimens in patients with DME [59]. Upon entering the study, patients in all groups were treated monthly until the maculas were dry, after which injections were given according to the T&E or PRN strategies. Visual acuity improvements in patients receiving T&E + laser, T&E, and PRN were similar (+5.9, +6.1, +6.2 letters). The mean numbers of injections were 12.4, 12.8, and 10.7, but patients treated with T&E required 46% fewer clinic visits compared to PRN. More than 70% of patients had treatment intervals extended to at least 2 months. Though trial design differences make it difficult to directly compare these data to those from the phase III registration trials, the results with T&E are encouraging. A multicenter, randomized trial comparing monthly therapy with T&E is needed, but its high cost will probably prevent it from being organized.

Several issues (compounding and packaging of bevacizumab, use of ETDRS visual acuity, and complex retreatment criteria) challenge surgeons' ability to apply Protocol T strategies to clinical practice. Nonetheless, many physicians treat patients according to the trial's major conclusions: for patients with baseline BCVA of 20/40 or better, each of the drugs performed the same, so surgeons use bevacizumab because of its lower cost; for eyes with VA of 20/50 or worse, surgeons use aflibercept because of its greater efficacy through 1 year. Additional trials are needed to validate these data and identify additional subgroups that may respond particularly well or poorly to treatment.

Visual acuity improvements attributed to the use of corticosteroids for center-involving DME are somewhat more difficult to interpret, and complication rates due to therapy are higher. DRCR.net Protocol I showed that pseudophakic eyes treated with intravitreal triamcinolone and prompt laser had 2-year improvements in BCVA comparable to eyes receiving ranibizumab together with immediate or deferred laser [19]. Protocol amendments allowed patients to cross over to ranibizumab from 1.5 to 3 years after enrollment (depending on the time of randomization), so long-term results attributed exclusively to the use of triamcinolone/laser are not available. Nonetheless, intravitreal triamcinolone remains an inexpensive treatment option for pseudophakic patients (or those scheduled for cataract surgery) who do not have a corticosteroid-induced elevation in intraocular pressure.

The dexamethasone insert (DEX, Ozurdex®, Allergan, Irvine, CA) produced mean BCVA gains of +6 to +7 letters in pseudophakic eyes at 3 years with patients receiving an average of 4.1 injections [5]. Though more than 25% of patients experienced elevations in intraocular pressure (IOP), fewer than 1% required incisional glaucoma surgery. These improvements lagged behind the +9 to +12 letter mean BCVA gains from the phase III anti-VEGF trials, but randomized, multicenter trials directly comparing DEX with anti-VEGF drugs have not been performed. DEX has

been used as first-line therapy for patients desiring a treatment with a longer duration of action than can be achieved with anti-VEGF injections.

6.6 Treatment Failures

Anti-VEGF therapy for center-involving DME usually resolves edema and improves VA, but a significant minority of eyes (approximately 25%) respond suboptimally. Some authors cite data from the pivotal phase III trials to claim that this poor response rate may be as high as 50%, but since nearly half of the eyes from these trials improved by at least +10 letters, this reference to poor responders may be overstated. Nonetheless, the term "treatment failure" is often used to describe suboptimally responsive eyes, even though a uniformly accepted definition of this term does not exist.

A post hoc analysis of VIVID and VISTA showed that 15% of eyes with poor responses (less than +5 letters improvement in VA or <10% improvement in CST) after the first 3 monthly injections experienced below-average improvements throughout the balance of the trial. Their BCVA improvements at the study endpoint averaged only +7.8 letters [6]. These data suggest that poorly responding eyes may be identified after as few as 3 anti-VEGF injections and that perhaps they should be considered for alternate therapies such as corticosteroids. The DRCR.net Protocol U trial is currently comparing 0.3 mg ranibizumab + DEX combination therapy against 0.3 mg ranibizumab monotherapy for the treatment of persistent DME [58].

Before switching strategies or adding a second medication, the surgeon should discuss with the patient whether or not the treatment has been perceived as a success and then temper expectations because changing therapies often does not improve functional results. A significant number of eyes in Protocol I had persistent edema at 1 year despite the fact that BCVA had improved from baseline. Because these patients were stable during the latter half of the first year, they received injections as-needed during the remainder of the trial. Visual acuity in this group remained stable, indicating that improved but incompletely resolved edema may constitute a visually successful and anatomically stable result.

Switching to another drug within the same pharmacologic group or to another group has been proposed for eyes that respond inadequately to initial therapy (Fig. 6.6) [78]. Most drug-switching studies have been retrospective and suffer from several methodologic shortcomings: nonstandardized visual acuity measurements, variable entry criteria, and differing retreatment strategies. In one study, 33 eyes that were refractory to bevacizumab, triamcinolone, and DEX were switched to ranibizumab. Over an average of 48 weeks, mean VA improved from 20/110 to 20/90 and CST improved from 384 μm to 335 μm [13]. In a 1-month prospective trial, 14 eyes that responded poorly to bevacizumab and ranibizumab were switched to aflibercept. The mean CST improved from 421 μm to 325 μm ($P < 0.0132$) [78]. In a retrospective chart review of 21 eyes that were unresponsive to bevacizumab or

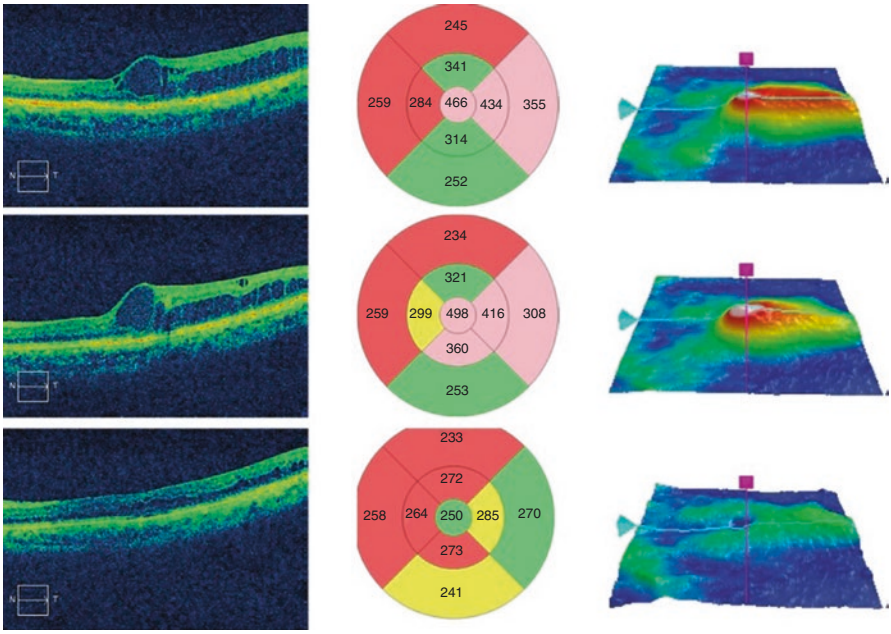

Fig. 6.6 This figure shows the optical coherence tomography scans of an eye with center-involving DME (*top row*) that was treated with intravitreal injections of bevacizumab and aflibercept. Despite a series of 5 monthly injections, the macular edema persisted (*center row*). After 2 injections of the dexamethasone insert, the macular edema had completely resolved (*bottom row*)

ranibizumab, the mean VA at the last follow-up examination (median of 5 months) improved from 0.42 LogMAR to 0.37 LogMAR ($P = 0.04$), and the mean CFT improved from 453 μm to 325 μm ($P < 0.001$) after switching to aflibercept [43].

Some eyes with neovascular age-related macular degeneration that respond poorly to monthly anti-VEGF injections improve when bevacizumab is injected every 2 weeks or is injected every 2 weeks with alternating injections of ranibizumab or aflibercept [68]. This strategy has not been reported for eyes with DME, but the author has seen improvement in a small number of eyes treated in this manner.

The phase III anti-VEGF registration trials allowed the use of macular laser photocoagulation for eyes with persistent edema at 3 months (RISE and RIDE) or 6 months (VIVID and VISTA). Between 20 and 30% of anti-VEGF-treated eyes subsequently received laser, but these trials did not include anti-VEGF monotherapy arms. Data suggest that early laser photocoagulation does not improve VA in eyes receiving ranibizumab and does very little to decrease the anti-VEGF treatment burden. Through 3 years in Protocol I, patients treated with ranibizumab + prompt laser required 3 fewer ranibizumab injections but 3 more laser treatments compared to those receiving ranibizumab and deferred laser [20]. Data from Protocol I and RESTORE also suggest that the addition of macular laser photocoagulation to ranibizumab therapy decreases long-term BCVA improvement by 2–3 letters

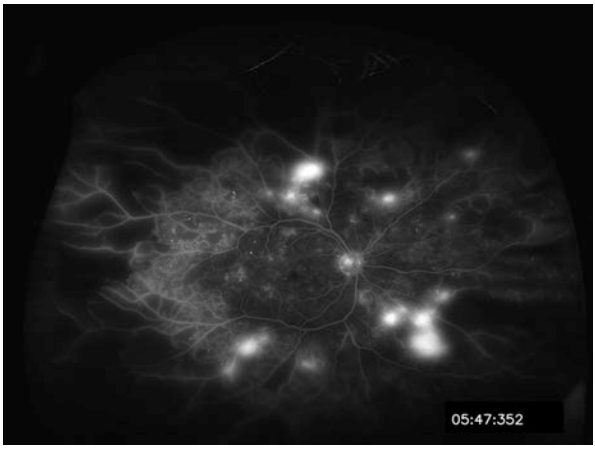

Fig. 6.7 This ultra-widefield fluorescein angiogram frame shows scattered areas of retinal neovascularization and broad areas of peripheral capillary non-perfusion. Targeted scatter laser photocoagulation to these areas has been proposed to treat persistent macular edema and retinal neovascularization but has not yet been demonstrated to be effective for either condition

compared to ranibizumab monotherapy. Nonetheless, macular laser photocoagulation for eyes with persistent edema despite 3–6 months of anti-VEGF therapy remains an accepted treatment option.

Ultra-widefield imaging systems have identified large areas of peripheral retinal non-perfusion in many eyes with DME (Fig. 6.7). Some investigators speculate that ischemia-induced upregulation of VEGF and pro-inflammatory chemokines from these areas contributes to pharmacotherapeutic resistance. Unfortunately, laser photocoagulation to these areas does not reliably improve macular edema. In one study, 52 eyes with DME were randomized to receive intravitreal bevacizumab or bevacizumab + targeted photocoagulation of the peripheral retina. Compared to eyes receiving bevacizumab, those treated with photocoagulation had better stability of the macular edema and improvement in BCVA ($P < 0.05$) [69]. Though results of this study appeared promising, good evidence supporting targeted photocoagulation to areas of peripheral non-perfusion has not yet been accrued. Since peripheral laser carries a low complication rate, treatment may be reasonable in selected patients.

Some investigators have attributed poorer VA results and failure to reduce the anti-VEGF burden to the inadequacies of surgeon-directed laser photocoagulation. Small studies report that navigated laser may not incrementally improve BCVA [42], but it may decrease the need for subsequent anti-VEGF injections [4, 42]. More work with navigated laser and subthreshold micropulse laser needs to be performed before firm treatment recommendations can be made.

Corticosteroids have become increasingly popular as second-line therapy for DME. Posterior subtenon's injections of triamcinolone have been given, but little evidence supports their use in DME. Sustained-release dexamethasone and fluocinolone inserts are more attractive alternatives to intravitreal triamcinolone because they cause less glaucoma. In a small retrospective study, eyes that responded poorly

to 6 injections of ranibizumab experienced improved VA after receiving dexamethasone inserts [81]. Good long-term data regarding the use of these devices as salvage therapy is not yet available, but either device is an accepted treatment for anti-VEGF resistant DME [72]. Data from the FAME trials showed that eyes with chronic DME respond better to fluocinolone than those with more recent DME, perhaps because chemokine synthesis increases as the DME becomes more chronic [15]. This supports the use of corticosteroid inserts in eyes that are resistant to anti-VEGF therapy.

Drug regimens that use combination therapy (anti-VEGFs + corticosteroids) have produced mixed results compared to monotherapy [80]. A prospective, 12-month trial randomized 40 previously treated eyes to receive bevacizumab monotherapy or bevacizumab/dexamethasone insert combination therapy. Visual acuity gains at 6 months were similar in the 2 groups (+5.4 vs. +4.9 letters), but monotherapy eyes achieved less macular thinning (−30 μm vs −45 μm; $P = 0.03$). Patients in the combination therapy group required 3 fewer bevacizumab injections, but this was offset by the need for a mean of 2.1 dexamethasone injections [47]. A study of 25 patients evaluated intravitreal triamcinolone combined with bevacizumab (IVB) in eyes that had been refractory to either as monotherapy [80]. The mean BCVA was 0.8 LogMAR before enrollment, 0.6 LogMAR at 6 months, and 0.6 LogMAR at 12 months ($P = 0.0001$ and $P = 0.003$ compared to baseline). The median CMT was 575 μm at baseline, 370 μm at 6 months, and 410 μm at 12 months ($P = 0.0001$ and $P = 0.0001$ compared to baseline). At 6 months, the BCVA of 13 (52%) patients was stabilized (± 0.2 LogMAR of initial BCVA), and 12 (48%) patients showed significant visual acuity improvement (>0.2 LogMAR improvement from baseline). At 12 months, 10 (40%) patients had stabilized vision, 13 (52%) showed visual acuity improvement, and 2 (8%) had loss of VA. At 6 months, 18 (72%) patients showed anatomic stabilization (a 10–50% decrease from initial CMT), and 7 (28%) demonstrated anatomic success (a decrease in CMT of more than 50% or to ≤250 μm at final visit). At 12 months, 13 (52%) patients showed anatomic stabilization, 10 (40%) had anatomic success, and 2 (8%) demonstrated anatomic failure (a decrease in CMT of less than 10%). The authors concluded that the combined application of IVB and triamcinolone may improve vision and decrease CMT in severe DME cases that have been refractory to previous monotherapies.

Regimens that combine corticosteroids and anti-VEGF drugs are attractive options for eyes that have failed monotherapy. True combination therapy in which DEX is administered every 3 months and an anti-VEGF is administered monthly, regardless of macular thickness, has not been adequately studied but may be considered for eyes that appear resistant to monotherapy.

Pars plana vitrectomy has been used to treat DME for over two decades, but trials directly comparing vitrectomy to anti-VEGF therapy are lacking. In a recent, prospective, short-term trial, 44 patients without vitreomacular traction were randomized to pars plana vitrectomy with internal limiting membrane removal or 3 monthly injections of bevacizumab [60]. The frequency of visual acuity improvements was similar in patients receiving vitrectomy (59.1%) and bevacizumab (72.7%) though vitrectomy resulted in greater resolution of macular edema (−161 μm vs. −108 μm). The study's primary temporal endpoint was at 120 days (60 days after the last bevacizumab injection), which is generally longer than is optimal for anti-VEGF

therapy. The VA remained stable between days 60 and 120, but the CMT increased slightly in the bevacizumab group between days 90 and 120. Seven eyes developed retinal breaks during vitrectomy, but none progressed to retinal detachments.

In the United States, vitrectomy is generally reserved for eyes with DME that have failed pharmacotherapy and laser photocoagulation. The DRCR.net trials showed that eyes with vitreomacular traction improved by a mean of +3 letters after vitrectomy [28], whereas eyes without traction showed no mean improvement in VA [25]. Visual acuity changes varied widely among individuals, meaning that though many eyes achieved excellent improvements in VA, a large number lost considerable VA.

The DRCR.net study enrolled eyes that, in the belief of the investigator, would not respond to other treatments. SD-OCT scanning was not available at the time of the study, so detailed evaluation of the outer retina was not possible. Recent studies have shown that eyes with defects in the external limiting membrane (ELM) and ellipsoid zone (EZ) have less improvement in VA after vitrectomy [11]. Physicians who perform vitrectomy for eyes that have failed other therapies may wish to preoperatively evaluate the integrity of the ELM and EZ lines to set realistic expectations regarding improvements in VA.

Many surgeons in Europe and Asia who use vitrectomy as primary or early therapy for DME contend that surgery is safe, effective, and less costly than anti-VEGF therapy. Since long-term anti-VEGF therapy is expensive, even if bevacizumab is used instead of ranibizumab or aflibercept, early vitrectomy has the potential to become a low-cost approach to the treatment of DME. The multicenter International Consortium Studying Vitrectomy for Diabetic Macular Edema (ICV-DME) [31] is evaluating the efficacy of vitrectomy in eyes with mostly intact ELM and EZ lines. Study proponents hope that results from this study will lead to a larger, randomized trial that directly compares vitrectomy with anti-VEGF therapy.

6.7 Cataract Surgery and DME

Patients with DME frequently have coexisting cataracts that also contribute to vision loss. Even small-incision phacoemulsification cataract extraction upregulates inflammatory molecules that may induce postoperative cystoid macular edema or exacerbate preexisting DME. Diabetic patients without retinopathy or with mild retinopathy and no macular edema can safely undergo cataract extraction without significant risk of postoperative macular edema. Postsurgical administration of topical corticosteroids or nonsteroidal anti-inflammatory drugs is usually sufficient to prevent macular edema.

In patients with foveal-threatening DME or previously treated DME, preoperative injections of anti-VEGF drugs or corticosteroids should be considered. The MEAD trial showed that patients receiving the dexamethasone insert who underwent cataract removal achieved similar improvements in VA as patients who had been pseudophakic at the study's inception [5].

The Pan American Collaborative Retina Study Group retrospectively evaluated the use of no supplemental intravitreal pharmacotherapy, intravitreal bevacizumab, and intravitreal triamcinolone in 138 patients with diabetic retinopathy

who underwent cataract surgery. Mean BCVA (LogMAR) improved from 0.82 at baseline to 0.14 at 6 months ($P < 0.001$) in group 1, from 0.80 to 0.54 ($P < 0.001$) in group 2, and from 1.0 to 0.46 ($P < 0.001$) in group 3. The mean central subfield thickness increased from 263.57 μm at baseline to 274.57 μm at 6 months ($P = 0.088$) in group 1, from 316.02 μm to 339.56 μm ($P = 0.184$) in group 2, and from 259.18 μm to 282.21 μm ($P = 0.044$) in group 3. The authors concluded that cataract surgery may be successfully performed in patients with preexisting DME when treated prophylactically with intravitreal bevacizumab or triamcinolone [26].

In another study of eyes with DR that underwent cataract surgery, the average macular thickness increased by 11% in eyes that did not receive prophylactic intravitreal injections but did not increase in eyes with CSME that received prophylactic bevacizumab [7].

The best approach to cataract surgery in eyes with DR depends upon the preoperative status of the fovea. For eyes without DME, prophylactic pharmacotherapy beyond the customary corticosteroid and nonsteroidal anti-inflammatory drops does not appear necessary. For eyes with DME, preoperative intravitreal injections of an anti-VEGF drug or corticosteroid within 1 week of surgery appear to be prudent.

6.8 Proliferative Diabetic Retinopathy

Recent advances in the treatment of DME have been remarkable, but those applicable to the management of PDR have been more modest. Panretinal photocoagulation remains the most commonly used treatment for PDR, but promising new data show excellent disease control after 2 years of intravitreal ranibizumab [79]. For highly compliant patients who wish to avoid the visual side effects of panretinal photocoagulation, intravitreal ranibizumab therapy appears to be an attractive option. Controlled trials for the treatment of PDR with bevacizumab and aflibercept have not been performed, but most investigators believe that these drugs will produce results comparable to those achieved with ranibizumab. Results with ranibizumab are limited to 2 years, so continued follow-up of these patients is required to show durability. The phase III anti-VEGF DME registration trials showed that the ETDRS severity scores decrease after 2 years of regular injections [8, 9], suggesting that the VEGF synthesis and treatment burden may decrease over time.

Compared with data published by the Diabetic Retinopathy Vitrectomy Study (DRVS), recent results support better visual and anatomic outcomes with vitrectomy and decreased complication rates [71]. Many surgeons now perform vitreoretinal surgery earlier in the course of disease management instead of waiting for the development of more advanced PDR complications [67]. Surgeons should also consider preoperative anti-VEGF injections to decrease neovascularization and minimize intraoperative bleeding. If the eye has significant vitreoretinal traction, however, surgeons must be willing to proceed quickly to vitrectomy if anti-VEGF therapy precipitates the “crunch” syndrome. Further management options for

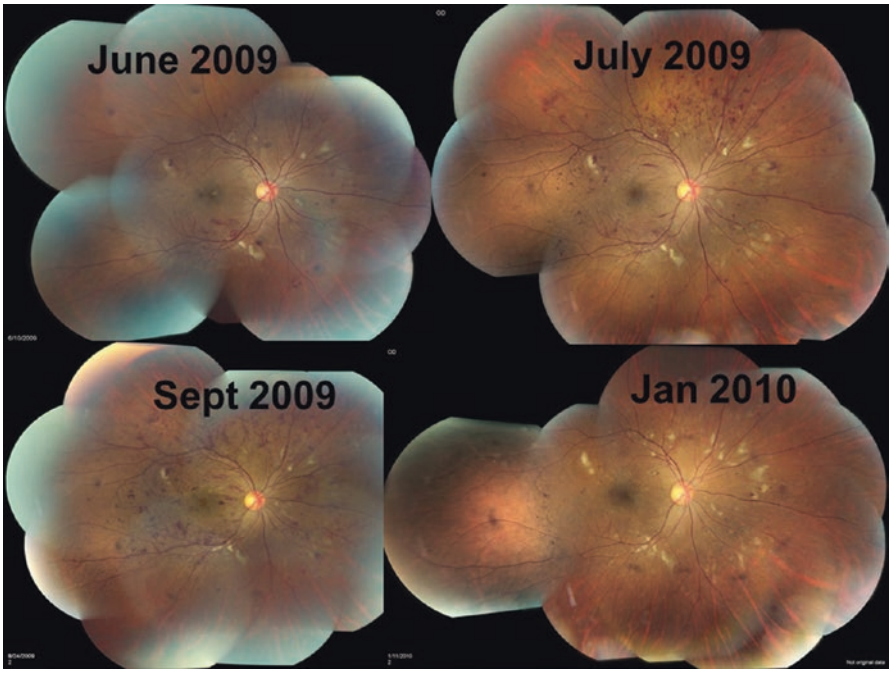

Fig. 6.8 This composite figure shows worsening of diabetic retinopathy during pregnancy. The patient was first examined at 5 months gestation (*upper left*) and was found to have moderate nonproliferative diabetic retinopathy without macular edema. Over the next 3 months (*upper right and lower left*), she developed severe nonproliferative diabetic retinopathy with macular edema appearing at 8 months gestation (*lower left*). The macular edema was not treated at this time, and by 3 months after delivery (*lower right*), the retinopathy improved and the macular edema resolved

established PDR are still needed, and an in-depth discussion of the surgical management of PDR is beyond the scope of this book.

6.9 Pregnancy

The development of DME during pregnancy poses a significant management challenge. Spontaneous miscarriages have followed the intravitreal injections of bevacizumab [57], making pregnancy a high-risk condition for the institution of intravitreal anti-VEGF therapy. Diabetic macular edema in pregnancy can be approached in two ways. Firstly, intravitreal dexamethasone inserts are generally effective and safe without known systemic adverse events [14]. No more than 2 injections per eye would be needed before delivery of the baby, after which all treatment options – including anti-VEGF drugs – become available. Secondly, intraocular pharmacotherapy could be withheld for the balance of the pregnancy and anti-VEGF injections started after delivery (Fig. 6.8). The RESTORE trial showed that delaying

anti-VEGF therapy for up to 1 year after laser does not cause permanent long-term vision loss. Newly developed PDR during pregnancy should be treated with PRP.

6.10 Conclusions

Intravitreal anti-VEGF therapy has become the standard of care for most cases of center-involving diabetic macular edema. Eyes that respond poorly to first-line therapy remain a therapeutic challenge, and consensus recommendations for the treatment of these eyes have not yet been developed.

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Chapter 7
Vitreolysis: Targeting the Vitreoretinal Interface

7.1 Introduction

The vitreous humor is the most abundant ocular tissue, comprising the majority (4 ml) of the eye's volume. Often referred to as the "vitreous gel," a reference to both its consistency and biomechanical composition, the vitreous extends from the posterior lens capsule back to the retina's internal limiting membrane (ILM). For centuries, ophthalmologists believed that the vitreous could not be safely manipulated, altered, or removed without precipitating catastrophic consequences such as retinal tears, retinal detachments, suprachoroidal hemorrhages, or endophthalmitis. In retrospect, it has become obvious that physicians lacked a sufficient understanding of vitreous anatomy and physiology and did not possess the proper technology with which to safely and effectively handle the vitreous [60].

The gel-like consistency of the vitreous impedes the diffusion of substances such as vascular endothelial growth factor out of the retina and the movement of oxygen from anterior structures into the retina. The high viscosity of the vitreous (up to six times that of water) slows the resorption of hemorrhage that may result from insufficiently treated proliferative diabetic retinopathy (PDR), and it impedes the elimination of intravitreally administered drugs, thereby improving the effectiveness and duration of pharmacologic therapy. Trial designers recognize the importance of an intact vitreous since they routinely exclude eyes that have already undergone vitrectomy from participating in intravitreal drug trials.

An intact vitreous provides a scaffold for fibrovascular growth in various proliferative retinopathies. Vitreomacular traction (VMT) due to partial vitreous contraction and collapse without complete separation of the posterior hyaloid from the ILM upregulates VEGF production, promotes the formation of macular edema, and limits the efficacy of intravitreal pharmacotherapy.

In eyes with diabetic retinopathy (DR), the vitreous is viewed as both an enemy that contributes to the disease and as an ally that potentiates treatment by providing a reservoir for drug placement. Successfully changing the vitreous by inducing a vitreous detachment or removing most of it has become an important therapeutic

M.W. Stewart, *Diabetic Retinopathy*, DOI 10.1007/978-981-10-3509-8_7

approach for many retinal and vitreoretinal interface diseases. Pars plana vitrectomy with the removal of VMT has been used for two decades to treat diabetic macular edema (DME) (Chap. 3).

This chapter will discuss pharmacologic approaches to eliminate the adverse effects of VMT on the formation of diabetic retinopathy.

7.2 Vitreous Anatomy

The vitreous comprises about 80% of the ocular volume [82], and its transparent matrix is composed primarily (98–99%) of water [6]. The gel structure is maintained by a complex branching network of collagen fibrils held apart by hyaluronic acid and other macromolecules. Type II collagen is the most common vitreous protein with highest concentrations found at the vitreous base and in the posterior vitreous cortex [41]. The collagen fiber network gives the gel strength and stability, which enables it to absorb shock from blunt trauma and resume its previous shape after being subjected to distorting forces.

Hyaluronic acid, the most common glycosaminoglycan within the vitreous, stabilizes the collagen network [7, 78]. The hyaluronic acid-collagen matrix correctly spaces fibrils to optimize transparency and decrease light scatter, and endows the vitreous with viscoelastic properties [7, 8, 13]. The vitreous is nearly acellular except for a small number of hyalocytes, phagocytic cells found mostly at the vitreous base and posterior pole [4, 8, 78, 94]. Hyalocytes also synthesize collagen fibrils and hyaluronic acid.

The interface between the vitreous and adjacent structures is composed of vitreous cortical fibrils and the basal laminae of the other tissue [30]. Changes at the vitreous base usually result in retinal tears and detachments, but the interface between the posterior hyaloid and macula is more important in patients with DR. The ILM of the retina consists of the footplates or basal laminae of the Müller cells [79] together with types I and IV collagen, proteoglycans, fibronectin, and laminin [36, 47, 52, 53]. It is not clear which of these molecules must be dissolved to facilitate a posterior vitreous detachment, and even “linker molecules” like lectins, integrins, and chondroitin sulfate may ultimately be responsible for the attachment of the vitreous cortex to the ILM. The ILM varies in thickness from 50 μm at the vitreous base to 300 μm at the equator to 1890 μm over the posterior pole before it thins to only 10–20 μm at the fovea [25, 30, 37]. The firmest vitreous adhesions occur where the ILM is thinnest – at the vitreous base and the fovea.

7.3 Posterior Vitreous Detachment

The vitreous both liquefies and aggregates as it ages. Vitreous liquefaction, or syneresis, begins at the age of 4 years, whereas aggregation of fibrils, or synchysis, begins in midlife and continues into old age (Fig. 7.1) [48, 49]. Changes in

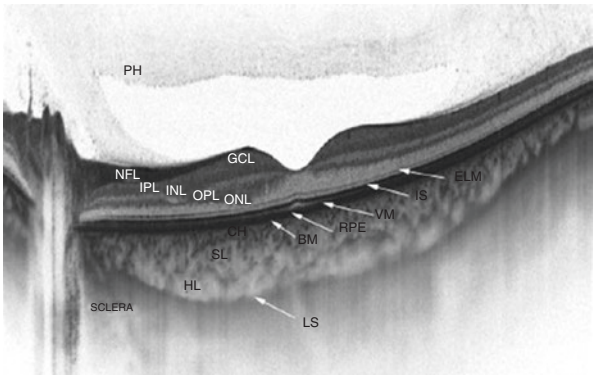

Fig. 7.1 This swept-source optical coherence tomography image shows excellent detail in the vitreous, retina, and choroid. The gel-like consistency of the vitreous appears hyperreflective (*top of image*). A large preretinal bursa (posterior to the "PH") due to liquefaction of the vitreous is present over the macula. *PH* posterior hyaloid, *NFL* nerve fiber layer, *GCL* ganglion cell layer, *IPL* inner plexiform layer, *INL* inner nuclear layer, *OPL* outer plexiform layer, *ONL* outer nuclear layer, *ELM* external limiting membrane, *IS* inner segment/outer segment line, *VM* cone outer segments, *RPE* retinal pigment epithelium, *CH* choriocapillaris, *SL* Sattler's layer, *HL* Haller's layer, *LS* junction between choroid and sclera

hyaluronic acid and its interaction with the collagen fibrils create areas of liquefaction that alternate with areas of increased collagen fiber concentration within the residual gel [45, 59]. Liquefaction in the central vitreous overlying the macula enables incident light to produce free radicals that weaken the residual collagen and decrease the concentrations of glycosaminoglycans and chondroitin sulfate [51]. This promotes posterior hyaloid separation from the ILM (posterior vitreous detachment).

Most posterior vitreous detachments (PVDs) result from a combination of vitreous synchysis and weakening of the adhesions between the ILM and posterior hyaloid. Liquefied premacular vitreous slowly migrates into the potential space between the posterior hyaloid and ILM, and since the adhesions have been weakened, the two surfaces slowly begin to separate, beginning in the peripheral macula (Fig. 7.2). Saccadic eye movements and continued loss of adherent molecules cause further cleavage with progressive enlargement of the preretinal space that marches toward the fovea. A final collapse of the vitreous gel with complete separation of the posterior hyaloid from the fovea and optic disc characterizes a PVD [22, 58, 77].

7.4 Anomalous Posterior Vitreous Detachment

Incomplete separation of the posterior hyaloid because of residual vitreomacular adhesion constitutes an anomalous PVD [84]. If the residual vitreous adhesion causes distortion of the macula by elevating the ILM, this is termed vitreomacular

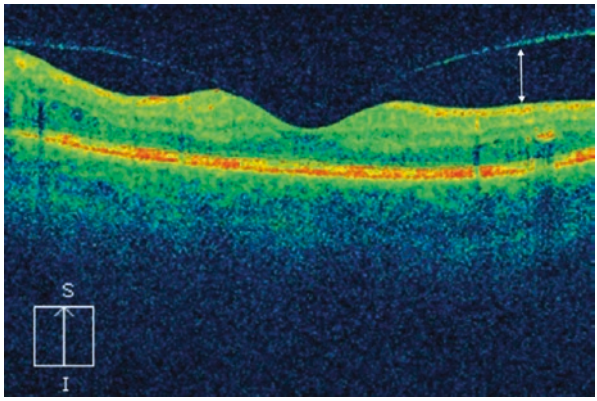

Fig. 7.2 This optical coherence tomography scan shows the age-related separation between the posterior hyaloid and the internal limiting membrane (*white arrow*). This begins in the peripheral macula and moves toward the fovea before the posterior hyaloid completely detaches from the macula

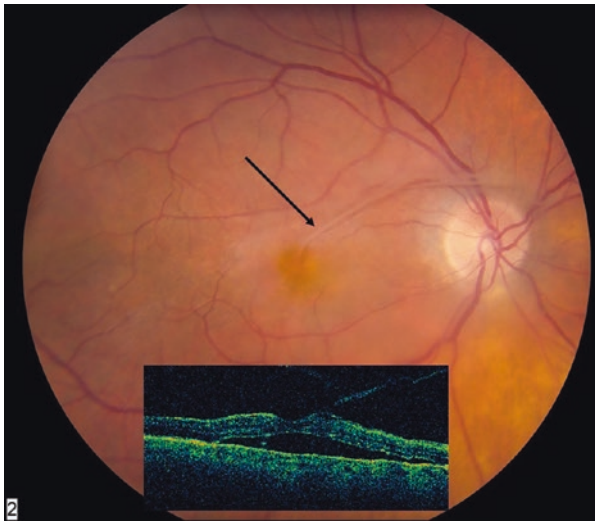

Fig. 7.3 In the color photograph, the taut, opaque posterior hyaloid can be seen just above the fovea (*arrow*). The optical coherence tomography scan (*insert*) shows the anomalous posterior vitreous detachment and the resultant traction macular detachment

traction. Excessive gel liquefaction is seen in many systemic conditions including diabetes [80] and together with strong vitreomacular adhesion may cause symptomatic vitreomacular traction or even macular holes. In patients with diabetic retinopathy, this can cause diabetic macular edema (DME).

Diagnosing an anomalous vitreous detachment can sometimes be done with slit-lamp biomicroscopy and a magnifying fundus lens (Fig. 7.3), but spectral domain optical coherence tomography (SD-OCT) is an easier and more accurate method of visualizing the partially detached posterior hyaloid [31, 54, 75, 97]. Correctly differentiating between a complete PVD and a completely attached posterior hyaloid can be difficult with OCT, and ultrasound is a more accurate tool to discriminate between these two conditions.

7.5 Association Between Posterior Hyaloid and Diabetic Retinopathy

Several studies have investigated the association between integrity of the vitreous and the development and progression of DR. Retinal vessels are normally excluded from the vitreous because development of neovascularization on the retinal surface – proliferative diabetic retinopathy (PVR) – requires a scaffold of collagenous material [21]. Elevated glucose levels in the vitreous induce the formation of advanced glycation end products [85] and promote cross-linking of collagen and the development of excess vitreous liquefaction, without promoting dehiscence of the posterior cortical vitreous. Intravitreal proteins that normally inhibit the development of neovascularization may lose this ability because they undergo nonenzymatic glycation [38, 81]. In eyes with DR, growth factors that are synthesized in the retina diffuse into the vitreous and encourage the development of neovascularization [33, 34, 61]. Oxygen transport from the ciliary body to the inner retina may be impeded, which worsens retinal ischemia and further increases the synthesis of growth factors.

Diabetes-mediated breakdown of the blood-retinal barrier leads to an extracellular accumulation of serum proteins, which increases the concentrations of fibronectin and other chemokines at the vitreoretinal surface by tenfold [53]. These molecules stimulate the migration and adhesion of proliferating vascular endothelial cells [9] and fibroblasts to the posterior hyaloid. Contraction of these cells, in the presence of an anomalous PVD, exerts tangential traction on the retina, further upregulates VEGF, and exacerbates DME [70]. Traction also decreases interstitial pressure, which, according to Starling's Law, promotes passage of fluid out of the vascular lumens [15].

A complete PVD should provide some protection against the development of neovascularization [65, 66], but since islands of residual cortical vitreous remain attached to the ILM, such protection is generally incomplete. Five studies that included over 2000 eyes have looked at the association of PDR and BDR development according to the status of the posterior hyaloid [2, 46, 69, 90, 91]. A pooled analysis of these data found that eyes with complete PVDs had a significantly lower prevalence of PDR (OR 0.1, 95% CI, 0.05–0.18), and the development of a PVD is frequently followed by resolution of DME [42, 100]. Two of the studies found that eyes with partial PVDs had six times the progression rate as those without a PVD and 15 times the progression rate as those with complete PVDs [2, 46, 69, 90, 91].

Removal of the posterior hyaloid may help resolve DME by relieving traction and releasing trapped preretinal growth factors. A liquefied vitreous may accelerate the removal of vascular endothelial growth factor from the inner retina. When surgeons propose treatments for DR that involve the induction of a PVD, they need to remember that a partial PVD may actually worsen the retinopathy.

7.6 Treatment of Vitreomacular Traction

Spectral domain OCT imaging has demonstrated that vitreomacular adhesion (VMA) occurs in the majority of patients over the age of 50 years. Vitreomacular adhesion that distorts the inner retinal contour is termed vitreomacular traction (Fig. 7.4). Macular changes may be limited to loss of the foveal depression, to macular splitting or schisis, or to traction foveal detachment. Patients usually experience decreased central visual function though some may be asymptomatic. The natural history of this process is highly variable, as some eyes achieve complete vitreoretinal separation, others progress to partial or full-thickness macular holes, and a third group remains stable for years.

Creating a complete PVD to relieve an anomalous partial vitreous detachment has been a topic of considerable interest to retinal surgeons over the last 5 years. Surgical intervention for eyes with symptomatic VMT can be done in two ways. Pars plana vitrectomy removes the core vitreous, after which the posterior hyaloid is detached with aspiration. Despite what appears to be complete removal of the posterior cortical vitreous, islands of cortex frequently remain attached to the ILM. These may sequester growth factors and promote fibrovascular growth. Many surgeons perform a dye-assisted peeling of the ILM to remove all vitreous fragments and minimize the chance of postoperative angiogenesis or traction.

Vitrectomy with ILM removal effectively and predictably removes traction but not without associated risks. Many phakic patients develop cataracts within 2 years that require removal. The risks of retinal tears, retinal detachments, and endophthalmitis are relatively low but still of concern. If the adhesive forces between the posterior cortical vitreous and the ILM are sufficiently strong, vitrectomy can convert VME into a full-thickness macular hole.

Pharmacologic vitreolysis involves the use of drugs that liquefy the vitreous (synchysis) and weaken the adhesion between the posterior hyaloid and the ILM [83]. Intravitreal hyaluronidase was used as early as 1946 [72] and collagenase was used in 1973 [67]. Chondroitinase, dispase, plasmin, and tissue plasminogen

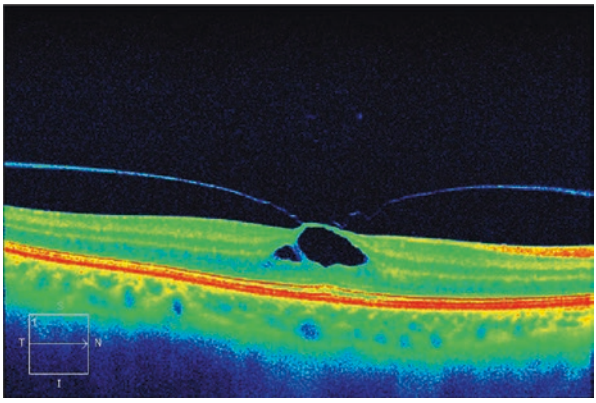

Fig. 7.4 This optical coherence tomography scan shows that the partially detached posterior hyaloid is exerting traction on the inner limiting membrane. The traction causes splitting of the inner retina

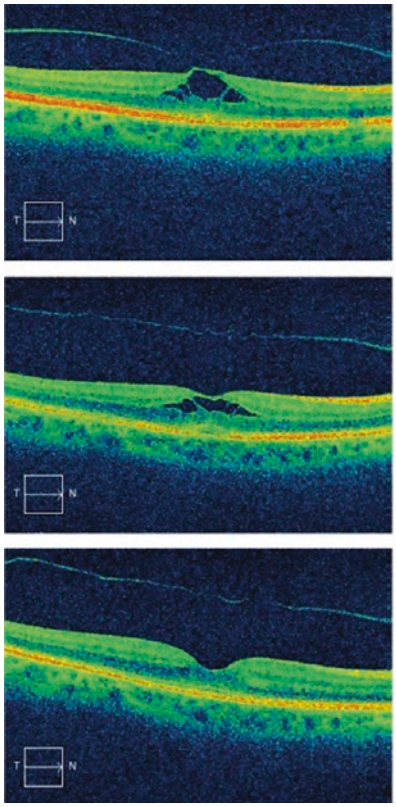

Fig. 7.5 The top optical coherence tomography scan shows vitreomacular traction in a symptomatic patient with visual acuity of 20/25. One month after the intravitreal injection of ocriplasmin (*middle scan*), the contour of the internal limiting membrane has inverted, and the splitting of the inner retina has diminished. One year after the ocriplasmin injection (*bottom scan*), the macula appears normal and the posterior hyaloid has completely separated

activator (tPA) have all been tested in animals and used sporadically in humans [67, 71, 93, 99]. Results with intravitreal hyaluronidase and dispase were disappointing because of insufficient vitreoretinal separation or partial digestion of the inner retina [32, 44, 50, 55, 56, 68, 93]. Only the fibrinolytic enzymes plasmin and tissue plasminogen activator (tPA) showed some success in PVD induction when injected into human eyes [3, 12, 17, 57, 63, 95]. Successful induction of PVDs has been achieved after intravitreal injections of ocriplasmin (Jetrea®, Thrombogenics, Leuven, Belgium) into eyes with symptomatic vitreomacular traction (Fig. 7.5) or stage 2 macular holes (Fig. 7.6). Eyes that responded best to ocriplasmin had the following characteristics: age >65 years of age, vitreomacular adhesion <1500 μm, phakic lens status. and the absence of epiretinal membrane. Macular holes of <400 μm diameter with persistent vitreomacular adhesion also responded well to ocriplasmin.

Many surgeons believe that pharmacologic vitreolysis produces a more complete and less traumatic PVD than does surgery [3]. Vitreolysis trials with ocriplasmin and other pharmacologic agents in patients with DME are underway. Table 7.1 lists the drugs that have been used to induce posterior vitreous detachments in animals and humans.

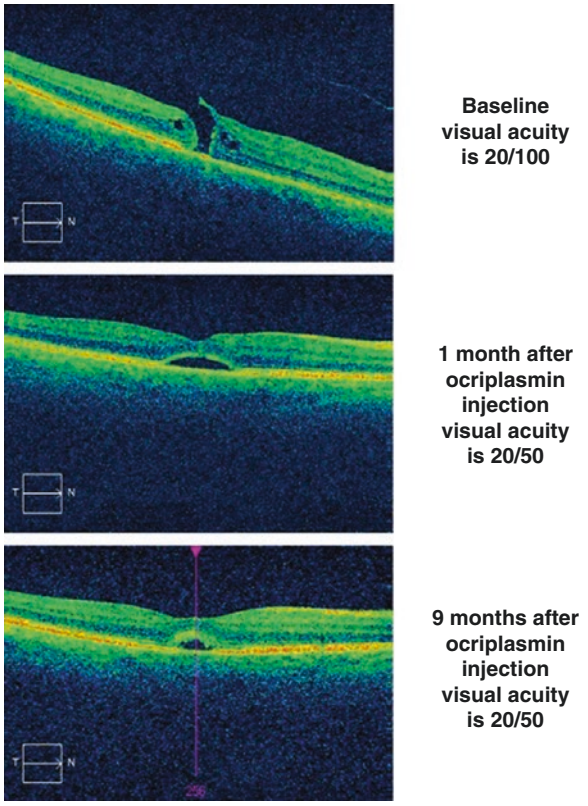

Fig. 7.6 The top scan shows a full-thickness macular hole with persistent traction on an inner retinal flap. One month after the intravitreal injection of ocriplasmin (*middle scan*), the hole has closed, but the photoreceptor layer is still separated from the retinal pigment epithelium (RPE). Nine months after the intravitreal injection of ocriplasmin (*bottom scan*), the photoreceptors have moved closer to the RPE

Table 7.1 The drugs that have been investigated for the induction of posterior vitreous detachment (PVD)

Drugs used to induce posterior vitreous detachments		
Drug	Characteristics	Clinical data
Streptokinase	• Binds to and activates plasminogen • Used to dissolve clots causing myocardial infarctions and pulmonary emboli	• Intravitreal injections used to dissolve post-vitrectomy fibrin • Used to induce PVDs in rabbit eyes • Pilot study showed creation of PVDs in humans
Hyaluronidase	• Cleaves glycosidic bonds of hyaluronic acid • Mimics liquefaction by lowering vitreous viscosity	• Mixed ability to induce PVDs in rabbits • Caused retinal necrosis in rabbits and monkeys
Nattokinase	• Activates plasminogen activator and inactivates an inhibitor	• Induces PVDs in rabbit eyes • Causes retinal hemorrhages and changes in electroretinogram
Chondroitinase	• Degrades chondroitin sulfate and depolymerases hyaluronic acid • Has liquefactant and interfactant properties	• Detached posterior hyaloid in monkeys after 5 to 15 min

Table 7.1 (continued)

Drugs used to induce posterior vitreous detachments		
Drug	Characteristics	Clinical data
Plasmin	• Nonspecific protease from plasminogen activation • No activity against type IV collagen	• In DR pilot study (7 eyes), vitreoretinal interface weakened • PVD induced in 10 of 25 DME eyes with improved VA and CRT • PVD induced in 4 of 12 eyes; most had improved VA
Tissue plasminogen activator (tPA)	• Catalyzes conversion of plasminogen to plasmin	• Induces PVDs in rabbits • PVDs reported in case studies and retrospective series • Prospective, randomized trial failed to show development of PVD
Autologous plasmin enzyme (APE)	• Easier and less expensive to isolate than plasmin	• 85% of eyes found to have PVD 1 h after injection • Compared to controls, decreased DME and improved VA after 1 month
Vitreosolve	• Nonenzymatic agent comprised of urea	• Phase III trial failed to show difference between vitreosolve and controls
Ocriplasmin	• Truncated version of plasmin • Acts on several molecules at vitreoretinal interface	• Phase III MIVT trials, 26.5% with vitreomacular traction achieved PVD • Fewer than 20% of DME eyes achieved vitreoretinal separation in MIVI-II • Phase III trial for DME currently underway

Several drugs have appeared promising, but only ocriplasmin has been fully developed and approved for the treatment of vitreomacular traction. A phase III trial is currently evaluating the efficacy of ocriplasmin in eyes with diabetic macular edema.
DR diabetic retinopathy, *VA* visual acuity, *CRT* central retinal thickness, *DME* diabetic macular edema

7.6.1 Streptokinase

Streptokinase, an enzyme produced by several *Streptococci* sp., binds to and activates human plasminogen. Streptokinase is a fibrinolytic that is used to dissolve clots in patients with acute myocardial infarctions [86] and pulmonary emboli [63]. No adverse effects have been noted after the intravitreal injection of 1000 IU.

Intravitreal streptokinase has been used to dissolve post-vitrectomy fibrin in patients with advanced PDR [11]. Posterior vitreous detachments have been induced in rabbit eyes after the injection of 1500 IU, and only mild toxic effects on the retina have been noted [19]. Pilot studies have been performed to evaluate the efficacy of streptokinase-plasmin injections into human eyes for the induction of PVDs [14].

Though the drug has demonstrated potential for vitreolysis, further studies are required to establish efficacy.

7.6.2 Hyaluronidase

Hyaluronidase cleaves glycosidic bonds of hyaluronic acid and other glycosaminoglycans. This mimics liquefaction by lowering the viscosity of the vitreous. Commercially available hyaluronidase includes a bovine testicular protein enzyme (Wydase®), which has been used in ophthalmology for over 40 years as a spreading or diffusing agent with local anesthetics.

Hyaluronidase has been used in experimental intravitreal models, but its ability to cause a PVD remains unclear. Intravitreal bovine hyaluronidase has produced mixed results in rabbits [35, 44], but retinal necrosis has been noted in both rabbit and monkey eyes [29, 36]. Hyaluronidase has been suggested as a drug to induce a PVD, but since it does not weaken the vitreoretinal interface, there is little evidence that it would work.

7.6.3 Nattokinase

Nattokinase is a 275-amino acid serine protease produced by *Bacillus subtilis* [64]. Nattokinase has potent fibrinolytic activity as a plasminogen activator and by inactivating a plasminogen activator inhibitor, and it hydrolyzes collagen fibrils [88, 89]. Nattokinase induces a PVD in rabbit eyes 30 min after injection but also causes retinal hemorrhage and ERG changes [92].

7.6.4 Chondroitinase

Chondroitinase degrades chondroitin sulfate and depolymerases hyaluronic acid. A low dose failed to induce PVD in pigs but higher doses detached the posterior hyaloid in monkeys within 5 to 15 min [32]. Chondroitinase remains an attractive molecule for vitreolysis because of both its liquefactant and interfactant properties, but much more studies need to be done to determine its clinical efficacy.

7.6.5 Plasmin

Plasmin is a nonspecific protease resulting from plasminogen activation. Plasmin mediates fibrinolysis and also acts on numerous glycoproteins such as laminin and fibronectin. Plasmin is not believed to alter the intact ILM because it has no activity

on type IV collagen. Intravitreal plasmin can create posterior vitreous detachments (dose dependent) in rabbit and pigs [23, 40, 43], and 0.4 IU can induce complete PVDs in postmortem human eyes [24]. Intravitreal autologous plasmin injections have been used as primary or adjunctive therapy for the treatment of pediatric macular holes [62] and advanced diabetic retinopathy [7–9]. It can be prepared in vitro by hydrolyzation with urokinase.

In a pilot study, seven eyes with advanced diabetic retinopathy (six with traction retinal detachment and one with treatment resistant DME) received intravitreal plasmin. The vitreoretinal interface was weakened in all eyes leading to either spontaneous PVD or easier surgical dissection. All eyes achieved resolution of DME without the need for additional laser [99].

In a prospective interventional case study, 25 eyes with clinically significant DME and vitreomacular traction received 0.2 IU/0.2 ml of autologous plasmin. PVD was achieved in ten (41.3%) eyes – complete in six and partial in four. All ten eyes had vitreous separation from the fovea. Visual acuity improved by one Snellen line in four eyes. The mean foveal thickness improved from 480 to 226 μm ($P = 0.05$) [20].

A prospective study evaluated the efficacy of intravitreal plasmin in 12 eyes with SD-OCT-diagnosed VMT that were injected over the course of 4 years. Five eyes were injected once and seven eyes each received three injections. Four eyes (33%) developed PVDs, two after single injections and two after multiple injections. Central macular thickness improved significantly ($P = 0.016$), and seven eyes experienced BCVA improvement of at least one line ($P = 0.017$). The only complication was an immediate elevation in intraocular pressure in one eye [74].

7.6.6 Tissue Plasminogen Activator (tPA)

Tissue plasminogen activator, a serine protease found on the surface of endothelial cells, catalyzes the conversion of plasminogen to plasmin. tPA was first reported to induce a PVD in 1995 [39], and several subsequent reports have described its mechanism of action – plasma activation that degrades the extracellular matrix proteins [25, 43, 96]. PVDs were noted in all rabbit eyes treated with intravitreal tPA, and retrospective series and case studies have reported the induction of PVD following tPA [28]. A prospective, randomized, case-control trial, however, failed to show surgical benefits after the intravitreal injection of 25 μg of tPA 15 min prior to vitrectomy [57].

7.6.7 Autologous Plasmin Enzyme (APE)

Autologous plasmin enzyme is easier and less expensive to isolate than is plasmin and has been used as an adjuvant during vitrectomy [99]. During vitrectomy, 85% of eyes were found to have PVDs after they had received intravitreal APE 1 h prior to surgery [73]. There appears to be a direct correlation between the exposure time

of the posterior hyaloid to plasmin and the development of vitreoretinal separation in pig eyes [26].

The efficacy of 0.2 ml intravitreal APE was tested in eyes with DME that had failed prior laser photocoagulation. Compared to the fellow control eye, intravitreal APE led to decreased macular edema and improved vision 1 month later [18].

7.6.8 Vitreosolve

Vitreosolve (Vitreoretinal Technologies, Inc.) is a nonenzymatic agent comprised of urea. It has been reported to "unravel" collagen to produce both liquefaction and vitreoretinal separation. Phase III trials failed to detect a clinically significant difference in PVD rates between controls and eyes treated with vitreosolve. As a result, subsequent development of vitreosolve has been discontinued.

7.6.9 Ocriplasmin

Microplasmin is a truncated version of plasmin, with retained protease activity and considerably more stability [74]. At doses of 125 μg, microplasmin induces PVDs in pig, cat, and human eyes and leaves a smooth surface on the ILM [15, 25, 76]. Studies in rabbit eyes showed no histologic toxicity and only transient decreases in electroretinographic a- and b-wave amplitudes [25, 98].

Ocriplasmin, a recombinant version of microplasmin, has been approved for the enzymatic lysis of persistent vitreomacular adhesions. Ocriplasmin acts on several molecules within the vitreoretinal interface – laminectin, fibronectin, and type IV collagen [10]. Like other serine proteases, ocriplasmin is highly autolytic and behaves according to a second-order pharmacokinetic profile that results in a very short intravitreal half-life [1, 16]. Vitreous gel liquefaction occurs after injection, but the total effect depends upon the baseline status, since ocriplasmin must diffuse through the gel to achieve optimum effect.

The prospective, uncontrolled MIVI trial reported that 25 ug to 125 ug doses of ocriplasmin induced posterior vitreous separation when given to eyes with VMT prior to vitrectomy [14]. A subsequent phase II dose-ranging trial reported that ocriplasmin induced a PVD in 31% of eyes with VMT or macular holes [5].

In the two randomized, phase III registration trials (MIVI-006 and MIVI-007), patients with symptomatic VMT were randomized to receive ocriplasmin or sham. At the 4-week primary temporal endpoint, 26.5% of ocriplasmin patients achieved a PVD (compared to 10.1% of sham patients; $P < 0.001$), and this did not change with follow-up through 6 months. Of patients with successful VMT resolution, 41.1% achieved BCVA improvements of at least two lines by 6 months. For the subgroup with full-thickness macular holes, the closure rate at 28 days was 40.6%

(compared to 10.6% of sham patients; $P < 0.001$). Results of the phase III registration trials indicate that single intravitreal injections of ocriplasmin are indicated in patients with symptomatic vitreomacular traction and small diameter macular holes with persistent vitreoretinal traction.

Despite the encouraging findings of the MIVI trials, a majority of patients fail to achieve successful PVD with single injections of ocriplasmin. Since a partial PVD may actually increase the risk of PDR development in patients with DR, a strategy to increase the success rate and control complications in those who fail pharmacologic vitreolysis would be needed in these patients. As discussed earlier in this chapter, PVD appears to decrease the incidence and severity of DME so pharmacologic vitreolysis in patients with DR may be beneficial.

Patients with diabetes were excluded from the phase III MIVI-006 and MIVI-007 registration trials, but they were included in other published trials [14, 87]. No DME studies with ocriplasmin have yet been published, and the closest has been the plasmin study discussed previously. Fewer than 20% of eyes with DME that received intravitreal microplasmin in the MIVI-II trial developed PVDs.

7.7 Conclusions

There is no current indication for vitreolysis in eyes with DME, but phase II clinical trials with ocriplasmin are in the planning stages. The area of vitreoretinal adhesion is frequently greater in eyes with DME than in those studied for VMT in the MIVI-006 and MIVI-007 trials perhaps making successful vitreolysis rates with single intravitreal injections more uncertain. Plasmin injections to induce PVDs in eyes with DME appeared promising but plasmin is difficult to prepare. Since ocriplasmin and plasmin have the same enzymatic profile and ocriplasmin should diffuse better (due to its smaller molecular weight), results with ocriplasmin may be as good as those reported with plasmin. Short-term visual acuity results after enzymatic vitreolysis in eyes with DME appear good, but long-term results are still unknown. The MIVI-006 and MIVI-007 prohibited repeated intravitreal injections, but a small number of eyes from the phase II MIVI-IIT trial that failed to respond to single intravitreal microplasmin injections received additional (up to three) injections. Two of seven eyes that ultimately achieved PVD and had no adverse events, such as lens dislocation, due to the repeated injections were reported [87].

A phase II randomized, double-masked, sham-controlled, multicenter study (CIRCLE Trial) will evaluate the efficacy and safety of up to three intravitreal injections of either 0.125 or 0.0625 mg of ocriplasmin in 230 patients with moderately severe to very severe NPDR. The primary endpoint is the proportion of eyes with total PVD by the 3-month visit. Several secondary endpoints will evaluate ocriplasmin's potential for reducing the risk of progression of NPDR to PDR. Preliminary results are expected in late 2017.

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Chapter 8
Investigational Medications

Pharmacologic treatment of diabetic retinopathy (DR) began with the use of intravitreal triamcinolone acetonide and has evolved rapidly over the past 16 years. Dozens of drugs are in the development pipeline and will probably give us several new therapeutic options within the next decade. Development of new pharmacotherapeutic agents is being driven by several factors. Our understanding of the molecular pathways responsible for DR has improved, particularly with the discovery of vascular endothelial growth factor (VEGF) and other cytokines and chemokines. Successful completion of the Human Genome Project has spun off techniques that have enabled the discovery of various genetic abnormalities associated with DR.

The success of intravitreal anti-VEGF and corticosteroid therapy for neovascular age-related macular degeneration (nAMD), diabetic macular edema (DME), and edema due to retinal vein occlusions has transformed the way companies, scientists, and clinicians think about treating these conditions. Development of ocular pharmacotherapies can be financially lucrative as ranibizumb and aflibercept have become the second and third highest reimbursed medications on the Medicare Part B list of reimbursed medications. Clinicians have embraced the high efficacy and favorable safety profiles of intraocular therapy, and they continue to move away from laser photocoagulation. New indications for currently available drugs, such as treatment of diabetic retinopathy in eyes with diabetic macular edema (DME) and treatment of proliferative diabetic retinopathy (PDR), continue to emerge. Patients have accepted intravitreal injections, and compliance with demanding treatment regimens is generally favorable.

Despite the numerous drawbacks associated with long-term intravitreal anti-VEGF use in DR, these drugs are now standard of care for the treatment of most patients. When considering a new therapeutic strategy for DR, one must understand whether the targeted mechanism is independent of VEGF or if it ultimately suppresses VEGF. If the action of a new therapeutic is to primarily impact the VEGF pathway or VEGF levels, then it is unlikely that this treatment will be superior to currently available intravitreal anti-VEGF therapies. This may impact the likelihood of successful drug development since reduction of treatment burden, though attractive to physicians and patients, is often not an acceptable regulatory endpoint

M.W. Stewart, *Diabetic Retinopathy*, DOI 10.1007/978-981-10-3509-8_8

in some areas. Therefore, the new therapeutic must be non-inferior to intravitreal anti-VEGF therapy or result in increased efficacy as an adjuvant therapy.

This chapter will discuss drugs that are in preclinical testing, the early stages of human testing (phase I, II, or III trials) for DR, are likely to be used in patients with DR after they have been successfully tested for other conditions such as nAMD and off-label use of drugs that have already been approved for other indications. The drugs discussed in this chapter fall into several categories: biologics, corticosteroids, and antibiotics, among others. These drugs have not been subjected to multicenter, randomized, controlled, masked trials against standard-of-care therapies and at this time are rarely used outside of laboratory studies and clinical trials.

8.1 Corticosteroid-Related Molecules

Corticosteroid-related drugs that are being investigated for the treatment of diabetic macular edema are listed in Table 8.1.

Table 8.1 Corticosteroid-related drugs that are being evaluated for the treatment of diabetic macular edema are listed, along with their clinical trial status and important biochemical characteristics and study results

Corticosteroid-related drugs being evaluated for the treatment of diabetic macular edema		
Drug	Clinical phase	Important characteristics
Danazol	Phase IIb	• Binds androgens and steroid-binding globulins • Approved for the treatment of endometriosis • In Phase IIa study, oral danazol outperformed placebo in: 1. Decreasing excess retinal thickness ($P = 0.05$) 2. Improving BCVA by 1 category (47% vs. 14%)
Dexamethasone-cyclodextrin Microparticulate Drops	Phase I completed	• Dissolves in tear film to form microparticles • Penetrates to retina in rabbits, anterior chamber in humans • In phase I trial, 19 eyes treated 3 or 6 times per day for 4 weeks and observed for 4 weeks: 1. LogMAR BCVA improved by −0.15 and −0.07 2. CMT improved by −113 μm and −24 μm
Difluprednate (Durezol®)	Phase I completed	Refractory DME after vitrectomy in 11 eyes, difluprednate was compared to subtenon's triamcinolone: 1. 4 times daily for 1 month then twice daily for 2 months 2. Mean improvement in CRT at 3 months of −159 μm 3. No change in BCVA at 3 months

Table 8.1 (continued)

Corticosteroid-related drugs being evaluated for the treatment of diabetic macular edema		
Drug	Clinical phase	Important characteristics
EGP-437	Completed phase Ib/IIa Extension study in progress	• Iontophoresis drug delivery to the retina • Open-label study of 19 patients with DME, RVO, and CME: 1. Drops delivered on days 0, 4, and 9 2. Pseudophakic patients and those with postoperative CME did best
Loteprednol etabonate (KP-121)	Phase II trial underway	• Topically administered • Mucus-penetrating platform (MPP) • QID dosing

BCVA best corrected visual acuity, *LogMAR* logarithm of the minimal angle of resolution, *CMT* central macular thickness, *DME* diabetic macular edema, *RVO* retinal vein occlusion, *CME* cystoid macular edema, *QID* 4 times per day

8.1.1 Danazol

Danazol binds to androgens and steroid-binding globulins, stimulating the formation of a cortical actin ring that enhances endothelial barrier function. Danazol has been approved by the US Food and Drug Administration (US FDA) for the oral treatment of endometriosis.

A randomized, 12-week, placebo-controlled study evaluated the safety and efficacy of twice daily oral danazol in patients with DME. Twenty-three patients with DME and central retinal thickness (CRT) >300 μm were enrolled. The study's primary functional endpoint was change in CRT, and secondary endpoints were changes in macular volume and best corrected visual acuity (BCVA). Compared to placebo treated eyes, those receiving danazol achieved significant decreases in excess CRT (−29% vs. −86%) and macular volume ($P = 0.05$) and modest improvements in BCVA (improvement by 1 category: 14% vs. 47%).

The FDA mandated that Ampio Pharmaceuticals perform a confirmatory study on patients with DME who are refractory to approved medications. Since patients will already have failed to respond to anti-VEGF medications, placebo will serve as the control group. The company projects that 80 patients (40 in each of the danazol and control groups) will be needed for a 12-month study. The FDA did not require that safety be a specified endpoint because danazol would be used at doses only 10% of those approved for use in patients with endometriosis [7].

8.1.2 Dexamethasone-Cyclodextrin Microparticle Drops

A drug delivery platform based on cyclodextrin microparticles that dissolve in the tear film to form water-soluble drug/cyclodextrin complex microparticles has been developed for ocular pharmacology [44]. Microparticulate 1.5% (wt/vol) dexamethasone-cyclodextrin eye drops can deliver the drug to the retina and vitreous

humor in rabbits [63, 80, 81, 122]. Cyclodextrin-based dexamethasone eye drop solutions penetrate well into the anterior segment of the human eye [75, 110]. Since early pharmacology studies in rabbits and humans suggested that dexamethasone-cyclodextrin microparticle eye drops may reach the human retina, clinical trials of dexamethasone eye drops were initiated for the topical treatment of DME.

Nineteen eyes of 19 patients with DME received dexamethasone-cyclodextrin eye drops three or six times a day for 4 weeks and were then observed for 4 weeks without treatment [127]. At weeks 0 (baseline), 4, and 8, logMAR visual acuity (mean ± SD) was 0.52 ± 0.41, 0.37 ± 0.40 (P = 0.0025 vs. baseline), and 0.45 ± 0.41, respectively; central macular thickness (μm) was 512 ± 164, 399 ± 154 (P = 0.0016 vs. baseline), and 488 ± 172 (P = 0.0116 vs. week 4), respectively; and intraocular pressure (mm Hg) was 15.2 ± 3.1, 17.4 ± 4.2 (P = 0.0015 vs. baseline) and 15.8 ± 4.0, respectively. At week 4, central macular thickness had decreased more than 10% in 12 of 19 eyes (63%), and the mean change was −20% (−65% to −10%). In 14 of 19 eyes (74%) visual acuity had improved more than 0.1 logMAR at week 4. No eye drop-related adverse effects were noted.

8.1.3 Difluprednate Ophthalmic Emulsion

The efficacy of topical difluprednate ophthalmic emulsion 0.05% (Durezol®, Sirion Therapeutics Inc., USA) on the treatment of refractory DME after vitrectomy was compared to sub-Tenon's injections of triamcinolone (STTA) [87]. Eleven eyes of ten subjects were treated with STTA (STTA group), and 11 eyes of seven subjects were treated with difluprednate ophthalmic emulsion 0.05% four times daily for the first month and then twice daily for 2 months (eye drop group).

The mean VA (±SD) in the eye drop group was similar at 3 months (0.67 ± 0.29 loMAR) as at baseline (0.67 ± 0.35 logMAR); mean retinal thickness (μm) decreased from baseline (500.6 ± 207.7) to 3 months (341.2 ± 194.8), with a mean minimum retinal thickness during the treatment period (300.6 ± 123.2) that was significantly lower than that at baseline (Mann-Whitney U test: P = 0.003). In the STTA group, mean VA (±SD) was 0.67 ± 0.35 logMAR, and mean retinal thickness was 543.3 ± 132.6 μm at baseline. After 3 months of treatment, mean VA improved to 0.49 ± 0.67 logMAR, and mean retinal thickness had decreased to 378.6 ± 135 μm. The mean minimum retinal thickness during the treatment period (349.9 ± 113.8 μm) was significantly lower than at baseline (Mann-Whitney U test: P = 0.003). The rate of improvement in retinal thickness did not differ between the eye drop group (73%) and STTA group (84%) (Fisher's exact test: P = 1).

8.1.4 EGP-437

Eyegate Pharmaceuticals is using iontophoresis to deliver the experimental drug EGP-437 (reformulated, topically active dexamethasone phosphate) to the retina of patients with DME. A multicenter, open-label, phase Ib/IIa trial has enrolled 19

patients with DME, retinal vein occlusions, and postsurgical cystoid macular edema. Treatments with an electrical impulse of 4.0 mA-min (3.5 mA) were administered on days 0, 4, and 9 with the primary outcome being the reduction in central subfield thickness (CST) on days 4, 9, and 14. The dexamethasone insert was administered to patients who did not respond favorably by day 14.

The interim results showed that some patients, particularly those with postoperative cystoid macular edema (CME) and those who were pseudophakic, responded positively [36]. Edema re-accumulated when the drug was cleared from the tissues. An extension study will include an additional 15 patients who will be dosed on 3 consecutive days.

8.1.5 Loteprednol Etabonate

Kala Pharmaceuticals is developing nanotechonology-based ophthalmic products to treat DME. They are initiating a phase II clinical trial (KPI-121-C-004) to evaluate KP-121 (LE-MPP), a topically administered loteprednol etabonate mucus-penetrating platform (MPP), for the treatment of macular edema due to DR and retinal vein occlusions [82]. This single-masked, randomized trial will investigate the efficacy and safety of 1% LP-MPP and 0.25% LPP administered QID to 20 patients.

8.2 Vascular Endothelial Growth Factor Inhibitors

Vascular endothelial growth factor inhibitor drugs that are being investigated for the treatment of diabetic macular edema are listed in Table 8.2.

Table 8.2 Vascular endothelial growth factor inhibitory drugs that are being evaluated for the treatment of diabetic macular edema are listed, along with their clinical trial status and important biochemical characteristics and study results

Vascular endothelial growth factor inhibitory drugs being evaluated for the treatment of diabetic macular edema		
Drug	Clinical phase	Important characteristics
Abicipar pegol	Phase II	• Designed ankyrin repeat protein • Currently in phase III trial for nAMD • In phase I/II trial of 18 patients with DME: 1. Estimated half-life of 13.4 days 2. BCVA improvement of +10 letters at 12 weeks after single injection
Conbercept	Phase III	• Fusion protein with receptor binding sequences attached to Fc fragment of IgG • Binding affinity of 0.75 pM for $VEGF_{165}$ • Binds VEGF-A, VEGF-B, placental growth factor • Approved for treatment of nAMD in China • In vitro suppression of glucose-induced endothelial cell migration and proliferation

(continued)

Table 8.2 (continued)

Vascular endothelial growth factor inhibitory drugs being evaluated for the treatment of diabetic macular edema		
Drug	Clinical phase	Important characteristics
Encapsulated cell technology (NCT-03)	Phase II nAMD trial terminated early due to poor efficacy	• Immortalized retinal pigment epithelial cells in semipermeable implanted chamber produce aflibercept-like fusion protein • Ciliary neurotrophic factor producing implant failed trials for dry AMD and retinitis pigmentosa. Failed phase II trial for nAMD • Future development is uncertain
Gene therapy (AVA-101)	Completed phase IIa nAMD trial	• Subretinally injected adenovirus DNA for slt-1 (soluble VEGFR1) into retinal pigment epithelial cells • In phase IIa trial with 21 nAMD patients: 1. Ranibizumab injected at baseline, AVA-101 injected at day 7 2. 11 patients received ranibizumab only (control) 3. At 52 weeks, BCVA in AVA (+2.2 letters) vs. ranibizumab (+9.3 letters) 4. Mean center point thickness improved by −27 μm and −85 μm • Future development is uncertain
Implantable drug delivery pump (PMP)	Phase I completed	• Miniature pump delivers drug to retina • Long-term safety seen in animals • Phase I trial of 11 patients with DME treated for 3 months: 1. No cases of endophthalmitis or strabismus
PAN-90806	Phase II trial underway	• Low molecular weight, topical anti-VEGF medication • Excellent drug concentrations in the retina 17 h after administration • Animal studies show CNVM control comparable to antibodies • Phase I/II trial data in 2016 • Phase I proliferative diabetic retinopathy trial underway
Ranibizumab sustained release reservoir	Currently in phase II trial for nAMD	• A refillable port delivery system that is implanted through the pars plana • 1-year phase I nAMD trial of 20 patients found: 1. Mean of 4.8 reinjections 2. BCVA improvement of +10 letters 3. Four implant-related SAEs
RTH258	Currently in phase III trial for nAMD	• High-affinity, single-chain antibody fragment • High injected concentration (6 mg/0.05 ml) produces extended duration of action • Phase II nAMD trial showed comparable efficacy to aflibercept. Extended duration of action suggests q3month dosing
Ziv-aflibercept	Off-label use in DME	• Intravenous formulation of aflibercept indicated for treatment of advanced solid tumors • Two DME patients had significant improvements in BCVA and macular thickness • Ongoing off-label treatment continues

nAMD neovascular age-related macular degeneration, *DME* diabetic macular edema, *BCVA* best corrected visual acuity, *VEGF* vascular endothelial growth factor, *CNVM* choroidal neovascular growth factor, *SAEs* serious adverse events

8.2.1 Abicipar Pegol

DARPins (*d*esigned *a*nkyrin *r*epeat *p*roteins) are small molecular weight (14–20 kDa) molecules with high solubility (>100 mg/L) in saline. They are a flexible design platform that allows for the creation of genetically engineered mimetic proteins that can target any molecule [17]. Several DARPin molecules (at least 15) are being developed for chorioretinal vascular conditions, oncologic indications, and inflammatory diseases. DARPin technology also allows for the design of dual action proteins.

Abicipar pegol (Allergan, Irvine, CA), formerly known as DARPin MP0112, binds all isoforms of VEGF-A. This high-affinity molecule ($K_D = 2$ pM for $VEGF_{165}$) has a surprisingly long intravitreal half-life in rabbits (6 days) that may be attributed to its pegylation. A phase I/II multicenter, open-label, dose-escalation trial evaluated the safety and bioactivity of abicipar in 18 patients with DME [21]. Patients receiving 1 mg injections experienced excellent reductions in macular thickness and mean improvement in VA (+10 letters) 12 weeks after single injections. Pharmacokinetic analyses based on anterior chamber drug concentrations suggest an extended intraocular half-life of 13.4 days. Multicenter, randomized, double-masked, phase III nAMD trials (CEDAR and SEQUOIA) are comparing q8wk and q12wk abicipar with q4wk ranibizumab. Given abicipar's high binding affinity, apparently long intraocular half-life, and encouraging results from the early phase trials, the developers are hoping to establish the efficacy of 3-month dosing. Phase III DME trials are being planned but have not yet begun.

8.2.2 Conbercept

Conbercept (KH902, Chengdu Kanghong Biotech Co., Sichuan, China) is a recombinant, fusion protein that, like aflibercept, acts as a decoy receptor. Conbercept (MW of 143 kDa) contains the second immunoglobulin (Ig)-binding domain from VEGF receptor 1 (VEGFR1), the third and the fourth binding domains from VEGFR2, and the Fc region of human IgG. The difference between aflibercept and conbercept is that aflibercept does not contain the fourth domain of VEGFR2 [53, 141]. Conbercept has a high affinity for VEGF because the fourth Ig domain of VEGFR2 is essential for receptor dimerization and it enhances the association rate of VEGF to the receptor [141]. Like aflibercept, conbercept binds all isoforms of VEGF-A, VEGF-B, and placental growth factor. At concentrations from 100 ng/ml to 100 μg/ml, conbercept was not cytotoxic to cultured human retinal endothelial cells (HRECs). A 500 ng/ml solution of conbercept significantly suppresses high glucose-induced migration and sprouting of HRECs by downregulating the expression of PI3K and inhibiting the activation of Src, Akt1, and Erk1/2 [25].

Four weeks after intravitreal injection, conbercept-treated rats had better retinal electrophysiological function, less retinal vessel leakage, and lower levels of PlGF, VEGFR2, PI3K, AKT, p-AKT, p-ERK, and p-SRC than PBS or Avastin-treated rats [58]. The distribution of claudin-5 and occludin in the retinal vessels of diabetic rats

treated with conbercept was smoother and more uniform than those of diabetic rats treated by PBS or Avastin. Conbercept has already been approved in China for the treatment of nAMD [79], and a phase III trial evaluating the efficacy of conbercept for the treatment of DME is currently enrolling patients.

8.2.3 Encapsulated Cell Technology

Encapsulated cell technology (ECT) was first reported by Bisceglie (1934) as a way to prevent rejection of foreign cell, tissues, or organisms. ECT involves the use of immortalized cells that have been programmed to overproduce a specified biochemical product. The cells are grown in a cylinder lined by semipermeable membranes that allow ingress of nutrients and egress of the synthesized product. The membrane prevents migration of the modified cells and shields them from the body's immune system. The cylinder is 10 mm long and is surgically implanted through the pars plana and sutured to the sclera.

Clinical studies using ECT to produce ciliary neurotrophic factor (CNF) have been completed in eyes with retinitis pigmentosa and atrophic AMD [70]. The ECT cylinder was well tolerated, but the trials failed to meet their primary therapeutic endpoints. Pharmacokinetic analyses showed that the half-life of CNF production by the cylinder was 54 months. Phase I trials with a cylinder that produced a high-affinity VEGF-binding protein similar to aflibercept have been performed, and a multicenter phase II trial [28] failed to meet its primary efficacy endpoint, thereby calling into question future development and use in patients with DME.

8.2.4 Gene Therapy

Avalanche Biotechnologies has developed a viral delivery system (AVA-101) to enable the eye to produce long-term anti-VEGF therapy. An adenovirus vector inserts the DNA for a naturally occurring slt-1 (soluble VEGF receptor-1) into retinal pigment epithelial cells. Infected cells synthesize and excrete the soluble VEGF inhibitory protein into the outer retina and choriocapillaris.

In a phase IIa trial, 21 patients with nAMD received AVA-101, with 0.5 mg ranibizumab injected both at baseline and 1 month and again as rescue therapy. Patients underwent core vitrectomy and subretinal injection of AVA-101 adjacent to the macula at day 7. Patients were evaluated monthly and were eligible for rescue ranibizumab therapy based on prespecified criteria. Eleven control patients received only 0.5 mg ranibizumab monthly.

At the 52-week endpoint, mean improvement in BCVA was +2.2 letters in the AVA-101 group compared to +9.3 letters in the ranibizumab group [51]. These differences were statistically significant, but they were largely driven by three subjects in the AVA-101 group who each lost at least four lines of vision. Mean center point

thickness improved by −27 μm in the AVA-101 group and −85 μm in the control group. There were no serious ocular adverse events in the AVA-101 group, and no systemic safety signals were noted. All patients in the AVA-101 group who were phakic at baseline developed cataracts, and three (14%) developed moderate vitreous hemorrhages. Gene therapy was well tolerated by patients, but the technology failed to provide a complete or durable anti-VEGF response.

8.2.5 Implantable Drug Delivery Pump (PMP)

Microelectromechanical system (MEMS) technology is a miniaturized system that is currently used in insulin pumps to deliver drug to the tissues. The Posterior MicroPump Drug Delivery System (PMP, Replenish Inc., Pasadena, CA) uses MEMS technology to deliver drug within the eye. Long-term safety after implantation into animal eyes has been demonstrated [47, 111]. The PMP can reliably deliver 100 programmed doses of an anti-VEGF drug, equivalent to over 8 years of therapy. The PMP was evaluated for 3 months in 11 patients with DME [59]. After episcleral implantation, similar to placement of a glaucoma drainage device, the PMP was well tolerated with no cases of endophthalmitis or strabismus.

8.2.6 PAN-90806

PanOptica, Inc. is developing a topical anti-VEGF medication for the treatment of nAMD and PDR. PAN-90806 is a low molecular weight, VEGF receptor blocker administered in eye drop form. Pharmacokinetic studies show excellent drug concentrations in the central retina and choroid as late as 17 h after administration. Animal studies are reported to show control of leakage and bleeding from choroidal neovascular membranes, comparable to that achievable with intravitreal anti-VEGF antibodies, with minimal systemic exposure to the drug. Preliminary results from each of four monotherapy treatment arms in a phase I/II trial were judged by an independent panel of experts to show promise for the treatment of nAMD [94]. Results from a phase II trial of PAN-90806 maintenance therapy after a single anti-VEGF injection are expected to be presented in 2017. The company is moving forward with a phase I trial for the treatment of PDR.

8.2.7 Ranibizumab Sustained Release Reservoir

A refillable ranibizumab port delivery system is being codeveloped by Genentech and ForSight Vision 4 to reduce the need for repeated intravitreal anti-VEGF injections. The pre-loaded implant is surgically implanted beneath the conjunctiva through a 3.2 mm scleral incision over the pars plana. The reservoir tip can be

accessed easily through the conjunctiva and refilled in the office as needed. The device continuously releases ranibizumab into the vitreous between refills.

A phase I trial for patients with nAMD was performed in Riga, Latvia [105]. At baseline, the reservoir was implanted, and eyes were given 500 μg ranibizumab injections, 250 μg into the vitreous and 250 μg into the reservoir for sustained release. Additional injections were given based on optical coherence tomography (OCT) evaluation of disease activity. The primary endpoint was 12 months with an observation period that extended through 36 months. The primary objective of the study was safety assessment with secondary objectives that included functional measurements.

Four of the patients had significant or serious adverse events (endophthalmitis, vitreous hemorrhage (2), and traumatic cataract), but 3 of these 4 had improved vision by the study's endpoint. The average visual acuity gains for the cohort were +10 letters; 10 eyes (50%) gained at least 3 lines and 2 (10%) lost at least 3 lines. The mean number of refills was 4.8 per patient.

The planned phase II trial will feature 750 μg injections, with hopes of extending the treatment interval to 4 months.

8.2.8 RTH258

RTH258 (formerly known as ESBA 1008) is a single-chain, VEGF-binding antibody fragment currently being developed by Alcon (Ft. Worth, TX) for the treatment of nAMD. It has been touted by its developer as having a longer duration of action than currently available anti-VEGF drugs, thereby requiring fewer injections.

A phase II clinical trial compared RTH258 to aflibercept in patients with nAMD [92]. The trial's primary objective was to compare the efficacy of 6 mg RTH258 against 2 mg aflibercept with the primary endpoint of the study being the mean change in BCVA from baseline to 12 weeks. Secondary endpoints included improvement in central subfield foveal thickness (CSFT) on SD-OCT. Preliminary reports stated that RTH258 produced BCVA gains that were non-inferior to aflibercept with a greater reduction in macular fluid. Patients treated every 3 months experienced a positive effect, suggesting a long duration of action. No new safety signal was seen.

The phase III clinical trial program was initiated in December 2014, with an enrollment goal of 1700 patients in more than 50 countries. These 2-year, double-masked, multicenter trials will randomize patients with untreated nAMD to one of two dosage levels of RTH258 or 2 mg aflibercept bimonthly [29]. The primary endpoint will be changed in BCVA at 48 weeks with several additional secondary functional and morphologic endpoints. No DME trials have yet been announced.

8.2.9 Ziv-Aflibercept

Ziv-aflibercept (Zaltrap®, Regeneron, Tarrytown, NY) is the systemic formulation of Eylea® that is indicated for the intravenous treatment of advanced colorectal carcinoma. Single use vials contain 4 ml (25 mg/ml) of

ziv-aflibercept in a buffered solution of polysorbate 20 (0.1%), sodium chloride (100 mM), sodium citrate (5 mM), sodium phosphate (5 mM), and sucrose (20%), with a pH of 6.2. Small series of patients with nAMD that received single injections of ziv-aflibercept had anatomic and visual acuity improvements at 1 month without evidence of toxicity [26]. Two patients with DME had improved VA (20/800 to 20/100; 20/800 to 20/200) and macular thickness (CST: −65 μm and −352 μm) 1 week after intravitreal injections [84]. Additional studies continue to evaluate the use of ziv-aflibercept for nAMD, DME, and retinal vein occlusions.

8.3 Tumor Necrosis Factor-α Inhibitors

Tumor necrosis factor (TNF)-α is a pro-inflammatory cytokine that is synthesized by T-lymphocytes, macrophages, neutrophils, and mast cells. It plays an important role in mediating the immune response, tumorigenesis, and inhibiting viral replication. TNF-α is upregulated in eyes with uveitis, nAMD, and diabetic retinopathy [130]. Several anti-TNF-α biologicals have been approved for the treatment of systemic inflammatory conditions including rheumatoid arthritis [97]. In animal models, TNF-α has been shown to contribute to the development of DR [46, 54, 66], and TNF-α inhibition limits breakdown of the blood-retinal barrier [67]. It is also possible that high-dose NSAIDs delay the onset of diabetic retinopathy via TNF-α suppression [107].

Tumor necrosis factor inhibitor drugs that are being investigated for the treatment of diabetic macular edema are listed in Table 8.3.

Table 8.3 Tumor necrosis factor inhibitory drugs that are being evaluated for the treatment of diabetic macular edema are listed, along with their clinical trial status and important biochemical characteristics and study results

Tumor necrosis factor inhibitory drugs being evaluated for the treatment of diabetic macular edema		
Drug	Clinical phase	Important characteristics
Adalimumab	Off-label use	• Was not effective when injected into five eyes with DME • When injected into seven eyes with DME, positive results were seen only when combined with bevacizumab
Etanercept	Phase I trial completed	• Prevents TNF-α binding to transmembrane receptor • Two injections, 2 weeks apart given to seven eyes with DME yielded no clinical response
Infliximab	Phase IIa trial completed	• 15 patients with DME received single 1.0 mg injections and 19 received 2.0 mg injections. 42% developed uveitis • In a double-blind, randomized, placebo-controlled study of patients with persistent DME, patients receiving infliximab had improved BCVA and retinal thickness
Pegsunercept	Preclinical	• Injections into rats led to reduction in pericyte loss and capillary degeneration

DME diabetic macular edema, *TNF* tumor necrosis factor, *BCVA* best corrected visual acuity

8.3.1 Adalimumab

Intravitreal injections of 2.0 mg adalimumab were ineffective in five eyes with DME that had been refractive to anti-VEGF therapy. No adverse side effects were noted in any of these eyes [136]. In a series of seven eyes of five patients with macular edema from various causes, favorable clinical responses were noted when adalimumab was combined with bevacizumab [9].

8.3.2 Etanercept

Etanercept is a soluble TNF-α receptor that acts as a competitive inhibitor to block TNF-α binding to transmembrane receptors. It reduces leukocyte adherence in retinal blood vessels [67], blood-retinal barrier breakdown, and NF-κB activation in the diabetic retina [66]. Two injections of etanercept (2.5 mg) were performed 2 weeks apart to seven eyes with refractory DME, but no clinical responses were noted at 3 months [131].

8.3.3 Infliximab

Visual acuity changes from baseline to 3 months in 15 patients with DME receiving single injections of 1.0 mg infliximab (1.49 LogMAR to 1.38 LogMAR), and 19 patients receiving 2.0 mg infliximab (0.76 LogMAR to 1.03 LogMAR) were disappointing. Furthermore, 42% of eyes developed severe uveitis with 37% requiring vitrectomy, thereby halting further research with intravitreal infliximab [136].

A double-blind, randomized, placebo-controlled, crossover study in patients with DME that had persisted after two sessions of laser photocoagulation showed that patients receiving intravenous infliximab (5 mg) had significantly improved visual acuity and reduced retinal thickness [118].

8.3.4 Pegsunercept

Intravitreal injection of the TNF-α-specific inhibitor, pegsunercept, led to a significant reduction in pericyte loss and capillary degeneration in diabetic rats [14, 15].

8.4 Nonsteroidal Anti-inflammatories

Nonsteroidal anti-inflammatory drugs that are being investigated for the treatment of diabetic macular edema are listed in Table 8.4.

Table 8.4 Nonsteroidal anti-inflammatory drugs that are being evaluated for the treatment of diabetic macular edema are listed, along with their clinical trial status and important biochemical characteristics and study results

Nonsteroidal inflammatory drugs being evaluated for the treatment of diabetic macular edema		
Drug	Clinical phase	Important characteristics
Aspirin	Phase II completed	• Low-dose aspirin provides little to no benefit in preventing diabetic retinopathy
Diclofenac	Phase IIa completed	• 57 patients with treatment naïve DME received single injections of diclofenac or bevacizumab: 1. Diclofenac patients had better improvements in BCVA: −0.08 LogMAR vs. +0.04 LogMAR 2. Bevacizumab improved edema better

DME diabetic macular edema, *BCVA* best corrected visual acuity, *LogMAR* logarithm of the minimum angle of resolution

8.4.1 Aspirin

In clinical studies, low-dose aspirin has shown only little or no benefit in preventing diabetic retinopathy [93]. However, further work is still needed to determine if high-dose aspirin can prevent the development of diabetic retinopathy.

8.4.2 Diclofenac

In a randomized trial, 57 eyes with treatment naïve DME received single intravitreal injections of either diclofenac (500 μg/0.1 ml) or bevacizumab. The primary outcome was the change in mean BCVA at 12 weeks, and secondary outcomes included changes in macular thickness, macular leakage, and safety. Eyes receiving diclofenac had better mean improvements in BCVA compared to bevacizumab (Δ −0.08 LogMAR vs. Δ +0.04 LogMAR, $P = 0.033$), but bevacizumab improved macular edema slightly better [124].

8.5 Other

Drugs that do not fit into the other listed categories that are being investigated for the treatment of diabetic macular edema are listed in Table 8.5.

8.5.1 Adenosine Kinase Inhibitor

Adenosine is centrally involved in the signaling cascade that regulates anti-inflammatory actions, angiogenesis, the oxygen supply/demand ratio, and ischemic pre- and post-conditioning [65]. Under these circumstances, the local levels of

Table 8.5 Nonsteroidal anti-inflammatory drugs that are being evaluated for the treatment of diabetic macular edema are listed, along with their clinical trial status and important biochemical characteristics and study results

Drugs not in the previously identified categories being evaluated for the treatment of diabetic macular edema		
Drug	Clinical phase	Important characteristics
Adenosine kinase inhibitor (ABT-702)	Preclinical	• Adenosine helps regulate anti-inflammatory actions, angiogenesis, and oxygen supply and demand • Adenosine is a major source of stored energy (ATP) • Intraperitoneal adenosine in rats decreased signs of inflammation in experimental diabetes
Angiopoietin-2 inhibition	Phase II Trial Underway (AVENUE)	• Competes with Ang-1 for Tie2 receptor • Bi-specific antibody (VEGF and Ang2) currently being studied
Antioxidants	Phase II Trials Completed	• Calcium dobesilate has been studied in several trials • Failed in most trials to prevent the development of macular edema
ASP8232	Phase II trial underway	• Inhibitor of vascular adhesion protein-1 • VIDI trial is evaluating ASP8232 monotherapy and in combination with ranibizumab
Darapladib	Phase II trial underway	• Inhibits lipoprotein-associated phospholipase CA2 • Protects against atherogenesis and vascular leakage in animal models
Epalrastat	Phase II trial completed	• Inhibits production of protein kinase C • Prevented progression of early retinopathy and neuropathy
Fasudil	Phase I trial completed	• Rho-kinase inhibitor used to treat cerebral vasospasm • Can suppress leukocyte adhesion and prevent neutrophil-mediated capillary endothelial cell damage • In a small prospective study, fasudil + bevacizumab improved BCVA and CRT at 4 weeks
iCo-007	Phase II trial completed	• iCo-007 and iCo-007 + ranibizumab were compared to laser (iDEAL study). No difference among groups for proportion of patients with 15-letter BCVA loss
Luminate (ALG-1001)	Phase IIb trial underway	• Integrin receptor antagonist • May be effective for VMT and DME. Promotes vitreolysis and interferes with angiogenesis • Phase I trial in patients with DME that were refractory to standard care. At 150 days: 1. BCVA improved from 20/200 to 20/125 2. CMT improved from 519 μm to 387 μm

Table 8.5 (continued)

Drugs not in the previously identified categories being evaluated for the treatment of diabetic macular edema		
Drug	Clinical phase	Important characteristics
Mecamylamine	Phase I/II trial completed	• Antagonist of n-acetyl choline receptors • 23 patients with DME were treated with BID drops for 12 weeks. At 16 weeks: 1. BCVA improved by +3.1 letters 2. No change in foveal thickness
Microspheres	Preclinical	• Local administration of sustained release particles that can be loaded with several molecules • Subconjunctival injections of sustained release celecoxib-loaded microspheres decreased VEGF production and blood-barrier breakdown in rat model of diabetes
Minocycline	Phase I/II trial completed	• Exhibits anti-inflammatory effect against glial activation • Six months oral administration in prospective, open-label study resulted in: 1. BCVA improvement of +5.8 letters 2. CST improvement of 8.1%
PF-04523655	Phase II trial completed	• Small interfering ribonucleic acid that inhibits expression of hypoxia-inducible gene RTP801 • May work independent of and possibly complimentary to anti-VEGF drugs • 184 patients were randomized to one of three doses of PF-0423655 or laser. At 12 months: 1. BCVA in highest dose improved nonsignificantly more than laser (+5.77 vs. +2.39 letters; P=0.08) 2. No evidence that macular fluid changes were dose related 3. Study was terminated based on predetermined futility criteria
Plasma kallikrein inhibitor	Phase II trials planned	• A serine protease that is part of the body's inflammatory response. Increases levels of bradykinin • Increased kallikrein activity seen in DME, hereditary angioedema, and cerebral hemorrhage • In a phase I study, 14 patients received single injections of three doses. At day 84: 1. BCVA improved by +4 letters 2. CST improved by −40 μm
Ruboxistaurin	Phase III trials completed	• Orally administered protein kinase C inhibitor • Improves DR in animal models and retinal hemodynamics in patients with DM • Studied in phase III trials but failed to meet primary endpoints

(continued)

Table 8.5 (continued)

Drugs not in the previously identified categories being evaluated for the treatment of diabetic macular edema		
Drug	Clinical phase	Important characteristics
Sirolimus	Phase II trials underway	• mTOR inhibitor that modulates HIF-1α-mediated activation of growth factors • In phase I trial, single subconjunctival or intravitreal injections were given to 50 eyes. At day 45, median improvements in subconjunctival and intravitreal eyes were: 1. BCVA: +4 letters in both groups 2. Decrease in retinal thickness: −23.7 μm and −52 μm
Squalamine	Phase I trials underway	• Small antiangiogenic molecule that interferes with several growth factors including VEGF • Has been evaluated in early phase nAMD trials and investigator-initiated DR trials
Tie2 agonist (AKB-9778)	Phase IIa trial completed	• Tie 2 is a transmembrane receptor that stabilizes vasculature and decreases leakage • 12-week randomized trial evaluated AKB-9778 monotherapy and in combination with ranibizumab: 1. AKB-9778 monotherapy was not effective 2. Compared to ranibizumab monotherapy, combination therapy: (a) Improved CST (−164 μm vs −110 μm; $P = 0.008$) (b) Improved BCVA (+6.3 letters vs. +5.7 letters)
Tocilizumab (TCZ)	Phase II trial underway	• Inhibits interleukin-6 • READ-4 trial randomizes patients to ranibizumab, TCZ, or combination therapy
Teprotumumab	Phase I trial underway	• Insulin-like growth factor inhibitor • Intravenous administration for DME

ATP adenosine triphosphate, *VEGF* vascular endothelial growth factor, *Ang2* angiopoietin-2, *BCVA* best corrected visual acuity, *CRT* central retinal thickness, *VMT* vitreomacular traction, *DME* diabetic macular edema, *CMT* central macular thickness, *BID* twice daily, *CST* central subfield thickness, *DR* diabetic retinopathy, *DM* diabetes mellitus, *HIF* hypoxia-inducible factor, *nAMD* neovascular age-related macular degeneration

extracellular adenosine are increased due to the increased need for energy supplied by adenosine triphosphate [132]. The increased extracellular adenosine at inflamed sites can protect against cellular damage by activating the A2A adenosine receptor (A2AAR), a Gs-coupled receptor [60].

The selective adenosine kinase inhibitor (AKI), ABT-702, was injected intraperitoneally twice weekly to streptozotocin-induced diabetic mice [35]. Retinal inflammation was evaluated using Western blot, real-time PCR, and immunostaining analyses, and the role of A2AAR signaling in the anti-inflammation regulation of ABT-702 was analyzed in amadori-glycated-albumin (AGA)-treated microglial

cells. At 16 weeks, when diabetic mice exhibit significant signs of retinal inflammation including upregulation of oxidative/nitrosative stress, A2AAR, ENT1, Iba1, TNF-α, ICAM1, retinal cell death, and downregulation of AK, the ABT-702-treated group showed decreased signs of inflammation compared to control animals receiving the vehicle. The involvement of adenosine signaling in the anti-inflammation effect of ABT-702 was supported by the tumor necrosis factor (TNF)-α release blocking effect of an A2AAR antagonist in AGA-treated microglial cells. These results suggest a role for adenosine kinase in regulating adenosine receptor signaling in the retina.

8.5.2 Ang2 Inhibition

Compromise of the blood-retinal barrier has been associated with elevated vitreous concentrations of angiopoietin-2 (Ang2) in patients with clinically significant macular edema (CSME) [98]. Ang2 promotes angiogenesis and vascular leakage in the presence of VEGF and pro-inflammatory cytokines but facilitates vascular regression in the absence of VEGF [12]. Intravitreal injection of Ang2 in nondiabetic rats increases retinal vascular permeability, and Ang2 also leads to a loss of VE-cadherin function [102]. Ang2 sensitizes endothelial cells to TNF-α-induced expression of ICAM-1, the critical player in the pathogenesis of inflammation-induced retinopathy [38]. Pharmacologic blockade of Ang2 might also prevent pericyte dropout in DR.

A bispecific – anti-VEGF and anti-Ang2 – antibody is currently in phase II testing for patients with DME (AVENUE Trial, Regeneron).

8.5.3 Antioxidants

Evidence from animal studies speaks both for and against the use of antioxidants to prevent experimental diabetic retinopathy [50, 73]. This use of antioxidants has not been supported by clinical trials [128].

Despite disappointing data from human studies, controversy over the advantages of calcium dobesilate (CaD) in the treatment of DR remains. Several reports suggest that CaD slows the progression of DR [13, 16, 40, 106, 112, 133]. One study [41] suggested that CaD might protect against endothelial cell dysfunction, reduce apoptosis, and retard the local proliferation of vascular endothelial cells, but others [78, 104] failed to show that CaD benefits the capillary resistance in diabetic patients or decreases the progression of DR. In a recent double-blind, multicenter trial [49], CaD could not prevent or reduce the development of macular edema in patients with nonproliferative DR during a 5-year follow-up period. In a trial of obese nondiabetic male smokers [114], CaD (1000 mg/d) did not improve endothelial function.

8.5.4 ASP8232

ASP8232 belongs to a novel class of orally administered vascular adhesion protein-1 inhibitors. It is being evaluated in a phase 2 multicenter, randomized, controlled trial (the VIDI study) for the treatment of DME. The safety and efficacy of ASP8232 + sham are being compared to ASP8232 + ranibizumab and placebo + ranibizumab. The trial has an enrollment target of 84 patients and is expected to reach the primary completion date in 2017 [11].

8.5.5 Darapladib

Darapladib, a specific inhibitor of lipoprotein-associated phospholipase CA2 (Lp-PLA_2), is protective against atherogenesis and vascular leakage in diabetic and hypercholesterolemic animal models. It effectively suppresses BRB breakdown in streptozotocin-diabetic brown Norway rats, comparable to that of intravitreal anti-VEGF therapy [24].

8.5.6 Epalrestat

Neuropathy, retinopathy, and nephropathy, which are all microvascular complications of diabetes, may be mutually and closely related, with diabetic neuropathy acting as a possible trigger for the onset or progression of the other complications. Incubation of rat aortic smooth muscle cells in the presence of a high glucose concentration significantly increases protein kinase C activity and expression of the protein kinase C bII isoform, and these increases are suppressed by epalrestat [86]. In a study of human coronary artery smooth muscle cells [137], epalrestat inhibited an increase in membrane-bound protein kinase C. An aldose reductase inhibitor also reduced hyperglycemia-induced apoptosis in cultured bovine retinal pericytes [88] and inhibited upregulation of genes in the transforming growth factor-β pathway and apoptosis in retinal vessels of diabetic rats [43]. Therefore, increased activity of the polyol pathway may also be closely related to increased activity of protein kinase Cb and transforming growth factor-β in microangiopathies.

Epalrestat, an aldose reductase inhibitor, was found to be effective for the treatment of both diabetic neuropathy and for early retinopathy [55–57]. Progression of diabetic retinopathy and nephropathy was significantly inhibited in a group treated with epalrestat compared with a control group (odds ratio = 0.323, $P = 0.014$) and was dependent on the severity of diabetic neuropathy at the end of the study (odds ratio = 2.131, $P = 0.025$) [57].

8.5.7 *Fasudil*

Fasudil (Asahi Kasei Pharma Corporation, Tokyo, Japan) is a rho-kinase (ROCK) inhibitor that is used to treat cerebral vasospasm after aneurysm rupture and stroke. It has also been used for primary pulmonary hypertension and memory deficits in patients with Alzheimer's disease. Experimental studies have demonstrated that fasudil can suppress leukocyte adhesion and prevent neutrophil-induced retinal capillary endothelial cell damage [10]. Fasudil may directly protect vascular endothelial cells by reversing endothelial nitric oxide synthase activity.

In a small, prospective study, patients with DME received single intravitreal injections of bevacizumab combined with fasudil (0.025 mg). Compared to baseline, eyes had significant improvements in mean BCVA (0.84 logMAR to 0.49 logMAR; $P = 0.003$) and mean CRT (448 μm to 347 μm; $P = 0.001$) at 4 weeks [91].

8.5.8 *iCo-007*

The RAF proto-oncogene serine/threonine-protein kinase, also known as proto-oncogene c-RAF or c-Raf, is a principal component of the first described mitogen-activated protein kinase (MAPK) pathway: ERK1/2 signaling. c-Raf acts as a MAP3 kinase, thereby initiating the entire kinase cascade. It has been hypothesized that several growth factors including VEGF, basic fibroblastic growth factor (bFGF), insulin-like growth factor (IGF)-1, epopoietin (EPO), hepatocyte growth factor (HGF), and integrin, signal through c-Raf. The antisense oligonucleotide iCo-007 inhibits c-Raf expression and blocks MAP kinase signaling. iCo-007 has a favorable ocular pharmacokinetic profile and an intraocular half-life of 6–8 weeks in rabbits and monkeys after intravitreal injection.

iCo-007 completed a phase 1, open-label, dose-escalation study in 15 patients with diffuse DME with a 6-month follow-up after a single intravitreal injection (doses ranging between 110 μg and 1000 μg). The study included patients with diffuse DME within 300 μm of the foveal center, OCT-measured macular thickness at baseline of >250 μm, and BCVA at baseline of 60 ± 15 ETDRS letters (20/63 to 20/500 Snellen). Enrolled patients were divided into four cohorts (a total of 15 patients, six in the last cohort).

No drug-related adverse effects were seen during the study. Pharmacokinetic results indicated that iCo-007 concentrations were below the detectable level of 2 ng/mL in the blood. At a secondary endpoint at week 24, mean reduction of excess retinal thickness compared to baseline was 40%, with a 69% improvement in BCVA [18].

A multicenter, phase II trial evaluated iCo-007 as monotherapy and in combination with ranibizumab or laser for patients with DME involving the foveal center (the iDEAL Study). When using multiple imputation analysis, there was no statistically

significant difference between 350 μg iCo-007 monotherapy and each of the 700 μg monotherapy, 350 μg plus laser, and 350 μg plus ranibizumab arms. At 8 months, in the 700 μg monotherapy arm, 64% of patients experienced a 15-letter or greater loss of vision, compared to 33% in the 350 μg monotherapy arm, 33% in the 350 μg plus laser arm, and 41% in the 350 μg plus ranibizumab arm. At 4 months, the corresponding numbers were 29%, 9%, 9%, and 14%, respectively [61].

8.5.9 Luminate (ALG-1001)

Integrin peptide therapy is a novel approach to the treatment of DME. Integrins are cell-surface receptors that participate in cell signal transduction, mediation of attachments between cells, and regulation of the cell cycle. Integrins interact extracellularly with important proteins, such as collagen and fibronectin, and by intracellularly regulating cell survival, proliferation, and trafficking.

Luminate (ALG-1001, Allegro Ophthalmics, San Juan Capistrano, CA) is a first-in-class integrin peptide therapy that targets integrin receptors involved in cell signaling and regulation and in the formation of new blood vessels. Luminate may be useful in the treatment of vitreomacular traction diseases by promoting vitreolysis and in macular vascular diseases by interfering with angiogenesis. ALG-1001 binds to all integrin receptors involved with retinal angiogenesis and has a long-lasting effect.

Early clinical trials showed that luminate inhibits new vessel growth and decreases vascular leakage. A phase I study evaluated the safety and efficacy of luminate in 15 subjects with advanced DME. Patients had BCVA of 20/100 or worse, some had early proliferative DR, and many were already refractory to standard-of-care therapy. After a period of 90 days without any DME therapy, the patients received three intravitreal 2.5 mg injections of luminate at monthly intervals as monotherapy. Follow-up continued for 3 months after the last injection.

No subjects in the study lost BCVA or experienced an increase in CMT. No serious or significant adverse events were seen during follow-up. Mean BCVA improved from 20/200 at baseline to 20/125 at 60 days (last treatment) and remained stable through 150 days. The mean central macular thickness of 519 μm at baseline decreased to 387 μm at 150 days [76].

Luminate is presently being evaluated in a phase IIb clinical trial against bevacizumab and focal laser for DME [6]. The enrollment goal for the phase IIb DME trial (150 patients) was met in late 2015, and top-line data may be released by the third quarter of 2016.

8.5.10 Mecamylamine

In a multicenter phase I/II trial, the safety and bioactivity of topical mecamylamine, an antagonist of n-acetyl choline (ACh) receptors, was tested in 23 patients with DME [23]. Mecamylamine (1%) was administered topically twice daily for

12 weeks. Patients underwent safety assessments and measurements of BCVA and foveal thickness at baseline, 1, 4, 8, 12, and 16 weeks.

Mecamylamine drops were well tolerated, and there were no drug-related safety problems. Mean improvement in BCVA at 1, 4, 8, 12, and 16 weeks was +2.8, +1.9, +2.4, +0.8, and +3.1 letters, respectively. There was little change in mean excess foveal thickness, but there was substantial heterogeneity in response as eight patients had improved BCVA, foveal thickness, or both. Nine patients experienced no significant changes and four patients worsened. Five patients had a significant improvement in BCVA, foveal thickness, or both between weeks 12 (last visit while receiving mecamylamine) and 16 (1 month after stopping mecamylamine). The study suggested that administration of topical mecamylamine, a nonspecific nACh receptor blocker, has heterogeneous effects in patients with diabetic macular edema.

8.5.11 Microspheres

Local administration of biodegradable microspheres may be an attractive alternative to multiple injections since they are able to deliver drug in a controlled fashion. Since most of the treatable retinal diseases are multifactorial, microspheres are particularly promising as they can be filled with more than one active substance and complemented with pharmacologically active additives.

Microsphere carriers have been loaded with budesonide and celecoxib for the treatment of diabetic retinopathy. Comparison between nano- and microspheres prepared from poly(lactic acid) (PLA; intrinsic viscosity, 1.1 dL/g) and loaded with budesonide was performed after subconjunctival injection of particles in rats. In this study, microspheres delivered the active substance in a more sustained fashion than nanospheres [72] because the nanospheres were removed more rapidly from the subconjunctival administration site.

Nanoparticles, microspheres, and budesonide in solution (75 mg) were administered to rat eyes, and drug concentrations in different tissues (retina, vitreous, lens, and cornea) were compared at various times after administration. On days 7 and 14, drug levels in the eyes treated with microspheres were higher than those treated with solution and nanoparticles. Sustained release of celecoxib from poly(lactic-co-glycolic acid) (PLGA) was evaluated in a diabetic rat model [8]. A posterior subconjunctival injection of 0.05 mL of the celecoxib-microsphere suspension inhibited diabetes-induced VEGF elevations and blood-retinal barrier leakage.

8.5.12 Minocycline

Microglial activation due to induced inflammation within the retina usually precedes the microvascular findings in DR [42, 74, 139]. Tetracycline reduces connective tissue breakdown [109], protein glycation [108] and excessive collagen synthesis [30] and limits microglial-mediated cell death, retinal cell apoptosis, and

capillary damage by inhibiting caspase [39, 74, 134]. Minocycline, a commonly used second-generation tetracycline, has been demonstrated in cell culture and animal models to have anti-inflammatory properties that are independent of its antibacterial property [37, 74].

Oral minocycline (100 mg twice daily for 6 months) was investigated in a single-center, prospective, open-label, phase I/II clinical trial of five participants with fovea-involving DME [31]. Minocycline hydrochloride (Ranbaxy Pharmaceutical Inc., Princeton, NJ; National Drug Code 63304 696 50) was reformulated by the NIH Research Pharmacy as capsules for oral administration. Main outcome measurements included changes in BCVA, CST, and central macular volume using SD-OCT. Mean BCVA improved continuously from baseline through 1, 2, 4, and 6 months by +1.0, +4.0, +4.0, and +5.8 letters, respectively, while CST decreased by 2.9%, 5.7%, 13.9, and 8.1% for the same time points. At month 6, the mean area of late leakage on fluorescein angiography decreased by 34.4% in study eyes. Mean changes in fellow eyes demonstrated similar trends. Improvements in outcome measures were not correlated with concurrent changes in systemic factors. The study drug was well tolerated and not associated with significant safety issues.

Two trials with oral doxycycline, however, produced conflicting results [115, 116].

8.5.13 PF-04523655

PF-04523655 is an O-methyl stabilized, 19-nucleotide, double-stranded, small interfering ribonucleic acid (siRNA) that inhibits expression of a hypoxia-inducible gene, RTP801, via RNA interference [121]. RTP801 expression is upregulated in streptozotocin-induced diabetic mice and rats [19] but is suppressed by intravitreal PF-04523655. Blocking the RTP801 hypoxia/stress pathway with PF-04523655 could provide a new treatment option that is independent of, and possibly complementary to, anti-VEGF therapies for the treatment of DME.

In a multicenter, prospective, masked, phase II clinical trial, 184 patients with DME were randomized to receive PF-04523655 (0.4 mg, 1 mg, or 3 mg) or focal/grid laser photocoagulation [89]. The main outcome measure was the change in BCVA from baseline to month 12.

All doses of PF-04523655 improved BCVA from baseline through month 12. The 3 mg PF-04523655 group showed a trend for greater improvement in BCVA from baseline compared to laser (respectively, +5.77 vs. +2.39 letters; $P = 0.08$), but the study was terminated early at month 12 based on predetermined futility criteria for efficacy and discontinuation rates. PF-04523655 was generally safe and well tolerated with few treatment-related adverse events.

There was no evidence that the changes in CST, macula volume, fluorescein angiogram leakage area, or diabetic retinopathy were related to the dose of PF-04523655. The lack of correlation between the changes in DME structural

measures and PF-04523655 doses suggests that PF-04523655 may be working through a mechanism that does not involve vascular permeability, such as increased pigment epithelial-derived factor (PEDF) expression combined with suppression of the RTP 801 gene. PF-04523655 showed a dose-related tendency for improvement in BCVA in DME patients, and studies with higher doses have been considered to determine the optimum dose [27].

8.5.14 Plasma Kallikrein Inhibitor

Plasma kallikrein, a serine protease, is an important component of the body's inflammatory response. Kallikrein circulates as an inactive enzyme (pre-kallikrein) that becomes activated at the site of vascular injury and initiates a cascade that increases the levels of bradykinin. This potent, vasoactive protein dilates blood vessels and increases vascular permeability, edema, and inflammation. Plasma kallikrein is regulated by a C1-inhibitor.

Increased plasma kallikrein activity is implicated in many diseases including DME, hereditary angioedema, and cerebral hemorrhage. Several components of the kallikrein-kinin system (KKS), including plasma kallikrein, factor XII, and kininogen, are found in the vitreous of patients with advanced diabetic retinopathy. Preclinical studies in rodents showed that plasma kallikrein activation in the vitreous increases retinal vascular permeability, whereas kallikrein inhibition reduces diabetes- and hypertension-induced retinal leakage.

Small molecule plasma kallikrein inhibitors delivered intravitreally and orally have the potential to treat these and other retinal vascular problems. KalVista Pharmaceuticals is developing plasma kallikrein inhibitors for the treatment of DME and hereditary angioedema. A phase I study at 5 US sites established the safety of an intravitreally injected kallikrein inhibitor (KVD001). Three cohorts (14 total patients) previously treated with anti-VEGF injections received single injections of 1, 3, and 10 μg. Thirty-six minor adverse events were recorded. At day 84, the mean BCVA and CST improved by +4 letters and −40 μm, respectively. Systemic drug absorption was low [125].

Two phase II trials are being planned: one combines a plasma kallikrein inhibitor with an anti-VEGF drug; the other employs plasma kallikrein inhibitor monotherapy for eyes with anti-VEGF-resistant DME [69].

8.5.15 Ruboxistaurin

Ruboxistaurin (RBX) is an orally administered, isoform-selective inhibitor of protein kinase C that positively affects animal models of diabetic retinopathy [3] and improves diabetes-induced retinal hemodynamic abnormalities in patients with diabetes [4]. Two randomized controlled studies and a combined analysis of two

additional studies have suggested that oral RBX reduces the rate of sustained moderate vision loss in patients with diabetes [100, 101, 120]. These studies did not meet their primary endpoints; consistent improvements in macular edema and reduction in need for laser photocoagulation were not seen.

8.5.16 Sirolimus

The mTOR inhibitors are uniquely suited to address both early and advanced manifestations of DR. The mTOR inhibitors have the potential to delay or prevent the progression of retinal microangiopathies by averting breakdown of the blood-retinal barrier by modulating HIF-1α-mediated downstream activation of growth factors. As DR progresses and proliferative lesions develop, inhibiting the PI3K/Akt/mTOR pathway may promote neovascular regression by downregulating pro-survival growth factors, modulating the inflammatory cascade, preventing angiogenesis, and promoting apoptosis of nascent vessels [62].

A randomized, open-label, dose-escalating, phase I study evaluated the safety and tolerability of sirolimus (Perceiva, Macusight, Union City CA) for the treatment of DME [33]. Single subconjunctival (SCJ: 220, 440, 880, 1320, or 1760 μg) or intravitreal (IVT: 44, 110, 176, 264, or 352 μg) injections were given to 50 eyes of 50 patients with retinal thickness of at least 300 μm and BCVA of 20/40 to 20/200. The primary endpoints were the frequency and severity of ocular and systemic adverse events at 90 days. Secondary endpoints were changes in BCVA and retinal thickness.

No dose-limiting toxicities were observed, and ocular adverse events were mostly mild and transient. Conjunctival hyperemia, hemorrhage, and edema were common after SCJ injections, and conjunctival hemorrhage was common after IVT injections. Three patients experienced ocular adverse events considered possibly related to study drug: conjunctival edema and reduced visual acuity were reported in one SCJ patient each, and iritis was reported in one IVT patient. No serious ocular adverse events were reported. No non-ocular adverse events were considered related to study drug. Systemic exposure to sirolimus was low with blood concentrations below levels necessary for systemic immunosuppression.

For the SCJ group, a median increase in BCVA started at day 7 (+5.0 letters) and was +3.0, +4.0, and +4.0 letters at days 14, 45, and 90, respectively. At day 45, median decrease in retinal thickness was −23.7 μm. For the IVT group, the median increase in BCVA was +2.0 letters at day 7; at days 14, 45, and 90, the median increase was maintained (+4.0 letters); the median decrease in retinal thickness was −52.0 μm at day 45.

The investigators concluded that locally administered sirolimus was well tolerated with minimal systemic exposure at all doses tested in this small phase I population. These findings support advancing the present sirolimus formulation into phase II studies.

8.5.17 Squalamine

Squalamine (Ohr Pharmaceuticals) is a small antiangiogenic molecule that interferes with the actions of several growth factors including VEGF, platelet-derived growth factor (PDGF), and basic fibroblastic growth factor (bFGF). It enters the cell and sequesters intracellular calmodulin to inactivate the growth factor receptors. It has been evaluated in early phase nAMD trials, and investigator-initiated trials have studied its effect on DR.

8.5.18 Tie2 Agonist (AKB-9778)

Tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains 2 (Tie2) is a transmembrane receptor located on vascular endothelial cells that has been associated with vascular permeability. Unlike activation of the VEGF receptors, activation of Tie2 stabilizes vasculature and decreases leakage. Angiopoietin 1 stimulates Tie2 phosphorylation, whereas angiopoietin 2 only partially stimulates Tie2 phosphorylation and, therefore, competes with angiopoietin 1 to suppress Tie2 phosphorylation.

A phase IIa, randomized, placebo and sham injection-controlled, double-masked clinical trial assessed the effect of the Tie2 agonist AKB-9778 alone or in combination with ranibizumab in subjects with DME. Patients (n = 144) with decreased VA from DME and CST ≥325 μm measured by SD-OCT were enrolled at 36 sites. Patients were randomized to receive AKB-9778 monotherapy (subcutaneous AKB-9778 15 mg twice per day (BID) + monthly sham intraocular injections), combination therapy (subcutaneous AKB-9778 15 mg BID + monthly 0.3 mg ranibizumab), or ranibizumab monotherapy (subcutaneous placebo injections BID + monthly 0.3 mg ranibizumab). Best corrected visual acuity and CST were measured at baseline and every 4 weeks. The primary outcome measure was the mean change from baseline CST at week 12. Other predetermined outcome measures included changes in BCVA, diabetic retinopathy severity score (DRSS), and safety assessments.

At week 12, mean change from baseline CST was significantly greater in the combination group (−164.4 ± 24.2 μm) compared with the ranibizumab monotherapy group (−110.4 ± 17.2 μm; $P = 0.008$) but was only 6.2 ± 13.0 μm in the AKB-9778 monotherapy group. The mean CST at week 12 and percentage of eyes with resolved edema was 340.0 ± 11.2 μm and 29.2%, respectively, in the combination group versus 392.1 ± 17.1 μm and 17.0%, respectively, in the ranibizumab monotherapy group. The mean change from baseline BCVA (ETDRS letters) was +6.3 ± 1.3 in the combination group, +5.7 ± 1.2 in the ranibizumab monotherapy group, and +1.5 ± 1.2 in the AKB-9778 monotherapy group. The percentage of study eyes that gained ≥10 or ≥15 letters in the AKB-9778 monotherapy group, ranibizumab monotherapy group, and combination group was 8.7%, 29.8%, and 35.4%, respectively, and 4.3%, 17.0%, and 20.8%. Improvements in DRSS in study

eyes were similar across groups, and the percentage of qualified fellow eyes with a ≥2-step change was 11.4% in all AKB-9778-treated subjects compared with 4.2% in the ranibizumab monotherapy group. AKB-9778 was well tolerated, with no clear differences in adverse events.

The authors concluded that activation of Tie2 by subcutaneous injections of AKB-9778 combined with suppression of VEGF reduces DME greater than that seen with anti-VEGF monotherapy [22].

8.5.19 Tocilizumab

The READ-4 study compares ranibizumab and tocilizumab (TCZ), an interleukin-6 inhibitor, for the treatment of DME. The study will randomize patients to receive ranibizumab, TCZ, or a combination, with the primary endpoint analysis at month 6. Enrollment for the study is expected to begin in the first quarter of 2016 [103].

8.5.20 Teprotumumab

The safety and efficacy of insulin-like growth factor (IGF-1) inhibitors are being evaluated in patients with DME. The intravenously administrated IGF-1 inhibitor teprotumumab (RV001) is being evaluated in an open-label phase 1 study in three centers in the United States [129].

8.6 Future Therapies

Experimental work has shown that leukostasis-related death of endothelial cells can be prevented by blocking or genetically eliminating either ICAM-1 or CD18 [1, 68] and by administering anti-inflammatory agents. High-dose aspirin reduces expression of CD11a, CD11b, and CD18 [68]; high-dose aspirin, etanercept (soluble TNFR-Fc, tumor necrosis factor-1 linked to a human FC region), and meloxicam (a cyclooxygenase 2 inhibitor) reduce leukostasis and suppress BRB breakdown in diabetic rats [68].

Repopulating diabetes-damaged acellular capillaries with vascular progenitor cells constitutes another therapeutic approach [64, 119]. Unfortunately, diabetic endothelial progenitor cells (EPCs) are deficient in homing ability and have limited engraftment capacity [20], but this can be increased by treating the cells with nitric oxide [117]. Vascular progenitors have been generated from CD34þ cord blood cells that have been induced nonvirally into pluripotent stem cells (iPSCs) [95]. iPSCs were trained to be vascular progenitors on fibronectin substratum with high levels of VEGF [96]. When CD31þ/CD146þ cells were injected into the vitreous of NOD/

Scid mice that had experienced ischemia-reperfusion injury to retina resulting in acellular capillaries, the cells homed to the ablumenal surface of the acellular capillaries, taking a pericyte position. When the cells were delivered intravenously, they were engrafted in a lumenal position, suggesting that they were assuming the role of endothelial cells. The hypoxic adjacent retina makes both stromal-derived factor-1 (SDF-1) and VEGF, which would provide the stimulus for homing of vascular progenitors to the acellular capillaries. Future work will focus on repopulating acellular capillaries with iPSC vascular progenitors in diabetic animals [83].

Neuroprotective factors such as pigment epithelium-derived factor (PEDF) [90, 99, 126, 138], somatostatin (SST) [71], nerve growth factor (NGF) [48], brain-derived neurotrophic factor [45], and epopoietin [85, 135, 140] have been used to treat experimental DR. Intraocular gene transfer of PEDF significantly increases neuroretinal cell survival after an ischemia-reperfusion injury [126]. Intravitreal injections of PEDF prevent neuronal derangements and vascular hyperpermeability in early DR [138]. Intravitreally administered somatostatin and somatostatin analogs protect the retina from alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-induced neurotoxicity [71]. Treating diabetic rats with NGF prevents ganglion cell and Müller cell apoptosis [48]. These promising results suggest that enhancing the expression and function of the neuroprotective factors synthesized by the retina could be a therapeutic strategy to treat DR.

The ability to clinically identify retinal neurodegeneration will be crucial for developing early treatment strategies with drugs that possess neuroprotective effects. At this stage of disease, however, patients are still asymptomatic, so invasive treatments such as intravitreal injections are not appropriate. Emerging experimental evidence indicates that many drugs, including some that are administered topically, can reach the retina in adequate concentrations [34]. In fact, the retinal neuroprotective effects of topically administered brimonidine, NGF, PEDF, insulin, and SST have already been reported in experimental models [5, 32, 39, 52, 77, 113, 123]. Topical administration limits drug actions primarily to the eye and minimizes unwanted systemic effects, which should result in higher patient compliance [2]. Effective topical therapies could revolutionize the care of diabetic patients, but clinical trials are needed to test the safety and effectiveness of neuroprotective agents.

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Chapter 9
Safety Considerations of Pharmacotherapy

9.1 Introduction

Current pharmacologic treatment of diabetic retinopathy (DR) is based on the intraocular administration of several medications. Injections into the vitreous have emerged as the preferred route of drug delivery for several reasons. Firstly, drug concentrations in the vitreous, retina, and choroid are higher after intravitreal injections than after any other route of administration. Secondly, the vitreous acts as a depot that slows drug elimination from the eye and prolongs its duration of action. Thirdly, the small volume of injected drug results in low serum concentrations and may minimize the likelihood of systemic adverse events.

The favorable safety profile attributed to intraocular pharmacotherapy has contributed to the rapid development and widespread adoption of corticosteroid formulations and drugs that bind vascular endothelial growth factor (VEGF). The pivotal phase III registration trials were sufficiently sized to demonstrate clinically efficacy and garner approval by the United States Food and Drug Administration (US FDA), but they were insufficiently powered to identify low-frequency ocular and systemic adverse events. Consequently, systematic reviews and meta-analyses have been performed to better understand the risks of therapy.

The safety profiles of intraocular corticosteroids differ significantly from those of the anti-VEGF drugs. The volume of administered corticosteroids is not sufficient to cause systemic adverse events, but steroid therapy is complicated by high rates of cataract development and glaucoma, and triamcinolone acetonide injections may cause a severe sterile endophthalmitis or pseudoendophthalmitis.

Compared to both the general population and other groups who receive intraocular pharmacotherapy, patients with diabetes may be at greater risk of developing thromboembolic events and impaired wound healing, which might be worsened by anti-VEGF therapy. Physicians, therefore, remain keenly interested in the ocular and systemic safety profiles of drugs used to treat DR. The aim of this chapter is to discuss known risks of drugs used to treat DR.

M.W. Stewart, *Diabetic Retinopathy*, DOI 10.1007/978-981-10-3509-8_9

9.1.1 Intravitreal Injections

Drugs are injected into the mid-vitreous through the pars plana (3–4 mm posterior to the limbus) (Fig. 9.1). Vascular endothelial growth factor inhibitory drugs may be injected through 30- or 32-gauge needles, whereas triamcinolone acetonide can be injected through 27- or 30-gauge needles. Large volumes (1 ml) of triamcinolone suspension frequently occlude 30-gauge needles, but the author has never experienced occlusion when injecting small intravitreal volumes (0.05 ml). Needles may be safely inserted to a depth of 12 mm and should be directed toward the mid-vitreous. The bioerodible, sustained release dexamethasone phosphate insert (Ozurdex®, Allergan, Irvine, CA) is injected through a pre-loaded 22-gauge insertion system, whereas the non-bioerodible, sustained release fluocinolone acetonide insert (Iluvien®, Alimera, Alpharetta, GA) is injected through a pre-loaded 25-gauge insertion system.

Neither preinjection nor postinjection antibiotics appear to alter low postinjection endophthalmitis rates [10, 48], but practice patterns regarding antibiotic use differ widely among surgeons. Most physicians use an eyelid speculum to stabilize the eyelids and improve exposure of the eye, but excellent results have been reported by having the surgeon or an assistant manually retract the eyelids (Fig. 9.2) [68]. Topical povidone-iodine, either 5% or 10%, should be used to sterilize the conjunctiva prior to the injection [17, 66]. True allergy to povidone-iodine is rare and most adverse reactions are due to a toxic keratitis. Patients who claim to be "allergic to iodine" because of reactions to shellfish (allergy to myosin) or intravenous contrast dye (hyperosmotic reaction) can be assured that there is no relationship between these adverse reactions and either iodine or povidone-iodine.

Various forms of anesthesia can be administered – topical proparacaine or tetracaine drops, lidocaine gel, subconjunctival lidocaine injection, or peribulbar lidocaine injection – to decrease the pain experienced by patients. Each form of

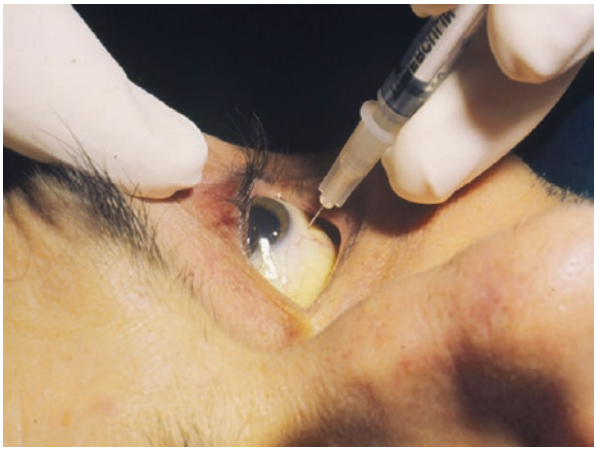

Fig. 9.1 This photograph of the author's technique of performing an intravitreal injection shows the inferior insertion position of the needle as the patient looks up

anesthesia has its associated advantages and drawbacks. For example, topical drops provide less predictable anesthesia but allows the procedure to be performed very quickly; subconjunctival lidocaine improves the depth of the anesthesia but prolongs the procedure and causes a subconjunctival hemorrhage in more than 50% of cases. The overall experience with the procedure appears to be independent of the method of anesthesia [11]. Surgeons, therefore, should find a technique with which they are most comfortable but should always consider individualizing anesthesia to meet the needs of each patient. Since the needles on the dexamethasone (22 gauge) and fluocinolone (25 gauge) insertion devices have considerably larger bores than those usually used for anti-VEGF injections (30 gauge), surgeons should consider the routine use of subconjunctival lidocaine when injecting these steroids.

Injections may be safely and efficiently performed in an outpatient clinic or, as is required in some European countries, within an operating suite. Some physicians contend that the incidence of endophthalmitis may be higher when injections are performed in the clinic rather than in the operating room [1], but the results from large pooled series of injections do not support this. Some clinics deal with the increasing number of intravitreal injections by employing specially trained nurses. In one New Zealand clinic, nurse specialists administered a total of 2900 injections over an 18-month period. Two patients (0.07%) developed postinjection endophthalmitis (1 microbial and 1 nonmicrobial), two patients (0.07%) developed vitreous hemorrhages, and five patients (0.17%) had elevated intraocular pressures (IOPs) [60]. The anticipated growth of intravitreal pharmacotherapy will likely result in an increase in the use of physician extenders.

9.2 Ocular Complications

Tables 9.1 and 9.2 list the complication rates of ocular adverse events due to anti-VEGF drugs and corticosteroids, respectively.

Fig. 9.2 This photograph of the author's technique for performing an intravitreal injection shows the manual retraction of the eyelids without the use of an eyelid speculum or surgical assistant

Table 9.1 The incidences of ocular adverse events that have been described in patients receiving treatment with vascular endothelial growth factor inhibitors

Ocular adverse events due to anti-VEGF drugs	
Complication	Incidence rate
Increased intraocular pressure	3–12%
Cataracts	<1% to 3.7%
Endophthalmitis	From: 0.02% per injection to 1%/patient over 3 years
Sterile inflammation	0–1.49%/injection
Retinal detachment	0.9%/injection to 3% per eye *most may be exacerbation of traction detachment
Vitreous hemorrhage	0.4%
Worsening traction detachment	5.2%

Table 9.2 The incidences of ocular adverse events that have been described in patients receiving treatment with intravitreal corticosteroids

Ocular adverse events due to corticosteroids	
Complication	Incidence rate
Cataracts	37–80%
Glaucoma	32–40%
Endophthalmitis	0.03–0.09% per Injection
Migration of dexamethasone insert into anterior chamber	

9.3 Elevated Intraocular Pressure (IOP) and Glaucoma

Population studies have defined the normal human IOP (encompassing 95% of the population) as 9–22 mmHg. Intraocular pressure is maintained through a complex balance of aqueous production, ocular rigidity, tissue deturgescence, and aqueous outflow. Most studies do not implicate diabetes as a risk factor for glaucoma, but intravitreal pharmacotherapy increases the risk of glaucoma in this population. In patients with diabetic macular edema (DME) that receive pharmacotherapy, elevated IOP usually results from increased resistance to aqueous outflow. Since eyes of diabetic patients frequently have complex vitreoretinal pathologies, IOP elevations should prompt evaluations for causes of secondary open-angle (corticosteroid-induced, anti-VEGF-induced, hemolytic, ghost-cell) and secondary angle-closure (neovascular) glaucoma.

The evaluation and treatment of glaucoma encompasses an entire subspecialty within ophthalmology that is beyond the scope of this book. Physicians who manage DME, however, need to be continually aware of the IOP and health of the optic nerve and nerve fiber layer. Intraocular pressure should be measured at each visit,

and the status of the optic nerve and nerve fiber layer should be assessed periodically with disc photographs and/or optical coherence tomography (OCT) measurements. Any IOP elevation should prompt the physician to consider testing (assessment of optic disc anatomy with biomicroscopy and stereoscopic disc photographs, OCT analysis of the nerve fiber layer, threshold visual fields, and pachymetry). If treatment of prolonged IOP elevation is outside the scope of practice of the treating physician, then referral to a glaucoma subspecialist or qualified comprehensive ophthalmologist should be considered.

Intraocular pressure often rises immediately after intravitreal injections but usually normalizes quickly. One study demonstrated that >95% of eyes had an IOP <35 mmHg immediately after injection and only 33% had an IOP elevation of 10 mmHg [8]. Sustained elevations in intraocular pressure occur in approximately 6% of eyes receiving anti-VEGF injections [30] and after a median of five injections. Preexisting glaucoma is a risk factor for sustained IOP elevation, probably because of an already compromised aqueous outflow. Patients receiving at least 29 injections have a 5.75 times risk of developing sustained IOP elevation compared to those receiving 12 or fewer injections [33]. Reasons for a higher risk with more injections may include prolonged VEGF blockade, development of an inflammatory trabeculitis, impaired aqueous outflow due to protein aggregates in the trabecular meshwork, large macromolecules obstructing the meshwork because of bulk deposition, or silicone droplets from the needles and syringes.

An exploratory post hoc analysis of DRCR.net Protocol I sought to determine the incidence of elevated IOP in eyes without preexisting glaucoma. During the 3-year trial, sustained IOP elevation was seen in 6 patients in the laser/sham group and 22 patients receiving ranibizumab (Lucentis®, Genentech, S. San Francisco, CA/Roche, Basel, Switzerland). The risk of developing a sustained IOP of >22 mmHg with an elevation of ≥6 mmHg above baseline was 10% in patients receiving ranibizumab, but only 3% in patients randomized to laser/sham (hazard ratio 2.9 [95% CI, 1.0–7.9]). Through 1 year in the DRCR.net Protocol T trial, patients treated with aflibercept (Eylea®, Regeneron, Tarrytown, NY), bevacizumab (Avastin®, Genentech, S. San Francisco, CA/Roche, Basel, Switzerland), and ranibizumab had the following incidences of elevated intraocular pressures: 12%, 9%, and 9% [20].

In a large retrospective study of 760 eyes, both transient (7%) and sustained (5.8%) elevations of intraocular pressure (defined as increases ≥6 mmHg, >20% above baseline, or >24 mmHg on two or more consecutive visits) were seen after treatment. The probability of an IOP rise increased with the number of injections.

Development of glaucoma is a greater concern in patients receiving intraocular corticosteroids. Patients susceptible to steroid-induced glaucoma are believed to have upregulation of corticosteroid receptors in trabecular meshwork cells [81], which leads to increased expression of the extracellular protein fibronectin, glycosaminoglycans, and elastin [37, 67]. Steroids also downregulate phagocytic activity, which allows obstructive material to accumulate in the meshwork [58, 59]. Increased pressure in patients receiving triamcinolone acetonide may be due to obstruction of aqueous outflow due to steroid deposits in the angle. Approximately 50% of patients receiving intravitreal triamcinolone will experience an elevation in pressure within 2 to 4 weeks [64].

In the DRCR.net trial comparing macular laser photocoagulation with 1 mg and 4 mg intravitreal triamcinolone acetonide, the incidences of IOP elevation in the 1 mg and 4 mg triamcinolone cohorts were 20% and 40%, respectively [19]. Incisional glaucoma surgery was eventually required in 1.6% of patients receiving 4 mg triamcinolone. In DRCR.net Protocol I trial, 42% of patients receiving 4 mg triamcinolone experienced elevated IOP [22].

Intraocular pressure increases with the dexamethasone insert can generally be managed with pressure-lowering medications and are usually transient [9, 16, 31]. In a report of 186 eyes that received the dexamethasone insert through 6 months, eight eyes (4.3%) had intraocular pressure increases to at least 30 mmHg and one eye had an IOP of 50 mmHg [51]. The elevated pressure was successfully treated in all eyes with medications. In the phase III MEAD trials, patients receiving the 0.7 mg dexamethasone insert had a 32% incidence of IOP >25 mmHg and a 6% incidence of IOP >35 mmHg [12]. Less than 1% of eyes required incisional glaucoma surgery.

In the 36-month FAME trial, 38.4% of patients receiving the 0.2 mg fluocinolone insert (Iluvien) developed increased intraocular pressure, and 18.4% had IOPs over 30 mmHg [14]. Approximately 4% of patients required incisional glaucoma surgery to control the pressure. In a series of 17 eyes that were treated with the fluocinolone insert after having previously responded poorly to anti-VEGF therapy, only three had elevated IOP by 12 months and all were successfully treated with topical therapy [46].

9.4 Cataracts

Cataracts due to intravitreal injections of anti-VEGF drugs are very unusual. Only 1 injection-related cataract occurred after 6000 injections in DRCR.net Protocol T [20], presumably from needle trauma to the crystalline lens capsule (Fig. 9.3).

Corticosteroids induce posterior subcapsular cataracts (Fig. 9.4) because upregulated fibroblast growth factor-1, epidermal growth factor, transforming growth factor-β, lens epithelium-derived growth factor, platelet-derived growth factor, and bone morphogenic proteins induce proliferation and migration of lens epithelial cells. Local activation of glucocorticoid receptors in the lens may also downregulate apoptosis [35, 36].

Prospective studies evaluating the treatment of DME with triamcinolone acetonide report that the incidence of cataracts appears to be dose-related [19] and 37–54% of eyes undergo cataract surgery within 2 years [19, 28]. In the Protocol I trial, 15% of patients randomized to triamcinolone required cataract surgery within the first year compared to 3.7% of patients receiving ranibizumab and 6% of patients randomized to sham/laser [22]. The incidence of cataract surgery in the triamcinolone group increased to 55% at 2 years [23].

In the cohort of phakic eyes receiving the 0.7 mg dexamethasone insert in the phase III MEAD trials, progression of cataracts was experienced by 67.9% of eyes

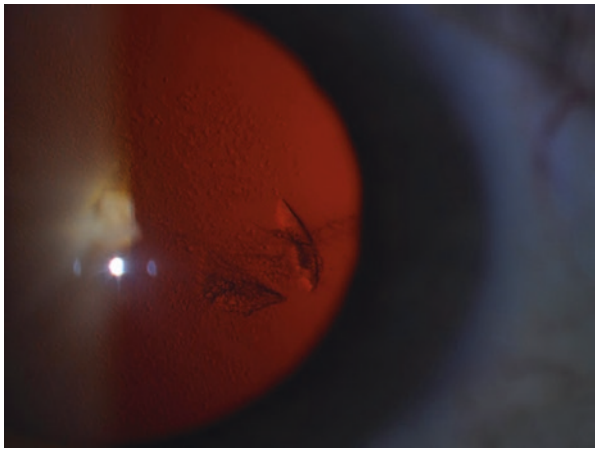

Fig. 9.3 This slit-lamp photograph shows a perforation of the posterior lens capsule following the intravitreal injection of a vascular endothelial growth factor inhibitory drug. Localized posterior subcapsular lens opacifications have developed

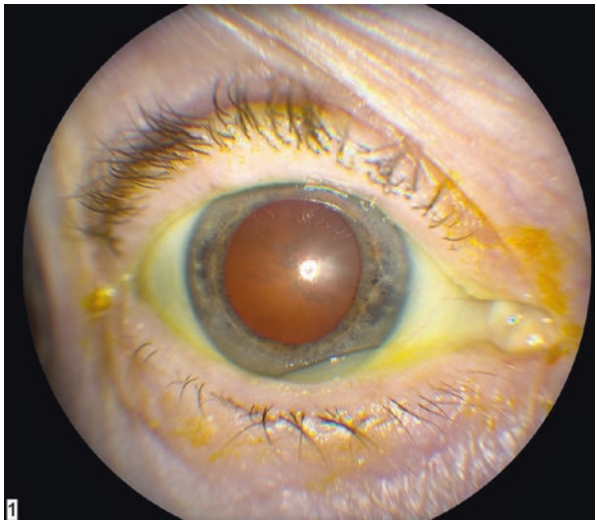

Fig. 9.4 This slit-lamp photograph shows a posterior subcapsular cataract that formed after treatment with intravitreal corticosteroids

over 3 years [12]. In another dexamethasone insert study, progression of lens opacities was seen in 26 of 112 (23.2%) phakic patients within 6 months and 7 (6.3%) underwent cataract extraction [51]. This rate was higher than had been reported in other studies [9, 16, 27, 31].

9.5 Endophthalmitis

Individual cases and clusters of patients with noninfectious inflammation that followed injections of each anti-VEGF drug have been reported. In a study of 61 eyes with AMD, DME, or RVO, increased aqueous flare was detected in eyes that

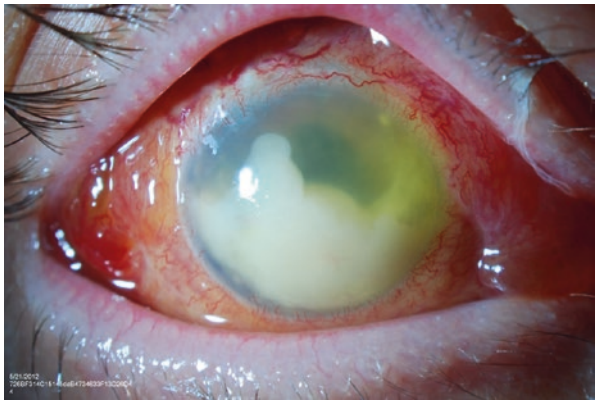

Fig. 9.5 This external photograph shows a large hypopyon in an eye with streptococcal endophthalmitis

received bevacizumab but not ranibizumab or aflibercept ($P = 0.006$) [11]. Rates of inflammation after bevacizumab range from 0.14% to 0.32% and have been attributed to bevacizumab's large size and Fc fragment [24, 26]. A review of postinjection inflammation reported rates of 0.09–1.49% with bevacizumab, 0–0.83% with ranibizumab, and 0–0.05% with aflibercept [3].

A large meta-analysis of intravitreal injections found that *Staphylococcus epidermidis* was the most common cause of infectious endophthalmitis [47], but a single-center report of over 65,000 injections reported that *Streptococci* was the most common organism (Fig. 9.5) [52]. Since most *Streptococci* originate from the oropharynx, some surgeons have begun wearing masks while injecting or minimizing conversation to minimize aerosol spread of organisms.

The single-injection rates of endophthalmitis in the phase II and III DME trials were low, but the cumulative per patient endophthalmitis rates in the RESOLVE [45] and DRCR.net Protocol I [23] trials were approximately 1%. On the other hand, no cases of infectious endophthalmitis were reported in either the phase III RESTORE trial, which followed RESOLVE [62], or the DRCR.net Protocol T trial. Two cases of noninfectious inflammation occurred in each drug group (out of approximately 2000 injections with each drug) in Protocol T [20].

In some countries surgeons compound several doses of bevacizumab from the same vial to use in different patients during a single day. This may be done inside or outside of an operating suite. In one study, surgeons aliquoted bevacizumab from six vials and stored the syringes for up to 1 week at 4° C [18]. Subsequent analysis demonstrated no evidence of bacterial contamination and no cases of endophthalmitis occurred after 973 injections. The authors concluded that this technique is safe if "standard precautions" are adhered to. A retrospective report described a technique in which up to ten doses of bevacizumab were prepared by the surgeon from each vial [53], and no cases of endophthalmitis occurred after 1184 injections. Though this compounding procedure may be performed commonly in some areas, it does not comply with United States Pharmacopeia Chapter 797 guidelines and should be viewed with skepticism [29].

Bilateral same-day injections are performed by some surgeons and patients favor this technique since it decreases the number of office visits. In a series of 342 bilat-

eral injections, one patient developed infectious endophthalmitis in only one eye. The authors claim that this technique is safe if the surgeon changes povidone-iodine, eyelid speculums, and all needles and syringes between injecting the two eyes [2].

Because bevacizumab must be compounded or fractionated from 4 ml multiuse vials into 20 to 30 single-dose 1 ml syringes, it may cause clusters of infectious endophthalmitis because of compounding errors. Several outbreaks of compounding-related endophthalmitis due to highly virulent organisms have occurred [29]. Visual outcomes usually depend upon the organism, but severe vision loss is common. Compounding is best performed by a trained technician working under a high-quality, laminar flow hood. By following the guidelines in Chapter 797 of the United States Pharmacopeia, these technicians are able to safely and inexpensively compound bevacizumab for intraocular use.

The overall incidence of endophthalmitis following intravitreal triamcinolone injections has been estimated at 0.8% for noninfectious causes and 0.6% from infectious agents [63]. The DRCR.net Protocol B trial reported only one case of endophthalmitis in 3159 triamcinolone injections (0.03% per injection) [19] though smaller series have reported higher incidence rates primarily because of the smaller numbers of injections. The cause of sterile or noninfectious endophthalmitis is unknown, but some investigators believe that it may be due to preservatives. Pseudoendophthalmitis occurs when the crystalline triamcinolone suspension diffuses rapidly throughout an eye that has previously undergone vitrectomy; fortunately this is a self-limited condition.

The incidence of endophthalmitis following insertion of the dexamethasone insert appears to be low. In the MEAD trial, 2928 DEX injections were performed, with only two cases of endophthalmitis [12]. One of these occurred after cataract surgery and was felt to be unrelated to the DEX.

9.6 Counterfeit Drugs

Bevacizumab ranks seventh in world drug sales (by dollars) with most of this stemming from oncologic use [56]. Because bevacizumab is manufactured as a clear liquid, it has become an easy target for counterfeiters. Counterfeit bevacizumab naturally lacks biological efficacy and the substitute solution may be contaminated with microbes or pro-inflammatory substances. Though intravenous injections of counterfeit bevacizumab may not cause observable adverse events, intraocular injections have caused both infectious endophthalmitis and severe sterile inflammation with retinal necrosis.

Series of patients receiving intravitreal injections of counterfeit bevacizumab have been reported in China [75], Mexico [25], and India [69]. Counterfeit bevacizumab has entered the supply chain in the United States though no injuries were reported [76].

Bevacizumab should always be purchased from a reputable supplier, and physicians and pharmacists must carefully scrutinize bottles for authenticity to be sure that they are not using counterfeit products.

9.7 Other Complications

Retinal detachments (RDs) are frequently cited as complications of intravitreal injections, but they occur only rarely. A systematic review put the RD rate at 3.9% per eye and 0.9% per injection [34], but most of these detachments were believed due to vitreoretinal traction from the underlying disorder rather than due to the injection procedure. Detachment rates from the phase III DME registration trials, from which eyes with significant fibrovascular proliferation were excluded, are generally much lower.

Intraocular hemorrhages (retinal, vitreous, or hyphema) have been reported after intraocular injections, but like RDs, these are mostly due to the underlying diabetic retinopathy. The incidence of vitreous hemorrhage in RISE and RIDE was only 0.4% in the treatment arms compared to 2.8% in the sham arm [54], suggesting that most hemorrhages were due to progression of the diabetic retinopathy. Subconjunctival hemorrhages are quite common (10%), particularly in patients taking aspirin (17%) [41]. Most studies do not report an increased incidence of intraocular hemorrhages in patients taking systemic anticoagulants [42, 44], and special precautions are not required when injections are performed in these patients.

Considerable in vitro and in vivo research has been done to determine whether anti-VEGF drugs are cytotoxic. Neither bevacizumab nor ranibizumab is toxic to the corneal endothelium when injected intravitreally into patients with DME [32]. Repeated intravitreal injections of ranibizumab for the treatment of nAMD do not appear to adversely affect retinal nerve fiber layer thickness [21], and intravitreal bevacizumab injections into rabbit eyes do not cause retinal apoptosis [71]. Consistent cytotoxicity patterns have not been observed for any of the drugs.

Rapidly worsening traction retinal detachments, the “crunch syndrome,” have been reported after injections of bevacizumab into eyes with preexisting fibrovascular proliferation due to proliferative diabetic retinopathy. These may occur because rapidly falling VEGF concentrations lead to increasing connective tissue growth factor levels and a switch from angiogenesis to fibrosis [40]. In one report, the incidence of traction retinal detachment after bevacizumab was 11 out of 211 injections (5.2%), with 82% of these occurring within 5 days of the injections [4].

Early reports suggested that anti-VEGF injections might decrease retrobulbar blood flow, constrict retinal arterioles, and worsen macular ischemia [55]. Macular perfusion studies, however, have not demonstrated a significant decrease in macular perfusion [49], and current observations suggest that anti-VEGF injections may actually improve retinal capillary blood flow.

Migration of the dexamethasone insert into the anterior chamber has been reported in eyes without an intact lens-iris diaphragm. Though 28-gauge bimatoprost SR inserts (constructed with the same design as the dexamethasone inserts) are designed to settle safely into the anterior chamber angle, the larger dexamethasone insert fits poorly and causes significant corneal edema. Dexamethasone inserts may be injected into eyes with in-the-bag intraocular lenses and YAG capsulotomies, but they should be avoided in aphakic eyes and those with sutured posterior chamber intraocular lenses.

9.8 Systemic Complications

Intravenous injections of VEGF inhibitors lower serum VEGF concentrations [74] and increase the risks of VEGF-related complications. Systemic arterial hypertension and proteinuria are the most commonly seen adverse effects, and delayed wound healing, gastrointestinal bleeding, and Antiplatelet Trialists' Collaborative (APTC) defined events such as myocardial infarction and stroke are less frequently seen (Fig. 9.6) [57, 61]. Patients with advanced solid carcinomas have double the risk of stroke when receiving intravenous bevacizumab.

The Pan-American Collaborative Retina Study Group (PACORES) found that 7 of 1173 patients (0.6%) developed transient systemic arterial hypertension between 7 h and 2 weeks after intravitreal bevacizumab injections [77]. Strokes and myocardial infarctions occurred in 1.2% and 0.4% of patients, respectively.

The phase II and III DME trials were designed to demonstrate the clinical superiority of anti-VEGF therapy over laser photocoagulation/sham injections, but they were insufficiently powered to detect infrequently occurring APTC events. Meta-analyses of large treatment populations have generally determined that intravitreal anti-VEGF therapy is not associated with an increased risk of systemic complications [78], but many of these studies have not focused on higher-risk populations such as patients with diabetes mellitus [13, 50, 65, 70, 72, 73]. Data from several studies

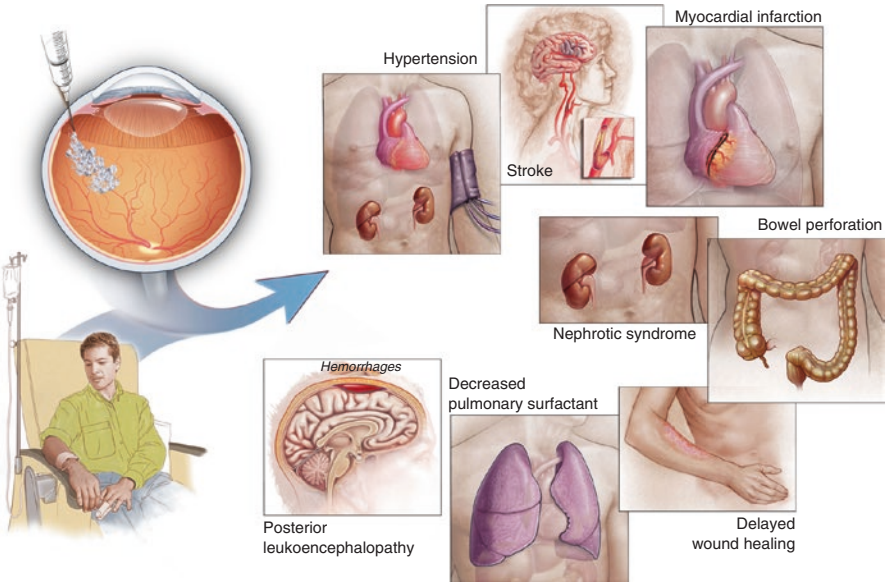

Fig. 9.6 Systemic adverse events related to vascular endothelial growth factor binding have been reported after intravenous and intravitreal drug injections. Systemic arterial hypertension, proteinuria, and Antiplatelet Trialists' Collaborative defined events (myocardial infarction and cerebrovascular event) are of particular concern in diabetic patients receiving intravitreal therapy

have shown that serum concentrations of VEGF fall after intravitreal injections of bevacizumab and aflibercept (but not ranibizumab), thus providing a plausible mechanism for the development of some VEGF-related systemic adverse events [5, 15, 75, 79, 80]. Associations between low VEGF concentrations and higher risks of APTC events, however, have never been established.

In the phase II RESOLVE trial, the incidences of hypertension and arteriothrombotic events were similar in the ranibizumab and sham groups [45]. The 1-year results in the RISE and RIDE trials showed a dose-dependent, increased incidence of stroke with ranibizumab. When also considering that the 0.3 mg and 0.5 mg ranibizumab doses were similarly effective for DME, the US FDA approved the 0.3 mg dose for DME. At 3 years in the RISE and RIDE trials, the incidences of APTC-related events in the sham/laser, 0.3 mg ranibizumab, and 0.5 mg ranibizumab groups were 7%, 11%, and 10%, and the overall incidences of death were 3%, 4%, and 6%. The causes of death were typical of a cohort with advanced diabetes mellitus. These data must be considered carefully since most patients in the sham/laser group crossed over to monthly 0.5 mg ranibizumab at 24 months and had 1 year of drug exposure by the study's conclusion. The use of the lower ranibizumab dose may be of particular importance in a diabetic population that is at risk of APTC events since over 50% of patients may require bilateral treatment.

Increased risk of death and cerebrovascular events was not seen in patients receiving 0.5 mg ranibizumab in the DRCR.net Protocol I trial [23]. Patients in this trial were not required to receive monthly injections, so total exposure to ranibizumab was considerably lower than in RISE and RIDE. In the phase III VISTA and VIVID trials, APTC events occurred in 3.3% of patients receiving aflibercept and 2.8% of patients treated with laser/sham [39].

A Cochrane meta-analysis evaluating the safety of anti-VEGF drug use for DME did not find an increased incidence in non-ocular serious adverse events [73]. The relative risks of anti-VEGF-treated patients experiencing serious systemic adverse events (2985 patients from 15 trials, relative risk (RR) 0.98, 98% confidence interval (CI) 0.83 to 1.17), arterial thrombotic events (3034 participants from 14 studies, RR 0.89, 95% CI 0.63 to 1.25), and overall mortality (3562 participants, RR 0.88, 95% CI 0.52 to 1.47) were all insignificant.

Primary analysis of the DRCR.net Protocol T trial detected no significant differences in adverse events between patients treated with aflibercept, bevacizumab, and ranibizumab, but a post hoc analysis found a higher incidence of cardiovascular events in patients treated with ranibizumab (17%), as compared to aflibercept (9%) and bevacizumab (9%) ($P = 0.01$) [43].

A meta-analysis of phase II and III trials in which aflibercept had been administered for several conditions found that the risk of death in aflibercept-treated patients with DME was 1.70 per 100 patient-years at risk compared to 0.55 per 100 patient-years at risk for patients in the sham/laser groups [38].

In the first year of the VISTA and VIVID trials, the incidences of ocular and non-ocular adverse events and serious adverse events including APTC-defined events and deaths were similar among all groups [39]. Serious non-ocular adverse events were uncommon (hypertension, 9.7%; cerebrovascular accidents, 1.1%; and myocardial infarction, 1.1%). The incidences of noninfectious intraocular

inflammation were <0.5% in each trial, and there were no incidences of infectious endophthalmitis. The incidences of congestive heart failure and anemia were higher in the aflibercept groups, but the incidences of myocardial infarction and osteoarthritis were higher in the laser groups. The total numbers of deaths due to vascular and non-vascular causes were similar among all groups. Through 148 weeks in VIVID and VISTA, 6.8% eyes in the aflibercept arms and 4.5% of eyes in the laser arms experienced significant ocular adverse events, and 47.6% and 48.7% of patients, respectively, experienced serious systemic adverse events.

A meta-analysis was performed to examine the systemic safety profile of anti-VEGF agents in patients with several chorioretinal vascular conditions [6]. Higher risks for death, CVA, and impaired wound healing were found only in patients with DME. The authors performed a subsequent systematic review and meta-analysis to evaluate the systemic safety of monthly (the most intensive dosing regimen) intravitreal anti-VEGF injections administered to patients with DME over the course of at least 2 years [7]. Four studies (two using monthly aflibercept and two using monthly ranibizumab) with a total of 1328 patients met the authors' entry criteria and were included in the analysis. In the combined analysis, patients receiving monthly injections had an increased risk for death (odds ratio of 2.98; 95% confidence interval: 1.94–5.22; $P = 0.04$) compared to those receiving sham injections or laser. However, no significantly increased risk of myocardial infarctions or arteriothrombotic events could be identified.

Determining the risk of developing APTC events with anti-VEGF therapy has been difficult since patients with recent (generally <6 months) myocardial infarctions or strokes have been excluded from phase III trials. Physicians, therefore, have very little guidance regarding the safety of anti-VEGF therapy in high-risk patients that have experienced recent thromboembolic events. When considering treatment for a patient with neovascular age-related macular degeneration, for which there are no good alternatives to anti-VEGF drugs, physicians will generally provide appropriate informed consent that includes disclosure of possible systemic risks and then will usually proceed with injections. Fortunately for patients with DME, intravitreal corticosteroids are an effective alternative to anti-VEGF drugs, without known systemic risks.

9.9 Conclusion

The first intravitreal injection was performed over 100 years ago, but only with the use of anti-VEGF drugs during the last 11 years has the performance of intravitreal therapy become a common procedure. Fortunately, anti-VEGF treatment of diabetic macular edema is effective and safe. Most treatment-associated adverse events are related to the injection itself and are minor. Patients with diabetes are at higher risk for APTC events, but there is no conclusive evidence that anti-VEGF injections increase the occurrence rates, and neither is there evidence of different rates among the drugs. Intraocular corticosteroids do not cause systemic adverse events, but rates of cataract progression and glaucoma development must always be considered.

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Chapter 10
Socioeconomic Cost of Diabetic Retinopathy and Therapy

10.1 Introduction

Diabetes mellitus (DM) significantly impacts quality of life [8, 16, 48] with affected patients unable to manage their diabetes as well when they are visually impaired [6]. Vision impairment compromises quality of life by limiting physical activity, promoting social isolation, and causing dependence on others for the performance of many of life's necessary functions [43]. Patients with diabetic macular edema (DME) also have higher rates of healthcare utilization (doctors' visits, hospitalizations, diagnostic testing, treatments, and medications) compared to diabetic patients without diabetic retinopathy [20, 26, 44]. A US health quality study reported that diabetes is associated with a utility of 0.53, whereas blindness has a utility of only 0.38, with only major stroke (0.31) and end-stage renal disease (0.35) ranking lower [19]. By identifying patients with diabetic retinopathy (DR), physicians are frequently able to stabilize and improve vision, thereby improving the quality of patients' lives. Improvement in visual acuity often enables patients to resume driving and obtain gainful employment.

For decades, the standard treatment for most patients with vision-threatening DR was laser photocoagulation, with pars plana vitrectomy reserved for advanced vitreoretinal pathologies. As discussed in other chapters in this text, laser reduces the risk of vision loss by approximately 50% in most patients with DME and proliferative diabetic retinopathy, but does not usually improve visual acuity. Maintaining good visual acuity also depends upon early diagnosis through effective screening programs, regularly scheduled examinations, and prompt examination and treatment of symptomatic and at-risk patients.

Intravitreal pharmacotherapy with corticosteroids and drugs that inhibit the actions of vascular endothelial growth factor (VEGF) is capable of significantly improving vision in a majority of treated patients. Physicians in several countries have studied the cost-effectiveness of diabetic retinopathy treatments, and their conclusions vary according to the drug costs in each country and the assumptions used to create each of the respective economic models. All generally agree, however, that

M.W. Stewart, *Diabetic Retinopathy*, DOI 10.1007/978-981-10-3509-8_10

treatment of DR with laser and pharmacotherapy is cost-effective. The cost of drugs in each country remains a major determinant of cost-effectiveness, and future models will have to contend with the major price differences between compounded bevacizumab and commercially provided ranibizumab and aflibercept. In some countries, this comparison is moot because intraocular injection of compounded bevacizumab is prohibited by regulatory authorities.

The costs associated with intravitreal pharmacotherapy can be considerable and they can impact private insurance companies and national health systems. This chapter examines the costs of therapy and the subsequent effects on insurance and healthcare policy.

10.2 National Costs

In the United States, diabetes accounts for 10% of healthcare spending and is the leading cause of new blindness in working aged adults [5]. Approximately 75,000 new cases of DME are diagnosed yearly and medical costs for these patients are 29% higher than for diabetic patients without DME. Inpatient care comprises nearly half of these costs [44].

The pharmaceutical costs associated with the treatment of DME in the United States are considerable. Insurance payments for intravitreal drugs administered to Medicare patients are paid through Part B. In 2010, when intravitreal pharmacotherapy was being used predominantly for the treatment of neovascular age-related degeneration, VEGF inhibitors accounted for $2 billion or one-sixth of the US Medicare Part B drug budget [49]. By 2013, Part B expenditures for ranibizumab and aflibercept totaled $2.5 billion [50]; during calendar year 2014, ranibizumab ($1.3 billion) and aflibercept ($1.3 billion) were the second and third most expensive drugs (behind rituximab) (Table 10.1). The majority of these payments were for the treatment of neovascular age-related macular degeneration, but an increasing proportion was for DME.

Table 10.1 The 2014 Medicare Part B expenditures for the top five drugs

Generic and brand names	Major indications	Total spending
Rituximab (Rituxan)	Non-Hodgkin lymphoma, chronic lymphocytic leukemia	$1.4 Billion
Ranibizumab (Lucentis)	Neovascular AMD, DME, edema due to RVOs	$1.3 Billion
Aflibercept (Eylea)	Neovascular AMD, DME, edema due to RVOs	$1.3 Billion
Infliximab (Remicade)	Rheumatoid arthritis, psoriasis, Crohn's disease	$1.2 Billion
Pegfilgrastim (Neulasta)	Myelosuppressive chemotherapy adjunct, myelosuppression	$1.2 Billion

Note that ranibizumab and aflibercept rank second and third
AMD age-related macular degeneration, *DME* diabetic macular edema, *RVO* retinal vein occlusions

The costs of healthcare in Canada have risen considerably faster over the past few decades than the gross domestic product, and the expenditure on vision care has risen even faster, from 1.8% of total health expenditures in 1975 to 2.2% in 2007 [4]. The annual cost of vision loss in Canada due to age-related macular degeneration (AMD), diabetic retinopathy (DR), cataract, glaucoma, and refractive error was estimated at $15.80 billion (2007), with $8.6 billion resulting from direct healthcare expenditures, $4.4 billion from productivity loss (lower employment, higher absenteeism, and premature death), $1.8 billion from dead weight losses (welfare payments and lost taxation), $0.7 billion for care of people with vision loss, $305 million due to aids and home modifications, and $11.7 billion due to lost well-being. This equates to an annual financial cost of $19,370 per affected person, or $33,704 if the value of well-being is included [7]. The prevalence of vision loss is expected to increase from 2.5% of the population in 2007 to 4.0% in 2032.

The number of people over the age of 11 years with diabetes mellitus in England was estimated at 2,334,550 in 2010 with 166,325 (7.12%) believed to have DME. The total cost of health and social care attributed to these patients was £116,296,038 [28].

The cost to the German statutory health insurance system of caring for the ocular complications of diabetes was about €2.23 billion in 2002. German patients with DME use almost twice the medical resources as those with only mild retinopathy [15].

10.3 Cost-Effectiveness of Treating Diabetic Macular Edema

The concept of "cost-effectiveness" only has meaning when a particular intervention is compared to a standard [24]. Policy makers in the United States have generally not established formal standards for cost-effectiveness though they probably exist in an unpublished form [34]. Some other nations base standards of effectiveness on the quality-adjusted life year (QALY), a measure of health-related quality of life in which every health state is assigned a value between 1 (perfect health) and 0 (death) [12]. For ophthalmic conditions, most models are based on health states determined by visual acuities. A cost of $50,000/QALY is generally accepted as a willing-to-pay cost-effectiveness standard [17, 53], but some authors have suggested an upper limit of $100,000/QALY [25] (Fig. 10.1). The United Kingdom's National Institute for Health and Care Excellence (NICE) generally approves interventions with cost-utility ratios of less than £20,000/QALY (approximately $32,000/QALY) but usually requires strong reasons to approve interventions costing more than $48,000/QALY. Some researchers have suggested that the United States should be willing to spend $100,000/QALY or more [52]. The World Health Organization recommends that interventions that cost less than the per capita gross domestic product per individual of a country per disability-adjusted life year are very cost-effective [51]. An intervention that costs less than 3 times the gross domestic product per disability-adjusted life year is considered cost-effective. According to these standards, ranibizumab remains cost-effective in the United States if 3.5 or fewer

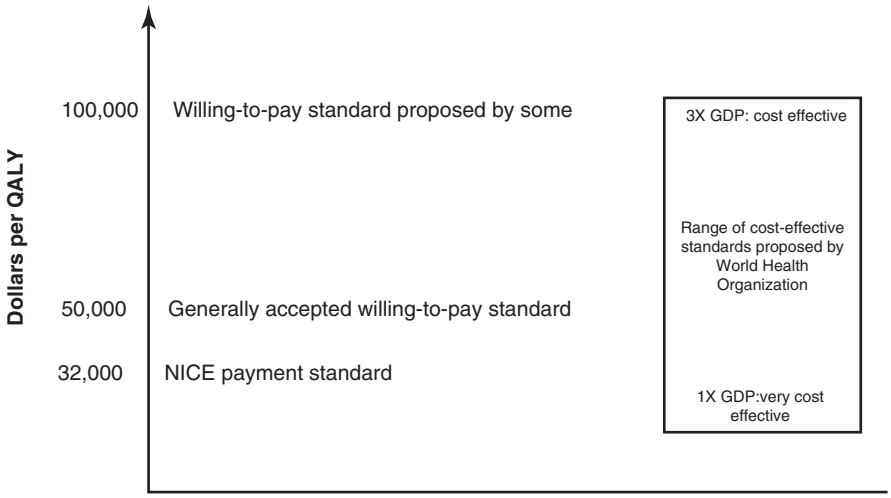

Fig. 10.1 The recommendations of cost-effectiveness standards made by several groups and government agencies. Some analysts have proposed that standards of over $100,000/QALY are still cost-effective. *QALY* quality adjusted life year, *NICE* United Kingdom's National Institute for Health and Care Excellence, *GDP* gross domestic product

injections are required each year from years 3 through 14. Data from The Diabetic Retinopathy Clinical Research network Protocol I suggests that fewer injections are likely to be given after the first year of DME treatment, thereby suggesting that ranibizumab is cost-effective over extended periods of time [11]. Though cost-effective analyses are common in US medical literature, legislation in the United States has banned the use of QALY for making insurance coverage decisions [35].

Laser photocoagulation and vitrectomy surgery are usually considered durable therapies for DR. Using data from the Early Treatment Diabetic Retinopathy Study [13], laser photocoagulation was estimated to produce a gain of 0.236 QALY and was considered to be highly cost-effective compared to no treatment [43]. Though repeat sessions of laser and return visits to the operating room are not uncommon for patients with advanced diabetic retinopathy, the total number of these interventions is generally far fewer than the number of required intravitreal anti-VEGF injections. Randomized, prospective trials suggest that patients require at least 15 anti-VEGF injections over the course of 3 years, and RISE and RIDE featured monthly injections for 36 months. In most vitreoretinal practices, physicians individualize therapy with pro re nata (PRN) or treat-and-extend (T&E) regimens. Compared to monthly injections, both strategies decrease the number of injections and T&E also decreases the number of clinic visits. Both regimens decrease the total direct costs for treating DME.

Treatment costs vary considerably depending upon the choice of drugs [42]. In the United States, compounded bevacizumab costs approximately $60 per dose, ranibizumab (0.3 mg) costs $1170 per dose, and aflibercept costs $1850 per dose [49, 50]. Triamcinolone (Kenalog®) costs only $49 per dose, the dexamethasone

insert costs $1371 per dose, and the fluocinolone insert costs $8800 per dose. The total lifetime cost for one patient treated with anti-VEGF therapy has been estimated at $133,126. The cost to achieve one line of visual acuity improvement per year ranges from $60 to $561 [46]. When analyzed according to the incremental cost-effective ratio (ICER) per cost of QALY gained, each of the anti-VEGF drugs decreases costs compared to no treatment [18].

No trials have directly compared all of the available treatment options and regimens, and few have detailed the costs of therapy. The cost-effectiveness of therapy varies according to the authors – their assumptions and biases. The United Kingdom's NICE evaluated an industry-sponsored study of ranibizumab for the treatment of DME and concluded that the cost-effectiveness of anti-VEGF therapy is not convincingly superior to laser photocoagulation [32]; two other studies, however, reached the opposite conclusion though they did not compare all treatment options or calculate lifetime costs [10, 29]. In the final step of NICE's assessment of the cost-effectiveness of ranibizumab for DME, NICE used a preplanned subgroup threshold analysis to find that the ICER for ranibizumab treatment fell below the willing-to-pay threshold only for eyes with central retinal thickness greater than 400 μm at baseline. By approving ranibizumab use only for this subgroup, NICE effectively halved the potential payment budget for the treatment of DME [33].

Data from the RESTORE trial were used to create one of the first cost-effective analyses for the treatment of DME [29]. Using a Markov model, the authors determined that ranibizumab monotherapy resulted in a 0.17 QALY gain at an incremental cost of £4191 relative to laser monotherapy; this yields an ICER of £24,028. A probabilistic analysis reported a 64% probability that ranibizumab was cost-effective at a threshold of £30,000 per QALY. Combined ranibizumab and macular laser photocoagulation results in a 0.13 QALY gain over laser monotherapy at an incremental cost of £4695 with an ICER of £36,106. They concluded that ranibizumab monotherapy is cost-effective, but the cost-effectiveness of ranibizumab combined with laser photocoagulation is less certain because of higher costs and lower effectiveness. If a 40-year time line were used with an average patient age of 47 years – as in the study by Sharma – then the RESTORE model would predict a 0.26 QALY and an ICER of £10,412 for ranibizumab compared to laser.

A Markov model was used to determine the cost-effectiveness of different treatments (laser photocoagulation, intravitreal injections of triamcinolone acetonide, intravitreal injections of anti-VEGF drugs, or a combination of both) for DME [36]. The model determined that all treatments except laser monotherapy substantially reduced costs, and all treatments except triamcinolone increased quality-adjusted life years (QALYs). Combined laser photocoagulation and anti-VEGF therapy produced the greatest benefit by gaining 0.56 QALYs at a cost of $6975 for an incremental cost-effectiveness ratio per QALY compared to laser plus triamcinolone. Anti-VEGF monotherapy was similarly cost-effective to laser and anti-VEGF combination therapy. The authors concluded that anti-VEGF monotherapy or in combination with laser photocoagulation was the most effective treatment strategy for DME, and both compare favorably with cost-effective interventions for other conditions. According to the authors, the decision to add laser photocoagulation to

anti-VEGF therapy should probably be made according to each patient's preference. In most cases, adding laser to an anti-VEGF regimen does not incrementally improve VA beyond that achievable with anti-VEGF monotherapy. Laser minimally extends the durability of anti-VEGF therapy but introduces additional risk of permanent macular complications. Because of its low unit dose cost, the cost-effectiveness of bevacizumab exceeds that of ranibizumab. Absolute visual acuity gains with ranibizumab, however, are generally greater in eyes with moderate to severe vision loss, and this needs to be considered when choosing therapy.

A computer simulation cost-effectiveness analysis was based on 2-year data from DRCR.net Protocol I [10]. The authors calculated several ICER values in terms of dollars per letter: $393 (sham + laser vs. triamcinolone + laser); $5943 (ranibizumab + prompt laser vs. sham + laser); and $20 (ranibizumab + prompt laser vs. ranibizumab + deferred laser). In pseudophakic patients, the ICER value for ranibizumab + deferred laser (compared to triamcinolone + laser) was $14,690. In phakic patients, ranibizumab + deferred laser produced an additional 6 letters of visual acuity at a cost of $19,216 compared to triamcinolone + laser. The authors concluded that intravitreal triamcinolone acetonide appears to be the most cost-effective treatment for DME in pseudophakic eyes.

Using results from several of the earlier clinical trials, a cost-benefit analysis compared several treatments [46]. Not surprisingly, the use of lower cost drugs such as bevacizumab and triamcinolone proved to be most cost-effective [45].

A 14-year cost-utility analysis for the treatment of DME with ranibizumab was performed with data from the RISE and RIDE trials [2]. This analysis not only assessed the costs of ranibizumab therapy, but it contrasted these to savings gained from lowered vision-related healthcare costs. The 14-year gain attributed to ranibizumab therapy was 0.9981 QALYs, which produced an 11.6% improvement in quality of life. The direct ophthalmologic cost to treat one eye was $30,116 and for both eyes was $56,336. The decrease in direct nonophthalmologic medical costs from lower incidences of depression, injury, skilled nursing home admissions, and other costs associated with vision were $51,758. This resulted in a net medical cost of $4578. The mean cost to society for bilateral ranibizumab therapy was −$30,807, consisting of $31,406 in savings due to decreased caregiver costs and $3978 in decreased wage losses. The third-party insurer's cost-utility ratio was $4587/QALY, and the societal cost perspective for bilateral therapy was −$30,807/QALY. This demonstrated that ranibizumab therapy not only produced a positive QALY of 0.9981 but also a positive financial gain of $30,807. The authors stated that ranibizumab produces a much greater gain than the 6–9% attributed to β-blocker treatment of systemic arterial hypertension, the 4–6% gain attributed to statin treatment of hyperlipidemia [1], and the 1% gain attributed to the use of bisphosphonates for osteoporosis [3].

A Markov model using RESTORE data was developed to determine the cost-effectiveness of DME treatment with ranibizumab monotherapy, ranibizumab in combination with laser photocoagulation, and laser monotherapy for the province of Quebec [14]. The ICERs for ranibizumab monotherapy and combination therapy compared to laser monotherapy were CA$24,494 and CA$36,414, respectively. The yearly incremental costs for ranibizumab monotherapy and combination therapy

without legal blindness were CA$15,822 and CA$20,616. The authors concluded that both ranibizumab treatment regimens were cost-effective and both increased the time without blindness.

The cost-effectiveness of ranibizumab + laser was compared to laser monotherapy with 5-, 7-, and 10-year time horizons in Mexico [41]. The ICERs for combination therapy vs. laser were $5019, $2375, and $622 at each time point, respectively. The authors concluded that ranibizumab was a cost-effective option at 98.7% vs. monotherapy before reaching the GDP per capita.

Using a US healthcare model with a 25-year horizon, ranibizumab (compared to laser) was found to have an ICER of $89,903 per QALY gained (2013 US dollars) [47]. By comparison, bevacizumab was estimated to cost $11,138/QALY. This suggests that ranibizumab would be cost-effective at a willing-to-pay threshold of $100,000/QALY (but not at $50,000/QALY) if patients received fewer than 0.45 injections annually after year 2, whereas bevacizumab is cost-effective at nearly all willing-to-pay levels.

A Markov model was used to evaluate the cost-effectiveness of the fluocinolone acetonide insert with a 15-year time horizon [31]. The ICER was $38,763, assuming that 40% of patients are treated unilaterally. The insert is cost saving when 100% of patients receive unilateral treatment.

10.4 Comparative Cost-Effectiveness Models

Several studies have compared the cost-effectiveness of the anti-VEGF drugs in different countries, but these have been plagued by competing interests, since many studies were written by employees of the drug manufacturing companies. Markov models are complex, and selecting the necessary inputs requires considerable expertise by the authors. Most of these studies have been published in peer-reviewed journals, but it is not clear that reviewers, many of whom were probably physicians, had the necessary expertise to adequately evaluate all inputs and assumptions [37]. The reader, therefore, is advised to closely scrutinize the methodology used in each of these studies to determine if the assumptions and comparisons made by the authors are appropriate.

A network meta-analysis was used by many of the national studies to determine the efficacies of ranibizumab, aflibercept, and laser photocoagulation [39]. Data were used from the RESTORE (PRN regimen) [30] and RETAIN (treat and extend regimen) [38] trials for ranibizumab and the VIVID and VISTA (monthly ×5 followed by bimonthly) [22] for aflibercept. Importantly, PRN and T&E regimens, which at that time had not been published, were not modeled for aflibercept.

Using a Markov model previously reviewed by NICE and based on data from the RESTORE study, the costs of treating DME in the United Kingdom were estimated [40]. A 3-year treatment period with a lifetime horizon was assumed. The lifetime costs for treating patients with DME were £20,019 (PRN), £22,930 (T&E), and £25,859 (aflibercept q8weeks). The authors claimed that ranibizumab produced an

incremental gain of 0.05 QALYs over aflibercept and produced cost savings of £5841 (PRN) and £2930 (treat-and-extend) compared to aflibercept.

A Markov model was used to compare the cost-effectiveness of PRN and T&E ranibizumab against bimonthly aflibercept (after 5 monthly injections) in Greece [23]. The authors concluded that ranibizumab offered cost savings of €2,824 and €22 for each regimen over aflibercept and produced greater gains in QALYs (+0.05) and more time without visual impairment. The net monetary benefits were €3,984 and €1,278. They stated that ranibizumab should be the dominant treatment option for the treatment of DME in Greece.

A Markov model was used to compare the cost-effectiveness of intravitreal aflibercept vs. ranibizumab in Turkey [9]. Results from VIVID and VISTA and RESTORE and REVEAL were used to construct the model. The total annual costs of aflibercept and ranibizumab therapy were 15,315 and 14,791 Turkish Lira, respectively, and QALYs were 7343 and 7295. Aflibercept was deemed to be cost-effective compared to ranibizumab at a cost-effectiveness threshold of 26,415 TL. The expected number of years with one blind eye was 0.416 with aflibercept and 0.647 with ranibizumab. The authors concluded that aflibercept is a cost-effective option for the treatment of DME in Turkey compared to ranibizumab.

A Markov model was used to compare the cost-effectiveness of ranibizumab and aflibercept in the Czech Republic [21]. The authors concluded that there is a 62% probability that ranibizumab brings more quality than aflibercept at a lower cost.

The DRCR.net performed a cost-effectiveness analysis based on its data from the Protocol T trial [54]. Whereas Protocol T found that for patients with VA of 20/40 or better, bevacizumab, ranibizumab, and aflibercept were equally effective, for patients with VA of 20/50 or worse, aflibercept produced better VA gains at 1 year, though the statistical significance compared to ranibizumab had vanished at 2 years. The network determined that during year 1 the ICERs of aflibercept and ranibizumab compared with bevacizumab were $1,110,000 and $1,730,000 per QALY, respectively. Extrapolating to 10 years, they were $349,000 and $603,000 per QALY, respectively. Aflibercept's ICER compared to ranibizumab was $648,000 per QALY at 1 year and $203,000 per QALY at 10 years. The 10-year ICERs of aflibercept and ranibizumab compared to bevacizumab at 10 years were $287,000 and $817,000, respectively. These numbers indicate that though aflibercept and ranibizumab are more effective than bevacizumab for the treatment of DME, their relative costs exceed the commonly accepted $100,000/QALY threshold. Interestingly, even if single doses of bevacizumab were drawn from each 4 ml vial and the rest of the vial was discarded, bevacizumab is still more cost-effective than aflibercept and ranibizumab.

The cost-effectiveness of ranibizumab for the treatment of PDR was recently performed based on the DRCR.net Protocol S data [27]. The authors found that the 2-year cost was $13,053 with a cost per QALY of $7988 when pan-retinal photocoagulation was the primary treatment, compared to a 2-year cost of $30,328 and a cost per QALY of $19,150 when ranibizumab was the primary treatment. When extrapolated to lifetime therapy, ranibizumab had a cost per QALY of $138,852 to $164,360.

10.5 Future Considerations

The treatment of DME has been shown repeatedly to be cost-effective, but the high aggregate costs due to the large and growing number of affected individuals have already influenced healthcare policy. The treatment landscape is complicated because of bevacizumab, since some countries forbid its use because of its off-label status despite its demonstrably low cost and high cost-effectiveness. Safety issues pertaining to compounding-related sterility and the penetration of counterfeit drugs into the market has created a patchwork of drug availability throughout the world. Physicians in some countries are able to safely use bevacizumab as first-line therapy, whereas bevacizumab cannot be used in other countries, and approved anti-VEGF therapy is limited by inequalities in cost and income.

Safe, universally available, cost-effective therapy is needed throughout the world, but easy solutions are not available. Pars plana vitrectomy has emerged as a low-cost alternative to pharmacotherapy, but despite 25 years of experience, effectiveness has not been consistently demonstrated, and level I evidence supporting its efficacy is lacking.

Drugs are being developed to target new pathogenic pathways, but there is no evidence that these will be less expensive than approved anti-VEGF drugs. Extended duration therapy with longer acting drugs or sustained release devices, if they prove to be effective, will likely decrease the number of clinic visits but these therapies will likely be generally priced higher because of their durations of action.

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