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EXPERT REVIEWS

Pathophysiology and management of diabetic retinopathy

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Diabetic retinopathy remains a major cause of worldwide preventable blindness. In this review, we evaluate the recent advances in understanding the molecular mechanisms of diabetic retinopathy, highlight the current management of diabetic retinopathy and new therapeutic approaches, and discuss the range of potential future therapeutic strategies in order to combat the disease. The microvasculature of the retina responds to hyperglycemia through a number of biochemical changes, including the activation of PKC, increased advanced glycation end-products formation, polyol pathway and oxidative stress, and activation of the renin–angiotensin system. There is an accumulating body of evidence that inflammation and neurodegeneration play a prominent role in the pathogenesis of diabetic retinopathy. Strict metabolic control, tight blood pressure control, laser photocoagulation and vitrectomy remain the standard care for diabetic retinopathy. Emerging therapies include intravitreal triamcinolone or anti-VEGF agents, ruboxistaurin, renin–angiotensin system blockers, fenofibrate, islet cell transplantation, PPAR-γ agonists and intravitreal hyaluronidase. However, more randomized, controlled clinical trials are required to clarify their role alone or in combination.

Keywords: advanced glycation end product • diabetic retinopathy • inflammation • neurodegeneration • oxidative stress • PPAR-γ • protein kinase C • renin–angiotensin system • treatment

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Learning objectives

Upon completion of this activity, participants should be able to:

- Identify factors that contribute to the development of diabetic retinopathy
- Prevent diabetic retinopathy effectively
- Describe emerging oral treatments for diabetic retinopathy
- Compare ocular treatments for diabetic retinopathy

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Diabetic retinopathy (DR) is the most common microvascular complication of diabetes and remains one of the leading causes of blindness worldwide among adults aged 20–74 years. The two most important visual complications of DR are diabetic macular edema (DME) and proliferative DR (PDR). The prevalence of DR increases with the duration of diabetes, and nearly all people with Type 1 diabetes, and more than 60% of those with Type 2, have some retinopathy after 20 years. In the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), 3.6% of younger onset patients (Type 1 diabetes) and 1.6% of older onset patients (Type 2 diabetes) were legally blind [1].

Pathophysiology of diabetic retinopathy

The retina consists of a number of cells, and normal vision depends on intact cell–cell communication among them. Diabetes damages all the major retinal cells of the retina, vascular cells (endothelial cells and pericytes) [2,3], neurons (photoreceptors, bipolar, horizontal, amacrine and ganglions) [4–7], glia (Müller cells and astrocytes) [5,6,8], microglia [9] and pigment epithelial cells [10]. Before damage, these cells are activated, which changes the production pattern of a number of mediators, such as growth factors, vasoactive agents, coagulation factors and adhesion molecules, resulting in increased blood flow, increased capillary permeability, proliferation of the extracellular matrix and thickening of basal membranes, altered cell turnover (apoptosis, proliferation, hypertrophy), procoagulant and proaggregant patterns and, finally, in angiogenesis and tissue remodeling. The mechanisms of diabetes-induced damage to retinal cells correlate with excessive circulating levels of glucose, lipids, hormones, amino acids and inflammatory molecules. The increased systemic, vitreal and retinal levels of these factors in diabetic patients induce several unrelated and inter-related biochemical pathways and molecules implicated in pathophysiology of the disease, which are briefly discussed in the following sections.

Diabetes Control and Complications Trial (DCCT) [11] and the UK Prospective Diabetes Study (UKPDS) [12] established that hyperglycemia is the initiating cause of retinal damage. Underlying biochemical mechanisms associated with hyperglycemia and identified in diabetic retinas include activation of PKC, increased formation of advanced glycation end products (AGEs), polyol formation, increased hexosamine fluxes, activation of the renin–angiotensin system (RAS) and production of excess reactive oxygen species (ROS). Numerous studies suggested that increase in fluxes through these pathways may lead to a cascade of events, such as promotion of apoptosis, inflammation and angiogenesis, which may, in turn, induce damage to a diabetic retina, leading to DR.

Advanced glycation end products

Among several pathogenic mechanisms that may provide the link between chronic hyperglycemia and the development of DR are the formation and accumulation of AGEs in the diabetic retina, which are known to impair retinal vascular cell function both *in vitro* and *in vivo* [13,14]. AGEs are known to be generated from early glycation products, such as Schiff bases, or their derivative, Amadori-type products, in which amino acids of proteins are nonenzymatically reacted to glucose and other reducing sugars. These early glycation products undergo further complex reactions, such as rearrangement, dehydration and condensation, to become irreversibly crosslinked heterogeneous derivatives, termed AGEs [15].

In diabetic subjects, the serum level of AGEs was associated with the severity of retinopathy [16]. Recently, we demonstrated that AGEs are specifically localized in vascular endothelial cells and stromal cells in epiretinal membranes of proliferative DR patients [17]. AGEs are thought to contribute to various microvascular and macrovascular complications of diabetes mellitus by engaging with the receptor for AGEs (RAGEs) [18,19]. More recently, Wang *et al.* showed that RAGE expression in the retinas of experimental diabetic rats was found to be upregulated predominantly in retinal Müller cells, making RAGE a central modulator in DR [20].

Several lines of evidence have suggested that the AGE–RAGE axis is implicated in most of the pathophysiological processes that lead to DR. AGE–RAGE interaction activates NADPH oxidase and enhances the formation of oxygen radicals, with subsequent activation and translocation of NF-κB, which releases proinflammatory cytokines and growth factors implicated in the pathogenesis of the complications of diabetes, including TNF-α, VEGF, MCP-1 and TGF-β, and adhesion molecules, including VCAM-1 and ICAM-1 [18,19,21].

Blood–retinal barrier breakdown, a characteristic sign of DR, was seen in nondiabetic animals that received intravenous injections of AGEs. Blood–retinal barrier dysfunction was associated with a concomitant increase in retinal VEGF, a central growth factor in PDR [22]. Recently, Warboys *et al.* demonstrated that AGEs produced an increase in retinal capillary permeability that required ROS generated by NADPH oxidase [23]. AGEs stimulated the growth and tube formation of human microvascular endothelial cells, the key steps of angiogenesis. The angiogenic activity of AGEs was mainly mediated by autocrine VEGF, which is upregulated by AGEs in microvascular endothelial cells [24,25]. In addition, through interaction with RAGE, AGEs decreased the number of pericytes, the earliest histopathological hallmark of DR, which would, in turn, relieve the restriction on endothelial cell replication and facilitate angiogenesis [24,25]. *In vivo* studies using the chorioallantoic membrane assay demonstrated that AGEs induce angiogenesis. In this assay, the AGE-induced vascular lumens were devoid of pericytes [26]. The expression of extracellular proteins, such as laminin, fibronectin and types I and IV collagen, was induced by AGEs. This was mediated by induction of fibrogenic cytokines and growth factors, including TGF-β and CTGF [27,28]. TGF-β levels are elevated in the vitreous humor of patients with PDR [29]. We also demonstrated that TGF-β is specifically localized in vascular endothelial cells in PDR epiretinal membranes [17]. Recently, we and others have demonstrated increased expression of the angiogenic and fibrogenic CTGF in human diabetic retina [30], which was consistent with previous reports showing the increased levels of CTGF in retinas of diabetic rats [31]. Expression of CTGF in diabetic epiretinal membranes [30] and elevated CTGF content in the vitreous humor of patients with active PDR have been reported [29,32,33]. Kuiper *et al.* demonstrated a strong correlation between CTGF levels in a vitreous humor sample and the degree of fibrosis in vitreoretinal disorders, suggesting an important role of CTGF in ocular fibrosis [33,34].

Inflammation

A large body of evidence supports the role of proinflammatory cytokines, chemokines and other inflammatory mediators in the pathogenesis of DR leading to persistent low-grade inflammation, and influx of leukocytes contributing to the damage of the retinal vasculature and neovascularization. Leukostasis is the major component of the inflammatory processes, which has been found to be significantly increased in the retinas of diabetic animals and might contribute to the capillary nonperfusion in DR [35,36]. Leukostasis has been postulated to be a factor in the death of endothelial cells and breakdown of the blood–retinal barrier. Diabetic retinal vascular leakage, capillary nonperfusion and endothelial cell damage are associated with leukocyte recruitment and adhesion to the retinal vasculature, which correlate with increased expression of retinal ICAM-1 and elevated expression of the β-integrin subunit, CD18, on neutrophils [35,36]. Joussen *et al.* showed that retinas from diabetic mice lacking ICAM-1 and CD18 are protected from the development of diabetes-induced increase in leukostasis, vascular permeability and degeneration of retinal capillaries. Therefore, these proteins/receptors are important in the development of early stages of DR [35].

In addition, the increased expression of many inflammatory proteins are regulated at the level of gene transcription through the activation of proinflammatory transcription factors, including NF-κB, SP-1, AP-1 and PPARs [37]. In addition, a large body of evidence suggested the involvement of several inflammatory molecules in the pathogenesis of DR, including proinflammatory cytokines, such as TNF-α, IL-1β and IL-6, and chemokines, such as MCP-1, IP-10, SDF-1 and IL-8, in addition to other key inflammatory proteins, including inducible nitric oxide synthase (iNOS), COX-2 and matrix metalloproteinase (MMP)-9/gelatinase B .

Increased levels of TNF-α has been found in vitreous fluid of diabetic patients [38], and a strong correlation between the plasma level of TNF-α and severity of DR has been found [39]. The association between serum level of TNF-α and PDR in Type 1 diabetic patients has also been demonstrated [39]. We have shown the expression of TNF-α in vascular endothelial cells and stromal cells in a PDR epiretinal membrane, supporting the mechanistic link between low-grade inflammation and PDR [17]. Several studies demonstrated that expression of TNF-α is increased in the retina of diabetic rats [9,40] and that blockade of TNF-α reduced leukocyte adhesion, suppressed blood–retinal barrier breakdown and reduced ICAM-1 expression [40]. High serum level of TNF-α in diabetic patients complicated with retinopathy and/or nephropathy has been shown to induce endothelial dysfunction [41]. In addition, increased levels of TNF-α in diabetic plasma has been shown to induce leukocyte–endothelial cell adhesion [42]. Increased vascular TNF-α expression in animal models of diabetes induced NADPH oxidase and production of ROS, leading to endothelial dysfunction [43,44]. *In vivo* studies demonstrated that TNF-α enhances angiogenesis [45]. In addition, a recent study showed that TNF-α is required for VEGF-induced endothelial hyperpermeability [46]. Increased levels of IL-1β are detected in the vitreous fluid of the patients with proliferative DR [38] and in the retina from diabetic rats [9,47]. An increased level of IL-6 is detected in vitreous fluid of patients with PDR and DME [48–50].

The CCL2/MCP-1 chemokine has been found to be increased in vitreous humor samples from patients with PDR and DME [49–53]. In addition, we have shown the expression of MCP-1 in myofibroblasts and in the vascular endothelial cells of PDR epiretinal membranes [51]. Several studies demonstrated that MCP-1 is a potent inducer of angiogenesis and fibrosis [54–56]. We, and others, found increased levels of CXCL10/IP-10 in vitreous humor samples from patients with PDR [51,52]. Several studies reported that IP-10 is a potent inhibitor of angiogenesis and may have an inhibitory effect on fibrosis [57,58]. Elevated levels of IP-10 in vitreous humor from patients with PDR, as well as its interaction with its receptor, CXCR3, may negatively regulate fibrosis/angiogenesis in proliferative vitreoretinal disorders [51]. CXCL12/SDF-1 is the predominant chemokine that has been shown to be upregulated in many damaged tissues as part of the injury response and mobilizes stem/progenitor cells to promote repair. Butler *et al.* reported increased SDF-1 levels in vitreous from patients with PDR [59]. We have demonstrated the expression of SDF-1 and its receptor CXCR4 in PDR epiretinal membranes [51,60]. SDF-1 is upregulated in ischemic tissue, establishing an SDF-1 gradient favoring

recruitment of endothelial progenitor cells from peripheral blood to sites of ischemia, thereby accelerating neovascularization. In addition, SDF-1 promotes the chemotaxis of bone marrow-derived $CD34^+$ stem cells and their differentiation into endothelial progenitor cells in ischemic tissue and in tumors [61–63]. Recently, Reddy *et al.* demonstrated that upregulation of SDF-1 in tumors results in the formation of enlarged lumen-bearing, functional blood vessels, implying that this chemokine may influence vascular remodeling via direct action on endothelial cells [62]. They also showed that SDF-1-mediated vasculogenesis may represent an alternative pathway that could be utilized by tumors to sustain growth and neovasculature expansion after anti-VEGF therapy. Several recent studies have demonstrated that the interaction of SDF-1 with its receptor, CXCR4, plays an important role in endothelial progenitor cell migration, differentiation, proliferation and survival [61–63]. IL-8 is an inflammatory and angiogenic mediator that is produced by many cells. The vitreous levels of IL-8 were significantly higher in patients with PDR in comparison to control subjects [53] and in patients with higher extents of large-vessel gliotic obliteration [64].

Increasing evidence strongly supports the role of COX-2 and its metabolic products, PGE2 and TXA2, as regulators of angiogenesis [65]. Recent studies revealed that diabetes is associated with the upregulation of COX-2 in both large vessels and microvessels [66]. Recently, we have demonstrated that COX-2 is specifically localized in vascular endothelial cells and stromal cells in PDR epiretinal membranes [67], which is consistent with the finding that hypoxia increases COX-2 mRNA and protein, with subsequent PGE2 induction in human vascular endothelial cells [68]. In the retina of diabetic animals, the induction of COX-2, as well as increased production of PGE2, has been reported [69,70]. Several studies demonstrated that PGE2 stimulated the expression of VEGF mRNA and protein, and the tube-like formation in endothelial cells [71], treatments of endothelial cells with VEGF, and induced the expression of COX-2 mRNA and proteins and increased PGE2 synthesis [71], suggesting a positive feedback loop for angiogenesis in endothelial cells. These findings suggest that COX-2 might provide the mechanistic link between chronic, low-grade inflammation and angiogenesis in DR.

We have shown increased expression of iNOS in the retinas of human subjects with diabetes [72,73], and others have also demonstrated the expression of iNOS in the retina of diabetic animals [69]. Recently, Leal *et al.* demonstrated that the iNOS isoform plays a predominant role in leukostasis and blood–retinal barrier breakdown [74]. The mechanism involves ICAM-1 upregulation and tight junction protein dowregulation. In addition, diabetic mice deficient in iNOS did not develop leukostasis, superoxide generation, degeneration of retinal capillaries, or cell loss in the ganglion cell layer [75].

Du *et al.* demonstrated that NOS and COX-2 act together to contribute to retinal cell death in diabetes and to the development of DR [69]. Recently, Chan *et al.* demonstrated that good glycemic control that followed poor glycemic control failed to reverse the elevations in the proinflammatory mediators IL-1β, TNF-α, ICAM-1, VCAM-1 and iNOS in the retinas of diabetic rats [76]. Their findings suggest that failure to reverse retinal inflammatory mediators support their important role in the resistance of retinopathy to arrest after cessation of hyperglycemia.

The development of PDR is a multistage event, including an angiogenic process, in which basement membrane degradation, endothelial cell migration and proliferation, followed by capillary tube formation, occur. Such migratory and tissue-remodeling events are regulated by proteolysis mediated by MMPs, among other proteases. Giebel *et al.* showed elevated levels of MMP-2/gelatinase A, and MMP-9/gelatinase B in the retinas of diabetic animals [77]. They demonstrated that elevated expression of MMPs in the retina may facilitate an increase in vascular permeability. Several studies showed the expression of MMP-2 and -9 in PDR epiretinal membranes [30,78]. Immunohistochemical studies demonstrated immunoreactivity for MMP-9 in vascular endothelial cells and myofibroblasts in PDR epiretinal membranes, and *in situ* zymography confirmed the presence of intense gelatinolytic activity in vascular endothelial cells and in scattered cells in PDR epiretinal membranes [30]. In addition, elevated levels of MMP-9 were measured in vitreous humor samples from patients with PDR [79–81]. Recently, we demonstrated that activated MMP-9 might be involved in hemorrhagic transformation in patients with PDR [81].

Peroxisome proliferator-activated receptor-γ

Peroxisome proliferator-activated receptor-γ is a member of the ligand-activated nuclear receptor superfamily and plays a critical role in a variety of biological processes, including adipogenesis, glucose metabolism, angiogenesis and inflammation. Recently, Tawfik *et al.* demonstrated that retinal expression of PPAR-γ was suppressed in experimental models of diabetes and in endothelial cells treated with high glucose [82]. Their findings suggest that PPAR-γ is involved in the pathogenesis of DR. Muranaka *et al.* demonstrated that diabetic mice deficient in PPAR-γ had increased retinal leukostasis and leakage compared with wild-type mice, indicating that endogenous PPAR-γ and its activation by specific ligands is critical in inhibiting leukostasis and leakage in diabetic retinas [83]. In addition, Aljada *et al.* demonstrated that the PPAR-γ agonists pioglitazone and rosiglitazone inhibited VEGF- and basic FGF-induced angiogenesis and also inhibited endothelial cell migration [84]. These studies suggest that PPAR-γ ligands may be useful in the treatment of DR.

Renin–angiotensin system

Induction of diabetes leads to significant increase in retinal expression and production of the RAS components, including angiotensin II, angiotensin II type 1 receptor and angiotensin II type 2 receptor [85]. Recent experimental studies have indicated the association of the RAS with DR. The RAS mediates retinal leukostasis [86] involved in the gene expression of VEGF via NADPH oxidase [87], and contributes to synaptophysin degradation and neuronal dysfunction in the retinas of diabetic animals [88].

Several studies investigated the molecular and cellular mechanisms by which RAS blockers reduce DR. In diabetic mice, diabetes-induced retinal expression of ICAM-1 and VEGF and leukocyte adhesion to the retinal vasculature were suppressed by

blocking angiotensin II type 1 receptor. Inhibition of NF-κB exhibited equivalent effects on these diabetes-induced retinal inflammatory parameters compared with angiotensin II type 1 receptor blockade. *In vitro*, glucose-induced NF-κB activation and upregulation of ICAM-1 and MCP-1 in brain-derived endothelial cells were suppressed by application of an angiotensin II type 1 receptor blocker. In addition, *in vivo* treatment with angiotensin II type 1 receptor blocker or NF-κB inhibitor attenuated diabetes-induced retinal expression of angiotensin II and angiotensin II type 1 receptors [85]. Zheng *et al.* demonstrated that the protective effect of angiotensin-converting enzyme inhibitors on DR was associated with decreased VEGF–PEDF ratio by reducing mitochondrial ROS production [89]. They also found that the decreased ROS production was a result of the angiotensin-converting enzyme inhibitor-induced upregulation of PPAR-γ and UCP-2 expression. Sugiyama *et al.* demonstrated that an angiotensin II receptor blocker may inhibit the development of DR by reducing the accumulation of AGEs and expression of VEGF in the retina [90]. Yamagishi *et al.* demonstrated that olmesartan, an angiotensin II type 1 receptor blocker, inhibited the AGE-elicited angiogenesis *in vitro* by suppressing NF-κB-mediated RAGE expression [91]. Furthermore, olmesartan was found to block the AGE-induced upregulation of VEGF mRNA levels in cultured microvascular endothelial cells. In addition, olmesartan inhibited AGE-evoked ROS generation and reduced the expression levels of MCP-1 in microvascular endothelial cells. Olmesartan also suppressed ICAM-1 expression and, subsequently, blocked T-cell adhesion to AGE-exposed endothelial cells. These findings suggest that olmesartan inhibits AGE-evoked inflammatory reactions in endothelial cells by suppressing ROS generation [92]. Similarly, Chen *et al.* demonstrated that suppressing the activity of endogenous RAS markedly decreased the retinal leukostasis in the retina of diabetic rats associated with a decrease in ICAM-1 gene expression [86]. Recently, Silva *et al.* demonstrated that the angiotensin II type 1 receptor blocker, losartan, prevented oxidative stress, mitochondrial dysfunction and neurodegeneration in the retina of diabetic hypertensive rats [93]. Similarly, treatment with angiotensin II type 1 receptor blocker reduced diabetes-induced neuronal dysfunction in the retinas of diabetic mice [88].

Oxidative stress

The retina has a high content of polyunsaturated fatty acids and has the highest oxygen uptake and glucose oxidation compared with any other tissue in the body. This phenomenon renders the retina more susceptible to oxidative stress. Oxidative stress is elevated in the retina in diabetes, and increased oxidative stress contributes to the development of DR. In the retina, mitochondrial dysfunction is present in hyperglycemic conditions and is an important source of superoxide production [93–95]. Potential sources of ROS are still unclear, although a number of studies showed that high glucose and the diabetic state stimulate flux through the glycolytic pathway, increase cytosolic NADH, increase tissue lactate-to-pyruvate ratios and increases tricarboxylic acid cycle flux, which may flood the mitochondria with electrons, thereby producing excess levels of ROS [96,97].

Oxidative stress, besides creating a vicious cycle of damage to macromolecules by amplifying the production of more ROS, activates other metabolic pathways that are detrimental to the development of diabetic retinopathy. Other sources of oxidative stress are the activation of NADPH oxidase, which may increase levels of superoxide, and, through induction of xanthine oxidase-inhibiting superoxide dismutase, decreased tissue concentration of endogenous antioxidants, such as glutathione, and impaired activities of antioxidant defense enzymes, such as superoxide dismutase and catalase [98,99]. An increase in ROS is considered a causal link between elevated glucose and other metabolic abnormalities important in the development of DR, including the polyol, AGE, PKC and hexosamine biosynthesis pathways, and alteration in the expression of VEGF and IGF-1 [99]. In diabetic mice, overexpression of the enzyme responsible for scavenging mitochondrial superoxide, manganese superoxide dismutase, prevents early lesions of retinopathy [100].

Protein kinase C

Biochemical mechanisms involved in hyperglycemia-induced vascular damage include alterations in cellular signaling by activation of PKC. PKC is a family of serine and threonine protein kinases, which phosphorylate specific target proteins, leading to their activation or deactivation, which is important to signal transduction. They are grouped into three categories based on structure, including conventional calcium and diacylglycerol (DAG)-dependent PKCs, novel calcium-independent but DAG-dependent PKCs, and atypical calcium-independent and DAG-unresponsive PKCs. Both conventional and novel PKC isoforms translocate to the membranous component of cells to exert biological actions in the presence of DAG [101]. Studies have shown that, in both diabetic animals and humans, there is an increase in DAG levels and PKC expression in various tissues, including endothelial, smooth muscle and mesangial cells [102]. Hyperglycemia induces *de novo* synthesis of DAG, which activates many of the PKC isoforms. In addition, PKC can also be activated by AGEs, products of polyol pathways and increased oxidative stress. Numerous studies suggest that increased activation of PKC-β isoforms play an important role in the development of diabetic retinal complication. Activation of PKC-β is associated with basement membrane thickening, changes in blood flow, leukocyte vascular adhesion, extracellular matrix expansion, vascular permeability and angiogenesis, which are all observed in DR [103]. Activated PKC, particularly PKC-β, activates NF-κB and NAPDH oxidases and modifies the expression of endothelial nitric oxide synthase and ET-1, leading to changes in blood flow [104]. PKC activation may also be involved in glucose-induced increases of CTGF and TGF-β expression, leading to extracellular matrix synthesis. Activated PKC also induces synthesis of type IV collagen and fibronectin. Thus, PKC activation induced by hyperglycemia or diabetes increases matrix protein synthesis, causing vascular dysfunction. Hyperglycemia-induced VEGF expression in the retina has been shown to be mediated by the PKC-dependent mechanism, and VEGF is believed to be the primary mediator of retinal vascular permeability in DR [103].

Polyol pathway

Some of the excess glucose in diabetes metabolizes through the polyol pathway. The polyol pathway is a two-step metabolic pathway in which glucose is reduced to sorbitol, which is then converted to fructose. Aldose reductase, the key enzyme involved in the polyol pathway, becomes activated in diabetes, which reduces glucose to sorbitol using NADPH as a cofactor, thereby reducing the NADPH level, resulting in less glutathione, and increases oxidative stress, a major factor in retinal damage. Retinas from diabetic patients with retinopathy showed a higher expression of aldose reductase protein in ganglion cells, nerve fibers and Müller cells than retinas from nondiabetic individuals. Excess accumulation of sorbitol has been found in various tissues, including the retinas of diabetic animals [105,106], and also in human retinas from nondiabetic eye donors exposed to high glucose levels (similar to the level in nondiabetic rat retinas incubated under identical conditions) [107]. In one of our studies, we measured the rate of polyols formation in *ex vivo* rat retinas that provided evidence of increased flux through the polyol pathway with an increase in the duration of diabetes and with hyperglycemia [108]. Sorbitol accumulates intracellularly, which may cause osmotic stress. The byproducts of the polyol pathway, fructose-3-phosphate and 3-deoxyglucosone, are powerful glycosylating agents that enter in the formation of AGEs, which are an important factor for the pathogenecity of DR. Thus, activation of the polyol pathway, by altering intracellular tonicity, generating AGEs precursors and exposing cells to oxidative stress by decreasing antioxidant defenses and generation of oxidant species, initiates and multiplies several mechanisms of cellular damage.

Hypoxia-inducible factor-1α

In DR, hypoxia appears to be the primary stimulus for neovascularization by upregulating the production of angiogenic stimulators and by reducing the production of angiogenic inhibitors, disturbing the balance between the positive and negative regulators of angiogenesis. VEGF and its cognate receptors are critical mediators of angiogenesis, mediating endothelial cell proliferation, migration and tube formation [109]. PEDF has been shown to be a highly effective inhibitor of angiogenesis as it specifically inhibited the migration of endothelial cells. It was also shown that PEDF contributes to most of the antiangiogenic activity in the vitreous humor [110]. Elevated intraocular levels of the angiogenic VEGF [111,112] and decreased intraocular levels of the antiangiogenic PEDF in patients with PDR have previously been demonstrated [113]. These data support the concept that induction of angiogenesis in PDR requires not only an elevation of angiogenic growth factors, such as VEGF, but also a decrease in angiogenesis inhibitors, such as PEDF.

All the hypoxia-dependent events in cells appear to share a common denominator, HIF-1, which is a heterodimeric transcription factor. HIF-1 is composed of HIF-1α and HIF-1β subunits, which are both members of the basic helix-loop-helix-PAS family of proteins. Whereas the β-subunit protein is constitutively expressed, the stability of the α-subunit and its transcriptional activity are precisely controlled by the intracellular oxygen concentration. Under normoxia, the level of HIF-1α protein is kept low through rapid ubiquitylation and subsequent proteasomal degradation. In cells under hypoxia, the ubiquitylation and subsequent degradation of HIF-1α protein is suppressed, resulting in accumulation of the protein to form an active complex with HIF-1β. Under hypoxic conditions, HIF-1 triggers the activation of a large number of genes encoding proteins that regulate angiogenesis, such as VEGF, erythropoietin (Epo), angiopoietins (Angs), TGF-β, CTGF, COX-2, iNOS, SDF-1, CXCR4 and integrins [114]. Recently, we demonstrated that HIF-1α was specifically localized in vascular endothelial cells in PDR epiretinal membranes [115]. We, and others, demonstrated that Ang-2 and VEGF were colocalized in vascular endothelial cells in PDR epiretinal membranes [115,116], and experimental studies demonstrated that VEGF and Ang-2 cooperatively contribute to angiogenesis [117]. In addition, VEGF and Ang-2 were upregulated in the retina of diabetic rats [118]. Elevated levels of VEGF and Ang-2 were detected in vitreous humor samples from patients with PDR, and vitreous concentration of VEGF correlated significantly with that of Ang-2. Both VEGF and Ang-2 levels in the eyes with active PDR were significantly higher than in those with inactive PDR [119]. Several studies demonstrated elevated levels of Epo in vitreous fluid from patients with PDR compared with patients without diabetes [120–122]. In addition, Kase *et al.* demonstrated that the Epo receptor was strongly expressed in endothelial cells and stromal cells in PDR epiretinal membranes [123].

Poly (ADP-ribose) polymerase

Poly (ADP-ribose) polymerase (PARP) is a nuclear enzyme that is found to be activated in retinas of diabetic animals, causing DNA breaks and exacerbating oxidative and nitrosative stress [124,125]. PARP activation depletes its substrate, NAD^+, slowing the rate of glycolysis and mitochondrial function, eventually leading to cell death, and also inhibits glyceraldehydes 3-phosphate dehydrogenase activity, which, in turn, increases the influx through hyperglycemia-induced activation of PKC, hexasominase pathway and AGE formation, which triggers the production of ROS and nitrogen species, playing a role in the pathogenesis of endothelial dysfunction and diabetic complications. Oxidative stress breaks down DNA, which activates PARP, in turn potentiating NF-κB activation, resulting in an increased expression of NF-κB-dependent genes, such as *ICAM-1*, *MCP-1* and *TNF*-α, with an increase in leukostasis and producing greater oxidative stress. PARP inhibition suppresses NF-κB activation and the expression of adhesion molecules in cultured endothelial cells under high glucose concentration [124]. In addition, PARP inhibition inhibited diabetes-induced death of retinal microvascular cells and the development of early lesions of DR [124]. More recently, Drel *et al.* demonstrated that PARP inhibition alleviated oxidative-nitrosative stress and counteracted glial activation, as well as neural apoptosis, in the retinas of diabetic rats [126].

Neurodegeneration

Growing bodies of evidence have emerged to indicate that impairment of retinal function precedes the earliest signs of vascular complications in DR. Retinal function tests, such as

electroretinography, dark adaptation, contrast sensitivity and color vision, have demonstrated that neuroretinal function is compromised before the onset of vascular lesions in humans [127]. Retinal neurons, particularly retinal ganglion cells, have been reported to degenerate, as shown by accelerated apoptosis in diabetic animals and in human diabetic subjects before retinal vascular cell death [4–7]. An excess amount of glutamate has been found in the vitreous humor of DR patients [128] and also in the retina of diabetic animals [129]. These findings suggest that the excitotoxic glutamate is involved in retinal neurodegeneration induced by diabetes. Glutamate can be increased owing to ischemia or from dysfunction of glutamate metabolism and homeostasis, such as glutamate uptake, oxidation and turnover in the process of neurotransmission, involving both neuronal and Müller cells [130–132]. In our study, we found an increased level of branched-chain amino acids within diabetic retinas, which further augments the extracellular level of glutamate and, thereby, may be implicated in exacerbating the neurotoxicity [Ola MS *et al.*, unpublished data].

Integrins

Functional cell surface integrins are complexes of an α- and a β-subunit. The difference in the subunit composition determines the specificity of the integrin complex for its substrate in the extracellular matrix. Integrins, such as $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$ and $\alpha_2\beta_1$, promote angiogenesis by mediating cell migration, proliferation and survival of angiogenic endothelial cells [133]. Recently, we demonstrated that only $\alpha_v\beta_3$ integrin was expressed in PDR epiretinal membranes and was specifically localized in vascular endothelial cells and stromal cells [17]; similar findings were noted by Ning *et al.* [134]. Integrin $\alpha_v\beta_3$ has been especially identified as a marker of angiogenesis because it is expressed on angiogenic blood vessels in human wound granulation tissue but not in normal skin. Its expression increased during angiogenesis on the chick chorioallantoic membrane. In the latter assay, a monoclonal antibody to $\alpha_v\beta_3$ blocked angiogenesis induced by basic FGF, TNF-α and human melanoma fragments but had no effect on pre-existing vessels [134]. Ligation of integrin $\alpha_v\beta_3$ is required for the survival and maturation of newly forming blood vessels. Antagonists of this integrin induce apoptosis of the proliferative angiogenic vascular cells, leaving pre-existing quiescent blood vessels unaffected [135]. Several studies demonstrated that $\alpha_v\beta_3$ integrin participates in the pathologic angiogenesis that occurs in the murine model of oxygen-induced ischemic retinopathy [136,137].

Growth hormone, IGF & somatostatin

Growth hormone (GH) and IGF-1 are implicated in the aberrant cell growth and pathological neovascularization that characterizes proliferative DR. Levels of IGF-1 are increased in the vitreous fluid of patients with proliferative DR. Intraocular IGF-1, but not systemic IGF-1, was found to induce increased retinal vascular permeability [138,139]. IGF-1 may exert its cell growth-promoting properties by stimulating a number of pathways, including PKB, NF-κB, AP-1 and HIF-1α. Other growth factors may participate in IGF-1-induced cell growth, including VEGF, PDGF and FGF. Hyperglycemic conditions were found to enhance the proliferative response of retinal endothelial cells to IGF-1 [140]. The importance of the GH/IGF system in DR and retinal neovascularization has been highlighted by the use of agents that inhibit the system. The naturally occurring GH inhibitor, somatostatin, has been suggested as a candidate for developing novel therapies. Somatostatin may exert its antiangiogenic effects through both antagonism of the GH axis and direct antiproliferative and apoptotic effects on endothelial cells. Therefore, the use of long-acting somatostatin analogs will lead to an upregulation of antiangiogenic signaling [141]. It was demonstrated that GH receptor antagonists, GH receptor antisense oligonucleotides, somatostatin analogues and receptor-neutralizing antibodies to IGF-1 reduced hypoxia-induced retinal neovascularization [142].

Evidence-based patient care

Five large, randomized, controlled trials provide the scientific basis for care in the diabetic patient to preserve vision.

Diabetes Control & Complications Trial (DCCT)

The DCCT randomized 1441 patients with Type 1 diabetes to receive intensive glycemic or conventional therapy. Over 6.5 years of follow-up, intensive treatment (median glycosulated hemoglobin [HbA1c]: 7.2%) reduced the incidence of DR by 76% and progression of DR by 54%, compared with conventional treatment [11]. Long-term observational DCCT data showed that despite gradual equalization of HbA1c values after study termination, the rate of DR progression in the former intensively treated group remained significantly lower than in the former conventional group ('metabolic memory') [143], emphasizing the importance of instituting tight glycemic control early in the course of diabetes.

Tight glycemic control has two clinical important adverse effects. First, there is risk of early worsening of DR. In the DCCT, this occurred in 13.1% of the intensive versus 7.6% of the conventional treatment group. However, this effect was reversed by 18 months, and no case of early worsening resulted in serious visual loss. In the DCCT, the long-term benefits of intensive insulin treatment greatly outweighed the risks of early worsening. Therefore, ophthalmoscopic monitoring before initiation of intensive treatment, and at 3-month intervals for 6–12 months thereafter, seems appropriate when intensive treatment is initiated in patients with long-standing poor glycemic control, particularly if retinopathy is at or past moderate nonproliferative stage. In patients whose retinopathy is already approaching the high-risk stage, it may be prudent to delay the initiation of intensive treatment until photocoagulation can be completed, particularly if HbA1c is high [144]. Second, tight glycemic control was associated with more frequent, severe hypoglycemic episodes compared with the conventional group [11].

UK Prospective Diabetes Study (UKPDS)

The UKPDS randomized 3867 patients with newly diagnosed Type 2 diabetes to receive intensive or conventional therapy. After 12 years of follow-up, the progression of DR was reduced by 21%

and the need for laser photocoagulation by 29% in the intensive versus the conventional treatment group [12]. The UKPDS also investigated the influence of tight blood pressure control. A total of 1148 hypertensive patients with Type 2 diabetes were randomized to recieve less tight (<180/105 mmHg) and tight blood pressure control (<150/85 mmHg). With a median follow-up of 8.4 years, patients assigned to tight control had a 34% reduction in progression of retinopathy and a 47% reduced risk of deterioration in visual acuity of three lines compared with the less tight control group [145].

Diabetic Retinopathy Study (DRS)

The DRS investigated whether scatter (panretinal) photocoagulation compared with indefinite deferral could reduce the risk of vision loss from PDR. After 2 years, photocoagulation was shown to significantly reduce severe visual loss (best corrected visual acuity of 5/200 or worse) from PDR. The benefit persisted through the entire duration of follow-up and was greatest among patients whose eyes had high-risk characteristics [146]. Recently, the Diabetic Retinopathy Clinical Research Network compared the effects of single-sitting with four-sitting panretinal photocoagulation on macular edema in subjects with severe nonproliferative or early proliferative DR with relatively good visual acuity and no or mild center-involved macular edema. The results suggest that clinically meaningful differences are unlikely in optical coherence tomography thickness or visual acuity following application in one sitting compared with four sittings [147].

Early Treatment Diabetic Retinopathy Study (ETDRS)

The ETDRS demonstrated that focal/grid laser photocoagulation reduced the risk of moderate vision loss (i.e., a doubling of the visual angle) from clinically significant macular edema by 50% or more [148]. ETDRS analyses also indicated that for patients with Type 2 diabetes, it is especially important to consider scatter photocoagulation at the time of the development of severe nonproliferative or early proliferative DR [149].

A recent, randomized, controlled trial compared modified ETDRS direct/grid photocoagulation technique with mild macular grid-laser technique, in which microaneurysms are not treated directly and small mild burns are placed throughout the macula for DME. At 12 months after treatment, the mild macular grid technique was less effective at reducing optical coherence tomography-measured retinal thickening than the current modified ETDRS laser photocoagulation approach. It was concluded that modified ETDRS focal photocoagulation should continue to be a standard approach for treating DME [150].

Diabetic Retinopathy Vitrectomy Study (DRVS)

The DRVS randomized 616 eyes with recent vitreous hemorrhage, reducing visual acuity to 5/200 or less for at least 1 month, to undergo early vitrectomy within 6 months or deferral of vitrectomy for 1 year. After 2 years of follow-up, 25% of the early vitrectomy group had visual acuity of 10/20 or better compared with 15% of the deferral group. In patients with Type 1 diabetes, who were, on average, younger and had more severe PDR, there was a clear-cut advantage for early vitrectomy, as reflected in the percentage of eyes recovering visual acuity of 10/20 or better (36 vs 12% in the deferral group). No such advantage was found in the Type 2 diabetes group (16% in the early group vs 18% in the deferral group) [151].

The DRS and the ETDRS showed that laser photocoagulation for DR is effective at slowing the progression of retinopathy and reducing visual loss but the treatment usually does not restore lost vision. Since these treatments are aimed at preventing vision loss and retinopathy can be asymptomatic, it is important to identify and treat patients early in the disease. To achieve this goal, patients with diabetes should be routinely evaluated to detect treatable disease. Guidelines for the frequency of diabetic eye examinations have been largely based on the severity of retinopathy [1].

Emerging therapies

Owing to the limitations of current treatment, new therapeutic approaches are being developed.

Islet cell transplantation

Recent studies demonstrated that improved islet transplant outcomes could be observed with enhanced islet isolation, glucocorticoid-free immunosuppression and provision of an adequate islet mass of more than 10,000 islet equivalents per kilogram of bodyweight. These improvements have resulted in benefits to Type 1 diabetic subjects, including long-term c-peptide secretion, improved glycemic control and reduced hypoglycemic episodes. Recently, it was demonstrated that islet transplantation yields improved HbA1c and less progression of retinopathy compared with intensive medical therapy during 3 years follow-up [152,153].

Fibrates

Fibrates are a widely prescribed lipid-lowering drug for the treatment of dislipidemia. Their main clinical effects, mediated by PPAR-α activation, are a moderate reduction in total cholesterol and low-density lipoprotein cholesterol levels, a marked reduction in triglycerides and an increase in high-density lipoprotein cholesterol. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study demonstrated that long-term lipid-lowering therapy with fenofibrate reduced the progression of DR and the need for laser treatment in patients with Type 2 diabetes, although the mechanism of this effect does not seem to be related to plasma concentration of lipids [154].

Ruboxistaurin

Ruboxistaurin (RBX; LY333531; Lilly Research Laboratories, IN, USA) is a PKC β-selective inhibitor with adequate bioavailability to permit oral administration once daily. In the PKCβ Inhibitor-Diabetic Retinopathy Study 2 (PKC-DRS2), oral administration of RBX (32 mg/day) reduced sustained moderate visual loss, need for laser treatment for macular edema and macular edema progression, while increasing occurrence of visual improvement in patients with

nonproliferative retinopathy [155]. In the PKCβ Inhibitor Diabetic Macular Edema Study (PKC-DMES), RBX treatment also showed a beneficial effect on DME progression relative to placebo [156]. More recently, Davis *et al.* demonstrated that RBX treatment appears to ameliorate DME-associated visual decline [157].

Anti-VEGF treatment

Currently, there are four anti-VEGF agents that have been used in the management of DR, including pegaptanib (Macugen®; Pfizer, Inc., NY, USA), ranibizumab (Lucentis®; Genentech, Inc., CA, USA), bevacizumab (Avastin®; Genentech, Inc., CA, USA), and VEGF Trap-Eye (Regeneron Pharmaceuticals, Inc., NY, USA).

Pegaptanib

Pegaptanib is a pegylated RNA aptamer directed against the VEGF-A 165 isoform. A Phase II clinical trial of intravitreal pegaptanib in patients with DME followed-up for 36 weeks resulted in better visual acuity outcomes, reduced central retinal thickness and reduced need for additional photocoagulation therapy [158]. A retrospective analysis of the same study on patients with retinal neovascularization at baseline demonstrated regression of neovascularization after intravitreal pegaptanib administration [159].

Ranibizumab

Ranibizumab is a recombinant humanized monoclonal antibody fragment with specificity for all isoforms of human VEGF-A. Two pilot studies of intravitreal ranibizumab demonstrated reduced foveal thickness and maintained or improved visual acuity in patients with DME [160,161].

VEGF Trap-Eye

Vascular endothelial growth factor Trap is a 115-kDa recombinant fusion protein consisting of the VEGF binding domains of human VEGF receptors 1 and 2 fused to the Fc domain of human IgG1. One pilot study showed that a single intravitreal injection of VEGF Trap-Eye was well tolerated and was effective in patients with DME [162].

Bevacizumab

Bevacizumab is a full-length, recombinant, humanized antibody active against all isoforms of VEGF-A. It is US FDA approved as an adjunctive systemic treatment for metastatic colorectal cancer. Several studies reported the use of the off-label intravitreal bevacizumab (IVB) to treat DME, complications of PDR and iris neovascularization. However, bevacizumab's safety for ophthalmic intravitreal use has not been tested in any large, randomized studies.

To date, all studies regarding IVB (1.25 mg) for DME therapy have demonstrated transient beneficial effects with a requirement for repeated injections [163–167]. Increased visual acuity with decrease in macular edema with a single injection of IVB lasts for 4–6 weeks, with deterioration of visual acuity and recurrence of macular edema at 8–12 weeks, necessitating another injection [166,168]. Fang *et al.* reported that the improvement in visual acuity and decrease in macular edema were maintained for 8 weeks in the nonpretreated eyes group and for 2–4 weeks in the pretreated eyes group [167]. In addition, Yanyali *et al.* reported that IVB in DME have no effect on visual acuity and macular edema in previously vitrectomized eyes [169].

Several studies demonstrated that IVB injection resulted in marked regression of retinal and iris neovascularization, and rapid resolution of vitreous hemorrhage in patients with PDR [170–173]. In addition, IVB injection was demonstrated to be an effective adjunctive treatment to panretinal photocoagulation (PRP) in the treatment of high-risk PDR [174–176] and neovascular glaucoma [172,173]. IVB injection before PRP was found to be beneficial in preventing PRP-induced visual dysfunction and foveal thickening and was associated with a greater reduction in the area of active leaking new vessels than PRP alone in patients with high-risk PDR [174–176].

The use of preoperative IVB injection a few days before planned pars plana vitrectomy for treatment of complications of PDR was also found to be efficacious and safe as an adjuvant treatment to facilitate surgery, reduce intraoperative bleeding, prevent rebleeding and accelerate postoperative vitreous clear-up [177–179]. However, tractional retinal detachment may occur or progress shortly following administration of IVB in these patients [180]. In addition, severe intraocular inflammation was reported following IVB injections [181].

Intravitreal triamcinolone acetonide

Intravitreal triamcinolone acetonide (IVTA) is reported to generate favorable results in the treatment of diffuse DME. However, the major limitation of IVTA is the recurrence of DME, which develops after a relatively short duration of action, necessitating repeated applications of IVTA, which carry risk and are inconvenient for patients [182,183]. This early disappearance of the effect of IVTA might be consistent with the results reported by Beer *et al.*, who estimated that measurable concentrations of triamcinolone could be expected to last no longer than 3 months in nonvitrectomized eyes [184].

In a prospective, randomized, controlled trial, eyes with persistent DME after focal/grid photocoagulation received either IVTA 4 mg or sham injection (saline injection into the subconjunctival space). After 2 years, 19 out of 34 eyes treated with repeated IVTA (56%) had a visual acuity improvement of five letters or more compared with nine out of 35 placebo-treated eyes (26%). An increase of intraocular pressure of 5 mmHg or above was observed in 23 out of 34 treated (68%) versus three out of 30 untreated eyes (10%) . Glaucoma medication was required in 15 out of 34 treated (44%) versus one out of 30 untreated eyes (3%). Cataract surgery was performed in 15 out of 28 treated (54%) versus zero out of 21 untreated eyes (0%). Two eyes in the IVTA group required trabeculectomy. There was one case of infectious endophthalmitis in the treatment group [185].

The Diabetic Retinopathy Clinical Research Network reported 2-year results of a multicenter, randomized clinical trial comparing preservative-free IVTA and focal/grid laser for DME. In this study, 840 eyes were randomized to focal/grid photocoagulation, or IVTA 1 mg or 4 mg. Retreatment was given for persistent or new edema at 4-month intervals. At 4 months, mean visual acuity was better in the 4-mg IVTA group than

in either the laser group or the 1-mg IVTA group. Mean visual acuity at 2 years after starting the treatment was better in the laser group compared with the steroid-injected groups. Optical coherence tomography results generally paralleled the visual acuity results. Cataract surgery performed before the 2-year visit was most frequent in the 4-mg IVTA group (51%) versus the 1-mg IVTA group (23%) and the laser group (13%). Increased intraocular pressure from baseline by 10 mmHg or more at any visit was most frequent in the 4-mg (33%) versus the 1-mg IVTA group (16%) and the laser group (4%) [186]. More recently, the Diabetic Retinopathy Clinical Research Network reported that the 3-year visual outcome results were consistent with the previously published 2-year results. The cumulative probability of cataract surgery by 3 years was 31, 46 and 83% in the laser, 1- and 4-mg IVTA groups, respectively. Intraocular pressure increased by more than 10 mmHg at any visit in 4, 18 and 33% of the eyes, respectively [187]. This randomized study indicated clearly that focal/grid photocoagulation is a better treatment than IVTA in eyes with DME involving the center of the macula with visual acuity between 20/40 and 20/320. The fact that the 4-mg IVTA group had a greater positive treatment response on visual acuity and retinal thickening at 4 months, whereas the photocoagulation group had a greater positive response later, raises the possibility that combining focal/grid photocoagulation with IVTA may produce greater benefit for DME than either focal/grid photocoagulation or IVTA alone [186].

Several small, randomized clinical trials demonstrated that the combination of laser photocoagulation (panretinal and macular) with IVTA was associated with improved best-corrected visual acuity and decreased central macular thickness and total macular volume when compared with laser photocoagulation alone for the treatment of PDR and macular edema [183,188,189].

Recently, two studies compared the morphological and visual acuity outcomes associated with a single intravitreal injection of triamcinolone acetonide versus bevacizumab for the treatment of DME. These studies concluded that one single intravitreal injection of triamcinolone showed better results in reducing DME and in the improvement of visual acuity than that of bevacizumab in the short-term management of DME. The reduction effect of bevacizumab on DME was weaker and shorter than that with triamcinolone. However, IVB had the advantage of intraocular pressure stability compared with the triamcinolone injection [168,190]. In addition to steroid-related side effects, injection-related side effects include retinal detachment, vitreous hemorrhage, bacterial endophthalmitis, noninfectious endophthalmitis and pseudoendophthalmitis [191].

Vitrectomy for persistent diffuse DME

Vitrectomy with removal of the premacular posterior hyaloid for persistent diffuse DME has gained rapid widespread acceptance. The large number of series evaluating the efficacy of vitrectomy (with or without internal limiting membrane peeling) has yielded conflicting results. In a prospective, randomized trial, Stolba *et al.* showed that vitrectomy with internal limiting membrane peeling was superior to observation in eyes with persistent diffuse DME that previously failed to respond to conventional laser treatment and positively influenced distance and reading visual acuity, as well as the morphology of the edema [192]. However, they suggested the need for larger follow-ups and larger series to confirm these findings. Other studies suggested that vitrectomy with and without internal limiting membrane peeling may provide anatomic and visual benefit in eyes with diffuse, nontractional, unresponsive DME refractory to laser photocoagulation [193–195]. Best-corrected visual acuity continued to improve until 1 year postoperatively and is maintained in the long term [194,195]. The preoperative best-corrected visual acuity was the best prognostic factor for final best-corrected visual acuity [194,195]. On the other hand, other studies showed that the benefits of vitrectomy for DME in terms of visual acuity and macular thickness were limited to patients who exhibited signs of macular traction, either clinically and/or on optical coherence tomography [196–199]. Macular detachment on optical coherence tomography was suggested to be an adverse predictive indicator [198].

The necessity of internal limiting membrane peeling is still unclear. Several studies reported that there was no difference in the absorption rate of macular edema or the functional outcome after vitrectomy with or without internal limiting membrane peeling [194,200,201].

Enzymatic vitreolysis

Enzymatic vitreolysis and clearance of the hemorrhage has been investigated as a minimally invasive, conservative, and economical treatment for vitreous hemorrhage. Intravitreous ovine hyaluronidase (Vitrase; ISTA Pharmaceuticals, Inc., CA, USA) can facilitate hemorrhage clearance by inducing liquefaction of the vitreous, which allows for red blood cell lysis and phagocytosis. Two large, multinational, randomized, double-masked, placebo-controlled Phase III clinical trials evaluated the efficacy of a single intravitreous injection of highly purified, preservative-free, ovine hyaluronidase for the management of persistent vitreous hemorrhage from PDR and other causes. It was demonstrated that ovine hyaluronidase resulted in a statistically significant effect on the primary efficacy end point sufficient (sufficient clearance of vitreous hemorrhage to see the underlying pathology and completion of treatment, when indicated, by month 3) at months 1 and 2 [202].

RAS blockers

Several studies suggested that RAS blockers might reduce the burden of DR. The findings of the Eurodiab Controlled Trial of Lisinopril in Insulin-dependent Diabetes (EUCLID) suggested that blockade of the RAS with the angiotensin-converting enzyme inhibitor, lisinopril, could reduce both incidence and progression of retinopathy in Type 1 diabetes [203]. Recently, the Diabetic Retinopathy Candesartan Trials (DIRECT) demonstrated that the angiotensin receptor antagonist, candesartan, reduced the incidence of retinopathy in patients with Type 1 diabetes [204], and might induce improvement of retinopathy in Type 2 diabetic patients with mild-to-moderate retinopathy [205].

PPAR-γ agonists

The PPAR-γ agonist rosiglitazone inhibited both the retinal leukostasis and retinal leakage observed in experimental diabetic rats. In addition, the decreased expression of the endogenous PPAR-γ in mice leads to the aggravation of retinal leukostasis and retinal leakage in diabetic mice [83]. Rosiglitazone maleate (Avandia; GlaxoSmithKline, NC, USA) is an orally administered medication used to improve glycemic control in patients with diabetes mellitus. This medication activates the PPAR-γ and leads to insulin sensitization in adipose and other tissues, with potential antiangiogenic activity. Recently, Shen *et al.* demonstrated that rosiglitazone may delay the onset of proliferative DR in patients with severe nonproliferative DR at baseline [206]. Several studies showed that the use of the glitazone class of drug was associated with DME [207,208]. However, another retrospective study concluded that rosiglitazone is not linked to DME [209].

Potential future drugs & their targets

Despite marked improvements in the treatment of DR, vision loss is still increasing at an alarming rate. Continuous efforts of researchers towards better understanding of the specific molecular and biochemical changes in DR will develop targeted pharmacological treatment strategies. Future therapies are likely to involve inhibiting several different pathways or discovering the master regulator molecule(s) and their inhibitors for DR treatment.

AGE inhibitors

Inhibitors of AGEs have been used in experimental studies to modulate the action of AGEs in the pathogenesis of DR. Aminoguanidine, an inhibitor of AGE formation, prevented DR and AGE accumulation at branching sites of precapillary arterioles, diminished pericyte dropout, reduced the progression of vascular occlusion and inhibited abnormal endothelial cell proliferation in diabetic rats [210]. Pyridoxamine treatment, an inhibitor of formation of AGEs and lipoxidation end products, protected against capillary dropout and limited laminin protein upregulation, extracellular matrix mRNA expression and AGE levels increase in the retinal vasculature of diabetic rats [211]. In addition, LR-90 treatment, a multistage inhibitor of AGEs, reduced the number of acellular capillaries and pericyte loss in the retinas of diabetic rats [212]. Systematic administration of the soluble form of RAGE inhibited blood–retinal barrier breakdown, leukostasis and expression of ICAM-1 in the retina of diabetic mice [213]. Thus, inhibition of AGEs formation, blockade of AGE–RAGE interaction and suppression of RAGE expression may be novel therapeutic targets to treat DR.

Aldose reductase inhibitors

Numerous studies showed that aldose reductase inhibitors diminish the prevalence of microaneurysms, basement membrane thickening, oxidative stress, VEGF expression, neuronal apoptosis and gliosis in the retina of animals with diabetes. Over the last two decades, several aldose reductase inhibitors have been developed, but owing to lack of specificity and side effects, clinical trials were disappointing. Currently, new aldose reductase inhibitors are being developed that show greater potency. The new structural class of aldose reductase inhibitors, such as epalrestat, fidarestat, ranirestat and ARI-809, have been studied in diabetic animals with great success [214–216].

Nonsteroidal anti-inflammatory drugs

High-dose aspirin suppressed breakdown of the blood–retinal barrier in diabetic rats. This suppression was accompanied by the inhibition of retinal expression of ICAM-1 and a concomitant reduction in the adhesion of leukocytes to the retinal vasculature. Similar reductions also resulted with other anti-inflammatory drugs, including etanercept, a soluble receptor of the cytokine TNF-α, as well as meloxicam, an inhibitor of COX-2 [40]. Diabetes-induced vascular damage in the rat was inhibited by topical application of nepafenac, a nonsteroidal anti-inflammatory prodrug that inhibits both COX-1 and COX-2 [217], and periocular injection of celecoxib-poly (lactide-co-glycolide) microparticles, directed against COX-2 [218]. Salicylate-based anti-inflammatory drugs inhibited the development of early stages of DR in rats [219]. Recently, Yang *et al.* demonstrated that baicalein treatment, an anti-inflammatory drug, suppressed diabetes-induced inflammatory process and inhibited vascular abnormality and neuron loss in the retinas of diabetic rats [220]. Treatment with sulindac, a NSAID, significantly inhibited the development and progression of DR in a 3-year study [221].

Antioxidants

There is accumulating evidence from animal studies that oxidative stress is associated with the development of retinopathy in diabetes, and antioxidants have beneficial effects on the development of retinopathy [95]. Nutritional supplements, consisting mainly of antioxidants that are routinely used in clinical settings for age-related macular degeneration (e.g., ascorbic acid, vitamin E, β-carotene, zinc and copper) protected diabetic rats from the development of DR and other metabolic abnormalities associated with its development [222]. However, the results from clinical trials are ambiguous.

HIF-1α inhibition

Several studies demonstrated that targeting the master modulator HIF-1α can suppress retinal neovascularization in animal models of retinal ischemia. siRNA targeting HIF-1α could specifically decrease the retinal expression level of HIF-1α and VEGF and retinal neovascularization in a murine model of oxygen-induced proliferative retinopathy [223,224].

SDF-1 blockers

In proliferative retinopathy, SDF-1 plays a major role and may be an ideal target for the prevention of proliferative retinopathy. Intravitreal injection of blocking antibodies to SDF-1 prevented retinal neovascularization in a murine model of proliferative retinopathy, even in the presence of exogenous VEGF [59].

Integrin antagonist

The orally bioavailable nonpeptide α_v antagonist (JNJ-26076713) is a potent compound that inhibits $\alpha_v\beta_3$ and $\alpha_v\beta_5$ binding to vitronectin in the nanomolar range. It blocks cell migration induced

by VEGF, FGF and serum, and angiogenesis induced by FGF in the chick chorioallantoic membrane model. JNJ-26076713 inhibits retinal neovascularization in an oxygen-induced model of retinopathy of prematurity after oral administration. In diabetic rats, orally administered JNJ-26076713 markedly inhibits retinal vascular permeability, a key early event in DME [225].

Neuroprotection

Chronic memantine treatment improved retinal function and protected retinal ganglion cell loss in diabetic rats. In addition, chronic memantine treatment decreased elevated vitreoretinal VEGF protein levels and blood–retinal barrier breakdown in diabetic rats [226]. Recently, Smith *et al.* demonstrated that the σ receptor 1 ligand(+)-pentazocine treatment conferred significant neuroprotection, reduced evidence of oxidative stress and preserved retinal architecture in diabetic mice [227]. We also showed that memantine and gabapentin reduced caspase-3 activity and levels of ROS in retinas of diabetic rats [Ola MS *et al.*, unpublished data].

siRNA & antisense digonucleotides

Recently, siRNA emerged as a powerful tool for therapeutic methods for diseases. siRNA technology allows the production of dsRNA molecules that can specifically prevent the production of particular gene product in a potent and efficient manner [228]. An attractive advantage for using siRNA is its ability to temporally knock down the expression of target genes specifically and potently. *HIF-1*α siRNA and *VEGF* siRNA specifically downregulated *HIF-1*α, *VEGF* mRNA and protein levels, both in human umbilical vein endothelial cells and in the retina of ischemic retinopathy model in mice with a decrease in neovascularization [224]. An antisense oligonucleotide targeting the GH receptor inhibits neovascularization in a mice model of oxygen-induced retinopathy [229].

Adenoviral vector for gene therapy

Intravitreous injection of adenovirus vectors expressing a soluble form of the VEGF receptor (sflt-1), which acts by sequestering VEGF, resulted in detectable levels of sflt-1 and reduced retinal neovascularization in a rat model of oxygen-induced retinopathy. Furthermore, pre-existing retinal vessels were not affected [230,231].

Expert commentary

The most important evidence-based therapies for DR include strict metabolic control, tight blood pressure control, laser photocoagulation and vitrectomy. Focal/grid photocoagulation is a better treatment than IVTA in eyes with DME. The current evidence suggests that IVTA or anti-VEGF agents are effective adjunctive treatment to laser photocoagulation or vitrectomy. However, triamcinolone is associated with risks of elevated intraocular pressure and cataract. Vitrectomy with removal of the posterior hyaloid without internal limiting membrane seems to be effective in eyes with persistent diffuse DME, particularly in eyes with associated vitreomacular traction.

Five-year view

Current evidence-based treatments rarely improve visual outcomes in patients with established DR. Emerging treatments, possibly used in combination with standard therapy, may offer effective and safe treatment that may allow us to improve visual outcomes and prevent the damaging consequences of DR. The availability of new strategies will result in a paradigm shift in treating the early stages of DR. Furthermore, therapeutic strategies may involve treating both microvascular and neuronal elements of the retina to preserve vision. Better understanding of the specific molecular and biochemical changes in DR will lead to the development of targeted therapeutic interventions. Future therapies include AGE inhibitors, aldose reductase inhibitors, PKC inhibitors, antioxidants, NSAIDs, NF-κB inhibition, HIF-1α inhibition, integrin antagonists, somatostatin analogs and therapies to inhibit neurodegeneration. Targeted therapeutics utilizing antisense oligonucleotides, siRNAs and gene therapy have, so far, demonstrated beneficial effects in animal models of ischemic retinopathy.

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Key issues

- Diabetic retinopathy remains a leading cause of blindness worldwide.
- Several pathogenic mechanisms provide the link between chronic hyperglycemia and the development of diabetic retinopathy, including formation and accumulation of advanced glycation end products, increased oxidative stress and the polyol pathway, and activation of PKC and the renin–angiotensin system.
- Inflammation and neurodegeneration play a prominent role in the pathogenesis of diabetic retinopathy.
- Current evidence-based treatments, including intensive glycemic and hypertensive control, laser photocoagulation and vitrectomy, rarely improve visual outcomes in patients with established diabetic retinopathy.
- Focal/grid photocoagulation is a better treatment than intravitreal triamcinolone acetonide in eyes with diabetic macular edema.
- Intravitreal triamcinolone acetonide or anti-VEGF agents are effective adjunctive treatment to laser photocoagulation or vitrectomy. However, triamcinolone is associated with risks of elevated intraocular pressure and cataract.
- Vitrectomy seems to be effective in eyes with persistent diffuse diabetic macular edema, particularly those associated vitreomacular traction.
- Emerging therapies include ruboxistaurin, renin–angiotensin system blockers, fenofibrate, islet cell transplantation, PPAR-γ agonists and intravitreal hyaluronidase.
- Better understanding of the underlying molecular and biochemical mechanisms involved in diabetic retinopathy will lead to the development of targeted therapeutic interventions.

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CME Pathophysiology and management of diabetic retinopathy

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Activity Evaluation
Where 1 is strongly disagree and 5 is strongly agree

	1	2	3	4	5
1. The activity supported the learning objectives.					
2. The material was organized clearly for learning to occur.					
3. The content learned from this activity will impact my practice.					
4. The activity was presented objectively and free of commercial bias.					

1. All of the following statements about the pathogenesis of diabetic retinopathy are accurate, except:
   - ☐ **A** Serum levels of advanced glycation end products positively correlate with the severity of retinopathy
   - ☐ **B** Retinal expression of PPAR-γ may be suppressed in diabetes
   - ☐ **C** Plasma levels of TNF-α positively correlate with the severity of retinopathy
   - ☐ **D** The retina is relatively protected from various forms of oxidative stress

2. Which of the following statements about established means to prevent and treat diabetic retinopathy is most accurate?
   - ☐ **A** Intensive diabetes therapy significantly reduces the risk for diabetic retinopathy in the short term
   - ☐ **B** Intensive diabetes therapy can reduce the need for laser photocoagulation
   - ☐ **C** Blood pressure control does not affect the progression of retinopathy
   - ☐ **D** Laser photocoagulation has a strong record of restoring lost vision in diabetic retinopathy

3. All of the following statements about emerging oral treatment to prevent or treat diabetic retinopathy are accurate, except:
   - ☐ **A** Fibrates may reduce progression of diabetic retinopathy regardless of their effect on serum lipids
   - ☐ **B** Clinical trials strongly support the use of antioxidants to prevent diabetic retinopathy
   - ☐ **C** Both angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists may reduce the incidence of diabetic retinopathy
   - ☐ **D** Rosiglitazone may delay the onset of proliferative diabetic retinopathy in patients with severe nonproliferative diabetic retinopathy

4. Which of the following statements about emerging ocular treatment for diabetic retinopathy is most accurate?
   - ☐ **A** Intravitreal triamcinolone acetonide is superior to focal/grid photocoagulation in the management of diabetic macular edema
   - ☐ **B** Intravitreal triamcinolone acetonide is effective as an adjunct treatment to laser photocoagulation
   - ☐ **C** Antivascular endothelial growth factor agents are not effective as adjunct treatment to vitrectomy
   - ☐ **D** Intravitreal triamcinolone acetonide has similar rates of adverse events compared with placebo