

Advances in Diabetic Retinopathy

a report by

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DOI: 10.17925/USE.2008.04.2.85

Diabetic retinopathy (DR) is a sight-threatening and chronic ocular disorder that eventually develops to some degree in nearly all people with diabetes. DR is characterized by the progressive development of well-defined morphological abnormalities in the retinal microvasculature that can remain relatively stable or progress to diabetic macular edema (DME) and/or proliferative DR (PDR), which are leading causes of both moderate and severe vision loss in working-age adults.^{1,2} A recent report from the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) showed that the 25-year cumulative progression to DR and PDR was 83 and 42%, respectively, for people with type 1 diabetes.³ The rate of progression of DR can vary markedly among individuals, and this has been attributed to a number of factors including genetics, the management of risk factors, and residual insulin production.⁴⁻⁶ The prevalence of DME has been estimated from large epidemiological population-based surveys to be approximately 20–26% in patients with type 1 diabetes.^{7,8} The occurrence of PDR and DME in type 2 diabetes is comparable to that observed for type 1 diabetes of similar diabetes duration,² and DR risk is increased in earlier-onset type 2 diabetes.⁹ While the prevalence of diabetes is increasing and DR is its most frequent vascular complication, medical treatments to prevent and reverse DR remain unmet clinical needs.

Pathogenesis of Diabetic Retinopathy

Diabetes-associated Retinal Dysfunctions Before the Onset of Clinical Diabetic Retinopathy

Abnormalities in both vascular and neuronal retinal functions in diabetes can be detected before the clinical diagnosis of DR, based on the appearance of its morphological characteristics (see *Figure 1*). One of the earliest retinal changes in diabetes is a decrease in retinal blood flow,¹⁰ which has been attributed to the prolongation of blood transit time through retinal arterioles and capillaries. Although this hemodynamic change provides early evidence of retinal vascular dysfunction, the long-term significance of diabetes-induced changes in blood flow is not yet available. Diabetes has been shown to increase retinal vascular permeability (RVP) and the adherence of leukocyte to the retinal endothelium, suggesting the local activation of inflammatory processes (reviewed in reference 11). Diabetes without DR has also been associated with prolonged retinal implicit time delays detected using a multifocal electroretinogram,¹² suggesting early and regional abnormalities in neuroretinal responses or conduction. Although these abnormalities in retinal function associated with diabetes may provide biomarkers for DR, it is not known whether the mechanisms that induce these early functional changes are the same as the mechanisms that drive the morphological changes associated with advanced stages of DR.

Non-proliferative Diabetic Retinopathy

The first clinical signs of DR include the appearance of microaneurysms and small retinal hemorrhages. Histological studies have revealed decreased numbers of retinal pericytes and the appearance of acellular capillaries. These changes indicate a breakdown of small-vessel and capillary endothelium integrity. Pericytes play a critical role in the maintenance of endothelial tight junctions and microvascular blood flow. Pericyte loss, along with the appearance of acellular capillaries, indicates microvascular damage that could eventually lead to areas of non-perfusion. The occurrence of retinal hemorrhages indicates disruption of endothelium and basal lamina, enabling blood components to diffuse into the neuroretina. The degree and severity of microaneurysms and intraretinal hemorrhages compared with well-established clinical photograph standards is a common marker used to define the level of non-proliferative (NP) DR, graded as mild, moderate, and severe. Additional retinal abnormalities including hard exudates and vitreoretinal abnormalities can also emerge during the progression of NPDR. Although these early retinal abnormalities are not typically associated with vision loss, it is believed that these pathological changes contribute to the sequel of advanced DR.

Proliferative Diabetic Retinopathy

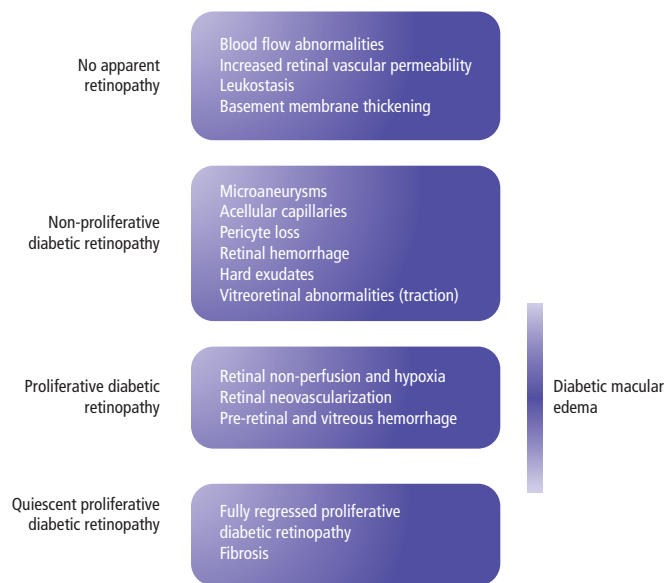
PDR is characterized by the appearance of new pathological blood vessels that grow from existing retinal vasculature and vitreous/pre-retinal hemorrhage. New blood vessels that develop from the retina in PDR tend to grow in an anterior direction into the vitreous and are prone to hemorrhage. Retinal neovascularization in DR is believed to be primarily driven by a hypoxic response caused by microvascular damage, reduced retina perfusion, and local ischemia that developed during NPDR. The newly formed blood vessels in PDR do not appreciably improve retinal perfusion and can exacerbate vision loss by causing vitreous hemorrhage and development of fibrous tissue that can lead to traction retinal detachments.



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Figure 1: Pathogenesis of Diabetic Retinopathy



Diabetic Macular Edema

DME is the most prevalent cause of vision loss in patients with diabetes.¹³ Despite its major clinical influence, the etiology of DME remains poorly understood. The condition is characterized by diffusion of serum proteins and lipids across the retinal endothelium into the intraretinal space, resulting in fluid retention, lipid deposition, and thickening of the macula.¹⁴ Vision can be transiently or permanently reduced. It has been postulated that an inability of the retina to compensate for this excessive increase in RVP is a primary cause of DME,¹⁴ which occurs most frequently in patients with advanced stages of DR.¹⁵ However, since DME occurs in patients at all stages of NPDR, PDR, or quiescent PDR (QPDR), the factors that cause intraretinal edema appear to be, at least in part, distinct from the causes of retinal neovascularization. The retinal vascular endothelium provides an essential role in partitioning the retinal interstitial fluid and vitreous fluid from factors in the circulating plasma. An increase in RVP is observed in early diabetes and there is an additional increase in RVP that correlates with the severity of DR.^{16,17} Although increased RVP has been implicated as a primary cause of DME, other aspects of fluid homeostasis, including water permeability, also play a critical role in the retention of fluid in the retina. For example, the retina expresses multiple aquaporin water channels, which mediate water transport across retinal barriers and are regulated by both diabetes and hypoxia.^{18,19} In fact, aquaporin 4 deficiency in mice has been reported to provide protective effects against ischemia-induced retinal damage.¹⁹ In addition to the diffusion and transport abnormalities of proteins, lipids, and water across the blood–retinal barrier that lead to an accumulation of intraretinal fluid in DME, vitreoretinal traction on the macula can also contribute to increased retinal thickness.²⁰

Targeting Risk Factors for Diabetic Retinopathy

Established risk factors for DR include hyperglycemia, hypertension, and diabetes duration. Intensive glycemic and blood pressure control are associated with a delay in onset and a slowing of the progression of DR (reviewed in reference 4).

Glycemic Control

The landmark report from the Diabetes Control and Complications Trial (DCCT) Research Group in 1993 demonstrated that intensive therapy reduced the onset of DR by 76% and the progression of DR in subjects with pre-existing mild DR by 54% compared with conventional therapy for people with insulin-dependent diabetes.²¹ This trial had a mean follow-up of 6.5 years and glycated hemoglobin (HbA_{1c}) levels were maintained at approximately 7 and 9%, respectively, for the intensive and conventional therapy groups. Similar findings were reported for people with type 2 diabetes enrolled in the UK Prospective Diabetes Study (UKPDS), which demonstrated that the two-step progression of DR grade was reduced by 21% in the intensive glucose control group (HbA_{1c} 7%) compared with the conventional treatment group (HbA_{1c} 7.9%).²² Moreover, DR progression was strongly associated with increased HbA_{1c} levels in this cohort of patients with type 2 diabetes.²³

More recent studies have evaluated the effects of additional glucose lowering on DR. The Action in Diabetes and Vascular Disease (ADVANCE) Collaborative Group compared standard glucose control (HbA_{1c} 7.3%) with intensive glucose control (HbA_{1c} 6.5%) in people with type 2 diabetes on both macro- and microvascular end-points.²⁴ This trial, with a median five years of follow-up, did not detect a significant benefit of intensive glucose control on new or worsening DR. Moreover, both the ADVANCE and the Action to Control Cardiovascular Risk in Diabetes (ACCORD) studies demonstrated increased hypoglycemic events with intensive blood glucose lowering in their respective cohorts.^{24,25}

Post-trial monitoring studies have been performed on both the DCCT and UKPDS patient populations to examine the potential influence of intensive glycemic control on the subsequent development of DR. The DCCT–Epidemiology of Diabetes Interventions and Complications (EDIC) study, which followed the DCCT cohort after completion of the trial with a follow-up of 6.5 years, demonstrated that the patient group originally on intensive glycemic control had a 75% reduction in advanced DR, including PDR and clinically significant ME compared with conventional control, even though the difference in HbA_{1c} levels for the intensively and conventionally treated groups had narrowed to 8.1 and 8.3, respectively.²⁶ The 10-year post-trial monitoring of the UKPDS patients with type 2 diabetes revealed a decrease in microvascular disease (including vitreous hemorrhage, retinal photocoagulation, or renal failure) in the sulfonylurea–insulin group compared with the conventional therapy group.²⁷ These findings have suggested the existence of a metabolic memory in the vasculature that prolongs the beneficial effects of intensive glucose control or that the prevention of early changes in DR delays disease progression.

Although these studies demonstrated that intensive glycemic control produced a robust reduction in DR onset and progression and the occurrence of sight-threatening PDR compared with conventional treatment, these intensive therapies did not eliminate this disease. Indeed, even though there has been improvement in glucose control over the past decade, DR remains a leading cause of vision loss. While additional glucose interventions that more closely replicate euglycemic conditions will likely continue to lower the incidence of DR, intensive and tight glycemic control can be difficult to maintain over the decades of exposure to diabetes. Intensifying glucose-lowering interventions to further lower the risk for vascular complications has been associated with adverse effects, including hypoglycemic events and potential cardiovascular risks.^{24,25} Therefore, other

approaches to target the underlying biochemical causes of DR and improve risk factor management provide important opportunities to reduce DR.

Blood Pressure Control

Several large clinical trials have shown that blood pressure lowering for individuals with both diabetes and hypertension reduces the development of DR. The UKPDS reported that tight blood pressure lowering in hypertensive patients with type 2 diabetes reduces the need for laser photocoagulation by 35% compared with conventional therapy.²⁸ However, unlike the continuation of beneficial effects observed during the post-trial monitoring in the glucose control cohort of the UKPDS,²⁷ a sustained beneficial effect on microvascular disease by intensive blood pressure control was not observed during the long-term follow-up in the UKPDS blood pressure study.²⁹

The adverse effects of high blood pressure on the retina have been attributed to both increased pressure and hemodynamic forces on retinal vessels, as well as increased local activation of the renin-angiotensin system. In fact, there is evidence for the upregulation of an ocular angiotensin system in DR, and angiotensin II has been shown to exert effects on the retina that could contribute to both edema and new vessel growth in the absence of hypertension.³⁰ *A post hoc* analysis of results from the Diabetic Retinopathy Candesartan Trials (DIRECT) program indicated that the angiotensin II type-1 (AT1) receptor blocker candesartan reduced incidence and increased regression of DR in normotensive subjects;³¹ however, this study did not achieve primary end-points for reducing DR progression.

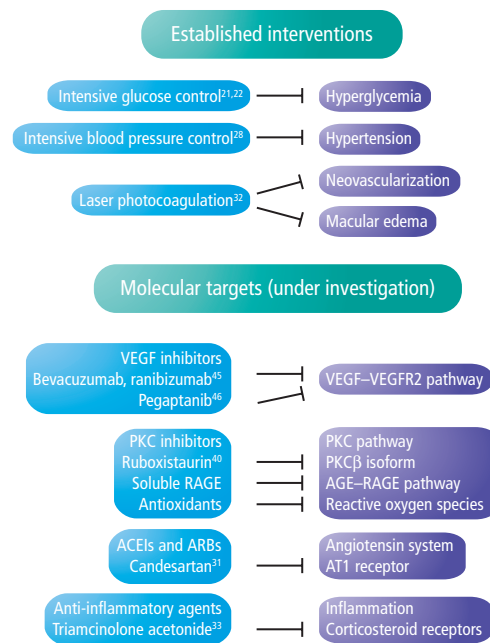
Targeting Mechanisms for Diabetic Retinopathy

Although intensive glucose and blood pressure control can reduce DR progression (see Figure 2), the long-term management of these risk factors over decades of diabetes duration can be difficult to maintain. Once sight-threatening PDR develops, laser photocoagulation is highly effective in reducing further vision loss.^{4,32} Focal/grid photocoagulation has been shown to be effective in treating DME.³³ However, laser photocoagulation is also associated with potential complications affecting visual field, color vision, and contrast sensitivity (reviewed in reference 34). Therefore, a number of other approaches are being investigated to reduce progression of DR and reverse its advanced stages.

Glucose-mediated Effects

The observations that hyperglycemia contributes to DR have prompted a number of laboratories to investigate the mechanisms that contribute to the detrimental effects of high glucose levels on cultured retinal vascular cells and retinal blood vessels. This work has demonstrated that glucose exerts a number of effects on signaling pathways and metabolic intermediates in microvascular tissues, which have been reviewed elsewhere.^{35,36} Several of the most highly developed theories for the adverse effect of elevated glucose levels on microvascular complications include the advanced glycation end-products (AGEs) theory and pathways downstream of glucose metabolism that lead to the activation of protein kinase C (PKC) and the generation of reactive oxygen species (ROS) (see Figure 2). Glucose can produce intermediates that form Amadori products on proteins, which can spontaneously rearrange to form stable AGE products that affect protein functions as well as generating ligands for a cell surface receptor of advanced glycation end-products (RAGE), which has been implicated in early DR.³⁷ Several strategies have emerged for blocking the AGE-RAGE pathway, including the interference of AGE-induced RAGE activation and reversing or preventing glycation.^{37,38} Glucose is also metabolized

Figure 2: Diabetic Retinopathy—Established and Emerging Targets



VEGF = vascular endothelial growth factor; VEGFR2 = VEGF receptor 2; PKC = protein kinase C; AT1 = angiotensin II type-1.

via a number of pathways that can activate intracellular signaling cascades. Elevated extracellular glucose leads to increased flux through these metabolic pathways and an accumulation of certain carbon intermediates and oxidation products. Increased glucose metabolism via the glycolytic pathway has been proposed to increase the *de novo* synthesis of diacylglycerol, a co-factor leading to the chronic activation of PKC. Isoform-selective PKC inhibition has been shown to ameliorate early vascular abnormalities in diabetic models.³⁹ The Protein Kinase C Diabetic Retinopathy Study (PKC-DRS), which examined the effect of ruboxistaurin (Arxant, Eli Lilly) DR progression on patients with moderate to severe NPDR, reported a trend for reduced progression to DME in patients taking ruboxistaurin compared with those on placebo.⁴⁰ Finally, glucose metabolism also results in the generation of ROS via a number of mechanisms involving glycolytic pathways and mitochondrial activity (reviewed in reference 36). Although there has been considerable progress delineating the mechanisms of glucose-induced ROS production and experimental evidence showing that overexpression of manganese superoxide dismutase in animal models can provide protective effects in diabetes,⁴¹ clinical evidence for the beneficial effects antioxidant supplementation on DR is not available.

Emerging Targets for Diabetic Retinopathy

A growing body of research is focused on the identification of factors that contribute to new vessel growth and edema in DR, and the development of interventions that target the inhibition of these factors. Much of the progress in this area has emerged from the analysis of the vitreous fluid from patients with DR. Factors that are released from the retina diffuse in an anterior direction into the vitreous chamber, where these factors can accumulate and feedback to effect retinal functions. A number of studies have utilized a candidate approach for the measurement of factors in vitreous that have been implicated in DR. Reports by Miller et al. and Aiello et al. in 1994 demonstrated elevated levels of vascular endothelial growth factor (VEGF) in

diabetic subjects with active PDR and associated elevated VEGF with ischemia-induced retinal neovascularization.^{42,43} As VEGF is a potent hypoxia-induced angiogenic and vascular permeability factor,⁴⁴ its potential role in DR has been an area of intense investigation. Intravitreal injections with anti-

Recent developments in proteomics, genomics, and metabolomics have created new opportunities to search for factors that are associated with diabetic retinopathy and could contribute to its progression.

VEGF antibodies and aptamers are being evaluated for the treatment of both PDR and DME.^{45,46} While targeting VEGF in advanced DR has shown some beneficial effects, this approach is limited to local ocular delivery due to the potential adverse effect of systemic VEGF inhibition of blood pressure control and thromboembolic and renal function.⁴⁷

Most existing targets for DR, described above, have been developed using a candidate approach and the subsequent characterization of their role in DR. Recent developments in proteomics, genomics, and metabolomics have created new opportunities to search for factors that are associated with DR and could contribute to its progression. Recently, using mass spectrometry, we have identified over 250 proteins in the human vitreous and 56 proteins that were abnormally abundant in people with diabetes compared with people without diabetes, including a subset of about 42 protein abnormalities in vitreous from people with PDR.^{48,49} The analysis of the vitreous proteome has revealed groups of proteins associated with biological pathways, including the kallikrein-kinin, complement, and

coagulation systems. These proteomics findings have suggested that the vitreous plays a number of active roles in intraocular physiology, beyond those previously described. One of the most striking changes that we identified in the PDR proteome was an increase in carbonic anhydrase 1 (CA-1), which is an abundant enzyme in red blood cells.⁴⁸ Intravitreal injection of purified CA-1 into rats increased RVP and retinal thickening. In addition, we demonstrated that both complement 1 inhibitor and a neutralizing antibody against plasma kallikrein inhibited CA-1-induced RVP. These results have revealed a novel pathway by which retinal hemorrhages may exacerbate RVP and retinal edema. Moreover, these studies have suggested that the plasma kallikrein-kinin system may play a role in the loss of blood-retinal barrier dysfunction in DR.⁵⁰ The current challenges from these proteomics data are to interpret the relevance of changes in vitreous proteins with regards to the pathogenesis of DR and to examine whether these newly identified vitreous proteins suggest novel therapeutic targets.

Conclusions

Although there have been significant advances in the management of DR risk factors, including hyperglycemia and hypertension, PDR and DME continue to be leading causes of vision loss and their treatment and prevention remains an unmet clinical need. Since DR is a progressive disease that can develop over decades of diabetes duration, the front-line treatments to reduce the incidence and slow the early progression of DR, as well as other diabetic microvascular complications, are intensive glucose and blood pressure control. Once DR develops, additional mechanisms, including hypoxia-induced VEGF production, contribute to retinal disease progression. New adjunct therapies to current standard of care laser photocoagulation treatment are needed to reduce or reverse NPDR and the progression to PDR and DME. One approach has focused on the downstream effects of glucose as a mediator of DR. Further analyses using new -omics approaches could reveal additional therapeutic targets for DR that are associated with biological processes that mediate DR, in addition to factors that are linked to hyperglycemia or hypertension. ■

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