ABSTRACT

Hyperglycemia is the principal underlying cause of diabetic microvascular complications, including diabetic retinopathy (DR). Several complex mechanisms are involved in the pathways leading from hyperglycemia to cellular dysfunction and damage. Hyperglycemia-induced protein kinase C (PKC) activation, in particular PKC-β activation, plays a key role in several of these pathways. Therefore, inhibition of PKC-β represents a potential therapeutic approach. Vascular endothelial growth factor (VEGF) is a major mediator of retinal neovascularization and vascular permeability in DR. Several anti-VEGF therapies are in various stages of development and study. These therapies include aptamers, antibody fragments, siRNAs, and intravitreal steroids. Further study will determine the role of these agents in monotherapy or combined therapy for the microvascular damage caused by diabetes. (Adv Stud Ophthalmol. 2006;3[1]:8-12)

THE MOLECULAR BIOLOGY OF DIABETIC RETINOPATHY: OPPORTUNITIES FOR THERAPEUTIC INTERVENTION*

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Diabetic retinopathy (DR) represents a spectrum of disease, ranging from patients who have diabetes but no evidence of DR through mild, moderate, and severe stages of nonproliferative diabetic retinopathy (NPDR) and progressing to proliferative diabetic retinopathy (PDR). Diabetic macular edema (DME) can occur at any point along this spectrum. In asymptomatic patients with no evidence of DR, there are already a variety of biological changes occurring in the retina. Ophthalmologists can intervene throughout the spectrum of DR to reduce its progression and resultant vision loss.

EFFECTS OF ACTIVATED PROTEIN KINASE C ON DIABETIC RETINOPATHY

Hyperglycemia is thought to be the principal underlying cause of diabetic microvascular complications, leading to cellular dysfunction and damage through several complex physiologic mechanisms. As Figure 1 illustrates, the hyperglycemic state stimulates synthesis of diacylglycerol, which is a potent activator of protein kinase C (PKC). PKC activation has multiple effects on the microvasculature, including induction of basement matrix protein synthesis, activation of leukocytes and endothelial cells, increased endothelial permeability, cytokine activation, and angiogenesis. Therefore, PKC inhibition may ameliorate hyperglycemia-induced cellular dysfunction and damage, even in the presence of high glycemic levels.

At least 13 isoforms of PKC have been identified. Of these isoforms, the β isoform is thought to play the most important role in mediating the microvascular complications of diabetes, including DR. PKC-β overexpression in genetically altered nondiabetic mice has
been associated with the development of the same pathologic changes that occur in DR. Figure 2 shows that the level of PKC activation in human monocytes was associated with the severity of DR. PKC-β activity was progressively higher in monocytes from patients with more severe retinopathy (King GL, Unpublished data, 2005).

**Effect of Vascular Endothelial Growth Factor on Diabetic Retinopathy**

The role of vascular endothelial growth factor (VEGF) in the development and progression of DR has been delineated through many animal and human investigations. In one study, intravitreal injection of clinically relevant concentrations of VEGF dramatically increased vascular permeability in rat retinas. Likewise, intraocular injection of VEGF into primate eyes has been shown to cause changes similar to those seen in severe NPDR, including aneurysms and capillary dropout. VEGF also has been shown to be expressed in the retinas of patients with diabetes; in some cases, early in the disease. A study measuring VEGF levels in the aqueous and vitreous fluids demonstrated high VEGF levels in the eyes of patients with active PDR compared to VEGF levels in those who had no proliferative disease, diabetes without PDR, or diabetes with quiescent PDR (Figure 3). These animal and human studies suggest that VEGF is an important mediator of retinal neovascularization and vascular permeability under ischemic retinal conditions, such as DR. VEGF acts through several pathways, including PKC activation (Figure 4). These findings suggest that blocking the action of VEGF may be an effective means of preventing many of the changes that occur in DR.

**Summary**

On the physiologic level, diabetic complications appear to be the result of hyperglycemia-induced dysfunction. Activated PKC β is thought to mediate much of the vascular dysfunction associated with diabetes. Increased expression of VEGF promotes neovascularization and increased vascular permeability, at least partly through the activation of PKC. Therapies that counter PKC-β and VEGF-mediated dysfunction may alleviate the microvascular damage and complications observed in patients with diabetes.

**Therapeutic Opportunities**

**PKC-β Inhibitors**

Several PKC-β inhibitors are being evaluated in preclinical and early stage clinical trials. More clinical data are currently available for ruboxistaurin (RBX) than for the other agents under investigation. RBX is a highly selective PKC-β inhibitor that is orally bioavail-

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*Figure 1. Hyperglycemia-Induced PKC Activation*

*Figure 2. Severity of DR was Associated with Increased Monocyte PKC Activity*

**Figure 1. Hyperglycemia-Induced PKC Activation**

**Figure 2. Severity of DR was Associated with Increased Monocyte PKC Activity**

ADP = adenosine diphosphate; ATP = adenosine triphosphate; DAG = diacylglycerol; PKC = protein kinase C; PLC = phospholipase C.

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DM = diabetic macular edema; DR = diabetic retinopathy; NPDR = non-proliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy; PKC = protein kinase C.
able and taken once daily. In animal studies, RBX has been associated with reductions in retinal neovascularization, diabetes-induced vascular permeability, and the normalization of retinal blood flow.\textsuperscript{5,11,12} Studies in rats have demonstrated that intravitreal injection of VEGF is associated with a large increase in retinal vascular permeability. This increase was blocked by the oral administration of RBX for 1 week.\textsuperscript{3}

The promising results from animal studies have led to various trials of RBX in humans, including 2 double-masked, randomized, placebo-controlled, multidose, multicenter clinical trials. The Protein Kinase C-β Inhibitor Diabetic Retinopathy Study (PKC-DRS) investigated the effect of RBX on DR progression in patients with moderate to severe NPDR who had not had prior cataract surgery or scatter photocoagulation treatment.\textsuperscript{13} Although RBX treatment did not prevent DR progression in this study, patients receiving RBX had a reduced risk of moderate vision loss. The Protein Kinase C-β Inhibitor Diabetic Macular Edema Study (PKC-DMES) examined the effect of RBX on DME progression in patients with mild to moderate NPDR and DME present at baseline and no prior focal or pan-retinal photocoagulation.\textsuperscript{14} The Cox proportional hazard model in the PKC-DMES trial showed a 36% reduced risk of DME progression to within 100 µ of the macula for patients receiving RBX treatment compared to patients receiving placebo (Figure 5). Although there was no apparent effect on retinopathy progression, Cox hazard analysis showed that RBX treatment was associated with a 70% risk reduction for moderate vision loss compared to placebo (Figure 6).

**ANTI-VEGF STRATEGIES**

Studies in murine models of proliferative retinopathy have shown that intravitreal injection of VEGF inhibitors can reduce neovascularization.\textsuperscript{15,16} In nonhuman primates, administration of a VEGF inhibitor prevented retinal ischemia-associated iris neovascularization.\textsuperscript{17} The results of animal studies have spawned several anti-VEGF approaches that are now in clinical trials with humans. For example, phase II study results for pegaptanib showing a possible beneficial effect for DME have recently been published,\textsuperscript{18} and phase III trials of pegaptanib for treatment of DME are beginning. Trials of ranibizumab, VEGF trap, and bevacizumab for DME are under way. Bevacizumab is already being used off-label in some cases.
**Intravitreal Steroids**

Steroids have multiple effects on the diabetic retina, including the potential to prevent the release of VEGF or increase its degradation. Several phase II and phase III clinical trials of intravitreal steroids are under way or completed. Preliminary data suggest that intravitreal steroids reduce retinal edema in a time-limited manner, but that this reduction is not consistently associated with improved visual acuity.19-21 There are several potential risks associated with intravitreal steroid administration, including endophthalmitis, cataract formation, and elevation of intraocular pressure.

The Diabetic Retinopathy Clinical Research Network is conducting a long-term study of more than 470 patients to investigate the effects of a preservative-free steroid formulation in the eye. Patients will be treated with laser photocoagulation or with 1-mg or 4-mg triamcinolone acetonide injections, and they will be followed for 3 years.22

**Summary**

Studies suggest that PKC-β and VEGF inhibition may prevent microvascular damage or complications that occur in the retina of patients with diabetes. RBX treatment has been associated with a reduced risk for moderate vision loss and reduced DME progression in patients with microvascular complications from diabetes. Clinical trials of several VEGF inhibitors and intravitreal steroids are also under way.

**Conclusions**

New therapies aimed at preventing or ameliorating the development of diabetic microvascular complications, including DR, are under investigation. Understanding the roles of key mediators, such as PKC β and VEGF, have helped develop strategies to block their action and may be instrumental in preventing diabetes-associated vision loss. Preclinical and clinical studies have led to the development of PKC-β inhibitors, anti-VEGF approaches, and intravitreal steroid use, all of which hold great promise. However, further clinical trials will determine the appropriate role of these new therapies in clinical care.
REFERENCES


