MOLECULAR BIOLOGY AND NATURAL HISTORY OF DIABETIC RETINOPATHY*

Lloyd P. Aiello, MD, PhD†

ABSTRACT

Diabetic retinopathy is the most common diabetic microvascular complication, affecting approximately 50% of patients with diabetes. During the next 2 decades, the prevalence of diabetes and the impact of diabetic retinopathy (DR) are expected to grow considerably in the United States and around the world. Beginning in the 1950s, a number of clinical trials have characterized the natural history of DR and the efficacy and safety of DR treatment strategies. Even before the onset of clinically evident retinopathy, diabetes is associated with a number of pathologic and biochemical alterations of the retina, including derangements of blood flow, pericyte loss, leukocyte adhesion, and capillary basement membrane thickening. The initial stages of nonproliferative DR (NPDR) are characterized by retinal hemorrhage and microaneurysms. This stage is followed eventually by proliferative DR (PDR), which is characterized by the formation of new retinal blood vessels. Diabetic macular edema (DME) can occur at any stage of DR although it is more likely with more advanced retinopathy. The severity of DR has traditionally been classified using a complex rating system that was developed by the Early Treatment Diabetic Retinopathy Study (ETDRS) investigators. More recently, the American Academy of Ophthalmology has developed a simplified 5-stage rating scale that is more readily applicable to routine clinical use.

The severity of DR is an important predictor of retinopathy progression and severe vision loss. The likelihood of progression to PDR within 1 year is approximately 1% for patients with mild NPDR, but about 15% for patients with severe NPDR. In 5 years the rates are 16% and 56%, respectively. Laser therapy can prevent visual loss, especially in patients with PDR, but is associated with a number of potential adverse events, including loss of peripheral vision and night vision, as well as alterations of color perception. More serious adverse events, such as laser burns to the fovea, lens, iris, or cornea, may also occur.

Recent advances in understanding the biochemical pathways that cause DR have led to the development of new treatment strategies to prevent the progression to severe vision loss. Protein kinase C (PKC) participates in many physiological processes that contribute to DR, including regulation of retinal blood flow and the production and action of retinal growth factors and cytokines. Emerging preclinical and clinical evidence suggests that pharmacologic agents that inhibit PKC may slow the progression of DR. Other potential therapeutic targets include vascular endothelial growth factor, which promotes the formation of new blood vessels, and agents that inhibit the formation or activity of free radicals. These novel treatment strategies are currently being evaluated in randomized controlled clinical trials. (Adv Stud Ophthalmol. 2005;2(1):8-13)

Diabetic ocular complications are a leading cause of severe visual loss, moderate visual loss, and new-onset blindness in the United States. They are associated with an annual incidence of 700,000 cases of proliferative diabetic retinopathy (PDR), 5000 cases of blindness, and a financial impact of $620 mil-

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An analysis of data from the Behavioral Risk Factor Surveillance System, a nationwide standardized survey conducted by the US Centers for Disease Control and Prevention and health agencies of 42 states, found that 6.5% of the US adult population (approximately 13 million persons) have been diagnosed by a healthcare provider as having diabetes. Another 5.2 million persons have unrecognized diabetes. According to the American Diabetes Association, diabetes is associated with direct medical costs of approximately $44.1 billion per year in the United States, as well as another $54 billion in indirect costs.

In 1997, medical expenses incurred by patients with diabetes totaled an average of $10,071 per patient, compared with $2,669 per person without diabetes. By 2002, the per-capita costs of diabetes had increased more than 30%, to $13,243. The World Health Organization (WHO) has estimated that by 2025, 22 million Americans may have diabetes, although the actual number may be considerably greater than this. The increasing impact of diabetes is not only a problem in the United States: the WHO has forecast a worldwide increase in the number of adults with diabetes of 122% (from 135 million to 300 million) between 1995 and 2025.

Diabetic retinopathy is the most common diabetic microvascular complication, affecting approximately 50% of patients. It has been estimated that more than 5 million Americans (approximately 2.5% of the US population) have some degree of diabetic retinopathy (DR), and that diabetes is the most common cause of new legal blindness among US adults. Other common complications of diabetes include diabetic peripheral neuropathy (40% of patients), diabetic nephropathy (35%), and cardiovascular disease (43%).

Beginning in the 1950s, a number of clinical trials have provided detailed information regarding the natural history of DR, the rate of DR progression, and the effectiveness of various treatment options. Some of the principal clinical trials of DR include pituitary ablation studies, the Diabetic Retinopathy Study, the Early Treatment Diabetic Retinopathy Study (ETDRS), the Diabetic Retinopathy Vitrectomy Study, the Diabetes Control and Complications Trial, and the United Kingdom Prospective Diabetes Study. In addition, recent advances in the understanding of both the natural history and the underlying molecular biology of diabetes have led to the development of potential new therapeutic approaches, and many of these approaches are currently being evaluated in clinical trials.

### STAGES OF DR

The retinal manifestations of diabetes encompass a broad spectrum of microvascular and clinical changes, but are generally divided into 2 broad subdivisions: DR and diabetic macular edema (DME). Even before the appearance of clinically evident DR, a number of pathological changes have begun in the retina, including alterations of retinal blood flow, the appearance of pericyte ghosts, pericyte loss, acellular capillaries, endothelial leukocyte adhesion, thickening of capillary basement membrane, and other biochemical alterations. The progression to nonproliferative diabetic retinopathy (NPDR) is associated with clinical findings of hemorrhage and microaneurysms, intraretinal microvascular anomalies (IRMAs), and venous beading. Further progression results in the appearance of PDR, which is characterized by neovascularization, vitreous hemorrhage, and/or preretinal hemorrhage. DME may develop at any point along this spectrum, although it is more common in more advanced stages of retinopathy.

It is important to be able to categorize the stages of retinopathy progression in order to compare research findings across different clinical trials. The severity of DR

<table>
<thead>
<tr>
<th>Proposed Disease Severity Level</th>
<th>Findings Observable on Dilated Ophthalmoscopy</th>
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<tbody>
<tr>
<td>No apparent retinopathy</td>
<td>No abnormalities</td>
</tr>
<tr>
<td>Mild nonproliferative diabetic retinopathy</td>
<td>Microaneurysms only</td>
</tr>
<tr>
<td>Moderate nonproliferative diabetic retinopathy</td>
<td>More than just microaneurysms but less than severe diabetic retinopathy</td>
</tr>
<tr>
<td>Severe nonproliferative diabetic retinopathy</td>
<td>Any of the following: more than 20 intraretinal hemorrhages in each of 4 quadrants; definite venous beading in 2+ quadrants; prominent intraretinal microvascular abnormalities in 1+ quadrant AND no signs of proliferative retinopathy</td>
</tr>
<tr>
<td>Proliferative diabetic retinopathy</td>
<td>One or more of the following: neovascularization, vitreous/preretinal hemorrhage</td>
</tr>
</tbody>
</table>

has traditionally been graded using a rating scale that was first developed in the ETDRS. In this rating scale, NPDR was categorized as mild, moderate, severe, and very severe; and PDR was categorized as less than high risk or high risk. DME was categorized clinically as absent, not clinically significant, or clinically significant. For clinical research purposes, a more detailed and complex rating scale was developed that included 13 categories and 2 subcategories. However, many clinicians feel that these complex rating scales were not well suited for routine clinical use because of the number of DR stages and the complexity of the ratings for each stage, as well as the need to use standard photographs for grading.

Recently there has been an effort by the American Academy of Ophthalmology to simplify the ratings of DR using an internationally recognized scale. This classification is summarized in Table 1. Although the risk of progression is similar for patients with no apparent DR and with mild NPDR, this rating scale distinguishes between the 2 conditions because it is often helpful for patients to realize that they have begun to develop clinically evident retinopathy. Severe NPDR is classified using the “4:2:1” rule (20 or more intraretinal hemorrhages in each of the 4 quadrants; or definite bleeding in 2 or more quadrants; or prominent IRMAs in 1 or more quadrants). Many clinicians would prefer a definition of severe NPDR that does include analysis of IRMAs. However, IRMAs have been found to be highly associated with advanced disease, and it is therefore important that clinicians learn to identify them.

The relationship between this new proposed international DR rating scale and the corresponding ETDRS levels are shown in Table 2. In this new classification system, DME is now divided into 2 categories, apparently absent (no apparent retinal thickening or hard exudates in the posterior pole), or apparently present (some degree of retinal thickening or hard exudates in the posterior pole). When present, DME may be subdivided as mild (some retinal thickening or hard exudates in the posterior pole but distant from the center of the macula); moderate (retinal thickening or hard exudates approaching the center of the macula but not involving the center); or severe (retinal thickening or hard exudates involving the center of the macula).

The level of retinopathy is highly correlated with subsequent disease progression. In the ETDRS, the rate of progression was examined for patients with mild, moderate, or severe NPDR at baseline. Approximately 1% of patients with mild NPDR at baseline developed high-risk characteristics for PDR within 1 year and 16% at 5 years.

Approximately 15% of patients with severe NPDR at baseline developed high-risk characteristics in 1 year while 56% developed high-risk PDR within 5 years. For patients with very severe NPDR at baseline, the numbers are even higher: 45% and 71%, respectively. Thus, information about the baseline level of retinopathy is important to help set schedules for follow-up eye examinations. Increasing severity of DR at baseline is also associated with increasing progression to sight-threatening disease and the need to see the patient more frequently.

### Laser-Based Treatment

Panretinal laser photocoagulation can be effective for the treatment of DR, especially for PDR. Severe vision loss (visual acuity <5/200) develops in 20% to 30% of untreated eyes, but in fewer than 5% of treated eyes. For DME, current treatments are not quite as beneficial. Focal photocoagulation produces approximately a 50% reduction in moderate visual loss on average, compared with deferral of photocoagulation, in eyes with clinically significant macular edema. However, vision already lost is usually not restored.

There are significant limitations to the available laser-based therapies. Laser treatment is not effective for every patient, and it partially destroys the retina in an effort to pre-
serve vision. Side effects of treatment include loss of peripheral vision or night vision, as well as altered color perception. Other potential complications include burns to the fovea, lens, iris, or cornea; burns spread through the macula; choroidal effusions; and angle-closure glaucoma. Therefore, novel therapies are needed for DR to prevent the development of PDR, the progression of DME, and the progression to NPDR.

Molecular Biology of DR

Understanding of the molecular biology underlying DR has increased substantially during the last decade. This knowledge has spawned the development of several novel therapeutic approaches. Four major metabolic pathways have been described by which hyperglycemia results in the activation of cellular signaling molecules that alter gene expression or protein function, resulting in retinopathy and other microvascular complications of diabetes (Figure). The aldose reductase pathway is a 2-step metabolic process in which intracellular glucose is first converted to sorbitol by the enzyme aldose reductase, and sorbitol is then converted to fructose by the enzyme sorbitol dehydrogenase. In the advanced glycation endproduct (AGE) pathway, the function of cellular proteins is disrupted by covalent modification and cross-linking of protein molecules by excessive glucose. In the reactive oxygen intermediate pathway, high levels of glucose result in the formation of toxic free radicals produced by mitochondrial energy metabolism. Each of these 3 pathways is hypothesized to result in the accumulation of reactive metabolites that contribute to widespread cellular injury.

A fourth pathway, activation of protein kinase C (PKC) is involved in many aspects of vascular regulation that are potentially important in retinal injury in diabetes, including vascular permeability, vasodilation, and growth factor activity. The identification of these biochemical pathways has led to the development of specific strategies to intervene and prevent the development of injury. For example, the aldose reductase pathway may be blocked by aldose reductase inhibitors; the AGE pathway may be blocked by soluble AGE receptors (soluble RAGE); reactive oxygen species may be inhibited by antioxidants; and the effects of PKC activation may be ameliorated by PKC inhibitors.

PKC has received considerable recent attention in the pathogenesis and treatment of diabetic microvascular complications because it produces a number of physiological effects that are thought to contribute to the features of retinal injury that were described previously (eg, pericyte loss, acellular capillaries, microaneurysms). PKC is a serine/threonine protein kinase that is found in the eye and in other tissues throughout the body. It is a G-protein coupled receptor that is activated by diacylglycerol. In hyperglycemia, the production of palmitate and oleate stimulate de novo synthesis of diacylglycerol. Diacylglycerol activates PKC (especially the PKC-β and PKC-δ isoforms), which produces its physiological effects by phosphorylating proteins. PKC affects retinal blood flow due to effects on endothelin-1 expression, angiotensin, and nitric oxide. It increases vascular permeability and stimulates the production and mediates the action of growth factors such as vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF-β). TGF-β is important in the increase in basement membrane protein synthesis, which is characteristic of early diabetes in the eye and elsewhere. VEGF is especially important in neovascularization and in retinal leakage that contributes to DME. PKC also stimulates the adhesion of leukocytes to the vascular endothelium.

Evidence from animal models and clinical studies demonstrates the importance of PKC in the develop-
ment of DR. The effects of PKC inhibition using ruboxistaurin were evaluated in diabetic rats. The vascular permeability of blood vessels within the retina increased markedly in untreated diabetic rats, and this increase was prevented when the animals were treated with a PKC-β inhibitor. More recently, the effects of PKC have been examined in transgenic animals that overexpress PKC. The overexpression of PKC in these animals simulates the effects of diabetes on PKC activity in the retina without inducing diabetes. Early diabetes-like changes were observed in the retinas of these animals, including microaneurysms, venous beading, pericyte ghosts, and acellular capillaries (King GL, personal communication). Confocal microscopy revealed the presence of venous loops, which are characteristic of early DR and are not seen in normal control animals. A significant relationship between PKC activity and DR has also been observed in patients with diabetes. In studies conducted at the Beetham Eye Institute, patients with various levels of DR severity have undergone evaluation for the activation of PKC-β in their monocytes (Aiello et al, unpublished observations). This research has demonstrated a very strong correlation between more advanced DR and greater PKC activation in the blood cells of these patients, suggesting a relationship between PKC activation and the development of NPDR or DME in patients with diabetes.

Recent research suggests that VEGF is also important in the development of DR. VEGF is a homodimeric glycoprotein that is relatively selective for endothelial cells. It affects both angiogenesis and vasopermeability. It is produced by numerous cell types within the eye and is diffusible within the eye. The expression of VEGF is increased by hypoxia, and is closely correlated with neovascularization and ischemic retinal disorders including DR. The concentration of VEGF in the vitreous fluid is very high in patients with diabetes who have active PDR, but not in nondiabetic patients or in patients who have diabetes but do not have active PDR. VEGF is believed to produce retinal complications in diabetes as the result of a multistep process. VEGF first binds to high-affinity receptors located on endothelial cells. This receptor binding activates several intracellular signaling pathways that directly or indirectly increase PKC activity, including mediators phospholipase C, diacylglycerol, and inositol triphosphate. Increased activation of PKC ultimately results in increased cellular proliferation and vascular permeability.

Numerous studies are being conducted to evaluate these treatments, including PKC inhibitors, VEGF inhibitors, and intravitreal steroids. Several of these studies are summarized in Table 3.

**SUMMARY AND CONCLUSIONS**

The natural history of DR has been well established in a number of large clinical trials conducted over the last several decades. The development of a simplified clinical rating scale for the evaluation of DR and DME should make it easier for clinicians to communicate their findings with each other. There have been a number of recent advances in understanding the mechanisms of DR, including the identification of growth factors and intracellular signaling pathways that stimulate neovascularization and increase vascular permeability. The identification of these biochemical pathways has suggested a number of new therapeutic approaches for the prevention or treatment of DR, many of which are currently being evaluated in multicenter controlled clinical trials.

<table>
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<th>Agent</th>
<th>Action</th>
<th>Indication</th>
<th>Status</th>
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<tr>
<td>DRS (ruboxistaurin)</td>
<td>Oral, once-daily</td>
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<td>Intravitreal steroid</td>
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<td>Triamcinolone</td>
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PKC = protein kinase C; PDR = proliferative diabetic retinopathy; DRS = Diabetic Retinopathy Study; NPDR = nonproliferative diabetic retinopathy; DMES = Diabetic Macular Edema Study; DME = diabetic macular edema; VEGF = vascular endothelial growth factor.
REFERENCES


