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Prophylactic Medical Treatment of Diabetic Retinopathy

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1. Introduction

Diabetic retinopathy (DR) is a leading cause of visual loss and blindness in adults in developed and developing countries (Friedman et al., 2011). Clinical trials have shown that intensive glycemic control reduces the incidence and progression of DR (Reichard et al., 1993; Stratton et al., 2001; DCCT, 2002). Other metabolic factors also affect the progression and development of DR. The UK Prospective Diabetes Study Group reported that tight blood pressure control is effective for reducing the incidence of DR (UK Prospective Diabetes Study Group, 1998a, 1998b). The EUCLID study group reported that inhibitors of angiotensin-converting enzyme drugs decreased the progression of retinopathy in patients without hypertension who had type 1 diabetes with little or no nephropathy (Chaturvedi et al., 1998). Lipid-lowering therapy with fenofibrate also might reduce the progression of DR (Chew et al., 2010; Keech et al., 2007). Among the metabolic factors, although glycemic control seems to be the most important, achieving acceptable glucose homeostasis is difficult, even when patients are highly compliant. Furthermore, DR continues to develop and progress even in patients who are treated intensively to achieve better glycemic control. Therefore, it is important to find medical options other than glycemic control to prevent diabetic ocular complications. The metabolic changes that accompany hyperglycemia, such as activation of the polyol pathway (Gabbay, 1973; Lorenzi, 2007; Robison et al., 1988; 1989), activation of protein kinase C (PKC) (Frank, 2002; Liang et al., 2005; Shiba et al., 1993), increased oxidative stress (Gurler et al., 2000; Jennings et al., 1991; Pan et al., 2008; Pinto et al., 2007), leukocyte adhesion to the endothelial cells (McLeod et al., 1995; Miyamoto et al., 1996; Schroder et al., 1991), and accumulation of advanced glycation end products (AGEs)

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(Chibber et al., 1997; Hirata et al., 1997; Kakehashi et al., 2008), are related to the development and progression of diabetic ocular complications. In particular, the polyol pathway is correlated strongly with oxidative stress, activation of PKC, and accumulation of AGEs that lead to induction of vascular endothelial growth factor (VEGF). A key enzyme in the polyol pathway, aldose reductase (AR), is found in the retina and lens (Akagi et al., 1983; Kern & Engerman, 1981). AR inhibitors (ARIs) slowed thickening of the basement membrane of the retinal capillaries and progression of diabetic cataract in experimental studies (Hu et al., 1983; Robison et al., 1983). Thus, the polyol pathway might be the most attractive target for adjunctive treatment to prevent development of diabetic ocular complications. Based on favorable observations from experimental studies using ARIs (Obrosova et al., 2005; Okuda et al., 1985; Robinson et al., 1996; Sun et al., 2006; Yeh et al., 1986), a clinical trial of the ARI, sorbinil, was conducted in 1990, but this drug did not affect the development of DR, and enthusiasm for the clinical application of ARIs waned. To test drugs to treat DR, good animal models of DR would be useful. In this context, the spontaneously diabetic Torii (SDT) rat, a newly established non-obese type 2 diabetes model rat, seems especially appropriate, since it shows advanced DR resembling that in humans (Kakehashi et al., 2006; Kakehashi, 2011a, b; Shinohara et al., 2000). A recent study showed a strong preventative effect of ARIs on the development of DR in this animal model (Kakehashi et al., 2011).

In this chapter, we discuss several studies of medical treatment to prevent DR including our previous and ongoing studies using SDT rats. Among the various medical treatments, we have focused on the ARIs, because we believe that they are the most potent and safest of the potential treatments.

2. ARIs

Many experimental studies of ARIs (Obrosova et al., 2005; Okuda et al., 1985; Robinson et al., 1996; Sun et al., 2006; Yeh et al., 1986) have reported favorable results, with the exception of a clinical trial of sorbinil as mentioned previously.

However, our recent study on the effects of the ARI, fidarestat (SNK-860; Sanwa Kagaku Kenkyusho, Nagoya, Japan), in SDT rats showed that the drug strongly inhibited the development of DR (Kakehashi et al., 2011). We evaluated four rat groups: untreated, low- and high-dose (8 and 32 mg/kg/day) fidarestat-treated SDT rats, and nondiabetic control Sprague-Dawley (SD) rats. We evaluated the DR and measured the retinal sorbitol and reduced glutathione (GSH), VEGF in the ocular fluid, and urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG). Compared with the untreated the incidence of DR was significantly lower in the low- and high-dose fidarestat groups group. Compared with the untreated group, the retinal sorbitol levels were lower in the control and low- and high-dose groups; the retinal GSH levels were higher in the control and the low- and high-dose groups; the VEGF levels were lower in the control and low- and high-dose groups; and the 8-OHdG levels were lower in the control and low- and high-dose groups. The results indicated that fidarestat prevents DR in SDT rats by suppressing oxidative stress and sorbitol production.

Based on these findings, we evaluated the recently developed ARI, ranirestat (AS-3201, Dainippon Sumitomo Pharmaceutical Co., Ltd., Osaka, Japan) for treating DR in SDT rats.

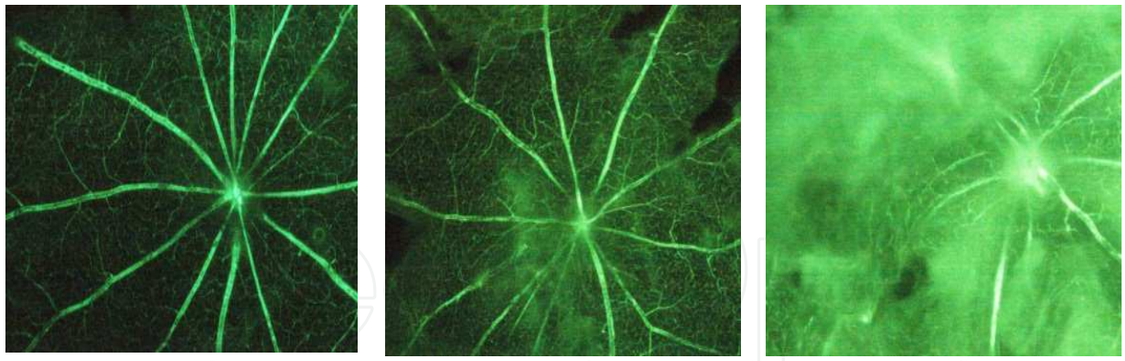
Male SDT rats and SD rats were obtained from CLEA, Inc., Tokyo, Japan. All SDT rats were confirmed to be diabetic based on a non-fasting blood glucose concentration exceeding 19.4 mmol/L. All rats were fed standard rat chow (CRF-1, Oriental Yeast, Inc., Tokyo, Japan) with or without ARI. Epalrestat (Kinedak, Ono Pharmaceutical Co., Ltd., Osaka, Japan), an ARI that is commercially available in Japan, was used as the positive control. The ranirestat-treated rats received the drug once daily; the epalrestat-treated rats were fed chow containing epalrestat at the onset of diabetes; and the SD rats and untreated SDT rats were fed chow without an ARI.

The animals were divided into six groups: normal SD rats (n=8), untreated SDT rats (n=9), ranirestat-treated SDT rats (0.1 mg/kg/day for 40 weeks, n=7; 1.0 mg/kg/day for 40 weeks, n=8; 10.0 mg/kg/day for 40 weeks, n=7), and epalrestat-treated SDT rats (100 mg/kg/day for 40 weeks) (n=8).

The body weight, blood glucose, and glycosylated hemoglobin (HbA1c) were measured once monthly. Blood samples were collected from the tail vein of the non-fasting rats to measure the plasma glucose and glycosylated hemoglobin levels. The body weight was greater in the SD than that in SDT rats with or without treatment ($p<0.01$). The mean plasma glucose levels and HbA1c levels in the SD rats were significantly ($p<0.01$) lower than in the SDT rats with or without treatment. However, there were no significant differences in the blood glucose levels and HbA1c levels between the treated and untreated SDT rats, indicating that an ARI did not affect the glycemic control. Therefore, we did not have to consider any effect of glycemia in interpreting results.

Fluorescein-dextran microscopy was performed after intracardiac injection of fluorescein-dextran (fluorescein isothiocyanate dextran, Sigma, St. Louis, MO, USA), using a modification of the method of D'Amato et al. (1993). With the animals under deep anesthesia induced by intraperitoneal injection of pentobarbital sodium (25 mg/kg body weight, Nembutal, Dainihonseiyaku, Osaka, Japan), 1 ml of phosphate buffered saline containing 50 mg of fluorescein dextran was injected into the left ventricle of each animal. After 5 minutes, the eyes were enucleated for fluorescein microscopy. The retinas were peeled from the eyecups, and the entire retinas were flat-mounted on a slide glass without fixation. A drop of aqueous mounting medium (Crystal/Mount, Biomeda Corp., Foster City, CA, USA) was applied over the retinas and allowed to dry. The flat-mounted retinas were examined by fluorescence microscopy (Nikon SMZ1500 with P-FLA fluorescence attachment, Nikon, Tokyo, Japan). As we reported previously (Kakehashi et al., 2006), DR was diagnosed when extensive fluorescein leakage was seen around the optic disc, and we classified the DR into three stages: no retinopathy (no dye leakage), mild retinopathy (mild dye leakage around the optic disc), and severe retinopathy (extensive dye leakage throughout the entire retina) (Fig. 1).

With the animals under deep anesthesia induced by intraperitoneal injection of pentobarbital sodium, the eyes were enucleated for conventional histopathologic studies and placed in a fixative (Superfix KY-500, Kurabo, Tokyo, Japan) to avoid iatrogenic retinal detachment. The fixed eyes were washed in 0.1% mol/L cacodylate buffer and embedded in paraffin. The paraffin block was cut into 4-μm sections and stained with hematoxylin and eosin for conventional histopathologic examination. As reported previously (Kakehashi et al., 2006), DR was diagnosed when large retinal folds mimicking tractional retinal detachment around the optic disc were observed.



Retinopathy (-) Retinopathy (+) Retinopathy (++)

Fig. 1. Classification of diabetic retinopathy. Retinopathy (-), no dye leakage; retinopathy(+), mild dye leakage around the optic disc; retinopathy (++), extensive dye leakage throughout the retina.

Compared with controls (7/9 eyes), DR did not develop in any ranirestat-treated groups (0.1 mg/kg/day; 1.0 mg/kg/day; 10.0 mg/kg/day for 40 weeks) (0/7, 0/8, 0/7 eyes, $p<0.01$, chi-square test). Mild DR developed in the epalrestat-treated group (100 mg/kg/day for 40 weeks) (2/8 eyes; $p=0.09$ by the chi-square test). Epalrestat did not prevent development of DR in SDT rats. The incidence of DR was significantly ($p<0.01$) lower in the ranirestat-treated groups compared with the untreated group.

Immunohistochemical procedures were based on the standard avidin-biotin horseradish peroxidase method using the appropriate antibody and developed with AEC Substrate Chromogen (DakoCytomation, Carpinteria, CA, USA). VEGF was immunostained with a monoclonal antibody for human VEGF (1:25 dilution, Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan). Carboxymethyl-lysine (CML) was immunostained with a monoclonal antibody for human AGEs (1:50 dilution for CML, Trans Genic Inc., Kumamoto, Japan). Bovine serum was used as the primary antibody for the negative control. The immunostaining grades were divided into three groups: minimal (-), moderate (+), and severe (++). Minimal staining was characterized by almost no retinal staining, moderate staining by light red retinal staining, and severe staining by intense dark red retinal staining. Fig. 2 shows immunostaining for CML and VEGF. Immunostaining for CML was very strong in the untreated eye (upper left) compared with minimal staining in the ranirestat-treated (10 mg/kg/day for 40 weeks) eye (lower left). Immunostaining for VEGF was moderate in the untreated eye (upper right) compared with minimal staining in the ranirestat-treated eye (lower right).

Strong immunostaining for CML was seen in eight of nine eyes of the untreated SDT rats. The staining significantly ($p<0.05$) decreased to the moderate level in the eyes treated with 1.0 mg/kg/day and 10.0 mg/kg/day of ranirestat, except for those treated with 0.1 mg/kg/day of ranirestat ($p=0.111$). Epalrestat did not have a significant ($p=0.241$) effect. Moderate immunostaining for VEGF was seen in eight of nine eyes in the untreated SDT rats, and this decreased significantly ($p<0.05$) to minimal staining in the rats treated with 1.0 mg/kg/day and 10.0 mg/kg/day of ranirestat, but not in those treated with the 0.1 mg/kg/day dose of ranirestat ($p=0.117$). Epalrestat did not have a significant effect ($p=0.247$).

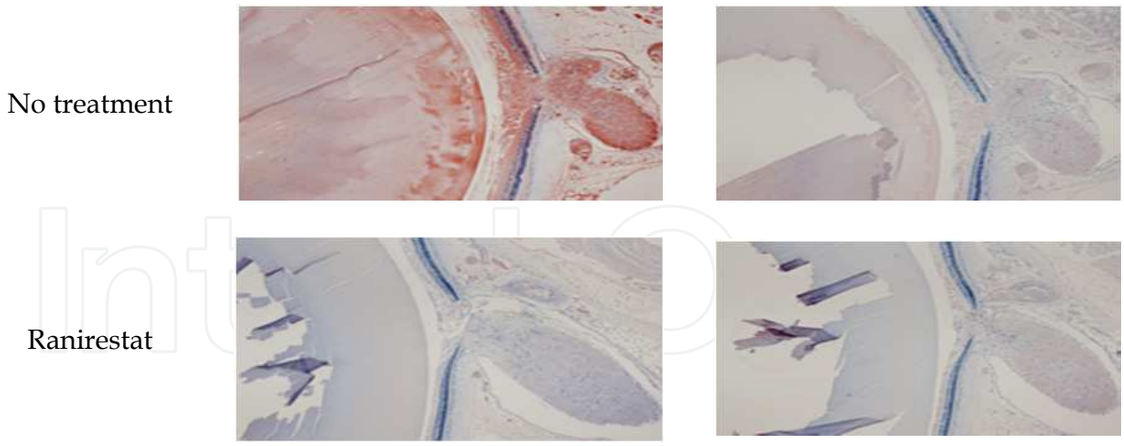


Fig. 2. Immunostaining for CML and VEGF. Immunostaining for CML shows strong staining in the untreated eye (upper left) compared with minimal staining in the ranirestat-treated eye (lower left). Immunostaining for VEGF shows moderate staining in the untreated eye (upper right) compared with minimal staining in the ranirestat-treated eye (lower right).

The retinal sorbitol and fructose levels were measured. ARI activity was determined after the liquid chromatography-tandem mass spectrometry method was performed. Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg, intra peritoneally; Abbott, Abbott Park, IL, USA), and the retinas were removed, promptly cooled with liquid nitrogen, and stored at −50°C. The samples for the sorbitol and fructose assay were prepared by protein precipitation followed by a solid-phase extraction procedure. The API 4000 Mass Spectrometer (AB SCIEX, Tokyo, Japan) was operated in the selected-reaction monitoring mode under optimized conditions to detect sorbitol- or fructose-negative ions formed by atmospheric pressure chemical ionization. At 15 weeks, the sorbitol levels in the retina of the SDT rats treated with ranirestat (10.0 mg/kg/day) were lower than in the untreated eyes. Eparlestat did not affect the retinal sorbitol levels. However, there were no significant differences in the sorbitol levels among the treated and untreated SDT rats at 40 weeks. Retinal fructose levels were lower at 15 weeks in SDT rats treated with ranirestat (10.0 mg/kg/day) than those that were untreated. Eparlestat did not affect the retinal fructose levels. At 40 weeks, there were no significant differences in the retinal fructose levels among the rat groups.

3. Other options for prophylactic medical treatment of DR

3.1 PKC β inhibitor

Liang and coworkers (2005) reported amelioration of vascular dysfunction in diabetic rats using an oral PKC β inhibitor. Those authors later reported that VEGF increases intraocular vascular permeability through activation of PKC in vivo and suggested that oral pharmacologic therapies involving PKC β-isoform-selective inhibitors might be effective for treating DR (Aiello et al., 1997). Based on their report, the oral PKC β inhibitor,

ruboxistaurin (RBX) (LY333531, Eli Lilly and Co., Indianapolis, IN), administered to patients with diabetes with no or mild DR, ameliorated diabetes-induced retinal circulation time abnormalities (Aiello et al., 2006). Another multicenter, double-masked, randomized, placebo-controlled study in patients with diabetes with moderate to very severe nonproliferative diabetic retinopathy showed that RBX was well tolerated and reduced the risk of visual loss but did not prevent DR progression (PKC-DRS Study Group, 2005).

We evaluated the effect of our PKC β inhibitor (JTT-010, Takatsuki, Japan) on the development of diabetic ocular complications in SDT rats. The rats had delayed oscillatory potentials on electroretinography, but DR developed eventually. We concluded that PKC β inhibitors may require concurrent administration of antihyperglycemic drugs to achieve maximal therapeutic effects on DR (Sasase et al., 2009).

3.2 Anti-AGE agents

Previous studies have suggested the concept of "metabolic memory" associated with accumulation of AGEs as DR develops (Chibber et al., 1997; Genuth et al., 2005; Hammes et al., 1999). Another anti-AGE agent, pyridoxamine, also prevented development of DR (Stitt et al., 2002). However, clinical trials of anti-AGE agents for the treatment of DR have not yet been conducted.

Based on those reports, we evaluated the effects of oral aminoguanidine and pyridoxamine on the development of cataract and DR in SDT rats (Toyoda et al., 2011) and reported that aminoguanidine prevented accumulation of CML and resulted in almost complete inhibition of DR, but pyridoxamine did not prevent DR. Aminoguanidine seems to be a stronger inhibitor of DR than pyridoxamine.

3.3 Anti-leukocyte adhesion agents

Leukocyte adhesion to the diabetic retinal vasculature is thought to be the critical early event in the pathogenesis of DR, resulting in breakdown of the blood-retinal barrier and in capillary nonperfusion (Gurler et al., 2000; Hu et al., 1983). Adamis and coworkers (1994) reported that the antileukocyte adhesion agents, anti-intercellular adhesion molecule-1 antibody (Miyamoto et al., 1999) and anti-CD 18 antibody (Barouch et al., 2000), were useful for treating experimental DR. They also showed an antileukocyte adhesion effect using a PKC β inhibitor (Nonaka et al., 2000) and receptor for the AGEs (Kaji et al., 2007). Nagai et al. (2007) reported an inhibitory effect of an angiotensin II type 1 receptor blocker on retinal leukostasis in diabetic mice.

We tested several commercially available antileukocyte adhesion agents in diabetic rats. We first evaluated the effectiveness of the sulphonylurea gliclazide for decreasing the adhesion of neutrophils to endothelial cells and leukocyte entrapment in the retinal microcirculation of streptozotocin (STZ)-induced diabetic rats. We showed that gliclazide attenuated retinal leukostasis irrespective of hyperglycemia in diabetic rats, whereas another sulphonylurea, glibenclamide, did not. This indicated that gliclazide, among the sulphonylurea drugs, might be selectively beneficial for preventing development of DR (Kinoshita et al., 2002). We also evaluated the effectiveness of topical nipradilol, a topical antiglaucoma αβ-blocker and

nitric oxide donor, on retinal microvascular leukocyte adhesion in diabetic rats. Topical nipradilol significantly reduced retinal leukostasis in the retinal microcirculation of STZ-induced diabetic rats, and we think that nipradilol may be a prophylactic agent that can inhibit development of early DR through its nitric oxide donor effects on the microcirculation. We believe that most antileukocyte adhesion agents can slow progression of DR but do not inhibit it completely. Good glycemic control or other additional medical treatments are needed to achieve a maximal effect on DR.

3.4 Anti-VEGF agents

Since the VEGF levels increase as DR progresses (Adamis et al., 1994; Aiello et al., 1994; Amin et al., 1997; Lutty et al., 1996), VEGF is very important in the development and progression of DR. VEGF is also a vascular permeability factor and plays an important role in the development of diabetic macular edema (DME) (Mathews et al., 1997). Based on these observations, several anti-VEGF agents such as an anti-VEGF antibody (Adamis et al., 1996), soluble VEGF-receptor chimeric proteins (Aiello et al., 1995), and antisense phosphorothioate oligodeoxynucleotides against VEGF (Robinson et al., 1996) have been tested in an animal model of retinal neovascularization mimicking DR. The results were very promising. Based on these favorable results in animal studies, several clinical studies have been undertaken to evaluate treatment of DME using an anti-VEGF antibody. The RESOLVE Study evaluated the safety and efficacy of intravitreal ranibizumab (Lucentis, Genentech, Inc., South San Francisco, CA) for treating patients with DME (Massin et al., 2010). Ranibizumab is a fully humanized monoclonal antibody fragment, which binds to multiple variants of VEGF-A, and is approved to treat age-related macular degeneration. The results indicated that intravitreal ranibizumab improves visual acuity and reduces macular thickness in DME. The Diabetic Retinopathy Clinical Research Network (DRCR.net) is evaluating and comparing several medical treatments for DME. The DRCR.net is a collaborative network formed in September 2002 that is dedicated to facilitating multicenter clinical studies of DR, DME, and associated conditions. Currently, there are more than 109 participating sites with over 320 physicians throughout the United States (http://drcrnet.jaeb.org/). The DRCR.net reported that intravitreal ranibizumab combined with laser treatment is more effective than laser treatment with or without triamcinolone. The anti-VEGF agents inhibit VEGF and increase capillary permeability. Macular edema is resolved by blocking increased capillary permeability. However, a drawback of the anti-VEGF agents is that they do not have long-term efficacy, so repeated injections are usually required. Moreover, a 2% prevalence of endophthalmitis over 12 months in the RESOLVE Study is worrisome. To minimize the number of intravitreal injections, gene therapy using a safe virus vector, such as an adeno-associated virus (Ideno et al., 2007), may be a future option for treating DME.

4. Comments

Good glycemic control is the most important factor for preventing the development and progression of diabetic ocular complications. However, it is also difficult to obtain good glycemic control in many clinical cases. In this chapter, we discussed several additional medical therapeutic options for diabetic ocular complications. PKC β inhibitors and

antileukocyte adhesion agents might help prevent DR, but neither was sufficient without good concurrent glycemic control or other additional medical treatments. Anti-VEGF agents have a strong therapeutic effect in DME; however, they do not have long-term efficacy and the possibility of complications, such as endophthalmitis, associated with injection of anti-VEGF agents into the vitreous is a concern. Thus, anti-VEGF agents do not seem to be safe therapeutic options for preventing diabetic ocular complications. Oral anti-AGE agents seem to be good prophylactic medical treatments for DR in experimental animal studies. Clinical trials of oral anti-AGE agents for treating DR are worthwhile. Among the several prophylactic medical treatments for DR, we focused on the ARIs in this chapter. Epalrestat, which is commercially available in Japan, has been widely used to treat diabetic neuropathy. Although the effect of eparlestat on DR has not been clearly recognized in a clinical trial, the safety and clinical effects of long-term oral administration of the drug for treating diabetic neuropathy have been established. Thus, we believe that our findings suggest the potential therapeutic usefulness of a new ARI, ranirestat, for preventing DR. Importantly, it appears that ranirestat might prevent DR even without strict glycemic control. We look forward to the development and evaluation of new ARIs with minimal side effects for use in future clinical studies to prevent DR.

5. References

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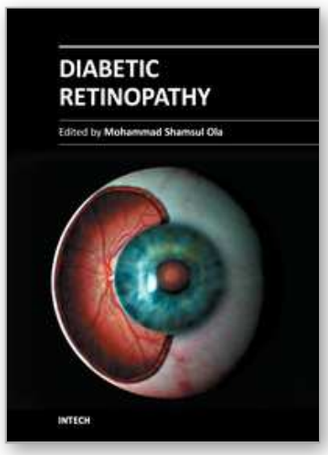

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The aim of this book is to provide a comprehensive overview of current concepts in pathogenesis, diagnosis and treatments of diabetic retinopathy. It provides a collection of topics written by excellent authors, covering discussions on advances in understanding of pathophysiology, immunological factors and emerging concepts, relating to clinical aspects and treatment strategies. The contents of the book will not only provide a resource for our knowledge but also improve diagnosis and treatment options for those patients who suffer vision loss due to diabetic retinopathy.

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