Role of nitric oxide, superoxide, peroxynitrite and PARP in diabetic retinopathy

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TABLE OF CONTENTS

1. Abstract
2. Diabetic retinopathy
3. NO, NOS and diabetic retinopathy
   3.1. NO and NOS in retina
   3.2. NO and NOS in diabetic patients with retinopathy
   3.3. NO and NOS studies in experimentally diabetic animals and retinal cells incubated in diabetic-like concentrations of glucose
      3.3.1. NO in retinas or retinal cells incubated in diabetic-like conditions
      3.3.2. NOSs in retinas or retinal cells incubated in diabetic-like conditions
      3.3.3. Interaction between NO and COX-2
4. Oxidative stress, superoxide and diabetic retinopathy
   4.1. Oxidative stress
   4.2. Oxidative stress markers in diabetic patients with retinopathy
   4.3. Oxidative stress in retinas of experimentally diabetic animals and retinal cells incubated in diabetic-like concentrations of glucose
5. Peroxynitrite and diabetic retinopathy
6. PARP and diabetic retinopathy
7. Perspectives
8. Acknowledgements
9. References

1. ABSTRACT

Diabetic retinopathy is the leading cause of blindness and visual disability in the industrialized world. The mechanisms of how diabetic retinopathy develops are still an open question. Alterations contributing to oxidative and nitrosative stress, including elevated nitric oxide (NO) and superoxide production, overexpression of different isoforms of nitric oxide synthase (NOS), nitrated and poly(ADP-ribosyl)ated proteins, downregulation of antioxidative enzymes, have been implicated in the pathogenesis of this ocular disease. The possible roles of these components in the development of diabetic retinopathy are reviewed here, and their values as therapeutic targets for inhibiting or delaying the development of diabetic retinopathy are highlighted.

2. DIABETIC RETINOPATHY

Diabetic retinopathy is the most common cause of acquired blindness and visual disability afflicting adults in the industrialized world. Most persons who have had Type I and Type II diabetes for 20 years have been found to have some retinopathy (1-3). The rising incidence of Type II diabetes in world undoubtedly will lead also to an increase in the numbers of patients having the retinopathy and suffering its effects on vision.

The retinopathy classically has been regarded as a disease of the retinal microvasculature, and has been divided into two stages: an early, nonproliferative stage, and a later, proliferative stage. Nonproliferative diabetic retinopathy currently is diagnosed ophthalmoscopically...
Oxidative and nitrosative stress in diabetic retinopathy

based on the presence of retinal vascular abnormalities, including retinal microaneurysms, intraretinal microvascular abnormalities (which include intraretinal new vessels), areas of capillary nonperfusion, retinal hemorrhages, cotton wool spots (infarctions within the nerve fiber layer), edema, dilation of retinal veins, and exudates. All these signs indicate regional failure of the retinal microvascular circulation, which presumably results in ischemia. Proliferative diabetic retinopathy is diagnosed based on the presence of new blood vessels on the surface of the retina. New vessels can extend into the vitreous cavity of the eye, and can hemorrhage into the vitreous, resulting in visual loss. Development of a fibrovascular membrane on the retinal surface also can cause tractional retinal detachment from the accompanying contractile fibrous tissue.

Laser photocoagulation therapy has been demonstrated to inhibit severe vision loss in patients who already have proliferative diabetic retinopathy and have diabetic macular edema (4-6). The onset and progression of the retinopathy also can be inhibited by improved glycemic control on type I or type II diabetes (7-9) or by tight blood pressure control on type II diabetes (10). Since good glycemic control and tight blood pressure control can be difficult to maintain in many patients, however, new therapies that can prevent or delay the retinopathy still are needed.

Recently, several laboratories have implicated oxidative and nitrosative stress in the development of this ocular disease. In this review, we focus on the possible roles of NO, NOS, superoxide, peroxynitrite and poly(ADP-ribose) polymerase (PARP) in the pathogenesis of diabetic retinopathy.

3. NO, NOS AND DIABETIC RETINOPATHY

3.1. NO and NOS in retina

NO is known to play a major role in a variety of biological processes including blood pressure homeostasis, immune regulation, and nervous system signal transmission (11-14). NO is generated from L-arginine by the catalytic activity of NO synthase (NOS). NOS is divided into three groups: constitutive NOS (cNOS), inducible NOS (iNOS), and neuronal NOS (nNOS).

NO can be made by every retinal cell type, and it is an important signaling molecule that regulates neurotransmitters release and modulates gap junction conductivity in retina (15). NO is selectively activated and diffusible as previously thought (15). In the retina, the NO produced by the constitutive NOSs, cNOS, and nNOS, contribute to regulate normal ocular hemodynamics and cell viability, and to protect retinal cells against different stresses (see the reviews of (16, 17)). NO derived from retinal constitutive NOSs have been found to be neuroprotective in retinal preconditioning ischemia and in primary open angle glaucoma (18, 19). Larger amounts of NO, such as that generated by iNOS, however, have been implicated in the development of several ocular diseases, including glaucoma, retinal ischemia and reperfusion, light-induced retinal degeneration, and ocular neovascularization (20-24). Elevated levels of NO and iNOS have been implicated in the development of diabetic retinopathy (17, 25-30).

3.2. NO and NOS in diabetic patients with retinopathy

An association of NO with the development of diabetic retinopathy has been demonstrated by several studies that investigated the levels of NO in serum, plasma, vitreous of diabetic patients. Increased serum levels of NO (estimated by measuring serum nitrite and nitrate (NO2\(^{-}\) + NO3\(^{-}\))) and inflammatory cytokines (including soluble IL-2 receptor, IL-8, and TNF-alpha) have been found in diabetic patients with retinopathy compared with diabetic patients without diabetic retinopathy or healthy controls (31, 32). In those studies, serum NO levels in the patients with proliferative diabetic retinopathy were significantly higher than the levels in the patients with nonproliferative retinopathy. Likewise, plasma NO and IL-8 levels were higher in patients with proliferative diabetic retinopathy than in controls (33, 34). It is interesting that basal plasma nitrate level were increased in diabetic type II patients having retinopathy, but there was no difference in basal plasma nitrite level between the these patients and nondiabetic subjects (35). Plasma nitrate levels also were suggested positively correlated with advanced microvascular complications, serum lipid peroxide, and advanced glycation end products in these patients (35).

Elevated metabolites of the L-arginine-NO pathway have been detected also in the vitreous of eyes from diabetic patients (27, 36-38). However, a study of patients having retinal detachment (rheumatogenous or tractional) due to proliferative diabetic retinopathy observed no statistical significant change in nitrite levels in vitreous of the diabetic patients compared to nondiabetic subjects (39). Greater than normal levels of the specific NOS by-product, N\(^{3}\)-hydroxy-L-arginine, have been detected in the ocular aqueous humor of diabetic patients (with and without diabetic retinopathy) compared with that in non-diabetic controls (40).

Increased iNOS immunostaining has been demonstrated in retinas of diabetic patients with nonproliferative retinopathy, and that immunostain was localized on retinal Muller cells. In contrast, there was no iNOS immunoreactivity in retinas from subjects without diabetes and any ocular diseases (25). The increased iNOS immunostaining was colocalized in the same area of increased VEGF immunostaining on retinas of diabetic patients (41). There are no published studies on nNOS and cNOS levels in the retinas from diabetes patients, but polymorphism studies indicated that polymorphic variability of cNOS as well as iNOS gene are associated with severe diabetic retinopathy in multiple ethnic populations (42-44).

3.3. NO and NOS studies in experimentally diabetic animals and retinal cells incubated in diabetic-like conditions

3.3.1. NO in retinas or retinal cells incubated in diabetic-like conditions

Elevated NO production (estimated by measurement of nitrites and nitrates) has been reported in
Oxidative and nitrosative stress in diabetic retinopathy

Figure 1. Vascular lesions of retinopathy in mice made by diabetic by injection of streptozotocin. Typical acellular capillary (arrow; Figure A) and pericyte ghost (arrow; Figure B) in retinal capillaries from mice diabetic for 10 months are shown. (PAS and hematoxylin staining).

Most retinal cells, including endothelial cells and Muller cells cultured in high glucose conditions (25 mM glucose) have been reported to generate elevated levels of NO compared to those cultured under normal (5 mM) glucose conditions (45, 50). Importantly, inhibition of this glucose-induced increase in NO production in these cells inhibited death of the same cells (50). Cultured retinal pericytes are an exception, because elevation of glucose in the media resulted in a decrease of NO production (51). Treatment of the pericytes cultured in high glucose with troglitazone, a potent agonist of peroxisome proliferator activated receptor-gamma, reversed glucose-induced inhibition of NO production.

Evidence that elevated NO level might cause retinal neurodegeneration was demonstrated by intravitreal injection of a NO-releasing compound (NOC 12) in rats. NOC 12 resulted in a decrease of cell density in the ganglion cell layer and reduction of retinal thickness of the rat retina (52). Both of these findings are similar to the neurodegeneration reported in retinas of diabetic rats (53). Since the NO level in the retinas after NOC 12 injection was not reported in this study, a direct correlation of NO after NOC 12 injection to the level of NO due to diabetes was not established.

Elevated NO level seems also to cause breakdown of the retinal blood barrier, a vascular lesion associated with edema in diabetic retinopathy. NG-monomethyl-L-arginine (L-NMMA), a general NOS inhibitor, and aminoguanidine, a nonselective iNOS inhibitor, were demonstrated to block the VEGF-induced and diabetes-induced vascular hyperpermeability in retina (12, 54, 55).

3.3.2. NOSs in retinas or retinal cells in diabetic-like conditions

The activity of NOS (assessed by the production of L-[3H]-citrulline) was found to be enhanced in retinas of diabetic rats compared to retinas from controls (56, 57). The L-arginine transport in retinas from streptozotocin-induced diabetic rats had a carrier of lower affinity and higher capacity than in retinas from control rats, which was correlated with the increased NOS activity and depletion of the intracellular pool of L-arginine (56). In diabetic Goto-Kakizaki rats (a model of Type II diabetes), elevated total NOS activity as well as elevated iNOS protein level were associated with elevated the blood retinal barrier permeability, compared to those in normal rats (57).

Increased iNOS expression in retinas of Type I or Type II diabetic rats has been found in several studies (26, 50, 57-59). Whether or not iNOS is present in vascular cells of the retina in diabetes is controversial. iNOS has been detected in retinal capillary endothelial cells of BBZ/WOR rats (an Type II diabetic model) and experimentally galactosemic rats (26, 60), whereas it has not been detected in the retinal vasculature of streptozotocin-induced diabetic rats (46). In vitro, elevated concentrations of glucose induced iNOS expression in cultured Muller cells (a nonvascular cells), but not in cultured endothelial cells (50).

In contrast, high glucose has been reported to decrease iNOS expression in cultured retinal pericytes, another type of capillary cells (51).

Beneficial effects of iNOS inhibitors on retinas of diabetic animals or retinal cells incubated in elevated concentrations of glucose have been reported in a number of studies (46, 49, 61-63). Aminoguanidine inhibited diabetes-induced increases in PKC activity, oxidative stress and NO production in retinas of rats diabetic or galactosemic for 2 months (46, 49). Subnormal responses of the retinal vasculature to changes in oxygen tension in diabetic rats and mice have been inhibited in iNOS deficient mice or using an iNOS inhibitor, L-NIL (62, 63). iNOS may contribute to high glucose induced retinal Muller cell death in vitro, since L-NIL inhibited the death of retinal Muller cells cultured in high glucose (50).

Importantly, aminoguanidine also has been shown to inhibit the diabetes-induced degeneration of retinal capillaries in multiple experimental diabetic animal species, including diabetic dogs (64), rats (65-67), and mice (Kern, unpublished results). Acellular (degenerate) capillaries and pericyte “ghosts” are two histological markers of early lesions of diabetic retinopathy (see example in Figure 1). Since aminoguanidine might also inhibit dicarboxyl-mediated cross-linking and protein modification (68, 69), and inhibit mRNAs under some circumstances (70), iNOS deficient mice were used to determine whether or not inhibition of iNOS was the critical step responsible for the aforementioned inhibition of retinopathy. Diabetic mice deficient in iNOS developed significantly less diabetes-induced capillary degeneration (Figure 2A) and retinal thinning than that found in wildtype diabetic mice, while having no effect on diabetes-induced abnormalities in the function of retinal neurons (demonstrated by electroretinogram) (30). Thus, iNOS seems to play critical role in the development, at least the vascular lesions, of diabetic retinopathy. Consistent with this, iNOS, but not eNOS, was found to be the source of NO responsible for peroxynitrite and nitrotyrosine formation in retina of the galactose-fed model of diabetic...
Oxidative and nitrosative stress in diabetic retinopathy by using mice deficient in iNOS and eNOS (60).

eNOS also has been found to be elevated in the retinas of diabetic animals (30, 71, 72). As a result of its vasodilatory actions, increased eNOS expression generally is considered to be beneficial. However, under certain pathophysiological conditions, eNOS itself can be a source of superoxide. eNOS uncoupling was shown in a variety of experimental and clinical vascular disease states, especially in diabetes. A highly specific VEGF-neutralizing Fli-Fc construct [VEGF Trap A(40)] was reported to suppress diabetes-induced leukocyte adhesion in retinal vasculature by inhibiting the expression of eNOS, a downstream mediator of VEGF activity (71). The diabetes-induced increase in expression of eNOS did not occur in retinas of iNOS-deficient mice (30), suggesting that the levels of iNOS or NO might directly or indirectly regulate expression of eNOS in the retina. Since diabetic iNOS-deficient mice do not develop the early vascular lesions of retinopathy, it is possible that the observed benefits form inhibiting iNOS on the development of retinopathy occur in part via normalization of eNOS level. Investigation of the role of eNOS in development of the retinopathy remains difficult because of confounding by other important actions regulated by this isoforms of NOS (e.g., blood pressure regulation).

Conclusions regarding nNOS levels in retinas of diabetic animals are contradictory. nNOS mRNA level and protein level have been reported to be elevated 2 weeks after induction of diabetes in rats (73), and Park et al. reported that nNOS protein level was increased in the retinas of rats after 12 weeks and 24 weeks of diabetes, specifically in bipolar cells (74). On the other hand, Goto et al. observed that diabetes disturbed the function of the nNOS-positive amacrine cells and reduced NO production via nNOS (75). Decreased retinal nNOS-containing neurons (by NADPH-diaphorase immunostaining) were found as early as one week after onset of diabetes, and remained decreased up to 32 weeks of diabetes. Inhibitors of advanced glycation, but not of NOSs or of institution of the numbers of nNOS-containing neurons (76).

3.3.3. Interaction between NO and COX-2

NO and cyclooxygenase-2 (COX-2; an inducible enzyme that catalyze arachidonic acids to prostaglandin) pathways have been identified to interact. Inhibition of NOS or iNOS (by L-NAME and L-NIL, respectively) in retinal Muller cells incubated in high glucose inhibited the increased production of NO and expression of iNOS as expected, but also inhibited the increased production of prostaglandin E2 (PGE2) and expression of COX-2. In contrast, inhibition of COX-2 by NS-398 was found to only block PGE2 production but without any effect on the levels of NO or iNOS (50). Consistent with the in vitro result, less PGE2 generation was found in retinas from diabetic iNOS deficient mice when compared to that in retinas from diabetic wildtype mice (30). Tropical application of a COX inhibitor, Nepafenac, via eyecrops inhibited diabetes-induced increases vascular lesions and PGE2, superoxide and COX-2 production, but not NO production (28). All these studies suggest that nitric oxide regulates COX-2 activity in the retina, and that inhibition of either the NO or COX pathways are targets to inhibit development of diabetic retinopathy.

4. OXIDATIVE STRESS, SUPEROXIDE AND DIABETIC RETINOPATHY

4.1. Oxidative stress

Retina is extremely rich in polyunsaturated lipid membranes, making it especially sensitive to oxygen and/or nitrogen activated species and lipid peroxidation (77, 78). Although low concentration of reactive oxygen species (ROS) might serve as intracellular signaling molecules to induce repair mechanisms against tissue injury, large amounts of ROS are considered toxic products that can cause cell death (79-81). A complex antioxidant defense system, including superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase, helps maintain the intracellular concentration of glutathione and NADPH necessary for optimal function of the cellular antioxidant defense mechanism (80, 82, 83). Downregulation of the defense system may also break the balance in the redox status and cause oxidative stress.

4.2. Oxidative stress markers in diabetic patients with retinopathy

Several oxidative stress markers have been investigated in the blood cells, serum, plasma and vitreous of diabetic patients. Abu el-Asrar et al observed that superoxide anion production by polymorphonuclear leukocytes (PMNs) from diabetic patients was significantly higher than that of nondiabetic controls, and significantly more superoxide was produced by PMNs incubated with serum from patients with retinopathy than retinopathy-free patients, suggesting some factors in diabetic serum stimulated a significant generation of superoxide anion in normal PMNs and related to the severity of retinopathy (85). Decreased activity of SOD was also found in the anterior chamber and vitreous in diabetic patients compared to that of nondiabetic controls, implicating that the activity of SOD may be involved in cataract development and diabetic retinopathy development (86).

4.3. Oxidative stress in retinas of experimental diabetic animals and retinal cells incubated in diabetic-like concentrations of glucose

NADH oxidase is believed to be a major source of superoxide in the vascular endothelium (87). The immunostaining of NADH oxidase on the blood vessels was significantly higher than normal in new onset (2-6 days) and chronic (4-18 months) diabetic rats, and in galactose fed mice (26, 60). In contrast, elevated production of superoxide in retinas of diabetic rats or retinal endothelial
Oxidative and nitrosative stress in diabetic retinopathy

Moreover, addition of SOD to the media of these cells inhibited the apoptotic death caused by elevated glucose (88). Treatment of diabetic rats with aminoguanidine, aspirin, or vitamin E, (three drugs known to inhibit the development of diabetic retinopathy in experimental diabetic animals (59, 64-66, 89, 90)) inhibited the hyperglycemia-induced increase in superoxide (88), raising the possibility that these therapies inhibit retinopathy by inhibiting a hyperglycemia-induced increase in superoxide production. 8-oxo, 2'-deoxyguanosine, a marker of oxidative DNA damage (91), was increased more than twofold in retinas (92) and other tissues (93) of diabetic rats. NF-kappa B, a transcription factor known to be sensitive to oxidative stress, became activated in endothelial cells and pericytes cultured in high glucose, and activated in the retinas of diabetic animals (48, 59, 94-96). The beneficial effects of antioxidants such as vitamin E, nicanartine, lipoic acid on vascular lesions of diabetic retinopathy in diabetic animals (45, 97, 98) confirm that oxidative stress is an important contributor to the development of diabetic retinopathy.

Increased superoxide in the retinas of diabetic rats may come also from impairment of antioxidant defense system. Decreased the activities of SOD, glutathione reductase, glutathione peroxidase and catalase were found in the retinas of 2 months duration of diabetes or experimental galactosemia rats (99, 100). To address whether SOD plays a critical role in the development of diabetic retinopathy, mice overexpressing MnSOD were made diabetic. Overexpression of MnSOD in these mice inhibited the diabetes-induced increase in generation of superoxide from retinal mitochondria, normalized diabetes-induced increase in mitochondria membrane permeability, and restored the activity of electron transport complex III in retina (101). Overexpression of MnSOD also prevented diabetes-induced decreases in retinal GSH and increases in 8-OHdG (102), diabetes-induced increase in degeneration of retinal capillaries (101) (Figure 2B). Brownlee and collaborators have suggested that hyperglycemia-induced overproduction of superoxide is the single unifying link to diabetic complications (103, 104).

Multiple other biochemical pathways associated with hyperglycemia also can increase the production of ROS. These include glucose auto-oxidation, increased polyol pathway, activation of PKC, increased hexosamine pathway flux, increased AGEs formation, stimulation of eicosanoid metabolism, and altered mitochondrial function (95, 105, 106). Elevated superoxide level in diabetes was found to activate H-Ras, a small molecular weight G-protein and its downstream signaling including Raf-1 and p-38 MAP kinase in retina (107, 108). ROS also can induce monocyte chemoattractant protein-1 expression in endothelial cells by activating p-38 MAP kinase (109).

5. PEROXYNITRITE AND DIABETIC RETINOPATHY

Peroxynitrite is a highly reactive oxidant formed by the combination of nitric oxide and superoxide. Peroxynitrite can initiate a variety of pathological processes,
Oxidative and nitrosative stress in diabetic retinopathy

including inhibition of key metabolic enzymes by nitration of protein tyrosine residues (110, 111), peroxidation of lipids (112, 113), reduction of cellular antioxidant defenses by oxidation of thiol pools (114, 115), and induction of DNA damage (116, 117) and apoptosis (118, 119).

Increased formation of nitrotyrosine (a measure of peroxynitrite production) in the retina was observed in several studies in diabetic mice and rats, and in retinal endothelial and Muller cells cultured in diabetic-like conditions of glucose (26, 30, 46, 120-123). Increased immunostaining of nitrotyrosine was observed on retinal blood vessels from diabetic animals (26, 46, 122). Increased nitrotyrosine levels were also observed in plasma, platelets, skin of diabetic patients (124-129). Interestingly, some of the therapies having beneficial effects on the development of diabetic retinopathy in animal models, such as aminoguanidine and aldose reductase inhibitors, also inhibited the diabetes-induced increase in generation of nitrotyrosine in retina (46, 130, 131), demonstrating that formation of nitrotyrosine is associated with the development of diabetic retinopathy. Consistent with this, diabetic iNOS deficient mice developed less vascular lesions in diabetic retinopathy also had less nitrotyrosine formation in retina than in wildtype diabetic controls (30). In addition, iNOS deficient diabetic mice had less leukostasis than wildtype controls (30), consistent with evidence that FP15, a peroxynitrite decomposition catalyst, inhibited leukocyte entrapment in the retinal microcirculation of diabetic rats (132). Normalization of glycemia after several months of hyperglycemia in diabetic rats failed to show any beneficial effects on nitrotyrosine levels (122, 133), indicating that this nitration is a long-lived modification.

Increased generation of peroxynitrite in diabetes may also alter the expression of VEGF in the retina. VEGF is a pro-angiogenic factor that is a critical player in several stages of the retinopathy. Peroxynitrite caused activation and nuclear translocation of STAT3 (a transcription factor that regulates VEGF expression) in endothelial cells (134). Peroxynitrite mediated VEGF’s angiogenic signal and function via a nitration-independent, but oxidation-mediated tyrosine phosphorylation mechanism in endothelial cells (135). Several proteins are reported to be nitrated in retinas of diabetic animals (111, 136), including phosphatidylinositol (PI)-3-kinase. Tyrosine nitration on the p85 subunit of PI-3-kinase blocked the activity of the kinase and Akt kinase, abnormalities which might contribute to endothelial cell death in diabetic retinopathy (136).

6. PARP AND DIABETIC RETINOPATHY

PARP is a nuclear enzyme that is involved in the cellular response to DNA injury, such as that from oxidative stress or nitrosative stress (137, 138). Activation of PARP has been demonstrated in the skin microvessels of type 2 diabetic patients (126), as well as several other organs in diabetic animals (96, 131, 139-145). As discussed above, the oxidative and nitrosative stress are greater in retinas of diabetic animals than those in retinas from normal animals (46, 146, 147). Thus, these abnormalities might cause DNA breaks and lead to PARP activation in retinas of diabetic animals. Activation of PARP (based on increased poly(ADP-riboseylation of retinal proteins) in retinas of diabetic rats has been demonstrated by western blots as well as immunohistochemistry (96, 131, 141). Poly(ADP-riboseylated proteins were found in the ganglion cell layer, inner nuclear layer, outer nuclear layer of the retina as well as endothelial cells and pericytes of retinas from diabetic rats (96). In wildtype C57Bl/6J mice, Zheng et al likewise demonstrated a significant increase in PARP activity in the retina of diabetic animals compared to nondiabetic controls (30), whereas Obrosova et al. were unable to find elevated PARP activity in the same stain of mice (148).

PARP inhibitors have been used on diabetic rats to investigate the role of PARP activity in the development of diabetic retinopathy (96, 141). Inhibition of PARP activity by a specific PARP inhibitor, PJ34, inhibited the diabetes-induced increase in the number of TUNEL-positive capillary cells (both endothelial cells and pericytes), and inhibited the accumulation of early vascular lesions of diabetic retinopathy such as pericyte ghosts and degenerate (acellullar) capillaries (96) (Figure 2C). Two other structural unrelated PARP inhibitors, 3-aminobenzamide and 1,5 isoquinolinediol, inhibited the diabetes-induced increase in retinal VEGF protein (141). In the same study, VEGF immunoreactivity was co-localized with PARP activation in the ganglion cell layer and inner nuclear layer in retinas of diabetic rats.

The PARP inhibitor, PJ-34, was also found to inhibit diabetes-induced leukostasis in the retina (96, 132). This inhibition of leukocyte adherence to the vessel wall occurred in part through inhibiting the diabetes-induced induction of ICAM-1 on retinal capillaries (96). ICAM-1 is known to play a critical role in the development of diabetic retinopathy, since mice deficient in ICAM-1 or its receptor, CD18, were protected from the development of diabetic-like retinopathy and increased vascular permeability (149). Thus, inhibition of PARP activity might inhibit degeneration of retinal capillaries in diabetes by inhibiting leukocyte-mediated occlusion of retinal vessels or by preventing release of toxic factors from leukocytes.

PARP activation commonly has been shown to result in subnormal levels of nucleotides, but the studies on levels of nucleotides in retinas of diabetic animal are less clear. Obrosova et al. demonstrated that 3-aminobenzamide and 1,5 isoquinolinediol restored diabetes-induced reduction of retinal NAD⁺ concentration in diabetic rats(141), but they found no alterations of mitochondrial or cytoplasmic NAD⁺/NADH in retinas of diabetic mice (148). In contrast, Diederer et al did not detect any differences in total NAD⁺, NADH or in the ratio of NAD⁺ to NADH in retinas between nondiabetic and diabetic rats (150). In vitro, neither retinal endothelial cells nor Muller cells showed NAD⁺ depletion after 5 days incubated with 25 mM glucose (Zheng & Kern, unpublished data).

The beneficial effect of PARP inhibitors on diabetic retinopathy also might occur via regulation of NF-
Oxidative and nitrosative stress in diabetic retinopathy

Figure 3. Postulated role of the contributions of oxidative stress and nitrosative stress to the development of diabetic retinopathy. (O$_2^-$, superoxide; ROS, reactive oxygen species; ONOO$,^-$ peroxynitrite). Solid lines have been experimentally demonstrated, dotted lines refer to unproven possibilities.

Kappa B and transcription of inflammatory genes. PJ-34 inhibited hyperglycemia-induced NF-kappa B activation in cultured retinal endothelial cells and inhibited the diabetes-induced induction of inflammatory proteins such as ICAM-1 and iNOS in retinas of diabetic rats (96) and Zheng & Kern, unpublished data). Consistent with this, inhibition of transcription coactivator p300 inhibited PARP expression, NF-kappa B activation, and fibronectin expression in endothelial cells incubated in elevated levels of glucose (151). This is important because inhibition of fibronectin expression using antisense oligonucleotides inhibited vascular degeneration as well as basement membrane thickening in galactose-fed rats (152).

What activates PARP in diabetic retinopathy is still an open question. So far, there is no strong data demonstrating overt DNA damage in retinas of diabetic animals. However, the TUNEL technique (which is based on labeling of DNA breaks in nuclei) has identified a small number of vascular and neuronal cells in retinas of diabetic rats that likely do have at least some DNA breaks. In vitro, Du et al demonstrated that overexpression uncoupling protein 1 or MnSOD in bovine aortic endothelial cells incubated in elevated concentrations of glucose blocked DNA breaks and the activation of PARP (153), suggesting that oxidative stress and resulting DNA damage might cause PARP activation. It is known that there are differences between macrovascular cells and microvascular cells (154-157, 158), so whether or not these findings apply to causes of PARP activation in the retinas of diabetic animals is still unclear. In contrast to the aforementioned hypothesis that mitochondrial-generated superoxide causes PARP activation (153), PARP inhibitors (PJ-34 and INO-1001) have been found to inhibit the diabetes-induced increase ROS production in kidney of db/db mice (142). Thus, it is not yet clear whether ROS is upstream or downstream of PARP activation in the retina of diabetes. In addition, diabetes-induced PARP activation was not completely inhibited in retinas of diabetic iNOS deficient mice (30), suggesting PARP activation occurs at least to some extent independent of nitrative stress in retinas of diabetic animals.

7. PERSPECTIVE

Taken together, the evidence suggests that oxidative stress and nitrosative stress play crucial roles in the diabetes-induced degeneration of retinal capillaries in diabetes, at least in part by activation of PARP (Figure 3). This capillary degeneration and other related lesions develop during the early or “background” stages of the retinopathy, but are believed to play an important role in the progression to the clinically significant, neovascular stage of the retinopathy. Inhibition of reactive oxygen and nitrogen species or inhibition of PARP and other
Oxidative and nitrosative stress in diabetic retinopathy

downstream pathways are promising therapeutic targets to inhibit the development of diabetic retinopathy.

8. ACKNOWLEDGMENTS

This work was supported by grants to TSK from the Medical Research Service of the Department of Veteran Affairs and NIH (RO1EY00300), and to LZ from the National Natural Science Foundation of China (NSFC 30870949 and NSFC 2009CB918300).

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Oxidative and nitrosative stress in diabetic retinopathy


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**Key Words:** Diabetic Retinopathy, Nitric Oxide, Nitric Oxide Synthase, Superoxide, Peroxynitrite, poly(ADP-ribose) polymerase, Review

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3987