[Frontiers in Bioscience 14, 3974-3987, January 1, 2009]

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

Ling Zheng[1,2], Timothy S. Kern[2,3,4]

[1] College of Life Sciences, Wuhan University, Wuhan, China, [2]Department of Medicine, [3] Department of Ophthalmology, Case Western Reserve University, Cleveland, OH, [4]Cleveland VAMC Research Service 151, Cleveland, OH

TABLE OF CONTENTS

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-ribosy)lated 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 micovasculature, and has been divided into two stages: an early, nonproliferative stage, and a later, proliferative stage. Nonproliferative diabetic retinopathy currently is diagnosed opthalmoscopically

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 reaction of different isoforms of NOS in the presence of oxygen and NADPH, including neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS).

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 trapped in the retinal cells which made it, not being as freely diffusible as previously thought (15). In the retina, the NO produced by the constitutive NOSs, eNOS 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 it sequelae are suggested also to have a pathogenic role 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 levels of plasma *nitrate* 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 (rhegmatogenous 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^G -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 nonproliferactive 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 eNOS levels in the retinas from diabetes patients, but polymorphism studies indicated that polymorphic variability of eNOS 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 concentrations of glucose

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

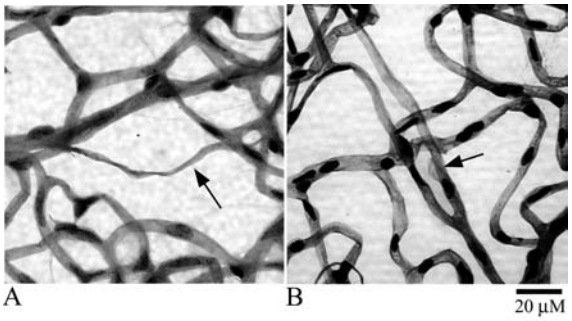

Figure 1. Vascular lesions of retinopathy in mice made by diabetic by injection of streptozotocin. Typical acellular capillary (arrow; Figure A) and pericyte ghost (arrow; Figure B) in retinal capillaries from mice diabetic for 10 months are shown. (PAS and hematoxylin staining).

retinas of experimentally diabetic rats (45, 46) and mice (30, 47, 48), and experimentally galactosemic rats (a model that develops a diabetic-like retinopathy due to chronic hexose elevation) (45, 49). The elevation of retinal NO in those animal models was not transient, remaining elevated for at least 1 year (45, 48).

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-[(3)H]-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 dicarbonyl-mediated cross-linking and protein modification (68, 69), and inhibit eNOS 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

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 Flt-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 good glycemic control by insulin, inhibited the decrease in the numbers of nNOS-containing neurons (76).

3.3.3. Interaction between NO and COX-2

NO and cyclooxgenase-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 E_2 (PGE_2) and expression of COX-2. In contrast, inhibition of COX-2 by NS-398 was found to only block PGE_2 production but without any effect on the levels of NO or iNOS (50). Consistent with the *in vitro* result, less PGF_2 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 eyedrops inhibited diabetes-induced increases vascular lesions and PGE_2, 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, but released significantly lower levels of superoxide compared to controls in response to phorbol myristate acetate stimulation (84). The same authors also found that incubation of normal PMNs with serum from diabetic patients resulted in significantly higher levels of superoxide than that incubated with serum from 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

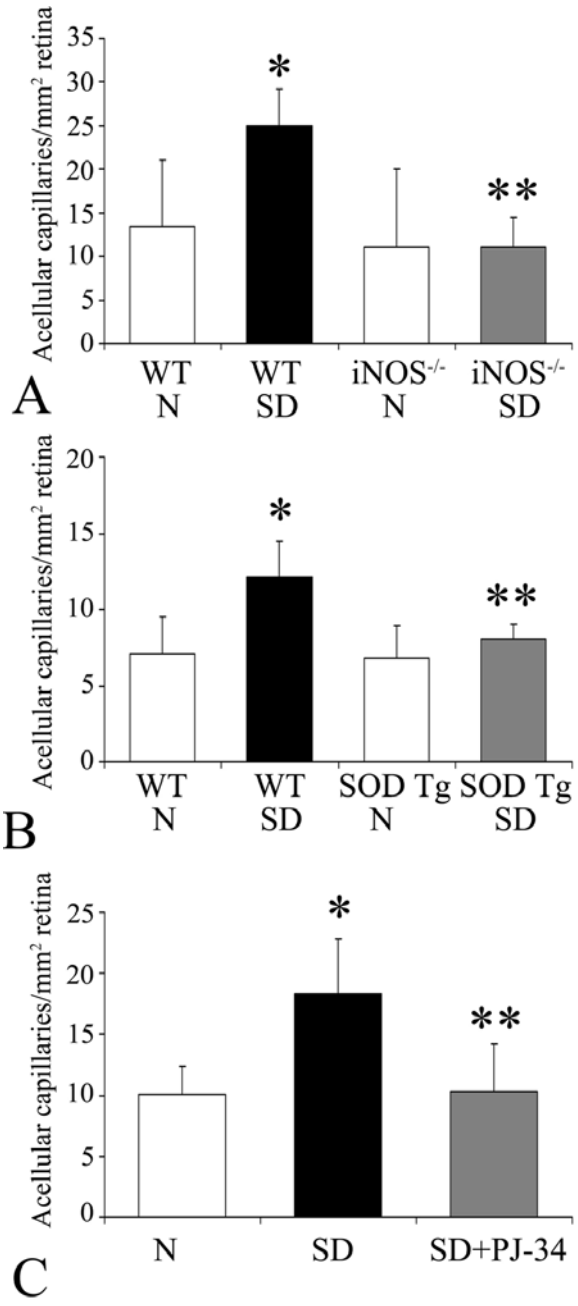

Figure 2. Inhibition of diabetes-induced acellular and degenerate capillaries by iNOS deficiency (A; reproduced with permission from *Diabetologia*), overexpression of MnSOD (B; reproduced with permission from *IOVS*), and inhibition of PARP inhibitor (C; reproduced with permission from *Diabetes*). (WT, wildtype; N, nondiabetic mice/rats; SD, diabetic mice/rats; iNOS$^{-/-}$, iNOS deficient mice; SOD Tg, MnSOD transgenic mice; SD+PJ-34, PJ-34 treated diabetic rats; * $p < 0.05$ compared to (wildtype) nondiabetic mice/rats; ** $p < 0.05$ compared to (wildtype) diabetic mice/rats)

and Muller cells cultured in diabetic-like concentrations of glucose was attributed largely to mitochondria (88). 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,

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 nitrotryrosine 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 nitrotryrosine 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 nitrative 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-ribosylation of retinal proteins) in retinas of diabetic rats has been demonstrated by western blots as well as immunohistochemistry (96, 131, 141). Poly(ADP-ribose)ylated 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 aniamls 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 (acellular) 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 cytoplamic NAD^+/NADH in retinas of diabetic mice (148). In contrast, Diederen *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-

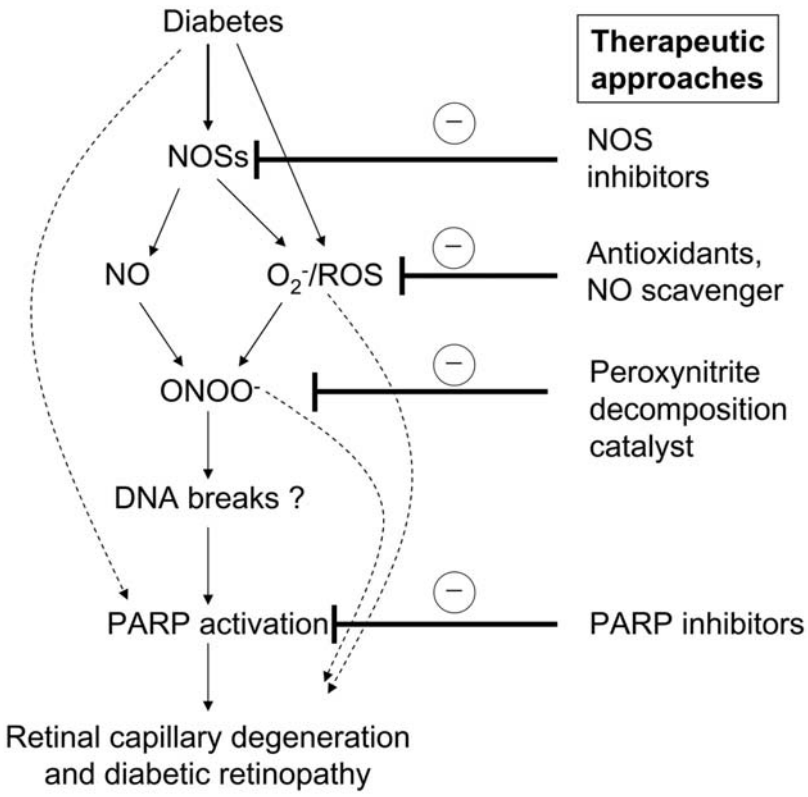

Figure 3. Postulated role of the contributions of oxidative stress and nitrosative stress to the development of diabetic retinopathy. (O_2^-, superoxide; ROS, reactive oxygen species; $ONOO^-$, peroxynitrite). Solid lines have been experimentally demonstrated, dotted lines refer to unproven possibilities.

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

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 (R01EY00300), and to LZ from the National Natural Science Foundation of China (NSFC 30870949 and NSFC 2009CB918300).

9. REFERENCE

1. R. Klein, B. E. Klein, S. E. Moss, M. D. Davis & D. L. DeMets: The Wisconsin Epidemiologic Study of Diabetic Retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is 30 or more years. *Arch Ophthalmol* 102, 527-532 (1984)

2. R. Klein, B. E. Klein, S. E. Moss, M. D. Davis & D. L. DeMets: The Wisconsin Epidemiologic Study of Diabetic Retinopathy. I. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. *Arch Ophthalmol* 102, 520-526 (1984)

3. B. E. Klein & R. Klein: Protecting vision. *N Engl J Med* 332, 1228-9 (1995)

4. DRS study group: Preliminary report on effects of photocoagulation therapy. *Am J Ophthalmol* 81, 383-96 (1976)

5. DRS study group: Photocoagulation treatment of proliferative diabetic retinopathy: the second report of diabetic retinopathy study findings. *Ophthalmology* 85, 82-106 (1978)

6. DRS study group: Indications for photocoagulation treatment of diabetic retinopathy: Diabetic Retinopathy Study Report no. 14. *Int Ophthalmol Clin* 27, 239-53 (1987)

7. DCCT Research Group: The effect of intensive treatment of diabetes on the development of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329, 977-986 (1993)

8. Y. Ohkubo, H. Kishikawa, E. Araki, T. Miyata, S. Isami, S. Motoyoshi, Y. Kojima, N. Furuyoshi & M. Shichiri: Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract* 28, 103-17 (1995)

9. UK Prospective Diabetes Study (UKPDS) Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352, 837-53 (1998)

10. UK Prospective Diabetes Study (UKPDS) Group: Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ* 317, 703-13 (1998)

11. P. L. Huang, Z. Huang, H. Mashimo, K. D. Bloch, M. A. Moskowitz, J. A. Bevan & M. C. Fishman: Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature* 377, 239-42 (1995)

12. R. G. Tilton, K. C. Chang, W. S. LeJeune, C. C. Stephan, T. A. Brock & J. R. Williamson: Role for nitric oxide in the hyperpermeability and hemodynamic changes induced by intravenous VEGF. *Invest Ophthalmol Vis Sci* 40, 689-96 (1999)

13. A. H. Neufeld, S. Kawai, S. Das, S. Vora, E. Gachie, J. R. Connor & P. T. Manning: Loss of retinal ganglion cells following retinal ischemia: the role of inducible nitric oxide synthase. *Exp Eye Res* 75, 521-8 (2002)

14. X. Garcia & F. Stein: Nitric oxide. *Semin Pediatr Infect Dis* 17, 55-7 (2006)

15. W. D. Eldred & T. A. Blute: Imaging of nitric oxide in the retina. *Vision Res* 45, 3469-86 (2005)

16. L. Schmetterer & K. Polak: Role of nitric oxide in the control of ocular blood flow. *Prog Retin Eye Res* 20, 823-47 (2001)

17. N. Toda & M. Nakanishi-Toda: Nitric oxide: ocular blood flow, glaucoma, and diabetic retinopathy. *Prog Retin Eye Res* 26, 205-38 (2007)

18. A. H. Neufeld, M. R. Hernandez & M. Gonzalez: Nitric oxide synthase in the human glaucomatous optic nerve head. *Arch Ophthalmol* 115, 497-503 (1997)

19. Y. Zhu, K. K. Ohlemiller, B. K. McMahan, T. S. Park & J. M. Gidday: Constitutive nitric oxide synthase activity is required to trigger ischemic tolerance in mouse retina. *Exp Eye Res* 82, 153-63 (2006)

20. M. Hangai, N. Yoshimura, K. Hiroi, M. Mandai & Y. Honda: Inducible nitric oxide synthase in retinal ischemia-reperfusion injury. *Exp Eye Res* 63, 501-9 (1996)

21. A. H. Neufeld, A. Sawada & B. Becker: Inhibition of nitric-oxide synthase 2 by aminoguanidine provides neuroprotection of retinal ganglion cells in a rat model of chronic glaucoma. *Proc Natl Acad Sci U S A* 96, 9944-8 (1999)

22. S. I. Kawai, S. Vora, S. Das, E. Gachie, B. Becker & A. H. Neufeld: Modeling of risk factors for the degeneration of retinal ganglion cells after ischemia/reperfusion in rats: effects of age, caloric restriction, diabetes, pigmentation, and glaucoma. *FASEB J* 15, 1285-7 (2001)

23. L. Piehl, F. Capani, G. Facorro, E. M. Lopez, E. R. de Celis, C. Pustovrh, A. Hager, H. Coirini & J. J. Lopez-Costa: Nitric oxide increases in the rat retina after continuous illumination. *Brain Res* 1156, 112-9 (2007)

24. H. She, T. Nakazawa, A. Matsubara, T. Hisatomi, T. A. Young, N. Michaud, E. Connolly, A. Hafezi-Moghadam, E. S. Gragoudas & J. W. Miller: Reduced photoreceptor damage after photodynamic therapy through blockade of nitric oxide synthase in a model of choroidal neovascularization. *Invest Ophthalmol Vis Sci* 48, 2268-77 (2007)

25. A. M. Abu El-Asrar, S. Desmet, A. Meersschaert, L. Dralands, L. Missotten & K. Geboes: Expression of the inducible isoform of nitric oxide synthase in the retinas of human subjects with diabetes mellitus. *Am J Ophthalmol* 132, 551-6 (2001)

26. E. A. Ellis, D. L. Guberski, B. Hutson & M. B. Grant: Time course of NADH oxidase, inducible nitric oxide synthase and peroxynitrite in diabetic retinopathy in the BBZ/WOR rat. *Nitric Oxide* 6, 295-304 (2002)

27. D. C. Tsai, S. H. Chiou, F. L. Lee, C. K. Chou, S. J. Chen, C. H. Peng, Y. H. Kuo, C. F. Chen, L. L. Ho & W. M. Hsu: Possible involvement of nitric oxide in the progression of diabetic retinopathy. *Ophthalmologica* 217, 342-6 (2003)

28. T. S. Kern, C. M. Miller, Y. Du, L. Zheng, S. Mohr, S. L. Ball, M. Kim, J. A. Jamison & D. P. Bingaman: Topical administration of nepafenac inhibits diabetes-induced retinal microvascular disease and underlying abnormalities of retinal metabolism and physiology. *Diabetes* 56, 373-9 (2007)

29. L. Zheng, B. Gong, D. A. Hatala & T. S. Kern: Retinal ischemia and reperfusion causes capillary degeneration: similarities to diabetes. *Invest Ophthalmol Vis Sci* 48, 361-7 (2007)

30. L. Zheng, Y. Du, C. Miller, R. A. Gubitosi-Klug, T. S. Kern, S. Ball & B. A. Berkowitz: Critical role of inducible nitric oxide synthase in degeneration of retinal capillaries in mice with streptozotocin-induced diabetes. *Diabetologia* 50, 1987-96 (2007)

31. S. Doganay, C. Evereklioglu, H. Er, Y. Turkoz, A. Sevinc, N. Mehmet & H. Savli: Comparison of serum NO, TNF-alpha, IL-1beta, sIL-2R, IL-6 and IL-8 levels with grades of retinopathy in patients with diabetes mellitus. *Eye* 16, 163-70 (2002)

32. S. Ozden, S. Tatlipinar, N. Bicer, V. Yaylali, C. Yildirim, D. Ozbay & G. Guner: Basal serum nitric oxide levels in patients with type 2 diabetes mellitus and different stages of retinopathy. *Can J Ophthalmol* 38, 393-6 (2003)

33. E. Cicik, H. Tekin, S. Akar, O. B. Ekmekci, O. Donma, L. Koldas & S. Ozkan: Interleukin-8, nitric oxide and glutathione status in proliferative vitreoretinopathy and proliferative diabetic retinopathy. *Ophthalmic Res* 35, 251-5 (2003)

34. D. C. Tsai, M. J. Charng, F. L. Lee, W. M. Hsu & S. J. Chen: Different plasma levels of vascular endothelial growth factor and nitric oxide between patients with choroidal and retinal neovascularization. *Ophthalmologica* 220, 246-51 (2006)

35. K. Maejima, S. Nakano, M. Himeno, S. Tsuda, H. Makiishi, T. Ito, A. Nakagawa, T. Kigoshi, T. Ishibashi, M. Nishio & K. Uchida: Increased basal levels of plasma nitric oxide in Type 2 diabetic subjects. Relationship to microvascular complications. *J Diabetes Complications* 15, 135-43 (2001)

36. G. Yilmaz, P. Esser, N. Kociok, P. Aydin & K. Heimann: Elevated vitreous nitric oxide levels in patients with proliferative diabetic retinopathy. *Am J Ophthalmol* 130, 87-90 (2000)

37. H. Oku, T. Kida, T. Sugiyama, J. Hamada, B. Sato & T. Ikeda: Possible involvement of endothelin-1 and nitric oxide in the pathogenesis of proliferative diabetic retinopathy. *Retina* 21, 647-51 (2001)

38. C. Hernandez, A. Lecube, R. M. Segura, L. Sararols & R. Simo: Nitric oxide and vascular endothelial growth factor concentrations are increased but not related in vitreous fluid of patients with proliferative diabetic retinopathy. *Diabet Med* 19, 655-60 (2002)

39. R. M. Diederen, E. C. La Heij, N. E. Deutz, A. G. Kessels, H. M. van Eijk & F. Hendrikse: Increased nitric oxide (NO) pathway metabolites in the vitreous fluid of patients with rhegmatogenous retinal detachment or diabetic traction retinal detachment. *Graefes Arch Clin Exp Ophthalmol* 244, 683-8 (2006)

40. L. O. Hattenbach, A. Allers, C. Klais, F. Koch & M. Hecker: L-Arginine-nitric oxide pathway-related metabolites in the aqueous humor of diabetic patients. *Invest Ophthalmol Vis Sci* 41, 213-7 (2000)

41. A. M. Abu El-Asrar, A. Meersschaert, L. Dralands, L. Missotten & K. Geboes: Inducible nitric oxide synthase and vascular endothelial growth factor are colocalized in the retinas of human subjects with diabetes. *Eye* 18, 306-13 (2004)

42. G. Kumaramanickavel, S. Sripriya, R. N. Vellanki, N. K. Upadyay, S. S. Badrinath, V. Rajendran, B. Sukumar, V. L. Ramprasad & T. Sharma: Inducible nitric oxide synthase gene and diabetic retinopathy in Asian Indian patients. *Clin Genet* 61, 344-8 (2002)

43. M. J. Taverna, A. Sola, C. Guyot-Argenton, N. Pacher, F. Bruzzo, A. Chevalier, G. Slama, G. Reach & J. L. Selam: eNOS4 polymorphism of the endothelial nitric oxide synthase predicts risk for severe diabetic retinopathy. *Diabet Med* 19, 240-5 (2002)

44. M. J. Taverna, F. Elgrably, H. Selmi, J. L. Selam & G. Slama: The T-786C and C774T endothelial nitric oxide synthase gene polymorphisms independently affect the onset pattern of severe diabetic retinopathy. *Nitric Oxide* 13, 88-92 (2005)

45. R. Kowluru, J. Tang & T. S. Kern: Abnormalities of retinal metabolism in diabetes and galactosemia. VII. Effects of long-term administration of antioxidants on retinal oxidative stress and the development of retinopathy. *Diabetes* 50, 1938-42 (2001)

46. Y. Du, M. A. Smith, C. M. Miller & T. S. Kern: Diabetes-induced nitrative stress in the retina, and correction by aminoguanidine. *J Neurochem* 80, 771-79 (2002)

47. R. A. Kowluru: Retinal metabolic abnormalities in diabetic mouse: comparison with diabetic rat. *Curr Eye Res* 24, 123-8 (2002)

48. R. A. Kowluru, P. Koppolu, S. Chakrabarti & S. Chen: Diabetes-induced activation of nuclear transcriptional factor in the retina, and its inhibition by antioxidants. *Free Radic Res* 37, 1169-80 (2003)

49. R. A. Kowluru, R. L. Engerman & T. S. Kern: Abnormalities of retinal metabolism in diabetes or experimental galactosemia VIII. Prevention by aminoguanidine. *Curr Eye Res* 21, 814-819 (2000)

50. Y. Du, V. Sarthy & T. S. Kern: Interaction between NO and COX pathways in retinal cells exposed to elevated glucose and retina of diabetic rats. *Am J Physiol* 287, R735-41 (2004)

51. J. Kim, Y. S. Oh & S. H. Shinn: Troglitazone reverses the inhibition of nitric oxide production by high glucose in cultured bovine retinal pericytes. *Exp Eye Res* 81, 65-70 (2005)

52. K. Takahata, H. Katsuki, T. Kume, D. Nakata, K. Ito, S. Muraoka, F. Yoneda, S. Kashii, Y. Honda & A. Akaike: Retinal neuronal death induced by intraocular administration of a nitric oxide donor and its rescue by neurotrophic factors in rats. *Invest Ophthalmol Vis Sci* 44, 1760-6 (2003)

53. A. J. Barber, E. Lieth, S. A. Khin, D. A. Antonetti, A. G. Buchanan & T. W. Gardner: Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. *J Clin Invest* 102, 783-91 (1998)

54. R. G. Tilton, K. Chang, K. S. Hasan, S. R. Smith, J. M. Petrash, T. P. Misko, W. M. Moore, M. G. Currie, J. A. Corbett, M. L. McDaniel & J. R. Williamson: Prevention of diabetic vascular dysfunction by guanidines. Inhibition of nitric oxide synthase versus advanced glycation end-product formation. *Diabetes* 42, 221-232 (1993)

55. T. M. Osicka, Y. Yu, V. Lee, S. Panagiotopoulos, B. E. Kemp & G. Jerums: Aminoguanidine and ramipril prevent diabetes-induced increases in protein kinase C activity in glomeruli, retina and mesenteric artery. *Clin Sci (Lond)* 100, 249-57 (2001)

56. A. Carmo, J. G. Cunha-Vaz, A. P. Carvalho & M. C. Lopes: L-arginine transport in retinas from streptozotocin diabetic rats: correlation with the level of IL-1 beta and NO synthase activity. *Vision Res* 39, 3817-23. (1999)

57. A. Carmo, J. G. Cunha-Vaz, A. P. Carvalho & M. C. Lopes: Nitric oxide synthase activity in retinas from non-insulin-dependent diabetic Goto-Kakizaki rats: correlation with blood-retinal barrier permeability. *Nitric Oxide* 4, 590-596. (2000)

58. A. Carmo, J. G. Cunha-Vaz, A. P. Carvalho & M. C. Lopes: Effect of cyclosporin-A on the blood--retinal barrier permeability in streptozotocin-induced diabetes. *Mediators Inflamm* 9, 243-8 (2000)

59. L. Zheng, S. J. Howell, D. A. Hatala, K. Huang & T. S. Kern: Salicylate-based anti-inflammatory drugs inhibit the early lesion of diabetic retinopathy. *Diabetes* 56, 337-45 (2007)

60. E. A. Ellis, N. Sengupta, S. Caballero, S. M. Guthrie, R. N. Mames & M. B. Grant: Nitric oxide synthases modulate progenitor and resident endothelial cell behavior in galactosemia. *Antioxid Redox Signal* 7, 1413-22 (2005)

61. B. A. Berkowitz, R. A. Kowluru, R. N. Frank, T. S. Kern, T. C. Hohman & M. Prakash: Subnormal retinal oxygenation response precedes diabetic-like retinopathy. *Invest Ophthalmol Vis Sci* 40, 2100-5 (1999)

62. B. A. Berkowitz, H. Luan, R. R. Gupta, D. Pacheco, A. Seidner, R. Roberts, J. Liggett, D. L. Knoerzer, J. R. Connor, Y. Du, T. S. Kern & Y. Ito: Regulation of the early subnormal retinal oxygenation response in experimental diabetes by inducible nitric oxide synthase. *Diabetes* 53, 173-8 (2004)

63. B. A. Berkowitz, R. Roberts, H. Luan, J. Peysakhov, D. L. Knoerzer, J. R. Connor & T. C. Hohman: Drug intervention can correct subnormal retinal oxygenation response in experimental diabetic retinopathy. *Invest Ophthalmol Vis Sci* 46, 2954-60 (2005)

64. T. S. Kern & R. L. Engerman: Pharmacologic inhibition of diabetic retinopathy: Aminoguanidine and aspirin. *Diabetes* 50, 1636-42 (2001)

65. H. P. Hammes, S. Martin, K. Federlin, K. Geisen & M. Brownlee: Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. *Proc Natl Acad Sci USA* 88, 11555-11558 (1991)

66. H. P. Hammes, M. Brownlee, D. Edelstein, M. Saleck, S. Martin & K. Federlin: Aminoguanidine inhibits the development of accelerated diabetic retinopathy in the spontaneous hypertensive rat. *Diabetologia* 37, 32-35 (1994)

67. T. S. Kern, J. Tang, M. Mizutani, R. A. Kowluru, R. H. Nagaraj, G. Romeo, F. Podesta & M. Lorenzi: Response of capillary cell death to aminoguanidine predicts the development of retinopathy: comparison of diabetes and galactosemia. *Invest Ophthalmol Vis Sci* 41, 3972-8 (2000)

68. D. Edelstein & M. Brownlee: Mechanistic studies of advanced glycosylation end product inhibition by aminoguanidine. *Diabetes* 41, 26-9 (1992)

69. M. Kochakian, B. N. Manjula & J. J. Egan: Chronic dosing with aminoguanidine and novel advanced glycosylation end product-formation inhibitors ameliorates cross-linking of tail tendon collagen in STZ-induced diabetic rats. *Diabetes* 45, 1694-1700 (1996)

70. W. Zhao, R. G. Tilton, J. A. Corbett, M. L. McDaniel, T. P. Misko, J. R. Williamson, A. H. Cross & W. F. Hickey: Experimental allergic encephalomyelitis in the rat is inhibited by aminoguanidine, an inhibitor of nitric oxide synthase. *J Neuroimmunol* 64, 123-33 (1996)

71. A. M. Joussen, V. Poulaki, W. Qin, B. Kirchhof, N. Mitsiades, S. J. Wiegand, J. Rudge, G. D. Yancopoulos & A. P. Adamis: Retinal vascular endothelial growth factor induces intercellular adhesion molecule-1 and endothelial nitric oxide synthase expression and initiates early diabetic retinal leukocyte adhesion *in vivo*. *Am J Pathol* 160, 501-9. (2002)

72. A. M. Joussen, V. Poulaki, N. Mitsiades, B. Kirchhof, K. Koizumi, S. Dohmen & A. P. Adamis: Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. *FASEB J* 16, 438-40. (2002)

73. M. Takeda, F. Mori, A. Yoshida, A. Takamiya, S. Nakagomi, E. Sato & H. Kiyama: Constitutive nitric oxide synthase is associated with retinal vascular permeability in early diabetic rats. *Diabetologia* 44, 1043-50 (2001)

74. J. W. Park, S. J. Park, S. H. Park, K. Y. Kim, J. W. Chung, M. H. Chun & S. J. Oh: Up-regulated expression of neuronal nitric oxide synthase in experimental diabetic retina. *Neurobiol Dis* 21, 43-9 (2006)

75. R. Goto, M. Doi, N. Ma, R. Semba & Y. Uji: Contribution of nitric oxide-producing cells in normal and diabetic rat retina. *Jpn J Ophthalmol* 49, 363-70 (2005)

76. E. Roufail, T. Soulis, E. Boel, M. E. Cooper & S. Rees: Depletion of nitric oxide synthase-containing neurons in the diabetic retina: reversal by aminoguanidine. *Diabetologia* 41, 1419-25 (1998)

77. W. G. Robison, T. Kuwabara & J. G. Bieri: The roles of vitamin E and unsaturated fatty acids in the visual process. *Retina* 2, 263-81 (1982)

78. A. Catala: An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay. *Int J Biochem Cell Biol* 38, 1482-95 (2006)

79. J. F. Engelhardt: Redox-mediated gene therapies for environmental injury: approaches and concepts. *Antioxid Redox Signal* 1, 5-27 (1999)

80. J. M. Mates, C. Perez-Gomez & I. Nunez de Castro: Antioxidant enzymes and human diseases. *Clin Biochem* 32, 595-603 (1999)

81. V. Calabrese, E. Guagliano, M. Sapienza, M. Panebianco, S. Calafato, E. Puleo, G. Pennisi, C. Mancuso, D. A. Butterfield & A. G. Stella: Redox regulation of cellular stress response in aging and neurodegenerative disorders: role of vitagenes. *Neurochem Res* 32, 757-73 (2007)

82. G. Slupphaug, B. Kavli & H. E. Krokan: The interacting pathways for prevention and repair of oxidative DNA damage. *Mutat Res* 531, 231-51 (2003)

83. C. Berndt, C. H. Lillig & A. Holmgren: Thiol-based mechanisms of the thioredoxin and glutaredoxin systems: implications for diseases in the cardiovascular system. *Am J Physiol Heart Circ Physiol* 292, H1227-36 (2007)

84. A. M. Abu El-Asrar, R. T. Soliman, S. A. Al-Amro & F. J. Al-Shammary: Production of superoxide anion by polymorphonuclear leukocytes from diabetic patients with or without diabetic retinopathy. *Doc Ophthalmol* 91, 243-54 (1995)

85. A. M. Abu El-Asrar, R. T. Soliman, S. A. Al-Amro & F. J. Al-Shammary: Serum factor from diabetic patients with or without retinopathy stimulates superoxide anion production by normal polymorphonuclear leukocytes. *Doc Ophthalmol* 91, 1-8 (1995)

86. A. Kernell, B. L. Lundh, S. L. Marklund, K. O. Skoog & B. Bjorksten: Superoxide dismutase in the anterior chamber and the vitreous of diabetic patients. *Invest Ophthalmol Vis Sci* 33, 3131-5 (1992)

87. M. Ushio-Fukai & R. W. Alexander: Reactive oxygen species as mediators of angiogenesis signaling: role of NAD(P)H oxidase. *Mol Cell Biochem* 264, 85-97 (2004)

88. Y. Du, C. M. Miller & T. S. Kern: Hyperglycemia increases mitochondrial superoxide in retina and retinal cells. *Free Radic Biol Med* 35, 1491-99 (2003)

89. R. A. Kowluru, J. Tang & T. S. Kern: Abnormalities of retinal metabolism in diabetes and experimental galactosemia. VII. Effect of long-term administration of antioxidants on the development of retinopathy. *Diabetes* 50, 1938-42 (2001)

90. W. Sun, C. Gerhardinger, Z. Dagher, T. Hoehn & M. Lorenzi: Aspirin at low-intermediate concentrations protects retinal vessels in experimental diabetic retinopathy through non-platelet-mediated effects. *Diabetes* 54, 3418-26 (2005)

91. P. Moller, S. Loft, C. Lundby & N. V. Olsen: Acute hypoxia and hypoxic exercise induce DNA strand breaks and oxidative DNA damage in humans. *FASEB J* 15, 1181-6 (2001)

92. R. A. Kowluru & S. Odenbach: Effect of long-term administration of alpha-lipoic acid on retinal capillary cell

death and the development of retinopathy in diabetic rats. *Diabetes* 53, 3233-8 (2004)

93. K. S. Park, J. H. Kim, M. S. Kim, J. M. Kim, S. K. Kim, J. Y. Choi, M. H. Chung, B. Han, S. Y. Kim & H. K. Lee: Effects of insulin and antioxidant on plasma 8-hydroxyguanine and tissue 8-hydroxydeoxyguanosine in streptozotocin-induced diabetic rats. *Diabetes* 50, 2837-41 (2001)

94. G. Romeo, W. H. Liu, V. Asnaghi, T. S. Kern & M. Lorenzi: Activation of nuclear factor-kappaB induced by diabetes and high glucose regulates a proapoptotic program in retinal pericytes. *Diabetes* 51, 2241-8. (2002)

95. H. P. Hammes, X. Du, D. Edelstein, T. Taguchi, T. Matsumura, Q. Ju, J. Lin, A. Bierhaus, P. Nawroth, D. Hannak, M. Neumaier, R. Bergfeld, I. Giardino & M. Brownlee: Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat Med* 9, 294-9. (2003)

96. L. Zheng, C. Szabo & T. S. Kern: Poly(ADP-ribose) polymerase is involved in the development of diabetic retinopathy via regulation of nuclear factor-kappaB. *Diabetes* 53, 2960-7 (2004)

97. H. P. Hammes, A. Bartmann, L. Engel & P. Wulfroth: Antioxidant treatment of experimental diabetic retinopathy in rats with nicanartine. *Diabetologia* 40, 629-634 (1997)

98. J. Lin, A. Bierhaus, P. Bugert, N. Dietrich, Y. Feng, F. Vom Hagen, P. Nawroth, M. Brownlee & H. P. Hammes: Effect of R-(+)-alpha-lipoic acid on experimental diabetic retinopathy. *Diabetologia* 49, 1089-96 (2006)

99. T. Nishida, S. Nakagawa & R. Manabe: Superoxide dismutase activity in diabetic rat retina. *Jpn J Ophthalmol* 28, 377-82 (1984)

100. R. A. Kowluru, T. S. Kern & R. L. Engerman: Abnormalities of retinal metabolism in diabetes or experimental galactosemia. IV. Antioxidant defense system. *Free Radic Biol Med* 22, 587-92 (1997)

101. M. Kanwar, P. S. Chan, T. S. Kern & R. A. Kowluru: Oxidative damage in the retinal mitochondria of diabetic mice: possible protection by superoxide dismutase. *Invest Ophthalmol Vis Sci* 48, 3805-11 (2007)

102. R. A. Kowluru, V. Kowluru, Y. Xiong & Y. S. Ho: Overexpression of mitochondrial superoxide dismutase in mice protects the retina from diabetes-induced oxidative stress. *Free Radic Biol Med* 41, 1191-6 (2006)

103. M. Brownlee: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414, 813-20. (2001)

104. M. Brownlee: The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 54, 1615-25 (2005)

105. D. Giugliano, G. Paolisso & A. Ceriello: Oxidative stress and diabetic vascular complications. *Diabetes Care* 19, 257-267 (1996)

106. T. Nishikawa, D. Edelstein & M. Brownlee: The missing link: a single unifying mechanism for diabetic complications. *Kidney Int Suppl* 77, S26-30. (2000)

107. V. Kowluru & R. A. Kowluru: Increased oxidative stress in diabetes regulates activation of a small molecular weight G-protein, H-Ras, in the retina. *Mol Vis* 13, 602-10 (2007)

108. R. A. Kowluru, A. Kowluru & M. Kanwar: Small molecular weight G-protein, H-Ras, and retinal endothelial cell apoptosis in diabetes. *Mol Cell Biochem* 296, 69-76 (2007)

109. H. Takaishi, T. Taniguchi, A. Takahashi, Y. Ishikawa & M. Yokoyama: High glucose accelerates MCP-1 production via p38 MAPK in vascular endothelial cells. *Biochem Biophys Res Commun* 305, 122-8 (2003)

110. P. C. Dedon & S. R. Tannenbaum: Reactive nitrogen species in the chemical biology of inflammation. *Arch Biochem Biophys* 423, 12-22 (2004)

111. X. Zhan, Y. Du, J. S. Crabb, X. Gu, T. S. Kern & J. W. Crabb: Targets of tyrosine nitration in diabetic rat retina. *Mol Cell Proteomics* 7, 864-74 (2008)

112. H. Rubbo, R. Radi, M. Trujillo, R. Telleri, B. Kalyanaraman, S. Barnes, M. Kirk & B. A. Freeman: Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. *J Biol Chem* 269, 26066-75 (1994)

113. H. Rubbo, R. Radi, D. Anselmi, M. Kirk, S. Barnes, J. Butler, J. P. Eiserich & B. A. Freeman: Nitric oxide reaction with lipid peroxyl radicals spares alpha-tocopherol during lipid peroxidation. Greater oxidant protection from the pair nitric oxide/alpha-tocopherol than alpha-tocopherol/ascorbate. *J Biol Chem* 275, 10812-8 (2000)

114. P. S. Wong, J. P. Eiserich, S. Reddy, C. L. Lopez, C. E. Cross & A. van der Vliet: Inactivation of glutathione S-transferases by nitric oxide-derived oxidants: exploring a role for tyrosine nitration. *Arch Biochem Biophys* 394, 216-28 (2001)

115. W. I. Lee & H. L. Fung: Mechanism-based partial inactivation of glutathione S-transferases by nitroglycerin: tyrosine nitration vs sulfhydryl oxidation. *Nitric Oxide* 8, 103-10 (2003)

116. S. Burney, J. L. Caulfield, J. C. Niles, J. S. Wishnok & S. R. Tannenbaum: The chemistry of DNA damage from nitric oxide and peroxynitrite. *Mutat Res* 424, 37-49 (1999)

117. F. Gu, W. G. Stillwell, J. S. Wishnok, A. J. Shallop, R. A. Jones & S. R. Tannenbaum: Peroxynitrite-induced

reactions of synthetic oligo 2'-deoxynucleotides and DNA containing guanine: formation and stability of a 5-guanidino-4-nitroimidazole lesion. *Biochemistry* 41, 7508-18 (2002)

118. K. T. Lin, J. Y. Xue, M. C. Lin, E. G. Spokas, F. F. Sun & P. Y. Wong: Peroxynitrite induces apoptosis of HL-60 cells by activation of a caspase-3 family protease. *Am J Physiol* 274, C855-60. (1998)

119. L. Virag, D. J. Marmer & C. Szabo: Crucial role of apopain in the peroxynitrite-induced apoptotic DNA fragmentation. *Free Radic Biol Med* 25, 1075-82 (1998)

120. A. B. El-Remessy, M. A. Behzadian, G. Abou-Mohamed, T. Franklin, R. W. Caldwell & R. B. Caldwell: Experimental diabetes causes breakdown of the blood-retina barrier by a mechanism involving tyrosine nitration and increases in expression of vascular endothelial growth factor and urokinase plasminogen activator receptor. *Am J Pathol* 162, 1995-2004 (2003)

121. I. G. Obrosova, P. Pacher, C. Szabo, Z. Zsengeller, H. Hirooka, M. J. Stevens & M. A. Yorek: Aldose reductase inhibition counteracts oxidative-nitrosative stress and poly(ADP-ribose) polymerase activation in tissue sites for diabetes complications. *Diabetes* 54, 234-42 (2005)

122. R. A. Kowluru, M. Kanwar & A. Kennedy: Metabolic memory phenomenon and accumulation of peroxynitrite in retinal capillaries. *Exp Diabetes Res* 2007, 21976 (2007)

123. C. Szabo, H. Ischiropoulos & R. Radi: Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. *Nat Rev Drug Discov* 6, 662-80 (2007)

124. M. Tannous, R. A. Rabini, A. Vignini, N. Moretti, P. Fumelli, B. Zielinski, L. Mazzanti & B. Mutus: Evidence for iNOS-dependent peroxynitrite production in diabetic platelets. *Diabetologia* 42, 539-44 (1999)

125. A. Ceriello, F. Mercuri, L. Quagliaro, R. Assaloni, E. Motz, L. Tonutti & C. Taboga: Detection of nitrotyrosine in the diabetic plasma: evidence of oxidative stress. *Diabetologia* 44, 834-8 (2001)

126. C. Szabo, A. Zanchi, K. Komjati, P. Pacher, A. S. Krolewski, W. C. Quist, F. W. LoGerfo, E. S. Horton & A. Veves: Poly(ADP-Ribose) polymerase is activated in subjects at risk of developing type 2 diabetes and is associated with impaired vascular reactivity. *Circulation* 106, 2680-6 (2002)

127. P. Pacher, I. G. Obrosova, J. G. Mabley & C. Szabo: Role of nitrosative stress and peroxynitrite in the pathogenesis of diabetic complications. Emerging new therapeutical strategies. *Curr Med Chem* 12, 267-75 (2005)

128. P. Pacher & C. Szabo: Role of peroxynitrite in the pathogenesis of cardiovascular complications of diabetes. *Curr Opin Pharmacol* 6, 136-41 (2006)

129. P. Pacher, J. S. Beckman & L. Liaudet: Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 87, 315-424 (2007)

130. Z. Dagher, Y. S. Park, V. Asnaghi, T. Hoehn, C. Gerhardinger & M. Lorenzi: Studies of rat and human retinas predict a role for the polyol pathway in human diabetic retinopathy. *Diabetes* 53, 2404-11 (2004)

131. V. R. Drel, P. Pacher, M. J. Stevens & I. G. Obrosova: Aldose reductase inhibition counteracts nitrosative stress and poly(ADP-ribose) polymerase activation in diabetic rat kidney and high-glucose-exposed human mesangial cells. *Free Radic Biol Med* 40, 1454-65 (2006)

132. R. Sugawara, T. Hikichi, N. Kitaya, F. Mori, T. Nagaoka, A. Yoshida & C. Szabo: Peroxynitrite decomposition catalyst, FP15, and poly(ADP-ribose) polymerase inhibitor, PJ34, inhibit leukocyte entrapment in the retinal microcirculation of diabetic rats. *Curr Eye Res* 29, 11-6 (2004)

133. R. A. Kowluru: Effect of reinstitution of good glycemic control on retinal oxidative stress and nitrative stress in diabetic rats. *Diabetes* 52, 818-23 (2003)

134. D. H. Platt, M. Bartoli, A. B. El-Remessy, M. Al-Shabrawey, T. Lemtalsi, D. Fulton & R. B. Caldwell: Peroxynitrite increases VEGF expression in vascular endothelial cells via STAT3. *Free Radic Biol Med* 39, 1353-61 (2005)

135. A. B. El-Remessy, M. Al-Shabrawey, D. H. Platt, M. Bartoli, M. A. Behzadian, N. Ghaly, N. Tsai, K. Motamed & R. B. Caldwell: Peroxynitrite mediates VEGF's angiogenic signal and function via a nitration-independent mechanism in endothelial cells. *FASEB J* 21, 2528-39 (2007)

136. A. B. El-Remessy, M. Bartoli, D. H. Platt, D. Fulton & R. B. Caldwell: Oxidative stress inactivates VEGF survival signaling in retinal endothelial cells via PI 3-kinase tyrosine nitration. *J Cell Sci* 118, 243-52 (2005)

137. L. Virag & C. Szabo: The therapeutic potential of poly(ADP-Ribose) polymerase inhibitors. *Pharmacol Rev* 54, 375-429. (2002)

138. P. Jagtap & C. Szabo: Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. *Nat Rev Drug Discov* 4, 421-40 (2005)

139. F. G. Soriano, L. Virag & C. Szabo: Diabetic endothelial dysfunction: role of reactive oxygen and nitrogen species production and poly(ADP-ribose) polymerase activation. *J Mol Med* 79, 437-48. (2001)

140. A. G. Minchenko, M. J. Stevens, L. White, O. I. Abatan, K. Komjati, P. Pacher, C. Szabo & I. G. Obrosova: Diabetes-induced overexpression of endothelin-1 and endothelin receptors in the rat renal cortex is mediated via

poly(ADP-ribose) polymerase activation. *FASEB J* 17, 1514-6 (2003)

141. I. G. Obrosova, A. G. Minchenko, R. N. Frank, G. M. Seigel, Z. Zsengeller, P. Pacher, M. J. Stevens & C. Szabo: Poly(ADP-ribose) polymerase inhibitors counteract diabetes- and hypoxia-induced retinal vascular endothelial growth factor overexpression. *Int J Mol Med* 14, 55-64 (2004)

142. C. Szabo, A. Biser, R. Benko, E. Bottinger & K. Susztak: Poly(ADP-ribose) polymerase inhibitors ameliorate nephropathy of type 2 diabetic Leprdb/db mice. *Diabetes* 55, 3004-12 (2006)

143. O. Ilnytska, V. V. Lyzogubov, M. J. Stevens, V. R. Drel, N. Mashtalir, P. Pacher, M. A. Yorek & I. G. Obrosova: Poly(ADP-ribose) polymerase inhibition alleviates experimental diabetic sensory neuropathy. *Diabetes* 55, 1686-94 (2006)

144. I. G. Obrosova, W. Xu, V. V. Lyzogubov, O. Ilnytska, N. Mashtalir, I. Vareniuk, I. A. Pavlov, J. Zhang, B. Slusher & V. R. Drel: PARP inhibition or gene deficiency counteracts intraepidermal nerve fiber loss and neuropathic pain in advanced diabetic neuropathy. *Free Radic Biol Med* 15, 972-81 (2008)

145. M. J. Stevens, F. Li, V. R. Drel, O. I. Abatan, H. Kim, D. Burnett, D. Larkin & I. G. Obrosova: Nicotinamide reverses neurological and neurovascular deficits in streptozotocin diabetic rats. *J Pharmacol Exp Ther* 320, 458-64 (2007)

146. R. A. Kowluru, R. L. Engerman, G. L. Case & T. S. Kern: Retinal glutamate in diabetes and effect of antioxidants. *Neurochem Int* 38, 385-390 (2001)

147. D. M. van Reyk, M. C. Gillies & M. J. Davies: The retina: oxidative stress and diabetes. *Redox Rep* 8, 187-92 (2003)

148. I. G. Obrosova, V. R. Drel, A. K. Kumagai, C. Szabo, P. Pacher & M. J. Stevens: Early diabetes-induced biochemical changes in the retina: comparison of rat and mouse models. *Diabetologia* 49, 2525-33 (2006)

149. A. M. Joussen, V. Poulaki, M. L. Le, K. Koizumi, C. Esser, H. Janicki, U. Schraermeyer, N. Kociok, S. Fauser, B. Kirchhof, T. S. Kern & A. P. Adamis: A central role for inflammation in the pathogenesis of diabetic retinopathy. *FASEB J* 18, 1450-2 (2004)

150. R. M. Diederen, C. A. Starnes, B. A. Berkowitz & B. S. Winkler: Reexamining the hyperglycemic pseudohypoxia hypothesis of diabetic oculopathy. *Invest Ophthalmol Vis Sci* 47, 2726-31 (2006)

151. H. Kaur, S. Chen, X. Xin, J. Chiu, Z. A. Khan & S. Chakrabarti: Diabetes-induced extracellular matrix protein expression is mediated by transcription coactivator p300. *Diabetes* 55, 3104-11 (2006)

152. S. Roy, T. Sato, G. Paryani & R. Kao: Downregulation of fibronectin overexpression reduces basement membrane thickening and vascular lesions in retinas of galactose-fed rats. *Diabetes* 52, 1229-34 (2003)

153. X. Du, T. Matsumura, D. Edelstein, L. Rossetti, Z. Zsengeller, C. Szabo & M. Brownlee: Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest* 112, 1049-57 (2003)

154. L. E. Craig, J. P. Spelman, J. D. Strandberg & M. C. Zink: Endothelial cells from diverse tissues exhibit differences in growth and morphology. *Microvasc Res* 55, 65-76 (1998)

155. H. S. Murphy, N. Bakopoulos, M. K. Dame, J. Varani & P. A. Ward: Heterogeneity of vascular endothelial cells: differences in susceptibility to neutrophil-mediated injury. *Microvasc Res* 56, 203-11 (1998)

156. J. King, T. Hamil, J. Creighton, S. Wu, P. Bhat, F. McDonald & T. Stevens: Structural and functional characteristics of lung macro- and microvascular endothelial cell phenotypes. *Microvasc Res* 67, 139-51 (2004)

157. A. Duffy, A. Liew, J. O'Sullivan, G. Avalos, A. Samali & T. O'Brien: Distinct effects of high-glucose conditions on endothelial cells of macrovascular and microvascular origins. *Endothelium* 13, 9-16 (2006)

158. D. Viemann, M. Goebeler, S. Schmid, U. Nordhues, K. Klimmek, C. Sorg & J. Roth: TNF induces distinct gene expression programs in microvascular and macrovascular human endothelial cells. *J Leukoc Biol* 80, 174-85 (2006)

Abbreviations: 8-OHdG: 8-hydroxydeoxyguanosine, AGEs: advanced glycation end products, COX: cyclooxgenase, eNOS: endothelial nitric oxide synthase, GSH: glutathione, ICAM-1: intercellular adhesion molecule-1, iNOS: inducible nitric oxide synthase, L-NMMA: NG-monomethyl-L-arginine, nNOS: neuronal nitric oxide synthase, NO: nitric oxide, NOS: nitric oxide synthase, PARP: poly(ADP-ribose) polymerase, PGE2: prostaglandin E2 , PKC: protein kinase C, PMNs: polymorphonuclear leukocytes, ROS: reactive oxygen species, SOD: superoxide dismutase , STAT3: signal transducer and activator transcription 3, VEGF: vascular endothelial growth factor

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

Send correspondence to: Timothy S. Kern, Department of Medicine, 434 Biomedical Research Building, Case Western Reserve University, 10900 Euclid Ave, Cleveland, OH 44106, Tel: 216-368-0800, Fax: 216-368-5824, E-mail: tsk@case.edu

http://www.bioscience.org/current/vol14.htm