ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 11 & 12, 2005

Forum Review

Diabetic Retinopathy: Mitochondrial Dysfunction and Retinal Capillary Cell Death

RENU A. KOWLURU

ABSTRACT

Oxidative stress is increased in the retina in diabetes; the levels of oxidatively modified DNA and nitrosylated proteins are elevated, and antioxidant defense enzymes are impaired. The levels of superoxides are elevated in the retina, and the mitochondria become dysfunctional with proapoptotic protein, Bax, translocating from the cytosol into the mitochondria, and cytochrome *c* leaking out from the mitochondria. This is accompanied by increased retinal capillary cell apoptosis, and the formation of acellular capillaries and pericyte ghosts, the early signs of retinopathy in animal models of diabetic retinopathy. Inhibition of superoxides inhibits glucose-induced mitochondrial dysfunction, activation of caspase-3, and cell death in retinal capillary cells. In animal models, long-term administration of lipoic acid or other antioxidants inhibits the development of diabetic retinopathy via inhibition of accumulation of oxidatively modified DNA and nitrotyrosine and capillary cell apoptosis in the retina. Understanding the role of mitochondria in the development of retinopathy in diabetes should help identify therapies that can neutralize superoxides and inhibit their dysfunction and, ultimately, the development of retinopathy. *Antioxid. Redox Signal.* 7, 1581–1587.

INTRODUCTION

Retinopathy, a progressive disease, is one of the most debilitating complications of diabetes. It is the leading cause of acquired blindness among young adults in developed countries. Animal models have shown that hyperglycemia-induced abnormalities in retinal metabolism, including elevated polyol pathway activity (44), increased nonenzymatic glycation (56), oxidative stress (6, 35), protein kinase C activity (34, 59), and the expression of vascular endothelial growth factor (VEGF) (1, 40), evidently contribute to the development of microangiopathy. But it has been difficult to recognize which abnormalities are critical, thus making the rationale for possible therapies limited.

OXIDATIVE STRESS IN DIABETES

Diabetes increases oxidative stress, which plays a key regulatory role in the development of its complications (7, 22, 35). Reactive oxygen species generated by high glucose are considered a causal link between elevated glucose and the other metabolic abnormalities important in the development of diabetic complications (8). Lipid peroxides are increased in the retina in diabetes, antioxidant defense enzymes are impaired, the levels of the intracellular antioxidant, the reduced form of glutathione (GSH), are decreased (23, 33, 35), superoxide levels are elevated (16), and mRNA of superoxide dismutase (SOD) and glutathione reductase are down-regulated (39). Oxidative stress is increased also in isolated retinal capillary cells (both endothelial cells and pericytes) incubated in high-glucose medium (31), and also in other nonvascular retinal cells, including Muller cells and photoreceptors (11, 16). These data have clearly suggested an important role of oxidative stress in the development of retinopathy in diabetes. Our studies have also shown that oxidative stress is important not only in the development of retinopathy in diabetes, but also in the resistance of retinopathy to arrest after initiation of good glycemic control (29). However, the mechanism by which oxidative stress can contribute to the pathogenesis of retinopathy in diabetes remains to be elucidated.

Kresge Eye Institute, Wayne State University, Detroit, MI.

OXIDATIVE STRESS, APOPTOSIS, AND DIABETIC COMPLICATIONS

Oxidative stress is closely linked to apoptosis in a variety of cell types, and the mechanisms by which it could increase cell death might involve increased membrane lipid peroxidation, increased oxidative injury to other macromolecules, or alterations in signal transduction (5, 41). Oxidative stress is shown to cause abnormal gene expression and altered signal transduction leading to apoptosis of myocardial cells (10), promote mitochondrial changes in the neurons resulting in sensory neuropathy (54), and trigger apoptosis in tubule, leading to nephropathy (58). The overexpression of the cell death protease gene in retinal pericytes in diabetes is reported to correlate with the altered gene profile of the scavenging enzymes, suggesting an important role of oxidative stress in pericyte dropout seen in diabetic retinopathy (39). Further, increased oxidative stress has been shown to play a critical role in advanced glycation end products-induced apoptosis of retinal capillary cells (14). Our studies have provided evidence that long-term administration of antioxidants to diabetic rats that inhibits the development of retinopathy also inhibits the activation of caspase-3 and nuclear factor-κB (NF-κB) in the retina, suggesting that perhaps the beneficial effect of antioxidants may be mediated, in part, via inhibition of oxidative stress-mediated apoptosis of retinal capillary cells (31, 36).

RETINAL CELL APOPTOSIS IN DIABETES

In the pathogenesis of retinopathy in diabetes, retinal microvascular cells (pericytes and endothelial cells) are lost selectively via apoptosis before other histopathology is detectable, or loss of vision is evident (27, 32, 42). Caspase-3 activity is increased in both endothelial cells and pericytes incubated in high-glucose medium for 5 days when capillary cell apoptosis can be detected (31). Further, in the same cells, increased DNA laddering and increased relative amounts of mono- and oligonucleosomes generated from apoptotic cells (measured using monoclonal antibodies directed against DNA and histones) are also observed, suggesting increased apoptosis of the cells in high-glucose medium (30). In diabetic rats, retinal caspase-3 activation is observed at a duration of diabetes when capillary cell death and histopathology can be detected (31, 36), suggesting that apoptosis of retinal capillary cells might be mediated via caspase-3 activation. Histopathology of diabetic retinopathy takes decades in humans and more than 1 year in rats to develop, but apoptosis is a rapidly consummated phenomenon, and the cell contains fragmented DNA for only a few hours (51). Therefore, the small number of terminal transferase dUTP nick-end labeling (TUNEL)-positive cells observed in diabetic retina (27, 32, 42) could have a major impact on the formation of acellular capillaries and pericyte ghosts. Defective endothelial cell replication seen in hyperglycemia could accelerate the process of retinal ischemia by exhausting the cell's replicative capacity, and as acellular capillaries in the retina are not perfused, ischemia (and subsequently, neovascularization) likely develops in diabetes as the acellular capillaries become more frequent. Thus, accelerated apoptosis can readily account for pericyte "dropout" and formation of "ghosts" in diabetic retinopathy. High-glucose treatment has been shown also to cause accelerated endothelial cell apoptosis by blocking the prosurvival effect of VEGF, and increased peroxynitrite via nitration of phosphatidylinositol 3-kinase and inhibition of Akt-1 kinase activity have been implicated in this process (18).

A possible role of apoptosis of other retinal cells, including ganglion cells, astrocytes, and Muller cells, in the pathogenesis of diabetic retinopathy, however, cannot be ruled out because increased apoptosis of these nonvascular cells is also seen early in the development of retinopathy in diabetes (4, 25, 38, 43).

ROLE OF MITOCHONDRIA

Mitochondria are considered the major endogenous source of superoxides, peroxynitrite, and hydroxyl radicals, and are also targets for the damaging effect of oxidants, suggesting the existence of a vicious cycle of oxidative damage (19). Mitochondrial DNA is very susceptible to oxidative damage, and increased oxidative damage to the inner membrane of the mitochondria leads to imbalances in the electron transport chain. This could result in increased superoxide and hydrogen peroxide production that, in turn, can further damage membrane proteins. Reactive oxidant intermediates can trigger mitochondria to release cytochrome *c*, and increased lipid peroxidation itself can damage mitochondrial membrane potential, provoking apoptosis (3, 13, 48). Once cytochrome *c* is released from mitochondria, it activates caspase-9, which then activates caspase-3, resulting in the execution of apoptosis. The mitochondrial pathway of apoptosis is also controlled by the Bcl-2 family proteins; proapoptotic Bax proteins enhance the release of cytochrome *c* by translocating to mitochondria and inducing mitochondrial permeability transition (13, 26).

Intramitochondrial oxidative stress is shown to increase in diabetes (37), and increased Bax immunostaining is seen in the vascular cells and ganglion cells, the cell types known to undergo apoptosis in diabetes (49) (Fig. 1). In dorsal root ganglion neurons and Schwann cells, diabetes-induced mitochondrial changes are associated with the activation of programmed cell death pathways. We have shown that mitochondrial dysfunction in the retina is an important contributor to the accelerated retinal capillary cell apoptosis seen in diabetes (30). However, how mitochondrial dysfunction is involved in retinal capillary cell apoptosis remains to be explored. One of the possible mechanisms postulated involves partial inhibition of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase, which diverts increased substrate flux from glycolysis to pathways of glucose overutilization, by mitochondrial reactive oxygen species (21).

Another possibility remains that increased nitric oxide (NO) could inhibit the activity of the electron transport system, resulting in increased superoxides. This suggests that the electron transport system can be both the source and a target of excess reactive oxygen species in the retina in diabetes.

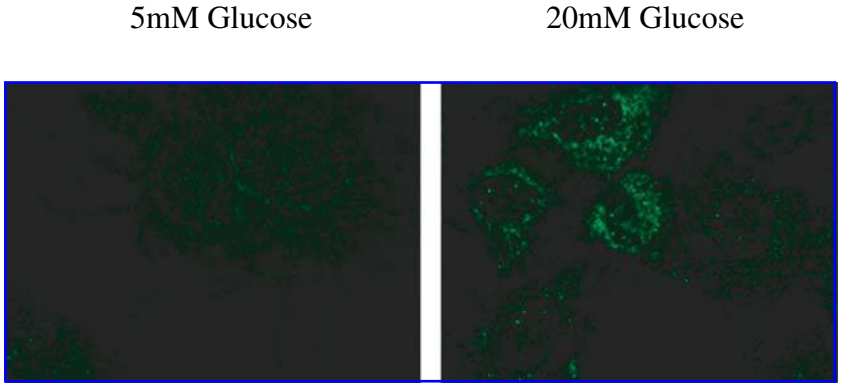

FIG. 1. Confocal image showing the effect of glucose on Bax expression in retinal endothelial cells. Retinal endothelial cells exposed to 5 m*M* glucose (**left**) and 20 m*M* glucose (**right**) for 3 days were fixed with methanol:acetone, blocked, and stained using polyclonal rabbit Bax antibody. Anti-rabbit IgG-specific fluorescein isothiocyanate was used as the secondary antibody. The microscopy was performed using a Zeiss LSM 310 inverted laser scan microscope equipped with a fluorescein filter. The experiment was repeated three times using different cell preparations.

Further, increased oxidative stress activates redox-sensitive nuclear translocation factor, NF-κB, and in diabetes NF-κB is activated in retina and endothelial cells and pericytes incubated in high-glucose medium (36, 53). Activation of NF-κB modulates the expression of the inducible form of NO, and this, in turn, results in increased free radical production (20). Reaction between superoxides and NO forms peroxynitrite, and peroxynitrite itself can interact with mitochondria, resulting in mitochondrial transition pore opening, thus releasing proapoptotic proteins (50). Peroxynitrite levels are elevated in retina early in diabetes and remain elevated at 14 months of diabetes in rats (29, 36), and increased nitrotyrosine can be localized in the retinal vasculature of diabetic rats (15).

Recent studies from our laboratory have shown that retinal mitochondria experience dysfunction in diabetes; diabetes of 8 months in rats (a duration when capillary cell apoptosis is seen in the retina) increases the release of cytochrome *c* into the cytosol and Bax into the mitochondria. In isolated retinal capillary cells, incubation in high-glucose medium results in the release of cytochrome *c* in the cytosol and Bax in the mitochondria, and these abnormalities are accompanied by increased cell apoptosis. Inhibition of superoxides by the SOD mimetic, MnTBAP [Mn(III) tetra(4-benzoic acid) porphyrin chloride], inhibits glucose-induced release of cytochrome *c* and Bax, and apoptosis in both endothelial cells and pericytes (30).

In order to clarify the mechanism involved in glucose-induced retinal capillary cell death, we evaluated the effect of inhibition of superoxides on the intracellular antioxidant, GSH, in the retinal capillary cells incubated in high-glucose medium. As shown in Fig. 2, GSH levels were decreased in the endothelial cells incubated in 20 m*M* glucose for 5 days as compared with those incubated in 5 m*M* glucose medium. Inhibition of superoxides by MnTBAP significantly ($p < 0.02$) inhibited a glucose-induced decrease in GSH levels. To investigate further the process of cell apoptosis, the effect of MnTBAP was determined on glucose-induced activation of caspase-3. Inclusion of MnTBAP in the 20 m*M* glucose medium inhibited the activation of caspase-3 in these retinal capillary cells (Fig. 3), and also the apoptosis of these cells (30). Thus, the results strongly suggest that glucose-induced apoptosis of retinal capillary cells is, in part, via mitochondrial dysfunction, and the process involves Bax translocation and release of cytochrome *c* that results in the activation of caspase-3, culminating in capillary cell apoptosis.

In contrast to our data and also findings by others showing oxidative injury during pericyte loss in diabetic retinopathy and involvement of an oxidative stress-mediated mechanism in the apoptotic loss of bovine retinal pericytes (2, 28), others

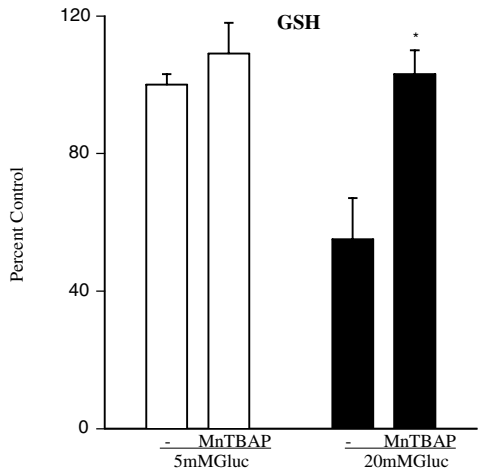

FIG. 2. GSH levels in the endothelial cells. GSH levels were measured in the endothelial cells incubated in 5 m*M* or 20 m*M* glucose in the presence or absence of 200 μ*M* MnTBAP (a cell-permeable SOD mimetic, obtained from Biomol, Plymouth Meeting, PA, U.S.A.), for 5 days using a glutathione assay kit from Cayman Chemicals (Ann Arbor, MI, U.S.A.) according to the manufacturer's instructions. The amount of 5-thio-2-nitrobenzoic acid produced was measured in the supernatant of the deproteinized cell suspension. Each measurement was made in duplicate, and the experiment was repeated with more than three separate cell preparations. Values obtained with 5 m*M* glucose were considered 100%, and are represented as the means ± SD. *$p < 0.05$ compared with control.

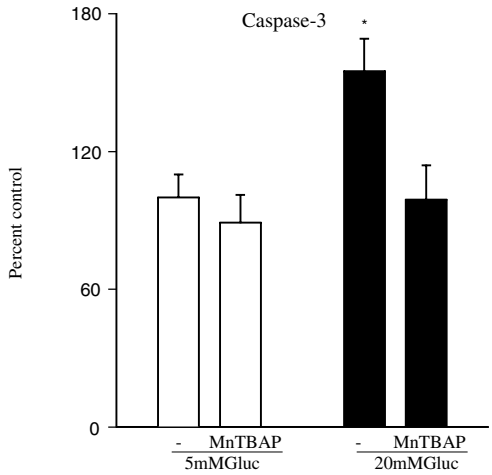

FIG. 3. Effect of superoxide inhibition on glucose-induced activation of caspase-3 activity. Caspase-3 enzyme activity was measured in the cell lysate by measuring the fluorescence emission released on proteolytic cleavage of the substrates [DEVD-AFC, *N*-acetyl-Asp-Glu-L-Val-Asp-7-amino-4 trifluoromethyl coumarin]. Each experiment was repeated with three or more cell preparations, and measurements were done in duplicate. Values obtained with 5 m*M* glucose were considered 100%, and are represented as the means ± SD. $*p < 0.05$ compared with control.

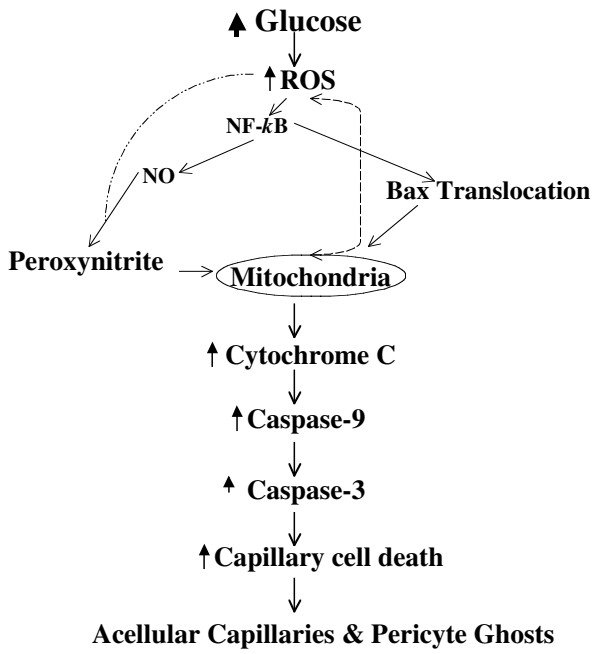

FIG. 4. Free radicals activate NF-κB and that is followed by translocation of Bax to the mitochondria. Cytochrome *c* is released from mitochondria into the cytoplasm, which activates caspase-9, followed by activation of caspase-3, culminating in cell death. Increased formation of peroxynitrite aggravates mitochondrial dysfunction, and dysfunctional mitochondria generate free radicals, resulting in a vicious cycle of oxidative damage. ROS, reactive oxygen species.

have failed to observe any glucose-induced oxidative damage in retinal pericytes despite lower GSH levels (55). The reasons for such discrepancies are not clear, but may include how the cells were prepared from the retina.

Based on the data from our laboratory and available literature, Fig. 4 shows a model that could explain, in part, the loss of retinal capillary cells in diabetes. Free radicals are increased in retinal vasculature in diabetes leading to the mitochondrial phase of apoptosis: NF-κB is activated, followed by translocation of Bax to the mitochondria. This results in release of cytochrome *c* from mitochondria into the cytoplasm, which activates caspase-9, followed by activation of caspase-3, culminating in cell death. We also surmise that increased peroxynitrite formed in diabetes exacerbates mitochondrial dysfunction. Dysfunctional mitochondria can generate further free radicals, resulting in a vicious cycle of oxidative damage. The model suggests that the effect of increased oxidative stress leading to retinal capillary cell death is an early event in the development of diabetic retinopathy.

However, retinal capillary cells could also undergo apoptosis in diabetes via a caspase-independent pathway. The role of apoptosis-inducing factor, a mitochondrial intermembrane flavoprotein that could mediate caspase-independent apoptosis and apoptotic mitochondrial events (12), in accelerated retinal capillary cell death in diabetes remains to be determined. Endothelial cells could undergo apoptosis also via a Fas-mediated pathway, because increased expression of FasL on circulating leukocytes in retina is observed in diabetes (24).

This article focuses on the role of mitochondrial oxidative stress in the capillary cell apoptosis, but the role of growth factors such as tumor necrosis factor and VEGF in the pathogenesis of retinopathy in diabetes cannot be ignored. Hyperglycemia induces VEGF expression in many retinal cell types, including retinal pigment epithelial cells, capillary cells, glial cells, Muller cells, and ganglion cells (17). Increased VEGF results in vascular permeability and leukostasis in the retina, and leukostasis can lead to vascular occlusion and ultimately tissue ischemia. In addition, the role of poly(ADP-ribose) polymerase (PARP) activation in the development of diabetic retinopathy via increased oxidative stress remains a strong possibility because PARP is considered to play an important role in diabetes- and hypoxia-induced VEGF production that can be inhibited by PARP inhibitors (47).

EFFECT OF ANTIOXIDANTS

Antioxidants have beneficial effects on retinal metabolic abnormalities postulated to be involved in the development of retinopathy. Vitamin E supplementation reduces retinal hemodynamics abnormalities seen in diabetic patients (9, 32, 35, 45, 46), and pyridoxamine inhibits the formation of diabetes-induced acellular strands in the retina (57). We have shown that administration of multi-antioxidants to diabetic rats inhibits the formation of acellular capillaries and pericyte ghosts (35). Our recent results have shown that long-term administration of lipoic acid, a disulfide derivative of octanoic acid that can quench radicals, chelate metals, and interact with thiols, to diabetic rats inhibits capillary cell apoptosis

and the histopathology characteristic of retinopathy in diabetes, and these beneficial effects are accompanied by inhibition of accumulation of oxidatively modified DNA and nitrotyrosine in the retina (32). Others have shown that lipoic acid inhibits the diabetes-induced decrease in retinal mitochondrial and cytosolic NAD^+/NADH ratios (45). This raises the possibility that the beneficial effects of lipoic acid on retinal capillary cell apoptosis and the development of retinopathy in diabetes are, in part, via inhibition of mitochondrial dysfunction that the retina is experiencing in diabetes. However, by contrast, some studies have failed to show any effects of antioxidants on retinal vascular lesions (52), and the differences for such discrepancies are not clear. Thus, whether oxidative damage has a causative role in the pathology of retinopathy in diabetes still remains an open question.

CONCLUSIONS

The data obtained from our laboratory and those of others show that mitochondria play a role in the development of retinopathy in diabetes. Thus, it is safe to speculate that hyperglycemia-induced retinal capillary cell death might be resulting from the mitochondrial cytochrome *c*-mediated caspase-3 activation pathway. Understanding the role of superoxides in the development of retinopathy in diabetes should help identify SOD mimetics that can neutralize superoxides and inhibit the development of retinopathy. Optimistically, SOD mimetics are already reported to have beneficial effects in ischemia–reperfusion injury and improve diabetes-induced decreases in blood flow and motor nerve conduction velocity. However, it is important to recognize that the treatment strategies that could inhibit superoxide production in diabetes represent one possible direction for clinical research, but mitochondrial superoxides represent an important part of the complex approach to inhibit the pathogenesis of diabetic retinopathy.

ACKNOWLEDGMENTS

The technical assistance of Sarah Odenbach and Saiyeda Noor Abbas is sincerely appreciated. This work was supported in part by grants from Juvenile Diabetes Research Foundation, The Thomas Foundation, and National Eye Institute.

ABBREVIATIONS

GSH, glutathione (reduced form); MnTBAP, Mn(III) tetra(4-benzoic acid) porphyrin chloride; NF-κB, nuclear factor-κB; NO, nitric oxide; PARP, poly(ADP-ribose) polymerase; SOD, superoxide dismutase; VEGF, vascular endothelial growth factor.

REFERENCES

1. Aiello LP. Vascular endothelial growth factor and the eye: biochemical mechanisms of action and implications for novel therapies. *Ophthalmic Res* 29: 354–362, 1997.
2. Ansari NH, Zhang W, Fulep E, and Mansour A. Prevention of pericyte loss by Trolox in diabetic rat retina. *J Toxicol Environ Health* 24: 467–475, 1998.
3. Anuradha CD, Kanno S, and Hirano S. Oxidative damage to mitochondria is a preliminary step to caspase-3 activation in fluoride-induced apoptosis in HL-60 cells. *Free Radic Biol Med* 31: 367–373, 2001.
4. Barber AJ, Lieth E, Khin SA, Antonetti DA, Buchanan AG, and Gardner TW. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. *J Clin Invest* 102: 783–791, 1998.
5. Baumgartner-Parzer SM, Wagner L, Pettermann M, Grillari J, Gessl A, and Waldhausl W. High-glucose-triggered apoptosis in cultured endothelial cells. *Diabetes* 44: 1323–1327, 1995.
6. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 40: 405–412, 1991.
7. Baynes JW and Thrope SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* 48: 1–9, 1999.
8. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 414: 813–820, 2001.
9. Bursell SE, Clermont AC, Aiello LP, Aiello LM, Schlossman DK, Feener EP, Laffel L, and King GL. High-dose vitamin E supplementation normalizes retinal blood flow and creatinine clearance in patients with type 1 diabetes. *Diabetes Care* 22: 1245–1251, 1999.
10. Cai L and Kang YJ. Oxidative stress and diabetic cardiomyopathy: a brief review. *Cardiovasc Toxicol* 1: 181–193, 2001.
11. Colantuoni A, Longoni B, and Marchiafava PL. Retinal photoreceptors of Syrian hamsters undergo oxidative stress during streptozotocin-induced diabetes. *Diabetologia* 45: 121–124, 2002.
12. Cregan SP, Dawson VL, and Slack RS. Role of AIF in caspase-dependent and caspase-independent cell death. *Oncogene* 26: 2785–2796, 2004.
13. De Giorgi F, Lartigue L, Bauer MK, Schubert A, Grimm S, Hanson GT, Remington SJ, Youle RJ, and Ichas F. The permeability transition pore signals apoptosis by directing Bax translocation and multimerization. *FASEB J* 16: 607–609, 2002.
14. Denis U, Lecomte M, Paget C, Ruggiero D, Wiernsperger N, and Lagarde M. Advanced glycation end-products induce apoptosis of bovine retinal pericytes in culture: involvement of diacylglycerol/ceramide production and oxidative stress induction. *Free Radic Biol Med* 33: 236–247, 2002.
15. Du Y, Smith MA, Miller CM, and Kern TS. Diabetes-induced nitrative stress in the retina, and correction by aminoguanidine. *J Neurochem* 80: 771–779, 2002.
16. Du Y, Miller CM, and Kern TS. Hyperglycemia increases mitochondrial superoxide in retina and retinal cells. *Free Radic Biol Med* 35: 1491–1499, 2003.
17. Duh E and Aiello LP. Vascular endothelial growth factor and diabetes: the agonist versus antagonist paradox. *Diabetes* 48: 1899–1906, 1999.
18. El-Remessy AB, Bartoli M, Platt DH, Fulton D, and Caldwell RB. Oxidative stress inactivates VEGF survival signaling in retinal endothelial cells via PI 3-kinase tyrosine nitration. *J Cell Sci* 118: 243–252, 2005.

19. Green DR and Amarante-Mendes GP. The point of no return: mitochondria, caspases, and the commitment to cell death. *Cell Differ* 24: 45–61, 1998.
20. Griscavage JM, Wilk S, and Ignarro LJ. Inhibitors of the proteasome pathway interfere with induction of nitric oxide synthase in macrophages by blocking activation of transcription factor NF-kappa B. *Proc Natl Acad Sci U S A* 93: 3308–3312, 1996.
21. Hammes HP. Pathophysiological mechanisms of diabetic angiopathy. *J Diabetes Complications* 17: 16–19, 2003.
22. Haskins K, Bradley B, Powers K, Fadok V, Flores S, Ling X, Pugazhenthi S, Reusch J, and Kench J. Oxidative stress in type 1 dabetes. *Ann N Y Acad Sci* 1005: 43–54, 2003.
23. Heath H, Rutter AC, and Beck TC. Changes in the ascorbic acid and glutathione content of the retinae and adrenals from alloxan-diabetic rats. *Vision Res* 2: 431–437, 1962.
24. Joussen AM, Poulaki V, Mitsiades N, Cai WY, Suzuma I, Pak J, Ju ST, Rook SL, Esser P, Mitsiades CS, Kirchhof B, Adamis AP, and Aiello LP. Suppression of Fas-FasL-induced endothelial cell apoptosis prevents diabetic blood–retinal barrier breakdown in a model of streptozotocin-induced diabetes. *FASEB J* 17: 76–78, 2003.
25. Joussen AM, Poulaki V, Le ML, Koizumi K, Esser C, Janicki H, Schraermeyer U, Kociok N, Fauser S, Kirchhof B, Kern TS, and Adamis AP. A central role for inflammation in the pathogenesis of diabetic retinopathy. *FASEB J* 18: 1450–1452, 2004.
26. Karpinich NO, Tafani M, Rothman RJ, Russo MA, and Farber JL. The course of etoposide-induced apoptosis from damage to DNA and p53 activation to mitochondrial release of cytochrome *c*. *J Biol Chem* 277: 16547–16562, 2002.
27. Kern TS, Tang J, Mizutani M, Kowluru R, Nagraj R, and Lorenzi M. Response of capillary cell death to aminoguanidine predicts the development of retinopathy: comparison of diabetes and galactosemia. *Invest Ophthalmol Vis Sci* 41: 3972–3978, 2000.
28. Kim J, Son JW, Lee JA, Oh YS, and Shinn SH. Methylglyoxal induces apoptosis mediated by reactive oxygen species in bovine retinal pericytes. *J Korean Med Sci* 19: 95–100, 2004.
29. Kowluru RA. Effect of reinstitution of good glycemic control on retinal oxidative stress and nitrative stress in diabetic rats. *Diabetes* 52: 818–823, 2003.
30. Kowluru RA and Abbas SN. Diabetes-induced mitochondrial dysfunction in the retina. *Invest Ophthalmol Vis Sci* 44: 5327–5334, 2003.
31. Kowluru RA and Koppolu P. Diabetes-induced activation of caspase-3 in retina: effect of antioxidant therapy. *Free Radic Res* 36: 993–999, 2002.
32. Kowluru RA and Odenbach S. Effect of long-term administration of alpha lipoic acid on retinal capillary cell death and the development of retinopathy in diabetic rats. *Diabetes* 53: 3233–3238, 2004.
33. Kowluru RA, Kern TS, and Engerman RL. Abnormalities of retinal metabolism in diabetes or experimental galactosemia. IV. Antioxidant defense system. *Free Radic Biol Med* 22: 587–592, 1996.
34. Kowluru RA, Jirousek MR, Stramm LE, Farid NA, Engerman RL, and Kern TS. Abnormalities of retinal metabolism in diabetes or experimental galactosemia. V. Relationship between protein kinase C and ATPases. *Diabetes* 47: 464–469, 1998.
35. Kowluru RA, Tang J, and Kern TS. Abnormalities of retinal metabolism in diabetes and experimental galactosemia. VII. Effect of long-term administration of antioxidants on the development of retinopathy. *Diabetes* 50: 1938–1942, 2001.
36. Kowluru RA, Koppolu P, Chakrabarti S, and Chen S. Diabetes-induced activation of nuclear transcriptional factor in the retina, and its inhibition by antioxidants. *Free Radic Res* 37: 1169–1180, 2003.
37. Kristal BS, Koopmans SJ, Jackson CT, Ikeno Y, Park BJ, and Yu BP. Oxidant-mediated repression of mitochondrial transcription in diabetic rats. *Free Radic Biol Med* 22: 813–822, 1997.
38. Kusner LL, Sarthy VP, and Mohr S. Nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase: a role in high glucose-induced apoptosis in retinal Muller cells. *Invest Ophthalmol Vis Sci* 45: 1553–1561, 2004.
39. Li W, Yanoff M, Jian B, and He Z. Altered mRNA levels of antioxidant enzymes in preapoptotic pericytes from human diabetic retinas. *Cell Mol Biol* 45: 59–66, 1999.
40. Lutty GA, McLeod DS, Merges C, Diggs A, and Plouet J. Localization of vascular endothelial growth factor in human retina and choroid. *Arch Ophthalmol* 114: 971–977, 1996.
41. Matsura T, Kai M, Fujii Y, Ito H, and Yamada K. Hydrogen peroxide-induced apoptosis in HL-60 cells requires caspase-3 activation. *Free Radic Res* 30: 73–83, 1999.
42. Mizutani M, Kern TS, and Lorenzi M. Accelerated death of retinal microvascular cells in human and experimental diabetic retinopathy. *J Clin Invest* 97: 2883–2890, 1996.
43. Mizutani M, Gerhardinger C, and Lorenzi M. Muller cell changes in human diabetic retinopathy. *Diabetes* 47: 455–459, 1998.
44. Naruse K, Nakamura J, Hamada Y, Nakayama M, Chaya S, Komori T, Kato K, Kasuya Y, Miwa K, and Hotta N. Aldose reductase inhibition prevents glucose-induced apoptosis in cultured bovine retinal microvascular pericytes. *Exp Eye Res* 71: 309–315, 2000.
45. Obrosova IG, Stevens MJ, and Lang HJ. Diabetes-induced changes in retinal NAD-redox status: pharmacological modulation and implications for pathogenesis of diabetic retinopathy. *Pharmacology* 62: 172–180, 2001.
46. Obrosova IG, Minchenko AG, Marinescu V, Fathallah L, Kennedy A, Stockert CM, Frank RN, and Stevens MJ. Antioxidants attenuate early up regulation of retinal vascular endothelial growth factor in streptozotocin-diabetic rats. *Diabetologia* 44: 1102–1110, 2001.
47. Obrosova IG, Minchenko AG, Frank RN, Seigel GM, Zsengeller Z, Pacher P, Stevens MJ, and Szabo C. Poly (ADP-ribose) polymerase inhibitors counteract diabetes- and hypoxia-induced retinal vascular endothelial growth factor overexpression. *Int J Mol Med* 14: 55–64, 2004.
48. Phaneuf S and Leeuwenburgh C. Cytochrome *c* release from mitochondria in the aging heart: a possible mecha-

nism for apoptosis with age. *Am J Physiol Regul Integr Comp Physiol* 282: R423–R430, 2002.
49. Podesta F, Romeo G, Liu WH, Krajewski S, Reed JC, Gerhardinger C, and Lorenzi M. Bax is increased in the retina of diabetic subjects and is associated with pericyte apoptosis in vivo and in vitro. *Am J Pathol* 156: 1025–1032, 2000.
50. Radi R, Cassina A, and Hodara R. Nitric oxide and peroxynitrite interactions with mitochondria. *Biol Chem* 383: 401–409, 2002.
51. Raff MC, Barres BA, Burne JF, Coles HS, Ishizaki Y, and Jacobson MD. Programmed cell death and the control of cell survival: lessons from the nervous system. *Science* 262: 695–700, 1993.
52. Robison WG, Jacot JL, Katz ML, and Glover JP. Retinal vascular changes induced by the oxidative stress of alpha-tocopherol deficiency contrasted with diabetic microangiopathy. *J Ocul Pharmacol Ther* 16: 109–120, 2000.
53. Romeo G, Liu WH, Asnaghi V, Kern TS, and Lorenzi M. Activation of nuclear factor-kappaB induced by diabetes and high glucose regulates a proapoptotic program in retinal pericytes. *Diabetes* 51: 2241–2248, 2002.
54. Russell JW, Golovoy D, Vincent AM, Mahendru P, Olzmann JA, Mentzer A, and Feldman EL. High glucose-induced oxidative stress and mitochondrial dysfunction in neurons. *FASEB* 16: 1738–1748, 2002.
55. Sharpe PC, Liu WH, Yue KK, McMaster D, Catherwood MA, McGinty AM, and Trimble ER. Glucose-induced oxidative stress in vascular contractile cells: comparison of aortic smooth muscle cells and retinal pericytes. *Diabetes* 47: 801–809, 1998.
56. Stitt AW. The role of advanced glycation in the pathogenesis of diabetic retinopathy. *Exp Mol Pathol* 75: 95–108, 2003.
57. Stitt A, Gardiner TA, Alderson NL, Canning P, Frizzell N, Duffy N, Boyle C, Januszewski AS, Chachich M, Baynes JW, Thorpe SR, and Anderson NL. The AGE inhibitor pyridoxamine inhibits development of retinopathy in experimental diabetes. *Diabetes* 51: 2826–2832, 2002.
58. Verzola D, Bertolotto MB, Villaggio B, Ottonello L, Dallegri F, Frumento G, Berruti V, Gandolfo MT, Garibotto G, and Deferran G. Taurine prevents apoptosis induced by high ambient glucose in human tubule renal cells. *J Investig Med* 50: 443–451, 2002.
59. Xia P, Inoguchi T, Kern TS, Engerman RL, Oates PJ, and King GL. Characterization of the mechanism for the chronic activation of DAG-PKC pathway in diabetes and hypergalactosemia. *Diabetes* 43: 1122–1129, 1994.

Address reprint requests to:
Renu A. Kowluru, Ph.D.
Kresge Eye Institute
Wayne State University
4717 St. Antoine
Detroit, MI 48201

E-mail: rkowluru@med.wayne.edu

Received after revision May 27, 2005; accepted June 27, 2005.

This article has been cited by:

1. D. Fu, M. Wu, J. Zhang, M. Du, S. Yang, S. M. Hammad, K. Wilson, J. Chen, T. J. Lyons. 2012. Mechanisms of modified LDL-induced pericyte loss and retinal injury in diabetic retinopathy. *Diabetologia* **55**:11, 3128-3140. [CrossRef]
2. Ghulam Mohammad, Mohammad Mairaj Siddiquei. 2012. Role of matrix metalloproteinase-2 and -9 in the development of diabetic retinopathy. *Journal of Ocular Biology, Diseases, and Informatics* . [CrossRef]
3. Junghyun Kim, Ki Mo Kim, Chan-Sik Kim, Eunjin Sohn, Yun Mi Lee, Kyuhyung Jo, Jin Sook Kim. 2012. Puerarin inhibits the retinal pericyte apoptosis induced by advanced glycation end products in vitro and in vivo by inhibiting NADPH oxidase-related oxidative stress. *Free Radical Biology and Medicine* . [CrossRef]
4. Yuzhen Yao, Rongrong Li, Yujie Ma, Xiaohui Wang, Chuanfu Li, Xiaojin Zhang, Rong Ma, Zhengnian Ding, Li Liu. 2012. #-Lipoic acid increases tolerance of cardiomyoblasts to glucose/glucose oxidase-induced injury via ROS-dependent ERK1/2 activation. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1823**:4, 920-929. [CrossRef]
5. Guangjun Jing, Joshua J. Wang, Sarah X. Zhang. 2012. ER Stress and Apoptosis: A New Mechanism for Retinal Cell Death. *Experimental Diabetes Research* **2012**, 1-11. [CrossRef]
6. M. K. Song, B. D. Roufogalis, T. H. W. Huang. 2012. Reversal of the Caspase-Dependent Apoptotic Cytotoxicity Pathway by Taurine from Lycium barbarum (Goji Berry) in Human Retinal Pigment Epithelial Cells: Potential Benefit in Diabetic Retinopathy. *Evidence-Based Complementary and Alternative Medicine* **2012**, 1-11. [CrossRef]
7. Meenal Pangare, Ayako Makino. 2012. Mitochondrial function in vascular endothelial cell in diabetes. *Journal of Smooth Muscle Research* **48**:1, 1-26. [CrossRef]
8. Renu A. Kowluru, Ghulam Mohammad, Julia M. Santos, Shikha Tewari, Qing Zhong. 2011. Interleukin-1# and mitochondria damage, and the development of diabetic retinopathy. *Journal of Ocular Biology, Diseases, and Informatics* . [CrossRef]
9. Sul Gee Lee, Chang Gun Lee, Il Han Yun, Dae Young Hur, Jae Wook Yang, Hyun Woong Kim. 2011. Effect of lipoic acid on expression of angiogenic factors in diabetic rat retina. *Clinical & Experimental Ophthalmology* no-no. [CrossRef]
10. Megha Barot, Mitan R. Gokulgandhi, Ashim K. Mitra. 2011. Mitochondrial Dysfunction in Retinal Diseases. *Current Eye Research* **36**:12, 1069-1077. [CrossRef]
11. Alex M. Laliberté, Thomas C. MacPherson, Taft Micks, Alex Yan, Kathleen A. Hill. 2011. Vision deficits precede structural losses in a mouse model of mitochondrial dysfunction and progressive retinal degeneration. *Experimental Eye Research* . [CrossRef]
12. Maria D Sanchez-Niño, Ana B Sanz, Elsa Sanchez-Lopez, Marta Ruiz-Ortega, Alberto Benito-Martin, Moin A Saleem, Peter W Mathieson, Sergio Mezzano, Jesus Egido, Alberto Ortiz. 2011. HSP27/HSPB1 as an adaptive podocyte antiapoptotic protein activated by high glucose and angiotensin II. *Laboratory Investigation* . [CrossRef]
13. Julia M. Santos, Shikha Tewari, Andrew F.X. Goldberg, Renu A. Kowluru. 2011. Mitochondrial biogenesis and the development of diabetic retinopathy. *Free Radical Biology and Medicine* . [CrossRef]
14. Min K Song, Basil D Roufogalis, Tom H.W Huang. 2011. Modulation of Diabetic Retinopathy Pathophysiology by Natural Medicines through PPAR-#-related Pharmacology. *British Journal of Pharmacology* no-no. [CrossRef]
15. Sul Gee Lee, Jung Lim Kim, Han Kee Lee, Gyu Won Ryu, Dae Young Hur, Il Han Yun, Jae Wook Yang, Hyun Woong Kim. 2011. Simvastatin suppresses expression of angiogenic factors in the retinas of rats with streptozotocin-induced diabetes. *Graefe's Archive for Clinical and Experimental Ophthalmology* **249**:3, 389-397. [CrossRef]
16. H. Kojima, A. Otani, A. Oishi, Y. Makiyama, S. Nakagawa, N. Yoshimura. 2011. Granulocyte colony-stimulating factor attenuates oxidative stress-induced apoptosis in vascular endothelial cells and exhibits functional and morphologic protective effect in oxygen-induced retinopathy. *Blood* **117**:3, 1091-1100. [CrossRef]
17. Chung-Jung Chiu, Allen Taylor. 2011. Dietary hyperglycemia, glycemic index and metabolic retinal diseases. *Progress in Retinal and Eye Research* **30**:1, 18-53. [CrossRef]
18. Frederick Pfister, Eva Riedl, Qian Wang, Franziska vom Hagen, Martina Deinzer, Martin C. Harmsen, Grietje Molema, Benito Yard, Yuxi Feng, Hans-Peter Hammes. 2011. Oral Carnosine Supplementation Prevents Vascular Damage in Experimental Diabetic Retinopathy. *Cellular Physiology and Biochemistry* **28**:1, 125-136. [CrossRef]
19. Seul Ki Lim, Min Jung Park, Jae Cheong Lim, Jong Chun Kim, Ho Jae Han, Gye-Yeop Kim, Benjamin F. Cravatt, Chang Hoon Woo, Seung Jin Ma, Kyung Cheol Yoon, Soo Hyun Park. 2011. Hyperglycemia induces apoptosis via CB1 activation through the decrease of FAAH 1 in retina pigment epithelial cells. *Journal of Cellular Physiology* n/a-n/a. [CrossRef]

20. Katja Krügel, Antje Wurm, Thomas Pannicke, Margrit Hollborn, Anett Karl, Peter Wiedemann, Andreas Reichenbach, Leon Kohen, Andreas Bringmann. 2011. Involvement of oxidative stress and mitochondrial dysfunction in the osmotic swelling of retinal glial cells from diabetic rats. *Experimental Eye Research* **92**:1, 87-93. [CrossRef]
21. Yu GAO, Jin-hui WU, Lin LIU. 2010. Streptozotocin-induced early diabetic retinopathy model in rats. *Academic Journal of Second Military Medical University* **30**:10, 1053-1059. [CrossRef]
22. A. R. Santiago, M. J. Garrido, A. J. Cristóvão, J. M. N. Duarte, R. A. Carvalho, A. F. Ambrósio. 2010. Evaluation of the Impact of Diabetes on Retinal Metabolites by NMR Spectroscopy. *Current Eye Research* **35**:11, 992-1001. [CrossRef]
23. Ghulam Mohammad, Renu A Kowluru. 2010. Matrix metalloproteinase-2 in the development of diabetic retinopathy and mitochondrial dysfunction. *Laboratory Investigation* **90**:9, 1365-1372. [CrossRef]
24. Qing Zhong, Renu A. Kowluru. 2010. Role of histone acetylation in the development of diabetic retinopathy and the metabolic memory phenomenon. *Journal of Cellular Biochemistry* **110**:6, 1306-1313. [CrossRef]
25. Kyle Trudeau, Anthony J.A. Molina, Wen Guo, Sayon Roy. 2010. High Glucose Disrupts Mitochondrial Morphology in Retinal Endothelial Cells. *The American Journal of Pathology* **177**:1, 447-455. [CrossRef]
26. Anh T. Tran, Deisy V. Bula, Kyle D. Kovacs, Judy Savageau, Jorge G. Arroyo. 2010. Apoptosis in Diabetic Fibrovascular Membranes After Panretinal Photocoagulation. *Ophthalmic Surgery, Lasers, and Imaging* . [CrossRef]
27. Faten Abdulhady Zakareia, Abdulmageed Abdulrahman Alderees, Khalid Abdualla Al Regaiy, Fawziah Abdallah Alrouq. 2010. Correlation of electroretinography b-wave absolute latency, plasma levels of human basic fibroblast growth factor, vascular endothelial growth factor, soluble fatty acid synthase, and adrenomedullin in diabetic retinopathy. *Journal of Diabetes and its Complications* **24**:3, 179-185. [CrossRef]
28. William I. Sivitz , Mark A. Yorek . 2010. Mitochondrial Dysfunction in Diabetes: From Molecular Mechanisms to Functional Significance and Therapeutic Opportunities. *Antioxidants & Redox Signaling* **12**:4, 537-577. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
29. Renu A. Kowluru, Pooi-See Chan. 2010. Metabolic memory in diabetes – from in vitro oddity to in vivo problem: Role of Apoptosis. *Brain Research Bulletin* **81**:2-3, 297-302. [CrossRef]
30. Masahide Fukuda, Yoriko Nakanishi, Masanori Fuse, Norihide Yokoi, Yasuhiro Hamada, Masafumi Fukagawa, Akira Negi, Makoto Nakamura. 2010. Altered expression of aquaporins 1 and 4 coincides with neurodegenerative events in retinas of spontaneously diabetic Torii rats. *Experimental Eye Research* **90**:1, 17-25. [CrossRef]
31. Ali. M. Sharifi, Habib Eslami, Bagher Larijani, Jamshid Davoodi. 2009. Involvement of caspase-8, -9, and -3 in high glucose-induced apoptosis in PC12 cells. *Neuroscience Letters* **459**:2, 47-51. [CrossRef]
32. Gillian Groeger, Ashley M. Mackey, Christopher A. Pettigrew, Lavinia Bhatt, Thomas G. Cotter. 2009. Stress-induced activation of Nox contributes to cell survival signalling via production of hydrogen peroxide. *Journal of Neurochemistry* **109**:5, 1544-1554. [CrossRef]
33. Ermelindo C. Leal, Célia A. Aveleira, Áurea F. Castilho, Andreia M. Serra, Filipa I. Baptista, Ken-Ichi Hosoya, John V. Forrester, António F. Ambrósio. 2009. High glucose and oxidative/nitrosative stress conditions induce apoptosis in retinal endothelial cells by a caspase-independent pathway. *Experimental Eye Research* **88**:5, 983-991. [CrossRef]
34. Z YUAN, W FENG, J HONG, Q ZHENG, J SHUAI, Y GE. 2009. p38MAPK and ERK promote nitric oxide production in cultured human retinal pigmented epithelial cells induced by high concentration glucose. *Nitric Oxide* **20**:1, 9-15. [CrossRef]
35. Sally A. Madsen-Bouterse, Renu A. Kowluru. 2008. Oxidative stress and diabetic retinopathy: Pathophysiological mechanisms and treatment perspectives. *Reviews in Endocrine and Metabolic Disorders* **9**:4, 315-327. [CrossRef]
36. Y GO, D JONES. 2008. Redox compartmentalization in eukaryotic cells. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1780**:11, 1273-1290. [CrossRef]
37. Nicholas A. Ramey, Choul Yong Park, Peter L. Gehlbach, Roy S. Chuck. 2007. Imaging Mitochondria in Living Corneal Endothelial Cells Using Autofluorescence Microscopy. *Photochemistry and Photobiology* **83**:6, 1325-1329. [CrossRef]
38. C Kaur, V Sivakumar, Z Yong, J Lu, WS Foulds, EA Ling. 2007. Blood–retinal barrier disruption and ultrastructural changes in the hypoxic retina in adult rats: the beneficial effect of melatonin administration. *The Journal of Pathology* **212**:4, 429-439. [CrossRef]
39. J KIM, J AHN, J KIM, Y YU, H KIM, J HA, S SHINN, Y OH. 2007. Fenofibrate regulates retinal endothelial cell survival through the AMPK signal transduction pathway. *Experimental Eye Research* **84**:5, 886-893. [CrossRef]
40. Nobuhiko Koike, Toshinari Takamura, Shuich Kaneko. 2007. Induction of reactive oxygen species from isolated rat glomeruli by protein kinase C activation and TNF-# stimulation, and effects of a phosphodiesterase inhibitor. *Life Sciences* **80**:18, 1721-1728. [CrossRef]

41. Pooi-See Chan, Renu A Kowluru. 2007. Role of retinal mitochondria in the development of diabetic retinopathy. *Expert Review of Ophthalmology* **2**:2, 237-247. [CrossRef]
42. Harrihar A Pershadsingh. 2007. #-Lipoic acid: physiologic mechanisms and indications for the treatment of metabolic syndrome. *Expert Opinion on Investigational Drugs* **16**:3, 291-302. [CrossRef]
43. Renu A. Kowluru, Pooi-See Chan. 2007. Oxidative Stress and Diabetic Retinopathy. *Experimental Diabetes Research* **2007**, 1-12. [CrossRef]
44. Sushil K. Jain. 2006. Superoxide dismutase overexpression and cellular oxidative damage in diabetes. *Free Radical Biology and Medicine* **41**:8, 1187-1190. [CrossRef]
45. Renu A. Kowluru, Vibhuti Kowluru, Ye Xiong, Ye-Shih Ho. 2006. Overexpression of mitochondrial superoxide dismutase in mice protects the retina from diabetes-induced oxidative stress. *Free Radical Biology and Medicine* **41**:8, 1191-1196. [CrossRef]
46. Ka Bian, Yan Ke, Yoshinori Kamisaki, Ferid Murad. 2006. Proteomic Modification by Nitric Oxide. *Journal of Pharmacological Sciences* **101**:4, 271-279. [CrossRef]
47. Martin J. Stevens . 2005. Redox-Based Mechanisms in Diabetes. *Antioxidants & Redox Signaling* **7**:11-12, 1483-1485. [Citation] [Full Text PDF] [Full Text PDF with Links]