Interleukin-1 β and mitochondria damage, and the development of diabetic retinopathy

Renu A. Kowluru · Ghulam Mohammad ·
Julia M. Santos · Shikha Tewari · Qing Zhong

Received: 6 October 2011 / Accepted: 5 December 2011 / Published online: 28 December 2011
© Springer Science+Business Media, LLC 2011

Abstract Mitochondrial dysfunction is considered to play an important role in the development of diabetic retinopathy. Recent evidence has also shown many similarities between diabetic retinopathy and a low grade chronic inflammatory disease. The aim of this study is to understand the interrelationship between proinflammatory mediator, IL-1 β and mitochondrial dysfunction in the accelerated loss of capillary cells in the retina. Using IL-1 β receptor gene knockout (IL-1R1 $^{-/-}$) diabetic mice, we have investigated the effect of regulation of IL-1 β on mitochondrial dysfunction and mtDNA damage, and increased retinal capillary cell apoptosis and the development of retinopathy. Retinal mitochondrial dysfunction and mtDNA damage were significantly ameliorated in IL-1R1 $^{-/-}$ mice, diabetic for ~10 months, compared to the wild-type diabetic mice. This was accompanied by protection of accelerated capillary cell apoptosis and the development of acellular capillaries, histopathology associated with diabetic retinopathy. Thus, mitochondrial damage could be one of the key events via which increased inflammation contributes to the activation of the apoptotic machinery resulting in the development of diabetic retinopathy, and the possible mechanism via which inflammation contributes to the development of diabetic retinopathy includes continuous fueling of the vicious cycle of mitochondrial damage, which could be disrupted by inhibitors of inflammatory mediators.

Keywords Diabetic retinopathy · Interleukin-1 β Mitochondria

Introduction

Diabetic retinopathy, a chronic microvascular complication of diabetes, remains the leading cause of acquired blindness in working adults. In the pathogenesis of this multifactorial disease many molecular and physiologic factors have been implicated; however, the exact mechanism of the development of diabetic retinopathy remains elusive [1, 2]. Recent research has shown that the retina in diabetes shows abnormalities consistent with other inflammatory diseases. The levels of proinflammatory cytokines including interleukin-1 β (IL-1 β), tumor necrosis factor are increased in the vitreous of the patients with proliferative diabetic retinopathy and also in the retina from diabetic rodents [3–7]. In experimental models of diabetic retinopathy, anti-inflammatory therapies have prevented its development, and suppression of inflammation and regulation of tight junction proteins by glucocorticoids have been considered as possible means to treat/prevent this blinding disease [8–10].

Retina and its capillary cells experience increased oxidative stress in diabetes, and the mitochondria become dysfunctional and their DNA is damaged. Cytochrome c leaks out of the mitochondria, accelerating the apoptosis of retinal capillary cells, and ultimately, resulting in the development of diabetic retinopathy [11–13]. Our previous work has shown that IL-1 β , via increase in oxidative stress and activation of NF- κ B, increases apoptosis of retinal capillary cells, and antioxidants, which inhibit the development of diabetic retinopathy in rodent models, also inhibit diabetes-induced increases in retinal IL-1 β [4, 14, 15]. How dysfunctional retinal mitochondria in diabetes increases IL-1 β remains unclear.

IL-1 β exerts its effects by binding to the specific receptors, and there are two main homologous receptors: type I IL-1 receptor (IL-1RI) and type II IL-1 receptor (IL-1RII).

R. A. Kowluru (✉) · G. Mohammad · J. M. Santos · S. Tewari · Q. Zhong
Kresge Eye Institute, Wayne State University,
4717 St. Antoine,
Detroit, MI, USA
e-mail: rkowluru@med.wayne.edu

Among these two, IL-1RI is the only receptor capable of mediating signaling, and IL-1RII acts as a ‘decoy’ receptor. Production and activity of IL-1 β are tightly regulated by IL-1Ra, an endogenous IL-1 β inhibitor which preferentially binds to IL-1RI without initiating signal transduction [16]. Mohr et al., using IL-1 β receptor knockout (IL-1R1 $^{-/-}$) mice has shown that these mice do not present retinal pathology associated with diabetic retinopathy and have implicated the role of caspase-1/IL-1 β signaling pathway in the development of diabetic retinopathy [17].

The purpose of this study is to determine the interrelationship between increased IL-1 β and mitochondrial dysfunction in the accelerated apoptosis of retinal capillary cells, resulting in the development of diabetic retinopathy. Here, we have investigated the effect of diabetes on mitochondria permeability, mtDNA damage and on the apoptosis and the development of acellular capillaries in the retina from IL-1R1 $^{-/-}$ mice diabetic for over 10 months.

Methods

Mice

Wild-type (WT; C57BL/6 J) or IL-1R1 $^{-/-}$ mice (B6.129S7-*Il1r1*^{tm1Imx}/J) were obtained from Jackson Laboratory (Bar Harbor, ME). A group of WT and IL-1R1 $^{-/-}$ mice were made diabetic by five consecutive injections of streptozotocin (55 mg/kg BW; WT-D and IL-D, respectively). These mice were maintained in diabetic conditions for ~10 months, and age-matched nondiabetic mice served as controls (WT-N and IL-N). Blood glucose levels were four to five times higher in diabetic mice compared to their age-matched nondiabetic mice, but the severity of hyperglycemia was comparable in both WT and IL-1R1 $^{-/-}$ diabetic mice as evidenced by similar average blood glucose values in these two groups (WT-D=395 $\pm$ 81 mg/dl and IL-D=409 $\pm$ 103 mg/dl). Mice were sacrificed by carbon dioxide asphyxiation. The retina from one eye was used to quantify biochemical and molecular parameters, and the other eye was incubated immediately in 10% buffered formalin for apoptosis/histopathology [18–20]. The treatment of the animals conformed to the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research, and the Institutional Guidelines.

Mitochondria isolation

Retinal mitochondria were isolated using mitochondria isolation kit (Pierce, Rockford, IL), as routinely performed in our laboratory [19, 20]. The mitochondria pellet, after washing with PBS, was suspended in the mitochondria lysis buffer (2% CHAPS in 25 mM Tris, 0.15 M NaCl, pH 7.2), and protein

concentration was quantified by the bicinchoninic acid assay (Sigma-Aldrich, St. Louis, MO).

Mitochondrial superoxide

Superoxide levels in the retina were quantified by incubating the freshly prepared retinal homogenate with 400 nM MitoTracker Red CM-H₂XROS (Molecular Probes, Eugene, OR) for 30 min at 37°C. The fluorescence was measured at 599 nm emission wavelength and 579 nm excitation wavelength using LS 55 Fluorescence Spectrometer (Perkin-Elmer, Waltham, MA) [20].

Mitochondria damage

Mitochondria permeability was determined spectrophotometrically by quantifying calcium chloride-induced decrease in absorbance at 540 nm [19]. In addition, the release of cytochrome c from the mitochondria into the cytosol was quantified by western blot technique [13]. Damage of mtDNA was quantified by quantitative extended length PCR using mitochondrial genome-specific primers (Table 1), and to calculate relative amplification, the intensity of the long product (13.4 kb) was normalized with the short product (210 bp), as routinely used in our laboratory [11, 12].

Quantification of IL-1 β

The amount of IL-1 β in the retina was quantified using ELISA kit (R&D Systems, MN) according to the manufacturer's

Table 1 List of primers

Target	Sequence
VEGF	
Forward	5'-TGGATGTCTACCAGCGAAGC-3'
Reverse	5'-ACAAGGCTCACAGTGATTTT-3'
ICAM-1	
Forward	5'-AGCACCTCCCCACCTACTTT-3'
Reverse	5'-AGCTTGACGACCCCTTCTAA-3'
β -actin	
Forward	5'-CCTCTATGCCAACACAGTGC-3'
Reverse	5'-CATCGTACTCCTGCTTGCTG-3'
Cytochrome b	
Forward	5'-ACCCGCCCATCCAACATCTCAT-3'
Reverse	5'-TTGAGGCTCCGTTTGCGTGT-3'
mtDNA-long	
Forward	5'-AAAATCCCCGCAAACAATGACCACCCC-3'
Reverse	5'-GGCAATTAAGAGTGGGATGGAGCCAA-3'
mtDNA-short	
Forward	5'-CCTCCCATTTCATTATCGCCGCCCTTGC-3'
Reverse	5'-GTCTGGGTCTCCTAGTAGGTCTGGGAA-3'

instructions, as previously reported by us [15]. Each sample was run in duplicate to ensure reproducibility of the data, and the sensitivity of the assay was ~ 3 pg/ml of IL-1 β .

Gene expression

Total RNA was extracted from the retina with Trizol reagent (Invitrogen), and cDNA was synthesized by High Capacity cDNA Reverse Transcription Kit (Applied Biosystem). Gene transcripts of VEGF and ICAM-1 were quantified by real time RT-PCR using the Sybr Green assay and gene-specific primers. Internal standard used was β -actin (Table 1). Gene expression of cytochrome b was quantified by semiquantitative conventional PCR. Change in mRNA abundance was calculated using the ddCt method as routinely used by us [20–22].

Retinal capillary cell apoptosis and histopathology

Retina was isolated from the formalin-fixed eyes and incubated with 3% crude trypsin in Tris–HCl buffer (pH 7.8) containing 0.2 M sodium fluoride for 90 min [15, 18, 20]. Trypsin-digested retinal microvessels were evaluated for terminal transferase dUTP nick end labeling (TUNEL) using a commercially available kit (In Situ Cell Death Kit, Roche Molecular Biochemicals, IN). Positive controls consisting of

retinal vessel exposed to DNase before initiation of the TUNEL reaction were run simultaneously. After counting the number of TUNEL-positive capillary cells, the slides were stained with periodic acid-Schiff and hematoxylin for histologic evaluation, and the number of acellular capillaries was counted in multiple midretinal fields and expressed per square millimeter of retinal area examined [18, 20, 23].

Statistical analysis

Data are reported as mean $\pm$ SD and analyzed with nonparametric statistics. Experimental groups were compared using Kruskal–Wallis test followed by Mann–Whitney test for multiple group comparison. Analyses yielded identical results when performed using ANOVA with Fisher or Tukey.

Results

Regulation of IL-1 β activation prevents diabetes-induced damage to the retinal mitochondria

Consistent with our previous results [20, 24], diabetes in WT mice increased mitochondrial superoxide levels in the retina by about two fold, and mitochondria permeability by 60–70% compared to the values obtained from WT-normal mice

Fig. 1 Retinal mitochondria damage is protected in IL-1R1 $^{-/-}$ diabetic mice: mitochondrial fraction was analyzed for **a** superoxide levels by employing fluorimetric method using MitoTracker Red CM-H₂XROS, **b** membrane permeability by quantifying calcium chloride-induced decrease in absorbance at 540 nm and **c** cytochrome c leakage into the cytosol by western blot technique using β -actin as loading control. Each measurement was made in duplicate in five to seven mice in each group, and the values are represented as mean $\pm$ SD. WT and WT-D= wild-type normal and diabetic mice, respectively, and IL-N and IL-D=IL-1R1 $^{-/-}$ normal and diabetic mice, respectively. Asterisk indicates $p<0.05$ compared to WT-N

(Fig. 1a and b). The amount of cytochrome c into the cytosol fraction was increased by over 50% (Fig. 1c). In the same animals, mtDNA was damaged, as evidenced by 60% decrease in the relative amplification of 13.4 kb and 210 bp products (Fig. 2a). This was accompanied by 30% decrease in the gene transcripts of mtDNA-encoded cytochrome b (Fig. 2b).

However, in IL-1R1^{-/-} diabetic mice, mitochondria were relatively healthy with significantly lower levels of mitochondrial superoxide, membrane that were less permeable with decreased leakage of cytochrome c into the cytosol and decreased damage to mtDNA compared to the values obtained from WT diabetic mice (Figs. 1 and 2). Retina from WT normal and IL-1R1^{-/-} normal and diabetic groups

had similar values for mitochondrial function, DNA damage and the expression of cytochrome b.

Mice deficient in IL-1 receptor gene are protected from diabetes-induced increase in the inflammatory cytokines

The concentration of retinal IL-1 β was significantly increased in WT-diabetes compared to WT normal. However, IL-1 β levels decreased by 30–40% in the retina from IL-1R1^{-/-} mice, and the values in IL-1R1^{-/-} normal and IL-1R1^{-/-} diabetic mice were similar to each other (Fig. 3a). Similarly, increase in retinal VEGF and ICAM, observed in WT-diabetic mice, was also prevented in IL-1R1^{-/-} diabetic mice (Fig. 3b and c), suggesting that these mice were also protected from diabetes-induced increase in proinflammatory mediators.

Capillary cell apoptosis is ameliorated in IL-1R1^{-/-} diabetic mice

Diabetes of ~10 months in WT mice significantly increased the apoptosis of capillary cells, and the number of TUNEL-positive cells in the trypsin digested retinal microvasculature were approximately twofold higher compared to those from WT-N mice (Fig. 4a). Consistent with this, the number of acellular capillaries was also significantly increased (Fig. 4b).

Regulation of IL-1 receptor gene protected the retinal microvasculature from diabetes-induced accelerated apoptosis, and this was accompanied by reduced number of acellular capillaries in IL-1R1^{-/-} diabetic mice compared to those in WT diabetic mice. The numbers of apoptotic capillary cells and acellular capillaries in IL-1R1^{-/-} diabetic mice were not different from those obtained from IL-1R1^{-/-} normal and WT normal mice (Fig. 4).

Discussion

In the development of diabetic retinopathy, inflammatory mediators are elevated in the retina, mitochondria become dysfunctional, the enzyme important in scavenging superoxide is decreased and mtDNA becomes damaged [6, 11–13, 15]. The present study demonstrates that amelioration of inflammatory mediators regulates mitochondrial damage and capillary cell apoptosis that the retina experiences in diabetes. Our data, using a mouse model of diabetic retinopathy with genetic manipulation for interleukin 1 receptor, show that retinal mitochondria of these mice are protected from diabetes-induced mitochondrial dysfunction and DNA damage. Furthermore, the retinal vasculature also escapes accelerated apoptosis, a phenomenon considered to precede the appearance of histopathology characteristic of diabetic retinopathy [25]. These results strongly suggest that

Fig. 2 Damage to the retinal mtDNA is ameliorated in mice with IL-1 receptor gene manipulated. **a** Damage to mtDNA was estimated by quantitative extended length PCR using GeneAmp XL PCR kit. **b** Gene expression of mtDNA-encoded Cytb was quantified in the retina by semiquantitative PCR using gene-specific primers. Measurements were made in duplicate in six or more mice in each group, and the values are represented as mean $\pm$ SD. Asterisk indicates $p < 0.05$ compared to WT-N

Fig. 3 Regulation of IL-1 receptor gene protects increase in the inflammatory cytokines in the retina: gene transcripts of **a** IL-1 β , **c** ICAM-1 and **d** VEGF were quantified in the retina by real time RT-PCR using the Sybr Green assay and gene-specific primers. **b** The amount of IL-1 β in the retina was quantified by an ELISA method. Each measurement was made in duplicate using retina from five to six mice in each of the four groups, and the values are represented as mean $\pm$ SD. Asterisk indicates $p<0.05$ compared to WT-N

Fig. 4 The number of apoptotic capillary cells in the retinal vasculature is significantly lower in diabetic mice with the IL-1 receptor gene manipulated. **a** Trypsin-digested retinal microvasculature was analyzed for capillary cell apoptosis by TUNEL staining. The arrow indicates TUNEL-positive capillary cell. **b** After TUNEL staining, the microvessels were stained with periodic acid-Schiff-hematoxylin and examined by light microscopy for the quantifying acellular capillaries–basement

membrane tubes lacking cell nuclei and maintaining at least one-fourth the normal capillary caliber over their length. The arrow indicates acellular capillary. Results are expressed as mean $\pm$ SD of at least six mice in each group. WT-N and WT-D=wild-type nondiabetic and diabetic mice, respectively, and IL-N and IL-D=IL-1R1 $^{-/-}$ are normal and diabetic mice, respectively. Asterisk indicates $p<0.05$ compared to WT-N and number sign indicates $p<0.05$ compared to WT-D

in diabetic environment, both inflammation and mitochondrial damage are interrelated.

Hyperglycemia elevates IL-1 β in the retina and its capillary cells; our previous work has shown that IL-1 β administration into the vitreous of normal rats increases oxidative stress and activates redox-sensitive nuclear transcriptional factor- κ B (NF- κ B), and antioxidants inhibit diabetes-induced increases in retinal IL-1 β [4, 15]. Furthermore, lipopolysaccharide-induced inflammatory response is shown to increase mitochondrial dysfunction in neuronal cells [26]. Here, our results demonstrate that the amelioration of IL-1 β activation prevents mitochondrial dysfunction and DNA damage, and further confirm a bidirectional mechanism between increased inflammatory cytokines and oxidative stress.

Diabetes damages mtDNA in the retina and its capillary cells, and mitochondrial genome-encoded electron transport chain proteins are compromised. The damage to mtDNA initiates a vicious cycle, and the transcription of protein encoded by mtDNA is decreased [11, 12, 22]. Protection of the damage to mtDNA and decrease in cytochrome b (an enzyme essential for the formation and activity of complex III) by ameliorating IL-1 β activation implies that the combination of increased inflammatory cytokine and mtDNA damage in diabetes further fuels the vicious cycle resulting in continued damage to the mitochondria, and decreased flux through the subnormal electron transport chain continues to supply additional superoxide.

Apoptosis of retinal capillary is considered as a predictor of histopathology associated with diabetic retinopathy [25]. Here, we show that IL-1R1 $^{-/-}$ mice, maintained diabetic for long durations, are protected from accelerated apoptosis of capillary cells implying that IL-1 β has an important role in the apoptosis. In support, our previous studies have shown that glucose-induced apoptosis of retinal endothelial cells is prevented by incubating the cells with IL-1 β antibody or IL-1Ra [4], and administration of IL-1 β in the vitreous of normal rats accelerates capillary cell apoptosis and generates acellular capillaries [15]. IL-1 β is reported to induce apoptosis of retinal neuronal cells during reperfusion ischemia [27], a phenomenon that is observed in the development of diabetic retinopathy as well [28]. Furthermore, glucose-induced apoptosis of retinal Müller cells is postulated to depend on the increased autocrine production of IL-1 β , and via a paracrine mechanism, in later stages of diabetes, this results in capillary cell death [29]. To make the bad situation worse, damaged mitochondria by releasing cytochrome c and impairing mitochondrial membranes provoke apoptosis [13]. Now, we show that the regulation of IL-1 β signalling protects retinal mitochondria from damage to their membrane and the release of cytochrome c into the cytosol is decreased, raising a strong possibility that the protection of diabetes-induced retinal capillary cell apoptosis (and the histopathology) in IL-1R1 $^{-/-}$ diabetic mice

could be, in part, due to the protection of the integrity of the mitochondria. In support, recent reports have shown that the mitochondria have a central role in the activation of inflammasomes, a crucial assembly platform which activates caspase-1, which in turn, processes IL-1 β and other inflammatory pathways [30, 31], and accumulation of damaged mitochondria is considered to contribute to increased inflammation via initiation of inflammasomes [32]. Thus, the possibility that the protection of the accelerated capillary cell apoptosis and mitochondrial damage in IL-1R1 $^{-/-}$ mice is via regulation of inflammasomes remains to be explored.

In summary, this study, for the first time, demonstrates that the mitochondrial damage is one of the key events via which increased inflammation could contribute to the activation of the apoptotic machinery resulting in the development of diabetic retinopathy. Thus, inhibition of inflammatory mediators have potential therapeutic value in preventing the fueling of the vicious cycle of mitochondrial damage, ultimately inhibiting the development and continued progression of diabetic retinopathy.

Acknowledgments The authors thank Yakov Shamailov and Dug Putt for their help in maintaining the mouse colony. This work was supported, in part, by grants from the National Institutes of Health, Juvenile Diabetes Research Foundation, The Thomas Foundation and Research to Prevent Blindness.

References

1. Kowluru RA. Diabetic retinopathy: mitochondrial dysfunction and retinal capillary cell death. *Antioxid Redox Signal*. 2005;7:1581–7.
2. Frank RN. Diabetic retinopathy. *N Engl J Med*. 2004;350:48–58.
3. Yuuki T, Kanda T, Kimura Y, Kotajima N, Tamura J, Kobayashi I, et al. Inflammatory cytokines in vitreous fluid and serum of patients with diabetic vitreoretinopathy. *J Diabetes Complications*. 2001;15:257–9.
4. Kowluru RA, Odenbach S. Role of interleukin-1beta in the pathogenesis of diabetic retinopathy. *British J Ophthalmol*. 2004;88:1343–7.
5. Demircan N, Safran BG, Soyulu M, Ozcan AA, Sizmaz S. Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. *Eye*. 2006;20:1366–9.
6. Krady JK, Basu A, Allen CM, Xu Y, LaNoue KF, Gardner TW, et al. Minocycline reduces proinflammatory cytokine expression, microglial activation, and caspase-3 activation in a rodent model of diabetic retinopathy. *Diabetes*. 2005;54:1559–65.
7. Kern TS. Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. *Exp Diabetes Res*. 2007;2007:95103.
8. Felinski EA, Antonetti DA. Glucocorticoid regulation of endothelial cell tight junction gene expression: novel treatments for diabetic retinopathy. *Curr Eye Res*. 2005;30:949–57.
9. Kowluru RA, Kanwar M. Effect of curcumin on retinal oxidative stress and inflammation in diabetes. *Nutr Metab (Lond)*. 2007;4:1–8.

10. Tang J, Kern TS. Inflammation in diabetic retinopathy. *Prog Retin Eye Res.* 2011;30:343–58.
11. Madsen-Bouterse SA, Mohammad G, Kanwar M, Kowluru RA. Role of mitochondrial DNA damage in the development of diabetic retinopathy, and the metabolic memory phenomenon associated with its progression. *Antioxid Redox Signal.* 2010;13:797–805.
12. Madsen-Bouterse S, Zhong Q, Mohammad G, Ho YS, Kowluru RA. Oxidative damage of mitochondrial DNA in diabetes, and its protection by manganese superoxide dismutase. *Free Rad Res.* 2010;44:313–21.
13. Kowluru RA, Abbas SN. Diabetes-induced mitochondrial dysfunction in the retina. *Inves Ophthalmol Vis Sci.* 2003;44:5327–34.
14. Kowluru RA, Tang J, 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.* 2001;50:1938–42.
15. Kowluru RA, Odenbach S. Role of interleukin-1beta in the development of retinopathy in rats: effect of antioxidants. *Invest Ophthalmol Vis Sci.* 2004;45:4161–6.
16. Hallegua DS, Weisman MH. Potential therapeutic uses of interleukin 1 receptor antagonists in human diseases. *Ann Rheum Dis.* 2002;61:960–7.
17. Vincent JA, Mohr S. Inhibition of caspase-1/interleukin-1beta signaling prevents degeneration of retinal capillaries in diabetes and galactosemia. *Diabetes.* 2007;56:224–30.
18. Kowluru RA, Kowluru V, Ho YS, Xiong Y. Overexpression of mitochondrial superoxide dismutase in mice protects the retina from diabetes-induced oxidative stress. *Free Rad Biol Med.* 2006;41:1191–6.
19. Mohammad G, Kowluru RA. Matrix metalloproteinase-2 in the development of diabetic retinopathy and mitochondrial dysfunction. *Lab Invest.* 2010;90:1365–72.
20. Kowluru RA, Mohammad G, Santos JM, Zhong Q. Abrogation of MMP9 gene protects against the development of retinopathy in diabetic mice by preventing mitochondrial damage. *Diabetes* 2011;60:3023–33.
21. Zhong Q, Kowluru RA. Epigenetic changes in mitochondrial superoxide dismutase in the retina and the development of diabetic retinopathy. *Diabetes.* 2011;60:1304–13.
22. Santos JM, Tewari S, Goldberg AFX, Kowluru RA. Mitochondria biogenesis and the development of diabetic retinopathy. *Free Rad Biol Med* 2011;51:1849–60.
23. Mohammad G, Kowluru RA. Novel role of mitochondrial matrix metalloproteinase-2 in the development of diabetic retinopathy. *Invest Ophthalmol Vis Sci.* 2011;52:3832–41.
24. Kanwar M, Chan PS, Kern TS, Kowluru RA. Oxidative damage in the retinal mitochondria of diabetic mice: possible protection by superoxide dismutase. *Invest Ophthalmol Vis Sci.* 2007;48:3805–11.
25. Kern TS, Tang J, Mizutani M, Kowluru R, Nagraj R, Lorenzi M. Response of capillary cell death to aminoguanidine predicts the development of retinopathy: comparison of diabetes and galactosemia. *Invest Ophthalmol Vis Sci.* 2000;41:3972–8.
26. Hunter RL, Dragicevic N, Seifert K, Choi DY, Liu M, Kim HC, et al. Inflammation induces mitochondrial dysfunction and dopaminergic neurodegeneration in the nigrostriatal system. *J Neurochem.* 2007;100:1375–86.
27. Yoneda S, Tanihara H, Kido N, Honda Y, Goto W, Hara H, et al. Interleukin-1beta mediates ischemic injury in the rat retina. *Exp Eye Res.* 2001;73:661–7.
28. Zheng L, Gong B, Hatala DA, Kern TS. Retinal ischemia and reperfusion causes capillary degeneration: similarities to diabetes. *Invest Ophthalmol Vis Sci.* 2007;48:361–7.
29. Busik JV, Mohr S, Grant MB. Hyperglycemia-induced reactive oxygen species toxicity to endothelial cells is dependent on paracrine mediators. *Diabetes.* 2008;57:1952–65.
30. Tschopp J. Mitochondria: sovereign of inflammation? *Eur J Immunol.* 2011;41:1196–202.
31. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature.* 2011;469:221–5.
32. Green DR, Galluzzi L, Kroemer G. Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science.* 2011;333:1109–12.