Mechanisms involved in the development of diabetic retinopathy induced by oxidative stress

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Background: Diabetic retinopathy (DR) is one of the main complications in patients with diabetes and has been the leading cause of visual loss since 1990. Oxidative stress is a biological process resulting from excessive production of reactive oxygen species (ROS). This process contributes to the development of many diseases and disease complications. ROS interact with various cellular components to induce cell injury. Fortunately, there is an antioxidan t system that protects organisms against ROS. Indeed, when ROS exceed antioxidant capacity, the resulting cell injury can cause diverse physiological and pathological changes that could lead to a disease like DR.
Objective: This paper reviews the possible mechanisms of common and novel biomarkers involved in the development of DR and explores how these biomarkers could be used to monitor the damage induced by oxidative stress in DR, which is a significant complication in people with diabetes.
Conclusion: The poor control of glucemy in pacients with DB has been shown contribute to the development of complications in eyes as DR.

Keywords: Biomarkers, Diabetic retinopathy, Oxidative stress, Retinal damage

Diabetic retinopathy

Diabetes mellitus (DM) is a complex metabolic disease that will affect a projected 380 million patients by the year 2025. Tragically, this disease will lead to blindness in approximately 4 million people around the world due to the development of diabetic retinopathy (DR).[1] In 1998, the prevalence and severity of DR was greater in non-Hispanic black and Mexican-American populations than in non-Hispanic whites with type 2 diabetes in the USA.[2] In 2008, the prevalence of both DR and vision-threatening DR in adults equal to or greater than 40 years old, especially among non-Hispanic black individuals, was high.[3]

Diabetic patients tend to have increased levels of reactive oxygen metabolites (ROM) in serum when compared with healthy subjects. A study carried out by Naruse *et al*.[4] demonstrated that ROM increased rapidly in serum as DR progressed. This increase was notoriously high during DR progression in patients with type 2 diabetes whose levels of lipoperoxide, catalase, glutathione peroxidase, and nitric oxide (NO) catabolites increased significantly in erythrocyte.[5]

Signs of retinal vascular activation and injury induced by diabetes and elevated glucose are associated with increased arginase activity and decreased levels of bioavailable NO,[6] which suggests that overactive arginase contributes to DR by reducing NO and increasing oxidative stress the activity of arginase, which competes NO synthase for the common substrate L-arginine. Inducible nitric oxide synthase is one of the three NOS isoforms that generate NO by conversion of L-arginine to L-citrulline.[7] NO generated from S-nitrosoglutation acts as a second messenger, which has been shown to control important cellular processes through S-nitrosylation by regulating of the expression or activity of certain proteins. Moreover, NO is necessary for physiological functions such as host defense, neurotransmission, and vasodilation, whereas the generation of reactive nitrogen species is implicated in the pathophysiology of degenerative diseases.

Mechanisms involved in DR

Diabetes results in an increased flux through the hexosamine biosynthetic pathway that increases

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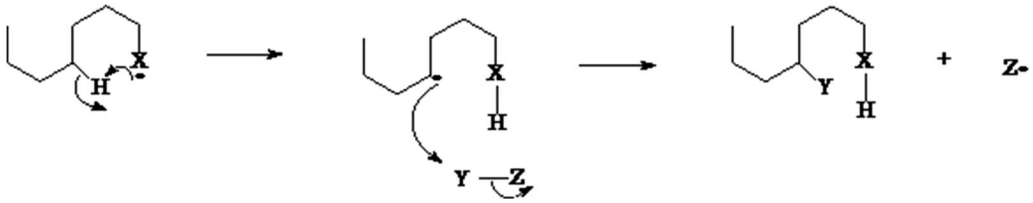

Figure 1 Pathway of FR generation through the addition of an electron to a stable molecule. The movement of electrons is accomplished by hydrogen attack on carbon atoms that substitute a released substrate.

posttranslational modifications of Ser/Thr residues on proteins by O-linked β-*N*-acetylglucosamine. *O*-GlcNacylation is involved in the regulation of many nuclear and cytoplasmic proteins in a manner similar to protein phosphorylation.[8] Diabetes is accompanied by an increased risk of developing chronic macro- and microvascular complications including DR.[9]

DR is an eye disorder affecting the human retina and is believed to be caused by an elevated amount of insulin in the blood[10] Intensive insulin therapy has been found to delay the progression of this clinical disorder, and there is level 1 evidence for intensive glycemic control to reduce the progression of DR in persons with type 2 diabetes.

DR is a complex condition; inflammation and oxidative stress are involved in crucial pathways that affect the pathogenesis of DR.[11] DR is characterized by pericyte and neuronal cell loss, formation of acellular-occluded capillaries, micro aneurysms, increased leukostasis, and thickening of the vascular basement membrane. Pericyte death is initiated when hyperglycemia persistently activates protein kinase C-δ (PKC-δ, encoded by Prkcd) and the p38 mitogen-activated protein kinase as the expression of a previously unknown target of PKC-δ signaling increases. This signaling cascade leads to PDGF receptor dephosphorylation and a reduction in downstream signaling by this receptor, resulting in pericyte apoptosis.[12] Thioredoxin interacting protein represents a novel gene and drug target to prevent pericyte loss and the progression of DR.[13] These alterations progressively affect the integrity of retinal microvessels, leading to the breakdown of the blood–retinal barrier and widespread hemorrhage and neovascularization, which appear on the surface of the retina along with microaneurysms, hemorrhages, and exudates.[14] Growing evidence suggests that local inflammation and oxidative stress are critical factors in the pathogenesis of DR.[15] Proinflammatory cytokines (TNF-alpha and IL-1), secretory phospholipase A2 IIA, and lipoprotein-PLA2 are implicated in vascular inflammation,[16] suggest that understanding cytokine-induced changes in lipid metabolism will promote the development of novel concepts and steer bench-to-bedside therapeutic developments.

Screening and gene expression

England is a world leader in DR screening, having offered 85.7% of eligible diabetic patients a screening program. The British Diabetic Association (Diabetes UK) has established standard values for any DR screening program of at least 80% sensitivity and 95% specificity. Papavasileiou *et al.*[17] suggested that a well-implemented program would provide timely treatment, reduce the need for vitrectomy and blind registration, and serve as a benchmark in planning the delivery of services in a similar population.

People with Down's syndrome, who have three copies of chromosome 21, almost never acquire DR. This protection appears to be due to the elevated levels in tumor cells of endostatin,[18] an anti-angiogenic protein, derived from collagen XVIII. Its gene is located on chromosome 21.

Uncoupling protein 1 (UCP1) reduces mitochondrial production of reactive oxygen species (ROS), and deleterious polymorphisms in the UCP1 gene are candidate risk factors for the development of DR. This protein is expressed in the retinas of people with type 1 DM. Brondani *et al.*[19] suggest that the 3826A/G polymorphism influences UCP1 expression and reduces mitochondrial production of ROS.

On the other hand, Payne *et al.*[20] suggested that the molecular pathways contributing to signal transduction in the retina exhibit a high energy demand with functional and structural consequences. Subsequent vascularization and elevated metabolic rates contribute to oxidative stress and influence age-related processes that increase oxidative load, resulting in chronically elevated levels of oxidative stress and ROS.

Ocular tissues are prone to damage from ROS due to constant exposure of the eye to sunlight, atmospheric oxygen, and environmental chemicals. Furthermore, free radical (FR)-catalyzed peroxidation of long-chain polyunsaturated acids (LCPUFAs) such as arachidonic acid and docosahexaenoic acid leads to generation of LCPUFA metabolites including isoprostanes and neuroprostanes that may further exert pharmacological and toxicological actions in ocular tissues.[21]

Reactive radical species

An FR can be generated through different pathways, but the most frequent mechanism in living organisms is the addition of an electron to a stable molecule (Fig. 1). Most of the molecules of an organism are

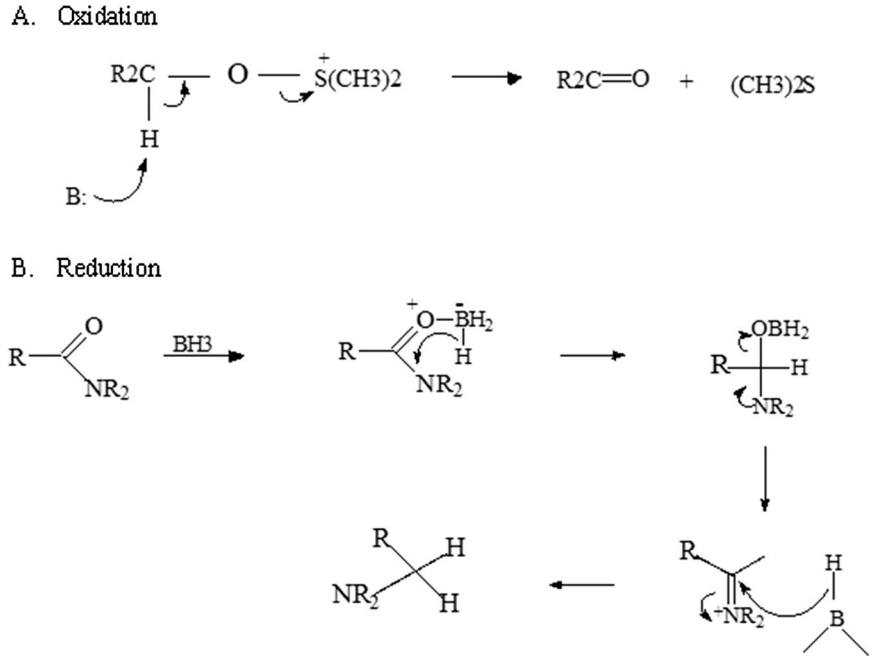

Figure 2 FRs interact with other molecules through redox reactions to obtain a stable electron configuration. In a redox reaction, electron transfer among the participating chemical species will occur. One molecule loses free electrons (A): oxidation process and the other gains electrons (B): reduction process. This balance is necessary in reactions or interactions between proteins that participate in any cellular biochemical pathways.

non-radicals, which mean that they have only an even number of electrons in their atomic orbits.

Once generated, FRs interact with other molecules through redox reactions to reach a stable electronic configuration. In a redox reaction, electron transfer between the participating chemical species will take place. One chemical species loses free electrons (oxidation process) and the other gains electrons (reduction process). The oxidation of one chemical species implies the reduction of another (Fig. 2).

The molecule losing electrons is known as a reducing agent, and the molecule gaining electrons is known as an oxidant agent. When an FR reacts with a non-radical molecule, it can lose or gain electrons, or simply join the molecule. In any case, the non-radical molecule turns into an FR and a chain reaction is triggered: one FR generates another FR. The reaction will stop only when two FRs meet.[22]

Several authors have classified FRs according to their functional groups.[23] The most common is an oxygen FR, in which oxygen is the functional center (Fig. 3).

Thiol radical groups contain sulfur (S), and other radicals contain carbon (C), phosphorous (P), or nitrogen (N) in their reactive groups. FRs are generated from normal metabolic reactions, and exogenous factors can increase their production.[24] This group of FR is formed by one superoxide anion, one hydroxyl radical, and FRs that come from organic compounds: alcoxyl, peroxyl, hydrogen peroxide, and singlet oxygen.[25]

Therefore, the general term ROS is used to indicate chemical species that act like oxidants but are not FRs such as hydrogen peroxide, hypochlorous acid, hydroperoxides, and epoxide metabolites.[26]

The major site of superoxide production is considered to be the respiratory chain in mitochondria, but the exact mechanism and the precise location of the physiologically relevant ROS generation within the respiratory chain have not yet been determined. The mechanism of ROS generation could be relevant because evidence indicates that oxidative stress is a crucial factor in the pathogenesis of clinical disorders.[27] However, recent studies suggest that the ubiquitination of the mitochondrial transcription factor A impedes its transport to the mitochondria, resulting in suboptimal mtDNA transcription and

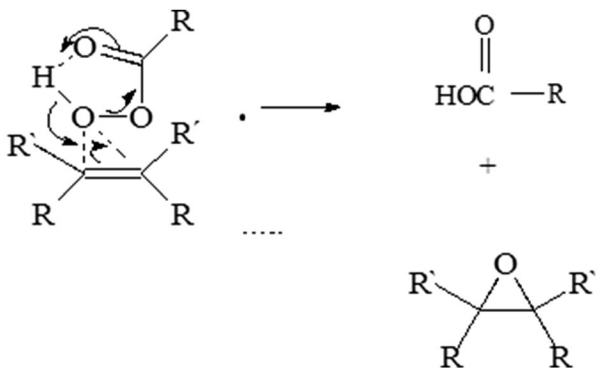

Figure 3 The most frequent FR produced is the oxygen radical, in which oxygen is the functional center. The movement of electrons is through atoms or molecules with electrophilic properties, and this substrate is critical because it participates in all cellular processes of inflammation.

Table 1 Clinical disorders and biomarkers related to DR

Disorders	Tissue	Effect or biomarkers	Reference
DR	Retina	Two single-nucleotide polymorphisms (rs1073203 and rs4838605) were found to be significantly associated with DR	McAuley *et al.*[29]
DR	Retinal capillary cell	Retinal capillary cell apoptosis and the number of degenerative capillaries were increased by three- to fourfold. Gene expression of mtDNA-encoded proteins was decreased, and VEGF, interleukin-1β, and NF-κB levels were elevated	Kowluru *et al.*[30]
DR	Retinal pigment epithelium (RPE)	Glycogen storage is increased in the RPE of diabetic patients and demonstrates the role of glycogen deposits in the pathogenesis of DR	Hernández *et al.*[31]
Retinal inflammation	Neural retina	Cells derived from the stromal fraction of adipose tissue are able to rescue the neural retina from hyperglycemia-induced degeneration	Rajashekhar *et al.*[32]
Retinal inflammation	Retina	Upregulation of oxidative/nitrosative stress, A2AAR, ENT1, Iba1, TNF-α, ICAM1, retinal cell death, and downregulation of AK	Elsherbiny *et al.*[33]
Diabetic retina	Retina	Brain-derived neurotrophic factor (BDNF) is reduced by high-mobility group box-1, thiobarbituric acid-reactive substances↑	Abu El-Asrar *et al.*[34]
Retinopathy induced by hyperoxia (80% O_2)	Retinal microglia	Activation of microglia and induction of microvascular injury through the release of Sema3A from adjacent neurons	Rivera *et al.* [35]
Experimental DR	Retina	SS31, a mitochondria-targeted antioxidant peptide in the retina of diabetic patients, could be a potential new treatment for DR	Huang *et al.*[36]
DR	Retina	Adiponectin on the retinal vasculature may help improve potential therapies for retinal vascular disorders	Omae *et al.*[37]
DR	Retina	Increased binding of Nrf2 to Keap1; its translocation to the nucleus is compromised, contributing to decreased GSH levels; regulation of Nrf2–Keap1 by pharmacological or molecular means could serve as a potential adjunct therapy to combat oxidative stress and inhibit the development of DR	Zhong *et al.*[38]
Diabetic retina	Cultured retina	The expression patterns of HO-1, Nox2, Nox4 in db/db mouse retinas, and the suppressive effects of NADPH oxidase inhibitors on the expression of HO-1, which is at least partially mediated by NADPH oxidase	He *et al.*[39]
Retinopathy animal model	Retina	Lack of glutathione peroxidase-1 was associated with increased oxidative stress, an increase in the retinal avascular area, and upregulation of retinal VEGF	Tan *et al.*[40]
Diabetic retina	Retina	Sigma receptor 1 is a non-opioid transmembrane protein that plays a key role in modulating retinal stress. It may be an important target in retinal disease	Ha *et al.*[41]
DR	Plasma	Decreased plasma purpose Dickkopf-1 levels, which may contribute to Wnt/β-catenin pathway activation, are associated with the presence and progression of DR	Qiu *et al.*[42]
DR	Retina	Strategies targeting T-cell lymphoma invasion and metastasis–Ras-related C3 botulinum toxin substrate 1 (TIAM1–RAC1) signaling could have the potential to halt the progression of DR in the early stages of the disease	Kowluru *et al.*[43]
DR	Retina	Hypoxia might be involved in DR development through the stimulation of two key events of RD, such as neoangiogenesis and apoptosis	Cervellati *et al.*[44]
Diabetic retina	Retina	Levels of circulating oxidized LDL immune complexes predict the development of DR	Fu *et al.*[45]
DR	Retina	Therapies targeting the retinal dopaminergic system may be beneficial in early-stage DR	Aung *et al.*[46]

Up (↑), down (↓).

mitochondria dysfunction. The inhibition of ubiquitination restores mitochondrial homeostasis and inhibits the development and progression of DR.[28] Oxygen-derived FRs such as hydroxyl and hydroperoxyl species have been shown to oxidize phospholipids and other membrane lipid components leading to lipid peroxidation. Lipid peroxidation has been reported to play a significant role in the progression of DR.[21]

Prevention and treatment

Recent studies describe three treatments for DR that are very effective in reducing vision loss from this disease. Even people with advanced DR have a 90% chance of maintaining their vision when they are treated before the retina is severely damaged. These three treatments are laser surgery, corticosteroid or anti-vascular endothelial growth factor (VEGF) injection into the eye, and vitrectomy. These treatment options do not cure DR. New insights into the pathophysiology of DR are needed to develop new methods to improve its detection, treatment, and prevention and to understand the underlying molecular mechanisms (see Table 1) that control the incidence and progression of the disease.

Table 2 Substances with antioxidant activity used in DR

Substance	Effects	Reference
Alpha-lipoic acid	ALA treatment has been shown to suppress expression of vascular endothelial growth factor, angiopoietin 2, and erythropoietin via blockade of superoxide formation	Nebbioso *et al.*[47]
Telmisartan	BDNF and glutathione are increased in the sera and the retina of a DR animal model, decreasing signs of apoptosis	Ola *et al.*[48]
Phlorizin	Significantly reduced fasting blood glucose and levels of advanced glycation end products and remarkably inhibited retina cell apoptosis and the expression of glial fibrillary acidic protein in the retinas	Zhang *et al.*[49]
Photobio-modulation	Daily 670-nm PBM treatment (6 J/cm^2) resulted in significant reduction in diabetes-induced death of retinal ganglion cells	Tang *et al.*[50]
Astaxanthin	Reduced apoptosis of retinal ganglion cells and improved the levels of superoxide anion, malondialdehyde (a marker of lipid peroxidation), 8-hydroxy-2-deoxyguanosine (indicator of oxidative DNA damage), and manganese superoxide dismutase activity in the retinal tissue	Dong *et al.*[51]
Hydrogen sulfide	Abated oxidative stress, alleviated mitochondrial dysfunction, suppressed NF-κB activation and attenuated inflammation in DR animal models	Si *et al.*[52]
Resveratrol	Alleviated hyperglycemia, induced weight loss, enhanced lipid peroxidation index, and oxidization to reduce the glutathione ratio and superoxide dismutase activity in the retina	Soufi *et al.*[53]
Hesperetin (flavonoids)	Showed inhibitory effects on caspase-3, which could be effective for the prevention of DR	Kumar *et al.*[54]
Green tea, rich source of epigallocatechin gallate	Protects the retina in DR due to an increase in the expression of glial fibrillary acidic protein, oxidative retinal markers, and glutamine synthetase levels	Silva *et al.*[55]
Methylene blue or apocynin	Pharmaceuticals targeting photoreceptor oxidative stress could offer a unique therapy for DR	Du *et al.*[56]
Tauroursodeoxycholic acid	Decreased protein carbonyl groups and ROS production	Gaspar *et al.*[57]

Many treatment options have recently been developed for the clinical management of DR complications. Table 2 shows experimental data and some relevant substances thought to ameliorate oxidative stress and prevent or retard the development of DR in animal models. However, clinical observations also suggest that reducing oxidative stress may help reverse pathological disturbances in DR. Early protection of retinal neurons in DR cases can protect against damage of retinal vessels, thereby helping ameliorate the progression of DR.

Over half of the people with young-onset diabetes, regardless of type, exhibit retinopathy within 10–12 years of disease duration, which emphasizes the need for regular eye screening and aggressive control of glucose and blood pressure to prevent ocular damage.[58]

Conclusions

Poor glycemic control and oxidative stress have been shown to contribute to the development of complications in the eye such as DR. Diabetic patients should be educated on eye complications that may arise from their condition. Regular eye screening with a fundus camera should be part of the routine management of diabetes.

Oxidative stress reduction and the restoration of the retinal antioxidant system using exogenous antioxidants or anti-inflammatory drugs are promising issues for further research.

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