



# Oxidative Stress, Diabetic Retinopathy, and Superoxide Dismutase 3

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## Abstract

Diabetic retinopathy (DR) is a multifaceted disease, combining the deleterious effects of hyperglycemia and the propensity for accumulation of reactive oxygen species. Studies indicate that auto-oxidation of glucose, reduced antioxidant activity, and metabolic aberrations contribute to the pathogenesis of DR. These abnormalities stem from a fundamental imbalance between ROSs and antioxidant scavengers. To correct this imbalance and downstream effects, we propose that superoxide dismutase 3 (SOD3) is a viable therapeutic target for DR.

## Keywords

Antioxidant · Diabetic retinopathy · Superoxide · Superoxide dismutase 3 · Neovascularization · Non-proliferative diabetic retinopathy · Proliferative diabetic retinopathy · Hydrogen peroxide · Vitamin C · Oxidative stress · Hypoxia · Macular edema

## 55.1 Antioxidants in the Retina

The pathological effect of oxidative stress to retinal dysfunction is a highly debated and under-examined aspect (Klein and Ackerman 2003). However, oxidant homeostasis was shown critical to normal neurological cell function. The retina is a highly metabolic organ, operating entirely by aerobic respiration (Panfoli et al. 2012). It consumes more oxygen than any other tissue (Panfoli et al. 2012), resulting in production of reactive oxygen species (ROSs), such as superoxide ( $O_2^-$ ), hydroxyl radical ( $\cdot OH$ ), and hydrogen peroxide ( $H_2O_2$ ) (Pham-Huy et al. 2008). The unpaired electrons in  $O_2^-$  and  $\cdot OH$  make these molecules incredibly reactive damaging cell membranes, cellular proteins, and DNA.  $H_2O_2$  is significantly less reactive but can easily permeate cell membranes and react with intracellular iron and other metallic molecules yielding more  $\cdot OH$ .

Retinal oxidative stress emanates from both endogenous and exogenous sources. The mitochondrial respiratory machinery is responsible for the largest contribution of superoxide to the intracellular space (Panfoli et al. 2012). The inner membrane of the mitochondria is responsible for formation of ATP by electron transport, ultimately releasing water and oxygen (García-Aguilar and Cuezva 2018). Hypoxia, hyperglycemia, or aberrations in mitochondrial function can disrupt oxidative phosphorylation and produce superoxide anions (Pham-Huy et al.

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2008). Superoxide is also generated by NADPH oxidase, which contributes significantly to the oxidant content in the extracellular space of the retina (Zeng et al. 2014). Additionally, tyrosine, histidine, methionine, and cysteine can undergo radiation damage by light and generate oxidative intermediates (Pattison et al. 2012). Recent findings suggest a respiratory mechanism in photoreceptor outer segments that may contribute to extracellular ROSs (Gosbell and Stefanovic 2006).

ROSs are also introduced from exogenous sources as a consequence of lifestyle and environment (Klein and Ackerman 2003). Besides environmental pollutants as a source of oxidants, studies have also correlated high-fat and sugar diets with high levels of ROSs (Al-Gubory et al. 2010). However, the largest exogenous contributor of ROSs is cigarette smoking (Klein and Ackerman 2003).

Under non-pathological conditions, the detrimental effects of ROSs can be mitigated by an effective cohort of antioxidant proteins. Superoxide dismutases (SODs), differentiated by their localization and metallic constituents, serve as the primary defense against free radicals catalyzing the dismutase of superoxide (Klein and Ackerman 2003). After conversion of superoxide anion to water and hydrogen peroxide, catalase, glutathione peroxidase, and glutathione reductase further dissociate hydrogen peroxide to oxygen and water (Klein and Ackerman 2003). Dietary antioxidants also play a large part in maintaining this delicate oxidant balance (Al-Gubory et al. 2010); among those are vitamin C and E, carotenoids, flavonoids, lipoic acid, glutathione (GSH), and L-arginine (Klein and Ackerman 2003).

The outer retina is rich in polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and consequently is highly susceptible to free radical damage by lipid peroxidation (Song et al. 2016). High levels of ROSs are debilitating and can lead to aberrations in phototransduction and disruption to cellular function of the retina and the retinal pigment epithelium (RPE) (Seddon et al. 1994). Consequently, further examining the critical roles of antioxidants and potentiating these molecules as therapies are

important steps for understanding and treating retinal pathologies.

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## 55.2 Diabetic Retinopathy and Antioxidants

The etiology that exists between oxidative stress and DR makes this disease a prime target for antioxidant-based therapies. DR is the major cause of blindness in working adults in the developed world (Sahajpal et al. 2018). Among patients with diabetes in the United States, 28.5% (about 4.2 million people) of them develop DR at some stage. Worldwide, there are approximately 93 million people diagnosed with DR. Based on the high prevalence of uncontrolled diabetes, obesity, and other high-risk factors for diabetes, it is estimated that by 2020, the United States may have approximately six million people effected by DR and about 22% of those individuals may be completely visually impaired (Sahajpal et al. 2018).

The interplay between hyperglycemia, oxidative stress, and antioxidants is integral to DR pathogenesis. Studies have yet to elucidate correlation between oxidative stress and hyperglycemia (Evans et al. 2002). It is more likely that high levels of ROSs are a consequence of many systemic aberrations rather than a single causality (Kowluru and Chan 2007). Many postulate that ROSs in DR patients can be derived from the auto-oxidation of glucose, a shift in redox balances, and a decrease in exogenous and endogenous antioxidants (Kowluru and Chan 2007). Levels of SODs, GSH, and vitamin E have shown to be consistently low in DR (Kowluru and Chan 2007). Furthermore, even after patients are able to control their steady-state glucose levels, the effect of DR appears more or less permanent, indicating oxidative damage to cells and molecules that cannot be repaired (Kowluru and Chan 2007).

The route from excess glucose to ROSs accumulation is attributed to certain features of DR. The polyol pathway, which uses aldose reductase to convert glucose to sorbitol, is upregulated in diabetes (Kowluru and Chan 2007). This mechanism exhausts retinal NADPH and consequently reduces its availability for GSH regeneration

(Kowluru and Chan 2007). The hexosamine biosynthesis is another pathway affected by hyperglycemia (Kowluru and Chan 2007). ROSs inhibit glyceraldehyde-3-phosphate dehydrogenase activity and redirect glycolysis intermediates toward hexosamine pathway, creating UDP-N-acetylglucosamine which serves as a substrate in posttranslational modifications such as the non-enzymatic addition of advanced glycation end products (AGEs) (Kowluru and Chan 2007).

Glucose auto-oxidation has been shown to change voltage across the inner mitochondrial membrane which interferes with proper electron transport across the complexes (Young et al. 2002). At complex III, electrons become “blocked” as a result of this change in the voltage equilibrium (Young et al. 2002). Electrons accumulate at Coenzyme Q and when transferred to O<sub>2</sub> create superoxide anions (Young et al. 2002) further disrupting the balance of ROSs and scavengers.

The current treatments available for DR are invasive and not entirely curative. Photocoagulation or anti-VEGF therapies remain the predominant treatments (Calderon et al. 2017). In photocoagulation, a laser is used to ablate or minimize abnormal retinal regions (Gast et al. 2016). For patients with proliferative DR with macular edema, photocoagulation has been effective in reducing vision loss from 30% to 15% over a 3-year period (Calderon et al. 2017). Combined with ranibizumab, and anti-VEGF drug, photocoagulation has been shown to substantially improve the prognosis of DR (Calderon et al. 2017). Nevertheless, there is a large cohort of patients that are not responsive to anti-VEGF therapies and instead have been prescribed corticosteroids (Calderon et al. 2017), which effectively downregulate VEGF and cytokines (Calderon et al. 2017).

Proper regulation of glycated hemoglobin (HbA1c) can substantially reduce progression of DR (Calderon et al. 2017); however, this and other aforementioned therapies are not entirely curative. Photocoagulation can be incredibly destructive and can lead to loss of peripheral vision (Calderon et al. 2017). It serves more as a damage control, rather than an effective fix. Anti-VEGF strategies require consistent dosage, and there is always a risk for drug tolerance.

Corticosteroids are equally effective but have shown to contribute to intraocular pressure (Calderon et al. 2017), which may compound the already deleterious effects of DR. As a consequence, the direction of DR therapies should be reformulated to address prevention and implement the retina's natural defenses, in lieu of damaging or repetitive invasive options.

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### 55.3 Propositioning Superoxide Dismutase 3 as a Therapeutic Target for DR

Antioxidants are a viable direction for DR therapies. SODs are effective superoxide scavengers making them promising therapeutic targets (Batinic-Haberle et al. 2014). SOD3 is an extracellular SOD that catalyzes the dismutases of superoxide in the extracellular matrix (ECM) and is expressed in almost all tissue at varying degrees (Faraci and Didion 2004). SOD3 is a secreted glycosylated protein and bound to the cell surfaces by heparan sulfate linkages (Faraci and Didion 2004). The majority of SOD3 is localized to the ECM; however, it can be found unbound in serum and intracellularly localized to the nucleus (Singh and Bhat 2012).

Apart from its enzymatic function, SOD3 has regulatory roles as well. Studies have shown SOD3 influencing cell proliferation and survival pathways (Laukkanen et al. 2015). Because it is bound to the cell membrane, SOD3 can interact with membrane receptor tyrosine kinases (RTKs) such as EGFR and AKT (Laukkanen et al. 2015). In an *in vitro* study, elevated SOD3 levels were correlated with increased phosphorylation of membrane-bound RTKs, activating cell proliferation (Laukkanen et al. 2015). In a dose-dependent manner, SOD3 has been shown to affect the expression levels of other antioxidant proteins, and its own level can be modulated based on vitamin C and butylated hydroxyanisole (BHA) intake (Singh and Bhat 2012). It has also been shown to reduce inflammation during wound healing and restore the oxidant balance after ischemic/reperfusion injury (Laurila 2009; Markus and Scheider 2010).

So how do these properties extend to DR therapies? The multifaceted nature of this protein makes it an ideal target. DR patients have consistently shown to express reduced levels of SOD3 and other antioxidant proteins (Laight et al. 2000). Intracellular GSH is reduced, and superoxide is significantly increased (Hakki Kalkan and Suher 2013). Primarily, increasing SOD3 will ameliorate ROSs damage by restoring the equilibrium of scavenger and ROSs in the extracellular space. As SOD3 has been shown to effect the levels of other antioxidants (Singh and Bhat 2012), both intracellular and extracellular microenvironments will benefit from the increase in retinal SOD3.

Subsequently, SOD1 has been shown to significantly mitigate the antioxidant stress caused by ischemic/reperfusion (I/R) injury in retinal microvasculature (Chen et al. 2009). SOD3 has demonstrated the same capacity in other tissues. Recent results suggest that this I/R injury model can be used to assess DR therapies as a similar mechanism of ROS-induced damage of capillaries is seen in DR patients (Hartsock et al. 2016). This sort of therapy would serve as a preventative measure, preventing the apoptosis of capillaries and pericytes seen in non-proliferative DR.

Finally, membrane-bound SOD3 plays a significant role in effecting membrane-bound receptors, such as RTKs (Laukkanen et al. 2015). RTKs themselves are critical in encouraging the cell toward survival, proliferation, or apoptosis. The receptors are also involved in the production of proangiogenic factors (Rahimi 2012). SOD3 can act as a gatekeeper on the membrane preventing ROSs' deleterious effects on these receptors.

The localization of SOD1 and SOD2 in the cytosol and mitochondria, respectively, has made them natural targets for potential treatments. Therapies with SOD2 and catalase (CAT) have shown to improve cone function in retinitis pigmentosa, another retinopathy whose pathogenesis has been closely linked to ROS-induced cytotoxicity (Usui et al. 2009). SOD3 has been shown to have better systemic and expansive effects. Levels of exogenous antioxidants, such as vitamin C and BHA, have shown to affect levels of SOD3 independent of the other SODs (Singh and Bhat 2012). Although studies have

confirmed a link between ROS and DR, it may appear counterintuitive to use an extracellular protein like SOD3 to tackle these predominately intracellular dysregulations, but the more pervasive nature of SOD3 and its many regulatory roles make it a logical target for addressing a multi-system disease like DR.

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## 55.4 Conclusions

DR is a debilitating disease caused by a complex array of factors from lifestyle choice to genetic predisposition. However, irrespective of how diabetes develops, ROSs are still a major and fundamental element to the pathogenesis of DR (Kowluru and Chan 2007). Consistently elevated levels of glucose cause an increase in ROSs and tilt the fragile balance in the retina toward cytotoxicity and tissue damage (Kowluru and Chan 2007). The current therapies either only treat a symptom or simply attempt to salvage the remaining healthy tissue. Therefore, it is critical to understand how these molecules cause extensive retinal damage and whether the use of SOD3 could prevent their negative effects.

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