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INVITED REVIEW

Induction of ischemic tolerance as a promising treatment against diabetic retinopathy

Ruth E. Rosenstein[1], **Diego C. Fernandez**[1, 2]

1 Laboratory of Retinal Neurochemistry and Experimental Ophthalmology, Department of Human Biochemistry, School of Medicine/CEFyBO, University of Buenos Aires/CONICET, Buenos Aires, Argentina
2 Present address: Johns Hopkins University, Department of Biology, Baltimore, MD, USA

Corresponding author:
Ruth E. Rosenstein, Departamento de Bioquímica Humana, Facultad de Medicina, /CEFyBO, UBA/CONICET. Paraguay 2155, 5°P, (1121), Buenos Aires, Argentina. ruthr@fmed.uba.ar.

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Abstract

Diabetic retinopathy is a leading cause of acquired blindness, and it is the most common ischemic disorder of the retina. Available treatments are not very effective. Efforts to inhibit diabetic retinopathy have focused either on highly specific therapeutic approaches for pharmacologic targets or using genetic approaches to change expression of certain enzymes. However, it might be wise to choose innovative treatment modalities that act by multiple potential mechanisms. The resistance to ischemic injury, or ischemic tolerance, can be transiently induced by prior exposure to a non-injurious preconditioning stimulus. A complete functional and histologic protection against retinal ischemic damage can be achieved by previous preconditioning with non-damaging ischemia. In this review, we will discuss evidence that supports that ischemic conditioning could help avert the dreaded consequences that results from retinal diabetic damage.

Key Words: *diabetic retinopathy; ischemic tolerance; retina; ischemic injury; ischemic conditioning*

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Diabetic retinopathy

About 380 million people across the globe are estimated to have diabetes, and its prevalence is expected to drastically increase in the near future (Shi et al., 2014). One of the most serious complications of diabetes is diabetic retinopathy (DR) (Szabadfi et al., 2014). Nearly all individuals with type 1 diabetes mellitus (T1DM), and more than 60% of individuals with type 2 diabetes mellitus (T2DM) have some degree of retinopathy after 20 years of diabetes (Robinson et al., 2012). DR is a leading cause of reduced visual acuity and acquired blindness. In inadequately controlled patients, the retinal microvasculature is constantly exposed to high glucose levels, which results in vascular damage and leakage, edema, capillary basement membrane thickening, neovascularization, hemorrhage, ischemia, and neuroglial alterations (Barber et al., 2011, Kowluru and Chan, 2007). Despite many differences between T1DM and T2DM, both forms of diabetes will lead to a similar retinopathy (Szabadfi et al., 2014). Current treatments for DR such as laser photocoagulation, corticosteroids, or anti-vascular endothelial growth factor (VEGF) agents are indicated for advanced DR, but have adverse effects. Therefore, new therapeutic treatments for DR are needed. In experimental studies, efforts to inhibit DR have focused either on highly specific therapeutic approaches for pharmacologic targets (Du et al., 2010) or using genetic approaches to change expression of certain enzymes (Zheng et al., 2007). Alternatively, identification of innovative treatment modalities that act by multiple potential mechanisms would be necessary. In this review, we will discuss evidence that supports that ischemic conditioning might pave the way for finding novel therapeutic strategies against retinal diabetic damage.

Ischemic conditioning

The resistance to ischemic injury, or ischemic tolerance, can be transiently induced by prior exposure to a non-injurious preconditioning stimulus. Ischemic preconditioning (IPC) itself initiates several adaptive reactions that lead to the establishment of what might be described as a "latent" protective phenotype; priming the tissue for the actual injurious ischemic event (Gidday, 2006). The first landmark paper on cardiac preconditioning in dogs by brief coronary ischemia was published in 1986 (Murry et al., 1986). Since then, many studies have strongly demonstrated that preconditioning stimuli trigger ischemic tolerance in different tissues (Gidday, 2006).

Ischemia is one of the key factors determining the pathophysiology of many retinal diseases, such as DR, glaucoma, and age-related macular degeneration, among others. Ischemic retinopathy develops when retinal blood flow is insufficient to match the metabolic needs of the retina (one of the highest oxygen consuming tissues), and induces irreversible morphologic and functional changes that result in blindness. Studies have focused on the ability of exogenous agents to treat retinal ischemic damage, but thus far, none of these strategies is completely effective. Roth et al. (1998) first demonstrated a complete functional and histologic protection against retinal ischemic damage by previous pre-

conditioning with non-damaging ischemia (5-minute ischemia). According to these authors, IPC is more effective in decreasing retinal ischemic injury than nearly any previously reported pharmacologic treatment. However, despite the robust neuroprotection induced by IPC, its use as a clinical strategy is limited because the onset of retinal ischemia is unpredictable, in contrast to the onset of reperfusion, which may be more predictable. Another endogenous form of protection in which a short series of repetitive cycles of brief ischemia-reperfusion were applied immediately at the onset of reperfusion, termed postconditioning (PostC), has been reported. PostC reduces myocardial injury to an extent comparable to that of IPC (Zhao et al., 2003). Other groups have confirmed the effectiveness of PostC in the central nervous system (Pignataro et al., 2008; Wang et al., 2008). We have shown that repetitive cycles of briefly interrupted reperfusion performed at the onset of full reperfusion, or even one 7-minute ischemia pulse applied 5 minutes after ischemia (PostC) induces a complete recovery from retinal I/R damage which is effective even when applied 60 minutes after the onset of reperfusion (Fernandez et al., 2009). The effectiveness of ischemic tolerance against acute ischemic events suggests that these strategies could contribute to the discovery of new therapeutic alternatives for chronic ischemic diseases, such as DR.

Ischemic conditioning and diabetic retinopathy

Since available treatments for DR are not completely effective, it is imperative to develop better approaches for its prevention and treatment. Unraveling which is the most critical mechanism to be targeted by new therapeutic strategies, is unlikely to be achieved in studies limited to the clinically observable retinal changes in human DR. Far more detailed and invasive studies are required, preferably in readily available animal models. The streptozotocin (STZ)-induced diabetes in rats shows many of the retinal alterations observed in human DR associated with T1DM (Wei et al., 2003). We have shown that the combination of diet-induced insulin resistance and a slight secretory impairment resulting from a low-dose STZ treatment mimics some features of human T2DM at its initial stages, and provokes significant retinal alterations (Salido et al., 2012). Based on the highly effective protection induced by IPC and PostC against an acute ischemic episode, and considering that DR is the most common ischemic disorder of the retina (Stitt et al., 2011), the effect of ischemic tolerance on retinal damage induced by both experimental T1DM and T2DM was analyzed. The results obtained are summarized below.

Ischemic tolerance on diabetic retinopathy associated to T1DM

T1DM was induced by an intraperitoneal injection of STZ, and ischemic tolerance was induced by increasing intraocular pressure (IOP) to 120 mmHg for 5 minutes; this maneuver started 3 days after STZ injection and was weekly repeated in one eye, while the contralateral eye was submitted to a sham procedure. Weekly ischemia pulses, which show no effect *per se*, prevent retinal alterations induced by experimental diabetes (Fernandez et al., 2011). It is well known that human diabetes induces significant alterations in the electroretinogram (ERG) and oscillatory potentials (OPs) (Coupland, 1987; Holopigian et al., 1992; Lovasik and Kergoat, 1993). In agreement, a significant and progressive ERG dysfunction is observed in eyes from rats injected with STZ, whereas weekly ischemia pulses prevent the decrease in ERG a- and b-wave, and OP amplitude induced by experimental diabetes (Fernandez et al., 2011).

Evans blue has been widely used for blood-retinal barrier (BRB) studies in several species (Ma et al., 1996; Zhang et al., 2005). Since intravenously injected Evans blue binds irreversibly to serum albumin, its distribution reflects albumin exchange between the intra- and extravascular compartments. In diabetic retinas submitted to a sham procedure, extravasated Evans blue is evident, whereas ischemia pulses prevent the effect of diabetes on BRB integrity. Astrocytes are closely associated with retinal vessels (Schnitzer, 1988), helping to maintain their integrity (Zhang and Stone, 1997), and increasing the vascular endothelium barrier properties (Gardner, 1995). Astrocyte dysfunction plays a pivotal role in inner BRB breakdown, resulting in the production of vasogenic edema (Chan-Ling and Stone, 1992; Gardner et al., 1997). Experimental diabetes induces a decrease in astrocyte glial fibrillary acid protein (GFAP) immunoreactivity, which is restored by ischemia pulses. A reduction in retinal astrocyte GFAP expression during diabetes may be linked to a reduced ability to maintain BRB characteristics in endothelial cells (Barber et al., 2000). Thus, changes in GFAP immunoreactivity could account for Evans blue leakage in diabetic eyes and its prevention by ischemic tolerance. The BRB plays an important role in the homeostatic regulation of the retinal microenvironment. Disruption of the BRB associated with increased vascular permeability results in edema and tissue damage, with consequent adverse effects on vision. Factors such as enhanced production of VEGF underlie the increased permeability of the BRB, and inhibition of VEGF is beneficial in humans and experimental models (Kaur et al., 2008; Jeganathan, 2011). In this vein, we have shown that ischemia pulses abrogate the increase in retinal VEGF levels induced by DR (Fernandez et al., 2011). Taken together, these results suggest that weekly ischemia pulses prevent retinal damage induced by experimental T1DM. However, the translational relevance of these results is limited by the fact that application of ischemia pulses started before the appearance of retinal changes provoked by diabetes. Therefore, we analyzed whether ischemia pulses could not only prevent but also reduce DR progression. For this purpose, we started the application of ischemia pulses at 6 weeks of diabetes onset, a time point in which a functional alteration is already evident. The delayed treatment resulted in a significant protection when compared with diabetic eyes submitted to a sham procedure, supporting that ischemic tolerance not only prevents but also restores the retinal function (Fernandez et al., 2011). On the other hand, we have shown that

axoglial alterations at the distal portion of the optic nerve could be the first structural change in the diabetic visual pathway (Fernandez et al., 2012a). Therefore, we analyzed the ability of ischemic conditioning on optic nerve axon protection against T1DM damage. In this sense, we demonstrated that ischemia pulses prevent a deficit in the anterograde transport from the retina to the superior colliculus, as well as an increase in astrocyte reactivity, ultraestructural myelin alterations, and altered morphology of oligodendrocyte lineage in the optic nerve distal portion at early stages of experimental diabetes (Fernandez et al., 2012b), which supports that ischemic tolerance protects optic nerve axonal function and structure against diabetic damage.

Ischemic tolerance on diabetic retinopathy associated to T2DM

In order to analyze the effect of ischemic tolerance on retinal damage associated with T2DM, adult male Wistar rats received a control diet or 30% sucrose in the drinking water, and 3 weeks after this treatment, animals were injected with vehicle or STZ (25 mg/kg) (Salido et al., 2012). Starting 3 weeks after vehicle or STZ, retinal ischemia was weekly induced by increasing IOP for 5 minutes in one eye, while control eyes were submitted to a sham procedure. At 12 weeks of treatment, animals that received a sucrose-enriched diet and STZ show significant differences in fasting and postprandial glycemia, and glucose, and insulin tolerance tests, as compared with control groups. Brief ischemia pulses in one eye and a sham procedure in the contralateral eye do not affect glucose metabolism in control or diabetic rats. Ischemic pulses reduce the decrease in the ERG a-wave, b-wave, and OP amplitude, and the increase in retinal VEGF levels in rats with experimental T2DM. Enhanced oxidative stress and inflammatory signals play important roles in the pathogenesis of diabetes mellitus and its complications (Zheng and Kern, 2009; Mokini et al., 2010). A significant increase in retinal lipid peroxidation, TNFα levels, and NOS activity, as well as a decrease in catalase activity was observed in diabetic animals, which are reduced by ischemic tolerance (Salido et al., 2013).

Concluding remarks

These results indicate that ischemic tolerance protects the retina against damage induced by experimental T1DM and T2DM. Notably, the protection induced by ischemia pulses is independent from the glycemic profile in both experimental models (Fernandez et al., 2011; Salido et al., 2013). Although different pathogenic mechanisms could be involved in retinal alterations induced by T1DM and T2DM, the application of ischemia pulses is effective in reducing retinal changes in both experimental models. The precise mechanisms responsible for the retinal protection induced by ischemia pulses remain to be established. Our results indicate that ischemic tolerance can behave as an antioxidant, antinitridergic, anti-inflammatory, and anti-VEGF therapy. Notwithstanding, the involvement of other mechanisms that have been implicated in retinal IPC (such as hypoxia-inducible factor-1 alpha and heme oxygenase-1 (Zhu et al., 2007), mitogen-activated protein kinase p38 (Dreixler et al., 2009a), protein kinase B/Akt (Dreixler et al., 2009b), and mitochondrial K^+/ATP channels (Roth et al., 2006), among others), cannot be excluded. Therefore, further studies to determine the mechanisms behind ischemic tolerance will provide a more complete picture of how neuroprotection is achieved in the context of retinal diabetic damage.

The relevance of these experimental studies to human DR is still an open question. Although care must to be taken when extrapolating data generated in rodents to humans, rodent retinas lacking the ability to develop bona fide proliferative DR, exhibit almost all of the biochemical, pathophysiologic, and histopathologic features of background retinopathy. Many exogenously delivered chemical preconditioning agents (*e.g.*, inflammatory cytokines, anesthetics, and metabolic inhibitors) can also induce ischemic tolerance, raising the hope that in the future, IPC and PostC could be pharmacologically mimicked *in vivo* (Gidday, 2006). Therefore, the present results support that induction of ischemic tolerance could constitute a fertile avenue for the development of new therapeutic strategies for DR treatment.

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