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Gene deletion and pharmacological inhibition of aldose reductase protect against retinal ischemic injury

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Abstract

Retinal ischemic injury is common in patients with diabetes, atherosclerosis, hypertension, transient ischemia attack and amaurosis fugax. Previously, signs of ischemic stress, such as pericyte loss, blood–retinal barrier breakdown and neovascularization, which can lead to occlusion of retinal vessels, have been prevented in diabetic *db*/*db* mice with aldose reductase (AR) null mutation. To determine the role in retinal ischemic injury of AR and sorbitol dehydrogenase (SDH), the first and second enzymes in the polyol pathway, mice with deletion of AR ($AR^{-/-}$) or SDH-mutation ($SDH^{-/-}$), or C57BL/6N mice treated with AR or SDH inhibitors were subjected to transient retinal artery occlusion (2 h of occlusion and 22 h of reperfusion) by the intraluminal suture method. Neuronal loss and edema observed in wildtype ($AR^{+/+}$) retinas after transient ischemia were prevented in the retinas of $AR^{-/-}$ mice or C57BL/6N mice treated with an AR inhibitor, Fidarestat. Fewer TUNEL-positive cells and smaller accumulations of nitrotyrosine and poly(ADP-ribose) were also observed in the retinas of $AR^{-/-}$ mice. However, $SDH^{-/-}$ mice and C57BL/6N mice treated with SDH inhibitor, CP-470,711, were not protected against ischemia-induced retinal damage. Taken together, AR contributes to retinal ischemic injury through increased edema and free radical accumulation. Therefore, AR inhibition should be considered for the treatment of retinal ischemic injury often observed in diabetic patients.

Keywords: sorbitol dehydrogenase; ischemia; Müller cells; retinal ganglion cell; edema; neuronal apoptosis

1. Introduction

Capillaries and arterioles are often occluded in diabetic retinas, leading to ischemic retinal damage (Davis, 1992). Retinal ischemia may contribute to depression of b-wave (Block and Schwarz, 1998), cell loss in retinal ganglion cells (RGC) and inner nuclear layers (INL) (Buchi, 1992), glial reactivation (Kim et al., 1998), blood–retinal barrier breakdown (Wilson et al., 1995), and edema (Da and Verkman, 2004). In fact, both diabetes and ischemia share similar pathological mechanisms (Zheng et al., 2007). The retinas of diabetic *db*/*db* mice with aldose reductase null mutation ($AR^{-/-}$) are protected against vascular abnormalities associated with diabetes, such as pericyte loss, blood–retinal barrier breakdown and neovascularization observed in wildtype ($AR^{+/+}$) diabetic *db*/*db* mice, suggesting that the activation of AR, the first and rate-limiting enzyme in the polyol pathway, contributes to diabetes-induced microvascular abnormalities (Cheung et al., 2005).

Abbreviations: AR, aldose reductase; SDH, sorbitol dehydrogenase; WT, wildtype; INL, inner nuclear layer; GSH, glutathione sulfhydryl; GFAP, glial fibrillary acidic protein; rCBF, relative cerebral blood flow; OLM, outer limiting membrane; ILM, inner limiting membrane; ONL, outer nuclear layer; OPL, outer plexiform layer; IPL, inner plexiform layer; NT, nitrotyrosine; PAR, poly(ADP-ribose).

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Although the activation of AR has been suggested to have a tissue-protective function by preventing damage from lipid peroxidation (Rittner et al., 1999), the activation of the polyol pathway in hyperglycemic conditions is thought to be the main cause of diabetes-induced tissue damage (Chung and Chung, 2005). In such conditions, glucose flux through the polyol pathway increases considerably, and may account for more than 30% of glucose utilization in some tissues (Petrash, 2004). Using NADPH as a cofactor, AR reduces glucose to sorbitol, which is further metabolized to fructose by sorbitol dehydrogenase (SDH), the second enzyme in the polyol pathway. Oxidative stress has been shown to increase glucose flux through the polyol pathway (Nishikawa et al., 2000), and to activate AR in ischemic conditions (Kariserova et al., 2006). Conversely, the activation of AR may cause oxidative stress (Chung et al., 2003). Increased AR activity may contribute to the formation of free radicals by depleting the pool of NADPH, which is also used by glutathione reductase to regenerate reduced glutathione (GSH). It has also been suggested that the activation of protein kinase C, the increased formation of advanced glycation end-production, and the activation of poly(ADP-ribose) are regulated by AR (Chung et al., 2003). Increased SDH activity would increase the NADH/NAD^+ ratio, leading to increased oxidative stress through the action of NADH oxidase (Chung et al., 2003). Increased oxidative stress has been implicated in the pathogenesis of ischemia/reperfusion retinal injury (Bonne et al., 1998). Administration of antioxidants, such as vitamin E and lipoate to rats during ischemia significantly reduced the depression of b-wave (Block and Schwarz, 1997). The flux through SDH and elevated level of fructose may also increase advanced glycation end-product formation (Cheung et al., 2005), which has been associated with ischemic injury (Bucciarelli et al., 2006).

To elucidate the role of the polyol pathway enzymes, AR and SDH, in retinal ischemia/reperfusion injury, $AR^{-/-}$ and SDH mutant ($SDH^{-/-}$) mice were subjected to a transient ischemia model using an intraluminal suture method (Lo et al., 2005). This method was earlier used to induce retinal ischemia/reperfusion injuries resulting in the suppression of a-wave and b-wave amplitudes of electroretinogram and the induction of glial fibrillary acidic protein (GFAP) expression in the retina (Block et al., 1997). Here, we showed that the deletion of AR and the pharmacological inhibition of AR prevented neuronal loss, edema formation and glial reactivation in retinas after transient retinal ischemia. However, blocking SDH did not contribute to either retinal protection or damage after similar stress, suggesting that ischemia-induced AR activation plays a key role in the pathogenesis of ischemic retinopathy.

2. Materials and methods

2.1. Animals

The AR wildtype (WT) ($AR^{+/+}$), $AR^{-/-}$ (Ho et al., 2000), SDH WT ($SDH^{+/+}$) and $SDH^{-/-}$ (Lee et al., 1997) mice in C57BL/6N genetic background were housed under diurnal lighting conditions and allowed free access to water and food (Ho et al., 2000). Experiments were performed on 8–10 weeks old mice in accordance with the experimental guidelines approved by The University of Hong Kong Committee on the Use of Animals for Teaching. Mice were sacrificed by cervical dislocation and their eyes were immediately enucleated and fixed in 4% paraformaldehyde overnight at 4 °C. They were then dehydrated with a graded series of ethanol and xylene and embedded in paraffin wax (Cheung et al., 2005; Mekada and Hazama, 1998). Sections (7 microm) were prepared for immunohistochemistry.

2.2. Transient retinal artery occlusion and drug treatment

To induce retinal ischemia, adult $AR^{+/+}$, $AR^{-/-}$, $SDH^{+/+}$, and $SDH^{-/-}$ mice (24–28 g, $n = 5–6$ in all groups) were subjected to transient retinal artery occlusion by the intraluminal suture method (Block et al., 1997; Lo et al., 2005, 2007). A nylon monofilament (Johnson & Johnson, Brussels, Belgium) was inserted through the common carotid artery and advanced into the right internal carotid artery to the bifurcation between the middle cerebral and anterior cerebral artery. The relative cerebral blood flow (rCBF) of middle cerebral artery territory was monitored by laser Doppler flowmetry (Perimed, Jarfalla, Sweden) to ensure successful occlusion and reperfusion of the retinal artery. Two hours later, the filament was pulled out to allow reperfusion for 22 h. In this procedure, the right eye was made ischemic and designated as 'ipsilateral' while the left eye served as a 'contralateral' control. The drug treatment involved administering Fidarestat, an orally administered AR inhibitor (Giannoukakis, 2003) (a kind gift from Dr. Chihiro Hibi at Sanwa Kagaku Kenkyusho Co. Ltd., Japan, 2 mg/kg body weight, oral administration by gavauge) or SDH inhibitor (SDHI), CP-470,711 (Chu-Moyer et al., 2002a,b) (5 mg/kg body weight, oral administration by gavauge) to C57BL/6N mice ($n = 5–6$ in each group) 15 min before reperfusion. Vehicle-treated (water for Fidarestat; 0.001N HCl for CP-470,711) mice ($n = 5–6$ in each group) were included as controls. All experiments were performed in a double-blinded fashion.

In the occlusion experiment, rCBF was normalized as 100% before ischemia. A successful blockage of the middle cerebral artery was confirmed with >75% drop in rCBF. Ischemia was defined at the time when <25% of the original rCBF remained. The reduction in rCBF produced by the occlusion was similar in all groups ($AR^{+/+}$, and similar reduction was observed in $SDH^{+/+}$: $15.1\% \pm 2.0\%$, $AR^{-/-}$: $14.0\% \pm 2.2\%$, $SDH^{-/-}$: $17.4 \pm 3.5\%$,mice treated with AR inhibitor: $14.8\% \pm 1.7\%$, mice treated with SDH inhibitor: $11.5\% \pm 2.9\%$). There was also no difference in the subsequent rise in rCBF after reperfusion ($AR^{+/+}$, and similar rise was observed in $SDH^{+/+}$: $124.6\% \pm 20.3\%$, $AR^{-/-}$: $193.1\% \pm 45.0\%$, $SDH^{-/-}$: $151.7 \pm 19.5\%$, mice treated with AR inhibitor: $141.3\% \pm 15.6\%$, mice treated with SDH inhibitor: $142.7\% \pm 13.5\%$).