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Research Report

Postconditioning with inhaled hydrogen promotes survival of retinal ganglion cells in a rat model of retinal ischemia/reperfusion injury

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ABSTRACT

Retinal ischemia/reperfusion (I/R) injury plays a crucial role in the pathophysiology of various ocular diseases. Intraperitoneal injection or ocular instillation with hydrogen (H_2)-rich saline was recently shown to be neuroprotective in the retina due to its anti-oxidative and anti-inflammatory effects. Our study aims to explore whether postconditioning with inhaled H_2 can protect retinal ganglion cells (RGCs) in a rat model of retinal I/R injury. Retinal I/R injury was performed on the right eyes of rats and was followed by inhalation of 67% H_2 mixed with 33% oxygen immediately after ischemia for 1 h daily for one week. RGC density was counted using haematoxylin and eosin (HE) staining and retrograde labeling with cholera toxin beta (CTB). Visual function was assessed using flash visual evoked potentials (FVEP) and pupillary light reflex (PLR). Potential biomarkers of retinal oxidative stress and inflammatory responses were measured, including the expression of 4-Hydroxynonenalv (4-HNE), interleukin-1 beta (IL1-β) and tumor necrosis factor alpha (TNF-α). HE and CTB tracing showed that the survival rate of RGCs in the H_2-treated group was significantly higher than the rate in the I/R group. Rats with H_2 inhalation showed better visual function in assessments of FVEP and PLR. Moreover, H_2 treatment significantly decreased the number of 4-HNE-stained cells in the ganglion cell layer and inhibited the retinal overexpression of IL1-β and TNF-α that was induced by retinal I/R injury. Our results demonstrate that postconditioning with inhaled high-dose H_2 appears to confer neuroprotection against retinal I/R injury via anti-oxidative, anti-inflammatory and anti-apoptosis pathways.

Abbreviations: I/R, ischemia/reperfusion; H_2, hydrogen; RGCs, retinal ganglion cells; HE, haematoxylin and eosin; CTB, cholera toxin beta; FVEP, flash visual evoked potentials; PLR, pupillary light reflex; 4-HNE, 4-Hydroxynonenalv; IL1-β, interleukin-1 beta; TNF-α, tumor necrosis factor alpha; ROS, reactive oxygen species; O_2, oxygen; N_2, Nitrogen; ILM, inner limiting membrane; GCL, ganglion cell level; OPL, outer plexiform layer; ONL, the outer nuclear layer.

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1. Introduction

Retinal ischemia/reperfusion (I/R) injury is a common clinical condition that occurs in a variety of ocular diseases, including diabetic retinopathy (Verma, 1993), retinal vascular occlusion (Archer, 1976), anterior optic neuropathy (Hayreh, 1978), and glaucoma (Nickells, 1996; Schmidt et al., 2004). It ultimately leads to retinal ganglion cell (RGC) death because these cells are particularly vulnerable to ischemia (Hayreh et al., 2004; Mukaida et al., 2004).

When tissues are exposed to ischemia followed by reperfusion, reactive oxygen species (ROS) are extensively generated in the early stage of reperfusion, and ROS can cause serious damage to various organs, including the liver (Zar et al., 1998), brain (Peters et al., 1998), heart (Kevin et al., 2003), and retina (Chen et al., 2012). Moreover, increasing evidence demonstrates that oxidative stress induced by ROS plays an key role in the pathophysiological mechanisms involved in retinal I/R injury (Alvarado et al., 1981; Valko et al., 2007). Interestingly, I/R injury can induce a variety of inflammatory mediators, including interleukin-1 beta (IL1-β) and tumor necrosis factor alpha (TNF-α), in the retina or the brain (Franks et al., 1992; Kaur et al., 2008; Zheng et al., 2007). Oxidative stress was found to mediate IL1-β and TNF-α production (Meldrum et al., 1998; Schwartz, 2001) and is now considered as one of the most important factors that mediates the process of apoptosis (Clutton, 1997; Coyle and Puttfarcken, 1993).

Ohsawa et al. (2007) reported that molecular hydrogen (H_2) is an efficient antioxidant when administered by gaseous rapid diffusion into tissues and cells (Ohsawa et al., 2007). H_2, as a novel neuroprotective gas, was confirmed in a variety of ocular disease models, including optic nerve crush, diabetic retinopathy, photochemical retinal injury and retinal I/R injury (Feng et al., 2013; Oharazawa et al., 2010; Sun et al., 2014; Tian et al., 2013). Intraperitoneal injection or ocular instillation with H_2-rich saline is the most common method of H_2 delivery, Therefore, we have developed a simple and effective method to produce the mixed gas with high-concentration H_2 for therapeutic inhalation. The high-dose inhaled method is a portable, easily administered, and safe, and it is more efficient means of delivering H_2.

We launched this study to test the hypothesis that inhaled 67% H_2 can promote the survival of RGCs and preserve visual function after retinal I/R.

2. Results

2.1. RGC density after retinal I/R injury

Fig. 1 shows representative samples of Haematoxylin and eosin (HE)-stained tissues from the H_2-treated and untreated retinal explants at 1 week after retinal I/R injury. The number of HE-stained cells in the ganglion cell layer (GCL) of the Control group was 37.13±3.38 cells/high power field (HPF) at 1 week. There was no difference between the Control group and the I/R-H_2 group (37.13±3.38 versus 31.92±2.88 cells/HPF; $P=0.127$; $P>0.05$; Fig. 1B). More HE-stained cells were observed in the GCL in the I/R-H_2 group than in the I/R group (31.92±2.88 versus 22.38±2.5 cells/HPF; $P=0.0023$; $P<0.01$; Fig. 1B). The total thickness of the retina was better preserved in the I/R-H_2 group compared to the I/R group (93.27±1.1 versus 104.3±2.88 μm; $P=0.0232$; $P<0.05$; Fig. 1C). Therefore, the I/R-H_2 group showed a statistically significant rescue effect in rat retinas compared to the I/R group.

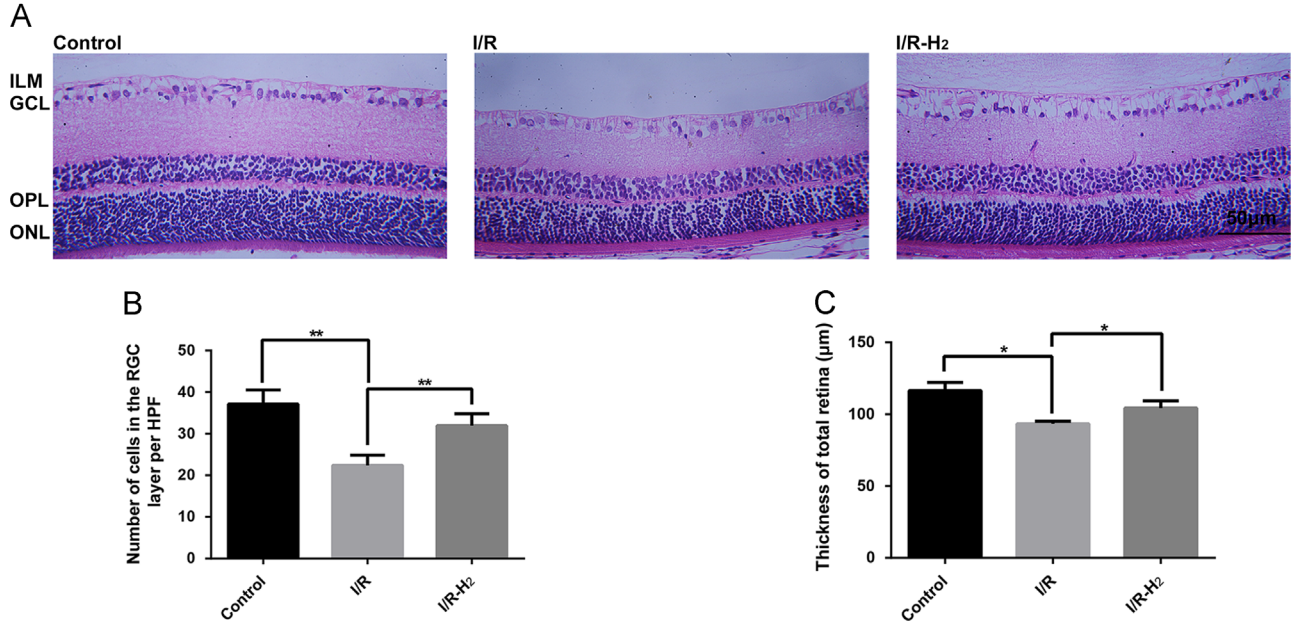

Fig. 1 – HE staining in representative retinas. (A) HE staining of representative retinal sections at 1 week after operation. (B) The number of cells in the GCL of the I/R-H_2 group was significantly higher than the number in the I/R group ($P<0.01$). (C) The thickness of the total retina in the I/R-H_2 groups was significantly increased compared with the I/R group. Data are expressed as percentage of mean±SEM, n=6 per group. Scale bar: 50 μm. *$P<0.05$, **$P<0.01$. HE, Haematoxylin and eosin; ILM, inner limiting membrane; GCL, ganglion cell level; OPL, outer plexiform layer; ONL, the outer nuclear layer.**