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Research report

Methane attenuates retinal ischemia/reperfusion injury via anti-oxidative and anti-apoptotic pathways

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ABSTRACT

Retinal ischemia/reperfusion injury (IRI) may cause incurable visual impairment due to neural regeneration limits. Methane was shown to exert a protective effect against IRI in many organs. This study aims to explore the possible protective effects of methane-rich saline against retinal IRI in rat. Retinal IRI was performed on the right eyes of male Sprague-Dawley rats, which were immediately injected intraperitoneally with methane-saturated saline (25 ml/kg). At one week after surgery, the number of retinal ganglion cells (RGCs), total retinal thickness, visual function were measured by hematoxylin and eosin staining, FluoroGold anterograde labeling and flash visual evoked potentials. The levels of 8-hydroxy-2-deoxyguanosine (8-OHdG), 4-Hydroxy-2-nonenal (4-HNE), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), caspase-3, caspase-9, B cell lymphoma/leukemia-2 (Bcl-2) and Bcl-2 associated X protein (Bax) in retinas were assessed by immunofluorescence staining, enzyme-linked immunosorbent assay and quantitative polymerase chain reaction. As expected, methane treatment significantly improved the retinal IRI-induced RGC loss, total retinal layer thinning and visual dysfunction. Moreover, methane treatment significantly reduced the levels of oxidative stress biomarkers (8-OHdG, 4-HNE, MDA) and increased the antioxidant enzyme activities (SOD, CAT, GPx) in the retinas with IRI. Meanwhile, methane treatment significantly increased the anti-apoptotic gene (Bcl-2) expression and decreased the pro-apoptotic gene (Bax) expression, accompanied by the suppression of caspase-3 and caspase-9 activity. Thus, these data demonstrated that methane can exert a neuroprotective role against retinal IRI through anti-oxidative and anti-apoptotic pathways.

1. Introduction

Retinal ischemia/reperfusion injury (IRI) is highly involved in the pathogenesis of several major vision-threatening diseases including glaucoma, retinal vascular occlusion, anterior ischemic optic neuropathy and diabetic retinopathy (Osborne et al., 2004), which ultimately leads to retinal neuronal loss through necrosis, apoptosis, and autophagy (Piras et al., 2011). Among the various retinal neurons, retinal ganglion cells (RGCs) are thought to be the most vulnerable to IRI (Hayreh et al., 2004). Once RGCs were degenerated progressively, the loss of them is irreversible, causing visual impairment. Therefore, neuroprotection is always the aim to reduce or prevent RGC damage with pharmaceutical intervention or molecular genetic techniques (Kuehn et al., 2005).

Methane, as the simplest alkane and the most abundant organic compound, is a by-product of carbon dioxide and hydrogen ingestion by methanogens in the colon. It is generally considered that methane is biologically inactive (Sahakian et al., 2010). However, much attention has been recently paid to the application of methane as a therapeutic gas recently (Liu et al., 2012). Boros et al. found that exhaled methane exerted the protective effects against intestinal IRI-induced oxidative stress and inflammation (Boros et al., 2012), and suggested that methane may be a gasotransmitter in mammalian physiology (Boros et al., 2015). Most recently, methane appears to exert a protective effect on liver, abdominal skin flap and myocardium with IRI (O. Chen et al., 2016, Z. Chen et al., 2016; Song et al., 2015; Strifler et al., 2016; Ye et al.,

Abbreviations: IRI, ischemia/reperfusion injury; RGCs, retinal ganglion cells; IOP, intraocular pressure; H&E, hematoxylin and eosin; HPF, high-powered field; GCL, ganglion cell layer; FVEP, flash visual evoked potentials; 8-OHdG, 8-hydroxy-2-deoxyguanosine; 4-HNE, 4-Hydroxy-2-nonenal; MDA, malondialdehyde; ELISA, enzyme-linked immunosorbent assay; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; Bcl-2, B cell leukemia/lymphoma-2; Bax, Bcl-2 associated X protein; RT-qPCR, real-time quantitative polymerase chain reaction

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2015), and even exert a neuroprotective role against diabetic retinopathy (Wu et al., 2015) and carbon monoxide poisoning (Fan et al., 2016), through multiple mechanisms including anti-inflammatory, anti-oxidant and anti-apoptosis. Nevertheless, the exact mechanism of therapeutic effect induced by methane treatment is not clear.

In this study, we use an established retinal IRI rat model by transient elevation of intraocular pressure (IOP) to investigate whether methane can play a neuroprotective role in retina against IRI and explore its potential mechanism.

2. Results

2.1. Methane treatment promoted survival of RGCs after retinal IRI

Fig. 1A shows the representative retinal sections of hematoxylin and eosin (H&E) staining at one week after surgery. As shown in Fig. 1B, there was no statistically significant difference in H&E-stained cell number per high-powered field (HPF) in the ganglion cell layer (GCL) between Sham group (123.50 ± 11.83 cells/HPF) and Sham + Methane group (125.46 ± 9.82 cells/HPF) ($p > 0.05$). Retinal IRI significantly reduced the cell number in IRI + Methane group (75.13 ± 9.75 cells/HPF) and IRI group (50.38 ± 12.45 cells/HPF) compared with Sham group ($p < 0.01$). However, there are more H&E-stained cells in IRI + Methane group compared with IRI group ($p < 0.01$). In addition, as shown in Fig. 1C, the total retinal thickness was remarkably reduced in IRI + Methane group (90.33 ± 6.5 μm) and IRI group (73.83 ± 7.41 μm) compared with Sham group (120.33 ± 5.16 μm) ($p < 0.01$), but methane treatment suppressed the reduction of retinal thickness induced by retinal IRI (IRI + Methane group vs. IRI group, $p < 0.01$). Fig. 1D shows the representative flat-mounted retinas by FluoroGold labeling at one week after surgery. As show in Fig. 1E, the numbers of FluoroGold-labeled cells of Sham group and Sham + Methane group were 1688.00 ± 177.65 cells/mm^2 and 1693.00 ± 186.80 cells/mm^2

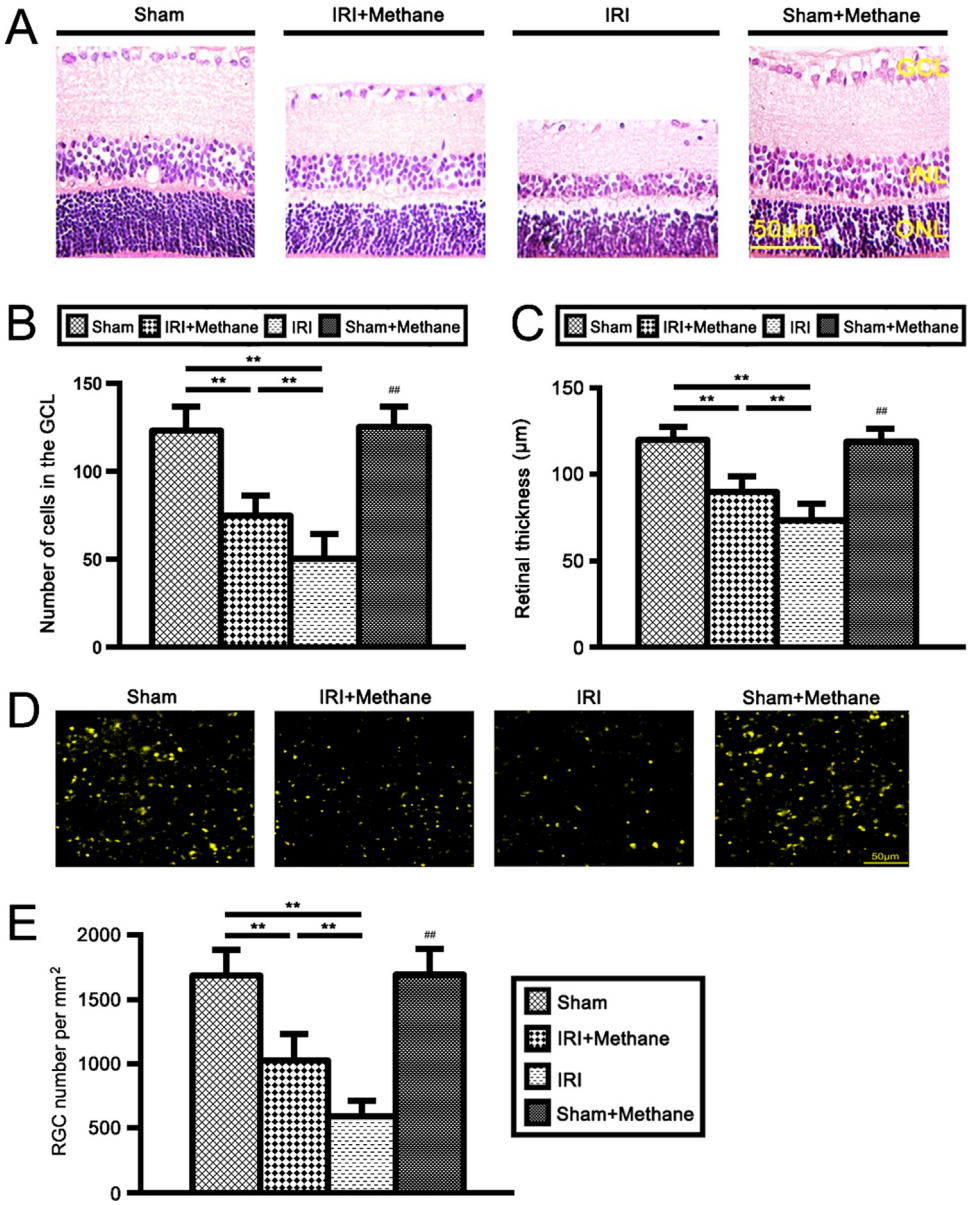

Fig. 1. Retinal histomorphometric evaluation after retinal IRI. (A) H&E staining of representative retinal sections at one week following IRI. (B) Cell number in the GCL of IRI + Methane group was significantly higher than that of IRI group ($^{**}p < 0.01$). (C) Retinal thickness of IRI + Methane group was significantly thicker than that of IRI group ($^{**}p < 0.01$). (D) Morphometry of RGCs in representative flat-mounted retinas by FluoroGold labeling. (E) The number of FluoroGold labeled RGCs was significantly higher than that of IRI group ($^{**}p < 0.01$). There was no significant difference between Sham group and Sham + Methane group ($^{\#\#}p > 0.05$).