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Protective effects of methane-rich saline on diabetic retinopathy via anti-inflammation in a streptozotocin-induced diabetic rat model

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ABSTRACT

As the commonest complication of diabetes mellitus (DM), diabetic retinopathy (DR) is a neuro-vascular disease with chronic inflammatory. Methane could exert potential therapeutic interest in inflammatory pathologies in previous studies. Our study aims to evaluate the protective effects of methane-rich saline on DR and investigate the potential role of related MicroRNA (miRNA) in diabetic rats. Streptozotocin-induced diabetic Sprague–Dawley rats were injected intraperitoneally with methane-rich or normal saline (5 ml/kg) daily for eight weeks. Morphology changes and blood-retinal barrier (BRB) permeability were assessed by hematoxylin eosin staining and Evans blue leakage. Retinal inflammatory cytokines levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL1-β) were evaluated by immunohistochemistry. Retinal protein expressions of glial fibrillary acidic protein (GFAP) and vascular endothelial growth factor (VEGF) were determined by western blotting. Retinal miRNA expressions were examined by miRNA-specific microarray, verified by quantitative RT-PCR and predicted by GO enrichment and KEGG pathway analysis. There was no significant changes in blood glucose level and body weight of diabetic rats with methane-rich or normal saline treatment, but the decreased retinal thickness, retinal ganglial cell loss and BRB breakdown were all significantly suppressed by methane treatment. DM-induced retinal overexpressions of TNF-α, IL-1β, GFAP and VEGF were also significantly ameliorated. Moreover, the methane treatment significantly up-regulated retinal levels of miR-192-5p (related to apoptosis and tyrosine kinase signaling pathway) and miR-335 (related to proliferation, oxidative stress and leukocyte). Methane exerts protective effect on DR via anti-inflammation, which may be related to the regulatory mechanism of miRNAs.

1. Introduction

Diabetic retinopathy (DR), the most prevalent microvascular complication of diabetes, is the leading cause of visual loss and blindness among working age adults in economically developed countries [1]. A number of factors are involved in the development of DR, including inflammation [2], oxidative stress [3], and apoptosis [4]. Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL1-β) are the pro-inflammatory cytokines of diabetes induced retinal neuroinflammation/neurodegeneration [5]. As a result of oxidative stress and inflammation, Glial fibrillary acidic protein (GFAP) (as a marker for astrocytes expression or Muller cells) increases in diabetic retina while the normal retina remains unchanged [6–8]. Now, the treatments of laser photocoagulation, vitrectomy, corticosteroids, and intraocular injection of anti-vascular endothelial growth factor (VEGF) which has been a standard therapy, are applicable only at advanced stages of the disease and are associated with significantly adverse effects [9,10]. Therefore, an effective treatment strategy that are preventive or can provide intervention in diabetes to delay or prevent the progression of DR are necessary.

MiRNA are an abundantly endogenous RNA, nonprotein-coding on average only 22 nucleotides, which are represent as critical transcriptional factors on regulating target mRNAs through sequence-specific base pairing with the 3′-untranslated region (3′-UTR) [11,12]. Binding to the 3′-UTR of downstream targets, usually resulting in translational repression and gene silencing [13]. So far,

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Table 1
Blood glucose levels and body weights of eight-week STZ-induced diabetic rats.

Group	n	Blood glucose (mmol/L)	Body weight (g)
Control	20	4.67 ± 0.56	393.00 ± 17.45
DM	20	27.79 ± 2.58**	292.23 ± 38.29**
DM-CH_4	20	28.76 ± 3.86**	267.29 ± 42.68**

STZ, Streptozotocin-induced; DM, diabetic mellitus; DM-CH_4, diabetic mellitus received methane-rich saline treatment; Values are means ± SD; **p < 0.01 versus Control.

over 1000 miRNAs have been verified by molecular cloning strategies and bioinformatic analysis [14]. MiRNAs are involved in the pathogenesis of DR through the modulation of multiple pathogenetic pathways [15]. Methane is the simplest alkane and the most abundant organic compound with the chemical formula CH_4 which has 87% volume component of natural gas [16]. Methane has the characteristics of free distribution which could penetrate the membranes and diffuse into the organelles [17]. Boros et al. [18] found that methane exerted the protective effects against the intestinal ischemic/reperfusion (IR) injury induced oxidative stress and inflammation. Ye Z et al. [19] found that methane attenuates the hepatic IR injury through anti-inflammatory and anti-apoptosis. Although the specific mechanisms and signaling pathways responsible for methane-induced protections remain poorly understood, the effect is obvious.

Thus, we used a DR rat model to evaluate the protective effect of methane treatment and investigate the potential mechanisms.

2. Materials and methods

2.1. Animals

Sixty adult male Sprague–Dawley rats weighing 180–200 g (Shanghai Slac Laboratory Animal CO. LTD, Shanghai, China) were used in our experiments. All animal protocols were performed in accordance with the Association for Research in Vision and Ophthalmology guidelines and approved by the Ethics Committee of Renji hospital affiliated to medical school of Shanghai Jiaotong University. All rats were housed under controlled conditions (22–24 °C, 12:12 h light/dark cycle) and provided with food and water ad libitum.

2.2. Induction of diabetes and experimental groups

Diabetes mellitus (DM) was induced by intravenous injection of streptozotocin (STZ) (60 mg/kg) in citrate buffer via tail vein. Only STZ-treated rats with blood glucose levels higher than 250 mg/dL at day 3 and day 7 after STZ injection were considered diabetic and included in the study. Blood glucose levels were measured by blood glucose meter (One Touch UltraEasy, USA) once a week after STZ administration until they were sacrificed. Body weights of rats were also monitored throughout the study. All rats were assigned randomly to three groups: (1) DM-CH_4 group: diabetic rats with intraperitoneal administration of methane-rich saline (5 ml/kg) once daily for 8 weeks after diabetic declared; (2) DM group: as the placebo group, diabetic rats with intraperitoneal administration of normal saline (5 ml/kg) once daily; (3) Control group: non-diabetic rats were only given sodium citrate in place of STZ.

2.3. Methane-rich saline production and determination of methane concentration

Pure methane stored in cylinder (Shanghai Jiliang Standard Gas Ltd., Shanghai, China) was dissolved in normal saline under high pressure (0.4 MPa) for 3 h to a supersaturated level. Then, the saturated methane-rich saline was stored under atmospheric pressure at 4 °C and was always freshly prepared one day before animal administration. As described in Ye Z et al. previous study [19], the methane concentrations of methane-rich saline, blood has been detected by gas chromatography (Gas chromatography-9860, Qiyang Co., Shanghai, China). Nevertheless, de-gassing of methane-rich saline administration is inevitable, the concentration of methane in blood retain effective.

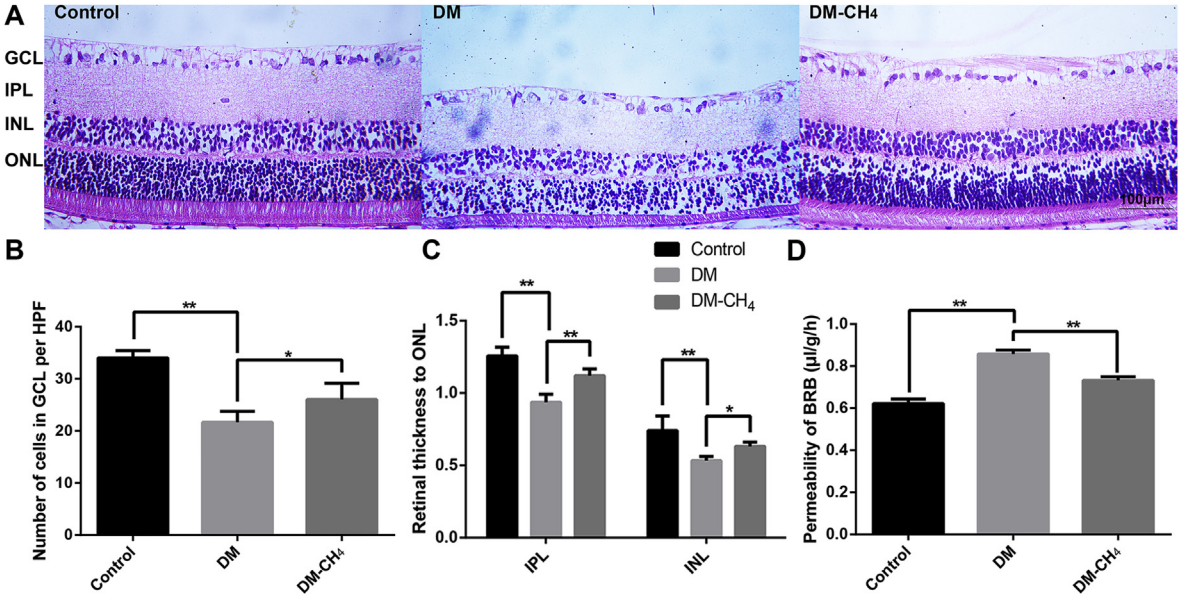

Fig. 1. Thickness of each retinal layer was measured in paraffin sections after HE staining and permeability of the BRB observed in rat retina. (A) Representative micrographs of the retina. (B) RGCs in the GCL were decreased in the DM, while the change was significantly improved in the DM-CH_4. (C) Relative thickness of IPL or INL which normalized to ONL was significantly reduced in the DM compared to the Control, but treatment with methane-rich saline could improve in DM-CH_4. (**p < 0.01, *p < 0.05, n = 6) (D) Permeability of the BRB observed in rat retina. (**p < 0.01, n = 3).