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RELEVANT VARIATIONS AND NEUROPROTECIVE EFFECT OF HYDROGEN SULFIDE IN A RAT GLAUCOMA MODEL

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Abstract—Glaucoma is an irreversible and blinding neurodegenerative disease of the eye, and is characterized by progressive loss of retinal ganglion cells (RGCs). Since endogenous hydrogen sulfide (H_2S) was reported to be involved in neurodegeneration in the central nervous system, the authors aimed to develop a chronic ocular hypertension (COH) rat model simulating glaucoma and therein test the H_2S level together with the retinal protein expressions of related synthases, and further investigated the effect of exogenous H_2S supplement on RGC survival. COH rat model was induced by cross-linking hydrogel injection into anterior chamber, and the performance of the model was assessed by intraocular pressure (IOP) measurement, RGC counting and retinal morphological analysis. Endogenous H_2S level was detected along with the retinal protein expressions of H_2S-related synthases cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST) in the COH rats. Retinal H_2S level and RGC survival were evaluated again after NaHS (a H_2S donor) treatment in the COH rats. The results showed that the COH model succeeded in simulating glaucoma features, and retinal H_2S level decreased significantly when the retinal protein expressions of CBS, CSE and 3-MST were downregulated generally in the COH rats. Furthermore, the decrease of retinal H_2S level and loss of RGCs were both improved by NaHS treatment in experimental glaucoma, without obvious variation of IOP. Our study revealed that the intracameral injection of cross-linking hydrogel worked efficiently in modeling glaucoma, and H_2S had protective effect on RGCs and might be involved in the pathological mechanism of glaucomatous neuropathy.

Key words: chronic ocular hypertension, glaucomatous neuropathy, retinal ganglion cells, hydrogel, hydrogen sulfide.

INTRODUCTION

Hydrogen sulfide (H_2S), long known for its characteristic odor of rotten eggs and toxic nature, was recently recognized as the third member of endogenous gasotransmitter family, along with carbon monoxide and nitric oxide (Abe and Kimura, 1996; Lowicka and Beltowski, 2007; Qu et al., 2008). This novel gaseous signaling molecule was reported to be implicated in physiological and pathological processes as diverse as neuroregulation, vasodilation, internal secretion, inflammation, etc. (Kaneko et al., 2006; Yang et al., 2008; Zhang and Bhatia, 2008; Papapetropoulos et al., 2009; Kimura, 2010; Tan et al., 2010; Tay et al., 2010). The endogenous H_2S in humans and other mammalians is mainly produced by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST) along with cysteine aminotransferase, which localized in various tissues with different distributions (Levonen et al., 2000; Meier et al., 2001; Sen et al., 2012; Kimura, 2014). So far as the ocular tissues were concerned, CBS, CSE and 3-MST were reported to be expressed in the retina of human as well as other mammalians, and take part in various retinal physiopathological processes (Persa et al., 2006; Pong et al., 2007; Mikami et al., 2011; Markand et al., 2013; Si et al., 2013).

Nowadays, the cytoprotective effect of H_2S is drawing accumulative interests of researchers to explore its therapeutic potentials. It was reported that H_2S played a role in neurodegenerative diseases in the central nervous system (CNS) (such as Alzheimer's disease and Parkinson's disease), presenting a decreased level in brain tissue and blood plasma, and the disease-associating symptoms and the biomolecular features

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Abbreviations: 3-MST, 3-mercaptopyruvate sulfurtransferase; CBS, cystathionine β-synthase; CNS, central nervous system; COH, chronic ocular hypertension; CSE, cystathionine γ-lyase; DAPI, 4′,6-diamidino-2-phenylindole; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethy-indocarbocyanin perchlorate; D-PBS, Dulbecco's phosphate-buffered saline; GCL, ganglion cell layer; H_2S, hydrogen sulfide; HCCS, HyStem Cell Culture Scaffold kit; INL, inner nuclear layer; IOP, intraocular pressure; IPL, inner plexiform layer; OD, optical density; ONL, outer nuclear layer; OPL, outer plexiform layer; RGCs, retinal ganglion cells; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling.

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were alleviated by application of exogenous supply of H_2S (Hu et al., 2010; Kamat et al., 2015). In the eye, it was discovered that the endogenous H_2S level in plasma and retina decreased in streptozotocin-induced diabetic retinopathy in rats, and treatment with exogenous H_2S donor, which increased the internal H_2S level, improved the impaired retinal structure and function reasonably (Si et al., 2013). Furthermore, supplement of H_2S was found to mediate therapeutic effect on retinal ischemia/reperfusion injury and NMDA-induced neuronal lesion (Biermann et al., 2011; Sakamoto et al., 2014).

Glaucoma is a leading cause of irreversible blindness worldwide, characterized by gradually impairing the visual field due to loss of retinal ganglion cells (RGCs) over time, in which the elevation of intraocular pressure (IOP) plays an essential role (Gupta et al., 2006; Quigley and Broman, 2006). Although the pathogenesis of glaucoma is still not fully understood yet, it is generally accepted that the apoptosis of RGCs and the secondary damage to the neighbor neurons lead to the impairment of visual function. Glaucoma is recognized as a neurodegenerative disease, and the oxidative stress, mitochondrial dysfunction and immune-inflammatory response were confirmed to be implicated in the pro-apoptotic mechanisms of RGCs in glaucoma (Pinazo-Duran et al., 2013), which were also the targets for the therapeutic potentials of H_2S. In the present study, we explored a new type of rat model of chronic ocular hypertension (COH) for evaluating the variations of endogenous level of H_2S together with the retinal protein expressions of CBS, CSE and 3-MST, and further assessed the protective effect of exogenous supplement of H_2S on RGCs in experimental glaucoma, trying to investigate the role of H_2S in the mechanism of glaucomatous neuropathy.

MATERIALS AND METHODS

Animals

All experimental procedures described here conformed to the National Institutes of Health guidelines for the Care and Use of Laboratory. The animal procedures were in accordance to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and with approval from the institutional review board of Ruijin Hospital, Shanghai, China. Male adult *Sprague-Dawley* rats weighing approximately 200–250 g were obtained from Shanghai Slack Laboratory Animals Ltd (Shanghai, China). The rats were fed *ad libitum* and maintained in an air-conditioned room at approximately 23 °C and 60% humidity, in a 12-h light–dark cycle for the duration of the experiments. All surgeries were performed under systemic anesthesia, and all efforts were made to minimize suffering. The details of number of animals used in separate experimental procedures are listed in Table 1.

Table 1. Number of animals used in separate procedures

Procedure	*N*	Procedure	*N*
Identification of purified RGCs	54	H_2S level measurement	24
CCK-8 assay	12	Immunohistochemistry staining	24
TUNEL assay	108	Western blotting	24
Intracameral injections for model building and IOP profile	60	H_2S level measurement (drug treated)	24
Retrograde labeling of RGCs	24	Retrograde labeling of RGCs (drug treated)	24
Hematoxylin–Eosin staining	24		

Induction of IOP elevation

Rats were anesthetized by intraperitoneal injection of xylazine 10 mg/kg (Sigma–Aldrich, St. Louis, MO) and ketamine hydrochloride 25 mg/kg (Sigma–Aldrich), respectively. Topical anesthesia to each eye was conducted by a drop of 0.5% proparacaine hydrochloride (Bausch & Lomb, Tampa, FL). The COH was induced in the right eyes of the rats. The IOP was elevated by injecting a pre-mixed *in situ* cross-linking hydrogel, HyStem Cell Culture Scaffold kit (HCCS; Sigma–Aldrich), into the anterior chamber. HCCS consisted of HyStem (a thiol-modified carboxymethyl hyaluronic acid) and Extralink (a thiol-reactive polyethylene glycol diacrylate), both dissolved in degassed water according to the manufacturer's instructions and mixed at the ratio of 4:1 immediately before the injection. The cross-linking gelation of the liquid mixture occurred *in situ* about five minutes in the anterior chamber. The anterior chamber puncture was performed in cornea from the peripheral area toward the central to create a long enough tunnel incision with a 31-gauge needle. Then a volume of 7 μL fresh liquid mixture of HyStem and Extralink was immediately injected into the anterior chamber through the incision with a Hamilton syringe (Hamilton Bonaduz AG, Switzerland). However, the left eyes were not determined as control eyes to avoid potential inflammatory reactions caused by contralateral COH eyes (Rojas et al., 2014) and to reduce the suffering of animals in consideration of humane care. Sham-operations, which were similar to the COH-inducing operations except for the injection of an equal volume of saline solution substitute for Hystem and Extralink mixture, were performed on the right eyes of else rats to be determined as control group and employed in all following experiments. A drop of 0.5% levofloxacin hydrochloride (Santen Pharmaceutical, Japan) was used for infection prevention after operations.

Drug administration

NaHS (5.6 mg/kg; Sigma–Aldrich) was injected intraperitoneally to treat rats, being started 3 days prior to the induction of COH and maintained for 4 weeks on a daily basis till the executions were done. Saline of the equivalent volume was used as treatment of control.

IOP measurement

IOP measurements were done under brief systemic anesthesia by isoflurane inhalation (2–4%;