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Protective effects and mechanism of tetramethylpyrazine against lens opacification induced by sodium selenite in rats☆

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ABSTRACT

Tetramethylpyrazine (TMP), extracted from the Chinese herbal medicine Ligusticum wallichii franchat (chuan xiong in Chinese), is a potent anti-free radical and calcium antagonist. Correspondingly, two important hypotheses in the causation of cataracts are free radical toxicity and calcium ion overload. In this study we investigated the effect of TMP on lens opacification induced by sodium selenite in rats, addressing the potential of TMP eye drops to prevent and treat cataracts. Results showed that the extent of lens opacification in the untreated Normal Control group (NC group) was significantly less than that of selenite-injected untreated rats (MC group) on days 3, 5, 7 and 10 ($p < 0.001$), while TMP treated selenite-injected rats (TMP group) had less lens opacification than the MC group on days 3, 5, 7 and 10 ($p < 0.05$). Compared with the NC group, the MC group had significantly decreased activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and catalase (CAT) and significantly elevated malondialdehyde (MDA) and calcium ion content ($p < 0.001$). Compared with the MC group, the activity of (SOD), (GSH-PX) and (CAT) were significantly higher while (MDA) and calcium ion levels were significantly lower in the TMP group at all time points ($p < 0.01$). The findings demonstrate that the selenite-induced cataract rat models were successfully built and the TMP eye drops can delay lens opacification induced by sodium selenite in rats. The mechanism by which TMP preserves lens transparency from selenite treated animals is associated with the lenses' ability to maintain normal levels of activity of SOD, GSH-PX and CAT and normal concentrations of MDA and calcium ion.

1. Introduction

A cataract, defined as any opacity in the lens in response to a variety of etiological factors, is the major cause of blindness in the world (Hiratsuka et al., 2001). This holds true in China and Latin America, where almost half of blindness and poor eyesight are caused by cataract (Huang et al., 2009; Liang et al., 2008; Limburg et al., 2008). So cataracts seriously impair vision and quality of life. Current cataract models include UV-induced cataract (Avetisov et al., 2008), glucocorticoid cataract (Ogiso et al., 1999), streptozotocin diabetic cataract (Shi et al., 2009), selenite cataract (Matsushima et al., 1997), galactose cataract (Ohta et al., 1999) and hereditary cataract (Yang et al., 2007). The selenite-induced nuclear cataract model is widely used since it can be quickly, easily and stably produced.

At present, the only effective treatment for advanced cataract is surgical removal and replacement of the cataract with an artificial intraocular lens. However, the expense and potential complications of cataract surgery call for development of pharmacological interventions to delay cataract onset and slow its progression. While the mechanism of cataract formation even now is not completely understood, oxidative damage is a significant factor (Lou, 2003; Truscott, 2005), making antioxidants promising agents to prevent oxidation-related cataractogenesis. Various antioxidants have been shown effective in experimental studies (Call et al., 2004; Kyselova et al., 2005; Varma et al., 2010).

Traditional Chinese medicine represents a worldwide resource for potential anti-cataract treatments. Recent studies show that tetramethylpyrazine (TMP), extracted from the Chinese herbal medicine Ligusticum wallichii franchat (chuan xiong in Chinese), is a significant anti-lipid peroxidation, anti-free radical, anti-apoptosis and calcium antagonist agent (Liu et al., 2005; Yang et al., 2008; Gao et al., 2008; Zhu et al., 2005). Our studies (Deng et al., 2006; He et al., 2007) indicate that TMP can penetrate both the ocular-surface barrier and the blood–aqueous barrier to reach the aqueous humor. Therefore, TMP is a potentially useful treatment for ocular

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and extraocular disorders. In the present study, we aimed to observe the effects and mechanisms of topical TMP against selenite-induced lens opacification.

2. Materials and methods

2.1. Experimental animal

We obtained 15-day-old Sprague–Dawley rats from the animal facility of the Sun Yat-sen University, Guangzhou, China. The experiments were conducted in compliance with both the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the EC Directive 86/609/EEC for animal experiments, and the study was approved by the Medical Ethics Committee belonging to Zhongshan Ophthalmic Center. Rats were reared under standard laboratory conditions (22 ± 2 °C, 60% ± 10% relative humidity and a 12-h light–dark cycle) and had free access to food and water throughout the experiment.

2.2. Induction of selenite cataract

Twenty-three 15-day-old rats were randomly divided into 4 groups: the untreated Normal Control group (5 rats, NC group); the Model Control group (6 rats, MC group) that were selenite-injected but untreated; TMP treated group (6 rats, TMP group) that were selenite-injected and treated with TMP (Batch No. 090516. M.W. 172.94, Nuotai Chemical Co. Ltd., Shanghai, China) eye drops (0.3% HPMC and 0.2%TMP, PH = 7.0); and the glutathione (GSH) treated group (6 rats, GSH group) that were selenite-injected and treated with glutathione eye drops (2% GSH, Batch No. 090401, ISEI Company Inc., Yamagata, Japan; pH = 5.30). According to the method used previously (Elanchezhian et al., 2009), 30 μmol/kg body weight sodium selenite (CAS No. 10102188, Sigma Chemical Co., St Louis, MO) was subcutaneously injected into the posterior region of necks of rats of the MC group, TMP group and GSH group; rats in the NC group were given the same amount of 0.9% saline in the same location.

2.3. Drug administration

Immediately after injection of sodium selenite and for the following 10 days, the right eyes of rats in these four groups were given corresponding eye drops: the NC group and the MC group were given 0.3% hydroxypropyl methylcellulose (Batch No. UL31012N03, HPMC, Dow Chemical Co., Midland, MI) eye drops; the TMP group rats were given TMP eye drops and the GSH group rats were given glutathione eye drops. Each rat was administered 5 μl eye drops at 8:00 a.m., 12:00 a.m., 4:00 p.m. and 8:00 p.m.

2.4. Slit-lamp microscope examination

Twenty minutes after mydriasis with 5 μl compound tropicamide eye drops (0.5%, Batch No. 090202, Beijing Double-Crane Pharmaceutical Co., Ltd., Beijing, China), at twice with 5 min interval, the right lenses of all rats ($n = 23$) were observed under slit-lamp microscope on days 0, 3, 5, 7 and 10, respectively. The extent of lens opacification was graded according to the method reported by Hiraoka and Clark (1995) as described in Table 1.

2.5. Sample preparation

Rats were sacrificed with an overdose of anesthesia with chlorpromazine (50 mg/kg) on day 10. The right lens of each rat was removed, weighed and added to nine times its mass of 0.9% ice-cold saline (pH = 7.2), and then homogenized by a handheld Tissue Tearor™ (PT 1200 C, JETPHARMA, SA, Balerna, Switzerland) at 10000 rpm totally for 2 min in ice bath, with an interval of 15 s after every homogenization for 30 s. And finally centrifuged (Zentrifugen D-78532, Hettich GmbH, Tuttlingen, Germany) at 2790 g for 10 min at 4 °C to obtain a clear supernatant.

Table 1
Grading standard of the extent of lens opacification in rats induced by sodium selenite.

Stage	Graded standard	Score
0	A normal transparent lens	0
I	The initial sign of nuclear opacity	1
II	A slight nuclear opacity	2
III	A diffuse nuclear opacity with cortical scattering	3
IV	A partial nuclear opacity	4
V	A nuclear opacity without lens cortex	5
VI	Mature cataract of entire lens	6

2.6. Absorbance values measured by photometry

All chemicals and reagents used in biochemical measurement were purchased from Jiancheng Bioengineering Institute (Nanjing, China) and the absorbance values were detected by a spectrophotometer (UV-1700, Shimadzu Corp., Kyoto, Japan) with ultra-micro cuvettes (105.201-QS, Hellma GMBH, Müllheim, Germany).

The activity of super-oxide dismutase (SOD) was measured with xanthine oxidase method according to instructions of the SOD assay kit. The wavelength was set at 550 nm and the results were expressed as U/mg protein. The activity of glutathione peroxidase (GSH-PX) was measured with modified glutathione exhaustion assay according to instructions of the GSH-PX assay kit, and the resulting yellow was read spectrophotometrically at 412 nm. The results were expressed as U/mg protein. The activity of catalase (CAT) was detected with ammonium molybdate method, and the faint yellow complexes were read at 405 nm. The results were described as U/mg protein if 1 U was defined as dismutation of 1 μmol hydrogen peroxide per sec. The content of malondialdehyde (MDA) was detected with barbituric acid reaction chromometry with reference to the MDA assay kit. The wavelength was set at 532 nm, and the results were expressed as nmol/mg protein. The calcium ion concentration was determined with methyl-thymol blue method using the Ca^{2+} assay kit, and the resulting yellow

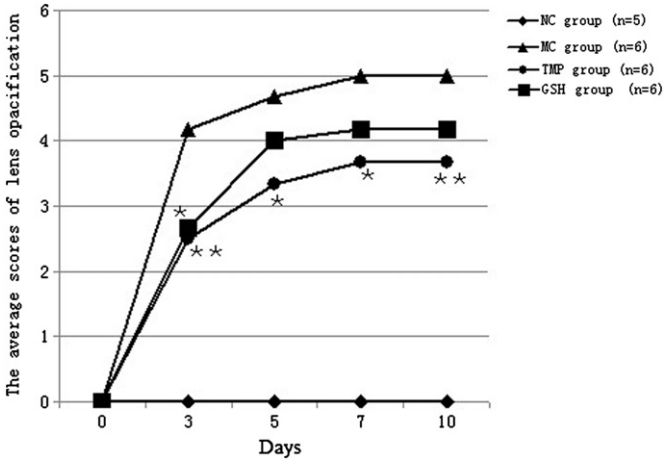

Fig. 1. The average scores of lens opacification for different groups on days 3, 5, 7 and 10. $^{*}p < 0.05$ or $^{**}p < 0.01$ (Tetramethylpyrazine [TMP] treated [TMP group] or glutathione [GSH] treated selenite-injected rats [GSH group] vs. untreated selenite-injected rats [MC group]).