Experimental Eye Research 143 (2016) 9–16

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Research article

Topical *trans*-resveratrol ameliorates steroid-induced anterior and posterior segment changes in rats

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ARTICLE INFO

Article history:
Received 16 April 2015
Received in revised form
1 September 2015
Accepted in revised form 24 September 2015
Available online 28 September 2015

Keywords:
Glaucoma
Intraocular pressure
Resveratrol
Retina
Steroid-induced
Trabecular meshwork

ABSTRACT

Steroid-induced hypertension and glaucoma is associated with increased extracellular meshwork (ECM) deposition in trabecular meshwork (TM). Previous studies have shown that single drop application of *trans*-resveratrol lowers IOP in steroid-induced ocular hypertensive (SIOH) rats. This IOP lowering is attributed to activation of adenosine A1 receptors, which may lead to increased matrix metalloproteinase (MMP)-2 activity. This study evaluated the effect of repeated topical application of *trans*-resveratrol for 21 days in SIOH animals on IOP, changes in MMP-2 level in aqueous humor, trabecular meshwork and retinal morphology and retinal redox status. We observed that treatment with *trans*-resveratrol results in significant and sustained IOP reduction in SIOH rats. This IOP reduction is associated with significantly higher aqueous humor total MMP-2 level; significantly reduced TM thickness and increased number of TM cells. Treatment with *trans*-resveratrol also significantly increased ganglion cell layer (GCL) thickness, the linear cell density in the GCL and inner retina thickness; and significantly reduced retinal oxidative stress compared to the SIOH vehicle-treated group. In conclusion, repeated dose topical application of *trans*-resveratrol produces sustained IOP lowering effect, which is associated with increased level of aqueous humor MMP-2, normalization of TM and retinal morphology and restoration of retinal redox status.

1. Introduction

Glaucomatous neuropathy, often associated with elevated intraocular pressure (IOP), is the leading cause for irreversible blindness due to apoptotic loss of retinal ganglion cells (RGCs) (Resnikoff et al., 2004). Topical and systemic use of steroids is associated with ocular hypertension, which may progress to glaucoma. One of the studies has shown that topical steroid treatment for vernal conjunctivitis leads to ocular hypertension in 28.3% of treated children, of which 5.5% progressed to glaucoma (Ang et al., 2012). Steroid-induced hypertension and glaucoma have become an area of interest due to significant increase in the incidence of steroid-induced ocular hypertension (SIOH) resulting from the increased use of steroids for ocular and systemic diseases (Garbe et al., 1997; Razeghinejad and Katz, 2012). SIOH and glaucoma are currently treated in the same way as the ocular hypertension and primary open angle glaucoma (POAG). However, currently used medications often provide a suboptimal reduction in IOP and are not known to have significant neuroprotective effects on RGCs (Vasudevan et al., 2011). In our previous studies, we investigated *trans*-resveratrol (3,5,4-tryhydroxystilbene) for IOP lowering activities. Resveratrol is a dietary polyphenols found in grapes, wine, peanuts, pines and many other plants. This phenolic plant compound exists as two geometric isomers, *trans*-resveratrol and *cis*-resveratrol. The former is shown to be biologically more active compared to its corresponding *cis*-isomer (King et al., 2006). We demonstrated that in rats with SIOH, topical administration of single drop of *trans*-resveratrol results in significant IOP reduction

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http://dx.doi.org/10.1016/j.exer.2015.09.014
0014-4835/

that peaks at 90 min post-instillation. Based on the area under curve (AUC) of time versus response curve, we observed that within the range of 0.05–0.35%, *trans*-resveratrol produces maximum IOP reduction at 0.2% concentration and the significant IOP reduction lasts for 12 h post-instillation (Razali et al., 2015b). Since this study evaluated effects of single drop application of *trans*-resveratrol, it remains to be determined if repeated dose application of *trans*-resveratrol over prolonged period can produce sustained IOP reduction.

Furthermore, by using adenosine receptor subtype specific antagonists, we observed that IOP lowering effect of *trans*-resveratrol in steroid-treated rats is mediated through adenosine A1 receptors (Razali et al., 2015b). Earlier studies have demonstrated that activation of adenosine A1 receptors at the trabecular meshwork (TM) leads to increased matrix metalloproteinases (MMP)-2 activity (Shearer and Crosson, 2002). MMPs are extracellular matrix (ECM) degrading enzymes and studies have shown that increased activity of MMPs enhances aqueous humor (AH) outflow and reduced activity of MMP-2 in the AH is associated with increased trabecular meshwork resistance in all types of glaucoma (Bradley et al., 1998; Maatta et al., 2002). Although in previous study we demonstrated adenosine A1 receptor-mediated IOP lowering effect of resveratrol, it remained unclear whether this effect is associated with significant changes in AH level of MMP-2. Additionally, in another study, we have demonstrated that prolonged topical administration of dexamethasone in rats is associated with significant morphological changes in trabecular meshwork and retina and increased retinal oxidative stress (Razali et al., 2015a). Hence, it is of significant interest to investigate whether prolonged administration of resveratrol can protect against trabecular meshwork and retinal morphological changes and can reduce the retinal oxidative stress.

Hence, in this study firstly we evaluated the IOP lowering effect of multiple drop application of *trans*-resveratrol over a period of 3 weeks in rats with SIOH. Secondly, we investigated if the IOP lowering effect of *trans*-resveratrol is associated with changes in MMP-2 levels in AH. Thirdly, we studied the effects of 3-week long administration of *trans*-resveratrol on trabecular meshwork morphology, retinal morphology and retinal oxidative stress.

2. Materials and methods

2.1. Animals

All procedures in this study complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the local regulatory and animal ethics requirements. *Sprague–Dawley* rats of either sex were maintained under standard laboratory conditions of 12-h cycle of light and dark and had access to pellet food and water ad-libitum. Animals found normal on general and ophthalmic examination were included in this study. All animals were acclimatized for one week to daily handling and tonometry.

2.2. Study design

Three groups of animals consisting of 24 animals each (48 eyes) were used. Group 1 consisted of normotensive rats that received polyvinylpyrrolidone (PVP) 3% (Sigma chemicals; Catalog no. 9003-39-8). Group 2 consisted of animals with experimentally elevated IOP that received PVP 3%. Group 3 also consisted of animals with experimentally elevated IOP, however, the animals in this group were treated with *trans*-resveratrol 0.2% (Sigma chemicals; Catalog no. R5010). All animals received treatment bilaterally, topically, twice-daily for 3 weeks. The treatment to all animals was administered at 7.00 AM and 7.00 PM daily. During 3 weeks of experimental period, all animals were subjected to IOP estimations twice weekly at 8.30 AM (90 min post-instillation) and before instillation at 7.00 PM (12 h post-instillation) using TonoPen XL. The choice of 0.2% concentration of resveratrol and twice daily frequency of treatment was based on our previous study that showed 12-h long IOP lowering effect of single drop application of resveratrol 0.2%. This IOP lowering effect of *trans*-resveratrol 0.2% was found to the significantly higher than all other concentrations evaluated within the range of 0.05–0.35% (Razali et al., 2015b). The timing of IOP estimation was also based on our previous study to coincide with peak and trough effects of single drop application of resveratrol on IOP (Razali et al., 2015b). During the entire experimental period, animals were also observed on a daily basis for adverse effects including circumciliary congestion and miosis.

At the end of 3 weeks treatment period, the animals were sacrificed using overdose of ketamine (50 mg/kg) and xylazine (5 mg/kg) intraperitoneally. Pooled aqueous humor from 2 eyes was gently collected as one sample and total MMP2 contents were quantified using ELISA (aqueous humor from 12 eyes gave $n = 6$). The same eyes were then enucleated for histological examination of trabecular meshwork ($n = 6$) and retina ($n = 6$). The retinal oxidative stress was estimated by quantifying SOD ($n = 6$), CAT ($n = 6$) and GSH ($n = 6$) by pooling samples from 2 eyes as one sample for each of the parameter, as was the case with MMPs estimation in aqueous humor.

2.3. Tonometry

IOP measurements were carried out using applanation tonometer (Tonopen XL), which was calibrated according to manufacturer's instructions. The IOP measurements were carried out in conscious rats after topical application of 0.5% propacaine hydrochloride as described previously (Moore et al., 1993). Prior to starting the experiment, the inter-observer and intra-observer variations in IOP measurements were determined. The IOP readings presented in this paper are mean of observations made by two independent and blinded investigators.

2.4. Experimental model

Experimental induction of IOP elevation was achieved in rats by topical instillation of steroids as described in our previous studies (Razali et al., 2015a,b). Briefly, single drop of dexamethasone 0.1% (Alcon Labs) was instilled in both eyes twice a day for 40 days. IOP measurements were carried out at day 0 as the baseline, once weekly for 2 weeks and twice a week subsequently during dexamethasone instillation. Oculohypertensive animals included for further studies were those showing IOP rise of more than 25% from baseline.

2.5. Preparation of trans-resveratrol for topical application

As described previously, *trans*-resveratrol 0.2% was prepared in a 3% (W/V) aqueous solution of PVP and filtered using a 0.22 μm Milipore filter (Razali et al., 2015b). The solution was prepared fresh every 3 days. Preparations were made in a dark room and kept in tubes wrapped with aluminium foil to prevent any alteration to the trans-resveratrol.

2.6. Collection of AH and estimation of total MMP-2

The eyes were washed with normal saline and dried. The cornea was punctured with a 30G needle and AH was collected with a micropipette. Estimation of total MMP-2 in the AH was based on standard sandwich ELISA (ABNOVA, Taiwan) using rat MMP-2 specific polyclonal antibodies. Further analysis was carried out