Experimental Eye Research 145 (2016) 401–411

Contents lists available at ScienceDirect

Experimental Eye Research

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

The elevation of intraocular pressure is associated with apoptosis and increased immunoreactivity for nitric oxide synthase in rat retina whereas the effectiveness of retina derived relaxing factor is unaffected

Selçuk Takır [a, c, *], Ebru Gürel-Gürevin [b], Ayça Toprak [a], Cihan Demirci-Tansel [b], B. Sönmez Uydeş-Doğan [a]

[a] Department of Pharmacology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey
[b] Department of Biology, Faculty of Science, Istanbul University, Istanbul, Turkey
[c] Department of Medical Pharmacology, Section of Internal Medical Sciences, Faculty of Medicine, Giresun University, Giresun, Turkey

ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form
5 February 2016
Accepted in revised form 2 March 2016
Available online 4 March 2016

Keywords:
Intraocular pressure
Glaucoma
Apoptosis
NOS
Retinal relaxing factor
Retinal artery
Mesenteric artery
Retina

ABSTRACT

Glaucoma is a progressive ocular disease that stands in the upper rank for the cause of blindness in worldwide. In the present study, we aimed to elucidate the possible disturbances occurred in the layers of retina due to an increase in intraocular pressure (IOP) and to verify the effectiveness of retina derived relaxing factor, i.e., RRF in this pathologic condition. The increase in IOP was induced by cauterization of the three of episcleral veins simultaneously in rats. After 8 weeks period, the retinas excised from the vein cauterized eyes were evaluated for the possible histopathological and ultrastructural alterations as well as for the relaxing effects on isolated bovine retinal and rat mesenteric arteries, in comparison with the retinas obtained from contralateral sham-operated eyes. In the retinas of IOP-elevated eyes, profound morphological deteriorations were determined in the ganglion and outer nuclear cell layers which were associated with an increased number of TUNEL positive cells in the ganglion and inner nuclear cell layers. Increased immunohistochemical stainings for three isoforms of nitric oxide synthase (NOS) were defined in almost all layers of the retinas of IOP-elevated eyes, in which eNOS was abundant particularly in the inner plexiform and ganglion cell layers. An irregular basal folding of retinal pigment epithelium (RPE) and an increased inter lamellar space of photoreceptor cell layer furtherly characterized the prominent degeneration of those layers in the retinas of IOP-elevated eyes. On the other hand, the relaxing effects of the retina obtained from IOP-elevated eyes were determined to be unchanged on the retinal and mesenteric arteries precontracted either with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 30 μM) or potassium chloride (K^+, 100 mM), when compared with the relaxations of control retina obtained from contralateral sham-operated eyes. Overall, these findings suggested that the elevation of IOP induces prominent structural changes in rat retina particularly in the ganglion and inner layers that is associated with marked apoptosis and increased immunoreactivity for NOS, while the functional effectiveness of retina derived relaxing factor, i.e., RRF is unaffected.

1. Introduction

Glaucoma, which stands in the upper rank for the cause of total visual loss (blindness) in worldwide, is a progressive ocular disease associated with an excavation in optic nerve head (ONH) and a degeneration in retinal ganglion cells (RGC) (Tham et al., 2014). An increase in the intraocular pressure (IOP) is suggested to induce the excavation in ONH, which is a well-recognized major clinical feature in glaucoma. This disturbs the axonal transport and triggers the degenerative processes in ganglion cells via apoptosis; whereas, the exact mechanism is still poorly understood (Almasieh et al., 2012; Quigley et al., 1995). In regard to the degeneration of RGC, different mechanisms have been reported to mediate this

* Corresponding author. Giresun University, Faculty of Medicine, Department of Medical Pharmacology, Section of Internal Medical Sciences, 28200 Giresun, Turkey.
E-mail address: selcuk_takir@yahoo.com (S. Takır).

http://dx.doi.org/10.1016/j.exer.2016.03.002
0014-4835/

impairment which is reliably verified in several experimental studies (Chen et al., 2011; Guo et al., 2005; Quigley et al., 1995). However, other studies have implicated a damage in the inner layers of the retina, as well (Pelzel et al., 2006; Wygnanski et al., 1995).

A decrease in the diameter of retinal vessels is also proposed to be a contributory defective mechanism in glaucoma (Wang et al., 2007), which probably induces homeostatic changes in the retinal blood flow (Grieshaber and Flammer, 2005; Venkataraman et al., 2010). In relation, the retinal arterial tone is suggested to be altered due to an imbalance between several vasoactive factors released from the retina and/or the endothelial layer of the retinal vessels (Grieshaber and Flammer, 2005; Venkataraman et al., 2010). Among these substances, endothelin-1, nitric oxide (NO), N-methyl-D-aspartate (NMDA), adenosine and calcitonin gene related peptide (CGRP) were reported to play a crucial role in the local regulation of ocular blood flow as well as in the maintenance of IOP (Ghanem et al., 2011; Grieshaber and Flammer, 2005; Venkataraman et al., 2010). A novel relaxing factor derived from the retina namely, retinal relaxing factor (RRF), has recently been identified to influence the retinal arterial tone by producing a substantial relaxation and thereby, decreasing the contractions to various spasmogens (Delaey and Van de Voorde, 1998; Takir et al., 2011, 2015). Inspite of several experimental studies that attempted to describe its nature and mechanism of action, the vascular effectiveness of RRF in pathological conditions which can unfavourably affect the retina and/or the retinal circulation is still scarce. Of note, among the ocular diseases, glaucoma is proposed to develop morphological and functional abnormalities in the retina as well as in the retinal vasculature (Grieshaber and Flammer, 2005; Venkataraman et al., 2010). Accordingly, in the present study, we aimed to evaluate the probable differences in the vasorelaxant influence of retina obtained from the eyes in normal and disease conditions, particularly related to the elevation of IOP by the episceral vein cauterization model in rats. We also attempted to investigate the retinal degeneration initiated by the elevation of IOP via determining the alterations occurred in the histological and ultrastructural pattern of retinal layers as well as specifying the contribution of apoptosis and nitric oxide synthase (NOS).

2. Material and methods

Male Wistar albino rats with an average age of 10–12 weeks (200–250 g) were used in the experiments. Animals were housed under standard temperature of 20 ± 2 °C and humidity of 55–60% on a 12:12 h light/dark cycle. Rats were prepared for the experimentation by daily handling for at least one week before starting to the surgical procedures and measurements of IOP. Overall, 51 rats were used in the experiments. 4 of the rats were used in the preliminary experiments to set the sustained increase in IOP with the episcleral vein cauterization method. While, 5 of the rats could not achieved a sustained elevation in the IOP for 8 weeks period and thus excluded from the experiments. 14 of the rats were used in histological, immunohistochemical and electron microscopy analyses and 28 of the rats were used in functional experiments performed in the myograph. All experimental and animal care procedures utilized were in accordance with the European Community and the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and approved by Local Animal Experimentation Ethics Committee of Istanbul University (16/02/2010, Decision No: 36).

2.1. Induction of ocular hypertension in rats

Induction of ocular hypertension (glaucoma) was performed on the right eyes of the rats that were anaesthetized with intraperitoneal injection of xylazine hydrochloride (Rompun®, Bayer, Turkey, 5 mg/kg) and ketamine hydrochloride (Ketalar®, Pfizer, Turkey, 50 mg/kg). After a 2–3 mm of conjunctival incision, one temporal and two dorsal episcleral veins located near the superior and lateral rectus muscles of the eyes were cauterized by a hand-held low temperature cautery (Advanced Meditech International, New York, USA) (Shareef et al., 1995). Care was taken to avoid thermal damage to the sclera. The contralateral left eyes were sham-operated and served as the corresponding control. Both eyes were flushed with saline and treated with ophthalmic gentamisin (Genta®, İ.E. Ulagay, Turkey, 0.3%) drop after the surgery. The mechanism of pressure elevation induced by episcleral veins cauterization involves obstruction of aqueous humour outflow and in part congestion in ocular vasculature, which might disturb overall venous circulation and cause the deterioration of choroidal, retinal as well as ONH perfusion (Goldblum and Mittag, 2002; Grozdanic et al., 2003; Morrison et al., 2015; Pang and Clark, 2007). Although, the retinal blood vessels were reported to appear normal following to the cautery procedure as assessed by funduscopic examination (Sawada and Neufeld, 1999), it is uncertain to what degree the retinal pathology may be affected by the vascular congestion and/or decreased blood flow, apart from the elevation of IOP in this experimental model. On the other hand, the advantages of the episceral vein cautery model is that the increase in IOP is rapid, stable, and long lasting, and the procedure is less invasive, efficacious, easy accessible, and induces no complications in the anterior chamber (Grozdanic et al., 2003; Ishikawa et al., 2015; Neufeld et al., 1999; Urcola et al., 2006).

2.2. Measurement of IOP

IOP was measured in both eyes of the rats under anaesthesia. A drop of proparacaine HCl (0.5%) was applied to each eye to desensitize the cornea before performing the measurements by a calibrated hand-held tonometer (Tono-Pen Avia, Reichert Technologies, New York, USA). To eliminate the probable influence of anaesthesia on IOP, the measurements were performed within 2 min. The tonometer was applied perpendicularly to the cornea. Five consecutive readings were taken for each eye and then averaged. The initial IOP values in rats eyes were established immediately before and after the cauterization or sham-operation process. Thereafter, the successive IOP measurements in both cauterized and sham-operated eyes were performed on the 1st, 4th and 7th days after the operation and then, once a week for the following 8 weeks (56 days) period. All measurements were performed between 10:00–12:00 a.m. in order to avoid a circadian variation in IOP readings.

2.3. Histological studies

The rat retinal tissues obtained from both the normal (control) and vein cauterized eyes were used for the histological evaluations. At the end of 8 weeks (56 days) period, the retinas were carefully excised from the control (sham-operated) and vein cauterized eyes, which displayed a sustained increase in IOP, then fixed in 10% neutral formalin for 24 h, dehydrated through graded alcohol and finally embedded in paraffin blocks. The paraffin-embedded sections (4 μm in thickness) were stained with Haematoxylin and Eosin (H&E) and further examined under a light microscope (Leica Microsystems, Mannheim, Germany). Histological images were obtained by using the Kameram 390 CU Software (Mikro Sistem