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Anterior and posterior segment changes in rat eyes with chronic steroid administration and their responsiveness to antiglaucoma drugs

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ABSTRACT

Steroid-induced ocular hypertension (SIOH) is associated with topical and systemic use of steroids. However, SIOH-associated anterior and posterior segment morphological changes in rats have not been described widely. Here we describe the pattern of intraocular pressure (IOP) changes, quantitative assessment of trabecular meshwork (TM) and retinal morphological changes and changes in retinal redox status in response to chronic dexamethasone treatment in rats. We also evaluated the responsiveness of steroid-pretreated rat eyes to 5 different classes of antiglaucoma drugs that act by different mechanisms. Up to 80% of dexamethasone treated animals achieved significant and sustained IOP elevation. TM thickness was significantly increased and number of TM cells was significantly reduced in SIOH rats compared to the vehicle-treated rats. Quantitative assessment of retinal morphology showed significantly reduced thickness of ganglion cell layer (GCL) and inner retina (IR) in SIOH rats compared to vehicle-treated rats. Estimation of retinal antioxidants including catalase, superoxide dismutase and glutathione showed significantly increased retinal oxidative stress in SIOH animals. Furthermore, steroid-treated eyes showed significant IOP lowering in response to treatment with 5 different drug classes. This indicated the ability of SIOH eyes to respond to drugs acting by different mechanisms. In conclusion, SIOH was associated with significant morphological changes in TM and retina and retinal redox status. Additionally, SIOH eyes also showed IOP lowering in response to drugs that act by different mechanisms of action. Hence, SIOH rats appear to be an inexpensive and noninvasive model for studying the experimental antiglaucoma drugs for IOP lowering and neuroprotective effects.

1. Introduction

Glaucoma, a leading cause of irreversible blindness, is characterized by loss of retinal ganglion cells (RGC), changes in the optic disc and loss of visual fields (Foster et al., 2002). Steroid-induced glaucoma, a form of secondary glaucoma, is known to be associated with topical and systemic use of steroids. Due to widespread use of steroids, steroid-associated ocular hypertension has become a special area of interest on its own as well as for providing relevant information applicable to other types of glaucoma such as primary open angle glaucoma (POAG).

Corticosteroids are believed to decrease the aqueous humor outflow as they increase the deposition of extracellular matrix within the trabecular meshwork (TM), particularly in the juxtacanalicular region, thereby increasing the resistance to aqueous humor outflow (Wilson et al., 1993; Clark et al., 1995). Steroids also cause changes in the TM cell morphology, TM cell population and their functions further contributing to increased TM resistance (Wilson et al., 1993; Matsumoto and Johnson, 1997; Clark et al., 1996; Clark and Wordinger, 2009). Such ultrastructural changes in TM in response to steroid treatment have been demonstrated in human and rabbit studies (Spaeth et al., 1977; Ticho et al., 1979; François et al., 1984; Johnson et al., 1997). However, the quantitation of associated retinal morphological changes and whether they are consistent with glaucomatous changes have not been investigated widely. This is particularly important in glaucoma-related investigations as the focus is now shifting from IOP lowering strategies to neuroprotective approaches. We undertook this study, firstly, to

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determine the pattern of IOP changes in response to chronic topical application of dexamethasone in rats. Secondly, we quantitatively analyzed the morphological changes in the TM and retina of the same animals and estimated the changes in retinal redox status. Thirdly, we studied the pattern of IOP changes in steroid-pretreated rats in comparison with normotensive rats after administering 5 established IOP lowering drugs belonging to 5 different classes in order to determine the responsiveness of steroid-treated eyes to drugs acting by different mechanism of action and hence their suitability as an animal model for investigating IOP lowering drugs. Since the TM and retinal changes observed in response to steroid treatment have resemblance to those in POAG, it seems logical to investigate suitability of steroid-treated animals as a model to study pathophysiological aspects of other types of glaucoma as well as for investigating neuroprotective effects of experimental drugs.

2. Materials and methods

2.1. Animals and study design

Sprague–Dawley rats of either sex were housed under standard laboratory conditions and were provided normal pellet diet and tap water ad libitum. All procedures complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research as well as the local regulatory and ethical requirements. All animals were subjected to general and ophthalmic examination and those found normal were included in this study. Animals were acclimatized for one week to daily handling and tonometry.

This experimental work was carried out in two separate sets of studies. In study 1, we evaluated the effect of chronic administration of topical dexamethasone on the morphology of TM and retina and retinal oxidative stress. In study 2 we evaluated the responsiveness of steroid-induced ocular hypertension (SIOH) to 5 different drug classes.

2.2. Study 1

2.2.1. Effect of chronic topical instillation of dexamethasone on IOP

10 *Sprague–Dawley* rats ($n=20$ eyes) of either sex weighing 80–100 g were topically treated with 10 μl of dexamethasone 0.1% (Alcon Labs) bilaterally, twice a day. IOP measurements were done at day 0 as the baseline, once a week for 2 weeks and subsequently twice a week. For IOP measurement, applanation tonometer was used in conscious rats after topical application of 0.5% propacaine hydrochloride as described previously (Moore et al., 1993). All IOP measurements were carried out by two blinded investigators and mean of the 2 was considered the final estimate. On day 40 of the topical steroid treatment, animals showing a rise of IOP amounting to at least 20% above baseline were subdivided into two groups. One of the groups continued to receive topical dexamethasone instillation until day 62 while for the other group topical steroid treatment was discontinued on day 40 to observe the effect of steroid withdrawal on IOP.

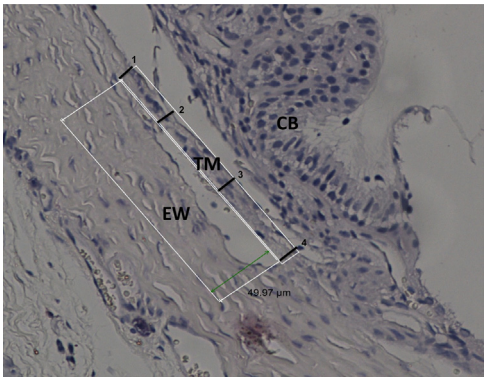

Fig. 1. Anterior chamber angle of a normal *Sprague–Dawley* rat. Lines 1–4 indicate the position of TM thickness measured. TM – trabecular meshwork; EW – external wall contacting the outer wall of the Schlemm's canal; CB – ciliary body. EW is the area 50 μm from the Schlemm's canal's connection with the TM.

2.2.2. Effect of steroid-induced ocular hypertension on TM and retinal morphology and retinal oxidative stress

Two groups of 15 animals each were used for this study. One of the groups consisted of 15 animals that had achieved an IOP rise of at least 20% from baseline after dexamethasone treatment, as described above, for 60 days. The other group consisted of the same number of normal rats that similarly received normal saline for 60 days.

On day 60 of the treatment, all animals in both groups were killed using overdose of ketamine (50 mg/kg) and xylazine (5 mg/kg) intraperitoneally and eyes were enucleated. In each group, one of the eyes from 6 randomly chosen animals was used for histopathological examination of TM ($n=6$) and retina ($n=6$). From the remaining 24 eyes in each group, retinae were isolated. In each group 4 retinae from 4 different animals were pooled to obtain a total of 6 samples per group to estimate the parameters of retinal oxidative stress.

2.2.2.1. TM morphology. The enucleated eyeballs were immediately fixed in 10% formalin. The eyeballs were then cut at midline from the optic nerve to the cornea to obtain 2 eyecups per eyeball. Both eyecups were paraffin embedded and capitally sectioned (3 μm thick) using microtome and stained with Haematoxylin and Eosin (H&E). The histopathological features of trabecular meshwork were examined by two pathologists using light microscope (Nikon) and the mean of two observations was considered as the final estimate.

The TM tissue thickness was measured at four different points: (1) 50 μm anterior to the top of Schlemm's canal, (2) at the top of Schlemm's canal, (3) midway of the canal, and (4) at the posterior end of the canal (Fig. 1). The thickness was measured perpendicular to the inner layer of uveal meshwork (Dietlein et al., 2000). Mean of the four different points represented the trabecular meshwork thickness. In addition, the number of cells in the TM and external wall contacting the outer wall of Schlemm's canal (EW) in the selected area (Fig. 1) was manually counted under 20× magnification. The mean of three readings from three separate slides was taken as a representative value for each specimen.

2.2.2.2. Retinal morphology and assessment of retinal ganglion cell loss. Retinal morphometric estimations were done as described previously by Takahata et al. (2003). Histological sections of formalin-fixed, paraffin-embedded retinae at 1 mm from the temporal edge of the optic disc were stained by H&E and viewed under light microscope. Microscopic images of 5 randomly selected fields of view from each section were calibrated at 40× magnification, saved in jpg format and evaluated with the aid of an image-analysis system (Nikon, Japan) in a masked fashion independently by 2 observers with subsequent estimation of the inter-observer and intra-observer differences. Each morphometric parameter was measured with the use of image-analysis software (Image Pro Plus 7.0, Media Cybernetics, USA).

According to common practice, no attempt was made to distinguish the types of RGC and displaced amacrine cells (Lam et al., 1999; Takahata et al., 2003). Morphologically distinguishable glial cells, vascular endothelial cells or any other cells with the diameter of the nucleus less than 7 μm were excluded from the cell count