Experimental Eye Research 93 (2011) 397–404

Contents lists available at ScienceDirect

Experimental Eye Research

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

Similar hydrodynamic and morphological changes in the aqueous humor outflow pathway after washout and Y27632 treatment in monkey eyes

Zhaozeng Lu [a], Yuyan Zhang [a], Thomas F. Freddo [b], Haiyan Gong [c,*]

[a] Huashan Hospital of Fudan University, Shanghai, PR China
[b] University of Waterloo, Waterloo, Ontario, Canada
[c] Department of Ophthalmology, Boston University School of Medicine, 715 Albany Street, Room L914, Boston, MA 02118, USA

ARTICLE INFO

Article history:
Received 14 October 2010
Accepted in revised form 27 May 2011
Available online 6 June 2011

Keywords:
monkey eye
aqueous outflow facility
Y27632
washout
Schlemm's canal
juxtacanalicular tissue
trabecular meshwork
light microscopy
electron microscopy
confocal microscopy

ABSTRACT

Our previous studies in bovine eyes demonstrated that the structural correlate to the increase in outflow facility after either Rho-kinase inhibitor Y-27632 (Y27) treatment or washout appeared to be separation between the juxtacanalicular tissue (JCT) and inner wall (IW) of the aqueous plexus, the bovine equivalent of Schlemm's canal (SC). While these findings suggest that Y27 and washout may increase outflow facility through a similar mechanism, the anatomy of bovine outflow pathway differs considerably from both the human and monkey outflow pathway; however, only the human eye does not exhibit washout. In light of this, we compared the effects of Y27 and washout on outflow facility, hydrodynamic patterns of outflow, and the morphology of the IW and JCT in monkey eyes, given that their anatomy is closer to human eyes.

Twelve freshly enucleated monkey eyes were used in this study. Eyes were perfused with Dulbecco's PBS containing 5.5 mM glucose (GPBS) to establish a baseline facility at 15 mmHg. Four eyes were perfused for a short-duration (30 min) as a control, 4 eyes for a long-duration (180 min) to induce washout, and 4 eyes with GPBS+50 μM Y27 for 30 min. All eyes were then perfused with fluorescent microspheres (0.5 μm; 0.002%) to label the hydrodynamic patterns of outflow and then perfusion-fixed. Confocal images of frontal sections were taken along the IW of SC. The total length (TL) and the tracer-decorated length (FL) of the IW were measured to calculate the average percent effective filtration length (PEFL = FL/TL). Sections with SC were examined by light and electron microscopy. The TL of the IW and the length exhibiting separation (SL) in the JCT were measured to calculate the average percent separation length (PSL = SL/TL).

Outflow facility increased 149.2% ($p < 0.01$) from baseline after washout during long-duration perfusion, and 114.9% ($p = 0.004$) after Y27 treatment, but did not change significantly after short-duration perfusion in control eyes ($p = 0.46$). Distribution of the tracer labeling appeared punctate along the IW of control eyes, while a more uniform pattern was observed after washout and Y27 treatment. PEFL in washout (83.4 ± 2.1%) and Y27 treated eyes (82.5 ± 1.6%) was 3.4-fold larger compared to controls (24.2 ± 4.2%, $P < 0.001$). The JCT appeared distended with loss of connections between JCT cells and between JCT cells and their extracelluar matrix in eyes with washout or after Y-27 treatment. PSL in the JCT was 2.3-fold larger in washout eyes (77.4 ± 3.3%) and 2.2-fold larger in Y27 treated eyes (75.2 ± 5.3%) versus controls (33.5 ± 5.3%, $p = 0.001$). Significant positive correlations were found between outflow facility and PEFL, facility and PSL and between PEFL and PSL.

Our data demonstrated that similar hydrodynamic and morphological changes occurred in the aqueous humor outflow pathway of monkey eyes after induction of washout and Y27 treatment. Both Y27 and washout increase outflow facility by redistributing aqueous outflow through a larger area in the JCT. These hydrodynamic changes are likely driven by morphologic changes associated with a decrease in cell–cell and cell–matrix connections in the JCT.

1. Introduction

The mechanism responsible for the generation of aqueous humor outflow resistance remains unknown in both normal and glaucomatous human eyes. The elevated intraocular pressure (IOP)

* Corresponding author. Tel.: +1 617 638 4434; fax: +1 617 638 5337.
E-mail address: hgong@bu.edu (H. Gong).

0014-4835/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2011.05.012

associated with primary open-angle glaucoma (POAG) occurs due to a malfunction or impairment of aqueous humor filtration within the trabecular meshwork[Epstein 1987; Johnson and Erickson, 2000; Lutjen-Drecoll, 1999]. Lack of a thorough understanding of the mechanism involved in regulating trabecular outflow resistance has hindered the development of an effective anti-glaucoma therapy that aims at increasing trabecular outflow. Although the mechanisms involved in governing outflow resistance are unclear, several studies have localized the primary resistive site to a region including the juxtacanalicular tissue (JCT) and inner wall endothelium of Schlemm's Canal (SC)[Grant 1958; 1963; Maepea and Bill, 1989; 1992].

Drugs that disrupt the cytoskeleton or its contractility have been shown to reduce aqueous humor outflow resistance and IOP. Such drugs include cytochalasin[Johnson 1997; Kaufman and Erickson, 1982; Tian et al., 1999; Tian and Kaufman, 2005], latrunculin [Ethier et al., 2006; Sabanay et al., 2006], H-7[Bahler et al., 2004; Sabanay et al., 2004], HA 1077[Honjo et al., 2001a,b] and Y27632 [Honjo et al., 2001a,b; Rao et al., 2001; Waki et al., 2001], and are therefore important candidates for the next generation of glaucoma therapy. Y27632 (Y27) is a protein kinase inhibitor selective for Rho-associated kinase (ROCK) isoforms ROCK-I and ROCK-II[Davies et al., 2000; Ishizaki et al., 2000; Uehata et al., 1997], which regulate the phosphorylation of the regulatory myosin light chain (MLC) to promote actomyosin-driven cell contractility. Inhibiting ROCK with Y27 decreases MLC phosphorylation by promoting MLC-phosphatase activity[Kaibuchi et al., 1999; Rosenthal et al., 2005], leading to cell relaxation and disassembly of actin stress fibers and focal adhesions in many cell types[Rao et al., 2001, 2001], including human trabecular meshwork and Schlemm's canal endothelial cells in vitro[Honjo et al., 2001a,b].In our previous study in bovine eyes, perfusion with 50 μM Y27 significantly increased outflow facility [Lu et al., 2008]. This result appeared to coincide with separation between the JCT and inner wall, which positively correlated with an increase in effective filtration length along the inner wall of SC.

"Washout" is the progressive increase in aqueous outflow facility observed during prolonged perfusion of non-human eyes[Barany and Scotchbrook, 1954; Barany and Woodin, 1955; Erickson-Lamy et al., 1990; Melton and DeVille, 1960; Van Buskirk and Brett, 1978; Yan et al., 1991]. Interestingly, the structural changes associated with the increase in outflow facility during washout were found to be similar to those after Y27 treatment (i.e. associated with separation between the JCT and inner wall, which was positively correlated with the increase in effective filtration length[Overby et al., 2002; Scott et al., 2007, 2009]). These findings suggest that inner wall/JCT separation may be a critical morphological mechanism for drug-induced increase in outflow facility. Similarly, Y27 and washout may share a common mechanism in increasing outflow facility.

Our previous studies in bovine eyes suggest a similar mechanism for the increase in outflow facility after washout and Y27 treatment [Lu et al., 2008; Scott et al., 2009]; however, the morphology of the bovine and human outflow pathways is different. The bovine eye has aqueous plexus in place of Schlemm's canal and a reticular meshwork rather than a trabecular meshwork as seen in the human eye[Johnson et al., 1990]. While the monkey eye is morphologically similar to the human eye[Epstein and Rohen, 1991; Hashimoto and Epstein, 1980; Toris et al., 2000], the physiology of the monkey outflow apparatus is comparable to the bovine outflow apparatus because they both exhibit washout[Erickson and Kaufman, 1981; Overby et al., 2002; Scott et al., 2007], whereas the human eye does not exhibit washout[Erickson-Lamy et al., 1990; Gong and Freddo, 2009; Scott et al., 2007]. This suggests that the functional anatomy of the human outflow apparatus is physiologically unique when compared with other species that do exhibit a washout effect, including the monkey. The human eye, when compared with bovine and monkey eyes, has a more developed elastic-like cribriform plexus extending from the tendons of the ciliary muscle to the endothelial cells of the inner wall of Schlemm's canal[Gong et al., 1996; Gong et al., 1989; Rohen et al., 1981]. This elastic-like network may provide structural support that inhibits inner wall/JCT distention and contribute to the regulation of steady outflow resistance and may be responsible for the lack of washout in human eyes[Erickson-Lamy et al., 1990]. In light of this, we investigated whether previously observed similarities in bovine eyes treated with Y27 or subjected to washout would differ in the monkey eye, which is significantly closer to the overall anatomy of the human outflow pathway. We hypothesized that Y27 and washout decrease outflow resistance through a similar mechanism by weakening the inner wall/JCT connectivity, inducing the separation between the inner wall and JCT and increasing the available outflow area through the resistive tissue of the JCT and the inner wall. To test our hypothesis, fluorescent microspheres and confocal microscopy were used to visualize the change in the hydrodynamic patterns of outflow after Y27 treatment or induced washout. After confocal microscopy examination, the same tissue was examined using light and electron microscopy to investigate how the facility-increasing effect correlated with changes in the hydrodynamic filtration patterns and morphology within the JCT and inner wall of the monkey eyes.

2. Materials and methods

2.1. Materials

Twelve enucleated rhesus monkey eyes were obtained from 1) Harvard Medical School New England Regional Primate Research Center, Southborough, MA; 2) University of Wisconsin, Laboratory Animal Center, Madison, WI and delivered on ice within 18 h after death. Eyes with any discernible damage or accumulated blood in the limbal area or anterior chamber were discarded. Dulbecco's phosphate-buffered saline (Life Technologies, Grand Island, NY) containing 5.5 mM D-glucose (GPBS) was used as mock aqueous humor in all perfusion. Y27632, a selective Rho-Kinase Inhibitor, was obtained from Calbiochem, San Diego, CA (Lot#: B64617). All studies adhered to the ARVO Statement for the Use of Animals and Human Parts in Ophthalmic and Vision Research and were in compliance with Boston University guidelines.

2.2. Perfusion procedure

The mechanical setup of the perfusion system was described previously[Sit et al., 1997a,b]. Briefly, the perfusion system consists of a perfusion chamber and a collection chamber. The perfusion chamber was linked to a pressure transducer connected electronically by a computer control system (Macintosh G4; Apple Computers, Cupertino, CA). Outflow facility ($C = Q/\mathrm{IOP}$) was measured at 10 Hz, averaged, and electronically recorded every 10 s by LabView version 7.0 (National Instrument, USA).

Monkey eyes were cleaned of extraocular tissue and submerged up to the limbus in Dulbecco's phosphate-buffered saline at 34 °C. A 23-gauge infusion needle was inserted intracamerally through the peripheral transparent cornea into each eye and connected to the perfusion chamber. Anterior chamber deepening, which can cause an artificial increase in outflow facility, was prevented by inserting the needle tip into the posterior chamber. A second 23-gauge needle was inserted intracamerally into the anterior chamber of each eye and connected to the collection reservoir. During perfusion the collection reservoir tube was clamped. All eyes were perfused at constant pressure (15 mmHg) with GPBS for at least 30 min to