Experimental Eye Research 169 (2018) 79–90

Contents lists available at ScienceDirect

Experimental Eye Research

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

The effects of graded intraocular pressure challenge on the optic nerve head

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ABSTRACT

Intraocular pressure (IOP) is an important risk factor for glaucoma, and the response of the ONH and surrounding tissues to elevated IOP are often investigated to better understand pathophysiology. In vivo structure including that of the optic nerve head (ONH) and surrounding tissue of the eye are often assessed using optical coherence tomography (OCT). With advances in OCT technology, both large vessels and capillaries can be imaged non-invasively (OCT Angiography). Because a significant portion of retinal thickness is comprised of vasculature, the purpose of the current study was to investigate OCT structural and vascular changes in healthy non-human primate eyes with systematic graded increases and decreases in IOP. Six healthy animals with no previous experimental intervention were used. The pressure in the anterior chamber was adjusted from 10 mmHg to 60 mmHg and back to 10 mmHg in 10 mmHg steps every 10 min. Using optical coherence tomography (OCT), retinal nerve fiber layer (RNFL) thickness, minimum rim width (MRW), Bruch's membrane opening (BMO) size and relative height, anterior lamina cribrosa surface (ALCS) depth, choroidal thickness, and angiography (OCTA) were quantified. With IOP challenge there were significant changes in all morphological measures quantified ($p < 0.01$) other than BMO size ($p = 0.30$) and RNFL thickness ($p = 0.29$). Specifically, the position of the BMO was sensitive to both an increase and decease in IOP. The inner retinal capillary density gradually decreased with increasing IOP, reaching statistical significance when pressure exceeded 50 mmHg, but returned when IOP was reduced. The average choroidal thickness around the ONH decreased for elliptical annuli 500–1000 μm and 1000–1500 μm, from the BMO, with increasing IOP ($p < 0.01$). For the 1000–1500 μm annulus, choroid thickness did not return to baseline with IOP reduction. Similarly, the MRW decreased with increase in IOP, but with pressure reduction did not return, and at the final 10 mmHg time point was thinner than at baseline ($p < 0.01$). The results from this experiment illustrate differences in ONH neural rim tissue, RNFL and vessel density changes with acute IOP challenge. Overall, vessel collapse could not completely account for changes in RNFL or ONH MRW thickness. The study supports the hypothesis neural rim compression may be an important part of IOP-induced damage.

1. Introduction

The glaucomas are progressive optic neuropathies with characteristic losses of visual function and structural alterations in the retina and optic nerve head (ONH). The pathophysiology of glaucoma is not understood completely, but intraocular pressure (IOP) has been identified as an important risk factor in all clinical forms (Anderson, 2003; Gordon et al., 2002; Leske et al., 2004; Quigley and Addicks, 1980). High IOP seems integral to the pathology of glaucoma, and the effects of IOP on posterior segment structures have been studied and modeled in healthy and glaucomatous eyes using both *in vivo* and *ex vivo* preparations.

From early experiments that involved both acute and sustained elevation of IOP in a primate model, the ONH was identified as the primary site for disturbances in axonal transport (Anderson and Hendrickson, 1974; Quigley and Anderson, 1976, 1977). Subsequently, in enucleated eyes implanted with platinum wire, displacement of the lamina was shown with increase in IOP (Levy and Crapps, 1984; Levy et al., 1981). As non-invasive *in vivo* imaging technologies evolved, the ONH and surrounding tissues have been quantified with increasing precision and accuracy, and the effects of acute changes in IOP on load bearing structures of the posterior segment investigated in healthy and disease eyes (Burgoyne et al., 1994, 1995; Fortune et al., 2009; Heickell et al., 2001; Ivers et al., 2016; Morgan et al., 2002; Quigley and Pease, 1996; Sharma et al., 2017; Strouthidis et al., 2011). These studies, along with histological evidence, have identified the peripapillary connective tissue, lamina cribrosa, and neural tissue as structures susceptible to IOP related stress (Burgoyne, 2011; Downs, 2015; Sigal et al., 2014).

Because OCT based peripapillary retinal nerve fiber layer (RNFL) thickness and ONH neural rim tissue measures, sample a majority of the

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https://doi.org/10.1016/j.exer.2018.01.025
Received 17 July 2017; Received in revised form 27 January 2018; Accepted 30 January 2018
Available online 01 February 2018
0014-4835/

retinal ganglion cell (RGC) axons, they are commonly used for the clinical diagnosis and management of glaucoma patients. In principle glaucomatous RGC axonal loss should result in proportional losses of the two measures, but ONH neural retinal rim measures have been shown to thin prior to RNFL in both longitudinal and cross sectional studies (Fortune et al., 2013; He et al., 2014; Patel et al., 2014). The discrepancy between ONH neural rim tissue thinning and that of RNFL could be due to differences in axonal characteristics, glial tissue, extracellular matrix or vasculature.

Recent advances in optical coherence tomography (OCT) imaging allow for non-dye based angiography (OCTA) of the major retinal vasculature and capillary networks. Using OCTA technology a decrease in peripapillary vascular density in both glaucoma patients and glaucoma suspects has been shown (Akil et al., 2017; Chen et al., 2016; Lee et al., 2016, 2017; Rao et al., 2017; Suh et al., 2016; Yarmohammadi et al., 2016). Although OCTA does not directly quantify flow, it provides useful insight to when blood flow has reduced beyond the capability of OCTA algorithms to detect.

The non-human primate has similar structure to that of humans, with similar relationships between ONH neural rim tissue and RNFL thickness (Patel et al., 2014), hence ideal for investigations of structural change with IOP and experimental glaucoma. IOP in this experimental model often ranges between 25 and 45 mmHg, and at these moderate to high IOPs, it is possible that capillary networks can be compressed adding variability to studies investigating rates of change in nerve fiber layer and capillary densities. However, changes in OCTA based peripapillary and ONH capillary density with IOP challenge remains unknown in primate eyes. Hence, the purpose of the present study was to quantify both structural and vascular changes, and the relationship between the two, in healthy non-human primate eyes with systematic, controlled graded increase and return in IOP. Some of the results of these studies have been presented in abstract form (Patel et al. ISER/BrightFocus Glaucoma Symposium, 2017).

2. Methods

2.1. Subjects

A total of six (five males and one female) rhesus monkeys (*Macaca mulatta*) with an average age of 5.2 yrs were subjects in the study (Table 1). One randomly selected healthy eye of each animal, with no prior experimental manipulation, was used for data collection and analysis (4 right eyes and 2 left eyes). In addition to the cannulation experiment, each animal was re-evaluated at least two weeks after the initial experiment to assess overall ocular health. Experimental and animal care procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Houston. The use of animals for these experiments confirmed to National Institutes of Health guidelines for the care and use of laboratory animals.

2.2. Animal preparation

Animals were anesthetized with an intramuscular injection of ketamine (20–25 mg/kg/hr) and xylazine (0.8–0.9 mg/kg/hr), administered at hourly intervals, and treated with a subcutaneous injection of atropine sulfate (0.04 mg/kg). Atropine sulfate was re-administered if the heart rate fell below 75 beats per minute, at a dose of 0.02 mg/kg. Throughout the experiment, body temperature was monitored and maintained at 37° Celsius using a thermal blanket (TC 1000 temperature controller CWE, Ardmore, PA). Heart rate and pulse were monitored continuously using a pulse oximeter, while blood pressure was monitored every 5 min using an automated inflatable cuff (petMAP + II, Ramsey Medical, Tampa, FL). The animal's pupils were dilated with 1% tropicamide. To prevent infection, 5% ophthalmic betadine (Alcon Laboratories, Fort Worth, TX) was applied to the eyelids, instilled on the ocular surface, and subsequently washed off with sterile balanced salt solution (BSS, Alcon Laboratories, Fort Worth, TX) after a period of two minutes. The head of the animal was stabilized using mouth and occipital bars. A sterile eyelid speculum was used to keep the eye open, and a plano power rigid gas permeable contact lens was placed on the eye to prevent corneal dehydration and maintain optical clarity throughout the experiment. Following anterior chamber cannulation and completion of data collection, the contact lens and needle were removed, and topical antibiotics (polymyxin B/trimethoprim and moxifloxacin) were instilled on the eye.

2.3. IOP control

The anterior chamber was accessed using a 27G butterfly needle, inserted approximately 0.5–1 mm from the temporal limbus and extending up to 2 mm into the anterior chamber. The needle was connected to a pressure control system with sterile microtubing filled with BSS. The pressure control system (Fig. 1) included a capacitive pressure transmitter (Keller PR-41X, Keller America, Newport News, VA) coupled with a syringe pump (Cole-Parmer, Vernon Hills, IL) that was monitored through a MATLAB (The Mathworks, Natick, MA) program (5 Hz sampling). To ensure accurate pressure control, the pressure sensor and syringe pump were adjusted to the same height as the cannulated eye. For this experiment, the initial IOP was set at 10 mmHg, and the eye was scanned after a 10 min duration at this pressure. Subsequently, the IOP was increased to 20 mmHg, and the eye was scanned again after 10 min. This increase in pressure by 10 mmHg and scanning pattern was continued up to 60 mmHg, after which the IOP was decreased in 10 mmHg steps to the baseline of 10 mmHg. At each setting, the eye was scanned after pressure had stabilized for the 10 min.

2.4. Optical coherence tomography

All OCT scans were acquired using beta version software (SP-X1601), on the Spectralis OCT2 system (Heidelberg Engineering, Heidelberg, Germany). Prior to scan acquisition at each pressure setting, a 20° vertical and horizontal section through the ONH was used to align the optics of the system to minimize tilt of the b-scan. Two scan protocols were used for ONH quantification, and all scans in each series were acquired using the auto-rescan feature to minimize noise in measurement from variable centration. For ONH structural analysis, a 24-line, 20° radial scan centered on the ONH, with averaging of 20

Table 1
Age, and biometric measures of the six subjects prior to anterior chamber cannulation. The mean arterial pressure median and range is for the duration of the imaging experiments.

Subject	Age (years)	Corneal Curvature (D)	Central Corneal Thickness (μm)	Lens Thickness (mm)	Axial Length (mm)	Anterior Chamber Depth (mm)	Mean Arterial Pressure (mmHg), median and range
Sub 1	4.1	50.65	443	3.53	19.68	3.38	89 (85–100)
Sub 2	5.3	55.06	419	3.33	17.68	3.30	93 (87–110)
Sub 3	5.2	53.10	460	3.38	19.52	3.70	88 (83–95)
Sub 4	5.2	55.43	447	3.52	18.37	3.57	85 (71–88)
Sub 5	5.1	55.64	477	3.31	19.10	3.71	84 (78–87)
Sub 6	6.3	49.57	472	3.23	19.48	3.46	85 (82–92)