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The effect of age on the response of retinal capillary filling to changes in intraocular pressure measured by optical coherence tomography angiography

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ABSTRACT

Purpose: To compare the effect of elevated intraocular pressure (IOP) on retinal capillary filling in elderly vs adult rats using optical coherence tomography angiography (OCTA).

Methods: The IOP of elderly (24-month-old, $N = 12$) and adult (6–8 month-old, $N = 10$) Brown Norway rats was elevated in 10 mmHg increments from 10 to 100 mmHg. At each IOP level, 3D OCT data were captured using an optical microangiography (OMAG) scanning protocol and then post-processed to obtain both structural and vascular images. Mean arterial blood pressure (MAP), respiratory rate, pulse and blood oxygen saturation were monitored non-invasively throughout each experiment. Ocular perfusion pressure (OPP) was calculated as the difference between MAP for each animal and IOP at each level. The capillary filling index (CFI), defined as the ratio of area occupied by functional capillary vessels to the total scan area but excluding relatively large vessels of > 30 μm, was calculated at each IOP level and analyzed using the OCTA angiograms. Relative CFI vs IOP was plotted for the group means. CFI vs OPP was plotted for every animal in each group and data from all animals were combined in a CFI vs OPP scatter plot comparing the two groups.

Results: The MAP in adult animals was 108 ± 5 mmHg (mean ± SD), whereas this value in the elderly was 99 ± 5 mmHg. All other physiologic parameters for both age groups were uniform and stable. In elderly animals, significant reduction of the CFI was first noted at IOP 40 mmHg, as opposed to 60 mmHg in adult animals. Individual assessment of CFI as a function of OPP for adult animals revealed a consistent plateau until OPP reached between 40 and 60 mmHg. Elderly individuals demonstrated greater variability, with many showing a beginning of gradual deterioration of CFI at an OPP as high as 80 mmHg. Overall comparison of CFI vs OPP between the two groups was not statistically significant.

Conclusions: Compared to adults, some, but not all, elderly animals demonstrate a more rapid deterioration of CFI vs OPP. This suggests a reduced autoregulatory capacity that may contribute to increased glaucoma susceptibility in some older individuals. This variability must be considered when studying the relationship between IOP, ocular perfusion and glaucoma in elderly animal models.

1. Introduction

Glaucoma, the second leading cause of blindness in the world (Bourne et al., 2016), involves both structural and functional damage to the axons of the optic nerve head (ONH) and their retinal ganglion cells (RGC) (Quigley, 2011). Elevated intraocular pressure (IOP), a widely recognized risk factor for this disease, has long been considered to contribute to optic nerve damage through biomechanical as well as vascular mechanisms (Liang et al., 2009; Roberts et al., 2010; Yang et al., 2017). However, to date, the exact relationship between these two mechanisms and their contribution to glaucomatous optic nerve damage is still unknown.

Age is another significant risk factor for glaucoma (Chrysostomou et al., 2010; Gordon et al., 2002; Leske et al., 2003). Tissue changes that accompany advancing age have been argued to contribute to glaucoma through biomechanical as well as vascular mechanisms (Burgoyne,

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2011; Coudrillier et al., 2012; Downs, 2015). Increases in stiffness of the connective tissues of the ONH could alter biomechanical responses to elevations in IOP that may increase axonal vulnerability. Additionally, age-related increases in laminar beam and basement membrane thickness may retard nutrient delivery from capillaries to axons within their nerve fiber bundles. Better understanding of these possibilities would benefit greatly from improved insights into the effects of IOP on vascular perfusion and how these may be affected by the aging process.

As a functional extension of optical coherence tomography (OCT), OCT angiography (OCTA) (Wang et al., 2010, 2007) is a technology that can generate 3D images of dynamic microcirculation within the whole retina without a need for contrast agents. Its high resolution and label-free features make OCTA a useful tool for studying glaucoma for pre-clinical as well as clinical studies. In earlier work, we used optical microangiography (OMAG), an OCTA imaging method, to demonstrate comparable responses to elevated IOP in the retina and ONH (Zhi et al., 2012). However, this work used a 1300 nm-centered light source that, while allowing penetration into the ONH, lacked the resolution needed to visualize details of capillary beds. With subsequent improvements, including use of a central wavelength of 840 nm, we later performed quantitative evaluations of capillary filling responses to a stepwise elevation of IOP, which allowed us to demonstrate, using 6 month old animals, preservation of retinal capillary bed filling down to an ocular perfusion pressure of 40 mmHg, which we interpreted as a manifestation of autoregulation (Zhi et al., 2015).

In this study, we used OMAG/OCT to evaluate the effect of elevated IOP on retinal capillary bed filling and compared the results obtained in young adult animals (age 6–8 months) to elderly animals (age 24 months). Because of the close anatomic association between the anterior optic nerve head and retinal vasculature (Morrison et al., 1999) and the comparable responses to elevated IOP between these two vascular beds (Zhi et al., 2012), we believe that observations acquired with this system have relevance for the glaucomatous process and how vascular factors may contribute to this disease in the elderly population.

2. Material and methods

2.1. System setup and animal preparation

The system setup used in this study has been previously described in detail (Zhi et al., 2012). Briefly, it is a customized SD-OCT system using a super luminescent diode (SLD) light source with central wavelength of 840 nm and the spectral bandwidth of 42 nm. The lateral and axial resolutions are ~15 μm and 7.2 μm, respectively. The total depth range is measured to be ~2.5 mm in air, and is estimated to be about 1.85 mm in tissue. The power of the OCT beam at the cornea is ~1.2 mW, providing a system sensitivity of ~100 dB. The phase stability of the system was measured at ~4 mrad. Integrated with the system is an apparatus used to alter IOP via anterior chamber cannulation with a tube connected to a reservoir filled with balanced salt solution and to a calibrated pressure transducer.

To investigate the association between age and retinal capillary filling upon the elevated IOP, we used 10 adult (6–8 month old) and 12 elderly (24-month old) Brown Norway rats in this study. All protocols conformed to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the Animal Care and Use Committees of the Oregon Health and Science University and the University of Washington.

The rats were first anesthetized with 5.0% inhalational isoflurane mixed with pure oxygen, which was then reduced to 2.0–3.0% for the period of IOP elevation and data acquisition. An active pump was employed to vent expired isoflurane and CO_2 (Zhi et al., 2015, 2012). Rectal temperature was monitored continuously and maintained at 38 °C with a recirculating water pad. Arterial blood pressure was monitored by tail cuff manometry CODA (Kent Scientific Corporation, Northwest Connecticut, USA). Other physiologic parameters, including percent oxygen saturation of functional arterial hemoglobin, heart rate, respiratory rate, and pulse and respiratory distention were monitored continuously during each experiment via a rat foot sensor and MouseOxPlus Oximeter (STARR Life Sciences Corporation, Oakmont, PA, USA).

The animal was positioned on a platform with six degrees of adjustment to facilitate the proper positioning under the OCT system. The pupil of the right eye was dilated with 1% tropicamide (Bausch & Lomb Inc.), and 0.5% proparacaine hydrochloride instilled for added corneal anesthesia. Following an initial 31 gauge needle track through the peripheral cornea, the anterior chamber was cannulated with a 1 in. long polyurethane tubing with inner and outer diameters of 0.005 and 0.010 in., respectively (Instech laboratories, Plymouth Meeting, PA). This cannula was connected via a larger polyurethane tubing (Component Supply Co.) to a reservoir filled with balanced salt solution (Morrison et al., 2016; Zhi et al., 2012) (BSS Plus, Alcon Laboratories Inc.) and to a calibrated pressure transducer, which was calibrated against an external manometer at the beginning of each experiment and IOP was monitored periodically using a TonoLab tonometer (Icare Finland Oy, Espoo, Finland). This entire system has also been separately tested to confirm that inflow pressure determined by this transducer delivers the indicated intraocular pressure using a separate cannula and transducer (Morrison et al., 2016). Topical BSS was applied intermittently to maintain corneal hydration throughout the course of each experiment. By adjusting reservoir height, IOP was increased from 10 to 100 mmHg in 10 mmHg increments, and then lowered to 10 mmHg directly. Following a 2-minute interval at each IOP level for stabilization, an OMAG data volume was captured for later processing to determine 3D retinal structure and microvascular filling.

2.2. OMAG data acquisition

Each data volume was captured using our OMAG scanning protocol (Wang et al., 2010; Zhi et al., 2012), which provided a field of view of 2.2 × 2.2 mm^2 (approximately 32°) over the fundus that includes the ONH. The protocol performed raster scanning of the probe beam. In the fast scanning axis, 512 A-lines were captured to form a B-frame (2D cross-sectional image). The B-frame was repeated 8 times at each spatial position. In the slow scanning axis, there were 400 equally spaced positions. Altogether, 3200 B-frames were captured for each 3D scan. For imaging, the frame rate of the system was 250 Hz, i.e. 250 frames per second (fps). Therefore, ~13 s were required to capture each 3D data volume for later data processing.

2.3. Microvascular imaging and capillary filling index

The 3D raw data were post-processed to generate two OCT volumes by using the OMAG algorithm (An et al., 2010a; Wang et al., 2010; Yousefi et al., 2011), one of which represents tissue structure, the other retinal vasculature. Briefly, at each spatial position (i.e. cross-section), the phase-compensation algorithm (An et al., 2010b) was applied to the eight repeated B-frames to minimize motion artifacts; then the complex signals were subtracted between adjacent B-frames, and consequently averaged to obtain final OMAG cross-sectional blood flow images. The 3D vascular image was obtained by processing the data volume at all the 400 positions. In parallel, OCT structural image was also obtained as the conventional OCT approach, but with improved signal to noise ratio since 4-repeated B-scans are available for averaging due to the OMAG scanning protocol.

To better quantitate the microvascular response to elevated IOP, we first used a semi-automated retinal layer segmentation software (Yin et al., 2014) to separate the retinal layer from the choroidal layer through identifying the anterior surface of the retina, i.e., inner limiting membrane (ILM), and retinal pigment epithelium (RPE) in the 3D