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Prolonged elevation of intraocular pressure results in retinal ganglion cell loss and abnormal retinal function in mice

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ABSTRACT

The purpose of this study was to assess the impact of prolonged intraocular pressure (IOP) elevation on retinal anatomy and function in a mouse model of experimental glaucoma. IOP was elevated by anterior chamber injection of a fixed combination of polystyrene beads and sodium hyaluronate, and maintained via re-injection after 24 weeks. IOP was measured weekly with a rebound tonometer for 48 weeks. Histology was assessed with a combination of retrograde labeling and antibody staining. Retinal physiology and function was assessed with dark-adapted electroretinograms (ERGs). Comparisons between bead-injected animals and various controls were conducted at both 24 and 48 weeks after bead injection. IOP was elevated throughout the study. IOP elevation resulted in a reduction of retinal ganglion cell (RGCs) and an increase in axial length at both 24 and 48 weeks after bead injection. The b-wave amplitude of the ERG was increased to the same degree in bead-injected eyes at both time points, similar to previous studies. The positive scotopic threshold response (pSTR) amplitude, a measure of RGC electrical function, was diminished at both 24 and 48 weeks when normalized to the increased b-wave amplitude. At 48 weeks, the pSTR amplitude was reduced even without normalization, suggesting more profound RGC dysfunction. We conclude that injection of polystyrene beads and sodium hyaluronate causes chronic IOP elevation which results in phenotypes of stable b-wave amplitude increase and progressive pSTR amplitude reduction, as well as RGC loss and axial length elongation.

1. Introduction

Glaucoma is a leading cause of blindness internationally and of increasing public health concern (Quigley and Broman, 2006). The only modifiable risk factor thus far conclusively identified is intraocular pressure (IOP), and the reduction of IOP is known to limit disease onset and slow disease progression (CNTGSG, 1998; Gordon et al., 2002; Leske et al., 2003). Ocular hypertension, defined as a mild, chronic elevation in IOP, can lead to progressive optic nerve and retinal ganglion cell (RGC) changes that impact visual function and optic nerve appearance (Gordon et al., 2002).

To better understand the role of elevated IOP on RGCs and the optic nerve, multiple laboratories have developed murine models of ocular hypertension and glaucoma (Aihara et al., 2003; Chen et al., 2011; Cone et al., 2010, 2012; Frankfort et al., 2013; Gross et al., 2003; Grozdanic et al., 2003; Ji et al., 2005; Ruiz-Ederra and Verkman, 2006; Samsel et al., 2011; Sappington et al., 2010; Urcola et al., 2006). Our preferred model, which makes use of the injection of polystyrene beads followed by sodium hyaluronate into the anterior chamber of a mouse eye as developed by Sappington and modified by Quigley, results in a chronic IOP elevation (Cone et al., 2010; Frankfort et al., 2013; Sappington et al., 2010). This model, and other variations of the "microbead occlusion" model, have similar characteristics including stable IOP increase, limited IOP variation, and neurodegeneration in mice (Chen et al., 2011; Cone et al., 2010; Della Santina et al., 2013; Frankfort et al., 2013; Sappington et al., 2010). The anatomic features of these models include abnormal axonal transport and neurotransmission, axonal loss, RGC loss, and age and species-dependent phenotypes (Chen et al., 2011; Cone et al., 2010, 2012; Crish et al., 2010; Della

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Santina et al., 2013; Frankfort et al., 2013; Samsel et al., 2011; Sappington et al., 2010). There are few detailed analyses of RGC and inner retinal functional changes in response to chronic IOP elevation in mice, and these studies suggest that RGC-specific functional changes occur, and likely include RGC subtype-specific effects (Della Santina et al., 2013; Feng et al., 2013; Frankfort et al., 2013; Holcombe et al., 2008).

The electroretinogram (ERG) can be used to assess retinal electrical function in living animals. The primary components of the ERG, the a-wave (produced by photoreceptors), and the b-wave (produced by bipolar cells) have been understood for many years. The positive scotopic response (pSTR; produced by RGCs), and the negative scotopic response (nSTR; likely produced by AII amacrine cells) have also been described (Abd-El-Barr et al., 2009; Saszik et al., 2002; Sieving et al., 1986). The entire system operates under the principle of synaptic convergence; light sensitivity responses increase after every synapse in the direction of electrical transmission (photoreceptors → bipolar cells → RGCs/AII amacrine cells). Thus, the components of the ERG have different levels of sensitivity, with the pSTR and nSTR detectable at the lowest light intensities, and the b-wave and a-wave detectable at relatively brighter light intensities (Abd-El-Barr et al., 2009). Lastly, since the retinal circuitry governing the ERG is well delineated, changes in the ERG can provide evidence not only for dysfunction of the retina as a whole, but for changes to specific pathways or cell types. For example, changes in the pSTR alone might signify an RGC-specific phenotype.

We have previously used a version of the microbead occlusion model to produce chronic IOP elevation in mice. With this model, we identified an IOP-dependent effect on RGC function as measured by the pSTR, a mild b-wave amplitude increase, and anatomic phenotypes of RGC loss and axial length elongation (Frankfort et al., 2013). We extended these studies and assessed long term (24 and 48 week post-injection) effects on RGC structure and function. We report persistent anatomic phenotypes of RGC loss and axial length, and physiologic phenotypes of stable b-wave amplitude increase and progressive pSTR amplitude reduction.

2. Methods

2.1. Animals

6 week old C57Bl/6J female mice were obtained from the Jackson Laboratories and maintained in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, Directive 2010/63/EU, NIH guidelines, and the Baylor College of Medicine IACUC requirements. Mice were maintained in a facility with a temperature of 68–72 °F and a light–dark cycle of 12 h.

2.2. IOP elevation and measurement

All mice were sedated with a weight-based intraperitoneal injection of ketamine (80 mg/kg), xylazine (16 mg/kg), and acepromazine (1.2 mg/kg). The left eye was dilated with a drop of tropicamide 1%. Additional topical anesthesia was achieved with a drop of proparacaine hydrochloride 0.5%. Anterior chamber injection of polystyrene beads was performed as previously described with minor modifications (Cone et al., 2010; Frankfort et al., 2013). A sterile 30 gauge needle was used to puncture the cornea lateral to the dilated pupil margin. A freshly pulled glass micropipette with an inner diameter of 60–75 μm was attached to a Hamilton syringe and advanced through the corneal incision into the anterior chamber. A fixed volume of polystyrene beads (1.5 μl containing 4.7×10^6 of 6 μm beads and 2.4×10^7 of 1 μm beads; Polysciences, Inc., Warrington, PA), followed by 3 μl of sodium hyaluronate (Provisc, Alcon Laboratories, Ft. Worth, TX) was injected into the anterior chamber over a period of 60 s. This 1.5 + 3 protocol is similar to the 2 + 3 protocol previously reported (Cone et al., 2010, 2012), but was developed independently as we found that it resulted in a lower but more stable IOP elevation compared to other bead volumes. Following bead injection, a drop of moxifloxacin 0.5% was placed on the cornea to prevent infection. To control for the procedure, additional animals received the same injection procedure except the beads were replaced with 1× phosphate-buffered solution (PBS or saline). The right eye was not injected in any animals to serve as an intra-animal control. A subset of animals (those which were not sacrificed at the 24 week time point for experiments and also did not display obvious anterior segment abnormalities such as posterior synechiae or an irregular pupil) received a repeat injection halfway through the expected study. This occurred after 24 weeks and the injection was performed via the same procedure (injection of either polystyrene beads or saline followed by sodium hyaluronate) to the same eye as originally injected. To optimize corneal integrity in these animals, a new corneal incision at least 90° away from the original incision was created with a 30 gauge needle and used as the injection site. Prior to use, beads were washed in 100% ethanol to reduce storage contaminants.

IOP was measured on the day prior to treatment, on the day after treatment and then once each week. IOP was measured for a period of 24 weeks for all animals ($n = 49$; 35 bead-injected and 14 saline-injected) and for a period of 48 weeks in the group of animals that received repeat injection after 24 weeks ($n = 24$; 17 bead-injected and 7 saline-injected). IOP was recorded using a rebound tonometer optimized for mouse use (Tonolab, Icare, Finland) as previously described (Frankfort et al., 2013; Pease et al., 2011). To reduce variability, IOP was measured from animals in the same order at the same starting time on the same day of the week. The IOP difference between the eyes was used to quantify the amount of IOP increase after treatment and calculated as previously described (Frankfort et al., 2013).

2.3. Axial length measurement/immunohistochemistry/cell counting

Eyes were immediately removed after euthanasia via anesthetic overdose followed by cervical dislocation and the optic nerves trimmed flush with the sclera. Whole eyes were placed cornea down on a slide and the anterior-posterior diameter of the eye (axial length) measured with a camera/controller system (Keyence Corp., Elmwood Park, NJ). The eyes were then rotated 90° along the anterior-posterior axis and re-measured. The average of the two measurements was recorded as the axial length as previously described (Frankfort et al., 2013).

Prior to immunohistochemistry, some eyes were retrogradely labeled with Neurobiotin as previously described (Frankfort et al., 2013; Pang and Wu, 2011). Eyes were then prepared for immunohistochemistry according to standard techniques. Briefly, retinas were dissected from freshly enucleated eyes in PB++ solution (1× PBS, 0.5% Triton X-100, 0.1% NaN_3), fixed in 4% paraformaldehyde for 1 h, washed with PB++ for several hours, and blocked in PB++ containing 10% donkey serum at 4° overnight to reduce non-specific binding. At this point, eyes for which sectioned retinas were required were cut into 40–60 μm thick sections with a microtome (Vibratome, Leica Microsystems, Bannockburn, IL). Flat mount or sectioned specimens were treated with PB++ containing 3% donkey serum as well as primary antibody (mouse anti-class III beta-tubulin (TUJ1, 1:500, Covance, Inc.)), if not treated with Neurobiotin, for several days at 4°. Retinas were then washed with