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Elevated intracranial pressure causes optic nerve and retinal ganglion cell degeneration in mice

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ABSTRACT

The purpose of this study was to develop a novel experimental system for the modulation and measurement of intracranial pressure (ICP), and to use this system to assess the impact of elevated ICP on the optic nerve and retinal ganglion cells (RGCs) in CD1 mice. This system involved surgical implantation of an infusion cannula and a radiowave based pressure monitoring probe through the skull and into the subarachnoid space. The infusion cannula was used to increase ICP, which was measured by the probe and transmitted to a nearby receiver. The system provided robust and consistent ICP waveforms, was well tolerated, and was stable over time. ICP was elevated to approximately 30 mmHg for one week, after which we assessed changes in optic nerve structure with transmission electron microscopy in cross section and RGC numbers with antibody staining in retinal flat mounts. ICP elevation resulted in optic nerve axonal loss and disorganization, as well as RGC soma loss. We conclude that the controlled manipulation of ICP in active, awake mice is possible, despite their small size. Furthermore, ICP elevation results in visual system phenotypes of optic nerve and RGC degeneration, suggesting that this model can be used to study the impact of ICP on the visual system. Potentially, this model can also be used to study the relationship between ICP and IOP, as well diseases impacted by ICP variation such as glaucoma, idiopathic intracranial hypertension, and the spaceflight-related visual impairment intracranial pressure syndrome.

1. Introduction

The optic nerve is made of retinal ganglion cell (RGC) axons and is located within the subarachnoid space (SAS). The RGC axons are exposed to constant pressure from two sources: intraocular pressure (IOP), which is transmitted within the eye posteriorly to the optic nerve head, and intracranial pressure (ICP), which is transmitted to the optic nerve at multiple points, including anteriorly to the optic nerve head. When either of these two pressures is increased in humans, deleterious consequences occur. Increased IOP may cause glaucoma, a neurodegenerative disease of the optic nerve and RGCs which is common among the elderly and is the second leading cause of blindness in the world (Gordon et al., 2002; Quigley and Broman, 2006). Increased ICP may result in a variety of conditions according to the magnitude of the elevation. At severe elevations, papilledema occurs and visual loss can be rapid and significant. At less extreme elevations, diseases such as idiopathic intracranial hypertension (IIH) and the spaceflight-related visual impairment intracranial pressure (VIIP) syndrome induce moderate, chronic visual changes (Acheson, 2006; Mader et al., 2011; Wall et al., 2014). In IIH, many patients also show evidence of optic nerve axon loss and RGC death (Keltner et al., 2014; Marzoli et al., 2013; Monteiro and Afonso, 2014; Padhye et al., 2013). Several reports have suggested that the balance between IOP and ICP is an important factor in optic neuropathies in general (Berdahl et al., 2008a, 2008b; Fleischman and Berdahl, 2014; Ren et al., 2010, 2011; Zhang and Hargens, 2014).

The anatomic effect of elevated IOP on the mouse visual system has been well-studied (Chen et al., 2011; Cone et al., 2010; Frankfort et al., 2013; Gross et al., 2003; Grozdanic et al., 2003; Ji et al., 2005; McKinnon et al., 2009; Ruiz-Ederra and Verkman, 2006; Samsel et al., 2011; Sappington et al., 2010). However, direct testing of the

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effects of elevated ICP on the visual system in mice has not been possible due to the lack of a suitable model for both the sustained elevation and accurate measurement of ICP. Thus, our current understanding of the effects of ICP in mice is limited to sedated animals in which ICP was elevated only for short periods of time, and we currently have no established system with which to potentially study the interaction between ICP and IOP in mice (Feiler et al., 2010; Ren et al., 2013). Fortunately, models of ICP monitoring have been developed in rodents as well as primates, so some of these critical relationships are experimentally accessible (Barth et al., 1992; Kusaka et al., 2004; Lin and Liu, 2010; Chowdhury et al., 2013; Silasi et al., 2009; Yang et al., 2014). However, the expansion of this approach into a genetically tractable organism such as mice would be beneficial.

We therefore developed a novel experimental system to modulate ICP in living, awake mice for an extended period of time. This system includes dual implantation of an infusion cannula and a radiowave based pressure monitoring probe through the skull and into the SAS. The infusion cannula is then used to increase ICP, which is measured directly by the nearby probe and transmitted to a receiver in real time. We validated this system and then used it to chronically increase ICP in wild type CD1 mice and study its effects on optic nerve structure and RGC number. We found that ICP elevation results in marked optic nerve axonal loss and disorganization, as well as prominent RGC loss. This system indicates that controlled manipulation of ICP levels is possible in mice. This method can be used to study the effects of ICP change and potentially the ICP − IOP relationship in a variety of ophthalmologic and neurologic conditions.

2. Methods

2.1. Animals

All protocols and procedures were approved by the Baylor College of Medicine Animal Care and Use Committee and comply with federal guidelines and the ARVO statement for animal research. Eight-week-old, female, CD1 mice were maintained according to a standard 12 h light–dark cycle.

2.2. Generation of ICP monitoring and modulation system

Animals were anesthetized with a weight-based intraperitoneal injection of ketamine (80 mg/kg), xylazine (16 mg/kg), and acepromazine (1.2 mg/kg). Subsequently, the hair on the scalp was shaved with a standard pair of clippers. A 1 cm midline incision starting at the base of the skull and directed anteriorly was fashioned using a #11 scalpel blade to expose the bony surface of the skull. A 1 mm hole was drilled 1 mm lateral and 1 mm posterior to bregma on the right side of the animal. The dura was nicked with a 30 gauge needle to ensure an egress of cerebrospinal fluid (CSF) and entrance into the SAS. A custom cannula made from a 1.57 mm diameter × 2.4 mm long nylon screw (Plastics One, Inc.) with a hole drilled through the center lengthwise using a 0.4 mm drill bit was positioned in the 1 mm hole and anchored into the skull using 3 M ESPE Durelon Carboxylate Luting Cement (3 M). The cannula was slightly oversized to facilitate a tight seal. The tip of a PA-C10 pressure-monitoring probe (Data Sciences International) was fed through the hole in the center of the cannula into the SAS to allow for measurement of ICP. The inner diameter of the cannula was chosen to allow for a tight seal with the PA-C10 pressure-monitoring probe. A second 1 mm hole was drilled 1 mm lateral and 1 mm posterior to bregma on the left side of the animal and the dura nicked as above. Prior to the surgery, a custom infusion cannula was made by placing a 23G syringe needle through a nylon screw, removing the hub, heat sealing it in place, and filing off the bevel was fashioned. This cannula was then positioned in the second 1 mm hole and anchored into the skull as above. The entire system was held in place with a mound of silicone caulk which included both cannulas and the anterior-most segment of the PA-C10 monitoring probe (Fig. 1). Subsequently, a 0.6 cm transverse incision was made 2–3 mm posterior to the base of the skull and a pocket made by blunt dissection subcutaneously over the back. The pressure probe transmitter was placed in this pocket between and past the scapulae of the animals and onto the mid-back, where it was sutured in place with 6.0 vicryl, interrupted sutures.

To evaluate the telemetry pressure monitoring system for stability, a cohort of animals (n = 6) was monitored for 3 weeks. These mice had their infusion cannula sealed off to ambient air by attaching a small length of PE50 tubing to the cannula and heat-sealing it with a soldering iron. ICP measurements were then recorded for 1 h at 1 day, 1 week, 2 weeks, and 3 weeks post-operatively. For ICP elevation experiments, 6 animals were exposed to elevated ICP and 6 animals received cannula placement without ICP elevation. In the ICP elevation group, the infusion cannulas were attached to a fluid bag filled with artificial CSF (124 mM NaCl, 2.5 mM KCl, 2.0 mM $MgSO_4$, 1.25 mM KH_2PO_4, 26 mM $NaHCO_3$, 10 mM glucose, 4 mM sucrose, 2.5 mM $CaCl_2$) via PE50 tubing. The fluid bag was hung to gravity to empirically raise ICP to 30 mmHg and remained at the same point for the duration of the study. In the control group, the infusion cannula was sealed off as for the stability experiments. In both groups, ICP was monitored for one hour each day for seven consecutive days. For all animals, sessions of ICP recording lasted one hour, and the mean value of each one hour block of time was calculated to get an average of ICP for that time point. ICP data for experiments were collected wirelessly by the PhysioTel® Small Animal Telemetry system and receivers and outputted to an excel spreadsheet using Ponemah Software 5.20 data analysis software (all from Data Sciences International; St. Paul, MN). This software automatically uploads raw data into a Microsoft Office Excel file for analysis.

2.3. Measurement of IOP

IOP was recorded using a rebound tonometer optimized for mouse use (Tonolab) under isoflurane anesthesia as previously described (Frankfort et al., 2013; Khan et al., 2015; Pease et al., 2011).

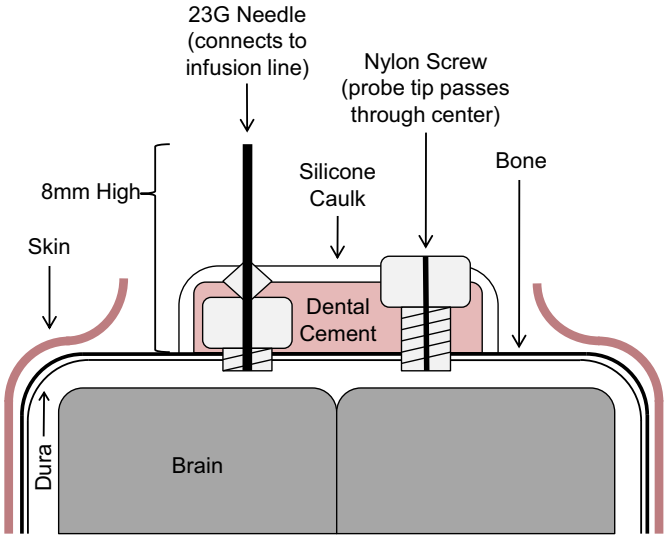

Fig. 1. Schematic of surgical complex for ICP modulation and measurement. Animal surgery for the elevation and measurement of ICP is described in section 2.2 of the Methods. A coronal view of the final surgical complex is shown.