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Modeling glaucoma in rats by sclerosing aqueous outflow pathways to elevate intraocular pressure

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ABSTRACT

Injection of hypertonic saline via episcleral veins toward the limbus in laboratory rats can produce elevated intraocular pressure (IOP) by sclerosis of aqueous humor outflow pathways. This article describes important anatomic characteristics of the rat optic nerve head (ONH) that make it an attractive animal model for human glaucoma, along with the anatomy of rat aqueous humor outflow on which this technique is based. The injection technique itself is also described, with the aid of a supplemental movie, including necessary equipment and specific tips to acquire this skill. Outcomes of a successful injection are presented, including IOP elevation and patterns of optic nerve injury. These concepts are then specifically considered in light of the use of this model to assess potential neuroprotective therapies. Advantages of the hypertonic saline model include a delayed and relatively gradual IOP elevation, likely reproduction of scleral and ONH stresses and strains that may be important in producing axonal injury, and its ability to be applied to any rat (and potentially mouse) strain, leaving the unmanipulated fellow eye as an internal control. Challenges include the demanding surgical skill required by the technique itself, a wide range of IOP response, and mild corneal clouding in some animals. However, meticulous application of the principles detailed in this article and practice will allow most researchers to attain this useful skill for studying cellular events of glaucomatous optic nerve damage.

1. Introduction

Intraocular pressure (IOP) is a widely accepted risk factor for glaucoma, and all current glaucoma therapy is directed toward controlling this risk factor. Because not all glaucoma patients respond adequately to IOP-lowering treatment, there is still a need to develop novel treatments designed to protect the optic nerve head (ONH) and retina in the face of elevated IOP. Understanding the cellular mechanisms involved in pressure-induced optic nerve damage will greatly facilitate accomplishing this goal.

By the end of the 1980's, the non-human primate glaucoma model, produced either acutely or chronically in monkeys, (Gaasterland and Kupfer, 1974; Quigley and Addicks, 1980; Quigley and Anderson, 1977) was primarily used to study this problem. However, the high cost of animals and their maintenance restricts their use for detailed cell biology studies, since such work requires large numbers of animals to account for individual biologic variability. Because of this, attention has been increasingly directed at the challenge of producing experimental IOP elevation in rodents. Although anatomic differences between the rodent and primate ONH exist, the general plan for this approach was to use the rodent models to understand basic concepts and likely important cell processes. This knowledge could then be used to develop specific hypotheses that could be tested in the primate model, using targeted experiments that test theories of mechanism as well as potential new treatments. This article will first discuss the basic anatomy of the rat ONH, and then describe one of the first-developed rodent glaucoma models–that produced by injection of hypertonic saline into the aqueous humor outflow pathways to produce sclerosis, increased resistance to aqueous humor outflow and elevated IOP.

1.1. Anatomy of the rat optic nerve head

Viewed “clinically”, from within the eye, the rat ONH is essentially obscured by a striking presence of retinal arteries and veins that may be initially assumed to radiate from the disc (Fig. 1A).

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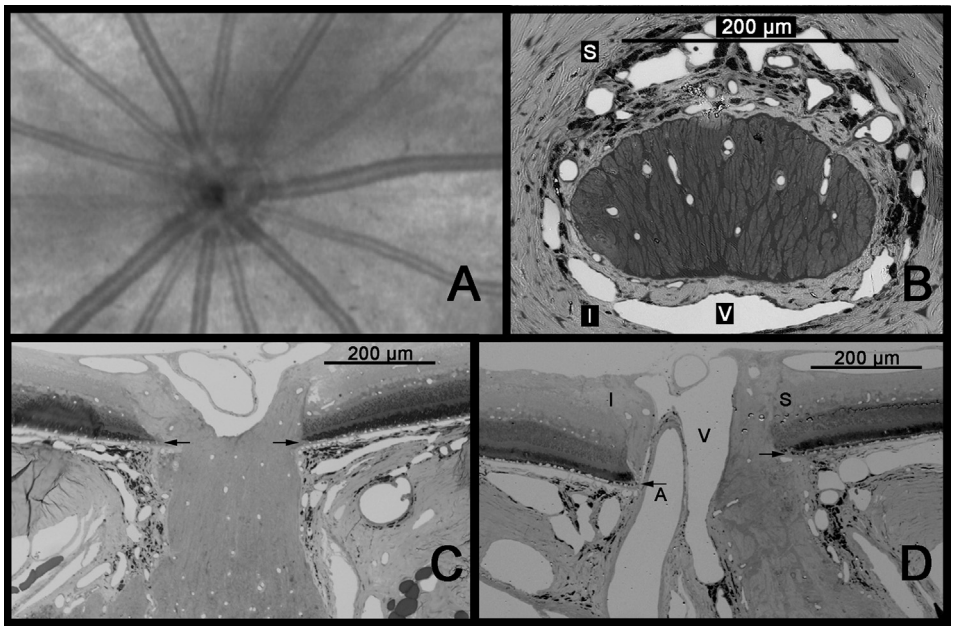

Fig. 1. Anatomy of the rat ONH. A. Anterior view of the rat fundus as seen by Optical Coherence Tomography (OCT). The ONH is dominated by spoke-like retinal arteries and veins that all but obscure the actual neural portion of the ONH. B. Cross-sectional view of the ONH at the level of the sclera. The nerve is horizontally oval and densely surrounded by vessels, including the central retinal vein (V), which is located inferiorly. The central retinal artery (not seen) lies inferior to the vein. C. Horizontal longitudinal section shows the optic nerve contacting the edge of Bruch's membrane at either extreme. D. A vertical longitudinal section shows that only the superior aspect of the nerve is in contact with the edge of Bruch's membrane, while inferiorly, the ONH is separated from Bruch's by the central retinal artery as well as the vein. Arrows = Bruch's membrane; S = superior; I = inferior; A = central retinal artery; V = central retinal vein (A. Courtesy of R. Wang and Z. Zhi).

However, these vessels actually enter the posterior sclera just inferior to the neural portion of the optic nerve head. This is a fundamental difference from the primate optic nerve, in which the retinal vessels are already within the nerve when they enter the eye. Histologically, in cross section, the neural portion of the rat optic nerve head has an oval shape at the level of Bruch's membrane and sclera, with its short axis oriented vertically (Fig. 1B). This means, when preparing longitundinal sections of the ONH, important for displaying, from anterior to posterior, all layers in a single section, differences in orientation will produce strikingly different appearances. Whereas a horizontal section (Fig. 1C) will show the neural tissue adjacent to the sclera and in contact with Bruch's membrane at either edge (similar to what is usually seen in the primate), a vertical section shows a more complex situation (Fig. 1D). The neural tissue appears to be narrower at the level of the sclera, representing the short axis of the oval. In addition, the nerve demonstrates intimate contact with the edge of Bruch's membrane only at its superior margin, as the retinal artery and vein, along with other vessels, lie inferior, separating it from the sclera and Bruch's.

Another important feature of the rat ONH lies in the structural composition of the lamina cribrosa. In contrast to the primate, dog, cat, and other larger species, the rat (and mouse) ONH does not have a robust collagenous lamina cribrosa. (Morrison et al., 1995a, b) While, in the rat, a supportive framework with a basket-like distribution similar to the collagenous lamina is detectable, this is composed almost entirely of astrocytes in association with capillaries. Since the similarity in their orientation to the collagenous lamina is quite striking, this has been referred to, in both mice and rats, as a “glial lamina” (Sun et al., 2009). This is appropriate, given that the astrocytes are oriented, as in the primate, across the scleral canal and perpendicular to the axon bundles (Johnson et al., 2000; Tehrani et al., 2014). In addition to their orientation, the astrocytes are in intimate contact with blood vessels and pial membranes of the ONH. Their nuclei are arranged in columns, similar to those in the anterior portions of the primate ONH (Anderson, 1969, 1970). Their processes also appear to surround axon bundles, and send numerous, actin-filled processes into the nerve bundles themselves (Morrison, 2005; Morrison et al., 2011; Tehrani et al., 2014). Ultrastructurally, these processes display intimate contact with individual axons, so that a single axon receives contact from numerous astrocyte processes, and, in turn, each astrocyte likely contacts numerous axons. These considerations indicate that, despite the lack of a collagenous connective tissue component, the glial lamina cribrosa of the rat contains many relationships that will help us use these animals to understand how the cell biology of the ONH responds to increases and fluctuations in IOP and affects axonal damage in human glaucoma.

Posterior to the sclera, the optic nerve gradually assumes a circular shape, primarily through expansion of its vertical dimension. In a group of 8 normal eyes, fixed and embedded in paraffin, the vertical height of the optic nerve at the opening of Bruch's membrane and 100 µm posterior to this was 85 ± 7 µm (SD) and 95 ± 13 µm, respectively. However, 350 µm posterior to Bruch's, the vertical width of the neural ONH had approximately tripled to 236 ± 49 µm. This expansion is part of a unique feature of the rodent ONH, termed the “transition zone”. Beginning at the posterior margin of the sclera, the transition zone extends posteriorly until the optic nerve becomes round and fully myelinated, approximately 400 µm posterior to Bruch's. However, a precise border is difficult to determine as ultrastructural evaluation reveals significant irregularities, with partial myelination of axons, and evidence of astrocytes phagocytosing organelle-containing axonal evulsions, suggesting a degradative pathway that may contribute in some way to glaucomatous axonal degeneration (Nguyen et al., 2011).

Total axon numbers in the myelinated portion of Brown Norway rat optic nerves have been determined using ultrastructural methods (Cepurna et al., 2005). Manual counting of all visible axons and extrapolating this to total axons revealed a mean of 121,960 ± 6680 in 5 month-old animals. Importantly, direct comparisons of right and left eyes in the same animal showed a