Experimental Eye Research 90 (2010) 210–215

Contents lists available at ScienceDirect

Experimental Eye Research

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

The presence and distribution of elastin in the posterior and retrobulbar regions of the mouse eye

Scott Gelman*, Frances E. Cone, Mary E. Pease, Thao D. Nguyen, Kristin Myers, Harry A. Quigley

Glaucoma Research Laboratory, Wilmer Ophthalmological Institute, Johns Hopkins University School of Medicine, Baltimore, MD USA

ARTICLE INFO

Article history:
Received 21 July 2009
Accepted in revised form 13 October 2009
Available online 22 October 2009

Keywords:
elastin
mouse
sclera
optic nerve
fibrillin
glaucoma

ABSTRACT

The Presence and distribution of elastin in the posterior and retrobulbar regions of the mouse eye was investigated. Mice of two strains (C57/BL6 and DBA/2J) were studied at 2 months and 8–12 months of age. Light, confocal, and transmission electron microscopy were used to identify elastin, using immunohistochemical techniques and ultrastructural evaluation. Elastin was found in the following ocular structures: conjunctiva, muscle tendons, sclera, choroid, and meninges. The elastin in the sclera was most dense in a ring surrounding the peripapillary optic nerve head, with its presence in the inner sclera declining with greater distance from the nerve head. Elastin fibers were oriented in the sclera along what would be expected to be the principal stress directions generated from the intraocular pressure, though actual biomechanical measurements have not yet been made in the mouse sclera. Elastin comprises a portion of the mouse sclera and its distribution in the peripapillary area is similar to that in human eyes.

1. Introduction

The internal structures of the eye are enclosed within the corneoscleral shell, made up of connective tissues that serve as both the primary refracting surface and load-bearing structure. The sclera has a central role in maintaining the mechanical integrity of the pressurized eye. Changes in intraocular pressure (IOP) cause scleral deformation that is transmitted to the optic nerve head, the site of glaucoma damage (Quigley et al., 1981), causing potentially damaging deformation (Sigal et al., 2005). An alteration in the structure or the material properties of the sclera will affect the deformation response to a given level of IOP. IOP and ocular blood flow provide both constant and pulsating stress in the eyewall that must be tolerated without permanent damage to its principal elements: collagen, elastin, and glycosaminoglycans.

Collagens I and III impart inherent tensile strength to the human sclera, complemented by elastin, which permits elastic deformation and recovery (Kielty et al., 2002). This function is critical to the maintenance of scleral integrity given the repeated cycles of loading produced by the choroidal vessels (Faury, 2001). Elastin is a complex of deposited tropoelastin on a template of fibrillin-rich microfibrils (Mecham and Davis, 1994). Tropoelastin is a 60–70 kDa protein composed of alternating hydrophobic and lysine-containing cross-linking domains (Gray et al., 1973). Of approximately 40 lysine residues in the secreted tropoelastin monomer, many are cross-linked by lysyl oxidase producing great stability and insolubility of the protein. Elastin has a typical longevity equal to the human lifespan (Shapiro et al., 1991) and its multi-step assembly pattern is tissue-specific, indicating that renovation or replacement may be difficult in adult tissues (Wagensweil and Mecham, 2007). Mice lacking both alleles of the one elastin gene die at or before birth, while those with one normal allele live a short time, but have defective arteries with increased numbers of elastic lamellae (Dietz and Mecham, 2000).

The microfibrils surrounding the elastin core may participate in extensibility of the complex (Wang et al., 2009). Fibrillin interacts with transforming growth factor β, regulating its activation, and abnormality in this function is related to development of Marfan syndrome (Neptune et al., 2003). Fibrillin-containing microfilaments are also found without elastin in the lens zonules.

Alterations in elastin may be either a result of glaucomatous damage to the eye or may participate in its causation. Hernandez and colleagues detected changes in elastin in human glaucoma eyes (Hernandez et al., 1989, 1992; Pena et al., 1998). In studies of both human and monkey eyes with glaucoma damage, our laboratory found an altered appearance of elastin without actual loss of fibers (Quigley, H.A., 1991a,b, 1994; Quigley, E.N., 1996). We speculated that elastin was seemingly disconnected from the remainder of the scleral and optic nerve head connective tissue matrix. Since

* Corresponding author at: Glaucoma Research Laboratory, Wilmer Ophthalmological Institute, Johns Hopkins University School of Medicine, The Smith Building, Room 002, 400 North Broadway, Baltimore MD 21287, USA. Tel.: +1 410 955 3337; fax: +1 443 287 2711.
E-mail address: sgelman2@jhmi.edu (S. Gelman).

0014-4835/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2009.10.007

African-derived persons have a greater prevalence of glaucoma compared to European-derived persons, it is interesting that they exhibit differences in elastin appearance in the optic nerve head region (Urban et al., 2007). Intriguingly, polymorphisms in the lysyl oxidase-like 1 gene are associated with exfoliation syndrome, the most common syndrome associated with open angle glaucoma (Thorleifsson et al., 2007). The protein coded by this gene participates in modification of elastin.

Animal models of glaucoma have added important information to our understanding of its pathogenesis, with recent use of both spontaneous (Jakobs et al., 2005) and induced (Grozdanic et al., 2003) mouse models of glaucoma. The relevance of mouse research in glaucoma depends upon the degree of homology of important ocular structures to the human eye. We did not find previous descriptions of the detailed structure of the sclera in the mouse, nor any prior mention of the presence or distribution of murine ocular elastin. Given its relevance to the biomechanical response of the eye to IOP, the present report presents the first description of the distribution of elastin in mouse sclera.

2. Methods

2.1. Animals used

A total of 41 mice of two strains were used: 10C57/Bl6 at 8 weeks of age, 10C57/Bl6 at 48 weeks of age, 10 DBA/2J at 8 weeks of age and 11 DBA/2J at 56 weeks of age. All animals were treated in accordance with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research using protocols approved and monitored by the Animal Care Committee of the Johns Hopkins University School of Medicine. Animals were housed with a 14 h light/10 h dark cycle with standard chow and water ad libitum.

2.2. Sacrifice and tissue processing

Animals were anesthetized with a mixture of ketamine, xylazine, and acepromazine at 50, 10 and 2 mg/kg, respectively, and both eyes were enucleated. Prior to fixation, right eyes were manually inflated to their normal IOP (~15 mm Hg), then their axial lengths were measured with a digital caliper (Instant Read-Out Digital Caliper, Electron Microscopy Sciences, Hatfield, PA). Left eye optic nerve and the entire right eye were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) and post-fixed in 1% osmium oxide in 0.1 M phosphate buffer (pH 7.2) for 2 h. The right eyes were divided into 4 distinct longitudinal portions (temporal, superior, nasal & inferior) containing sclera, choroid and retina, along with a circular retrobulbar sample (centered on the optic disc). These were then processed for transmission electron microscopy (TEM) by dehydration in graded alcohol, exposure to 1.0% uranyl acetate in 100% ethanol for 1 h and embedded in epoxy resin.

The left eyes, after optic nerve removal, were inflated to 15 mm Hg by insertion of a needle into the posterior chamber connected to a reservoir, containing 2% paraformaldehyde (PFA) in 5% sucrose in 0.1 M phosphate buffer (pH 7.2), whose height set the pressure. The eye was then fixed by immersion in 2% paraformaldehyde (PFA) in 5% sucrose in 0.1 M phosphate buffer (pH 7.2) for 1 h. Eyes were taken through graded sucrose solutions, 5%, 10%, 12.5%, and 15%, for 30 min each, and then placed in 20% sucrose overnight. After 30 min of 2:1 20% sucrose solution to optimal cutting temperature (OCT) compound (Sakura Finetek USA, Torrance, CA), eyes were embedded in fresh sucrose-OCT. Cryosections, 8 μm thick, were collected from the peripapillary region onto slides (SuperFrost Plus; Fisher Scientific, Pittsburgh, PA) and stored at −80 °C before immunolabeling.

2.3. Histochemistry

The primary antibody was rabbit anti-elastin TP592 (Elastin Products Company, Owensville, MO) at a dilution of 1:250. Sections were washed in Tris-buffered saline (TBS) containing 0.1% Triton X-100 for 10 min, dehydrated in 3% hydrogen peroxide for 30 min and again washed in TBS 3 × 10 min. Non-specific binding sites were blocked with 2% normal goat serum in TBS for 1 h followed by a 10 min TBS wash. Avidin and biotin blocking kit (Vector Laboratories, Burlingame, CA) was used before incubation of primary antibody in 1%BSA/TBST overnight at 4 °C. After washing in TBS (3 × 10 min), sections were incubated in biotin-labeled goat anti-rabbit secondary antibody (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) at 1:500 in 1% BSA/TBS for 1 h. Slides were washed and incubated with the AEC Substrate Kit (Vector Laboratories, Burlingame, CA). Negative control experiments included non-immune serum of the same species as the primary antibody at the same protein concentration in incubation buffer alone. Labeled sections were mounted with VectaMount AQ Mounting Medium (Vector Laboratories, Burlingame, CA). Images of all slides were captured digitally using standardized microscope and camera settings (Axioskop and Axiocam with Axiovision ver. 3 software; Carl Zeiss, Thornwood, NY).

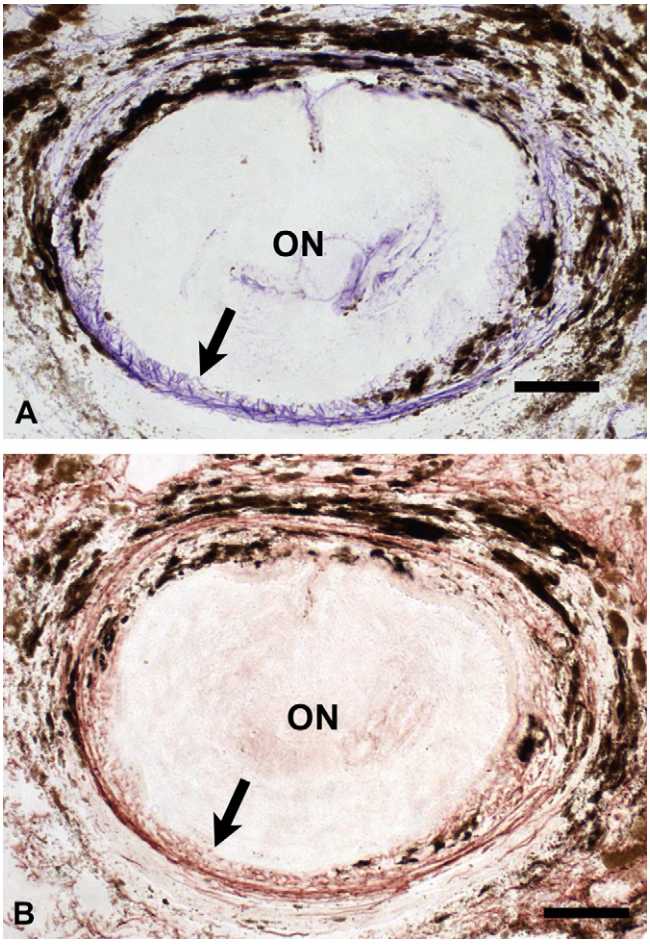

Fig. 1. Peripapillary sclera of young C57/BL6 mouse in cross-section. The upper micrograph shows a dense ring of purple, Luna-stained elastin fibers (arrow) is present immediately adjacent to the optic nerve head. A serial section from the same sample (bottom) that is stained with rabbit anti-elastin primary antibody demonstrates that the material stained by the Luna technique is similar in configuration to that identified by anti-elastin (arrow). (Top × 20, bar = 40 μm; bottom × 20, bar = 50 μm).