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Change in the appearance of elastin in the lamina cribrosa of glaucomatous optic nerve heads

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Abstract Elastin fibers in the lamina cribrosa of glaucoma eyes have a curled appearance when the tissue is fixed at atmospheric pressure. To evaluate the effect of preparation conditions on elastin appearance, we set the eye pressure at one of four levels during tissue fixation in 18 glaucoma and 16 normal eyes. Glaucomatous damage was associated with an abnormal appearance of elastin at each pressure level. The majority of glaucoma eyes (11/18) had curled elastin profiles. In glaucoma eyes with definite nerve damage, elastin was graded moderately or severely abnormal in 47% (7/15), while in normal controls the rate of similar curling was 6% (1/16). The severity of abnormal elastin appearance was lower in glaucoma eyes that were fixed and frozen at higher intraocular pressures, but the difference was not statistically significant.

Introduction

Glaucoma is differentiated from other optic neuropathies by its characteristic excavation of the optic nerve head [5, 7, 19, 21]. This physical change is important in clinical diagnosis and appears related to the pathogenetic events that lead to nerve fiber loss [4, 18, 24]. During the excavation process, alterations occur in the extracellular matrix of glaucomatous optic nerve heads [6, 9, 12, 17, 19, 21–23, 25].

The major components of the extracellular matrix at the lamina are collagens, glycosaminoglycans, and elastin [8, 10, 11, 13, 15, 16]. These have been studied to some degree in glaucoma eyes, and a major change in their position and relationships occurs. There is a decrease in the density of collagen, but no detectable shift in the diameter distribution of collagen fibrils [22]. There is a change in the distribution of some collagen types, particularly collagen type IV, in glaucoma nerve heads [6, 12, 17]. This seems to indicate either new synthesis of collagen or a translocation of existing material. The glycosaminoglycans have been studied in detail only in normal eyes [14, 26].

The distribution of elastin was first detailed in the lamina cribrosa by Anderson [1], though its presence has been known for many years [5]. Hernandez and coworkers showed an impressive amount of elastin in the area surrounding the nerve head [10, 11] and suggested that a loss of elastin or changes in its ultrastructural appearance occur in glaucoma [12]. We described an abnormal, curled appearance of elastin in glaucomatous nerve heads [23].

In this report, we attempt to confirm that the histological appearance of elastin is altered in glaucoma eyes and to determine if this alteration is related to the conditions under which the eye is prepared. Elastin may be able to change shape after aldehyde fixation; hence, it is possible that its appearance depends upon the pressure of the eye at the time of preparation. No prior study, to our knowledge, has examined this feature. We compare the elastin appearance in normal and glaucomatous eyes that were fixed and sectioned after preparation at four different levels of intraocular pressure.

Methods

Human eyes were obtained from eye banks and from national distribution systems. Those with no history of ocular disease and a normal optic nerve by histological study were classified as normal controls. Those with a history of treatment for glaucoma according to the hospital chart or family members were considered to have had glaucoma. Among the 18 eyes of 10 glaucoma patients included here, 9 eyes of 5 persons had a detailed ophthalmic history that confirmed the diagnosis of open-angle glaucoma. In 11 eyes of 6 persons with a glaucoma history, atrophy of the optic nerve or optic nerve head confirmed the presence of glaucoma. Thus, either detailed history or confirmatory histology were available for every eye. This research conformed to the principles embodied in the Declaration of Helsinki.

The normal control group consisted of 16 eyes of 9 persons. Their optic nerve area was calculated by a previously published histological method [20]. Every control eye had an optic nerve area that exceeded the lower limit of normal in our previously reported control nerves (6.6 mm^2).

The male/female ratio was equal in both glaucoma and normal eyes. Each group had one black person and the remainder were white. The glaucoma and normal groups had no statistically significant difference in mean age (t-test, $P > 0.05$). The time from death to fixation averaged 4 days in the glaucoma group and 3 days in the normal group, with only one eye in each group fixed more than 5 days after death. These times are considerably longer than in previous reports from this and other laboratories, as the tissues were shipped to us unpreserved, typically by overnight mailing services.

For initial tissue preservation, a 23-gauge needle was inserted into the pars plana, connected to a reservoir with 4% paraformaldehyde with 5% sucrose in 0.1 M phosphate buffer, pH 7.4. The height of the reservoir was set to achieve an immediate pressure within the eye of either 12, 34, 53, or 70 mmHg. Fixation continued for 1–3 h. A cross-section of the optic nerve was removed and marked for identification of the superior and nasal aspects with a razor blade. This specimen was processed for thick-section, epoxy embedding as previously described [20, 21]. Optimal cryopreservation was obtained by stepwise infiltration with increasing gradients of sucrose in 0.1 M phosphate buffer to a final concentration of 20% sucrose. Then, specimens were placed into two parts of 20% sucrose solution and one part of embedding medium (OCT, Miles, Elkhart, Ind.) [2]. While the eye was maintained at the set pressure, it was frozen in isopentane that was cooled by liquid nitrogen.

The optic disc was removed from the frozen globe and the shape of the surrounding sclera was cut to identify its superior pole. This was cryosectioned serially from the vitreous cavity to the retrobulbar optic nerve and stained by the Luna method [23].

The sections from both normal and glaucoma eyes were examined and the areas that included the lamina cribrosa were selected. The labels of these slides were coded. An additional set of sections from eyes prepared at atmospheric pressure [23] were also coded and read along with the new set to mask the reader more fully. One of us (H. Q.) then evaluated the appearance of the elastin in the lamina cribrosa on a four-level scale: normal (0) and mild (1), moderate (2) and severe (3) abnormality. An eye was defined as having mild curling (grade 1) if more than one beam of the lamina had curled elastin fibers (Fig. 1). Moderate curling (grade 2) was assigned to eyes in which the majority of laminar beams were involved. Severe curling (grade 3) denoted laminas in which nearly all beams had a curled elastin appearance.

All specimens were graded twice in masked fashion. Of 34 eyes, 19 had the same grading on both occasions. In the other 15 cases, an adjudication was made by the reader on a third, masked reading. In these, the two initial readings differed by one grade in 10 eyes and by two grades in only 5 eyes. No specimen differed in the two readings by three grades.

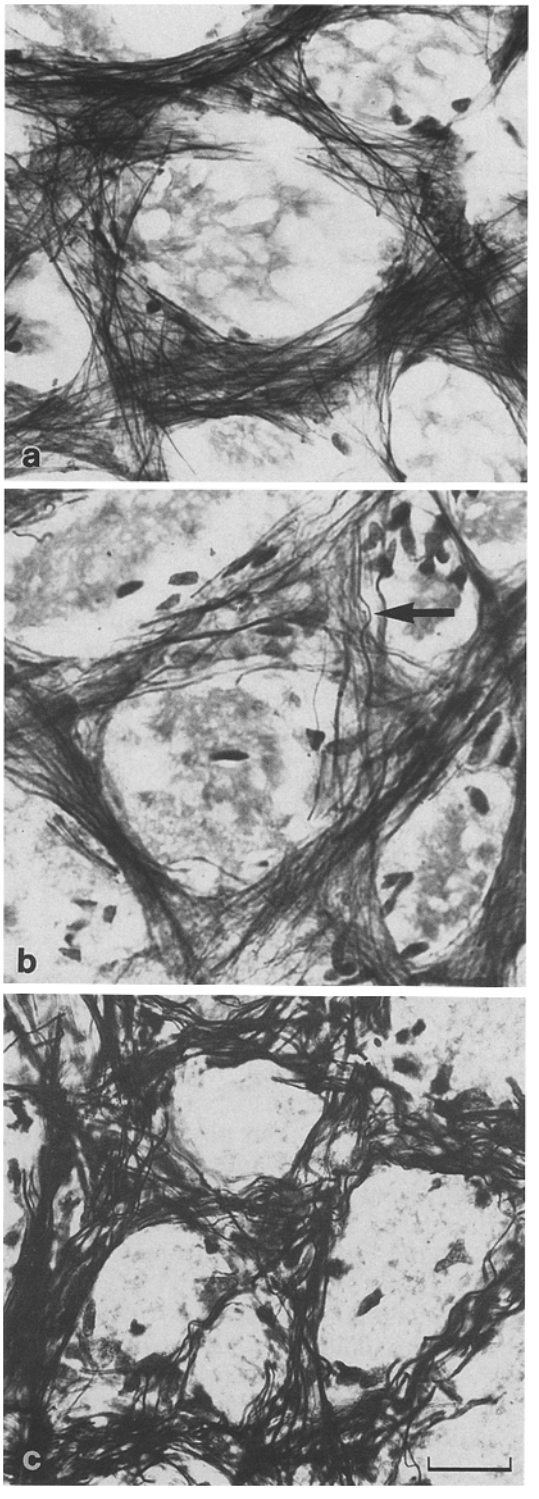

Fig. 1a–c The appearance of elastin fibers stained by the Luna method in beams of the human lamina cribrosa. **a** In a normal lamina, the fibers are straight and course across the nerve head opening from side to side. **b** In a glaucoma specimen with mild abnormality, some elastin fibers in a few areas can be seen to be curled. **c** In a severely affected glaucoma specimen, most of the elastin fibers are curled

Results

Among all eyes with a history of glaucoma, 11 of 18 (61%) were judged to have an abnormally curled appearance of their elastin (Table 1). Three glaucoma eyes of two persons were found to have no atrophy in the sections of their optic nerves. When these are excluded, the abnormal elastin rate was 10/15 (67%) in glaucoma eyes with confirmed damage. Four of the 16 normal eyes (25%) had an abnormal elastin grading. The difference between glaucoma with damage and normal was significant (chi-square test, $P<0.05$).

The glaucoma and normal eyes differed even more strikingly in their rates of moderate or severe curling of elastin (grades 2 and 3). Thirty-nine percent of all glaucoma eyes (7/18) and 47% (7/15) of damaged glaucoma eyes fell into these categories, while only one of the 16 normal eyes did (6%). When one eye was selected at random from each glaucoma and normal person, these results were not affected substantively (Table 1), though the numbers were too small to demonstrate a statistical difference.

Eyes were fixed at four levels of pressure: 12, 34, 53, and 70 mmHg (Table 2). Five of the seven eyes with moderate and severe curling were fixed at the two lower pressure levels. All four of the eyes with mild curling were in the two higher pressure levels. Among normal eyes, only one eye was graded as moderate or severe curling and it, too, was in the lower pressure fixation group. The numbers of glaucoma eyes that were available remained small despite nearly 2 years of collection. Hence, we collapsed the grading and eye pressure groups into a 2×2 matrix that showed a trend toward more eyes with abnormal elastin appearance in the lower pressure groups, but no statistically significant difference (Table 3).

When only one eye was selected at random from each patient, the same trend was observed. In the two lower pressure groups, three of five glaucoma eyes had moderate or severe curling of elastin, while in the two higher pressure groups, only one of five eyes had moderate or severe curling.

The time between death and fixation of the eye was unrelated to the elastin appearance.

In the area of the sclera immediately surrounding the lamina cribrosa there was no detectable difference in the appearance of elastin. This insertion zone is rich in elastin fibers that are oriented circumferentially and into which the most peripheral laminar beams are inserted.

Table 1 Elastin grading in glaucoma and normal eyes. Eyes prepared at different pressures are grouped together

Elastin grading	Analyzed by eyes		Analyzed by persons	
	Glaucoma	Normal	Glaucoma	Normal
Severe	4 (22%)	1 (6%)	3 (30%)	1 (11%)
Moderate	3 (17%)	0	1 (10%)	0
Mild	4 (22%)	3 (19%)	3 (30%)	2 (22%)
Normal	7 (39%)	12 (75%)	3 (30%)	6 (67%)
Total	18	16	10	9

Table 2 Elastin grading in glaucoma eyes by pressure at fixation

Elastin grading	Pressure at fixation			
	12 mmHg	34 mmHg	53 mmHg	70 mmHg
Severe	2	1	0	1
Moderate	1	1	1	0
Mild	0	0	1	3
Normal	2	2	2	1

Table 3 Elastin grading in glaucoma eyes with condensed groupings

Elastin grading	Pressure at fixation	
	12 or 34 mmHg	53 or 70 mmHg
Moderate or severe	5	2
Mild or normal	4	7

Difference not statistically significant, chi-square test, $P=0.33$

Discussion

In this and a previous report [23], we have studied the histochemical appearance of the elastin in the lamina cribrosa from a total of 37 glaucoma eyes. The present protocol imposed stricter preparation conditions than in our first study and introduced masked grading of elastin. In eyes with glaucoma damage, elastin within beams of the lamina cribrosa is often curled in appearance compared to its normally straight configuration. Examination of the laminar beams in glaucoma eyes by transmission electron microscopy shows a disruption of their normal architecture [22, 23]. This is manifest as an increase in space between collagenous fibrils. In addition, the elastin complexes are more separated from the surrounding collagen than normally.

We have speculated that elastin fibers are straight in the normal lamina because they are subjected to tension by the eyewall through their attachment to tissues at the margin of the optic nerve head. The predominant orientation of both collagen fibrils and elastin fibers at the lamina is in the direction of the circumference of the globe. It is reasonable that the disruption of laminar architecture induced by glaucoma leads to a dissociation between elastin and the other components of the extracellular matrix. This might remove the force trans-

mitted to elastin by ocular wall tension, leading to a curled appearance.

It was possible that the curled elastin in glaucoma eyes in our original study resulted from fixation at atmospheric pressure [23]. However, normal and glaucoma eyes were similarly prepared, while the curled elastin was seen predominantly in the glaucoma eyes. Furthermore, within individual glaucoma nerve heads, curled elastin was found more often in areas of the nerve head with nerve fiber loss, and not in zones without loss of nerve bundles. Hence, the curled appearance seemed related more to glaucoma damage than to tissue preservation. The present examples show that the abnormal elastin configuration is present in many glaucoma eyes, whether they are prepared at normal or at considerably elevated pressures.

There was a tendency for the curling of elastin to be less pronounced in eyes prepared at higher pressures, but the difference was not statistically significant with the sample size available. It is impractical to study substantially more eyes, as the acquisition of those presented here took more than 2 years. A trend toward less elastin curling in eyes with higher pressures might indicate that the post-mortem elastin appearance is responsive to changes in eyewall tension. In general, the curled elastin appearance was still present under a variety of preparation conditions.

It is likely that the curled appearance of elastin is a specific change caused by glaucoma. Hernandez and coworkers have reported a change in the ultrastructural appearance of the dense component of elastin in glaucoma eyes [9]. They also report a decrease in the number of elastin fibers in qualitative evaluations of immunofluorescent-stained material [12]. We found no change in the number of elastin molecules per unit area in experimental glaucoma in monkey optic nerve heads [22]. Furthermore, we detected no difference in the number of elastin fibrils in laminar beams with the masked, semiquantitative grading system reported here. Possibly the disruption of laminar architecture in glaucoma alters the immunofluorescent labelling of elastin or its ultrastructural appearance. It is clear that further studies of elastin in glaucoma are merited.

An alteration in elastin structure might affect the ability of the nerve head to restore its original position after modest deformation. With every pulsation of the ocular blood vessels, the eyewall measurably expands and contracts. The nerve head contains the lowest amount of connective tissue per unit area of any portion of the eyewall. Hence, it is probably the weakest zone of the sclera. It contains an extraordinary complement of elastin, while the remainder of the sclera has nearly none at all. This may be an adaptation to the need for elastic recoil in response to momentary shifts in eye pressure. A loss of the contribution by elastin to this ability would lead to lower tissue compliance.

If the elastin complex loses its normal attachments to the extracellular matrix during glaucoma injury [23], the behavior of the nerve head may be profoundly altered. Zeimer and Ogura measured the biomechanics of the optic nerve head in glaucoma eyes [28] under post-mortem, laboratory conditions. The compliance of glaucoma nerve heads was lower than that of normal eyes when small movements in the disc were induced by pulses of increased pressure. Decreased compliance of the nerve head of glaucoma eyes was also suggested by our studies of experimental glaucoma in vivo [3]. In monkey eyes, changes in intraocular pressure cause measurable movements in the surface topography of the optic disc. Such movements are smaller in eyes with severe glaucoma damage than in less damaged eyes. A similar observation has been made by Shirakashi et al. in movements of the nerve head surface in monkey eyes with chronic glaucoma after spontaneous reductions of intraocular pressure [27].

The susceptibility to injury in glaucoma may be substantially dependent upon the function of the extracellular matrix components of the optic nerve head. Conceivably, functional properties of elastin vary from one individual to another, representing a major determinant of the resistance of the optic nerve head to glaucoma injury. Changes induced in the function of elastin as glaucoma procedes may make further injury more likely if compliance of the nerve head declines. Clinical provocative testing of the nerve head compliance may provide important information about susceptibility to glaucoma damage.

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