Ultrastructural changes of the extracellular matrix of the trabecular meshwork with age

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SUMMARY

The morphology of the extracellular matrix was studied in a series of human trabecular meshworks obtained from specimens of different ages without any known ocular pathology. Our aim was to identify ultrastructural changes related to an increase in intraocular pressure with aging. 24 trabecular meshworks obtained from individuals with ages ranging from 23 to 99 were specifically prepared for subsequent transmission electron microscopic observation using standard techniques. Our results show that the extracellular matrix of the trabecular beams undergoes progressive changes with increasing age in all its components (collagen fibres, elastin-like plaques and basal membrane). These changes may be related to an increase in intraocular pressure since they can alter the aqueous outflow from the anterior chamber to Schlemm's canal and can interfere with the drainage, facilitating mechanism of the ciliary muscle.

Key Words : Trabecular meshwork – Extracellular matrix – Ultrastructure – Transmission electron microscopy – Aging

INTRODUCTION

The trabecular meshwork and Schlemm's canal constitute the system by which most of the aqueous humour is drained from the anterior chamber of the eye. It is generally accepted that an increase in the resistance of this aqueous outflow occurs with age (McMenamin and Lee, 1980; Rohen et al., 1981; McMenamin et al., 1986; Alvarado et al., 1986; Miyazaki et al., 1987; Hirano et al., 1988; Gong et al., 1989).

Histologically, the trabecular meshwork is made up of different layers of trabecular beams that delimit orifices through which the aqueous humour circulates. Two kinds of trabecular beam have been identified: inner or uveal beams, which are meridionally oriented, and outer or sclerocorneal beams, which are perpendicular to the former. The outer portion of the trabecular meshwork consists of a compact tissue known as juxtacanalicular tissue, which constitutes the inner wall of Schlemm's canal.

Ultrastructurally, trabecular beams consist of a central collagenic nucleus covered by a single layer of flattened endothelial cells. A well-delimited basal membrane composed of sparingly electrondense material lies under these cells. In the central nucleus, collagen bundles with a periodicity of 640 Å lie parallel to the major axis of the trabecular beam. Electrondense aggregates corresponding to the so-called elastin-like fibres (Lütjen-Drecoll et al., 1981; Rohen et al., 1981) are also found. These elastin-like fibres correspond to the tendons of the ciliary muscle (Rohen et al., 1981). This explains the action of this muscle, expanding the trabecular orifices, in facilitating aqueous humour drainage (Rohen et al., 1981; Hamanaka 1989; Kater et al., 1990; Kater et al., 1992; Tamm et al., 1992; Tamm et al., 1993). Around the elastin-like fibres there is an amorphous, granular material of medium electron density that forms a sheath-like structure. Finally, collagen fibres and elastin-like fibres with their corresponding sheaths are embedded

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in an electronlucent ground substance with a high content of proteoglycans (Grierson and Lee, 1975; Richardson, 1982; Tawara et al., 1989; Gong et al., 1992).

Observations indicating that the main area of resistance to aqueous humour drainage through the trabecular meshwork lies in the juxtacanalicular tissue (Bill and Svedbergh, 1972; Inomata et al., 1972; Ethier et al., 1995) have led many authors to focus on this specific area when studying the changes in the extracellular matrix that may account for the alterations observed in aqueous humour drainage in aged eyes and in primary open-angle glaucoma (McMenamin and Lee, 1980; Rohen et al., 1981; Rohen, 1983; Alvarado et al., 1986). Here, we analyzed the ultrastructural changes observed in the uveal and sclerocorneal trabecular beams with aging in an attempt to relate these changes to the mechanisms that may increase intraocular pressure with age.

Materials and methods

In this study we observed 24 non-glaucomatous trabecular meshworks by transmission electron microscopy. The samples were obtained from the sclerocorneal rings remaining from donor corneas after trephination of the central button for subsequent transplantation and from cadaver eyes obtained from donors of the Department of Human Anatomy of the University of Barcelona. Donor corneas were supplied by Hospital Clínic Eye Bank in Barcelona. In all cases donor age ranged from 23 to 99.

All sclerocorneal rings were obtained within 12 hours post-mortem and were immediately fixed in Karnovsky's solution for 2 hours. They were then sectioned meridionally into 1mm wide segments containing a trabecular meshwork fragment, postfixed in 1% osmium tetroxide, dehydrated in graded ethanol and embedded in Araldite.

Following the above, all samples were cut with an Ultracut E (Reichert-Jung) ultramicrotome into ultrathin sections, contrasted with lead citrate and uranyl acetate, following Reynold's method, and finally observed with a Hitachi H-600 AB transmission electron microscope at the Electron Microscopy Service of the University of Barcelona.

Results

In the youngest samples (age 23-37) (Fig. 1) the trabecular beams were characterized by a marked predominance of transversally sectioned normal collagen fibres in the central nucleus, widely separated by electronlucent spaces.

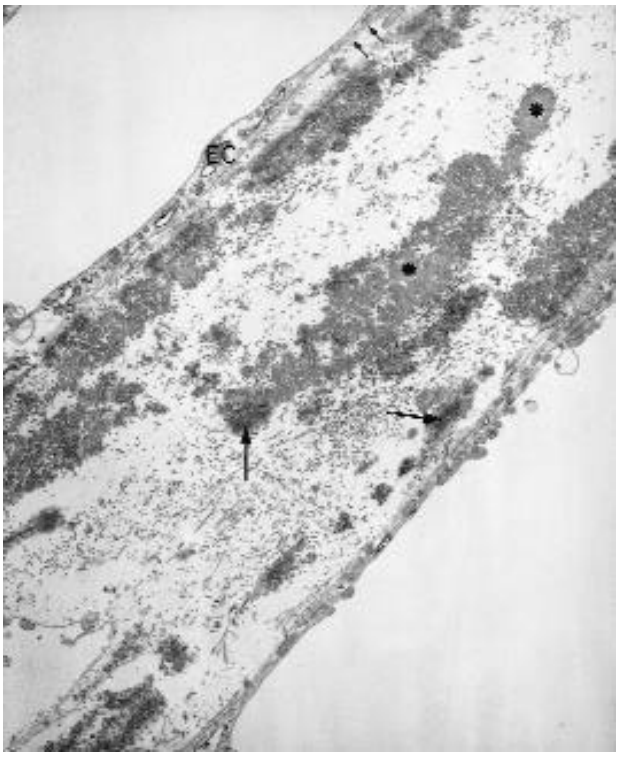

Fig. 1.- Transmission electron micrograph. Trabecular beam. Endothelial cells (EC). Basal membrane (small arrows). Elastin-like plaques (large arrows). Compacted collagen (asterisks). x 6,600.

Occasionally, some of the collagen fibres appeared longitudinally sectioned, thus showing a typical striation with a 640 Å period. Elastin-like plaques, varying in number and size, were also observed. These were randomly distributed in the central nucleus of the beams and under the basal membrane. These plaques consisted of an amorphous electronlucent central region surrounded by a fibrous area of high electron density. This electrondense area was additionally surrounded by a sheath of amorphous granular material of medium electron density.

We also observed areas with coalesced and degenerated collagen fibres embedded in an amorphous granular material similar to the sheaths of the elastin-like plaques. These areas covered only a small portion of the total area of the central nucleus of the trabecular beams. Finally, a subendothelial well-delimited basal membrane located under a continuous monolayer of flattened endothelial cells was observed.

The trabecular beams of samples aged 44 to 57 (Fig. 2) showed a progressive increase in the number and size of elastin-like plaques and an enlargement of the area of the central nucleus covered by compacted collagen surrounded by elastin-like sheath material. In this age group, the existence of subendothelial clumps of long-spacing collagen was also characteristic. Such long-spacing collagen consisted of fine longitudinal fibres interlaced at right angles with thick electrondense transversal fibres showing a 1000

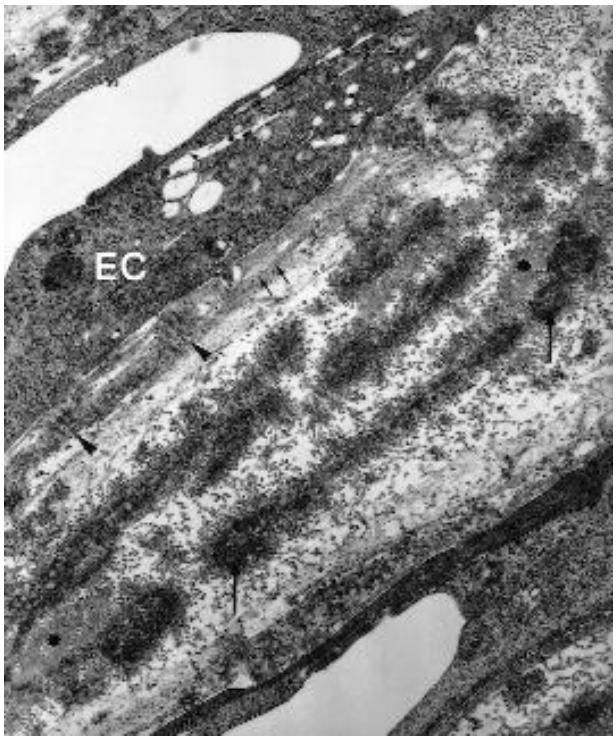

Fig. 2.- Transmission electron micrograph. Trabecular beam. Endothelial cells (EC). Basal membrane (small arrows). Elastin-like plaques (large arrows). Compacted collagen (asterisks). Long-spacing collagen (arrowheads). x 11,000.

Å periodicity (Fig. 3). The long-spacing collagen clumps increased in number with increasing donor age.

The samples aged between 64 and 99 showed a marked disorganisation of the trabecular beams, some of them being fused together (Fig. 4). The area covered by elastin-like plaques and compacted collagen showed a progressive increase in this age group. In some cases, these features occupied the entire area of the central nucleus (Fig. 5). The basal membrane was also severely degenerated, showing a thickening and a proliferation of small clumps of long-spacing collagen that gave rise to a characteristic shagreen-like appearance (Fig. 6). A marked loss of the endothelial layer of the trabecular beams was also observed, leading the basal membrane to enter in direct contact with the aqueous humour in many areas (Fig. 5).

Discussion

Transmission electron microscopy allowed us to observe significant progressive ultrastructural changes in the extracellular matrix of the central nucleus of the trabecular beams with aging. These changes were seen in almost all extracellular components.

First, we observed an increase in the number and size of elastin-like plaques with age, as

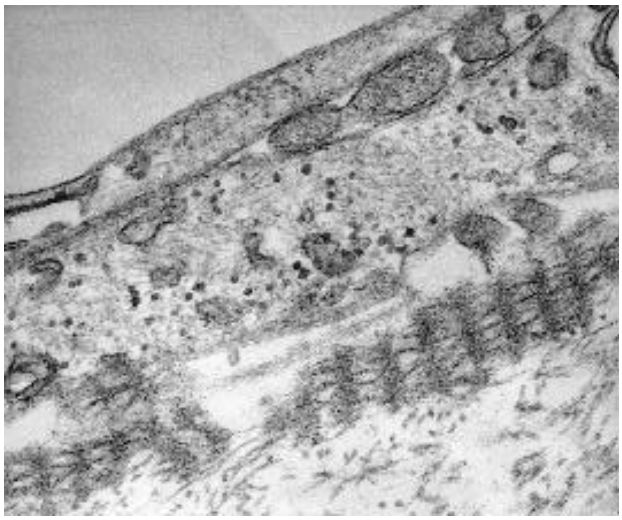

Fig. 3.- Transmission electron micrograph. Detail of subendothelial clumps of long-spacing collagen. x 46,000.

reported elsewhere (Fine, 1966; McMenamin and Lee, 1980; Alvarado et al., 1986; Miyazaki et al., 1987). This may be interpreted as a progressive alteration of the tendons that insert the longitudinal portion of the ciliary muscle to the trabecular beams. Thus, the capacity of this muscle to open the trabecular orifices is reduced and subsequently the physiological drainage of the aqueous humour is affected.

The progressive increase in degenerated and compacted collagen fibres, surrounded by amorphous and granular material with an intermediate electron density, similar to the sheaths of elastin-like plaques, becomes more significant with age. In the oldest specimens, these changes may cover the entire area of the central nucleus of the trabecular beams. The progressive compaction of collagen fibres may cause sclerosis of

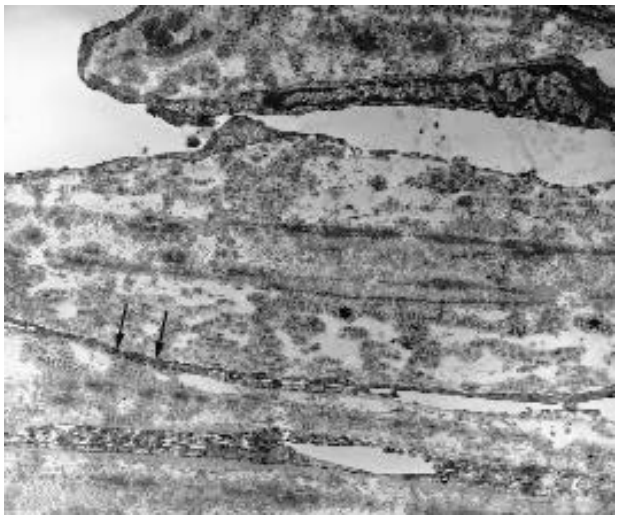

Fig. 4.- Transmission electron micrograph. Fused trabecular beams (arrows). Several clumps of subendothelial long-spacing collagen can be identified (asterisks). x 6,600.

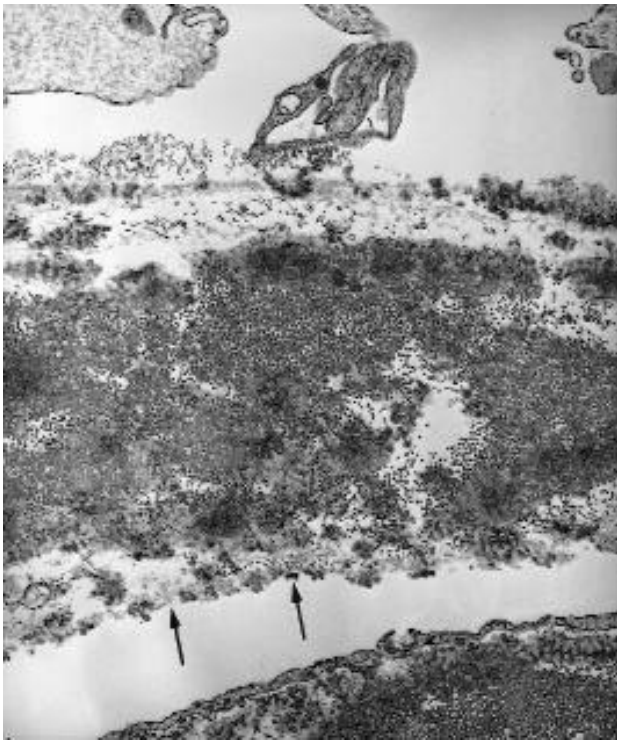

Fig. 5.- Transmission electron micrograph. Central nucleus of a trabecular beam totally occupied by compacted collagen surrounded by elastin-like sheath material. A marked loss of endothelial trabecular cells can be also observed (arrows). x 11,000.

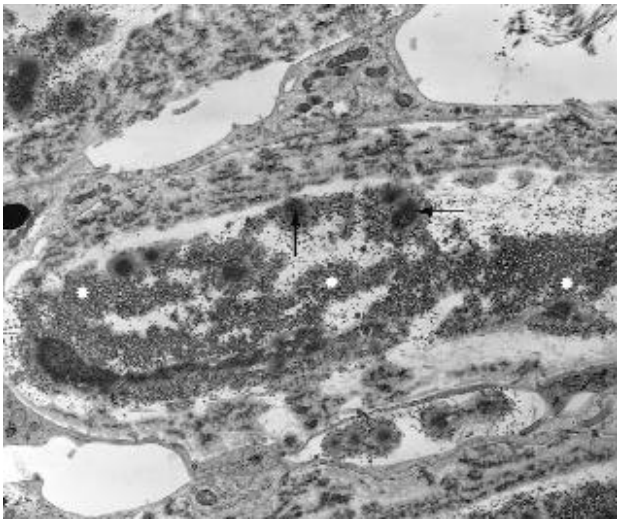

Fig. 6.- Transmission electron micrograph. Trabecular beam showing a degenerated basal membrane with a proliferation of small clumps of long-spacing collagen. The central nucleus also shows an increase in the number of elastin-like plaques (arrows) and an enlargement of the area covered by compacted collagen (asterisks). x 6,600.

the trabecular beams, wich would reduce the efficiency of the ciliary muscle as a regulating element in the transtrabecular drainage of the aqueous humour. In fact, this mechanism relies on the elasticity of the trabecular meshwork, which in turn depends on the presence of collagen III in the central nucleus of the trabecular beams (Murphy et al., 1987). The elastic properties of the trabecular meshwork enable the trabecular orifices to be opened when the longitudinal portion of the ciliary muscle contracts. As reported by Gong et al. (1992), coalescence of collagen fibres may be caused by a progressive reduction in the stabilizing proteglycans chondroitin sulphate and dermatan sulphate (Tawara et al., 1989). Another finding supporting the degeneration of trabecular collagen with age is the progressive appearance of long-spacing collagen clusters at the central nucleus of aged beams with a predominant subendothelial location. This has also been reported elsewhere (McMenamin and Lee, 1980; McMenamin et al., 1986; Hirano et al., 1988).

Finally, we observed a loss of endothelial cells covering the trabecular beams with aging, as previously described by Alvarado et al. (1981), McMenamin et al. (1986) and Miyazaki et al. (1987). This process facilitates the fusion of adjacent layers of beams (Grierson et al., 1982) causing a general compaction and subsequent stiffening of the trabecular meshwork, thus altering the functions of the ciliary muscle and reducing the area of the trabecular orifices through which the aqueous humour flows. In addition, direct contact between the basal membrane and the aqueous humour causes its degeneration and thickening. The presence of cumuli of long-spacing collagen and an electrondense reticulum in the basal membrane are possibly suggestive of the degeneration of collagen I, III and IV, since these types are characteristic components of the trabecular meshwork basal membrane (Murphy et al., 1987; Marshall et al., 1990; Marshall et al., 1991).

In conclusion, it would seem that the changes observed in senile trabecular meshworks may interfere in the physiologic drainage of the aqueous humour, thus facilitating the increase in intraocular pressure usually observed in aged individuals. It would appear that alterations in the aqueous humour outflow mechanism may be caused by changes in the drainage-facilitating function of the ciliary muscle (an increase in number of elastin-like plaques, compaction and degeneration of collagen fibres, fusion of trabecular beams) and by changes that increase the resistance of the trabecular meshwork to the aqueous outflow (fusion and thickening of trabecular beams secondary to a loss of the endothelial layer and a degeneration of the basal membrane). These changes have been related to the progressive decrease in the detoxifying action of enzymes, such as superoxide dismutase, common in the ageing process (Green, 1995; De la Paz and Epstein, 1996).

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