Tenon's Capsule: Ultrastructure of Collagen Fibrils in Normals and Infantile Esotropia

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No detailed information about the ultrastructure of Tenon's capsule has been published. The purpose of the present study was to compare the ultrastructural features of collagen fibrils from Tenon's capsule in a nonstrabismic control group (seven children) to those in an infantile esotropic group (10 children). Small biopsy specimens from Tenon's capsule were taken during various operations to be examined by electron microscopy. On electron microscopy, the capsule was found to be composed of groups of collagen fibrils arranged irregularly in different orientations, forming a three-dimensional network that provides tissue resistance to stress. The cross-sectioned collagen fibrils were studied by an image analyzer. In both study groups, all fibrils had a round, regular contour. In the esotropic group, the Tenon's collagen fibrils were thicker, as reflected by their significantly greater mean diameter: 101 ± 5 nm (mean ± standard deviation) compared to 86 ± 5 nm in the control group. Also, significantly greater heterogeneity was found in the collagen fibril thickness of each individual in the esotropic group compared to the control group. Moreover, the mean number of collagen fibrils per unit area was significantly higher in the esotropic group: 98 ± 13 fibrils per 10^6 nm^2 compared to 73 ± 5 fibrils per 10^6 nm^2 in the control group. These ultrastructural changes may be stress-induced secondary alterations of the Tenon's collagen fibrils resulting from prolonged deviation of the eye in infantile esotropia. The significantly denser collagen fibrils may cause a decrease in the elasticity of Tenon's capsule in infantile esotropia. Invest Ophthalmol Vis Sci 33:651-656, 1992

Tenon's capsule is a fascia made of dense connective tissue that covers the eyeball and the extraocular muscles. This fascia was first described in 1806 by the Parisian ophthalmologist and anatomist Jacques Rene Tenon. There are several synonyms for this fascia, such as the fascia bulbi and the tunica vaginalis oculi, but the most common name is Tenon's capsule.

The four extraocular rectus muscles penetrate Tenon's capsule posterior to the equator, whereas the two oblique muscles penetrate anterior to the equator. The anterior Tenon's capsule extends from the four muscle penetrations to the limbus. The posterior Tenon's capsule lies between the four rectus muscle penetrations and the optic nerve. Not only does Tenon's capsule support and protect the globe, but it also connects the eyeball to the orbit. It plays an important role in the physiology of ocular motility by serving as a cavity within which the eyeball may move.

Parks and other authors stress the importance of Tenon's capsule in squint surgery. An inadequate surgical technique may cause restriction in ocular motility due to scarring and adhesions of the capsule to the extraocular muscles.

We decided to study the ultrastructural features of Tenon's capsule because, in reviewing the literature of the last 20 years, we could not find one study on this topic. Long-standing deviation of the eye in one position, such as in infantile esotropia, may cause shortening and loss of elasticity of the conjunctiva and Tenon's capsule. Because, to the best of our knowledge, no data about the electron microscopic basis of these changes had been previously published, elucidation of the ultrastructural features of the capsule seemed appropriate.

The main purposes of this research were (1) to study the ultrastructural features of the collagen fibrils of Tenon's capsule in children without strabismus (control group); (2) to study the ultrastructural features of these collagen fibrils in children with infantile esotropia (esotropic group); and (3) to compare the ultrastructural features of these collagen fibrils in both groups.
Materials and Methods

Subjects

The study included 17 children in two study groups.

The control group included seven children without strabismus who were operated on under general anesthesia for congenital cataract (n = 3), traumatic cataract (n = 2), retinal detachment (n = 1), or intraocular foreign body (n = 1). Their mean age at operation was 5.9 ± 5.6 yr (mean ± standard deviation; range, 1 mo to 12 yr). The mean refraction of four children in the control group was +1.9 ± 1.75 diopters (mean ± SD; range, −2.0 to +3.25 D).

Small biopsy specimens were taken from Tenon’s capsule during the above-mentioned operations, which were associated with tenotomy as an integral part of the surgical procedure.

The esotropic group included 10 children operated on because of infantile esotropia. Their mean age at operation was 2.9 ± 2.2 yr (range 1.5–8 yr). Their mean refraction was +2.25 ± 2.0 D (range −1.5 to +5.5 diopters), and their mean angle of deviation was 54 ± 15 prism D (range 30–80 prism D). Five had dissociated vertical deviation, five had overaction of inferior oblique muscles, four had amblyopia, two had nystagmus, and one had anomalous head posture.

In all cases, the diagnosis of infantile esotropia was made by an ophthalmologist in the pediatric ophthalmology clinic of Rambam Medical Center. In all cases, manifest esotropia was diagnosed prior to the age of 6 mo. The children were treated and followed up in our pediatric ophthalmology clinic. Only those patients with clinical characteristics of essential infantile esotropia were included in the study.

Seven patients underwent bilateral recession of the medial rectus muscles combined with bilateral recession of the conjunctiva and Tenon’s capsule over the medial rectus muscles. Three patients underwent recession of the lateral rectus muscle and recession of the medial rectus muscle combined with recession of the conjunctiva and Tenon’s capsule. Small biopsies from Tenon’s capsule were taken during these operations. All operations were performed under general anesthesia.

The study was approved by the Helsinki Committee of Rambam Medical Center. In all subjects, preoperative parental informed consent was obtained after the nature of the biopsy procedure had been fully explained.

Tissue Processing

Biopsies from Tenon’s capsule always were taken during the same procedure and by the same surgeon (E.M.) during the various operations.

In each case, a 2 × 2 mm biopsy specimen was taken from the anterior Tenon’s capsule overlying the medial rectus muscle 8–10 mm from the limbus. On removal, the specimens were immediately cut into small pieces and fixed with ice-cold 3% glutaraldehyde in cacodylate buffer, pH 7.3, for 1 hr, postfixed with 2% osmium tetroxide, dehydrated through a series of graded alcohols (50–100%), and embedded in epon. At least four blocks were cut from each biopsy specimen. Sections 1 µm thick were stained with toluidine blue and examined under the light microscope. Thin sections were stained with uranyl acetate and lead citrate and were examined with a Zeiss (Oberkochen, Germany) 9S electron microscope.

Electron Microscopy and Individual Image Analysis

Electron micrographs of Tenon’s capsule, enlarged 3800 times, were taken from areas away from blood vessels. Micrographs of cross-sectioned and longitudinally sectioned collagen fibrils, enlarged 56,000 times, also were taken from these regions. For image analysis and morphometry, micrographs of the cross-sectioned fibrils then were enlarged an additional 1988 times (so the final enlargement was 111,333.6 times).

Image analysis was performed using the Stereology program of the Morphomat 30 image analyzer (Zeiss, Oberkochen, Germany). A histologic window with a constant area of 2,420,297 nm² was defined in each electron micrograph. Each histologic window included 163–304 collagen fibrils. For each patient, image analysis of three histologic windows was performed. Final image analysis was based on the pooled data of the three histologic windows because preliminary analysis showed very little variability between the characteristics of the three windows of each individual.

The pooled area of three windows is denoted as Ar. For each pooled image (j), the number of fibrils (Nj) as well as the area (Aij) and the diameter (Dij) of each fibril (ij) was measured. Five individual summary fibril characteristics were calculated for each pooled image, as follows:

1. Point density (PDYj): The number of fibrils per 10⁶ nm² of the tissue section, ie,

\[
PDYj = \frac{(Nj \times 10^6)}{Ar}
\]

2. Mean fibril diameter (nm) of the individual (Dj):

\[
Dj = \sum_{i=1}^{Nj} Dij/Nj
\]

3. Mean fibril area (nm²) of the individual (Aj):

\[
Aj = \sum_{ij} Aij/Nj
\]
4. Within-individual standard deviation (nm²) of fibril area (SD Aij):

\[ SD \ Aij = \sqrt{\frac{\sum_{j=1}^{Nj} (Aij - \tilde{A}j)^2}{Nj - 1}} \]

5. Volume density (VDYj): The fraction of the tissue volume occupied by the fibrils, i.e.,

\[ VDYj = \frac{\sum_{i=1}^{Nj} Aij}{Ar} \]

Statistical Comparison Between the Study Groups

Mean values ± SD (followed by ranges in parentheses) pertaining to each of the five individual summary fibril characteristics were computed for each of the study groups. Statistical comparison of the distributions of the summary fibril characteristics between the control group and the esotropic group was performed by Wilcoxon’s rank-sum test because of inequality of the variances between the two groups.

Results

Light Microscopy

Examination of Tenon’s capsule by light microscopy showed dense connective tissue with abundant collagen fibers, irregularly arranged around a few fibroblasts.

Electron Microscopy

Tenon’s capsule (general view): On electron microscopy with 3800-power magnification, we did not notice a remarkable difference between the basic structure of Tenon’s capsule in the control group and in the esotropic group. In both groups the tissue was composed of groups of collagen fibrils arranged irregularly in different orientations (Fig. 1). The longitudinal, horizontal, and oblique groups of collagen fibrils formed a three-dimensional network. The longitudinally sectioned collagen fibrils showed a delicate, wavy appearance. The collagen fibrils formed the major component of the tissue, with only a few fibroblasts interspersed between.

Collagen fibrils: On high magnification (>56,000), most cross-sectioned collagen fibrils in both groups had a round, regular contour (Figs. 2 and 3). Variability of diameter and of area of the collagen fibrils was evident.
Comparison of the Two Study Groups

The number of collagen fibrils per unit area, the size of the individual fibril, and the within-individual variability of fibril size all were larger in the esotropic group (Fig. 3) than in the control group (Fig. 2).

**Point density:** There was complete separation of the two study groups regarding the point density of each individual PDYj (Fig. 4). The mean PDYj of the esotropic group was higher than that of the control group. The group mean PDYj was 98 ± 13 fibrils per $10^6$ nm$^2$ (79–117 fibrils per $10^6$ nm$^2$) in the esotropic group, compared to 73 ± 5 fibrils per $10^6$ nm$^2$ (68–78 fibrils per $10^6$ nm$^2$) in the control group. The difference in the distributions between the two groups was highly significant ($P < 0.001$).

**Collagen fibril diameter:** The group mean of mean fibril diameter of each individual (Dj) in the esotropic group was larger than that in the control group—101 ± 5 nm (94–108 nm) versus 86 ± 5 nm (77–91 nm). The difference in the distributions between the two groups was highly significant ($P < 0.001$).

**Collagen fibril area:** As expected from the difference in the fibril diameter, fibril area of the esotropic group was larger than that of the control group. The group mean of the mean fibril area of each individual (Aj) was 7352 ± 749 nm$^2$ (6432–8536 nm$^2$) in the esotropic group, compared to 5299 ± 561 nm$^2$ (4313–5934 nm$^2$) in the control group. Moreover, there was complete separation of the two study groups with respect to the mean fibril area of each individual (Fig. 5). The difference in the distributions between the two study groups was highly significant ($P < 0.001$).

The within-individual standard deviation of fibril area (SD Aij) in the esotropic group was significantly higher than that in the control group ($P < 0.001$). The group mean of this characteristic was 767 ± 54 nm$^2$ (670–848 nm$^2$) in the esotropic group, compared to 587 ± 79 nm$^2$ (428–673 nm$^2$) in the control group. In addition, there was almost complete separation of the two study groups in regard to the individual SD of the collagen fibril area (Fig. 6). The finding indicates that more heterogeneity existed in the collagen fibril area of each individual in the esotropic group than in the control group.

**Volume density:** The VDYj of the esotropic group was higher than that of the control group. The group mean VDYj was 0.72 ± 0.08 (0.62–0.81) in the esotropic group, compared to 0.39 ± 0.03 (0.34–0.43) in the control group. The difference in the distributions between the two study groups was highly significant ($P < 0.001$).

The greater volume density in the esotropic group resulted from their greater number of fibrils per unit area and from their larger mean fibril area. Thus, simi-
lar to these two latter fibril characteristics, there was a complete separation between the two study groups regarding individual values of volume density (data not shown).

Periodicity of collagen fibrils: In longitudinal sections, the periodical banding of the fibrils was uniform, and the length of each period was 60–64 nm. No difference of periodicity existed between the control group and the esotropic group.

Discussion

In reviewing the literature of the last 20 years, we could not find a single study concerning the ultrastructure of Tenon's capsule. The main contribution of the present study is to present for the first time detailed information on the ultrastructural features of the collagen fibrils of Tenon's capsule in nonstrabismic eyes and in eyes with infantile esotropia.

Using transmission electron microscopy, Tenon's capsule was found to be composed of groups of collagen fibrils arranged irregularly in different orientations (Fig. 1). The three-dimensional network formed by groups of collagen fibrils may provide tissue resistance to stress. This basic formation also may enable Tenon's capsule to play an important role in supporting, protecting, and connecting the eyeball to the orbit. A different arrangement of the collagen fibrils is found in the corneal lamellae, which are composed of very regularly spread, uniform collagen fibrils that provide corneal transparency.

In the control group, which we believe represents normal Tenon's capsule tissue, mean collagen fibril diameter was 86 ± 5 nm and mean area was 5299 ± 561 nm². Quantitative analysis of electron micrographs has shown that the diameter of collagen fibrils may range from 20–120 nm in most connective tissues. The mean diameter of the collagen fibrils in normal human skin is considered by most authors to be 90–100 nm. Thus, normal collagen fibrils of the reticular derrmis are slightly larger than those of Tenon's capsule. In contrast, most collagen fibrils of the normal cornea measure 27–35 nm and are much smaller than those of normal Tenon's capsule. Mean point density of the Tenon's collagen fibrils in the control group was 73 ± 5 fibrils per 10⁶ nm², and mean volume density was 0.39 ± 0.03.

In the esotropic group, the Tenon's collagen fibrils were significantly thicker than those in the control group, as reflected by their larger cross-sectional areas. In addition, there were more fibrils per unit of Tenon's tissue volume in the esotropic group, indicated by their higher point density compared to the control group. As a result, the Tenon's collagen fibrils of the esotropic group were more densely arranged, as reflected by their higher volume density. In addition, there was greater heterogeneity in fibril thickness of the esotropic group, as demonstrated by their higher within-individual SD of fibril area.

Generally, most collagen fibrils are characterized by fairly uniform shape and diameter in most mature normal tissues. However, abnormal variations in the size and shape of the fibrils have been described in various diseases. The exact causes of increased fibril diameter and greater heterogeneity of fibril thickness in the esotropic group are unknown. Our hypothesis is that the ultrastructural changes of Tenon's capsule are secondary changes associated with infantile esotropia. Prolonged deviation of the eye in one position may exert tension or stress on the capsule, stimulating fibrosis or healing processes associated with increased diameter and greater heterogeneity in fibril thickness.

After corneal wounds heal, the diameter of the regenerated collagen fibrils is greater than normal. Also, fibrils of large and small diameter have been found in the dermis of patients undergoing wound repair. Also, some studies have shown there may be a relationship between mechanical stress and ultrastructural features of fibrils in different connective tissues. Matthew et al studied quantitatively the ultrastructure of collagen fibrils in the healing extensor digitorum longus tendon of the rat. Collagen fibrils with significantly larger diameters were synthesized only in response to increased levels of stress, in contrast to smaller fibrils, which were synthesized in areas of low and high stress levels.

Similarly, Stransky et al reported on heterogeneity in the diameter of collagen fibrils in idiopathic carpal tunnel syndrome. They demonstrated the presence of fibrils with small diameters comparable to that in normal tissue, as well as fibrils with large diameter that could not be observed in normal tissue. Thus, we suggest that the ultrastructural alterations in the collagen fibrils of Tenon's capsule in infantile esotropia may be stress-induced secondary changes, similar to those described in other connective tissues. Lack of size uniformity and significantly larger collagen fibrils forming the fibrotic lateral rectus muscle also have been described in patients with infantile esotropia and myopia.

Von Noorden and Helveston point out that long-standing esotropia may cause shortening and decreased elasticity of the conjunctiva and Tenon's capsule. Our study may provide the ultrastructural basis for their clinical observations. It is known that the collagen fibers, in contrast to the elastic fibers of the connective tissue, are not extensible. Therefore, the increase in the number and thickness of the collagen fibrils, resulting in their greater density, may cause a
decrease in the connective tissue elasticity of Tenon’s capsule in infantile esotropia.

The main surgical procedures for the treatment of infantile esotropia are bilateral recession of the medial rectus muscles or recession of the medial rectus muscle combined with resection of the lateral rectus muscle. The effectiveness of medial rectus muscle recession may decrease if the conjunctiva and Tenon’s capsule are returned to their original position at the end of the operation. On the other hand, recession of the conjunctiva and Tenon’s capsule can significantly enhance the effectiveness of medial rectus muscle recession by decreasing the restrictive effect caused by shortened and inelastic Tenon’s capsule. As mentioned above, the decreased elasticity of Tenon’s capsule in infantile esotropia may be explained according the ultrastructural findings reported here.

Further research with a larger number of subjects is needed to determine more definitely the characteristics of collagen fibrils in Tenon’s capsule in nonstrabismic patients and in those with infantile esotropia.

Key words: Tenon’s capsule, ultrastructure, image analysis, collagen fibrils, infantile esotropia

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