Effect of Latanoprost and Timolol on the Histopathology of the Rabbit Conjunctiva

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PURPOSE. Long-term medical treatment of glaucoma has an effect on the conjunctiva, possibly affecting the outcome of subsequent filtering surgery. The type and extent of these tissue changes caused by frequently used medications is important. An animal study using rabbits was performed to assess the tissue changes caused by timolol, latanoprost, and a combination of both substances.

METHODS. Rabbits were treated with timolol, latanoprost, or a combination of these drugs for 18 months. Conjunctival specimens were examined by light microscopy, quantitative transmission electron microscopy, and immunohistochemistry with antibodies against matrix metalloproteinase (MMP)-3 and tissue inhibitors of metalloproteinase (TIMP)-2.

RESULTS. By electron microscopy, the area of subepithelial collagen was significantly larger (P < 0.03; Mann–Whitney test) in timolol-treated eyes (71.6%) than in control (52.7%) and latanoprost-treated eyes (57.7%). An increase of amorphous material was present in timolol-treated eyes (25.6% versus 7.6% in the controls) as well as a smaller area of empty spaces (2.5% versus 39.4% in control eyes). Latanoprost-treated eyes had no significant increase of empty spaces but showed a marked staining for MMP-3 in the conjunctiva. This staining was not present in control or timolol-treated eyes. Morphologically, degenerative changes of fibrocytes were seen in timolol-treated eyes only.

CONCLUSIONS. A significant increase of subepithelial collagen density was present in timolol-treated eyes, whereas this finding was not apparent in latanoprost-treated eyes. Latanoprost-treated eyes showed an upregulation of MMP-3, which may be the reason for reduced extracellular matrix accumulation in such eyes. The morphologic feature of increased subepithelial collagen density and extracellular matrix changes may relate to failure of filtering blebs. (Invest Ophthalmol Vis Sci. 2001;42: 679–687)

Most treatment modalities for both primary and secondary forms of glaucoma are designed to constantly lower intraocular pressure, whereas only a few others, for some special forms of glaucoma, are intended to increase the blood flow in the area of the optic nerve head. There is evidence that long-term topical treatment of glaucoma induces changes of the conjunctival tissue including an increase in the number of mononuclear inflammatory cells both in the epithelium and stroma, and an increase in collagen deposition. It has been proposed that alteration in the function of the fibrocytes is caused by the topical medications. These changes relate to the amount, frequency of application, and type of medication as well as whether the drugs include preservatives.

Trabeculectomy is the most frequently performed surgical procedure to treat glaucoma. Several studies suggest that the surgical outcome is more favorable in patients with no or only short-term previous topical antiglaucoma therapy. Therefore, some surgeons tend to treat patients for a short time before surgery with a reduced amount of topical medication. Others replace topical antiglaucoma therapy by topical steroids and systemic antiglaucoma medication, in an effort to diminish the pathologic conjunctival effects and thus improve the surgical outcome. This is a rational approach, because the fibrocytes of the conjunctiva, Tenon’s capsule, and episclera are those cells that form the scar tissue surrounding the scleral flap, which ultimately causes surgical failure.

An animal study using rabbits was conducted to study such conjunctival alterations morphologically. The two substances timolol and latanoprost were chosen. Timolol has been available for many years, whereas latanoprost has only recently been introduced into the market, so that information regarding its side effects is limited. Morphologic changes to the conjunctiva of rabbits exposed to either timolol or latanoprost were investigated. To complete the study, the effect of different combinations of the two drugs on the conjunctiva was additionally examined.

MATERIALS AND METHODS
Thirty-two female pigmented chinchilla outbred rabbits initially weighing 1.9 to 2.5 kg were studied. The experiment was performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The project was approved by the local animal research review committee of the authors’ institution. The animals were maintained in a 12-hour day and 12-hour night cycle. They were fed and had access to water ad libitum.

Treatment
The animals were randomly and equally divided into four groups of eight rabbits (Table 1). Left eyes were treated, and right eyes served as untreated controls.

Latanoprost eye drops (50 µg/ml, Xalatan; Pharmacia & Upjohn, Uppsala, Sweden) were applied once a day (group 1). Timolol eye drops (5.0 mg/ml; Chibro-Timoptol; Alcon Pharma, Friedberg, Germany) were applied twice daily (group 2). A fixed combination of timolol and latanoprost was provided by the manufacturer (Pharmacia & Upjohn) and applied once daily (group 3). In group 4, timolol was applied twice daily and latanoprost once. The time interval between application of these two different eye drops was at least 30 minutes (group 4). The time interval between applications for animals receiving therapy twice daily was 12 hours.

Histopathology
After 18 months, the rabbits were killed, and all left eyes were enucleated with sufficient amounts of perilimbal conjunctiva. Eight right...
eyes were randomly selected as controls (two from each of the four groups) and similarly enucleated and processed.

For orientation, a silk suture was placed at the 12-o’clock position through the cornea. The eyes were immediately bisected in a sagittal vertical plane. One half of each globe was fixed in 4% paraformaldehyde buffered at pH 7.2 for light microscopic examination and immunohistochemistry. The second half was placed in a solution containing 3% glutaraldehyde and 1% paraformaldehyde for further processing for transmission electron microscopy.

The slides prepared included sections of both superior and inferior conjunctiva, stained with hematoxylin-eosin (HE).

**Immunohistochemistry**

For light microscopic immunohistochemistry, the labeled streptavidin-biotin method (LSAB Plus kit; Dako, Glostrup, Denmark) was applied on paraffin-embedded tissue sections according to the manufacturer’s instructions. Briefly, the sections were incubated for 30 minutes each with monoclonal mouse anti-rabbit matrix metalloproteinase (MMP)-3 (stromelysin) and mouse anti-tissue inhibitor of metalloproteinase (TIMP)-2 antibodies (Chemicon, Temecula, CA) in a concentration of 5 μg/ml, with the biotinylated link antibody and horseradish peroxidase (HRP)–conjugated streptavidin. Proteolytic predigestion using proteinase K was performed for 6 minutes. 3-Amino 9-ethyl carbazole was used as a chromogenic substrate and Mayer’s hemalum as a counterstain.

Negative controls included incubation of sections with irrelevant monoclonal antibodies and omission of the primary antibodies.

**Transmission Electron Microscopy**

From the second half of each globe, specimens that included superior conjunctiva, limbus, and sclera were excised adjacent to the 12-o’clock position. The specimens were further processed by postfixation in 2% buffered osmium tetroxide and embedded in epoxy resin (Epon 812; Fluka, Buchs, Switzerland) according to a standard technique. Semithin sections were stained with toluidine blue, and ultra-thin sections were stained with uranyl acetate, together with lead citrate and examined with an electron microscope (EM 906E; Leo, Oberkochen, Germany).

**Quantitative Analysis**

For quantitative analysis of the extracellular parameters (collagen fibers, amorphous substance, and electron-lucent spaces), an automated image-processing system (DigiVision; Leo) with an integrated software package (Analysis; Soft Imaging Systems, Münster, Germany) was used. From each specimen, two sections were investigated in areas close to the surgical limbus. The measurements were performed according to a defined random sampling procedure, using the bars of the supporting grid square as points of reference, by which 10 consecutive areas adjacent to the right side of a grid bar were analyzed. Ten measurements per specimen were performed in the subepithelial stroma and in the deeper stroma at a distance of approximately 100 μm from the epithelial basement membrane. Only areas including extracellular matrix but avoiding areas with cells or blood vessels were examined.

The size of the area analyzed was 66.8 μm² at a magnification of ×4000. The different gray values of the structural parameters measured (collagen fibers, amorphous material, optically empty spaces) were transformed into red, green, and white, and the percentage areas occupied by the different color-coded phases were automatically calculated.

The investigator (US) performing light and electron microscopy, area ± SD measurements, and immunohistochemistry was masked regarding the treatment protocol of the eyes.

For statistical analysis of the automatically measured areas, the mean ± SD of the individual 10 measurements was calculated for each layer and tissue component. These mean values were then compared between the control and the treated groups regarding each different component. For this purpose, the nonparametric Mann–Whitney test was used (StatView, ver. 4.5; Abacus Concepts, Berkeley, CA). Differences were considered significant at *P* < 0.05.

**RESULTS**

**Histopathology**

Examination of the sections stained with HE and toluidine blue (1-μm sections from plastic-embedded tissue) concentrated on the conjunctival histology at the 12-o’clock position, because this is the area where filtering surgical procedures are usually performed.

The control conjunctiva consisted of a normal goblet-cell-containing epithelium with an intact epithelial basement membrane, loose collagenous connective tissue, and no evidence of acute or chronic inflammation in the substantia propria. In semithin sections, an increase of the density of the collagen fibers in the substantia propria was present in eyes treated with timolol and treated with combinations of timolol and latanoprost (groups 2, 3, and 4), but not in eyes treated with latanoprost alone (group 1).

**Immunohistochemistry**

In control eyes, the conjunctival epithelium and the conjunctival stroma did not stain with the antibody against MMP-3. In eyes treated with latanoprost (group 1), the conjunctival epithelial cells showed increased staining for MMP-3. In the stroma, a moderate number of fibrocytes were stained, whereas the extracellular matrix did not show any positive reaction (Fig. 1). In eyes treated with timolol (group 2), the epithelium and the stroma were negative for MMP-3. In eyes with combined or separate therapy with latanoprost and timolol (groups 3, 4), the epithelium stained mildly positive, whereas several fibrocytes in the stroma were positive. In addition, the subepithelial stromal layer exhibited moderate diffuse staining for MMP-3.

In control eyes, the conjunctival epithelium and the conjunctival stroma did not stain with the antibody against TIMP-2.

<table>
<thead>
<tr>
<th>Group (n = 8)</th>
<th>Substance</th>
<th>Concentration (mg/ml)</th>
<th>Preservative</th>
<th>Concentration (mg/ml)</th>
<th>Applications per Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>Latanoprost</td>
<td>0.05</td>
<td>BAC</td>
<td>0.2</td>
<td>One</td>
</tr>
<tr>
<td>2</td>
<td>Timolol</td>
<td>5.0</td>
<td>BAC</td>
<td>0.1</td>
<td>Two</td>
</tr>
<tr>
<td>3</td>
<td>Combination</td>
<td>5.0/0.05</td>
<td>BAC</td>
<td>0.1</td>
<td>One</td>
</tr>
<tr>
<td>4</td>
<td>Timolol</td>
<td>5.0</td>
<td>BAC</td>
<td>0.1</td>
<td>Two</td>
</tr>
<tr>
<td></td>
<td>Latanoprost</td>
<td>0.05</td>
<td>BAC</td>
<td>0.2</td>
<td>One</td>
</tr>
</tbody>
</table>

BAC, benzalkonium chloride.

**TABLE 1. Topical Ocular Treatment of Four Groups of Rabbits**
In eyes treated with latanoprost (group 1), the conjunctival epithelial cells did not stain with the antibody, although in the stroma, a moderate amount of fibrocytes also stained positive (arrowhead). (E) In eyes treated with timolol (group 2), the epithelium and the stroma were negative. (G) In eyes with combined therapy with latanoprost and timolol (group 3), the epithelium (arrow) stained mildly positive, whereas several fibrocytes in the stroma (arrowhead) were positive. In addition, the subepithelial stromal layer exhibited moderate diffuse staining. (B) Control: The epithelium and the conjunctival stroma did not stain with the antibody against TIMP-2. (D) In eyes treated with latanoprost (group 1), the conjunctival epithelial cells (arrow) did not stain with the antibody. In the stroma (arrowhead), only a few fibrocytes stained mildly with the antibody. (F) In eyes treated with timolol (group 2), the epithelium and the stroma were negative. (H) In eyes with combined therapy with latanoprost and timolol (group 3), the epithelium was negative, whereas several fibrocytes in the stroma (arrows) were positive. In addition, the subepithelial stromal layer exhibited moderate to marked diffuse staining. Immunohistochemistry for MMP-3 or TIMP-2; counterstaining with Mayer’s hemalum. Original magnification, ×63.

Table 2. Extent of Cellular and Extracellular Changes in Conjunctival Specimens

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Goblet Cells</th>
<th>Subepithelial Collagen Density</th>
<th>Fibroblasts</th>
<th>Amorphous Material in ECM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal</td>
<td>None</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>None to mild</td>
<td>Normal</td>
<td>None to mild</td>
</tr>
<tr>
<td>2</td>
<td>Reduced</td>
<td>Moderate to marked</td>
<td>Degenerated</td>
<td>Marked</td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
<td>Mild to moderate</td>
<td>Normal</td>
<td>None to mild</td>
</tr>
<tr>
<td>4</td>
<td>Reduced</td>
<td>Moderate</td>
<td>Degenerated</td>
<td>Mild</td>
</tr>
</tbody>
</table>

Observations were made by transmission electron microscopy after 18 months of treatment. ECM, extracellular matrix.
generated. There was a moderate to marked increase of collagen density in the subepithelial area intermixed with large amounts of amorphous substance. In focal areas, inflammatory cells and plasma cells were present. Capillaries had multilaminar basement membranes. The fibrocytes appeared plump with prominent and enlarged rough endoplasmic reticulum and were associated with pericellular amorphous material (Fig. 4).

In group 3, in which eyes were treated with a fixed combination of latanoprost and timolol once daily, the number of goblet cells in the epithelium was not significantly reduced, and the mitochondria in the epithelial cells were only mildly affected. In the subepithelial stroma, there was only a mild increase of collagen fibers. Fibrocytes appeared to be without signs of degeneration or activation (Fig. 5).

Eyes that were treated separately with timolol and latanoprost (group 4) showed a moderate to marked reduction in the number of epithelial goblet cells and flattened epithelial cells. The intercellular spaces appeared enlarged and mostly filled with an amorphous material. Mitochondria were frequently enlarged. In the conjunctival stroma, there was a mild increase of collagen density and a moderate amount of amorphous material. The fibrocytes were frequently degenerated. Subepithelial capillaries had multilaminar basement membranes (Fig. 5).

Quantitative Analysis

The quantification of changes of the conjunctival stroma in the areas of collagen fibers, amorphous material, and optically clear spaces in areas without cells or vessels showed significant differences among the four groups and the control eyes (Table 3; Fig. 6).

**Figure 2.** Electron micrographs of conjunctiva from control eyes. (A) There were regular epithelial cells with two goblet cells of normal size and loosely arranged subepithelial collagen fiber bundles (>). (B) Aspect from the deeper substantia propria: Regular fibrocytes (arrow) were present with collagen fibers arranged in loose bundles with no interfibrillar ground substance (>). Bar, (A) 7 μm; (B) 2 μm.

**Figure 3.** Electron micrographs of conjunctiva from eyes treated with latanoprost 0.005% once daily for 18 months. There was a variability among different specimens showing no increased subepithelial collagen density (A, >) or mild increased collagen density (B, >). (C, D) Fibrocytes and collagen fibers in the substantia propria appear without abnormalities and with no amorphous material (<) between the collagen bundles. Bar, (A, B) 10 μm; (C) 5 μm; (D) 1 μm.
In the subepithelial layer, the percentage of area occupied by collagen fibers was significantly higher in groups 2, 3, and 4 than in the control and group 1. Although the amount of collagen fibers was approximately 53% in the control samples, there was an increase of approximately 36% in groups 2, 3, and 4. There was no significant difference between the control eyes and group 1 ($P = 0.72$; Mann–Whitney). The amount of amorphous material was low in the control eyes (approximately 8%), and there was no significant difference in comparison to group 1 ($P < 0.29$). However, there was a significant increase when the control eyes were compared with groups 2, 3, and 4 ($P < 0.03$; $P < 0.05$; $P < 0.03$). Similarly, approximately 40% of the area measured appeared as optically clear spaces in control eyes, with a mild reduction in group 1 ($P < 0.08$) and a significant decrease when compared with groups 2, 3, and 4 ($P < 0.05$; $P < 0.05$; $P < 0.05$).

In the deeper layers of the conjunctival substantia propria, the amount of collagen fibers in the control eyes was no different in comparison with collagen in the subepithelial layer. Again, there was no difference between the control group and group 1 ($P < 0.48$), but there were significant increases in groups 2, 3, and 4. In addition, the differences between group 1 and groups 2, 3, and 4 were significant ($P < 0.03$; $P < 0.05$; $P < 0.03$). The increase of amorphous material between the control eyes and group 1 was not significant ($P = 0.16$), but it was significant when compared with groups 2, 3, and 4. Furthermore, the differences between group 1 and groups 2, 3, and 4 were significant ($P < 0.02$; $P < 0.03$; $P < 0.02$). There was no reduction of optically empty spaces between the control group and group 1 ($P < 0.29$). The significant reduction of optically empty spaces occurred when the groups 2, 3, and 4 were compared with the control eyes ($P < 0.03$; $P < 0.05$; $P < 0.03$). The difference between group 1 and groups 2, 3, and 4 again was significant ($P < 0.02$; $P < 0.05$; $P < 0.02$).

**Figure 4.** Electron micrographs of conjunctiva from eyes treated with timolol 0.5% twice daily for 18 months, showing (A) a reduction of goblet cells of the epithelium, (B) distended spaces (**) between individual epithelial cells, and degenerated mitochondria (**arrow**), and (C) a marked increase of collagen density (**) in the area beneath the epithelium with a large amount of amorphous material. This abnormal material (**D, **) was also interspersed between the collagen fiber bundles of the deeper stroma in association with fibrocytes. Some fibrocytes in the substantia propria (**E**) contained prominent, distended, rough endoplasmic reticulum (**arrow**). (**F**) Stromal capillaries (**Ca**) show partially multilaminar basement membranes (**arrow**). Bar, (**A**) 10 μm; (**B**) 3 μm; (**C**) 2 μm; (**D, E, and F**) 1 μm.
DISCUSSION

Several studies suggest that eyes with a history of long-term topical antiglaucoma treatment have a less favorable outcome after filtering procedures. It has been proposed that such drugs cause changes of the conjunctival tissue.

It is of interest to investigate and define the kind and extent of conjunctival changes that are caused by long-term use of antiglaucoma drugs and combinations of these. In contrast to such long-term treatment, toxic effects of drugs or preservatives occur after short-term treatment with relatively high doses, which may be 10 or 100 times higher than the actual concentrations applied for glaucoma therapy. Toxicity studies are usually performed by pharmaceutical companies before new drugs are introduced into the market. In the context of this study, we sought to investigate the tissue changes that may occur when the drugs are used in long-term treatment of glaucoma.

Conjunctival changes after long-term topical antiglaucoma therapy include a decrease in the number of epithelial goblet cells, an increase in subepithelial collagen deposition, and a higher number of macrophages, fibrocytes, lymphocytes, and mast cells in the substantia propria. Antiglaucoma drugs cause, in addition to adverse effects on the conjunctiva, a delay in corneal epithelial regeneration and a decrease of the mucous layer of the tear film.

An additional effect on the conjunctival alterations may be caused by the preservatives, which are usually applied together with the pressure-lowering drugs. In an in vitro study, Tenon's fibroblast proliferation was not altered by exposure to β-blockers without preservatives, but by exposure to β-blockers with preservatives. In an experiment using rabbits, a mild increase of subepithelial fibrosis was detected in eyes treated with glaucoma drugs and preservatives. In addition, an increase in collagen type IV staining and staining for α-smooth muscle actin was present in that area.

TABLE 3. Quantification of Extracellular Conjunctival Tissue Changes

<table>
<thead>
<tr>
<th>Group</th>
<th>Collagen Fibers</th>
<th>Amorphous Material</th>
<th>Empty Spaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subepithelial layer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>52.7 ± 1.2</td>
<td>7.6 ± 6.3</td>
<td>39.4 ± 5.0</td>
</tr>
<tr>
<td>1</td>
<td>57.7 ± 4.4</td>
<td>20.8 ± 5.9</td>
<td>22.4 ± 6.5</td>
</tr>
<tr>
<td>2</td>
<td>71.6 ± 1.1*</td>
<td>25.6 ± 1.0*</td>
<td>2.5 ± 0.4*</td>
</tr>
<tr>
<td>3</td>
<td>68.0 ± 2.1*</td>
<td>26.5 ± 2.2*</td>
<td>3.4 ± 1.2*</td>
</tr>
<tr>
<td>4</td>
<td>70.7 ± 1.4*</td>
<td>25.1 ± 0.9*</td>
<td>4.5 ± 1.2*</td>
</tr>
<tr>
<td>Deep layer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>50.0 ± 3.1</td>
<td>1.9 ± 1.9</td>
<td>48.0 ± 4.9</td>
</tr>
<tr>
<td>1</td>
<td>55.5 ± 4.4</td>
<td>7.8 ± 2.7</td>
<td>36.8 ± 6.9</td>
</tr>
<tr>
<td>2</td>
<td>77.4 ± 1.1*</td>
<td>15.8 ± 1.3*</td>
<td>6.6 ± 0.2*</td>
</tr>
<tr>
<td>3</td>
<td>76.2 ± 1.8*</td>
<td>13.9 ± 0.6*</td>
<td>9.9 ± 2.4*</td>
</tr>
<tr>
<td>4</td>
<td>72.2 ± 3.8*</td>
<td>22.9 ± 4.2*</td>
<td>4.5 ± 1.3*</td>
</tr>
</tbody>
</table>

The subepithelial layer is the area directly beneath the conjunctival epithelium, and the deep layer is in the substantia propria. Treatments were as follows: group 1, latanoprost; group 2, timolol; group 3, fixed combination; and group 4, lantanoprost and timolol separately. Data are expressed as mean percentages ± SD.

* Significant difference as compared to control (Mann-Whitney test; P < 0.05).
substantiated using quantification in this context. Using this technique, it was possible to apply statistics to the morphologic changes observed. In the subepithelial layer, it was apparent that the amount of collagen fibers did not significantly increase in latanoprost-treated eyes, despite increases in all other treatment groups. This increase amounted to roughly 32% in comparison with control eyes. Corresponding to this increase of collagen density, the amount of empty spaces was reduced by approximately 45% in latanoprost-treated eyes compared with controls, but by approximately 90% in the eyes treated with timolol or combination therapy (mean values). Of note, the increase in collagen fiber density in timolol-treated eyes was associated with a decrease of fibrocytes, leading to the phenomenon that fewer producer cells produced an increased amount of extracellular matrix. The reason for this phenomenon remains obscure.

This effect was similarly present in the deeper conjunctival stroma. The amount of amorphous material present between collagen fibers increased by approximately 300% in latanoprost-treated eyes in comparison with controls (not significantly), but by approximately 600% to 1000% in the other groups (significantly). With these results, a specific effect of the drugs applied could be shown on the stromal fibrocytes in the absence of inflammatory cells. It became apparent from the data, that the tissue changes were less prominent with latanoprost in comparison with timolol or combination therapy.

The role of MMPs in glaucoma has not yet been fully elucidated. MMPs are a group of proteolytic enzymes active against all components of extracellular matrix. In pterygia samples and cultured pterygium epithelial cells, an increase of both MMPs and TIMPs was found. This finding may indicate that the regulation of MMPs and TIMPs plays a role in the formation of excessive fibrous tissue by fibroblasts.

Kawashima et al. investigated the amount of MMP and TIMP in normal conjunctiva and conjunctival scar tissue from patients. They detected by immunohistochemistry MMPs and TIMPs in the scar tissue, but not in the control tissue. They proposed that both MMPs and TIMPs may play a role in the remodeling of fibrous scar tissue after surgical interventions.

Huang et al. examined normal aqueous humor by zymography and found several active forms of MMPs present in the aqueous, as well as their endogenous inhibitors (TIMPs). In a different study of aqueous humor samples from eyes with primary open-angle glaucoma (POAG), eyes with secondary forms of glaucoma, and control eyes, an increase of TIMPs was found only in the aqueous humor of POAG-affected eyes. When the aqueous samples were used in tissue cultures, they increased collagen synthesis significantly. The authors concluded that this effect may contribute to an increased deposition of collagen in the trabecular meshwork and thus play a role in the pathogenesis of POAG.

In a study seeking to explain the pressure-lowering effect of latanoprost (a prostaglandin F<sub>2</sub><sub>a</sub> analogue), tissue sections of monkey eyes were treated with latanoprost. In those sections, the anterior part of the ciliary muscle contained less collagen types IV and VI, as detected by immunostaining. In addition, the amount of MMP-2 and MMP-3 was increased. It was concluded that latanoprost may have a degradative effect on the extracellular matrix of the ciliary muscle and thus facilitate the flow of aqueous through the ciliary muscle spaces into the uvea and sclera. In a similar study in which monkey eyes were treated with prostaglandin F<sub>2</sub><sub>a</sub> isopropyl ester, it was found that the amounts of collagen types I, III, and IV were significantly reduced in the ciliary muscle and adjacent sclera. The authors proposed that this specific effect of this prostaglandin may contribute to the increased uveoscleral outflow.

In a study by Baun et al., no increase of inflammatory cells, goblet cells, or fibrocytes was detected by examination of 18 conjunctival samples from patients with a history of treatment of up to 4 years. It may be speculated, that such changes need longer term treatment to develop to an extent that has been described earlier.

Timolol was the first β-blocker introduced to the market for treatment of glaucoma. Broadway et al. examined conjunctival biopsy specimens from 10 patients treated with β-blockers for a mean period of 18 months. Those specimens contained more mast cells in the superficial conjunctiva and a decrease of such cells in the deeper substantia propria. Otherwise, no differences were seen in the cellular make-up of the specimens.

In the present study, not only were conjunctival changes examined by light microscopy, but, in addition, electron microscopy was used to evaluate abnormalities both of conjunctival fibrocytes and extracellular elements including collagen fibers, amorphous material, and empty spaces. To the best of our knowledge, this is the first report in which morphologic changes observed by electron microscopy have been further
Recently, a different group\textsuperscript{35} exposed the posterior uveal tract to latanoprost acid and measured a marked increase of MMP-1 after incubation. This increase in MMP-1 biosynthesis may in addition contribute to the enhanced uveoscleral outflow effect of latanoprost. Weinreb et al.\textsuperscript{36} found that ciliary smooth muscle cells in tissue culture secrete several isoforms of MMP and that this secretion can be increased by exposure to specific prostaglandins.

No other studies have been performed to investigate the effect of topical antiglaucoma medications on the amount of MMP and TIMP in the conjunctiva.

In agreement with previous studies, MMP and TIMP were not found in the control eyes in the present study. In latanoprost-treated eyes, an increase of MMP-3 activity was found in the conjunctival epithelium, which may be a direct effect of latanoprost. In addition, a mild increase of TIMP was found in stromal fibrocytes. These findings correlate with the absence of extracellular tissue changes in that group. Timolol-treated eyes had no increase of MMP-3 activity in the conjunctiva and also no immunoreactivity for TIMP-2. This correlates with the morphologic changes of an increase of stromal collagen fibers and amorphous material, which probably consists of proteoglycans.

It can only be speculated how the results of our study regarding the presence of MMP-3 and TIMP-2 immunolocalization may be interpreted in light of the previously mentioned studies. Because upregulation of MMP expression by prostaglandins was shown earlier, it could be assumed that a similar effect was present in the current study. If so, this experiment suggests that treatment with latanoprost is not only effective in lowering intraocular pressure but reduces morphologic changes of the conjunctiva as well, even though the drug is preserved with benzalkonium chloride.

Eyes treated with latanoprost and timolol (groups 3, 4) showed a significant increase of collagen fibers and amorphous material, whereas the amount of empty spaces was reduced. There was a diffuse moderate to marked increase in immunoreactivity for both MMP-3 and TIMP-2 in the subepithelial conjunctival stroma. The increase of MMP-3 is probably similarly related to the application of latanoprost, although it appears that this substance could not completely inhibit the effect of timolol. The difference between the eyes that received a fixed combination therapy of latanoprost and timolol and those that received the drugs separately was not statistically significant. There was a tendency for the adverse effects to be less in the eyes receiving the fixed combination.

In summary, the results of this study showed that prolonged treatment with latanoprost caused no to mild changes of the morphology of the conjunctiva when compared with application of timolol. Moreover, latanoprost-treated eyes had an increase of MMP-3 in the conjunctiva, presumably a direct effect of the drug. This upregulation of MMP-3 in the conjunctival epithelium may have a protective effect on the substantia propria. It remains to be seen, whether the morphologically beneficial effect of latanoprost is clinically relevant.

References


