Morphological Changes in the Anterior Eye Segment after Long-Term Treatment with Different Receptor Selective Prostaglandin Agonists and a Prostamide

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PURPOSE. To investigate long-term changes in the anterior segment of primate eyes treated for one year with different prostaglandin agonists and a prostamide. The results were compared with those obtained after vehicle treatment and in untreated controls.

METHODS. Sixteen young cynomolgus monkeys were unilaterally topically treated for 1 year with either bimatoprost 0.03% (prostamide), sulprostone 0.05% (EP1/EP3 agonist), AH13205 0.1% (EP2 agonist), or latanoprost 0.005% (FP agonist), which all lower IOP in this species at the doses applied. Four animals were treated with the vehicle only. In all cases the left eye was treated, the right eye remained untreated. Six monkeys served as untreated controls. Sections from 4 quadrants each of the circumference of the eyes of 16 drug-treated, 4 vehicle-treated and 6 untreated control animals were investigated qualitatively and quantitatively using light- and electronmicroscopy. The area of widened spaces between ciliary muscle bundles, the number of nerve fiber bundles at the muscle tips, and the area of widened spaces between ciliary muscle bundles, the number of nerve fiber bundles at the muscle tips, and the width and length of the ciliary muscle were quantitated.

RESULTS. The general morphology of the ciliary muscle and trabecular meshwork was normal in appearance and shape in all animals, whereas some localized morphologic changes were observed in the drug-treated animals. The changes were found to be similar in all four treatment groups. In the ciliary muscle, there was a significant increase in optically empty spaces between muscle bundles in the anterior portion of the longitudinal and the reticular ciliary muscle compared with untreated and vehicle-treated control animals. Within these spaces, significantly more myelinated nerve fiber bundles were found in drug-treated compared with normal control animals. Ultrastructurally the spaces were partly covered by endothelial-like cells which, in some areas, were in contact with the basement membrane of the microvasculature. In all treatment groups, there were also changes in the trabecular meshwork region. Significant regional differences among the different quadrants of the eyes and quantitative differences between treatment groups were observed. The ciliary epithelium had a normal appearance in all treatment groups.

CONCLUSIONS. After one year of treatment with different prostaglandins and a prostamide, uveoscleral outflow pathways are enlarged and appear organized. Conventional outflow routes were also affected. Long-term treatment with AH13205, latanoprost, sulprostone, or bimatoprost also induces sprouting of nerve fibers. (Invest Ophthalmol Vis Sci. 2003;44:4419–4426) DOI:10.1167/iovs.02-1281

It is well established that topical treatment with prostaglandin (PG) F2α lowers intraocular pressure due to enlargement of uveoscleral outflow pathways.1–3 The mechanism underlying this effect is still not fully understood. First, ultrastructural studies by Tamm et al. showed that after 5 days of treatment with PGF2α there were signs of lysis of extracellular matrix (ECM) components between ciliary muscle (CM) bundles and widening of the intermuscular spaces.4 Lindsey et al.5 using immunohistochemical methods found that collagens type I, III, and IV were affected. Long-term treatment with AH13205, latanoprost, 0.035%, once daily for 5 days and that matrix metalloproteinases (MMP) 1, 2, and 3 were increased. The authors suggested that PGF2α may regulate uveoscleral outflow by MMP mediated alterations in CM matrix metabolism.6 All these studies however, were performed after short-term treatment with PGF2α for up to 5 days. Whether the long-term IOP effect of the drug is due to the same mechanism remains unknown. In a preliminary study, Svedbergh et al. investigated the histologic changes in aphic cynomolgus monkey eyes treated with latanoprost (0.05%, once daily) for 6 months.7 These authors did not see any differences in morphology between latanoprost and vehicle-treated eyes or between treated and untreated control monkeys. Unfortunately, the data are presented only in an ARVO Abstract so that the detailed descriptions of the materials and methods, e.g., the methods for handling the eyes after enucleation and the exact results are not available.

In the present study we investigated the morphologic changes in the anterior eye segment of cynomolgus monkeys unilaterally treated with the prostaglandin analogs sulprostone (EP1/EP3 agonist),8 AH13205 (EP2 agonist),9 latanoprost (FP agonist prodrug),10 and the prostamide bimatoprost11 for 1 year using qualitative and quantitative light- and electronmicroscopy as well as immunohistochemistry. These drugs were chosen because they lower IOP in this species at the concentration selected, while principally acting via different receptors.11–16 On a second messenger level, these receptors are known to stimulate (EP2) or inhibit (EP1) cAMP formation, or elevate intracellular calcium (EP1, FP, prostamide). We sought to investigate the long-term effects of these pharmacologically distinct ocular hypotensive drugs on tissues associated with aqueous humor outflow. The morphologic qualitative and quantitative investigation of the eyes was performed for all four treatment groups together, in a masked fashion, by two independent observers. Because morphologic changes were also seen in the untreated contralateral eyes in all treatment groups, the data were compared with those obtained from untreated and vehicle-treated matched control animals.
**METHODS**

Sixteen cynomolgus monkeys (*Macaca fascicularis*), divided into four treatment groups of four animals each, were topically treated once daily for 1 year with either bimatoprost 0.03% (AGN192024), sulprostone 0.03%, AHI3205 0.1%, or latanoprost 0.005%. In previous studies it has been demonstrated that these drugs lower IOP at the concentrations selected. The animals were unilaterally treated in the left eye; the right eye remained untreated. Six untreated and four vehicle-treated (poloxamer) animals were used as controls. The drug was delivered in a 25-μL volume using a micropipette to conscious, chair-restrained animals. The eyes were examined ophthalmologically at the beginning of the experiment and after 3, 6, 9 and 12 months by a veterinarian ophthalmologist. No pathologic changes were observed. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Tissue Fixation**

All animals were deeply anesthetized with sodium pentobarbital and transcardially perfusion fixed with paraformaldehyde (PFA) 4% for 10 minutes. After marking the temporal quadrant, the eyes were enucleated and small wedge-shaped pieces were removed from the four quadrants of the globe. The anterior cornea was also removed and the globe was then immersion fixed in Ito’s solution. With this protocol, the fixative can sufficiently penetrate into the globe, but the CM remains attached to the insertion of the posterior and anterior tendons and thereby retains its configuration.

**Light and Electronmicroscopy**

The Ito-fixed eyes were rinsed in cacodylate buffer. The specimens were postfixed in 1% OsO₄, dehydrated in an ascending series of alcohols, and embedded in resin (Epon; Fa Roth, Karlsruhe, Germany) according to standard methods. Semithin sections were cut with a microtome (Ultracut OmU3; Reichert, Vienna, Austria) and stained with toluidine blue. Ultrathin sections were stained with uranylacetate and lead citrate and viewed in an electron microscope (EM 902; Carl Zeiss, Oberkochen, Germany). From each quadrant of the eye (~20 semithin sections were analyzed qualitatively. Because the changes within adjacent areas of one quadrant were similar, the best section (one section without folds and clean staining) of each quadrant was used for quantitative evaluation.

For each treatment group, quantitative evaluation of the area of enlarged intermuscular spaces and the length and width of the CM was performed on sagittally oriented semithin sections. Measurements were obtained from one section of each of the four quadrants of the circumference of the treated eyes and the respective contralateral areas, fixed as described above. Quantitative measurement of the widened spaces in the CM was performed with a PC-based morphometric system (Quantimet 500; Leica, Cambridge, UK), in a masked fashion by two persons independently in a measuring window of ~200,000 μm² (333 × 600 μm) at a magnification of ×400. Figure 1 demonstrates the location of the measurement window. The data obtained by the two observers were so similar that the data obtained by the first author are listed in the tables.

Widening of intermuscular spaces could increase the width of the anterior CM. Therefore, measurement of the width (perpendicular distance between inner apex and outer longitudinal edge) of the CM was performed in each quadrant at a magnification of ×50 using a drawing microscope (Model 43463; Wild, Heerbrugg, Switzerland), as schematically depicted in Figure 1 and described in a previous paper. The width of the CM is also increased in the event of a contractile response. CM contraction, in addition to increasing the CM width, concomitantly decreases the length of the muscle. To distinguish whether the observed increase in CM width was the result of a widening of intermuscular spaces or a contraction of the muscle, the length of the CM (anteroposterior distance from the posterior tip to the anterior insertion of the scleral spur) was also determined.

The number of nerve fiber bundles was counted in the longitudinal, reticular, and circular portions in the most anterior part of the CM in each quadrant in a measurement field of approximately 200,000 μm² (magnification ×400); the same field as used for the measurement of the enlarged spaces (Fig. 1). The number of nerve fiber bundles was determined in each quadrant of each eye and averaged separately for the longitudinal/reticular and circular portions of the CM.

**Statistics**

Statistical analysis comparing data from treated animals with untreated and vehicle-treated control animals was done using the Wilcoxon, Mann and Whitney *U* test.

**RESULTS**

**Bimatoprost (Prostamide)**

*Ciliary Body, Ciliary Muscle.* The general morphology of the CM in the treated monkeys did not differ significantly from the normal untreated animals. The tips were at the level of the scleral spur and the circular portion formed an inner edge giving the muscle the typical triangular appearance (Fig. 2A). However, at the tips of the muscle, especially in the longitudinal and reticular portion, the bundles were separated from each other forming enlarged intermuscular spaces (Figs. 2A and 2B). These spaces were particularly large surrounding blood vessels. Measurements of the area of the spaces between the anterior CM bundles, performed by two independent observers in a masked fashion, revealed that there were significantly larger spaces in the treated animals compared with untreated and vehicle controls (Table 1). These changes were not uniform in the entire circumference and regional differences within each eye were observed. The anterior-posterior extension of the enlarged spaces varied between animals and the general morphology of the CM was preserved.
the apex was significantly larger in the treated eyes than in the vehicle-treated and untreated controls whereas the length of the CM remained unchanged (Table 2).

Within the enlarged spaces between the anterior muscle fiber bundles there were bundles of myelinated nerve fibers in some instances reaching toward the trabecular meshwork (Fig. 2B). Quantitative evaluation of these nerve fiber bundles revealed that their numbers had approximately doubled in treated animals compared with control animals. This increase was mainly due to a significant increase in the number of nerve fiber bundles in the longitudinal and reticular portion of the muscle tips (Table 3), whereas in the circular portion of the muscle, the number of nerve fiber bundles was similar to control animals (Table 4).

The spaces between muscle bundles at the very tips of the muscle consisted of long straight running tubes which were incompletely covered by elongated endothelial-like cells (EEC; Fig. 2A). The cells lining the optically empty spaces did not form a continuous basement membrane, but were separated from the adjacent CM cells by the basement membrane of the muscle and small amounts of fibrillar collagen and amorphous material. The EECs also bridged the spaces and were in contact with the basement membrane of blood vessels always found within the intermuscular spaces (Figs. 2A and 2B). The basement membranes of the vessels appeared thickened in some areas (Fig. 2B).

The nerve fiber bundles at the tips of the muscle consisted of myelinated but also of nonmyelinated nerve fibers. Single nerve terminals within the bundles contained dense core vesicles. Anteriorly extracellular material around the nerves was virtually absent. Further posteriorly, the nerve fiber bundles were surrounded by small amounts of extracellular material and elongated cells similar to and in connection with the EEC. In the more posteriorly located nerve fiber bundles, besides axons with a normal appearance, there were also fibers with numerous inclusion bodies (Fig. 4).

Ciliary Processes. The ciliary processes and the ciliary epithelium were histologically normal. There was no formation of Greff’s vesicles and no swelling of the processes of the anterior pars plicata. The epithelial cells of the entire pars plicata appeared unchanged (Fig. 2A).

### Table 1. Area of Empty Spaces in the Longitudinal and Reticular Portion of the Ciliary Muscle

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treated Eye (μm²)</th>
<th>Contralateral Eye (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bimatoprost</td>
<td>9408 ± 4276*</td>
<td>9414 ± 2470*</td>
</tr>
<tr>
<td>AH13205</td>
<td>9252 ± 3523*</td>
<td>6393 ± 2696*</td>
</tr>
<tr>
<td>Sulprostone</td>
<td>7263 ± 4074*</td>
<td>8808 ± 2518*</td>
</tr>
<tr>
<td>Latanoprost</td>
<td>8465 ± 4696*</td>
<td>11900 ± 8666*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>3105 ± 1190</td>
<td>3159 ± 943</td>
</tr>
<tr>
<td>Untreated</td>
<td>2536 ± 1240</td>
<td>(1056–4514)</td>
</tr>
</tbody>
</table>

The area of spaces between muscle bundles of the longitudinal and reticular portion of the ciliary muscle, in a measuring field of 200,000 μm², showed significant differences between the drug-treated groups and the untreated and vehicle-treated control groups. In the circular portion there were virtually no enlarged spaces in the drug-treated as in the untreated and vehicle-treated control eyes. In all eyes of each drug-treated group there were pronounced regional differences. Values are given as mean ± SEM (μm²) averaged from all four quadrants and all eyes from each treatment group; the range is given parenthesis. * P < 0.05 compared with vehicle and untreated control.
**Trabecular Meshwork.** In histologic sagittal sections, the trabecular meshwork had a normal triangular appearance. There was no obvious rarefication of the trabecular lamellae and the lamellae were posteriorly in contact with the ciliary muscle (Fig. 5A). In 13.3% of the 80 sections per eye evaluated semiquantitatively, the cribriform region appeared normal (Table 5). At the ultrastructural level the endothelial cells of Schlemms canal (SC) were connected to the subendothelial cells and to ECM. The endothelium formed some giant vacuoles as in untreated eyes (Fig. 5B). In most sections (66.7%; Table 5) the cribriform region appeared widened and in some sections, the inner wall of SC was in contact with either a scleral septum or the outer wall of SC (Fig. 5A). In these areas the endothelial cells of the inner wall of SC were disconnected from the subendothelial layer, giant vacuoles were absent and the area underneath the endothelium of SC appeared optically empty (Fig. 5C). Some cribriform cells were not oriented in a typical parallel fashion but perpendicularly to the inner-wall endothelium (Fig. 5C). In other areas, the inner wall was not disconnected from the subendothelial cells because cells of the corneoscleral trabecular meshwork running perpendicularly to the inner-wall endothelium had formed contacts with the endothelial cells (Fig. 5D). In none of the sections was an increase in ECM observed in the cribriform meshwork. In contrast, in most sections there seemed to be a loss of ECM from this region. In 20% of the sections there were also some changes in the lamellated portion of the trabecular meshwork. The gaps between the lamellae were enlarged. Trabecular cells in contact with each other bridged the gaps.

**TABLE 3. Number of Nerve Bundles in the Longitudinal and Reticular Portion of the Ciliary Muscle**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treated Eye (n)</th>
<th>Contralateral Eye (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bimatoprost</td>
<td>10.50 ± 2.15*</td>
<td>10.00 ± 1.83*</td>
</tr>
<tr>
<td>AH13205</td>
<td>9.81 ± 2.43*</td>
<td>8.45 ± 1.95*</td>
</tr>
<tr>
<td>Sulprostone</td>
<td>10.57 ± 1.95*</td>
<td>10.00 ± 1.58*</td>
</tr>
<tr>
<td>Latanoprost</td>
<td>9.51 ± 2.03*</td>
<td>9.60 ± 2.39*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>5.25 ± 2.38</td>
<td>4.47 ± 2.06</td>
</tr>
<tr>
<td>Untreated</td>
<td>5.70 ± 2.43</td>
<td>5.70 ± 2.43</td>
</tr>
</tbody>
</table>

Quantitative evaluation of the nerve fiber bundles in a measuring field of 200,000 μm² in the most anterior portion of the longitudinal and reticular portion of the muscle showed significant differences between the drug-treated groups and the untreated control groups. Values denote the average number of nerve fiber bundles in each quadrant. * P < 0.05 compared with vehicle and untreated control.

**Prostaglandin E₂ Analogos**

**AH13205 (EP₂ Agonist).** The main finding in the CM, namely enlargement of spaces between muscle bundles in the anterior longitudinal and reticular portion of the muscle, was almost identical with that seen in the bimatoprost-treated animals (Table 1). The width of the muscle was significantly larger than that seen in the controls, but somewhat less than in the bimatoprost-treated eyes (Table 2). There was also an increase of nerve fiber bundles within the spaces, mainly in the longitudinal and reticular portion of the CM (Table 3). The ciliary processes appeared normal. Changes in the trabecular meshwork (TM) were similar to those described for bimatoprost-treated eyes, except that changes of category 3 were detected more frequently (Table 5).

**Sulprostone (EP₂/EP₃ Agonist).** In the sulprostone-treated eyes spaces between muscle bundles in the anterior longitudinal and reticular portion of the CM were found as well, but the area was smaller than that seen in AH13205 and bimatoprost-treated animals (Table 1). The width of the muscle was significantly larger than that in the untreated controls, but somewhat less than in the prostamide-treated eyes (Table 2). The increase of nerve fiber bundles within the spaces in the longitudinal and reticular portion of the CM was similar to that seen in the bimatoprost-treated eyes (Table 3). The ciliary processes were unchanged. Sulprostone treatment produced changes in the TM similar to treatment with AH13205 (Table 5).

**Latanoprost (FP Agonist)**

CM morphology and size of the spaces between muscle bundles in the anterior longitudinal portion were comparable to

**TABLE 4. Number of Nerve Bundles in the Circular Portion of the Ciliary Muscle**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treated Eye (n)</th>
<th>Contralateral Eye (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bimatoprost</td>
<td>2.00 ± 0.97</td>
<td>2.94 ± 1.56</td>
</tr>
<tr>
<td>AH13205</td>
<td>2.50 ± 0.91</td>
<td>2.75 ± 0.90</td>
</tr>
<tr>
<td>Sulprostone</td>
<td>2.94 ± 1.25</td>
<td>2.57 ± 0.82</td>
</tr>
<tr>
<td>Latanoprost</td>
<td>2.60 ± 0.95</td>
<td>3.13 ± 1.02*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.67 ± 0.79</td>
<td>2.33 ± 0.88</td>
</tr>
<tr>
<td>Untreated</td>
<td>2.20 ± 1.03</td>
<td>2.20 ± 1.05</td>
</tr>
</tbody>
</table>

In the anterior circular portion of the muscle, the number of nerve fiber bundles in the treated eyes in a measuring field of 200,000 μm² was not different from the untreated and vehicle-treated controls. Values denote the average number of nerve fiber bundles in each quadrant. * P < 0.05 compared with vehicle and untreated control.
FIGURE 3. Electronmicrographs of sagittal sections through the anterior longitudinal portion of bimatoprost-treated eyes with enlarged spaces between muscle bundles. (A) The spaces between the muscle fiber bundles (M) are partly ensheathed by elongated endothelial-like cells (arrows). (B) The basement membranes of the capillaries (C) within enlarged intermuscular spaces are thickened (arrow). Some endothelial-like cells are in contact with the basement membrane. Scale bars: (A) 2 μm; (B) 1 μm.

those of the other treatment groups (Table 1). The same was true for the width of the anterior muscle (Table 2), as well as for the number of nerve fibers quantitatively evaluated in the longitudinal and reticular portion of the muscle (Table 3). In contrast to the other treatment groups, the number of nerve fiber bundles was increased in the circular portion of the CM of latanoprost-treated animals to some extent (Table 4). The ciliary processes were normal as in the other treatment groups.

Loss of ECM from the trabecular meshwork was more prominent than in the other groups (Table 5). At many places, the cribriform region was bridged by cytoplasmic processes of trabecular cells (Fig. 6A). At the ultrastructural level, at many places, there was not only a disconnection of subendothelial cells from the inner-wall endothelium, but the endothelial cells and the cribriform cells were disconnected from the subendothelial elastic network (Fig. 6B). The cribriform cells were elongated and showed several cytoplasmatic filaments arranged in a parallel fashion, similar to myofibroblast. In addition to loss of collagen from the subendothelial region, there was also loss of the electron dense core of the elastic-like fibers (Fig. 6B). In the inner portion of the trabecular meshwork there was loss of connective tissue from the beams in 53.3% of the sections analyzed (Fig. 6A, Table 5). Ultrastructurally, at some places, remnants of connective tissue strands were incompletely covered by trabecular cells (Fig. 6C).

Contra-lateral Eyes of Treated Animals
In the untreated contra-lateral eyes of drug-treated animals the morphologic changes of the CM and TM were similar to those seen in the treated eyes. At the muscle tips the area of the spaces between muscle bundles was significantly larger than in the untreated or vehicle-treated controls (Table 1). The number of nerve fiber bundles was almost doubled in the contra-lateral eye of the treated animals compared with the untreated controls. As in the treated eyes, this increase in nerve fibers was due to an increase in fibers in the longitudinal and reticular portion of the muscle (Table 3), whereas the number in the circular portion was unchanged. The width of the anterior CM was significantly larger in the contra-lateral eyes of the treated monkeys than in the untreated and in the vehicle-treated controls (Table 2). On the other hand, the CM of the contra-lateral eyes of sulprostone-treated monkeys was somewhat shorter than the controls (Table 2).

The ultrastructural changes of the CM tips and the TM were similar to those seen in the treated eyes (Table 5). The ciliary processes were unchanged.

Eyes of Untreated and Vehicle-Treated Animals
No differences, neither qualitative nor quantitative, were seen between vehicle-treated and untreated controls.

DISCUSSION
Our study demonstrates that, despite the different pharmacologic profiles of the drugs investigated, long-term treatment for 1 year resulted in enlarged intermuscular spaces in the ciliary body, presumably representing uveoscleral outflow routes. This observation was made for all four ocular hypotensive drugs: one prostamide and three pharmacologically selective prostaglandin analogs. Widening of intermuscular spaces, quantified directly using light microscopy, was accompanied by an increase in CM width without concomitant decrease in length. Based on these findings, it appears that the increase in width was caused by a widening of the intermuscular spaces and was not the result of a potential contraction of the CM. Histologically the spaces were small such that the general morphology of the CM of animals treated for one year was similar in appearance and shape to normal untreated animals.

In contrast to our findings in animals treated with high doses of PGF_2α-isopropylester for 5 days, in the present study we did not find macrophages at the muscle tips or in the

FIGURE 4. Electronmicrograph of nerve fibers (N) in the enlarged spaces between longitudinal muscle fiber bundles. Note that in some axons there are myelin figures (arrows), presumably degenerating mitochondria. Scale bar, 1 μm. EEC, elongated-like endothelial cells.
intermuscular spaces, but the spaces appeared more organized than in the short-term treated eyes. The fluid pathways appeared lined by an incomplete layer of EEC similar to fluid pathways in the choroid. In the choroid such pathways have been discussed as a kind of lymphatic.20 Because the entire ciliary muscle is formed by a three-dimensional network of connected branching muscle cells, elongated tube like spaces are never seen in normal eyes in this region of the muscle.19 Therefore, remodeling of the intermuscular connections and subsequent organization of fluid flow pathways would best explain our findings.

In the CM of the treated eyes these pathways seemed to lead toward capillaries present within the enlarged spaces. Because only fluid will enter the microvessels, it is possible that particles within the aqueous are scavenged in the basement membrane, thereby leading to the observed thickening. In vitro studies by Ocklind21 and Lindsey et al.22 suggested that the increase in the width of the intermuscular spaces leading to an increase in uveoscleral outflow may be the consequence of a stimulation of MMP synthesis by CM cells in response to treatment with prostaglandin F2α. If this is also true for treatment with bimatoprost, AH13205, and sulprostone, such synthesis of MMPs might still be elevated after long-term treatment. Alternatively, the pathways may not be constantly reformed but may be established initially, remain open, and then become organized as a result of fluid flow. It is interesting to note that long-term treatment with the drugs used in this study did not lead to a posterior extension of the fluid pathways beyond what is already visible after short-term treatment with PGF2α-isopropylester for 5 days.3 Thus, remodeling of the

**FIGURE 5.** Sagittal sections through the trabecular meshwork of bimatoprost-treated monkey eyes. (A) Corneoscleral trabecular lamellae are nearly unchanged, the cribriform layer is widened and Schlemm’s canal (SC) partly collapsed (arrow). (B) Electronmicrograph of the inner wall of SC of an untreated eye. The inner-wall endothelium (E) forms a giant vacuole (V). The endothelial cells are connected to subendothelial cells or extracellular material underneath the inner-wall endothelium. (C, D) Electronmicrographs of the inner wall of SC of bimatoprost-treated eyes. (C) In some areas the inner-wall endothelium (E) is disconnected from the subendothelial cells and the cribriform region (asterisk). The subendothelial region appears optically empty. Some cribriform cells are oriented perpendicularly to the inner-wall endothelium (arrow). (D) Sometimes the inner-wall endothelium (E) was connected to trabecular cells of the lamellated meshwork running perpendicularly to the inner-wall endothelium (arrow). V = vacuole. Scale bars: (A) 10 μm; (B) 1 μm; (C, D) 2 μm.
TABLE 5. Summary of Morphological Changes Observed in Trabecular Meshwork and Schlemms Canal

<table>
<thead>
<tr>
<th>Category</th>
<th>Treated Eye (%)</th>
<th>Contralateral Eye (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Controls</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Bimatropost</td>
<td>13.3</td>
<td>66.7</td>
</tr>
<tr>
<td>Sulprostone</td>
<td>15.1</td>
<td>46.1</td>
</tr>
<tr>
<td>AH13205</td>
<td>25.0</td>
<td>37.5</td>
</tr>
<tr>
<td>Latanoprost</td>
<td>20.0</td>
<td>26.7</td>
</tr>
</tbody>
</table>

Semiquantitative evaluation of semithin sagittal sections was performed in a total of 80 sections per eye, 20 sections per quadrant. The data are summarized from all eyes. Three categories were distinguished and the percentage of sections showing the related morphology are given.

Category of morphological changes: 1. Trabecular meshwork appeared normal; 2. Connesoceral trabecular lamellae nearly unchanged, cribriform region widened due to disconnection of the inner wall endothelium from the subendothelial cells. At places contact between inner wall endothelium and outer wall of SC; 3. In addition to the changes in the cribriform region described under 2, there were changes in the lamellated part of the trabecular meshwork (loss of connective tissue from the lamellae).

ECM in response to these drugs is restricted to longitudinal and reticular portions of the CM. While these fluid pathways appear to form rather rapidly, long-term treatment does not lead to an extension of the channels but to better organization.

In our study we also found changes in the conventional outflow routes. These changes were not present in all parts of the circumference of the eyes, but in areas where they were prominent, loss of ECM components could have been induced by MMPs similar to what has been suggested for the CM. Trabecular cells bridging gaps and covering partly missing trabecular beams indicate that there are also repair mechanisms and organization of fluid pathways in the conventional outflow routes. In monkey eyes loss of ECM from the cribriform region and disconnection of the cells from the ECM leads to extension of the inner wall with increase in conventional outflow. On the other hand, SC is partly collapsed. Because this collapse shows circumferential variations, it is difficult to predict whether treatment with any of these compounds induced changes in conventional outflow in our monkey eyes as a net effect.

Widening of intermuscular spaces and loss of extracellular material in parts of the circumference of the TM was also found in animals treated with the PGE2 analog sulprostone (EP1/EP3 agonist) and AH13205 (EP2 agonist). In vitro treatment of CM cells with 11-deoxy-PGE1, a nonspecific PGE1 analog, increases production of MMP 1, 2, 3 and 9.25 It is, therefore, not unreasonable to assume that the widening of spaces in the anterior part of the CM seen in eyes treated with sulprostone and AH13205 is induced by a similar mechanism.

After prostamide and prostaglandin treatment, the changes were significantly different from those found after long-term treatment with other classes of IOP-lowering drugs. After long-term treatment with the β-blocker timolol and after treatment with epinephrine-pronounced changes in the ciliary processes were seen.26 In none of the animals investigated in this study were changes in the ciliary epithelium observed. The morphology of the trabecular meshwork was also completely different after long-term treatment with the prostamide and prostaglandin analogs compared with long-term treatment with other antiglaucoma drugs. The rarefication of the trabecular meshwork was much more prominent in timolol-treated eyes. On the other hand, after long-term treatment with pilocarpine or phosholin iodide in monkeys, there was an increase in ECM in the cribriform region which was not seen in the treatment groups of this study.20–26

In all treatment groups the contralateral-untreated eyes showed similar morphologic changes in the CM and TM as the treated eyes. Contralateral effects had also been observed in animals treated long-term with timolol, epinephrine, pilocarpine, and phosholin iodide.20–26 This could be due to a

![Figure 6](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933434/)
contralateral contamination but more likely results from systemic absorption. Even if the drugs used in this study might be more quickly metabolized than the other drugs, rapid diffusion and active transport of active drug into ocular tissues from the systemic system is a distinct possibility.

An unexpected finding in all treatment groups was the increase in nerve fiber bundles in the longitudinal and reticular portion of the CM and TM. In the muscle the increased nerve fiber bundles were especially numerous at places where the intermuscular spaces were enlarged. The mechanism underlying this sprouting of nerve fibers is not yet known. It is possible that a retrograde degeneration of nerve fibers induces sprouting. Alternatively, these drugs may act as neurotrophic factors themselves. However, further studies are needed to clarify this.

In conclusion, long-term treatment with different prostaglandins and a prostamide leads to an enlargement of uveoscleral outflow routes and to morphologic changes in the TM perhaps suggestive of increased uveoscleral and conventional outflow. In the affected CM areas, sprouting of nerve fibers may be the consequence of tissue remodeling. The finding that the four drugs investigated, which are all ocular hypotensive agents in the same species but principally act through different receptors, produced similar changes in the aqueous outflow routes was not quite expected. To determine whether these actions involve a final common or parallel pathways requires further investigation.

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