LABORATORY STUDY

# Synthesis pattern of matrix metalloproteinases (MMPs) and inhibitors (TIMPs) in human explant organ cultures after treatment with latanoprost and dexamethasone

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**Abstract**

*Purpose* **To determine changes in production of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) in the ciliary body, the trabecular meshwork and the retinal pigment epithelium induced by both prostaglandins and corticosteroids.**

*Methods* **Explant organ cultures were removed by a scleral incision 3 mm posterior to the limbus. Retinal pigment epithelium was grown to confluence. Organ and cell cultures were treated with latanoprost and/or dexamethasone for 72 h. The activity of MMP-2, -3 and -9 was assessed using zymography. The synthesis pattern of MMPs and TIMP-1 and -2 was identified using immunostaining.**

*Results* **Treatment of explant organ cultures with 10 μg/ml of latanoprost induced a mean upregulation of MMP-2 by 36%, MMP-3 by 112% and MMP-9 by 156% as seen by zymography. Dexamethasone 500 nm reduced the amounts of secreted MMP-2 by 13%, MMP-3 by 69%. MMP-9 was not detectable in the media of corticosteroid-treated explant organ cultures. The addition of 10 μg/ml of latanoprost to dexamethasone-treated cultures increased MMP-2 by 14%, MMP-3 by 43% and MMP-9 by 49%. Using immunohistochemistry we found staining with antibodies against MMP-2, -3, -9 and TIMP-1 and -2 within the ciliary body, and only to a lesser degree in the trabecular meshwork. Latanoprost treatment caused an increase of 29% in MMP-2 ($p < 0.0001$), 98% in MMP-3 ($p < 0.0001$) and 108% in MMP-9 ($p < 0.0001$). Dexamethasone reduced the staining for MMP-2 by 32% ($p < 0.0001$), for MMP-3 by 33% ($p < 0.0001$) and for MMP-9 by 83% ($p < 0.0001$). Almost no change in staining for MMPs was detectable in the trabecular meshwork. Neither latanoprost treatment nor dexamethasone induced significant changes ($p < 0.93$) in the secretion of TIMPs. In the media of non-treated retinal pigment epithelium (RPE) cells the only MMP detected was MMP-2. RPE cells in culture did not respond to either treatment with a change in their MMP secretion.**

*Conclusion* **We detected a profound upregulation of both MMP-3 and MMP-9 and a mild induction of MMP-2 through latanoprost in the ciliary body, but not the trabecular meshwork or RPE cells. Corticosteroids, on the other hand, downregulated MMP expression in both tissues. This inhibiting effect of corticosteroids on MMP production was reversed by latanoprost.**

*Key words* Dexamethasone, Human explant organ cultures, Latanoprost, Matrix metalloproteinases, Tissue inhibitors of matrix metalloproteinases (TIMPs)

An ocular hypotensive effect has been proven for a number of prostaglandins (PG).[1] Among them is latanoprost a new 17-phenyl-substituted PG analogue used for topical therapy in glaucoma. The main effect of this drug is thought to be through an elevated uveoscleral outflow, explained by lytic effects or the lysis of extracellular matrix material by matrix metalloproteinases (MMPs) induced by latanoprost. *In vitro* studies with cultured human ciliary smooth muscle cells and *in vivo* studies in monkeys support this hypothesis.[2-4] In ciliary smooth muscle cell cultures latanoprost caused a 71% increase in stromelysin secretion (MMP-3).[2] This enhanced

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Received: 10 June 1999
Accepted in revised form: 15 October 1999

production of lytic enzymes is supposed to increase the aqueous humour outflow through enzymatic digestion of extracellular matrix material such as proteoglycans and collagens.[2–4] Because previous studies found enhanced MMP production only in ciliary smooth muscle cells,[2–4] it has been proposed that latanoprost enhances uveoscleral outflow but not outflow through the trabecular meshwork.[5] However, pilocarpine, which inhibits or even blocks the uveoscleral outflow, enhances the pressure-lowering effect of latanoprost in humans,[6] indicating that latanoprost might reduce outflow resistance in the trabecular meshwork also. But the effects of latanoprost on the trabecular meshwork are not yet clarified. Reports concerning the presence of prostaglandin F (PGF) receptors on trabecular meshwork cells are controversial. Krauss *et al*.[5] could not detect receptors for PGF in the trabecular meshwork, suggesting that latanoprost may act only on the ciliary body. However, Anthony *et al*.[7] recently reported the presence of PGF receptors on trabecular meshwork cells.

One of the major concerns in corticosteroid treatment is the induction of steroid-induced glaucoma. It is well known that corticosteroids inhibit MMP production[8] and it has been proposed that this reduction in MMP activity might lead to enhanced deposition of extracellular matrix material in the trabecular meshwork and thus induce an elevated juxtacanalicular outflow resistance.[9–11] Therefore considering that on the one hand there is reduced MMP activity through corticosteroids[8] and, on the other hand, an increase in MMP production through latanoprost,[2–4] latanoprost seems to be a logical choice of treatment in steroid-induced glaucoma. To test this hypothesis we have studied the effect of latanoprost either with or without the addition of dexamethasone on MMP production in both the ciliary body and the trabecular meshwork.

To date, only MMPs have been studied in these tissues. Since both MMPs and tissue inhibitors of matrix metalloproteinases (TIMPs) are regulated through the AP1-complex[12] induced by latanoprost, we hypothesised that one or more MMPs and TIMPs would be upregulated by latanoprost. The specific questions addressed were: (1) Which MMP or TIMP responds to latanoprost treatment? (2) Is the increased activity of lytic enzymes, as reported by zymography,[2,3] a consequence of increased secretion, increased conversion of pro-enzyme to active forms and/or altered production of TIMP-1 or TIMP-2? (3) Which cells respond to latanoprost: ciliary smooth muscle or trabecular meshwork cells? (4) Is latanoprost capable of reversing the effect of dexamethasone on endogenous MMP production? To address these questions we used explant organ cultures.

Finally, we wanted to see whether MMPs are induced only in cells of the anterior compartment, as, for instance, the retinal pigment epithelium (RPE) has been shown to express low levels of PGF-2α receptors[13] and is known to be the main source of MMPs[14] in the posterior compartment of the eye. An enhanced local production of MMPs by the RPE might be in part responsible for some of the adverse side effects[15,16] seen with the use of latanoprost, as there is the development of a cystoid oedema. Further, if latanoprost does indeed induce MMP production in the RPE cells, it would have to be used with care in patients with choroidal neovascularisation, since MMPs have been implicated in the development of choroidal neovascularisation.[17]

## Materials and methods

### *Preparation and culturing of the explant organ cultures*

Explant organ cultures from human eyes enucleated ($n$ = 40) within 4 h post-mortem were removed immediately after enucleation. The age of the donors was between 45 and 78 years (mean ± SD, 56 ± 12 years). Eyes from donors with a history of eye surgery, diabetes, sepsis and immune diseases were excluded from the study. All experiments were done four times on separate occasions. In each experiment all samples were done in duplicate.

Preparation of explant organ cultures was done as previously described.[18] In brief, explants were removed by a scleral incision 3 mm posterior to the limbus and lifted carefully. Special care was taken not to disturb the iris roots, the ciliary body and the scleral spur. Associated iris was then partially removed with tweezers without disruption of the ciliary body and the trabecular meshwork. This complete corneoscleral explant was used for culture.

The eye caps were rinsed with 10 ml phosphate-buffered saline and incubated concave side up in 10 ml Dulbecco's modified Eagle medium (hyClone, Cramlington) with 1× antibiotic (penicillin/streptomycin) (hyClone, Cramlington) mixture added.

The corneoscleral explants were allowed to stabilise for 1 week under a 95% air to 5% $CO_2$ atmosphere, in a $CO_2$ incubator at 37°C with medium changes every 2 or 3 days. After 1 week the medium was changed and the explant organ cultures were exposed to dexamethasone (Merck, Darmstadt) (with a final concentration of 500 nM), latanoprost (Pharmacia & Upjohn, Uppsala) (with a final concentration of 10 μg/ml) or a combination of both. Additional latanoprost and/or dexamethasone was added to the culture medium once a day over the following 3 consecutive days. Untreated explants cultured at the same time served as control for every experiment. Explants treated with Timoptic (10 μg/ml) served as an additional control for handling and medium additions. Timoptic, a beta blocker widely used to treat glaucoma, was chosen as a control, as an induction of c-*Fos*, that would result in an increase in MMP secretion, has not been reported.

### *Transmission electron microscopy*

To assess the viability of the explants, our samples were studied by transmission electron microscopy after 3 days, 6 days, 9 days and 12 days of culture. Both treated and non-treated organ cultures were examined at each time point. Fresh eye caps served as controls. Wedges 2 mm at

the circumference were cut from the corneoscleral explants and fixed for 4 h in 2.0% glutaraldehyde, 2% paraformaldehyde, 5 mM calcium chloride, 130 mM sucrose and 100 mM sodium cacodylate (pH 7.4). The samples were then washed three times in this buffer, post-fixed for 1 h in 1% osmium tetroxide in the same buffer, washed in the same buffer, dehydrated through sequential ethanol and embedded in resin (Aar-100, Agar Scientific, Essex, UK). Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a Philips CM 100 electron microscope.

### *Preparation and culturing of the retinal pigment epithelium*

RPE cells were prepared as previously described.[19] Human RPE cells were isolated and cultured as follows: human eyes were obtained within 8 h post-mortem. The anterior segment was removed and the RPE-choroid tissue was dissected from the neural retina and the sclera. RPE cells were isolated by incubating pieces of RPE-choroid tissue in Hank's solution containing collagenase (100 unit/ml, type IV, Sigma) for 1 h at 37 °C. After incubation RPE cell sheets were dissected free from the choroid under a dissecting microscope. The cell sheets were washed in Hank's solution three times, then incubated in RPMI (Gibco, UK) supplemented with 10% fetal calf serum, seeded in uncoated six-well plastic plates (Costar, Cambridge, Mass.), at 37 °C in a humidified atmosphere with 5% $CO_2$. Great care was taken to keep RPE cell sheets free from possible contamination by choroidal cells. After 24 h non-adherent cells were removed and fresh medium was added. After a confluent cell layer was established the medium was changed to RPMI without supplemental fetal calf serum for 3 days to exclude the possibility that MMPs detected by zymography are derived from the fetal calf serum. Prior to treatment with either dexamethasone (500 mM) or latanoprost (10 μg/ml) the medium was changed once again to ensure that all fetal calf serum was washed out. After 72 h of incubation, the medium was subjected to zymography.

### *Analysis of secreted proteinases*

After the third day of incubation zymography was used to analyse the content of gelatinases in the medium. With this technique, both proenzyme and proteolytically activated forms of the enzyme can be visualised.

Eleven per cent SDS gels were prepared using a 37.5:1 stock of acrylamide to *bis*-acrylamide (Boehringer) and either gelatin for the detection of MMP-2 and MMP-9 of β-casein for the detection of MMP-3 was included in the gel at a concentration of 1%. After electrophoresis, the gel was shaken in a 2.5% solution of Triton X-100 (Sigma) for 1 h at room temperature to remove SDS and then developed by incubation in a reaction buffer (50 mM Tris, pH 7.5, 10 mM $CaCl_2$) for 48 h at 37 °C.

After incubation, the gels were stained with Coomassie brilliant blue (Sigma). The different MMP species were detected as clear bands and identified by their molecular weights. To verify that the gelatinase activities were specific to metalloproteinases, in some gelatinase experiments 10 mM ethylenediaminetetraacetic acid (EDTA), a metalloproteinase inhibitor, was included in the 2.5% Triton X-100 solution as well as in the Tris-$CaCl_2$ buffer during proteinase reactions as previously described.[20,21]

The relative amounts of gelatinases were semiquantified by volume densitometry (NIH image, version 1.61).

### *Immunohistochemical procedures*

After 72 h of incubation with latanoprost (10 μg/ml), dexamethasone (500 nM), or culturing without additives, the explant organ cultures were removed from the medium, fixed with 10% buffered formaldehyde for 24 h and paraffin-embedded. To address the question of the viability of our explants, fresh, non-cultured explants were processed along with our cultured samples.

Lymph nodes served as positive controls. Samples stained without the primary antibody were used as negative controls.

The immunostaining procedures were performed as described in detail by Hsu *et al.*[22] and Tang *et al.*[23] with minor modifications. In brief, paraffin sections (5 μm thick) were dewaxed and rehydrated. The samples were then placed in sodium citrate buffer, treated with microwaves (800 W) for 35 min, and washed in phosphate-buffered saline (PBS) for 15 min. After that the samples were rinsed in PBS for 5 min. The endogenous peroxidase activity was blocked with 3% $H_2O_2$ in $H_2O$ (5 min). Samples were then rinsed twice with PBS and incubated for 45 min at room temperature with polyclonal rabbit anti-human MMP-2 (against the enzyme's hinge domain; 7 μg/ml), polyclonal rabbit anti-human MMP-3 (against the enzyme's C-terminal domain; 2 μg/ml), monoclonal mouse anti-human MMP-9 (neat), polyclonal rabbit anti-human TIMP-1 (4 μg/ml) and polyclonal rabbit anti-human TIMP-2 (against the enzyme's C terminal region; 4 μg/ml) (all antibodies were obtained from Novus Molecular, San Diego, CA, USA). Immunostaining was performed using a labelled streptavidin-biotin-peroxidase complex technique. 3-Amino-9-ethylcarbazol (AEC) was used as chromogen to visualise the positive reactions (LSAB plus Kit, Dakopatts Immunoglobulins, Denmark). Finally the sections were air-dried and mounted with clear mount (Zymed, San Francisco, CA, USA).

Three non-serial histological sections of each tissue were examined using a Zeiss Axioplan light microscope. The sections were then scanned into Adobe Photoshop 4.0 at a magnification of ×25 using a Sony 3CCD video camera. To ensure that all slides were scanned at the same magnification a 1 mm grid was also scanned each time and saved in Adobe Photoshop. Adobe Photoshop converts scanned images into three colour layers: red,

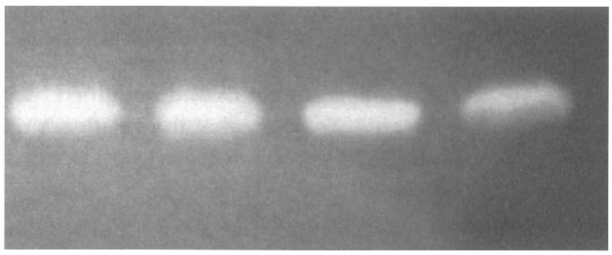


**Fig. 1.** *The zymography done with the media of retinal pigment epithelium (RPE) cell cultures. In all lanes (lane 1, dexamethasone-treated; lane 2, dexamethasone- and latanoprost-treated; lane 3, latanoprost-treated; lane 4, control) only one band, resembling the non-cleaved form of MMP-2, at an apparent molecular weight of 65 kDa was seen, indicating that neither drug had an influence on the matrix metalloproteinase (MMP) production of RPE cells in culture.*

green and blue. The reaction of the antibody with the antigen gave a red reaction result. Therefore the measurements of areas stained by the red reaction product were made on the red layer, using thresholding to isolate the stained region. NIH image (1.61) was used to measure the stained areas and the results were expressed in square pixels. The mean number of pixels of each tissue was calculated and compared among the different organ culture treatments.

### *Statistical anslysis*

Statistical analysis of the results was performed using the Dunnett test. A $p$ value < 0.05 was considered significant.

## Results

### *Viability of organ cultures*

After 10 days of culturing occasional ciliary cells (less than 5%) showed changes in their cytoplasmic appearance, indicating that the culture method was suitable for the ciliary body.

To further establish whether the amounts and distribution of secreted MMPs and TIMPs of our non-treated organ cultures were comparable to that of fresh eyes we stained fresh (non-cultured) eye caps along with our samples. The distribution and intensity of both TIMPs and MMPs were almost identical in the fresh and the cultured non-treated tissues, indicating that our cultured explant organ cultures were not only morphologically comparable to fresh eye caps but that they were still functioning.

### *MMP production by retinal pigment epithelium*

Only the non-cleaved MMP-2 proenzyme of molecular weight 65 kDa was detectable in the media of non-treated RPE cells. Drug treatment with either 500 nM dexamethasone or 10 μg/ml latanoprost did not have any effect on the amounts of MMPs detected in RPE cell cultures. In addition no change in the relative amounts of non-cleaved and cleaved zymogen was seen (Fig. 1).

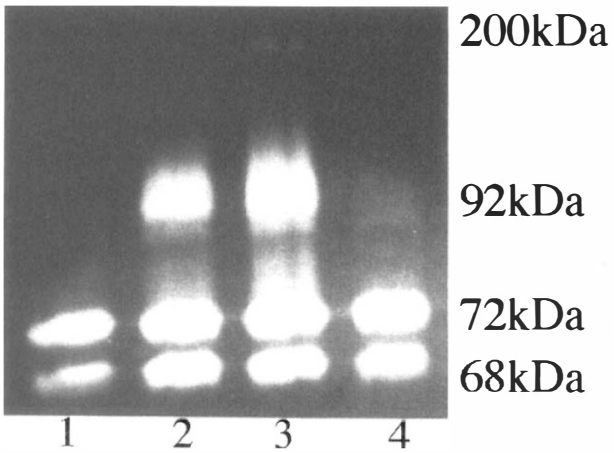



**Fig. 2.** *MMP-2 and MMP-9 in culture media from organ cultures as seen using gelatin polyacrylamide gels. Major bands migrated at the apparent molecular weights of 58 kDa and 65 kDa, resembling the cleaved and non-cleaved forms of MMP-2, and 92 kDa, resembling MMP-9. Increased intensity of bands at 92 kDa (MMP-9) were detectable in lane 2 (dexamethasone- and latanoprost-treated organ cultures) and lane 3 (latanoprost-treated organ cultures). Lane 1 (dexamethasone-treated organ cultures) as well as lane 4 (control) hardly showed any detectable band at 92 kDa.*

### *Gelatinase activities in the media of explant organ cultures*

The endogenous MMPs primarily produced by these cultures were MMP-2 and MMP-3. Latanoprost induced a massive increase in MMP-9 and some increase in MMP-2 and MMP-3 production. Dexamethasone almost ablated the activity of MMP-9 and MMP-3. MMP-2 production was inhibited too, but still detectable (Table 1). Using 10 mM EDTA in the gelatinase experiments led to total inhibition of the MMPs.

#### *MMP-2*

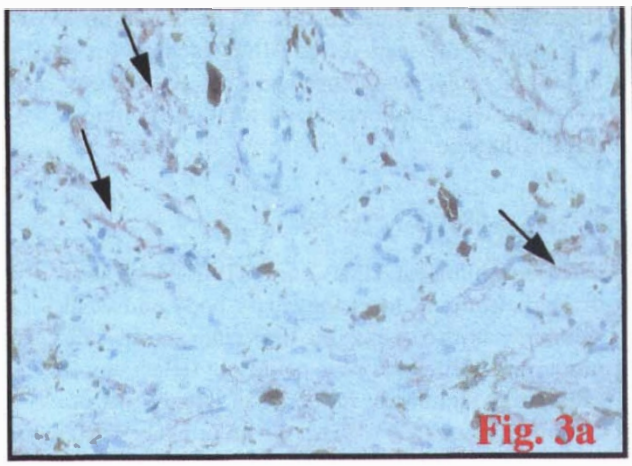
The culture medium of every untreated culture contained gelatinase activity at apparent molecular weights of 65 and 58 kDa, representing the proenzyme (65 kDa) and the proteolytically activated form (58 kDa). The ratio between the two bands (58:65 kDa) was between 1.7 and 2; drug treatment had no effect on the ratio (Fig. 2).

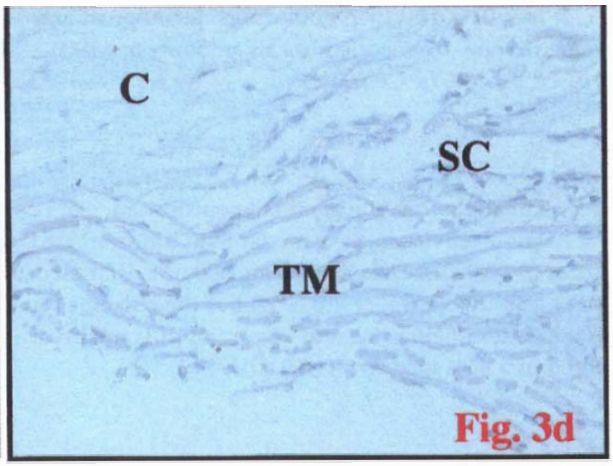
In comparison with untreated cultures 10 μg/ml of latanoprost increased the mean total (cleaved and uncleaved) of MMP-2 by 36% ($p$ < 0.0001). Dexamethasone 500 nM reduced the amounts of MMP-2

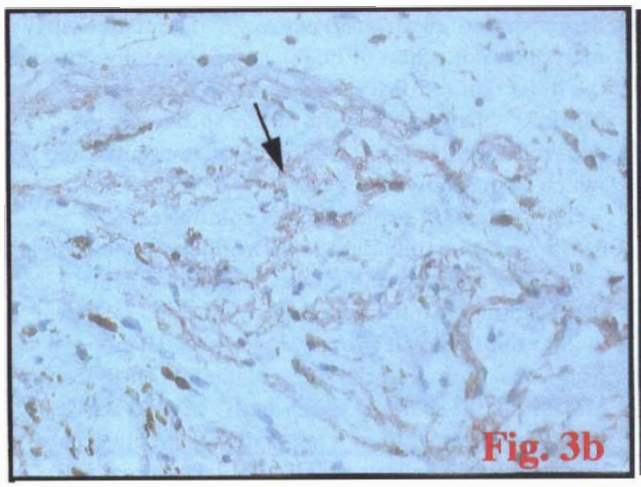
**Table 1.** *Summary of the results of zymography*

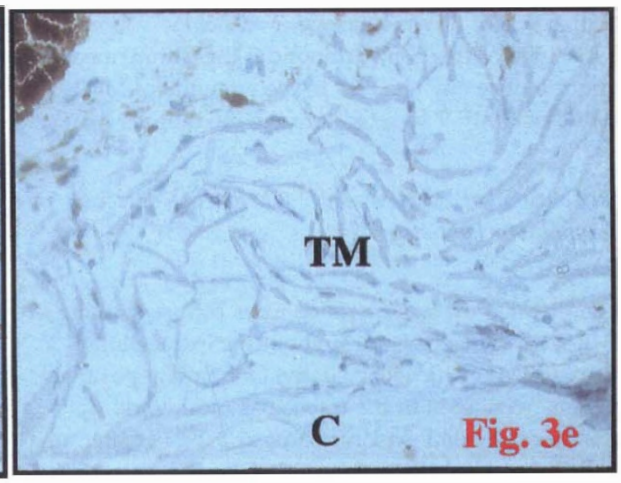
| Zymography | MMP-2 | MMP-3 | MMP-9 |
|---|---|---|---|
| Control | + | + | + |
| Latanoprost | ↑ 36% | ↑ 112% | ↑ 156% |
| Dexamethasone | ↓ 13% | ↓ 69% | – |
| Latanoprost + dexamethasone | ↑ 14% | ↑ 43% | ↑ 49% |
| RPE cells | + | – | – |

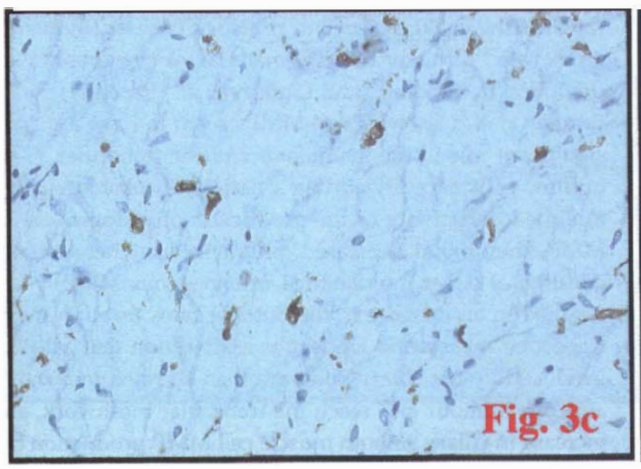
Latanoprost treatment results in an increase in all MMPs measured, as opposed to dexamethasone which led to a decrease in all MMPs measured. The inhibiting effect of dexamethasone on MMP production was reversed by latanoprost. RPE, retinal pigment epithelium.



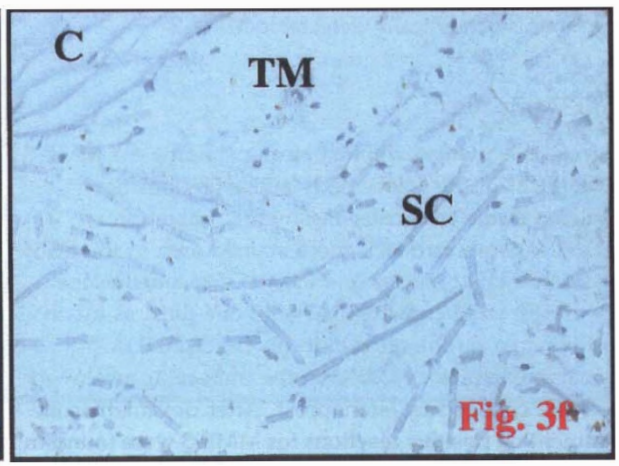




**Fig. 3.** *Immunohistochemical staining of MMP-3 (arrows) in the ciliary body (left panels) and the trabecular meshwork (TM) (right panels) of cultures treated for 3 days with nothing (a, d), latanoprost (b, e) or dexamethasone (c, f). In contrast to the trabecular meshwork, where in all samples hardly any staining for MMP-3 could be detected, latanoprost-treated ciliary smooth muscle cells showed an increase in MMP-3 production (b), whereas a decrease in MMP-3 was found in the ciliary body incubated with dexamethasone (c). No staining was seen in the cornea (C) or the sclera (SC). Magnification* ×250.

by 13% ($p < 0.0001$). When both drugs, latanoprost and dexamethasone, were added, mean amounts of MMP-2 increased by 14% ($p < 0.0001$).

*MMP-3*

Cultures treated with 10 μg/ml latanoprost had 156% more MMP-3 activity in their media than did untreated cultures ($p< 0.0001$). Dexamethasone 500 nM decreased MMP-3 activity by 69% ($p < 0.0001$) but the combination of 500 nM dexamethasone and 10 μg/ml of latanoprost resulted in a 43% increase ($p < 0.001$).

*MMP-9*

MMP-9 was barely detectable in non-treated organ cultures, and in the presence of dexamethasone there was no detectable activity. Latanoprost 10 μg/ml upregulated MMP-9 by 156% ($p < 0.0001$), and by 49% in the presence of dexamethasone ($p< 0.0001$).

Addition of 10 μg/ml Timoptic to the culture medium did not induce any changes in the amounts of MMP-2, MMP-3 or MMP-9 secreted into the medium.

***Distribution of MMPs and TIMPs as detected by immunohistochemistry***

*MMP-2*

In non-treated explant organ cultures diffuse staining of MMP-2 was detectable throughout the extracellular matrix (ECM) of the ciliary body. Only a few positive reactions were seen in the trabecular meshwork. Latanoprost-treated explants showed a 29% greater area of MMP-2 stained tissue than was found in untreated cultures ($p < 0.0001$), whereas dexamethasone treatment was associated with a 32% decrease in positive area ($p < 0.0001$). Latanoprost had no effect on the total area staining for MMP-2 in the trabecular meshwork but dexamethasone-treated explants had no immunohistochemically detectable MMP-2.

*MMP-2* (Fig. 3)

Untreated explants exhibited strong staining for MMP-3 in the ECM of the ciliary body and a few positive reactions in the trabecular meshwork. Latanoprost-treated explants had 98% more stained area in the ECM than non-treated tissues ($p < 0.0001$). Dexamethasone (500 nM)-treated explants had only one-third as much positive area as untreated cultures ($p < 0.0001$).

Stained areas for MMP-3 in the trabecular meshwork were not changed by latanoprost. After dexamethasone treatment no positive reactions for MMP-3 were found in the trabecular meshwork.

*MMP-9*

MMP-9 was diffusely distributed in the ciliary body; the area of cells expressing MMP-9 was doubled in latanoprost-treated explants ($p < 0.0001$) and markedly suppressed by dexamethasone. As described for MMP-2 and MMP-3, the scattered positive reactions for MMP-9 in the trabecular meshwork were not altered after treatment with latanoprost and totally lost after dexamethasone.

*TIMP-1* (Fig. 4) *and TIMP-2*

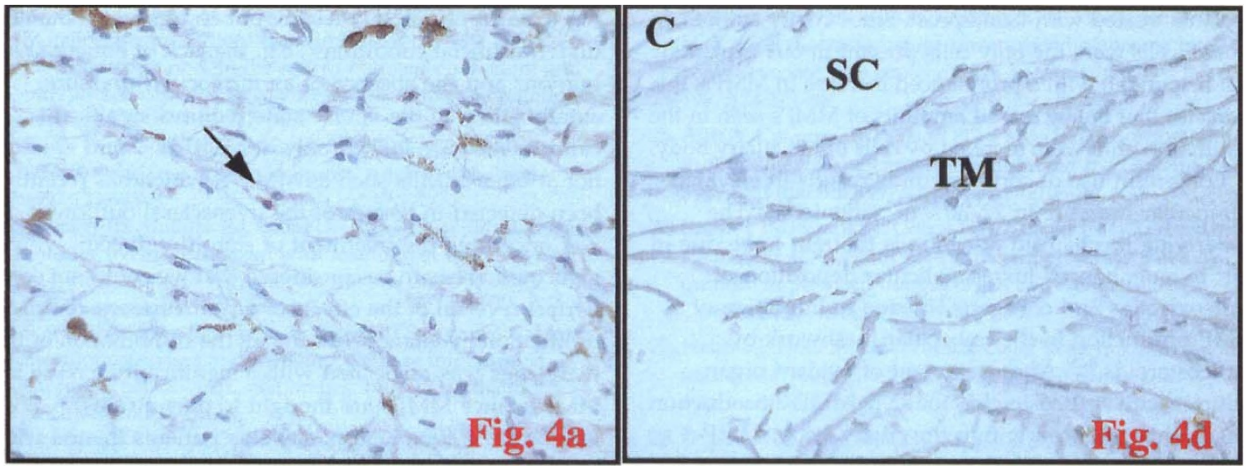
Diffuse staining for TIMP-1 and TIMP-2 was seen throughout the ECM of the ciliary body and weak staining was also seen in the trabecular meshwork. Using immunohistochemistry we could not detect any statistically significant influence of latanoprost or dexamethasone (500 nM) on the amounts of TIMP-1 and TIMP-2 expressed in organ cultures.

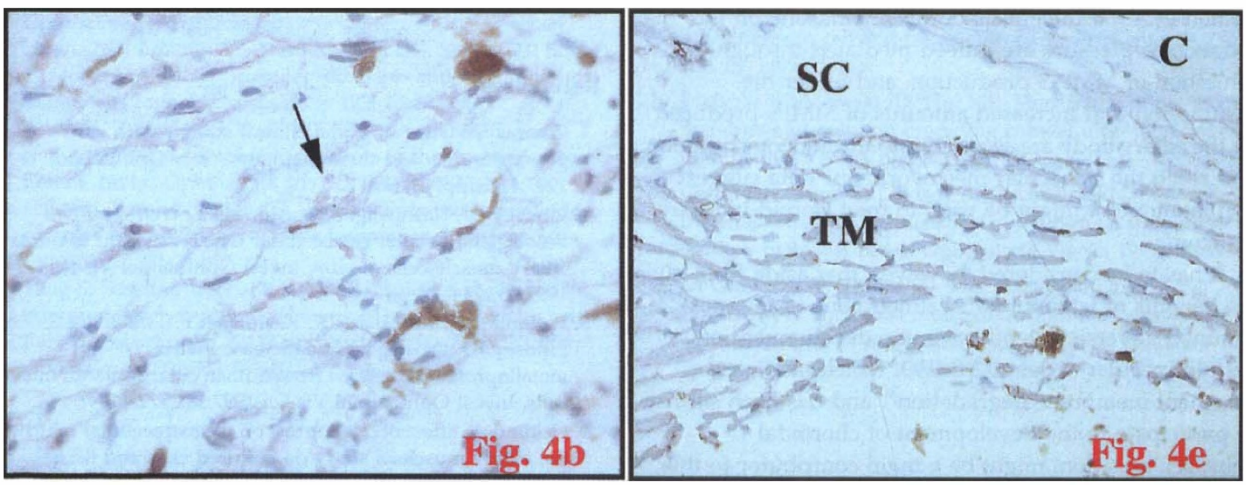
**Discussion**

The ocular hypotensive activity of latanoprost has been demonstrated in various studies.[24,25] The underlying mechanism of action of $PGF_{2\alpha}$ is thought to be through the induction of MMPs, especially MMP-2, and MMP-3.[2–4] One of the goals of our study was to determine which MMP responds to latanoprost and which tissue shows increased MMP production.

In non-treated explant organ cultures MMP-2 and MMP-3 were mainly detectable in ciliary smooth muscle cells. Only a little staining with antibodies against MMPs was seen in the trabecular meshwork.

Exposure of explant organ cultures to latanoprost resulted in a pronounced upregulation of MMP-3 and MMP-9 synthesis in the ciliary body. MMP-2 was upregulated also, but to a lesser degree. Using immunohistochemistry, no effect of latanoprost was detected on the amounts of MMPs present in the trabecular meshwork, or of TIMP-1 and TIMP-2 in either the ciliary body or the trabecular meshwork. These results indicate that in the presence of latanoprost the ratio between agonist (MMPs) and antagonist (TIMPs) is shifted towards the collagenases and proteoglycanases, inducing an elevated turnover of ECM material, as previously described by Ocklind[4] and Lindsey *et al*.[2] Recently, Bradley *et al*.[26] showed that MMPs seem to have a significant role in the maintenance of the trabecular outflow resistance. By adding a variety of substances that inhibited the activity or the production of endogenous MMPs they found decreased outflow rates, whereas the addition of either interleukin-1 or exogenous MMPs resulted in an increase in the outflow rates through the trabecular meshwork. Under the assumption that MMPs produced by the ciliary body are then secreted into the aqueous humour and reach the trabecular meshwork, an increase in ciliary smooth muscle cell MMP production by latanoprost might ultimately induce, as seen by Bradley *et al*.[26] when adding exogenous MMPs to their culture model, an increase in the outflow rates of the trabecular meshwork. The hypothesis that MMPs produced by ciliary smooth cells are indeed secreted into the aqueous humour is supported by the fact that we found increased amounts of MMPs in the culture medium of organ



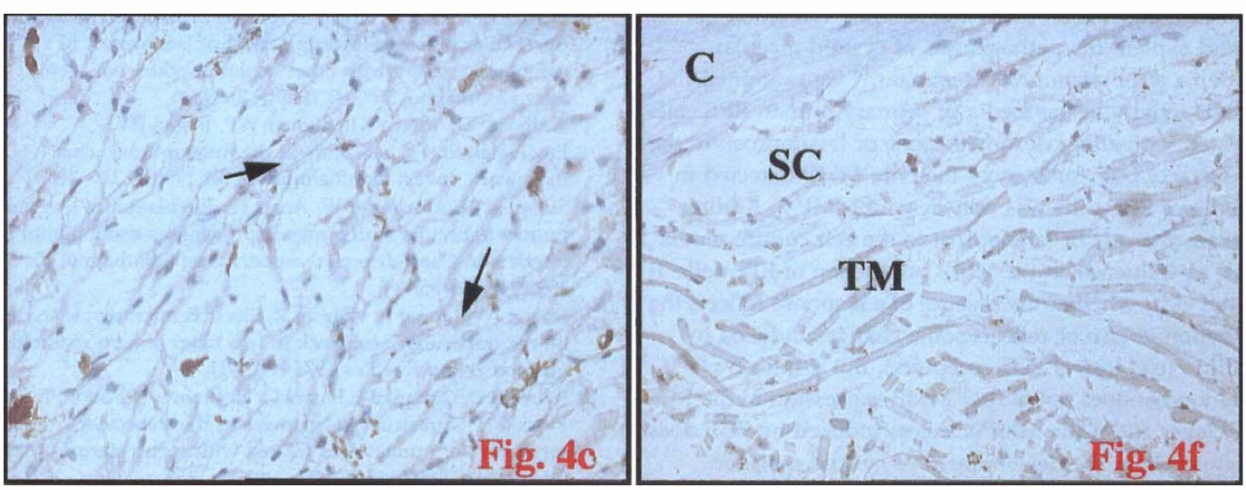




**Fig. 4.** *TIMP-1 expression in the ciliary body (left panels) and the trabecular meshwork (right panels) of cultures treated for 3 days with nothing (a, d), latanoprost (b, e) or dexamethasone (c, f). MMP expression was not altered by any of the drugs. Magnification* ×250.

explants treated with latanoprost. Since ciliary smooth muscle cells were the only cells present in our explants that responded with a pronounced increase in MMPs, this indicates that the increased amounts of MMPs seen in the culture medium were secreted by cells of the ciliary body.

Long-term use of corticosteroids results in elevated intraocular pressure in 22–30% of patients.[27,28] The underlying mechanism of action is thought to be due in part to an enhanced juxtacanalicular deposition of proteoglycans and collagens.[9–11] due to inhibition of MMP production in the trabecular meshwork by corticosteroids.[8,29] After treatment of explant organ cultures with corticosteroids for 72 h, MMP-2 production was slightly reduced within the ciliary body. MMP-3 production was almost absent in the ciliary body and ablated in the trabecular meshwork. It has been proposed that this loss of MMP-3 activity might ultimately lead to the elevated outflow resistance found in corticosteroid-treated eyes.[8] If the effects of dexamethasone on the intraocular pressure are indeed mediated through an inhibition of MMP-3 production, and under the assumption that increased amounts of MMPs produced by the ciliary body are secreted into the aqueous humour and reach the trabecular meshwork, our data suggest that latanoprost might be used to treat steroid-induced glaucoma.

It has to be considered, however, that aside from all the benefits of latanoprost treatment, one of the most common adverse side effects is the development of cystoid macular oedema. MMP-9, which mediates basement membrane degradation[30] and has been shown to participate in the development of choroidal neovascularisation, might be a main contributor to this adverse side effect, by altering the blood–retina barrier. The RPE has $PGF_2$ receptors[13] and is the primary source of MMPs[14] in the posterior compartment of the eye. Considering that an induction of MMPs in RPE cells could explain the pathogenesis of cystoid macular oedema after latanoprost treatment,[15] we examined the MMP activity in the media of primary human RPE cells treated with either dexamethasone or latanoprost. In the absence of both substances the only MMP detected in media of RPE cells was non-cleaved MMP-2. Adding dexamethasone or latanoprost to the cell culture media had no influence on the MMP production of RPE cells. It is not clear whether the absence of a response reflects the situation *in vivo* or reflects some changes induced by culture (e.g. loss of PGF receptors). But if indeed latanoprost does not induce MMP expression in the RPE cell layer and if the MMP-9 excess induced by PGF does play a role in the development of cystoid macular oedema, the results of our study indicate that the MMPs causing this adverse side effect are released from cells of the anterior segment. The fact that aphakic patients are at a higher risk of developing cystoid macular oedema confirms that notion.[15]

In conclusion, despite the fact that the evidence from transmission electron microscopy as well as the prompt response to both dexamethasone and latanoprost indicate that the ciliary body in our explants is vital, it has to be emphasised that since our results were obtained under 'artificial conditions', e.g. the lack of aqueous humour and the absence of an intraocular pressure, extrapolation to the *in vivo* state requires significant caution. Also we looked only at MMP-2, -3 and -9 and not at other MMPs such as MMP-1, which has recently been detected in tissues of the uveoscleral outflow.[31]

Concerning the treatment of steroid-induced glaucoma, it has to be considered that we could not see a perfect reversal of the effects of dexamethasone on MMP production by latanoprost. In fact the combination of the two drugs was associated with a significant increase in MMPs. Since MMPs are thought to play a role in intraocular inflammation,[32] uveitis patients treated with latanoprost for their steroid-induced glaucoma have to be watched carefully, as latanoprost has been shown to be capable of inducing a flare-up of the intraocular inflammation.[16]

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