Effect of Latanoprost on the Expression of Matrix Metalloproteinases and Their Tissue Inhibitors in Human Trabecular Meshwork Cells

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PURPOSE. To determine the effect of latanoprost on the expression of human matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in the trabecular meshwork (TM).

METHODS. Total RNA was isolated, and qualitative RT-PCR was performed to detect the mRNA of MMPs and TIMPs in human TM tissue and explant cultures of TM endothelial cells. Cultures of TM cells were treated with vehicle control or latanoprost acid for 24 hours. Real-time RT-PCR of cell cultures from five different donors was performed to determine relative changes in expression. GAPDH served as an endogenous control.

RESULTS. The mRNA of MMP-1, -2, -3, -11, -12, -14, -15, -16, -17, -19, and -24 and of TIMP-1 to -4 was present in TM tissue and cultures of TM cells. MMP-9 was not found. In control TM endothelial cells, the relative expression of MMP mRNA were MMP-2 and -14 > MMP-16, -19, and -24 > MMP-15 > MMP-11 and -17 > MMP-1 and -3 > MMP-12. The relative expressions of TIMP mRNA were TIMP-1 > TIMP-2 > TIMP-3 > TIMP-4. Latanoprost increased MMP-1 (in four of five cultures), MMP-3 (in four of five cultures), MMP-17 (in three of five cultures), MMP-24 (in all five cultures), TIMP-2, -3, and -4 expression (in three of five cultures); MMP-11 and -15 were downregulated.

CONCLUSIONS. Contrary to the expected result, latanoprost seems to have a significant effect on TM cells. The transcription of the genes for MMP-1, -3, -17, and -24 is increased by latanoprost treatment. TIMP-2, -3, and -4 are also upregulated. The upregulation of these TIMPs may compensate for the increase of those MMPs. The absence of MMP-9 and concurrent upregulation of a greater number of TIMPs may explain the limited effect of latanoprost on TM outflow. (Invest Ophthalmol Vis Sci. 2006;47:3887–3895) DOI:10.1167/iovs.06-0036

In the United States, glaucoma is the second leading cause of irreversible visual impairment.1 The only rigorously validated treatment is the lowering of intraocular pressure (IOP).2–4 Latanoprost is an effective IOP-lowering medication.5 Latanoprost increases uveoscleral outflow up to 60% and is believed to have little effect on aqueous humor production or outflow through the trabecular meshwork (TM).6–10 PGF2α analogues cause a decrease in extracellular matrix (ECM) around ciliary body smooth muscle (CBSM) cells after 4 to 8 days of treatment.11–13 The accelerated ECM turnover is the result of increased expression and activity of metalloproteinases (MMPs).14–18 We determined that the mRNAs of MMPs-1, -2, -3, -11, -12, -14, -15, -16, -17, -19, and -24 as well as tissue inhibitor of metalloproteinase (TIMP)1–4 are present in ciliary body and CBSM cells.19 In response to latanoprost treatment, the expression of MMP-9 is induced, and there is an increase in mRNA of MMP-3, MMP-17, and TIMP-2.19

However, outflow model studies20 and outflow facility studies using anterior segment perfusion systems with glycosaminoglycan degradation enzymes21–24 and MMPs25,26 strongly indicate that ECM turnover is essential in regulating outflow resistance in the trabecular meshwork as well. More recent evidence suggests that prostaglandin (PG) analogues may also cause morphologic changes in the TM as well as increased pressure-sensitive outflow.15,27–29 Components of the PGF2α-signaling pathway (e.g., PG FPR receptors and PG transporter) are present in both outflow tracts (Rhee DJ, et al. IOVS 2001;42:ARVO E-Abstract 753).30–32 Five MMPs—MMP-1, -2, -3, -9, and -14—and three TIMPs—TIMP-1, -2, and -3—have been described in the TM.16,33–35 The effect of latanoprost on MMP transcription in TM was unknown. The origins of the tissue specific response to latanoprost may be in the complement of expressed MMPs, the pattern of induced MMPs, differing levels of transcriptional response, activation of pro-MMPs, or inhibition of MMP activity. Understanding the basis of the tissue specific response may elucidate a fundamental control mechanism of IOP regulation in TM.

MMPs are zinc-dependent endopeptidases that are collectively capable of degrading all ECM components.36 There are approximately 21 unique human MMPs classified into five subfamilies according to their primary structures, substrate specificity, and cellular localization: collagenases, stromelysins, gelatinases, membrane-type (MT)-MMPs, and a miscellaneous group.37 TIMPs are kinetic inhibitors of MMP enzymatic activity and, in some cases, are involved in the activation of MMPs from their inactive zymogen form to their active form.38–39 TIMP-2 to -4 can bind pro-MMP-2, whereas TIMPs-1 and -3 can bind pro-MMP-9.40 Four mammalian TIMPs have been described. All four TIMPs can inhibit all known MMPs, although TIMP-1 poorly inhibits MMP-14, -15, -16, -19, and -24.40–41 MMPs can be regulated at transcription, translation, activation,
and through inhibition at the active site by TIMPs. For most MMPs, with the notable exception of MMP-2, transcriptional regulation, and kinetic inhibition by TIMPs predominates.36

In many tissues, the ratio of MMPs to TIMPs ultimately determines the rate of ECM turnover.54,59 Our long-term goal is to determine the MM-TIMP ratios that are associated with the observed increased outflow. In this investigation, we determined the presence of transcripts of MMPs and TIMPs expressed at baseline and determined the relative changes in transcription after incubation with latanoprost in human TM cells to ascertain which MMPs and TIMPs are critical to regulating outflow resistance.

**Materials and Methods**

Except for explant culturing of TM cells, we have described many of the experimental procedures elsewhere.19

**Tissue and Explant Culture**

All TMs were dissected from human donor corneoscleral buttons removed by the Lyons Eye Bank of Delaware Valley (Philadelphia, PA). All procedures were performed in accord with the Declaration of Helsinki. We isolated TM tissue from 21 separate donors aged 30 to 67 years. Ages of the donors of the TM tissue samples to determine the presence of MMPs and TIMPs were 42, 46, 48, 49, 50, 51, 52, 54, 55, 56, 58, 59, 60, 61, 62, 63, 66, 65, and 67 years. Explant cultures of TM cells from separate donors aged 30, 40, 54, 56, 64, and 65 years, were used for qualitative reverse transcription-polymerase chain reaction (RT-PCR) of MMPs and TIMPs, and those of donors aged 40, 54, 56, 64, and 65 years were used for real-time (or quantitative) (q)RT-PCR. TM cells were cultured and maintained according to a previously published protocol.42 All the cells used were from passage-4 cultures.

**Latanoprost Incubation and RNA Isolation**

TM cells (1 × 10⁶) were plated into T-75 flasks, grown to confluence, and then maintained for 1 week. The cells were then incubated with serum-free media for 48 hours and exposed to 0.06% ethanol (the vehicle control), 0.03 µg/mL, 0.3 µg/mL, or 30 µg/mL of latanoprost free acid for 24 hours. In practice, latanoprost is given as a 0.005% (50 µg/mL) topical solution resulting in a peak aqueous concentration of 0.028 µg/mL, 28 ng/mL 1 to 2 hours after administration; the half-life in aqueous humor is 2 to 3 hours.45 The cells were then scraped for RNA extraction (RNAqueous-4PCR kit; Ambion, Austin, TX) in accordance with the manufacturer’s protocol.

Purity and concentration of the RNA were tested by spectrophotometry.

**RT-PCR and Sequencing of PCR Product**

To determine the presence of MMPs and TIMPs, qualitative RT-PCR was performed on total RNA isolated from TM tissue and untreated explant cultures of TM cells (SuperScript One-Step RT-PCR with Platinum Taq; Invitrogen, Carlsbad, CA) in accordance with the manufacturer’s protocol. RT-PCR was initially performed using oligonucleotide primers specific to the mRNA of MMP-1, -2, -3, -7, -8, -9, -10, -11, -12, -13, -14, -15, -16, -17, -19, -20, -23, -24, -25, -26, -28 and TIMPs-1 though -14. Two tubes were run in parallel with the second tube containing only Platinum Taq Polymerase to assure that the source of the RT-PCR product was mRNA. For each reaction, 100 ng of template RNA was used. Forty cycles of amplification was performed (model 2700 Thermocycler; Applied Biosystems [ABI], Foster City, CA).

The mRNA concentration for MMPs and TIMPs in TM tissue is low, so reamplification for 35 cycles was performed. Ten percent of the RT-PCR product was used as DNA template for the PCR reaction. This reamplification was not necessary for cultures of TM cells. Gel electrophoresis of the PCR products was performed. When the single correct band size was located, the bands were cut from the gel and purified. DNA sequencing was then performed to confirm that the product obtained was representative of the specific MMP or TIMP. Results from the sequencing were analyzed using the BLAST program.

**qRT-PCR: Primer-Probe Design**

We have previously reported the DNA sequences and verification of a primer/probe combination for MMPs and TIMPs using Primer Express (Applied Biosystems).79 DNA sequencing of the amplicons verified the specificity of the primers and served as a second confirmation as to the presence of mRNA of the MMP or TIMP of interest.

Real-time or quantitative RT-PCR was then performed (Prism 7000; ABI; the TaqMan One Step RT-PCR Mastermix with GAPDH as an endogenous control; ABI). The thermocycling was performed in three stages: Stage one was cDNA synthesis of 48°C for 30 minutes. Stage two was denaturation at 95°C for 10 minutes. The third amplification step was 40 cycles of 95°C for 15 seconds followed by annealing at 60°C for 1 minute. Results were quantified (Prism 7000 SDS Software; ABI). Twenty nanograms of total RNA was used to compare expression levels of MMPs and TIMPs.

**Relative Quantitation**

We have previously described the relative quantitation methods.19 For relative quantitation normalized to GAPDH, standard curves for MMPs and TIMPs and their counterpart GAPDHs were constructed with a serial dilution of total RNA of four different cell lines: HT-1080 cells (human fibrosarcoma cell line; ATCC, Manassas, VA) for MMP-1, -2, -14, -15, -19, and -24; CCF-STTG1 cells (human astrocytoma; ATCC) for MMP-3, -11, and -17, CBSM cells for MMP-12, and TM endothelial cells for Timp-1, -2, -3, -4. Using three C T values (i.e., threshold cycle, defined as the fractional cycle number at which the amount of amplified target reaches a fixed threshold) to each input amount of dilution, standard curve equations for MMPs, TIMPs, and their counterpart GAPDHs were generated. Comparative ΔC T method was used for relative quantitation for those genes whose absolute value of the slope of log input versus ΔC T was less than 0.1. The relative standard curve method was used for genes not satisfying the criteria. The coefficient of variation (CV; percentage) was used for data reproducibility. Reproducibility of standard curves of GAPDHs obtained from those cell lines used for different genes was as follows: 4.45 to 5.57 of CV (percentage) for HT-1080, 2.27 to 5.61 of CV for CCF-STTG1, and 0.98 to 2.38 of CV for cultured TM cells. All triplicates of C T used for the experiment showed much less than 1% CV. According to the manufacturer’s protocol, triplicates showing less than 2% CV are acceptable for this assay (ABI).

**Results**

**mRNA Expression of MMPs and TIMPs**

Eleven of 21 MMPs were expressed in the TM tissue and the cultures of TM cells (Table 1) as determined by qualitative RT-PCR and subsequent DNA sequence confirmation (Fig. 1). MMP-9 was not detected either before or after latanoprost treatment. All four TIMPs in the TM tissue and the cultures of TM cells (Table 1) were expressed by qualitative RT-PCR and their subsequent DNA sequences were confirmed (Fig. 1). In the cultured TM cells, the relative level of expression of the 11 MMPs and 4 TIMPs were determined by the C T values (C T) s in the control group. MMP-2 and -14 had very high expression levels, with C T of 24 to 26 at 20 ng of total RNA (MMP-2 ≫ MMP-14). MMP-16, -19, and -24 had intermediate expression levels with C T of 29 to 31 (MMP-24 ≈ MMP-19 = MMP-16), and MMP-15 had intermediate expression levels with C T of 31 to 35. MMP-1, -3, -11, and -17 had low expression levels, MMP-11 and -17 with C T of 33 to 34 (MMP-11 ≈ MMP-17), and MMP-1 and -3 with C T of 34 to 36 (MMP-1 ≈ MMP-3). MMP-12 had a very low expression level with C T of 3888 Oh et al. IOVS, September 2006, Vol. 47, No. 9
38. The CTs of GAPDH were 22 to 23 at the same total RNA concentration, 20 ng. TIMP-1, -2, and -3 appeared to be expressed high levels. At 20 ng of total RNA, TIMP-1 had CTs of 26 to 27. TIMP-2 and -3 had almost the same expression level showing CTs of 27 to 28. TIMP-4 had a low expression level showing CTs of 33 to 36. The CTs of GAPDH were 22 to 23 at the same total RNA concentration.

### Table 1. The Relative mRNA Expression Levels of MMPs and TIMPs in Cultures of Human TM Endothelial Cells and the Effect of Latanoprost Acid on Their Expression at a Pharmacologic Level (0.03 μg/mL)

<table>
<thead>
<tr>
<th>Name</th>
<th>Protease</th>
<th>Expression Level*</th>
<th>Effect of Latanoprost Acid**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagenases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-1</td>
<td>Collagenase-1</td>
<td>++</td>
<td>4 UP, 1 DOWN</td>
</tr>
<tr>
<td>Gelatinases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-2</td>
<td>Gelatinase A</td>
<td>+++++</td>
<td>3 NO, 2 DOWN</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Gelatinase B</td>
<td>−</td>
<td>No expression</td>
</tr>
<tr>
<td>Stromelysins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-5</td>
<td>Stromelysin-1</td>
<td>++</td>
<td>4 UP, 1 NO</td>
</tr>
<tr>
<td>MMP-11</td>
<td>Stromelysin-3</td>
<td>++</td>
<td>1 NO, 4 DOWN</td>
</tr>
<tr>
<td>MT-MMPs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-14</td>
<td>MT1-MMP</td>
<td>+++++</td>
<td>2 UP, 3 NO</td>
</tr>
<tr>
<td>MMP-15</td>
<td>MT2-MMP</td>
<td>+++</td>
<td>1 UP, 1 NO, 3 DOWN</td>
</tr>
<tr>
<td>MMP-16</td>
<td>MT3-MMP</td>
<td>+++</td>
<td>1 UP, 3 NO, 1 DOWN</td>
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<tr>
<td>MMP-17</td>
<td>MT4-MMP</td>
<td>+++</td>
<td>3 UP, 1 NO, 1 DOWN</td>
</tr>
<tr>
<td>MMP-24</td>
<td>MT5-MMP</td>
<td>+++</td>
<td>5 UP</td>
</tr>
<tr>
<td>Other MMPs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-12</td>
<td>Metalloelastase</td>
<td>+</td>
<td>2 UP, 2 NO, 1 DOWN</td>
</tr>
<tr>
<td>MMP-19</td>
<td>RASI</td>
<td>+++</td>
<td>2 UP, 1 NO, 2 DOWN</td>
</tr>
<tr>
<td>TIMPs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMP-1</td>
<td></td>
<td>+++++</td>
<td>4 UP, 1 DOWN</td>
</tr>
<tr>
<td>TIMP-2</td>
<td></td>
<td>+++</td>
<td>5 UP, 2 NO</td>
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<tr>
<td>TIMP-3</td>
<td></td>
<td>+++</td>
<td>3 UP, 1 NO, 1 DOWN</td>
</tr>
<tr>
<td>TIMP-4</td>
<td></td>
<td>++</td>
<td>3 UP, 1 NO, 1 DOWN</td>
</tr>
</tbody>
</table>

The criteria for decision of expression level are as follows: UP: 10% above control; NO: control ± 10%; DOWN: 10% below control.

*: ++++; very high expression (24–26); +++; high expression (26–29); ++; intermediate expression (29–33); +: low expression (33–36); -: not detected at RT-PCR. The numbers in parenthesis are Ct values; the ranges encompass all Ct values from all 5 cell lines. The Ct values for GAPDH (i.e. endogenous control) were between 22–23 for all MMPs and TIMPs tested from all experiments.

**: Individual difference in the effect of latanoprost on MMPs: DOWN: down-regulation; NO: no effect; UP: up-regulation. Numbers indicate number of donors.

38. The Ct’s of GAPDH were 22 to 23 at the same total RNA concentration, 20 ng. TIMP-1, -2, and -3 appeared to be expressed high levels. At 20 ng of total RNA, TIMP-1 had Ct’s of 26 to 27. TIMP-2 and -3 had almost the same expression level showing Ct’s of 27 to 28. TIMP-4 had a low expression level showing Ct’s of 33 to 36. The Ct’s of GAPDH were 22 to 23 at the same total RNA concentration.

### Figure 1. Qualitative RT-PCR results using primers developed by Primer Express (ABI): DNA ladders (Invitrogen) were run concurrently to verify DNA size and the gel was imaged (Gel Doc 2000, BioRad, Hercules, CA). (A) Qualitative RT-PCR for MMPs in TM endothelial cells cultured from a 56-year-old donor. Odd-numbered lanes correspond to no-template controls, and lane 2: MMP-1 (100); lane 4: MMP-2 (88), lane 6: MMP-3 (96), lane 8: MMP-11 (84), lane 10: MMP-12 (80), lane 12: MMP-14 (104), lane 14: MMP-15 (75), lane 16: MMP-16 (148), lane 18: MMP-17 (77), lane 20: MMP-19 (120), and lane 22: MMP-24 (136). All PCR DNA products were sequenced. (B) Qualitative RT-PCR for TIMPs in TM endothelial cells cultured from a 56-year-old donor. Odd-numbered lanes correspond to no-template the control, and lane 2: TIMP-1 (107), lane 4: TIMP-2 (104), lane 6: TIMP-3 (142), and lane 8: TIMP-4 (94). Numbers in parentheses are expected band sizes in base pairs for the corresponding (A) MMPs and (B) TIMPs. (A, B) Left column: a 25-base-pair DNA ladder.
Changes in mRNA Expression of MMPs by Latanoprost Acid

A change of more than 10% in expression between the control and experimental values was defined as an alteration of expression; relative efficiencies of target and reference genes are considered equal if the absolute value of the slope of log input versus $\Delta C_v$ was $< 0.1$. Thus, the relative quantitation method classifies 10% variability as unchanged (ABI PRISM 7700 Sequence Detection System User Bulletin #2, 1997). A change of 10% above/below the control represents a 0.1-fold change in expression. There was substantial individual variability among the donors tested, which had been encountered in previous studies of MMP and TIMP expression with latanoprost in the ciliary body.18,19 Therefore, the ultimate determination of the effect of latanoprost on expression of an individual MMP or TIMP was determined by the trend of the majority (>60%) of the samples at 0.03 µg/mL (Table 1). We used the same method to determine the trend at the other concentrations (Fig. 2).

Collagenases. Of the three known collagenases, collagenase-1 (MMP-1) was the only detected enzyme of this subfamily. At a pharmacologic level (0.03 µg/mL) of latanoprost, the expression of MMP-1 increased an average of 31% in four of five donors. At 3 µg/mL, these four donors showed a further dose-dependent increase in expression (Fig. 2A). At 30 µg/mL, transcription decreased in all four. In the remaining donor, its expression was decreased at the level of 0.03 µg/mL.

Gelatinases. Of the two gelatinases, gelatinase A (MMP-2) was detected. Its expression in three donors was unchanged at 0.03 µg/mL and slightly decreased in two donors; and as the concentration of latanoprost increased, the overall trend was not altered (Fig. 2B). Overall, the expression of MMP-2 is unchanged or slightly decreased in response to latanoprost.

Stromelysins. Of the three known stromelysins, stromelysin-1 and -3 (MMP-3 and -11, respectively) mRNAs were detected. At 0.03 µg/mL, four of five donors showed an average of 54% more MMP-3 mRNA expression, which remained elevated in all five donors at 0.3 µg/mL. At 30 µg/mL, the expression decreased in three donors, whereas the relative expression in the other two donors continued to increase (Fig. 2C). MMP-11 expression was decreased in four of the five donors an average of 16% and not observed to change in one donor at 0.03 µg/mL. As the concentration of latanoprost increased in these four donors, there was a dose-dependent decrease in expression in three donors, but a dose-dependent elevation in one (Fig. 2C). One donor was unchanged at 0.03 µg/mL, then increased expression at 0.3 µg/mL, then decreased far below baseline at 30 µg/mL. MMP-11 is decreased by latanoprost.

Membrane Type-Matrix Metalloproteinases. At 0.03 µg/mL, the expression of MT1-MMP (MMP-14) was unchanged in three donors, and expression increased in the two other donors. At 0.3 µg/mL, expressions in two donors were unchanged, but it increased in one while expression decreased in

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**Figure 2.** Changes in mRNA expression of MMPs (A–D), MT-MMPs (E–G), and TIMPs (H–K) in TM endothelial cells treated with latanoprost. In response to latanoprost at the therapeutic level (0.03 µg/mL), in cultures of TM endothelial cells, MMP-1, -3, -17, and -24 were upregulated, MMP-11 and -15 were downregulated. MMP-2, -14, -16 and TIMP-1 appear to be unchanged from control. From 15 to 50 ng of total RNA was used according to expression level of MMPs.
two donors (Fig. 2E). At 30 μg/mL, transcription in four of five donors decreased, and one remained unchanged. Overall, MMP-14 transcription was unchanged by latanoprost.

The expression of MT2-MMP (MMP-15) decreased in three donors at 0.03 μg/mL an average of 26%; the decreased expression in these three donors continued at higher concentrations. The expression in one donor was unchanged at all concentrations of latanoprost and the remaining was consistently elevated at all concentrations (Fig. 2E). Overall, latanoprost decreases MMP-15 expression.

The expression of MT3-MMP (MMP-16) was unchanged in three donors at 0.03 μg/mL. As the concentration increased, MMP-16 expression increased in two of these three, and the third remained unchanged. In the remaining two donors, MMP-16 expression was consistently increased in one and decreased at all three concentrations (Fig. 2F) in the other. Overall, latanoprost had little effect on MMP-16 transcription. At peak pharmacologic doses, MMP-16 was unchanged from control.

At 0.03 μg/mL, MT4-MMP (MMP-17) was increased an average of 14% in three donors. In one donor, the expression was decreased, and in the remaining its expression was unchanged (Fig. 2G). As the concentration increased, MMP-17’s expression decreased. Overall, latanoprost slightly increased MMP-17 expression.

The expression of MT5-MMP (MMP-24) was increased in all five donors at 0.03 and 3 μg/mL (Fig. 2F) an average of 74% and 76%, respectively. At 30 μg/mL, MMP-24 expression was decreased in three of the 5 donors and unchanged from control in the remaining two.

Miscellaneous MMPs. Metalloelastase (MMP-12) expression increased at 0.03 μg/mL in two donors, decreased expression in one donor and unchanged in the remaining two donors. As the concentration of latanoprost increased, no clear trend became apparent. Overall, it is likely that latanoprost does not significantly affect MMP-12 transcription (Fig. 2D).

At 0.03 μg/mL, the expression of RASI-1 (MMP-19) decreased in two donors, was unchanged in one, and increased in the remaining two donors; higher concentrations of latanoprost resulted in a decrease in expression in 4 of five donors (Fig. 2D). It is likely that MMP-19 expression is unchanged at pharmacologic doses; however, at suprapharmacologic doses, there was decreased expression.

In summary, latanoprost increased transcription of MMP-1 -3, -17, and -24, whereas MMP-11 and -15 decreased. MMP-2, -14, -16, and -19 were unchanged from control. There was variability in the responses of mRNA expression of MMPs to latanoprost among donors.

Tissue Inhibitors of Matrix Metalloproteinases. The expression of TIMP-1 did not change in four of the five donors at 0.03 μg/mL (Fig. 2H). In the remaining one, expression was decreased. At 0.3 μg/mL, three were within 10% of baseline and two were decreased; at 30 μg/mL, three were decreased, and two unchanged.

At 0.05 μg/mL, TIMP-2 expression increased an average of 30% in three of five donors; expression in the two other donors...
was unchanged (Fig. 2I). As the concentration increased, expression of TIMP-2 decreased.

TIMP-3 expression increased an average of 26% in three of the five donors with 0.03 \( \mu \text{g/mL} \). Of the remaining two, the expression was unchanged in one donor and decreased in the remaining. The increase of expression continued in two of five donors at 0.3 \( \mu \text{g/mL} \) (Fig. 2J). At 30 \( \mu \text{g/mL} \), TIMP-3 was downregulated.

The expression of TIMP-4 increased an average of 49% in three of the five donors at 0.03 \( \mu \text{g/mL} \), but decreased in one and was unchanged in the remaining donor (Fig. 2K). At 0.3 \( \mu \text{g/mL} \), TIMP-4 remained increased in three of the five donors. At 30 \( \mu \text{g/mL} \), TIMP-4 transcription decreased in four donors.

In response to pharmacologic doses of latanoprost, mRNA expression of TIMP-2, -3, and -4 increased, and expression of TIMP-1 were unchanged. As the concentration increased to suprapharmacologic doses, the relative expression of all TIMPs generally decreased.

**DISCUSSION**

**mRNA Expression of MMPs and TIMPs**

To date, this is the most comprehensive analysis of mRNA expression of MMPs and TIMPs in human TM. The presence of mRNA and protein of MMP-1, -2, -3, -9, and -14 as well as TIMPs-1 and -2 have previously described in human TM. The TIMP-3 and -4 protein, but not the mRNA, have also been reported in TM. We found the mRNA of seven other MMPs as well as TIMPs-3 and -4. To assure the validity of our expression pattern, we used primers previously validated in the literature and confirmed the DNA sequence of the PCR products. We tested both TM tissues and explant cultures of TM cells. A second set of primers (those for the quantitative RT-PCR) also confirmed the expression of these genes in the TM cells.

At mRNA level, TM cells express the same 11 MMPs and 4 TIMPs expressed in CBSM cells. The relative levels of MMP-1, -11, and -24, mRNA were lower in TM cells than in the CBSM cells. However, TIMP-3 expression was higher in TM cells. Overall, TM cells had less expression of MMPs and more expression of TIMPs than CBSM cells. MMP-9 mRNA, gelatinase B, was not detected, although MMP-9 protein has been reported in cultured TM cells and TM anterior segment organ culture by immunohistochemistry and zymography. Detecting the RNA of MMP-9 has been elusive. Consistent with other reports, we were not able to detect MMP-9 mRNA in either TM tissue or TM cell cultures; nor did we find MMP-9 mRNA in vehicle control or latanoprost-treated TM cells. In CBSM cells, latanoprost treatment induces MMP-9 expression to detectable levels. If MMP-9 has a functional role in the TM, the protein may come from other tissues since the transcription of MMP-9 is at extremely low levels in TM cells.
Changes in MMPs and TIMPs by Latanoprost

Initially, it was believed that the ocular hypotensive effect of latanoprost was based solely on increased outflow through the uveoscleral pathway.\(^{46-47}\) TM cells produce PGE\(_2\) and PGE\(_{2a}\); these endogenous PGs may normally regulate aequorin dynamics.\(^{48}\) Activation of FP receptors may play a role in a PG-mediated regulation of IOP by also promoting conventional outflow.\(^{49}\)

In TM cells, pharmacologic doses of latanoprost increased mifepristone affinity of MMP-1,-3, -17, and -24, whereas MMP-11 and -15 decreased. MMP-1, -3, and -17 were expressed in low amounts in control samples, whereas MMP-24 was expressed at a high level. MMP-1 is a strong effector of ECM degradation with a broad substrate specificity.\(^{50,51}\) MMP-3 is involved with a decrease in outflow resistance to both tert-butyldihydroquinone and argon laser.\(^{26,50}\) MMP-17 can activate aggrecanase-1 (ADAMTS4) which degrades glycosaminoglycans, and its up-regulation is associated with increased ECM degradation.\(^{51,52}\)

MMP-17 may activate MMP-2 (progelatinase A).\(^{53,54}\) By activating aggrecanase-1, MMP-17 most likely increases ECM turnover. MMP-24 mRNA was uniformly increased by latanoprost. MMP-24 can activate pro-MMP-2.\(^{55,56}\) Through activation of pro-MMP-2, MMP-24 is most likely involved in increasing ECM remodeling.\(^{55-57}\) Although four MMPs with strong tendencies to degrade matrix are upregulated by latanoprost, the lack of extensive ultrastructural change is probably due to the concur rent elevation of TIMPs.

TIMP functions are primarily believed to be inhibitory. TIMP-3 is the only TIMP that is sequestered in ECM to heparin sulfate and possibly chondroitin-sulfate containing proteoglycans.\(^{58}\) In glaucoma, there is a shift in proteoglycans side chains to chondroitin-sulfates.\(^{59}\) TIMP-3 is an excellent inhibitor of MT1-MMP and MT2-MMP.\(^{60}\) TIMP-4 also participates in ECM homeostasis through the balance of MMPs to TIMPs.\(^{61}\) The upregulation of TIMPs-2, -3, and -4 mRNA probably balances the latanoprost-induced increase in MMPs. In CBSM cells, we reported increased MMP-3 and MMP-17 transcription after latanoprost, whereas TIMP-3 was the only inhibitor increased by latanoprost.\(^{19}\) Thus, the ratio of MMPs to TIMPs is likely to remain similar, whereas in CBSM cells, the ratio is shifted toward greater MMPs. The greater shift in MMP to TIMP ratios of that occurs in CBSM cells may be the reason for the differing levels of ECM degradation and resultant aqueous outflow. Further studies at the protein level should be completed for confirmation.

In the TM, immunohistochemical staining of MMP-2, -3, and -9 and TIMP-1 and -2 were found to a lesser degree than in the CB, possibly due to greater cellularity.\(^{16}\) After latanoprost treatment their reactivity was not significantly altered in the TM.\(^{16}\) Pang et al.\(^{66}\) found that PG did not affect TM cell MMP-1 and -3 levels at protein level.

We observed variability in the response to latanoprost among cultures of TM cells from different donors, similar to other studies of MMP transcription after latanoprost incubation.\(^{18,19,63}\) We observed less variability with TM cells than with CBSM cells.\(^{19}\) The variation could represent normal human variations of mRNA that is present in very low amounts under normal conditions, donor medication history, variability inherent to the analysis methods, and normal genetic variation in response to latanoprost.\(^{64}\) Latanoprost altered at least some of the MMPs/TIMPs in all cell lines; there was no apparent age-dependent pattern of response.

MMP-2 and-14 mRNA were the most highly expressed MMPs in TM as well as CBSM cells, but the transcription of these MMPs were not affected by latanoprost.\(^{15}\) Further study comparing the relative levels of zymogen (i.e., pro-MMP-2) versus active MMP-2 and -14 are needed to determine the importance of MMP-2, because it is primarily regulated at activation.

MMP-3 and -17 mRNA, which are upregulated in CBSM cells by latanoprost, are also increased in TM with the addition of MMP-1 and -24.\(^{15}\) Significant induction of MMP-9 and the difference in the response of TIMP-2 and -4 to latanoprost may be the key steps to the observed augmentation of uveoscleral outflow and relatively small/limited effect on conventional outflow. Latanoprost appears to shift the balance of MMPs and TIMPs toward greater MMPs in CBSM cells resulting in the observed changes in ECM, while the balance in TM cells is shifted to a lesser degree. Examination of MMP/TIMP mRNA expression at only one time point is a limitation of this study. Additionally, latanoprost-mediated changes in MMP/TIMP activity may be regulated by posttranscriptional events, extracellular activation, kinetic inhibition, and/or turnover.

References


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