Prostaglandins Increase Matrix Metalloproteinase Release From Human Ciliary Smooth Muscle Cells

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Purpose. To identify matrix metalloproteinases (MMPs) released by ciliary smooth muscle cells in vitro and to determine whether MMP release is altered by exposure to prostaglandins (PGs).

Methods. Human ciliary smooth muscle cells were grown to confluence in monolayer cultures and treated with $PGF_{2\alpha}$, 11-deoxy-PGE_1, or PhXA85 (the nonesterified analogue of PhXA41) for 12 to 72 hours. The activity of MMP in the medium was assayed using gelatin and casein zymography. Identification of the specific MMP associated with each band was made by Western blot analysis. Band intensity, which reflects activity, was measured with a scanning laser densitometer.

Results. Three major bands appeared in the gelatin zymographs at positions corresponding to molecular weights of 62 kDa, 68 kDa, and 97 kDa. A single band at 50 kDa predominated in the casein zymograms. Substitution of EDTA for calcium and zinc in the development solution eliminated the appearance of these bands, indicating that they reflect MMP activity. Immunoblotting, using MMP-specific antibodies, confirmed that the three bands in the gelatin zymographs were MMP-1, MMP-2, and MMP-9, respectively; the single band in the casein zymographs was MMP-3. Treatment with 200 nM $PGF_{2\alpha}$, 11-deoxy-PGE_1, or PhXA85 for 72 hours increased the combined density scores for MMP-1 and MMP-2 by 37%, 64%, and 27%; the density scores for MMP-9 by 268%, 253%, and 125%; and the density scores for MMP-3 by 35%, 71%, and 22%, respectively.

Conclusions. These results indicate that ciliary smooth muscle cells can secrete MMP-1, MMP-2, MMP-3, and MMP-9. In addition, exposure to $PGF_{2\alpha}$, 11-deoxy-PGE_1, or PhXA85 increases production of all four MMPs. These observations support the hypothesis that increased MMP production by ciliary muscle cells has a role in increasing uveoscleral outflow facility after topical PG administration. Invest Ophthalmol Vis Sci. 1997;38:2772–2780.

Physiological studies in animals and humans indicate that the intraocular pressure reduction induced by prostaglandin (PG) $F_{2\alpha}$ or the $PGF_{2\alpha}$ analogues, including latanoprost or $PGF_{2\alpha}$-isopropylester, reflects increased uveoscleral outflow without significant changes in conventional outflow or aqueous production and implicate the ciliary muscle as having an important role.[1–7] The biochemical events mediating this response, however, remain poorly understood.

Matrix metalloproteinases (MMPs) are a family of neutral, zinc-dependent enzymes that can hydrolyze specific peptide sequences found in extracellular matrix (ECM) structural proteins.[8,9] These molecules are secreted as inactive proenzymes (zymogens) and become activated by proteolytic truncation. Recently, it has been reported that exposure of human ciliary smooth muscle cells to PGs induces the secretion of promatrix metalloproteinase-1 (pro-MMP-1, also known as prointerstitial collagenase) and pro-MMP-3 (prostromelysin-1).[10] This response may contribute to the PG-mediated increase of uveoscleral outflow, because the uveo-

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scleral outflow path includes the extracellular spaces separating ciliary muscle fibers,[11,12] and because these extracellular spaces contain abundant ECM.[13-16] Thus increased degradation of ciliary muscle ECM by MMPs could decrease the hydraulic resistance to uveoscleral flow.[17]

In addition to MMP-1 and MMP-3, it is likely that ciliary smooth muscle cells may release other MMPs. For example, human vascular smooth muscle cells can secrete MMP-1 and MMP-3, as well as MMP-2 (gelatinase A) and MMP-9 (gelatinase B).[18,19] Each MMP type targets specific peptide sequences in ECM macromolecules for cleavage. For example, MMP-1 cleaves a single site in collagen type Iα1 and Iα2 chains, as well as in the homotypic collagen type III chains.[20,21] Matrix metalloproteinase-3 can cleave sites in collagen types III, IV, V, and IX and in fibronectin and laminin[9]; MMP-9 cleaves sites in collagen types IV and V; and MMP-2 cleaves sites in collagen types IV, V, VII, and X.[8,9] The action of MMP can initiate enzymatic degradation of ECM components by non-MMP extracellular proteases.[22] Hence, release of MMP types by ciliary smooth muscle cells could have important consequences for the structure of adjacent ECM.

In the current study, we evaluated the release of MMP-1, MMP-2, MMP-3, and MMP-9 by ciliary smooth muscle cells in vitro. Also, we investigated whether release of these MMPs was altered by exposing the cells to $PGF_{2\alpha}$, 17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ (17PT-$PGF_{2\alpha}$), 11-deoxy-PGE1, or PhXA85 (the free acid form of latanoprost).

MATERIALS AND METHODS

Human Ciliary Smooth Muscle Cultures

Nine pairs of human eyes from donors 31 to 76 years old were obtained from the San Diego Eye Bank within 24 hours after death. Enucleation was completed within 6 hours after death, and eyes were stored at 4°C for less than 24 hours before cultures were generated. Donors had no known history of glaucoma or other eye diseases.

Ciliary smooth muscle cell cultures were prepared as previously described.[14] Briefly, each eye was bisected horizontally 3 mm posterior to the limbus. The anterior segment was placed cornea-side-down under a dissecting microscope, and the lens was removed by cutting the zonule. Strips of the outermost portion of ciliary muscle were explanted into 35-mm culture dishes (Falcon, Lincoln Park, NJ). The medium was Dulbecco's modified Eagles medium and Ham's F12 nutrient mixture (DMEM–F12; University of California, San Diego Cell Culture Facility), supplemented with 10% fetal bovine serum (Gemini, Woodland, CA) and 1 ng/ml recombinant human basic fibroblast growth factor (bFGF; R & D Systems, Minneapolis, MN). The cultures were incubated in a humidified atmosphere of 95% air–5% CO_2. The medium was changed every 3 to 4 days. Primary cells reached confluence within 40 to 60 days. Subsequent passages reached confluence within 14 days. Confluent cultures were trypsinized and subcultured in the same medium at a ratio of 1:3. For the current study, we used confluent third- and fourth-passage cells.

These cells expressed several proteins specific to ciliary smooth muscle cells in vivo, including α-smooth muscle actin, desmin, and calponin, an actin-binding protein that regulates contraction in smooth muscles.[14,23] Also, these cells biosynthesize collagen types I, III, IV, which assemble into a characteristic ECM adjacent to the cell monolayer.[13,14,24]

Prostaglandins

Prostaglandin $F_{2\alpha}$, 17PT-$PGF_{2\alpha}$, and 11-deoxy-PGE_1 were purchased from Cayman Chemical Company (Ann Arbor, MI), and PhXA85 (the nonesterified analogue of PhXA41) was provided by Dr. J. Stjernschantz of Pharmacia Upjohn, Uppsala, Sweden. Topically applied PG esters (e.g., latanoprost) become hydrolyzed as they pass through the cornea and into the aqueous humor.[7,25] Thus, PhXA85, the nonesterified analog of latanoprost, is the appropriate choice for studies of latanoprost action in vitro. The concentration of PGs tested, 20 nM and 200 nM, was selected on the basis of the concentration of 78 nM $PGF_{2\alpha}$ (28 ng/ml) detected in aqueous humor collected from monkey eyes after topical treatment with an intraocular-pressure-lowering dose of $PGF_{2\alpha}$.[26] All PGs were dissolved in ethanol and diluted with DMEM–F12 to 2 mM. The final concentration of ethanol in the vehicle control was 0.001%, the same concentration of ethanol in the highest dose of PG treatment used in the study. All reagents were stored at −70°C to minimize oxidation until use.

Experimental Design

Cells were maintained in a medium containing 10% serum in DMEM–F12 supplemented with 1 ng/ml bFGF for 1 week after they had become confluent. The cultures then were switched to serum-free medium containing DMEM–F12 supplemented with 1 mM insulin, 5 mg/ml transferrin, 100 mM ascorbic acid, 1 mM β-aminopropionitrile, and 1 ng/ml bFGF for 4 days before experimental treatments were initiated. Serum-free medium was used to minimize nonspecific induction of MMPs, because serum contains agents known to stimulate MMP biosynthesis.[8,27] The 4-day preincubation period was based on the time required for the expression of c-*fos* to reduce to a basal plateau.[28] In the treatment phase, PGs or vehicle were added daily to the cultures for 1 to 3 days. Medium

samples were collected and stored at −80°C until analyzed.

Zymographic Analysis

Medium samples were thawed on ice, concentrated using Centricon-30 ultrafiltration units (30-kDa cutoff; Amicon, Beverly, MA), and adjusted to a final concentration ratio of 10:1. Aliquots of these samples underwent zymographic analysis with gelatin gels or with casein gels. This technique exploits the ability of MMP-1, MMP-2, and MMP-9 to degrade gelatin and the ability of activated MMP-3 to degrade casein. Gelatin (0.1%) or β-casein (0.2%) was mixed into liquid acrylamide before casting polyacrylamide gels. After sodium dodecyl sulfate–polyacrylamide gel electrophoresis was completed, the gels were twice washed for 30 minutes at room temperature with 2.5% Triton X-100 to remove the sodium dodecyl sulfate and were incubated 8 to 12 hours at 37°C in 150 nM NaCl, 50 mM Tris (pH 8), 10 mM $CaCl_2$, and 1 μM $ZnCl_2$. After staining with Coomassie blue, digestion of the substrate (gelatin or casein) at the position of the enzymes was observed as clear areas in the otherwise uniformly dark-staining gel. The size of these clear areas, which is proportional to MMP activity,[29] was directly measured using a scanning laser densitometer (Ultrascan II Laser Scanning Densitometer, Pharmacia, Bromma, Sweden). Each lane was analyzed by scanning along five parallel stripes, 800 μM apart, with the middle stripe positioned over the center of the lane. The results of these scans were averaged to yield a mean plot of optical density versus displacement. From these plots, the area under each peak was determined by integration to yield a single densitometry score for each band.

Western Blot Analysis

Medium samples were concentrated, using Centricon-30 ultrafiltration units, and were adjusted to a final concentration ratio of 10:1. Samples and prestained molecular weight standards containing myosin H-chain, phosphorylase *b*, bovine serum albumin (BSA), ovalbumin, carbonic anhydrase, β-lactoglobulin, and lysozyme (Calbiochem, San Diego, CA) were separated by electrophoresis on 7.5% or 12% polyacrylamide gels and transferred to a nitrocellulose membrane by Western blot. Rabbit polyclonal antibodies RP2C1, RP3G72, RP2S1, and RP1G92 to human MMP-1, MMP-2, MMP-3, and MMP-9, respectively, were a gift from Dr. J. P. Alexander (Casey Eye Institute, Oregon Health Science University, Portland). They were raised against unique peptide segments or purified MMPs, and specificity was confirmed by Western blot analysis of tissue homogenates.[30,31] The membrane was blocked by incubation with 3% bovine serum albumin–phosphate-buffered saline (PBS), incubated with primary antibody diluted at 1:1000 for 1 hour at 37°C, washed in 0.01% Tween–PBS, incubated with biotinylated antirabbit immunoglobulin (Ig) G antibody (Sigma Chemical Co.), diluted at 1:500 for 1 hour at 37°C, washed, incubated with peroxidase-conjugated avidin (Sigma Chemical Co.) for 15 minutes, washed, and developed with diaminobenzidine (Sigma Chemical Co.). The molecular weights of the immunostained bands were estimated by comparing their migration with the migration of the standards.

Immunocytochemical Analysis

Antibodies used for immunocytochemical analysis were mouse monoclonal antihuman calponin (hCP, diluted 1:1000; Sigma Chemical Co.), and mouse monoclonal antihuman α-smooth muscle actin (1A4, diluted 1:100; Sigma Chemical Co.). The specificities of these antibodies were characterized by Western blot analysis and immunoprecipitation.[32,33] Rhodamine-conjugated goat antimouse IgG (diluted 1:400; Sigma Chemical Co.) was preabsorbed with homogenized human uvea to reduce nonspecific binding and used as secondary antibody. The cultures were rinsed three times with PBS, fixed with 4% formaldehyde in PBS, washed, permeated with 0.1% Triton X-100 in PBS for 15 minutes, washed, blocked with 10% goat serum in PBS, washed again with PBS, incubated with primary antibodies for 60 minutes in a moist chamber, washed, exposed to secondary antibodies for 30 minutes in the dark, washed, and mounted with gelvatol. Controls for nonspecific secondary antibody binding were incubated with PBS instead of with primary antibodies.

RESULTS

Cell Culture

Cultured ciliary muscle cells formed confluent monolayers of spindle-shaped cells, organized into a characteristic "hill-and-valley" distribution—that is, areas of higher and lower cell density (Fig. 1) typical of ciliary smooth muscle cells in vitro.[14,34] Immunocytochemical evaluation with antibodies to calponin and α-smooth muscle actin produced positive staining in more than 95% and 90% of the cells, respectively (not shown). These results indicate high purity and differentiation of the ciliary smooth muscle cells within these cultures.[23]

Zymographic Analysis

Three major bands appeared in the gelatin zymographs at positions corresponding to apparent molecular weights of 62 kDa, 68 kDa, and 95 kDa (Fig. 2, lane 1). A faint band was observed at 50 kDa, which was more intense in the casein zymograms (Fig. 2, lane 3). Hence, in the quantitative studies of PG-mediated

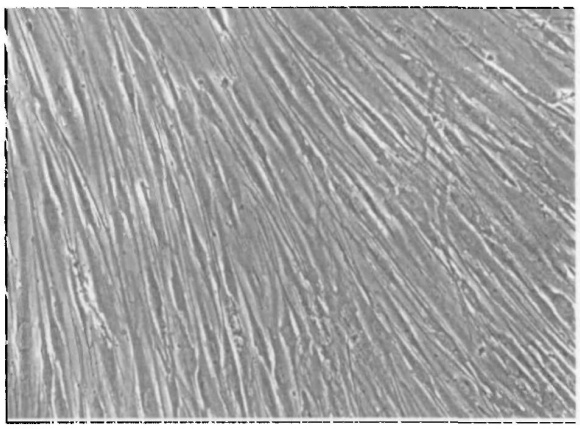

FIGURE 1. Phase image of third-passage human ciliary smooth muscle cells cultured from a 59-year-old eye. The cells formed a monolayer of aligned spindle-shaped cells that grew in a characteristic "hill-and-valley" pattern—that is, with condensed regions and sparse regions. Unstained; magnification, ×200.

MMP induction described later, this band was studied using casein gels. Substitution of EDTA for calcium and zinc in the development solution inhibited the appearance of these bands (Fig. 2, lanes 2 and 4), indicating that they reflected MMP activity.

Western Blot Analysis

Immunoblots for the analysis of MMP-1, MMP-2, MMP-3, and MMP-9 were prepared by electrophoresis of medium samples in 7.5% polyacrylamide gels and electrotransfer to nitrocellulose membranes. Incubation of blots with antibody RP2C1 (MMP-1) yielded a single, sharp band with an apparent molecular weight of 62 kDa (Fig. 3, lane 2). Similarly, incubations with antibodies RP3G72 (MMP-2), RP2S1 (MMP-3), or RP1G92 (MMP-9) yielded single sharp bands with apparent molecular weights of 68 kDa (lane 3), 50 kDa (lane 4), and 95 kDa (lane 5), respectively. These results confirmed that the three major bands in the gelatin zymographs were MMP-1, MMP-2, and MMP-9 and that the predominant band in the casein zymographs was MMP-3.

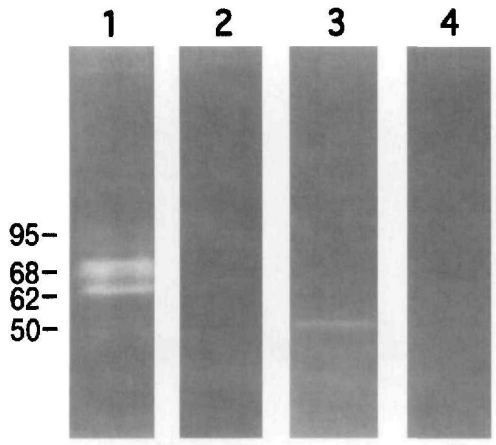

FIGURE 2. Zymograms of culture medium from confluent human ciliary smooth muscle cells maintained in chemically defined medium. Gelatin substrate zymograms (lanes 1 and 2) and casein substrate zymograms (lanes 3 and 4) were developed in the presence of calcium and zinc (lanes 1 and 3) or with divalent cations removed and EDTA added (lanes 2 and 4). Bands in the gelatin zymogram were observed at 50 kDa, 62 kDa, 68 kDa, and 95 kDa (*left*). The 50-kDa band was stronger in the casein zymogram (lane 3).

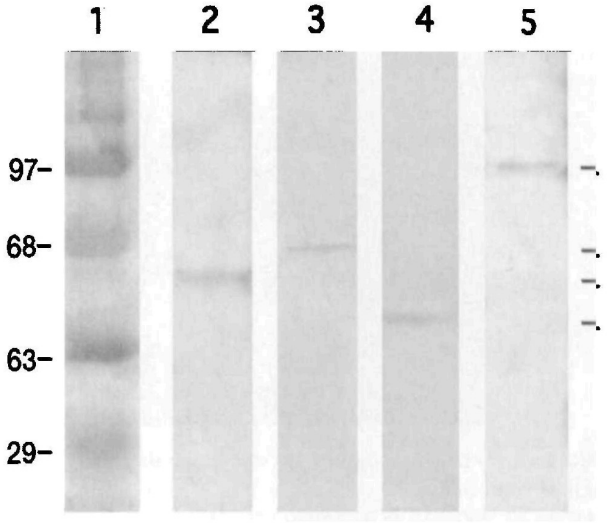

FIGURE 3. Western blots of culture medium from human ciliary smooth muscle cells probed with antibodies recognizing MMP-1 (lane 2), MMP-2 (lane 3), MMP-9 (lane 4), or MMP-3 (lane 5). Single, sharp bands were seen at 62 kDa, 68 kDa, 50 kDa, and 95 kDa, respectively (*right*). Dye-coupled molecular weight standards (lane 1) include carbonic anhydrase (29 kDa), ovalbumin (43 kDa), bovine serum albumin (68 kDa), and phosphorylase *b* (97 kDa; *left*). MMP = matrix metalloproteinase.

Prostaglandin's Influence on Matrix Metalloproteinase Production

Gelatin and casein zymographs of media samples from cultures treated with 200 nM $PGF_{2\alpha}$, 11-deoxy-PGE_1, or PhXA85 for 72 hours showed marked increases in the size and intensity of all bands (Figs. 4 and 5). Because of these increases, the nearby bands at 62 and 68 kDa often overlapped, and they could not be distinguished clearly. Hence, these two bands were analyzed together to yield a single score. As seen in Figure 4, PG treatment increased the size of the 62-kDa and the 68-kDa bands. Exposure of cultures to 200 nM $PGF_{2\alpha}$, 11-deoxy-PGE_1, or PhXA85 for 12, 24, or 72 hours induced increasing amounts of MMP-1/2, MMP-9, and MMP-3 (Fig. 6). In the cultures treated for 72 hours, the mean score increases compared with vehicle control scores were 37%, 64%, and 27% for MMP-1/2; 268%, 253%, and 125% for MMP-9; and

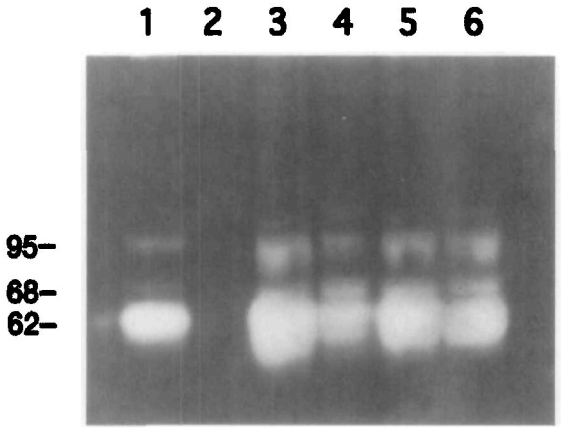

FIGURE 4. Gelatin zymogram of culture media from human ciliary smooth muscle cells exposed to vehicle control (lane 1), or to 200 nM $PGF_{2\alpha}$ (lane 3), 17-phenyltrinor $PGF_{2\alpha}$ (lane 4), 11-deoxy-PGE_1 (lane 5), or PhXA85 (lane 6). Lane 2 was empty. Strong bands were observed at 62 kDa, 68 kDa, and 95 kDa (*left*). Note increased band sizes in lanes containing medium from PG-treated cultures. PG = prostaglandin.

35%, 71% and 22% for MMP-3, respectively. Increased densitometric scores were sometimes seen after treatment with 20 nM PGs; however, the magnitude of these increases was considerably lower (not shown).

DISCUSSION

These results indicate that human ciliary smooth muscle cells biosynthesize MMP-2 and MMP-9, as well as MMP-1 and MMP-3. In addition, exposure of the cells to various PGs induces an increase in the amounts of

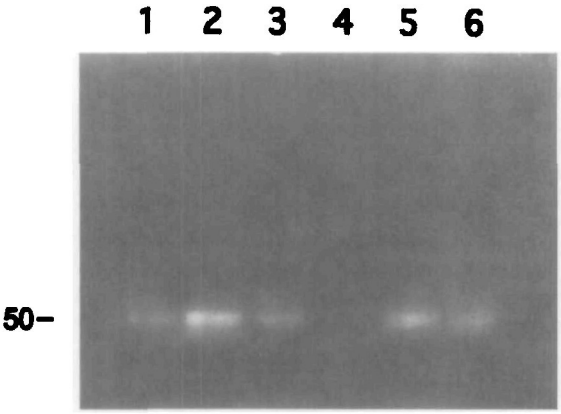

FIGURE 5. Casein zymogram of culture media from human ciliary smooth muscle cells exposed to vehicle control (lane 1), or to 200 nM $PGF_{2\alpha}$ (lane 2), 17-phenyltrinor $PGF_{2\alpha}$ (lane 3), 11-deoxy-PGE_1 (lane 5), or PhXA85 (lane 6). Lane 4 was empty. The predominant band migrated to at an apparent molecular weight of 50 kDa (*left*) and was increased in size in lanes containing medium from PG-treated cultures. PG = prostaglandin.

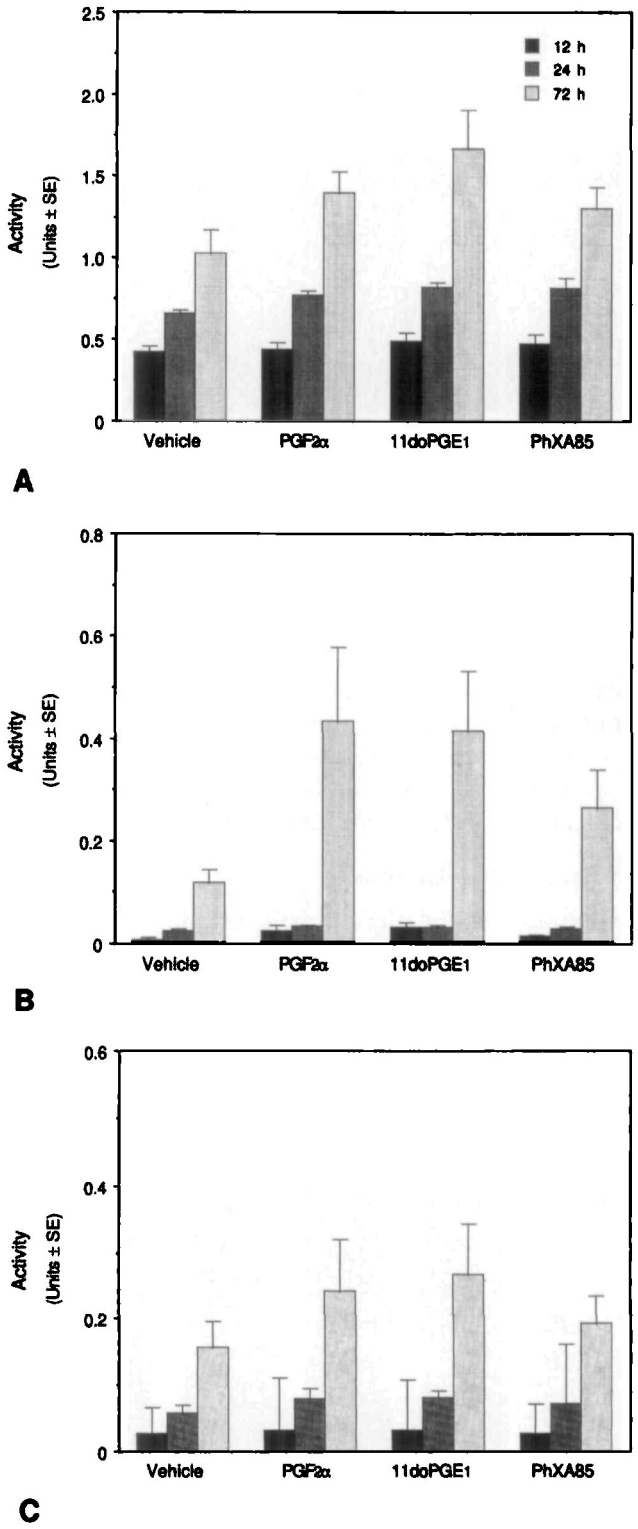

FIGURE 6. Increased matrix metalloproteinase activity scores from zymograms of ciliary smooth muscle cultures incubated with 200 nM $PGF_{2\alpha}$, 11-deoxy-PGE_1, PhXA85, or vehicle control for 12, 24, or 72 hours. Because of overlap, the bands at 62 kDa and 68 kDa in the gelatin zymographs were analyzed together (**A**; $n = 5$ cultures). Also analyzed were the 95-kDa band in the gelatin zymographs (**B**; $n = 5$) and the 50-kDa band in the casein zymographs (**C**; $n = 6$). PG = prostaglandin.

each of the MMPs. These results are consistent with the increased pro-MMP-1 and pro-MMP-3 in PG-treated ciliary smooth muscle culture media observed by enzyme-linked immunosorbent assay.[10]

Coordinate production of the MMP-1, MMP-2, MMP-3, and MMP-9 observed in the current study is not surprising in that each has an AP-1 regulatory element within their promoters.[35,36] This regulatory element promotes transcription when it is bound by a heterodimeric regulatory complex containing *Fos* and *Jun* family proteins.[36,37] Treatment of ciliary smooth muscle cells with $PGF_{2\alpha}$ induces c-*fos* expression.[28] Together, these results support the view that $PGF_{2\alpha}$ induction of MMPs is mediated by AP-1 activation. The quantitatively similar results of 17PT-$PGF_{2\alpha}$ and PhXA85 may reflect that both are specific FP receptor agonists. The induction of MMPs by 11-deoxy PGE_1 suggests that other regulatory elements also may regulate MMP induction in ciliary muscle cells, in that this agonist does not bind to FP receptors but appears to have high affinity for EP_3 receptors as well as moderate affinity for EP_2 receptors.[38,39] Results of studies in other cell types indicate that agonist-mediated activation of these various PG receptors induces different effects on G-protein activation, adenylyl cyclase activity, and intracellular calcium changes.[40,41] Thus, multiple signaling pathways may mediate alteration of MMP expression stimulated by PGs in human ciliary muscle cells.

After secretion, stepwise activation of pro-MMPs can occur by proteolytic truncation.[8,9] Considering its apparent molecular weight in the current study, it appears that MMP-2 is activated within the ciliary muscle cell cultures. Results of prior studies have shown that MMP-2 from human synovial fibroblasts is converted from an inactive state, with apparent molecular weight of 72 kDa, to an active state, with apparent molecular weight of 68 kDa.[42] Similar molecular weight changes were seen with activation of MMP-2 from other cell types.[43,44] Hence, the 68-kDa MMP-2-immunoreactive band observed in the current experiments probably was the activated form of MMP-2. Because MMP-2 can degrade collagen types I and IV, the altered amount and organization of these ECM macromolecules observed in ciliary muscle cultures exposed to PGs is consistent with increased MMP-2 activity.[45,46] Likewise, the apparent molecular weight of the MMP-3 immunoreactive band observed in the current study may reflect its activation status. In previous studies, it has been reported that the apparent weight of pro-MMP-3 is 57 to 60 kDa, and that fully activated MMP-3 has an apparent molecular weight of 41 to 45 kDa.[42,47] Intermediate sizes of MMP-3 are associated with partial activation.[42,48] Hence, the MMP-3 immunoreactive band at the 50 kDa observed in the current study probably is partially activated MMP-3. Because MMP truncation can be mediated by active MMP-3, further studies will be needed to confirm the activation status of MMP-3 in this experimental system.

In contrast to MMP-2 and MMP-3, the observed apparent molecular weights of MMP-1 and MMP-9 suggest that they were not activated. The sizes of pro-MMP-1 and active MMP-1 from human synovial fibroblasts have been reported as 52 to 56 kDa, depending on glycosylation, and 41 to 45 kDa, respectively.[42] We found an MMP-1 immunoreactive protein with an apparent molecular weight of 62 kDa. This is consistent with a 62-kDa MMP-1 immunoreactive band previously found in media from cultured human trabecular meshwork cells.[30] In view of these observations, it is possible that the 62-kDa band observed in the current study is pro-MMp-1. Likewise, in previous studies, it has been reported that the apparent sizes of the precursor and active forms of MMP-9 released by various human cell types range from 92 to 95 kDa and 82 to 84 kDa, respectively.[18,30,49,50] Thus, in contrast to MMP-2 and MMP-3, the observed sizes for MMP-1 and MMP-9 suggest that these MMPs are largely present as precursor pro-MMPs. Further studies will be needed to confirm these points.

An important concern is the relevance of the observed MMP response to what may be happening within the ciliary muscle in situ after topical PG treatment. Previously, 78 nM $PGF_{2\alpha}$ (28 ng/ml) was measured in aqueous humor collected from monkey eyes after topical treatment with an intraocular-pressure-lowering dose of $PGF_{2\alpha}$.[26] Recently, it has been found that the peak concentration of latanoprost-free acid in aqueous humor samples obtained from human eyes that had received one dose of topical latanoprost before undergoing routine cataract surgery was 100 nM (J. Stjernschantz, personal communication, 1997). In the current study, clear evidence of MMP induction was seen with 200 nM PG treatment. At lower concentrations, MMP induction was unclear because of the sensitivity of the assay. However, significant increases of pro-MMP-1 and pro-MMP-3 secretion were observed by enzyme immunoassay after 10-nM $PGF_{2\alpha}$ treatment.[10] This is consistent with the significant increase in c-*fos* induction after exposure to 10 nM $PGF_{2\alpha}$.[28] Thus, it is reasonable to expect that MMP induction occurs after treatment with an intraocular-pressure-lowering dose of $PGF_{2\alpha}$ or of a clinically relevant dose of latanoprost.

Several factors may contribute to the modulation of pro-MMP activation in vivo. First, urokinase plasminogen activator, which can activate isolated pro-MMPs,[42] has been identified in aqueous humor.[51–53] It is unknown whether PG treatment can alter the concentration or activity of this enzyme. Second, MMP activity can be blocked by the permanent binding of tissue inhibitors of metalloproteinases (TIMPs)[19,54];

TIMP-1 is secreted into the medium of trabecular meshwork cells in vitro:[30] Although aqueous humor passing through the trabecular meshwork generally exits the eye through Schlemm's canal, it is possible that some of the TIMP released by the trabecular meshwork near the iris root may gain access to aqueous humor entering the uveoscleral outflow path.[55] Thus, it will be important to evaluate as well the influence of PGs on MMP inactivation by TIMPs. Human aqueous humor collected from patients undergoing elective surgery for senile cataract appears to contain abundant pro-MMP-2 as well as smaller amounts of active MMP-2, MMP-9, and TIMPs.[52]

The current results suggest that exposure to PGs induces the amount of at least four different MMPs by ciliary smooth muscle cells. If this occurs in situ, these MMPs would be well positioned to degrade adjacent ECM and to reduce the hydraulic resistance around the ciliary muscle fibers to facilitate uveoscleral flow. Ciliary muscle release of MMPs also may be important in normal intraocular pressure regulation, in that PGs are produced by cultured human trabecular meshwork cells,[56,57] by intact trabecular meshwork tissue,[58] and by perfused human anterior segments in vitro.[59]

Key Words

glaucoma, human ciliary muscle, matrix metalloproteinases, prostaglandins, uveoscleral outflow

References

1. Camras C, Alm A, Watson P, Stjernschantz J, and the Latanoprost Study Groups. Latanoprost, a prostaglandin analog, for glaucoma therapy. Efficiency and safety after 1 year treatment in 198 patients. *Ophthalmology.* 1996;103:1916–1924.
2. Bito LZ, Stjernschantz J, Resul B, Miranda OC, Basu S. The ocular effects of prostaglandins and the therapeutic potential of a new $PGF_{2\alpha}$ analog, PhXA41 (latanoprost), for glaucoma management. *J Lipid Mediat.* 1993;6:535–543.
3. Gabelt BT, Kaufman PL. Prostaglandin $F_{2\alpha}$ increases uveoscleral outflow in the cynomolgus monkey. *Exp Eye Res.* 1989;49:389–402.
4. Crawford K, Kaufman PL. Pilocarpine antagonizes prostaglandin $F_{2\alpha}$-induced ocular hypotension in monkeys. Evidence for enhancement of uveoscleral outflow by prostaglandin $F_{2\alpha}$. *Arch Ophthalmol.* 1987;105:1112–1116.
5. Nilsson SF, Samuelsson M, Bill A, Stjernschantz J. Increased uveoscleral outflow as a possible mechanism of ocular hypotension caused by prostaglandin $F_{2\alpha}$-1-isopropylester in the cynomolgus monkey. *Exp Eye Res.* 1989;48:707–716.
6. Toris CB, Camras CB, Yablonski ME. Effects of PhXA41, a new prostaglandin $F_{2\alpha}$ analog, on aqueous humor dynamics in human eyes. *Ophthalmology.* 1993;100:1297–1304.
7. Stjernschantz J, Selén G, Sjöquist B, Resul B. Preclinical pharmacology of latanoprost, a phenyl-substituted $PGF_{2\alpha}$ analog. *Adv Prostaglandin Thromboxane Leukot Res.* 1995;23:513–518.
8. Birkedal-Hansen H, Moore WG, Bodden MK, et al. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med.* 1993;4:197–250.
9. Woessner J Jr. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J.* 1991;5:2145–2154.
10. Lindsey JD, Kashiwagi K, Boyle D, et al. Prostaglandins increase pro-MMP-1 and pro-MMP-3 secretion by human ciliary smooth muscle cells. *Curr Eye Res.* 1996; 15:869–875.
11. Bill A. Uveoscleral drainage of aqueous humor: Physiology and pharmacology. *Prog Clin Biol Res.* 1989; 312:417–427.
12. Wood RL, Koseki T, Kelly DE. Uveoscleral permeability to intracamerally infused ferritin in eyes of rabbits and monkeys. *Cell Tissue Res.* 1992;270:559–567.
13. Weinreb RN, Lindsey J, Luo XX, Wang T-H. Extracellular matrix of the human ciliary muscle. *J Glaucoma.* 1994;3:70–78.
14. Weinreb RN, Kim DM, Lindsey JD. Propagation of ciliary smooth muscle cells in vitro and effects of prostaglandin $F_{2\alpha}$ on calcium efflux. *Invest Ophthalmol Vis Sci.* 1992;33:2679–2686.
15. Tamm E, Lutjen-Drecoll E, Rohen JW. Age-related changes of the ciliary muscle in comparison with changes induced by treatment with prostaglandin $F_{2\alpha}$. An ultrastructural study in rhesus and cynomolgus monkeys. *Mech Ageing Dev.* 1990;51:101–120.
16. Marshall GE, Konstas AG, Abraham S, Lee WR. Extracellular matrix in aged human ciliary body: An immunoelectron microscope study. *Invest Ophthalmol Vis Sci.* 1992;33:2546–2560.
17. Lindsey J, Kashiwagi K, Kashiwagi F, Weinreb R. Prostaglandin action on ciliary smooth muscle extracellular matrix metabolism: Implications for uveoscleral outflow. *Surv Ophthalmol.* 1997;42(Suppl):S53–S59.
18. Galis ZS, Muszynski M, Sukhova GK, et al. Cytokine-stimulated human vascular smooth muscle cells synthesize a complement of enzymes required for extracellular matrix digestion. *Circ Res.* 1994;75:181–189.
19. Galis ZS, Muszynski M, Sukhova GK, Simon-Morrissey E, Libby P. Enhanced expression of vascular matrix metalloproteinases induced in vitro by cytokines and in regions of human atherosclerotic lesions. *Ann N Y Acad Sci.* 1995;748:501–507.
20. Fields G, Van Wart H, Birkedal-Hansen H. Sequence specificity of human skin fibroblast collagenase. Evidence for the role of collagen structure in determining the collagenase cleavage site. *J Biol Chem.* 1987;262:6221–6226.
21. Fields G. A model for interstitial collagen catabolism by mammalian collagenases. *J Theor Biol.* 1991; 153:585–602.
22. Matrisian LM. The matrix-degrading metalloproteinases. *Bioessays.* 1992;14:455–463.
23. Kashiwagi K, Lindsey JD, Kashiwagi F, Tsukahara S, Weinreb RN. Calponin distribution in human ciliary

muscle and other anterior segment tissues. *Invest Ophthalmol Vis Sci.* 1997;38:349–356.
24. Tamm E, Baur A, Lutjen–Drecoll E. Synthesis of extracellular matrix components by human ciliary muscle cells in culture. *Curr Eye Res.* 1992;11:333–341.
25. Basu S, Sjoquist B, Stjernschantz J, Resul B. Corneal permeability to and ocular metabolism of phenyl substituted prostaglandin esters in vitro. *Prostaglandins Leukot Essent Fatty Acids.* 1994;50:161–168.
26. Camras CB, Bhuyan KC, Podos SM, Bhuyan DK, Master RW. Multiple dosing of prostaglandin $F_{2\alpha}$ or epinephrine on cynomolgus monkey eyes. II Slit lamp biomicroscopy, aqueous humor analysis, and fluorescein angiography. *Invest Ophthalmol Vis Sci.* 1987; 28:921–926.
27. Werb Z, Tremble PM, Behrendtsen O, Crowley E, Damsky CH. Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. *J Cell Biol.* 1989;109:877–889.
28. Lindsey JD, To HD, Weinreb RN. Induction of c-*fos* by prostaglandin $F_{2\alpha}$ in human ciliary smooth muscle cells. *Invest Ophthalmol Vis Sci.* 1994;35:242–250.
29. Kleiner D, Stetler–Stevenson W. Quantitative zymography: detection of picogram quantities of gelatinases. *Anal Biochem.* 1994;218:325–329.
30. Alexander JP, Samples JR, Van Buskirk EM, Acott TS. Expression of matrix metalloproteinases and inhibitor by human trabecular meshwork. *Invest Ophthalmol Vis Sci.* 1991;32:172–180.
31. Alexander JP, Bradley JM, Gabourel JD, Acott TS. Expression of matrix metalloproteinases and inhibitor by human retinal pigment epithelium. *Invest Ophthalmol Vis Sci.* 1990;31:2520–2528.
32. Frid MG, Shekhonin BV, Koteliansky VE, Glukhova MA. Phenotypic changes of human smooth muscle cells during development: Late expression of heavy caldesmon and calponin. *Dev Biol.* 1992;153:185–193.
33. Skalli O, Ropraz P, Trzeciak A, et al. A monoclonal antibody against alpha-smooth muscle actin: A new probe for smooth muscle differentiation. *J Cell Biol.* 1986;103:2787–2796.
34. Tamm E, Flugel C, Baur A, Lutjen–Drecoll E. Cell cultures of human ciliary muscle: Growth, ultrastructural and immunocytochemical characteristics. *Exp Eye Res.* 1991;53:375–387.
35. Jiang W, Bond JS. Families of metalloendopeptidases and their relationships. *FEBS Lett.* 1992;312:110–114.
36. Karin M, Yang–Yen HF, Chambard JC, Deng T, Saatcioglu F. Various modes of gene regulation by nuclear receptors for steroid and thyroid hormones. *Eur J Clin Pharmacol.* 1993;45:S9–S15.
37. Curran T, Franza B Jr. Fos and jun: the AP-1 connection. *Cell.* 1988;55:395–397.
38. Woodward DF, Lawrence RA, Fairbairn CE, Shan T, Williams LS. Intraocular pressure effects of selective prostanoid receptor agonists involve different receptor subtypes according to radioligand binding studies. *J Lipid Mediat.* 1993;6:545–553.
39. Woodward D, Fairbairn C, Krauss H–P, Lawrence R, Protzman C. Radioligand binding analysis of receptor subtypes in two FP receptor preparations that exhibit different functional rank orders of potency in response to prostaglandins. *J Pharmacol Exp Ther.* 1995;273:285–291.
40. Regan JW, Bailey TJ, Pepperl DJ, et al. Cloning of a novel human prostaglandin receptor with characteristics of the pharmacologically defined EP_2 subtype. *Mol Pharmacol.* 1994;46:213–220.
41. Negishi M, Sugimoto Y, Ichikawa A. Molecular mechanisms of diverse actions of prostanoid receptors. *Biochim Biophys Acta.* 1995;1259:109–119.
42. Nagase H, Suzuki K, Morodomi T, Enghild JJ, Salvesen G. Activation mechanisms of the precursors of matrix metalloproteinases 1, 2, and 3. *Matrix Suppl.* 1992; 1:237–244.
43. Goldberg GI, Marmer BL, Grant GA, et al. Human 72-kilodalton type IV collagenase forms a complex with a tissue inhibitor of metalloproteases designated TIMP-2. *Proc Natl Acad Sci U S A.* 1989;86:8207–8211.
44. Yager DR, Zhang LY, Liang HX, Diegelmann RF, Cohen IK. Wound fluids from human pressure ulcers contain elevated matrix metalloproteinase levels and activity compared to surgical wound fluids. *J Invest Dermatol.* 1996;107:743–748.
45. Lindsey J, Kashiwagi K, Kashiwagi F, Weinreb R. Prostaglandins alter extracellular matrix adjacent to human ciliary muscle cells in vitro. *Invest Ophthalmol Vis Sci.* 1997;38:2214–2223.
46. Woessner J Jr. Literature on vertebrate matrix metalloproteinases and their tissue inhibitors. *Matrix Suppl.* 1992;1:425–501.
47. Wilhelm SM, Collier IE, Kronberger A, et al. Human skin fibroblast stromelysin: Structure, glycosylation, substrate specificity, and differential expression in normal and tumorigenic cells. *Proc Natl Acad Sci U S A.* 1987;84:6725–6729.
48. Nagase H, Suzuki K, Enghild JJ, Salvesen G. Stepwise activation mechanisms of the precursors of matrix metalloproteinases 1 (tissue collagenase) and 3 (stromelysin). *Biomed Biochim Acta.* 1991;50:749–754.
49. Shapiro SD, Fliszar CJ, Broekelmann TJ, et al. Activation of the 92-kDa gelatinase by stromelysin and 4-aminophenylmercuric acetate. Differential processing and stabilization of the carboxyl-terminal domain by tissue inhibitor of metalloproteinases (TIMP). *J Biol Chem.* 1995;270:6351–6356.
50. Nagase H, Fields GB. Human matrix metalloproteinase specificity studies using collagen sequence-based synthetic peptides. *Biopolymers.* 1996;40:399–416.
51. Bernatchez SF, Tabatabay C, Belin D. Urokinase-type plasminogen activator in human aqueous humor. *Invest Ophthalmol Vis Sci.* 1992;33:2687–2692.
52. Ando H, Twining SS, Yue BY, et al. MMPs and proteinase inhibitors in the human aqueous humor. *Invest Ophthalmol Vis Sci.* 1993;34:3541–3548.
53. Smalley DM, Fitzgerald JE, Taylor DM, Cone RE, O'Rourke J Tissue plasminogen activator activity in human aqueous humor. *Invest Ophthalmol Vis Sci.* 1994;35:48–53.
54. Denhardt DT, Feng B, Edwards DR, Cocuzzi ET, Malyankar UM. Tissue inhibitor of metalloproteinases (TIMP,

aka EPA): structure, control of expression and biological functions. *Pharmacol Ther.* 1993;59:329–341.
55. Barsotti M, Bartels S, Freddo T, Kamm R. The source of protein in the aqueous humor of the normal monkey eye. *Invest Ophthalmol Vis Sci.* 1992;33:581–595.
56. Weinreb RN, Polansky JR, Alvarado JA, Mitchell MD. Arachidonic acid metabolism in human trabecular meshwork cells. *Invest Ophthalmol Vis Sci.* 1988;29:1708–1712.
57. Weinreb RN, Mitchell MD. Prostaglandin production by cultured cynomolgus monkey trabecular meshwork cells. *Prostaglandins Leukot Essent Fatty Acids.* 1989; 36:97–100.
58. Polansky JR, Weinreb RN. Anterior segment inflammation and trabecular meshwork cell response. *New Trends Lipid Mediators Res.* 1990;5:56–64.
59. Shaw BR, Lindsey JD, Weinreb RN. Prostaglandin release in a perfused human anterior segment model. *J Glaucoma.* 1993;2:39–43.