Increased Human Scleral Permeability with Prostaglandin Exposure

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PURPOSE. To investigate the effect of prostaglandins (PGs) on the permeability of human sclera in vitro.

METHODS. Twenty-three pairs of human eye bank eyes were studied. Circular pieces of sclera were cultured in low-serum DMEM/F-12 media. Scleral hydration was assessed by measuring wet and dry weight of scleral cultures incubated with medium for 3 days and with Hanks’ buffered saline solution (HBSS) for 4 hours. To assess scleral permeability, organ-cultured scleral tissues were exposed to 100 to 500 nM PGF2\(\alpha\) (HBSS) for 4 hours. Compared to tracer movement across untreated scleral cultures (1.5 \(\times\) 10\(^{-6}\) cm/sec for 10 kDa dextran, 0.7 \(\times\) 10\(^{-6}\) cm/sec for 40 kDa dextran, and 0.4 \(\times\) 10\(^{-6}\) cm/sec for 70 kDa dextran), exposure to PGF\(_{2\alpha}\), 17-phenyltrinor PGF\(_{2\alpha}\), or PhXA85 each increased scleral permeability in a dose- and time-dependent manner. Increases in permeability were greater with the 10 kDa dextran than with the 40 or 70 kDa dextran. The magnitude of these effects was greatest with exposure to PhXA85 and similar with exposure to PGF\(_{2\alpha}\) or 17-phenyltrinor-PGF\(_{2\alpha}\). MMP expression also was significantly increased after PG exposure. These increases were generally time and dose dependent and greater with MMP-2 and -3 than with MMP-1.

CONCLUSIONS. There is increased permeability of human sclera exposed to various PGs in organ culture. This increased permeability is accompanied by increased expression of MMPs. (Invest Ophthalmol Vis Sci. 2001;42:1514–1521)

Considerable evidence has accumulated that uveoscleral outflow may contribute more to total outflow than previously appreciated, particularly in healthy young monkey and human eyes.\(^1\)–\(^4\) A poorly understood component of uveoscleral outflow is transscleral fluid movement at the distal portion of the uveoscleral outflow pathway. The observation that 125I-labeled albumin injected into the anterior chamber later appeared within orbital fat tissue is consistent with the sclera being an integral part of the uveoscleral outflow pathway.\(^5\)–\(^6\) Histologic analysis of sclera after injection of various tracers into the anterior chamber indicated the presence of transscleral fluid flow through the scleral stroma as well as through narrow spaces around penetrating nerves and blood vessels.\(^7\)–\(^10\) Fluid flux through the scleral stroma is supported by measurements of transscleral permeability across the superior temporal human sclera, a region devoid of perforating blood vessels or nerves.\(^10\) Physicochemical evaluations of labeled hydrocortisone movement across sclera provided further support for the existence of transscleral fluid movement through the scleral stroma.\(^11\) However, there is little information regarding how endogenous signals regulate this transscleral fluid movement.

The possibility that various prostaglandins (PGs) could modulate transscleral fluid movement is suggested by several observations. First, topical treatment of monkey eyes with PGF\(_{2\alpha}\)-isopropyl ester for 5 days reduced collagen type I and collagen type III immunoreactivity within sclera by 43% and 45%, respectively.\(^12\) Second, scleral collagen is predominantly type I collagen and accounts for about one half of the total dry weight of sclera.\(^13\) Finally, evidence that compaction of extracellular matrix affects transscleral permeability suggests that collagen density within sclera is an important determinant of permeability.\(^2\) Hence, it is plausible that PG-mediated reduction of scleral collagens could significantly alter permeability.

Increased matrix metalloproteinases (MMPs), a family of secreted neutral proteinases that can initiate specific degradation of key extracellular matrix components, may be one mechanism by which scleral collagen is reduced after topical PG.\(^14\)–\(^15\) There is immunohistochemical evidence that MMP-1, which can initiate degradation of fibrillar collagens, such as collagen type I and collagen type III, is present in normal human sclera.\(^16\) In the sclera of monkey eyes that have received topical PGF\(_{2\alpha}\)-HE treatment, there is increased MMP-1, -2, and -3 immunoreactivity.\(^13\) It is unclear, however, whether this increased scleral MMP immunoreactivity in the sclera is a direct response, reflecting increased production within sclera, or is an indirect consequence of increased MMP release by ciliary muscle cells.

The present study was undertaken to investigate these questions by determining whether exposure of organ cultures of human sclera to various PGs increases scleral permeability and whether this is associated with increased release of MMPs.

METHODS

Human Sclera Organ Cultures

Twenty-three pairs of human eyes from donors 45 to 80 years old were obtained from the San Diego Eye Bank within 24 hours after death.
Enucleation was completed within 6 hours postmortem, and the eyes were stored in a moist chamber at 4°C for <24 hours before generation of the organ cultures. Donors had no known history of glaucoma or other eye disease. The eyes were placed in Dulbecco’s modified Eagle’s medium and Ham’s F12 nutrient mixture (DMEM-F12) medium containing 50 U/ml penicillin and 50 μg/ml streptomycin for 15 minutes. This was repeated twice before dissection. Tenon’s capsule and episclera were removed from the surface of the sclera using a sterile cotton-tip applicator. Curved scissors were used to excise circular pieces of sclera. The chosen areas were selected to avoid the perforating anterior ciliary vessels and the vortex veins. The uveal tissues and retina were gently removed from the vitreous side of the sclera using a cotton-tip applicator. The circular pieces of scleral tissue were placed into 12-well culture plates containing DMEM-F12 supplemented with 1% fetal bovine serum and 1 ng/ml recombinant human basic fibroblast growth factor. Because serum contains agents known to stimulate MMP biosynthesis, low serum concentration was used to minimize nonspecific induction of MMPs. The cultures were incubated at 37°C in a humidified atmosphere of 95% air-5% CO₂.

**Prostaglandin Treatments**

The culture medium was changed to fresh medium supplemented with PGF₁₂, 17-phenyltrinor-PGF₂α, PhXA85 (Cayman Chemical Co., Grand Rapids, MI), or vehicle control. 17-Phenyltrinor-PGF₂α, and PhXA85 bind with greater specificity to the FP receptor (the endogenous PG receptor) than preferentially recognizes F-type prostaglandins¹⁹,²⁰ than PGF₂α. Each of these PGs can increase uveoscleral outflow facility in monkeys as well as reduce collagen and increase MMPs in cultures of human ciliary smooth muscle cells.¹⁷,²¹ Each PG was tested at concentrations of 100, 200, and 500 nM. PG concentrations were chosen based on their receptor binding profiles as well as the observation that the peak concentration of PhXA85 observed in aqueous humor after topical application of a clinical dose of latanoprost to human eyes is approximately 100 nM.²¹,²² Exposure durations of 24, 48, and 72 hours were chosen based on previous experiments that found increased MMPs in ciliary smooth muscle cells exposed to PGs for 24 to 72 hours.²³ Exposure treatments were initiated by addition of the test PGs prepared from 10 mM stock solutions in ethanol and appropriately diluted with DMEM-12 nutrient mixture.

**Viability after Prolonged Exposure to Prostaglandins**

To assess viability in vitro, sclera organ cultures were incubated with 500 nM of each PG, the highest dose in this study, for 1, 2, or 3 days. Ethidium-homodimer was then added to the cultures to a final concentration of 1 μM, and the cultures were returned to the incubator for 30 minutes (Molecular Probes, Eugene, OR). Because this dye cannot penetrate living cells, it is only bound to the DNA of dead cells in the cultures. The sclera cultures were then rinsed with phosphate-buffered saline (PBS) without phenol red and then exposed to 2% parafformaldehyde in PBS for 10 minutes. The cultures were then permeabilized by passage through graded methanols (50%, 70%, 90%, 95%, and 100%), rehydrated, rinsed, in PBS, and then exposed to 5 mg/ml Sytox green for 15 minutes. This stain cannot pass through the plasma membrane of living cells but readily stains DNA within dead cells. The cultures were then rinsed twice with PBS. The cultures were then homogenized in PBS using a polytron homogenizer (P-10; Brinkmann, Westbury, NY). The homogenates were centrifuged, and the supernatants were collected.

Cell viability in these samples was determined by first measuring Sytox green fluorescence using a spectrofluorometer (model SFM 25; Kontron, Zürich, Switzerland) with the excitation and emission wavelengths set at 500 and 525 nm, respectively. The amount of ethidium homodimer was then measured using a 550 nm excitation wavelength. This wavelength excited ethidium homodimer at 85% of maximal efficiency but minimally excited Sytox green. The emission wavelength analyzed was 650 nm because it retained 71% of maximal efficiency for ethidium homodimer and eliminated >99% of the cross-talk signal coming from Sytox green. The photomultiplier voltage was optimized to 480 V to obtain all readings on one setting. The signals from the ethidium homodimer were normalized with signals from the Sytox green by dividing the ethidium homodimer results by the Sytox green results. Positive (live) controls were fresh cultures not exposed to any treatment, and negative (dead) controls were cultures first treated with 2% paraformaldehyde for 10 minutes and permeabilized with graded methanols before evaluation. The viability of each sample was determined by interpolation from a standard curve that was generated by plotting positive and negative control values.

**Scleral Hydration Analysis**

Thirty scleral specimens were obtained from human eye bank eyes for the determination of scleral hydration. These studies were performed to ensure that maintaining sclera in the Ussing perfusion system did not hyrate the sclera, which may alter scleral permeability. Ten circular scleral preparations from 3-day-old preparations were incubated in DMEM/F-12 media only or with media for 3 days followed by HBSS for additional 4 hours. The preparations were then weighed using an analytical balance (accuracy 0.0001 g; Mettler, Geissen, Germany), dried to constant weight at 100°C for 24 hours, placed immediately in a tissue desiccator to cool for 30 minutes, and reweighed. Another 20 circular scleral preparations from fresh and 3-day-old moist, chamber-stored globes perfused with HBSS and without perfusion were used to evaluate potential effects of storage. The level of hydration in each piece of sclera was calculated by following equation:

\[
\text{mg H}_2\text{O/mg dry tissue} = (\text{wt. dry} - \text{dry weight})/\text{dry weight}
\]

**Permeability Analysis**

After 1- to 3-day incubation with test PG or vehicle control, the scleral tissue was clamped into the in vitro perfusion apparatus. (Ussing apparatus, model CHM2; World Precision Instruments Inc., Sarasota, FL). The two chambers were manufactured from clear Lexan plastic. Each had a 9-mm-diameter opening and was used to sandwich a 14-mm-diameter piece of scleral tissue. This assembly was held together with a screw clamp. Each unstirred chamber contained 0.75 ml and could be filled, drained, and purged through three ports. Three rhodamine-dextran polymers (Molecular Probes; MW = 10,000, 40,000, and 70,000) were diluted in phenol red-free HBSS (250 μg/ ml). The “uveal-side” chamber was filled with phenol-free HBSS, and “orbital-side” chamber was filled with rhodamine-dextran diluted in phenol-free HBSS. Permeability was assessed in this direction because the orbital-side was smoother than the uveal-side and thus the potential for measurement-altering small leaks around the edge of the tissue piece was less. Solutions were prepared and warmed to 37°C before use. After assembly and filling, the system was placed in the 37°C incubator. The apparatus was checked after 30 minutes to verify that no leaks were present. Any leaks of the dextran solution were readily apparent because of the dark red color of the solution. Leaks of the phenol red-free Hanks’ from the uveal-side chamber were recognized by reduction of the level of the fluid visible through the clear walls of the chamber. Four hours later, a 750-μl sample was removed through a valved port connected to the “uveal-side” chamber and stored at −80°C. Samples were protected from light at all times before fluorescence measurement.

**Scleral Permeability Coefficient**

Diffusion from the “orbital” chamber to the “uveal” chamber was characterized by means of a permeability coefficient (Pₛ), which is the ratio of steady state flux (the mass of solute crossing a planar unit surface normal to the direction of transport per unit time) to the concentration gradient.¹⁰ In this study, the concentration of “uveal-side” chamber, Cᵥ, was <1% of the concentration in the “orbital” chamber, Cₒ, which did not change measurably over the course of the
C flux converted from hours to seconds. The term \( t \) represents the volume of the each chamber (0.75 ml); and \( t \) is the duration of steady state flux converted from hours to seconds. The term \( \frac{C_{u0.5} - C_{u2}}{C_{o}} \) is the permeation rate of dextran across each excised sclera (\( \mu g/h \)).

\[
P_{u} (cm/sec) = \frac{(C_{u0.5} - C_{u2})V}{AtC_{o}}
\]

where \( C_{o} \) and \( C_{u} \) are the concentration in the "uveal" chamber at 0.5 hour and at 1 hour, respectively; \( C_{u0.5} \) is the initial drug concentration (0.25 mg/ml); \( A \) is the surface area of exposed sclera (0.65 cm²); \( V \) is volume of each chamber (0.75 ml); and \( t \) is duration of steady state flux converted from hours to seconds. The term \( \frac{(C_{u0.5} - C_{u2})}{C_{o}} \) is the permeation rate of dextran across each excised sclera (\( \mu g/h \)).

The fluorescence of rhodamine-dextran was measured using a spectrofluorometer at room temperature. The excitation and emission wavelengths were 550 and 580 nm, respectively. Standard curves of fluorescence versus concentration were obtained by serial dilution of rhodamine-dextran dissolved in diffusion medium (phenol red-free HBSS).

**Enzyme Immunosorbent Assays**

At the conclusion of the 1- to 3-day incubations with PGs or vehicle, media samples were collected from the scleral cultures for ELISA analysis. Measurements of MMP-1, -2, and -3 concentration were performed with commercially available ELISA kits (Biotrak; Amersham Pharmacia Biotech Inc., Piscataway, NJ). These assays are based on a two-site ELISA ‘sandwich’ format and detected both latent and active MMPs. For the MMP-1 assay, purified MMP standards and samples were incubated in microtiter wells precoated with anti-MMP-1 antibody. The wells were then washed, incubated with second polyclonal antibody to MMP-1, washed, incubated with anti-rabbit horseradish peroxidase, washed, and developed by tetramethyl benzidine. After development at room temperature, the absorbency was measured at 650 nm using a microtiter plate reader (SpectraMax 250; Molecular Devices, Sunnyvale, CA). The procedures for the MMP-2 and -3 assays were the same except the antibodies were to MMP-2 and -3, respectively.

**Statistical Evaluation**

Experimental differences between control culture results and a single treatment group were evaluated using the Student’s \( t \)-test. When results from several treatment groups were compared with a single control, significance was evaluated using analysis of variance and the Student’s-Newman-Keuls test. \( P < 0.05 \) was considered as statistically significant.

**RESULTS**

**Scleral Hydration**

To evaluate whether changes in scleral hydration occur with the culture conditions, water content in the scleral cultures was determined in scleral cultures exposed to HBSS for 4 hours at room temperature, to complete culture medium for 3 days at 37°C, or to complete culture medium for 3 days followed by 4 hours in HBSS. The mean water content, or scleral hydration, of fresh sclera was 3.05 ± 0.11 mg water/mg dry weight (\( n = 5 \)). As shown in Figure 1, scleral cultures incubated 4 hours in HBSS alone, in medium for 3 days or in medium for 3-days followed by 4 hours in HBSS were insignificantly different from the fresh cultures. This indicated that these culture conditions did not alter hydration within the scleral cultures.

**Viability**

Survival of cells in the organ culture was assessed by measuring the exclusion of ethidium homodimer, a vital stain that binds to DNA. The standards for maximal viability was freshly obtained donor sclera and for complete loss of viability were donor sclera that had been exposed to 2% paraformaldehyde before ethidium homodimer exposure. As shown in Figure 2, viability for all cultures was approximately 83% on day 1, 81% on day 2, and 80% on day 3. Differences of viability among cultures exposed to 500 nM PGF\(_{2\alpha}\) or 17-phenyltrinor-PGF\(_{2\alpha}\) were <1% on all 3 days. This suggests that incubation with these PGs for 3 days had minimal influence on cell survival in the scleral cultures.

**Scleral Permeability**

Scleral permeability was measured by assessing the flux of dextran labeled dextrans across the scleral cultures in a Ussing chamber. Dextran standards of different sizes were evaluated to model the...
potential differences among aqueous proteins of different sizes. As shown in Figure 3, flux across the scleral cultures incubated without PGs was $1.5 \times 10^{-2} \text{ cm/sec}$ for 10 kDa dextran, $0.7 \times 10^{-2} \text{ cm/sec}$ for 40 kDa dextran, and $0.4 \times 10^{-2} \text{ cm/sec}$ for 70 kDa dextran. Moreover, these fluxes did not change among cultures incubated without PGs for 1, 2, or 3 days. In contrast, incubation with PGF$_2\alpha$ significantly increased the flux of 10 kDa tracer. These increases ranged from 21% to 124%, were dose dependent, became larger as exposure time increased up to 3 days, and were significant for all concentrations and tested time points. The flux of 40 kDa dextran also increased with increasing PGF$_2\alpha$ and exposure time; however, these increases ranged from 7% to 21%. These permeability increases were statistically significant only on day 5 in the case of 100 nM PGF$_2\alpha$ but were significant for 200 nM or 500 nM PGF$_2\alpha$ on days 1, 2, and 3. Similar to the 40 kDa dextran, the flux of 70 kDa dextran increased with PGF$_2\alpha$ dose and exposure time with increases ranging from 5% to 28%. These increases were significant at 100 nM on day 2, at 200 nM on days 2 and 3, and at 500 nM on all 3 days.

Incubation of scleral cultures with 17-phenyltrinor-PGF$_2\alpha$ also increased permeability of the scleral organ cultures to the labeled dextrans in dose- and time-dependent manners. Permeability increases of the 10 kDa tracer ranged from 5% to 183% (Fig. 4). These increases were significant for all conditions except for 100 nM 17-phenyltrinor-PGF$_2\alpha$ on day 1. Permeability increases of the 40 kDa tracer ranged from 4% to 31% and were significant at all concentrations tested on days 2 and 3. Permeability increases of the 70 kDa tracer ranged from 9% to 24% and were significant at all tested concentrations on all 3 days. Overall, the increases observed with 17-phenyltrinor-PGF$_2\alpha$ were similar to the increases observed with PGF$_2\alpha$. The exception to this was the larger permeability increase observed at 3 days with 100 nM 17-phenyltrinor-PGF$_2\alpha$ than with 100 nM PGF$_2\alpha$.

PhXA85 generally induced moderately larger increases in scleral permeability than either PGF$_2\alpha$ or 17-phenyltrinor-PGF$_2\alpha$ (Fig. 5). Like the effects of other tested compounds, these increases were dose and time dependent. Increases of 10 kDa tracer flux ranged from 45% to 213% and were significant for all concentrations tested on all 3 days. Increases of 40 kDa dextran flux ranged from 6% to 41% and were significant for all concentrations on all 3 days except at 100 nM on day 1. Increases of 70 kDa dextran flux ranged from 13% to 48% and were significant for all concentrations on all 3 days except 100 nM on day 1.

**FIGURE 3.** Scleral permeability after PGF$_2\alpha$ exposure. Permeability determined by the transscleral movement of 10- (A), 40- (B), or 70-kDa (C) dextrans across treated sclera. Data presented as mean ± SD ($\times 10^{-2}$ cm/sec). *$P < 0.05$ by Student’s-Newman-Keuls test.

**FIGURE 4.** Scleral permeability after 17-phenyltrinor-PGF$_2\alpha$ exposure. Permeability determined by the transscleral movement of 10- (A), 40- (B), or 70-kDa (C) dextrans across treated sclera. Data presented as mean ± SD ($\times 10^{-2}$ cm/sec). *$P < 0.05$ by Student’s-Newman-Keuls test.
MMP Release Induced by Prostaglandin Treatments

One possible explanation for the observed increases in scleral permeability after exposure to the PGs is reduction in collagen content by MMP-mediated degradation. Hence, the medium of scleral cultures incubated with PGF$_2\alpha$, 17-phenyltrinor-PGF$_2\alpha$, or PhXA85 were assayed for changes in the concentration of MMP-1, -2, and -3. Among cultures incubated in control medium for 1, 2, or 3 days, there were no significant change in the concentration of MMP-1, -2, or -3 (Figs. 6 through 8, respectively).

Evaluation of MMP-1 in the medium of the treated cultures showed moderate increases in cultures exposed to PGF$_2\alpha$, 17-phenyltrinor-PGF$_2\alpha$, or PhXA85 (Fig. 6). These increases ranged up to 37%, increased with time of exposure, and were significant only for the higher concentrations and longer incubation times examined. Overall, there were slight increases of MMP-1 with increasing dose, and the effects of the different PGs tested were similar.

In contrast to MMP-1, increases in MMP-2 were much larger and ranged from 124% to 267%. These increases were significant in every condition examined and showed marked increases with increasing time of exposure. Overall, there were slight increases of MMP-2 with increasing PG concentration. The magnitude of the effects was least with 17-phenyltrinor-PGF$_2\alpha$, intermediate with PGF$_2\alpha$, and greatest with PhXA85.

MMP-3 concentration also increased in the medium of cultures exposed to PGF$_2\alpha$, 17-phenyltrinor-PGF$_2\alpha$, or PhXA85. These increases ranged up to 96% and were larger than those seen with MMP-1 but smaller than those seen with MMP-2. These increases were clearly time dependent, being generally insignificant on day 1 and significant on days 2 and 3. Dose dependence was clearly present with PhXA85 at every time point and less clear with PGF$_2\alpha$ or 17-phenyltrinor-PGF$_2\alpha$.

**DISCUSSION**

The present results show that PGs can act directly on scleral tissue to increase transscleral permeability. Viability analysis indicates that this response is not associated with altered cell survival in the experimental system nor is there any evidence of toxicity due to the PG treatments. These permeability changes are likely to be normal physiological responses because they are both dose and time dependent. That the PG treatments also increased release of MMP-1, -2, and -3 in these cultures suggests that the permeability changes reflect direct

![Figure 5](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933588/)

**Figure 5.** Scleral permeability after PhXA85 exposure. Permeability determined by the transscleral movement of 10-, 40-, or 70-kDa dextrans across treated sclera. Data presented as mean ± SD (*×10^{-6} cm/sec*). *P < 0.05 by Student’s-Newman-Keuls test.

![Figure 6](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933588/)

**Figure 6.** Concentration of MMP-1 in the medium of scleral organ culture exposed to PhXA85 (A), PGF$_2\alpha$ (B), or 17-phenyltrinor-PGF$_2\alpha$ (C) for 1 to 3 days as determined by ELISA. Cultures analyzed were from cultures generated from a 76-year-old male donor, a 66-year-old male donor, and a 45-year-old female donor (*n* = 3). Data presented as mean ± SD. *P < 0.05 by Student’s-Newman-Keuls test.
response of scleral tissue to PG exposure and that the mechanism of increased transscleral permeability reflects intrascleral collagen remodeling.

The permeability relationships of the various sizes of labeled dextran observed in the present control scleral cultures is similar to the permeability relationships of these tracers observed in sclera freshly dissected from donor eyes. For example, the present study found that permeability of the 40 kDa dextran through the sclera organ cultures was 1.7-fold less than 10 kDa dextran. This is similar to the previous observation that in freshly dissected sclera, 40 kDa dextran permeability is 1.4- to 3.8-fold less than 10 kDa dextran. Likewise, the present observation that permeability of 70 kDa dextran in the sclera organ cultures was 3.7-fold less than 10 kDa dextran was similar to the previous observation that 70 kDa dextran permeability was 2.6- to 4.2-fold less than 10 kDa dextran in freshly dissected donor eye sclera. These similarities suggest that hydrodynamic constraints to macromolecule movement through the scleral organ cultures were similar to freshly dissected donor sclera. Hence, the scleral cultures represent a reasonable model system in which to study modulation of transscleral macromolecule movement by PGs.

The greater increase in 10 kDa dextran permeability through PG-treated scleral cultures than was observed with 40 or 70 kDa dextran suggests that PGs may alter the size of intrascleral supramolecular passages. The collagen fibrils in sclera are organized into bundles that vary in their organization, according to position near the outer or inner wall of the sclera. Overall, the bundles vary in width and thickness, often give off branches, and intertwine with each other. At the outermost layers, there is substantial irregular intermingling of collagen fibrils in adjacent bundles. Like sclera, synthetic hydrogels contain substantial water content and long polymer units characterized by chemical cross-links and polymer entanglements. Within pH-sensitive hydrogels, lower pH increases the size of pore channels through the matrix, whereas higher pH causes the gel network to swell, with a resulting decrease in the size of pore channels. Analysis of a pH-sensitive hydrogel confirmed that protein permeability is enhanced under conditions that increase the size of the pore channels. Moreover, the magnitude of permeability increase was greater with lower molecular weight proteins than with higher molecular weight proteins. This relationship among protein size, macromolecule permeability, and pore size also has been seen in hydrogels in which pore size was altered by changing the size of polymer subunits used to synthesize the hydrogel. Hence, the greater permeability increases with the smaller dextran tracers that was observed in the PG-treated scleral cultures.

**FIGURE 7.** Concentration of MMP-2 in the medium of scleral organ culture exposed to PhXA85 (A), PGF2α (B), or 17-phenyltrinor-PGF2α (C) for 1 to 3 days as determined by ELISA. Cultures analyzed were from cultures generated from a 76-year-old male donor, an 80-year-old male donor, and a 66-year-old male donor (n = 5). Data presented as mean ± SD. *P < 0.05 by Student’s-Newman-Keuls test.

**FIGURE 8.** Concentration of MMP-3 in the medium of scleral organ culture exposed to PhXA85 (A), PGF2α (B), or 17-phenyltrinor-PGF2α (C) for 1 to 3 days as determined by ELISA. Cultures analyzed were from cultures generated from a 76-year-old male donor, a 66-year-old male donor, and a 45-year-old female donor (n = 3). Data presented as mean ± SD. *P < 0.05 by Student’s-Newman-Keuls test.
cultures is consistent with enlargement of the intrasceral supramolecular passages. Consistent with this hypothesis is the observation of reduced collagen type I and collagen type III immunoreactivity within the sclera of monkey eyes after topical treatment with PGF2α-isopropyl ester.1,2

The mechanism of increased permeability within the PG-treated scleral cultures is suggested by the increased amounts of MMP-1, -2, and -3 detected within the medium of the treated scleral cultures. Sclera contains collagen types I, III, VI, VIII, XII, and possibly a small amount of collagen type V as well as fibronectin.13,30–34 Of these ECM components, MMP-1, -2, and -3 are known to cleave sites within collagen types I, III, V, and fibronectin.13,34 Increased MMP-1, -2, -3, and -9 have been found in cultures of human ciliary smooth muscle cells exposed to PGF2α, 17-phenyltrinor-PGF2α, and PhXA85.23,24 These treatments also induce reorganization of collagen type I, collagen type III, laminin, and collagen type IV within the human ciliary muscle cultures.23,37 Immunohistochemical investigation of monkey eyes found evidence of FP receptors in sclera.36,37 However, in situ hybridization experiments in the same study did not detect FP receptor transcripts in sclera. This observation may reflect limited sensitivity of the in situ hybridization technique. The present results are consistent with the presence of FP receptors within sclera because there was enhanced MMP release from PG-treated scleral cultures and these cultures did not contain other ocular tissues. Moreover, FP receptors have been detected on fibroblasts from other tissues.20 The concentrations of PGF2α and 17-phenyltrinor-PGF2α treatments were greater than the EC50 for activation of the FP receptor.21 It is possible that, if present, EP1 receptors (a PG-receptor most sensitive to E-type PGs but that can recognize PGF2α) also may have been activated by the PGF2α or 17-phenyltrinor-PGF2α treatments in this study because EC50 for these agonists is 320 and 650 nM, respectively.31 However, the response to PhXA85 is likely to reflect FP receptor activation, for which the EC50 is 100 nM and not activation of EP1 or other PG receptors because the EC50 concentrations for PhXA85 activation of PG receptors other than the FP receptor are at least 10-fold higher than the highest PhXA85 concentration tested.21 Hence, it is likely that the increased MMPs observed in the PG-treated scleral cultures were released by FP-receptor-mediated activation of scleral cells. These MMPs could have initiated collagen remodeling within the scleral stroma that enlarged intrasceral supramolecular passages and thereby facilitated transscleral protein permeability. As the MMPs in the present experiments could accumulate in the closed culture system, whereas they might dissipate upon secretion in situ, the concentration of the MMPs measured may be greater than the concentrations that might occur in scleral interstitial fluid in situ. However, this model is supported by the observation of significant increases in MMP immunoreactivity in the sclera of monkey eyes after topical PGF2α-isopropyl ester treatments.17,19 Transscleral fluid movement though scleral stroma may be important for uveoscleral outflow. Investigation with tracers found evidence of large molecule movement from the anterior chamber to the interstitial sclera.9,40,41 In addition, tracers were found in the extracellular spaces surrounding normal blood vessel and nerve penetrations of sclera.8 Also, there is evidence that transscleral pores extend from the interior to the exterior of the globe.42 Hence, in the intact eye, there may be several pathways for molecules within the suprachoroidal space to move to the fluid compartment associated with extraorbital fat. The relative contributions of these different pathways remain to be characterized. Also further study is needed to determine whether PG-induced increases in transscleral permeability contribute to increased uveoscleral outflow facility and decreased intraocular pressure observed after topical PG treatments. Recent consideration of the basis for intraocular pressure suggests that with moderate changes in uveoscleral outflow facility, increased turnover of aqueous humor may occur with minimal influence on intraocular pressure.43

The increased scleral permeability after PG exposure may have implications for facilitating delivery of therapeutics to the posterior segment of the eye. For example, growth factors that may facilitate retinal neuron survival range from 10 to 40 kDa.44–46 Because of their size, these molecules cannot readily cross the cornea. Hence, a noncorneal absorption route through sclera may facilitate usefulness of such therapeutics. Recently, it has been found that proteins as large as 150 kDa can cross sclera.47 As noted by these authors, other factors may limit the utility of molecules delivered to the posterior pole by transscleral diffusion such as orbital clearance, intraocular pressure, uveoscleral outflow, choroidal blood flow, and the outer and inner blood retinal barriers. Nevertheless, the prospect of increased transscleral permeability by PG cotreatment may allow sufficient transscleral transport to provide delivery of therapeutics to posterior pole tissues. This may be particularly important for hypertensive or glaucomatous eyes, because elevated intraocular pressure may reduce scleral permeability.48

In conclusion, this study indicates that PGs increase the permeability of human sclera in organ culture. Also, this increase in permeability is accompanied by increased release of MMPs from scleral tissue. These changes are consistent with the reduced collagens observed in monkey eye after topical PG treatment and suggest that remodeling of the scleral extracellular matrix may explain the increased permeability.

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References