Effects of Oxidative Stress in Trabecular Meshwork Cells Are Reduced by Prostaglandin Analogues

Alice L. Yu, Rudolf Fuchsbofer, Anselm Kampik, and Ulrich Welge-Lüssen

PURPOSE. The trabecular meshwork (TM) of glaucomatous eyes is characterized by cell loss, increased accumulation of extracellular matrix (ECM), and cellular senescence. One factor increasingly discussed in the pathogenesis of primary open-angle glaucoma (POAG) is oxidative stress. The goal of this study was to determine whether oxidative stress is able to trigger these typical glaucomatous changes in vitro and whether these oxidative stress–induced TM changes can be reduced by the application of prostaglandin analogues.

METHODS. Cultured human TM cells were exposed to 200 to 800 μM hydrogen peroxide (H₂O₂) for 1 h. Cell loss was detected by cell-viability assay. Levels of fibronectin and MMP-2 mRNA were determined by real-time PCR analysis. Senescence-associated β-galactosidase (SA-β-Gal) activity was investigated by histochemical staining. The effects of prostaglandin analogues and benzalkonium chloride (BAC) on these glaucoma typical TM changes were investigated by preincubation of nonstressed or H₂O₂-treated cells with 1:100 diluted commercial solutions of bimatoprost, travoprost, and latanoprost or their corresponding BAC concentrations.

RESULTS. H₂O₂ induced cell death and fibronectin mRNA expression, but decreased the amount of MMP-2 mRNA. H₂O₂ increased SA-β-Gal activity. Additional pretreatment with BAC further increased the typical glaucomatous TM changes in vitro. These effects were reduced by preincubation with prostaglandin analogues in H₂O₂-treated and, to a lesser extent, in nonstressed cells. No reduction occurred in the presence of prostaglandin F receptor antagonists in H₂O₂-treated cells.

CONCLUSIONS. Oxidative stress is able to induce characteristic glaucomatous TM changes in vitro, and these oxidative stress–induced TM changes can be minimized by the use of prostaglandin analogues. Thus, prevention of oxidative stress exposure to the TM may help to reduce the progression of POAG.


The trabecular meshwork (TM) of patients with primary open-angle glaucoma (POAG) is characterized by specific morphologic and biochemical changes such as loss of TM cells, accumulation of extracellular matrix (ECM), and accelerated senescence.1,2 It is assumed that these TM changes lead to an increased outflow resistance and thus to elevated intraocular pressure (IOP). The reasons for these changes are not clear. Various factors may play an elementary role in the pathologic course of the disease, such as genetic factors, increased levels of glutamate, changes in nitric oxide metabolism, and vascular changes.3–5 One factor, which is increasingly important in the pathogenesis of POAG, is oxidative stress.6 Abu-Amero et al.7 in patients with POAG demonstrated the occurrence of mutations in the mitochondrial genome and a reduced mitochondrial respiratory activity in comparison to control subjects. Moreover, it has been ascertained that the antioxidative capacity in the aqueous humor of patients with POAG is markedly reduced compared to nonglaucomatous eyes.8 In another study, it has been shown that oxidative damage in the TM is correlated with visual field defects.9,10 Based on these observations, it can be postulated that POAG is a degenerative disease and may be promoted, among other factors, by oxidative stress. In various cellular systems, oxidative stress is able to induce a broad number of biological changes. It is known that oxidative stress can induce cell death,11 ECM production,12 and accelerated senescence.12–14 All these changes can be found in several age-related diseases (e.g., in atherosclerosis as a systemic disease),15–17 but also in diseases of the human eye, such as age-related macular degeneration (AMD)18,19 and POAG.1,2,20 Therefore, in this study we hypothesized that oxidative stress may be responsible for the TM changes in POAG.

The knowledge about oxidative stress–induced TM changes may help to find new therapeutic strategies for POAG. In other eye diseases such as in AMD, the use of oral antioxidants can show a positive preventive effect on the disease progression, as shown in the Age-Related Eye Disease Study (AREDS).21 Whether oral antioxidants may help to minimize or prevent the progression of POAG is unclear and needs further fundamental studies. However, there is evidence demonstrating that prostaglandin analogues may have antioxidative capacities.22 Prostaglandin analogues are first-line therapy in glaucoma due to their efficiency in reducing IOP and the low rate of severe side effects.23–26 They are able to reach the TM directly via conjunctival and scleral pathways.27 In cultured conjunctival cells, prostaglandin analogues were able to reduce the generation of free oxygen species created by benzalkonium chloride (BAC).22 BAC is the most widely used preservative in commercially available eye drops. In the commercial solution of all three prostaglandin analogues used in this study—namely bimatoprost, travoprost, and latanoprost—BAC is present in different concentrations.

The goal of this study was to examine whether oxidative stress is able to induce characteristic TM changes such as loss of TM cells, ECM accumulation, and accelerated senescence in cultured human TM cells. Based on these results, we tested the protective effects of three commercially available prostaglandin analogues on the oxidative stress–induced TM changes in POAG. Furthermore, we investigated whether these protective effects of prostaglandin analogues could be blocked by pretreatment with prostaglandin F receptor (FP receptor) antagonists.

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4872
MATERIALS AND METHODS

Cell Culture

Primary cell cultures of human TM were obtained from the eye bank of the Ludwig-Maximilians-University. Monolayer cultures were established from eyes of five human donors (42–49 years, obtained 4–8 hours postmortem) without any history of eye diseases. Methods of securing human tissue were humane, included proper consent and approval, complied with the Declaration of Helsinki, and were approved by the local ethics committee. TM cells were prepared, grown, and classified as described previously.28–51

To test the effects of oxidative stress on TM cells, we incubated confluent cultures of passages 3 to 5 for 24 hours in serum-free Ham’s F-10 medium (Invitrogen-Gibco, Karlsruhe, Germany) at 37°C and 5% CO2. The medium was replaced by fresh serum-free Ham’s F-10 medium and the cells were exposed to 200, 400, 600, or 800 μM hydrogen peroxide (H2O2) for 1 hour. After exposure to H2O2, the cells were placed in serum-free Ham’s F-10 medium for 24 hours. In control cultures, the medium was changed at the same time points, but no H2O2 was added.

Cell Treatment with BAC, Prostaglandin Analogy, and FP Receptor Antagonists

The following prostaglandin analogues were used: bimatoprost preserved with 0.005% BAC (Lumigan; Pharm-Allergen, Ettlingen, Germany), travoprost preserved with 0.015% BAC (Travatan; Alcon, Freiburg, Germany), and latanoprost preserved with 0.02% BAC (Xalatan; Pfizer, Karlsruhe, Germany). To test the effects of all three prostaglandin analogues, we diluted each of them and their corresponding BAC concentrations diluted 1:100 in Ham’s F-10 medium. At confluence, the cells were treated with drug-containing medium for 15 minutes, washed twice with phosphate-buffered saline (PBS) and cultured in Ham’s F-10 medium supplemented with 1% fetal bovine serum (FBS: Invitrogen-Gibco) for 24 hours. This procedure was repeated three times. After the third drug pretreatment, the cells were placed in serum-free Ham’s F-10 medium for 24 hours. To test the effects of all three drugs in H2O2-stressed cultures, we then exposed the pretreated cells to H2O2 in serum-free Ham’s F-10 medium for 1 hour. After H2O2 exposure, the cells were placed in serum-free Ham’s F-10 medium for 24 hours. The treated cells were compared with control cultures (without H2O2 exposure) pretreated with the same 1:100 diluted prostaglandin analogues or their corresponding BAC concentrations and replaced with the same medium at the same time points.

To test the involvement of the prostaglandin F receptor (FP receptor) in the effects of prostaglandin analogues on oxidative-stress-mediated TM changes, we preincubated confluent cells with the FP receptor antagonists PGF2α, dimethyl amine, PGF2α, dimethyl amine, or AL8810 (all from Cayman Chemicals, Ann Arbor, MI) for 20 minutes at a concentration of 10–6 M each time before the three prostaglandin analogues and their corresponding BAC concentrations were added.

RNA Isolation and Real-Time PCR

Total RNA was isolated from 10 cm Petri dishes by the guanidium thiocyanate-phenol-chloroform extraction method (Stratagene, Heidelberg, Germany). Structural integrity of the RNA samples was confirmed by electrophoresis in 1% Tris-acetate-EDTA (TAE)-agarose gels. Yield and purity were determined photometrically. After RNA isolation, mRNA was transcribed to cDNA via reverse transcription. This cDNA was then used for specific real-time PCR. Quantification of human mRNA was performed in 40 cycles in a thermocycler (LightCycler System; Roche Diagnostics, Mannheim, Germany). The primers selected were fibronectin forward primer 5′-ctgcccagaattcattgtaa-3′ and reverse primer 5′-ccacgctgctgagacg-3′; MMP-2 forward primer 5′-tttaattggaagctgcttcg-3′ and reverse primer 5′-aggacattcaataaggtagtgc-3′; and GAPDH forward primer 5′-agccacctgctgacagac-3′ and reverse primer 5′-gccaacagccacaaaacctt-3′. Primers and probes were found (ProbeFinder, ver., 2.04; Exiqon A/S, Vedbaek, Denmark). The standard curve was obtained from probes of three different untreated human TM cell cultures. To normalize differences in the amount of total RNA added to each reaction, we processed GAPDH simultaneously in the same sample, as an internal control. The level of fibronectin and MMP-2 mRNA was determined as the relative ratio (RR), which was calculated by dividing the level of fibronectin and MMP-2 mRNA by the level of the GAPDH housekeeping gene in the same samples. All experiments were performed at least in triplicate in TM cultures from three donors.

Cell Viability Assay

Cell viability was quantified based on a two-color fluorescence assay, in which the nuclei of nonviable cells appear red because of staining by the membrane-impermeable dye propidium iodide (Sigma-Aldrich, Deisenhofen, Germany), whereas the nuclei of all cells were stained by the membrane-permeable dye Hoechst 33342 (Intergen, Purchase, NY). Confluent cultures of TM cells growing on coverslips in four-well tissue culture plates were either not stressed or exposed to H2O2, with or without pretreatment with BAC or prostaglandin analogues. For evaluation of cell viability, the cells were washed in PBS and incubated with 2.0 μg/ml propidium iodide and 1.0 μg/ml Hoechst 33342 for 20 minutes at 37°C. Subsequently, cells were analyzed with a fluorescence microscope (DMR; Leica Microsystems, Wetzlar, Germany), and representative areas were documented (IM 1000 software; Leica Microsystems, Heerbrugg, Switzerland). The labeled nuclei were then counted in fluorescence photomicrographs, and dead cells were expressed as a percentage of total nuclei in the field. The data are based on counts from nine experiments of TM cultures of three donors performed in duplicate wells, with three to five documented representative fields per well.

Senescence-Associated β-Galactosidase (SA-β-Gal) Activity

The proportion of TM cells positive for SA-β-Gal activity was determined as described by Dimri et al. Briefly, treated TM cells were washed twice with PBS and fixed with 2% formaldehyde and 0.2% glutaraldehyde in PBS (pH 6.0) at room temperature (RT) for 4 minutes. The cells were then washed twice with PBS and incubated under light protection for 8 hours at 37°C with fresh SA-β-Gal staining solution (1 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside [X-Gal], 40 mM citric acid/sodium phosphate [pH 6.0], 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl, and 2 mM MgCl2 diluted in PBS). Cells were then examined for the development of blue color and photographed at low magnification (×200) using a light microscope. The results were expressed as the mean value ± SD of results in 12 experiments in TM cultures from four donors.

RESULTS

H2O2 and Cell Death

To determine the cytotoxic concentrations of hydrogen peroxide, we first treated cultured human TM cells with 200, 400, 600, and 800 μM H2O2 for 1 hour (Fig. 1E). Nonviable cells were detected by a cell viability assay 24 hours after stress exposure. In this assay, untreated control cells demonstrated almost no dead cells stained red by propidium iodide (Fig. 1B). Incubation of cultured human TM cells with 200 and 400 μM H2O2 showed only slightly elevated proportions of nonviable cells of 13.3% ± 5.6%, and 16.7% ± 5.7% (Fig. 1E). In contrast, exposure to 600 and 800 μM H2O2 significantly increased the proportion of nonviable TM cells to 46.8% ± 26.9%, and 87.6% ± 10.6% of total TM cells (Figs. 1D, 1E). Therefore, the subsequent experiments on further effects of hydrogen peroxide were conducted with subtoxic concentrations of 200 and 400 μM H2O2.
Effect of H$_2$O$_2$ on Fibronectin and MMP-2 mRNA Expression

Human TM cells were treated with 200 and 400 μM H$_2$O$_2$ for 1 hour (Fig. 2). Real-time PCR analysis was conducted 24 hours after stress exposure. The signals generated in untreated control cells were set to 100%. There was a significant increase of the fibronectin mRNA expression to 1.6 ± 0.1- and 1.8 ± 0.2-fold after treatment with 200 and 400 μM H$_2$O$_2$ compared with untreated control cells (Fig. 2A).

Treatment with 200 μM H$_2$O$_2$ for 1 hour showed no significant effects on MMP-2 mRNA expression (0.8 ± 0.1-fold; Fig. 2B). Treatment with 400 μM H$_2$O$_2$ for 1 hour led to a decrease in MMP-2 mRNA to 0.4 ± 0.1-fold of the mRNA amount of untreated control cells (Fig. 2B).

H$_2$O$_2$ and SA-β-Gal Activity

Human TM cells were treated with 200 and 400 μM H$_2$O$_2$ for 1 hour (Figs. 3B, 3C). The proportion of TM cells positive for SA-β-Gal activity was determined 24 hours after stress exposure. Untreated control cells showed 3.5% ± 0.6% SA-β-Gal-positive TM cells (Figs. 3A, 3C). Exposure to 200 and 400 μM H$_2$O$_2$ markedly increased the number of SA-β-Gal-positive TM cells to 17.6% ± 8.9% and 45.0% ± 5.4% of total cells, respectively (Figs. 3B, 3C).

Figure 1. Cell-viability assay of cultured human TM cells 24 hours after treatment with 200, 400, 600, and 800 μM H$_2$O$_2$ for 1 hour. Representative fluorescence photomicrographs of Hoechst 33342-stained TM cells in (A) untreated controls or (C) cells treated with 800 μM H$_2$O$_2$ for 1 hour. (B, D) Non-viable cells in the corresponding field. (E) Quantification of the number of non-viable cells. The percentage of dead cells was scored by counting at least 700 cells in fluorescence photomicrographs of representative fields. Data presented as a mean ± SD of nine experiments with three different cell cultures from different donors (*P < 0.05). Co, control.

Figure 2. Real-time PCR analysis of (A) fibronectin and (B) MMP-2 mRNA expression 24 hours after treatment of cultured human TM cells with 200 and 400 μM H$_2$O$_2$ for 1 hour. Results were normalized to GAPDH as a reference. The steady state mRNA level of fibronectin and MMP-2 genes in the untreated control cells was considered to be 100%. Data are the mean ± SD of results in nine experiments with three different cell cultures from different donors (*P < 0.05). Co, control.

Figure 3. H$_2$O$_2$-induced SA-β-Gal activity in cultured human TM cells. (A) Morphology and SA-β-Gal activity of untreated cultured TM cells. Only single cells were stained to indicate SA-β-Gal activity. (B) In contrast, TM cells of the same passage exposed to 400 μM H$_2$O$_2$ for 1 hour showed a marked increase in SA-β-Gal activity. (C) Quantification of the number of SA-β-Gal-positive cells. The percentage of SA-β-Gal activity was analyzed 24 hours after exposure to 200 and 400 μM H$_2$O$_2$ for 1 hour and scored by counting at least 300 cells in phase-contrast photomicrographs of representative fields. Data (mean ± SD) are based on the sampling of 6 to 10 photomicrographs per condition in nine experiments with three different cell cultures from different donors (*P < 0.05). Co, control.
Effects of BAC and Prostaglandin Analogues on Fibronectin mRNA Expression

In the subsequent approach, we investigated the effects of BAC, bimatoprost, travoprost, and latanoprost on the fibronectin mRNA expression in nonstressed cells (Fig. 4A). The concentrations of BAC in bimatoprost, travoprost, and latanoprost are 0.005%, 0.015%, and 0.02%, respectively. With real-time PCR analysis, the signals generated in untreated control cells were set to 100% (Fig. 4A). Treatment of TM cells with 0.005%, 0.015%, and 0.02% BAC diluted 1:100 in H2O2 for 1 h in H2O2-stressed cells results were normalized to GAPDH as a reference. The steady state mRNA level of fibronectin and MMP-2 genes in untreated control cells was considered to be 100%. Data are the mean ± SD of results in 12 experiments with four different cell cultures from different donors (*P < 0.05). Co, control.

Effects of BAC and Prostaglandin Analogues on MMP-2 mRNA Expression

Similar to the experimental studies on fibronectin mRNA expression, the effects of BAC, bimatoprost, travoprost, and latanoprost on MMP-2 mRNA expression were also investigated in nonstressed and H2O2-stressed cells (Fig. 4B). With real-time PCR analysis, the signals generated in untreated control cells were set to 100% (Fig. 4B). Treatment with 0.005% and 0.015% BAC, diluted 1:100, showed MMP-2 mRNA levels (0.8 ± 0.3-fold) nearly similar to those in untreated control cells (Fig. 4B). Treatment with 0.02% BAC, diluted 1:100, reduced the MMP-2 mRNA expression to 0.6 ± 0.1-fold of the amount of untreated control cells (Fig. 4B). Incubation with 1:100 diluted bimatoprost, travoprost, and latanoprost preserved with their corresponding BAC concentration showed similar fibronectin mRNA levels (0.7 ± 0.2, 0.9 ± 0.2, and 1.2 ± 0.1-fold) as untreated control cells (Fig. 4A).

To simulate oxidative stress, we first treated cultured human TM cells with the three BAC concentrations or prostaglandin analogues, all diluted 1:100, and then exposed them to subtoxic concentration of 400 µM H2O2 (Fig. 4A). Hydrogen peroxide treatment alone doubled the fibronectin mRNA amount compared with the untreated control cells (2.0 ± 0.2-fold; Fig. 4A). Preincubation of cultured TM cells with 0.005%, 0.015%, and 0.02% BAC diluted 1:100 before H2O2 exposure significantly increased the fibronectin mRNA expression by 2.5 ± 0.5, 3.1 ± 0.3, and 3.1 ± 0.6-fold compared with untreated control cells (Fig. 4A). Pretreatment of TM cells with bimatoprost and travoprost preserved with their corresponding BAC concentrations before H2O2 exposure showed results (1.2 ± 0.2- and 1.0 ± 0.1-fold) similar to those in untreated control cells, whereas pretreatment with latanoprost increased the amount of fibronectin mRNA by 1.8 ± 0.3-fold compared with that in untreated control cells (Fig. 4A).
MMP-2 mRNA expression by 1.5 ± 0.1-, 3.1 ± 0.4-, and 1.4 ± 0.7-fold compared with untreated control cells (Fig. 4B).

Effects of BAC and Prostaglandin Analogues on SA-β-Gal Activity

To investigate the effects of BAC and prostaglandin analogues on cellular senescence, we determined the proportion of TM cells positive for the SA-β-Gal activity 24 hours after stress exposure (Fig. 5). Untreated control cells showed 3.5 ± 0.6% of SA-β-Gal positive TM cells (Fig. 5). Treatment with 0.005%, 0.015%, and 0.02% BAC, diluted 1:100, slightly increased the number of SA-β-Gal-positive TM cells (6.8 ± 0.9%, 7.5 ± 1.9%, and 9.4 ± 1.3%; Fig. 5). Treatment with bimatoprost, travoprost, and latanoprost did not induce any marked changes in SA-β-Gal activity (3.1% ± 2.4%, 4.8% ± 3.0%, and 5.4% ± 2.4%) compared with that in untreated control cells (Fig. 5).

Exposure to 400 μM H₂O₂ markedly increased the proportion of SA-β-Gal-positive TM cells (55.0 ± 7.9%; Fig. 5). This increase was even more pronounced with pretreatment of H₂O₂-stressed cells with 0.005%, 0.015%, and 0.02% BAC, diluted 1:100 (52.2 ± 6.6%, 56.3 ± 10.2%, and 53.7 ± 13.2%; Fig. 5). In contrast, treatment of TM cells with bimatoprost, travoprost, and latanoprost diluted 1:100 and subsequent H₂O₂ exposure increased the number of SA-β-Gal-positive cells to 24.8% ± 4.8%, 21.1% ± 5.7%, and 29.9% ± 6.7% of total cells (Fig. 5).

Effects of BAC and Prostaglandin Analogues on Cell Death

Last, we investigated the effects of BAC and prostaglandin analogues on TM cell death by cell-viability assay (Fig. 6). With this assay, untreated control cells showed almost no dead cells stained by propidium iodide (5.2% ± 1.2%; Fig. 6). Incubation with 0.005%, 0.015%, and 0.02% BAC, diluted 1:100, increased the number of dead cells to 11.4% ± 3.0%, 20.7% ± 4.2%, and 20.1% ± 4.1% (Fig. 6). Treatment of cells with bimatoprost,
travoprost, and latanoprost, diluted 1:100, showed only slightly elevated proportions of nonviable cells of 10.1% /H11006 1.8%, 10.7% /H11006 2.4%, and 13.8% /H11006 2.1% of total cells (Fig. 6).

To test the protective effects of prostaglandin analogues on H2O2-stressed cells, we treated the cells were treated with 600 /H9262 MH 2O2. This concentration was chosen, because we had shown that 600 /H9262 MH 2O2 induced approximately 50% of dead cells (Fig. 1). Exposure to 600 /H9262 MH 2O2 significantly increased the number of dead cells (45.4% /H11006 3.7%; Fig. 6). Pretreatment of H2O2-stressed cells with 0.005%, 0.015%, and 0.02% BAC, diluted 1:100, further increased the number of dead cells to 53.7 ± 4.1%, 62.0 ± 4.4%, and 69.6 ± 2.2% (Fig. 6). This effect was markedly reduced by pretreatment of H2O2-stressed cells with 1:100 diluted bimatoprost, travoprost, and latanoprost (26.1 ± 3.5%, 19.0 ± 3.4%, and 34.3 ± 3.4%; Fig. 6).

**Effects of Prostaglandin Analogues on Oxidative Stress-Mediated TM Changes in the Presence of FP Receptor Antagonists**

To test the involvement of FP receptors in the effects of prostaglandin analogues on oxidative stress-mediated TM changes, we preincubated confluent cells with the FP receptor antagonists PGF2α, dimethyl amide, PGF2α, dimethyl amine, or Al-8810. Preincubation of TM cells with PGF2α, dimethyl amide, PGF2α, dimethyl amine, or Al-8810 and subsequent treatment with bimatoprost, travoprost, latanoprost, or 0.005%, 0.015%, and 0.02% BAC concentration had no marked effects on the fibronectin and MMP-2 mRNA levels, SA-β-Gal activity or cell death in nonstressed cells compared with untreated control cells (data not shown). In contrast, in H2O2-stressed cells, preincubation with FP receptor antagonists reduced the protective effects of all three prostaglandin analogues on fibronectin (Fig. 7A) and the MMP-2 (Fig. 7B) expression, SA-β-Gal activity (Fig. 8), and cell death (data not shown).

**DISCUSSION**

Oxidative stress is thought to be involved in morphologic and biochemical alterations of the trabecular meshwork (TM) of glaucomatous eyes. One characteristic finding in POAG is the loss of TM cells. In our in vitro experiments, we demonstrated that 600 and 800 μM H2O2 markedly increased the number of dead cells in human TM cultures. In other ocular cell types, H2O2 had significant cytotoxic effects at similar concentrations. In cultured human retinal pigment epithelium cells, we have previously that H2O2 is able to promote apoptosis. However, whether oxidative stress may induce apoptosis in cultured human TM cells awaits further investigation. Besides oxidative stress, other hypotheses suggest that increased loss of TM cells in POAG may also be attributed to intense phagocytic activity of TM cells or detachment of cells from the meshwork and migration toward Schlemm's
ases (MMP) expression. We showed that subtoxic concentrations of H₂O₂ did not induce the mRNA expression of fibronectin in cultured human TM cells. Oxidative stress-induced increase of fibronectin has also been demonstrated in other cellular systems such as in human fibroblasts, mesangial cells, and lens epithelial cells. In glaucomatous eyes, fibronectin accumulates in so-called plaque in the juxtacanalicular region of the TM. It is suspected that these pathologic TM changes increase the outflow resistance in POAG. Thus, our results support the hypothesis that oxidative stress may contribute to elevated IOP in part by increased synthesis of extracellular matrix (ECM) components such as fibronectin. Besides that, increased ECM accumulation can also be achieved by inhibiting the ECM degradation via decreased matrix metalloproteinases (MMP) expression. We showed that subtoxic concentrations of H₂O₂ did not decrease the MMP-2 mRNA amount in cultured human TM cells. This observation is consistent with those in previous studies on ocular lens epithelial cells, in which oxidative stress induced a downregulation of MMP-2 via reduced gene expression. We are aware of the limitation of our study’s showing only the protective effects of prostaglandin analogues on oxidative stress–induced senescence in TM cells. These results are in agreement with the hypothesis that BAC increases the production of ROS and thus may have effects in cultured human TM cells similar to those of oxidative stress.

Treatment of nonstressed cells with bimatoprost, travoprost, and latanoprost, diluted 1:100, had no significant effects on fibronectin mRNA expression, MMP-2 mRNA expression, and SA-β-Gal activity compared with untreated control cells. In a previous study, no effects on MMP-2 was detected after treatment of cultured TM cells with latanoprost. To our knowledge, no in vitro studies exist that demonstrate the effects of prostaglandin analogues on fibronectin expression or SA-β-Gal activity changes in cultured TM cells. However, a slight increase of cell death was detected after treatment with 1:100 diluted bimatoprost, travoprost, and latanoprost. Similarly, it has been reported that preserved antiglaucoma drugs including prostaglandin analogues, diluted 1:100, have cytotoxic effects in cultured human TM cells and human conjunctiva-derived epithelial cells. These effects, however, were lower than their corresponding BAC concentrations alone. Therefore, we suspect that the antioxidative action of the prostaglandin analogues counteracts the pro-oxidative effects of BAC. This hypothesis may explain the observed lower levels of cytotoxicity of all three prostaglandin analogues, compared to their corresponding BAC concentrations.

Another characteristic finding of TM changes in POAG is the accelerated senescence of this tissue. Oxidative stress and the associated production of reactive oxygen species (ROS) are regarded as the major factors in triggering cellular senescence—in particular, so-called stress-induced premature senescence (SIPS). Comparable studies have demonstrated that the TM of patients with POAG has a fourfold increased proportion of senescence-associated β-galactosidase (SA-β-Gal)-positive cells compared with that in age-matched control eyes. Our results showed that treatment of cultured TM cells with 400 μM H₂O₂ induced a more than 10-fold increase in SA-β-Gal activity. In other cellular systems, oxidative stress–induced cellular senescence has been demonstrated to be mediated via increased TGF-β release. Until now, the pathomechanism for oxidative stress–induced senescence in TM cells is still unclear.

For treatment of POAG, two principle mechanisms exist to lower IOP: either by reduction of aqueous humor secretion or by elevation of aqueous humor outflow. Prostaglandin analogues belong to the group of IOP-lowering drugs, which increase aqueous humor outflow. The commercial solutions of prostaglandin analogues contain different concentrations of BAC as a preservative. In this study, we showed that treatment with higher levels of BAC concentrations can increase fibronectin mRNA expression, decrease the level of MMP-2 mRNA, and induce SA-β-Gal activity and cell death in nonstressed TM cells. These results are in agreement with the hypothesis that BAC increases the production of ROS and thus may have effects in cultured human TM cells similar to those of oxidative stress.

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To investigate the antioxidative effects of prostaglandin analogues, we preincubated TM cells with BAC, bimatoprost, travoprost, and latanoprost, diluted 1:100, before exposure of cells to $\text{H}_2\text{O}_2$. Although pretreatment with BAC alone before $\text{H}_2\text{O}_2$ exposure further increased the $\text{H}_2\text{O}_2$-induced TM changes, preincubation with bimatoprost, travoprost, and latanoprost attenuated the $\text{H}_2\text{O}_2$-induced effects on fibronectin and MMP-2 mRNA expression, SA-β-Gal activity and cell death of TM cells. These results are in accordance with previous findings showing that prostaglandin and its analogues have antioxidative properties.22,27,54–56 For example, in neurons, prostaglandin analogues protect against oxidative stress-induced apoptotic cell death.24 In cultured renal proximal tubular epithelial cells, prostaglandin analogues diminish 2,3,5-tris(glu-thiono-S)hydrosultione-induced cytotoxicity.22,25,52 In a recent study with cultured conjunctival cells, it was shown that prostaglandin analogues reduce the generation of ROS and thus may have antioxidative properties.

Last, our in vitro experiments demonstrated that pretreatment of cells with FP receptor antagonists reduced the protective effects of prostaglandin analogues on oxidative stress-mediated TM changes. In a recent publication dealing with the effects of prostaglandin analogues on contraction of cultured human TM cells, it was clearly shown that this side effect of prostaglandin analogues is receptor mediated.27 Therefore, we believe that our observed protective effects of prostaglandin analogues can also be mediated via FP receptors.

Antioxidative activities of prostaglandin analogues can result either from direct mechanisms against ROS or indirectly through activation of the endogenous antioxidative defenses, or reduction in the overproduction of ROS.22,27,53–55 However, these specific effects of prostaglandin analogues could also be imitated by molecular interactions of BAC with active compounds of the drug. These molecular processes between BAC and prostaglandin analogues may block the interaction of free BAC with TM cells.22,27 Further studies are needed to evaluate possible antioxidative capacities or mechanisms of prostaglandin analogues.

In summary, we showed that oxidative stress induces TM changes characteristic of POAG in vitro and that these oxidative stress-induced changes were reduced by pretreatment with prostaglandin analogues. Further clinical investigations are needed to clarify whether the reduction of oxidative stress may help to minimize the progression of POAG and whether prostaglandin analogues may provide additive beneficial characteristics for POAG besides lowering IOP.

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