Effects of Topical Antiglaucoma Medications on Corneal Epithelium as Evaluated by Gene Expression Patterns

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Purpose: To examine the expression pattern of the stress-related genes c-fos and c-jun, which encode the 2 major components of activator protein (AP)-1, and cyclooxygenase (COX)-2 in rat corneal epithelium treated with topical antiglaucoma medications and benzalkonium chloride (BAK) preservative.

Methods: Eighty-eight male Wistar rats were used. We instilled antiglaucoma eye drops (0.5% Timoptol, 0.005% Xalatan, or 0.12% Rescula), their chemical constituents (active ingredients), or BAK preservative (0.005%, 0.01%, or 0.02%) in 1 eye of each rat. Fellow eyes served as controls. The eyes were enucleated after various intervals. In situ hybridization and immunohistochemistry were used to detect expression of c-fos, c-jun, and COX-2.

Results: Expression of c-fos, c-jun, and COX-2 was minimally observed in uninjured rat corneal epithelium. Thirty minutes to 1 hour after applying the antiglaucoma eye drops, signals for c-fos and c-jun mRNA were detected in the corneal epithelium. Ninety minutes after applying 0.005% Xalatan, 0.12% Rescula, or their chemical constituents, but not 0.5% Timoptol, COX-2 was detected in corneal epithelium. Expression of c-fos and c-jun seemed more marked with prostaglandins than with timolol. Thirty minutes to 1 hour after instillation of 0.02% BAK preservative, signals for c-fos and c-jun mRNA were detected in the corneal and conjunctival epithelium. COX-2 was not induced by 0.5% Timoptol or BAK preservative. COX-2 mRNA was not affected by applying 0.005% or 0.01% BAK preservative. Proteins of these components were also detected, indicating that each mRNA expression was followed by protein synthesis.

Conclusions: Corneal and conjunctival epithelial cells are transcriptionally activated transiently at an early phase after topical administration of antiglaucoma medications and BAK preservative. Stimulatory effects of prostaglandin drugs on corneal epithelial cells were more marked than those with timolol. Expression of COX-2 may potentially be involved in inflammatory response in the corneal epithelium.

Topical β-blocker (timolol, 0.5% Timoptol) and prostaglandin (PG)$F_{2\alpha}$ analogs (latanoprost, Xalatan; isopropyl unoprostone, Rescula) are widely used in the management of glaucoma and ocular hypertension. Because antiglaucoma drugs are used for long time periods, chronic side effects are a major concern. Among these side effects, ocular surface disorders attributable to the drug itself or to drug preservatives are relatively common.[1–4] Benzalkonium chloride (BAK), the most commonly used preservative in ophthalmic preparations,[5,6] is used at an average concentration of 0.01% (range, 0.005%–0.02%) in topical multidose solutions.[6] BAK is a quaternary ammonium compound[7] that has been shown to adversely affect both the cornea and conjunctiva.[8,9] One in vitro study concluded that ocular damage associated with BAK is dose dependent.[10] Therefore, ocular surface complications seen in glaucoma patients might be attributable to taking antiglaucoma drugs. The purpose of this study was to examine the expression pattern of stress-related genes c-fos and c-jun, which encode the 2 major components of activator protein (AP)-1, and cyclooxygenase (COX)-2 in rat corneal and conjunctival epithelium treated with topical antiglaucoma medications with or without BAK preservative.

AP-1, which regulates gene expression by binding to the AP-1 binding site located upstream of various target genes, is a dimer composed of various fos and jun family proteins.[11–13] Fos-Jun heterodimers, as well as Jun protein homodimers, bind to DNA at AP-1 sites in the promoters of many genes and thereby regulate transcription. In addition to their roles as transcriptional factors (TFs), they influence the transcriptional activity of TFs through protein–protein interactions.[14,15] c-fos, c-jun, and their family members are rapidly and transiently upregulated in quiescent cells in response to extracellular stimuli such as mitogens, growth factors, and mechanical stimulation.[16]

PGs, commonly known as lipid mediators of inflammation, are produced from arachidonic acid by COX enzymes, which exist in several isoforms. COX-1 is constitutively

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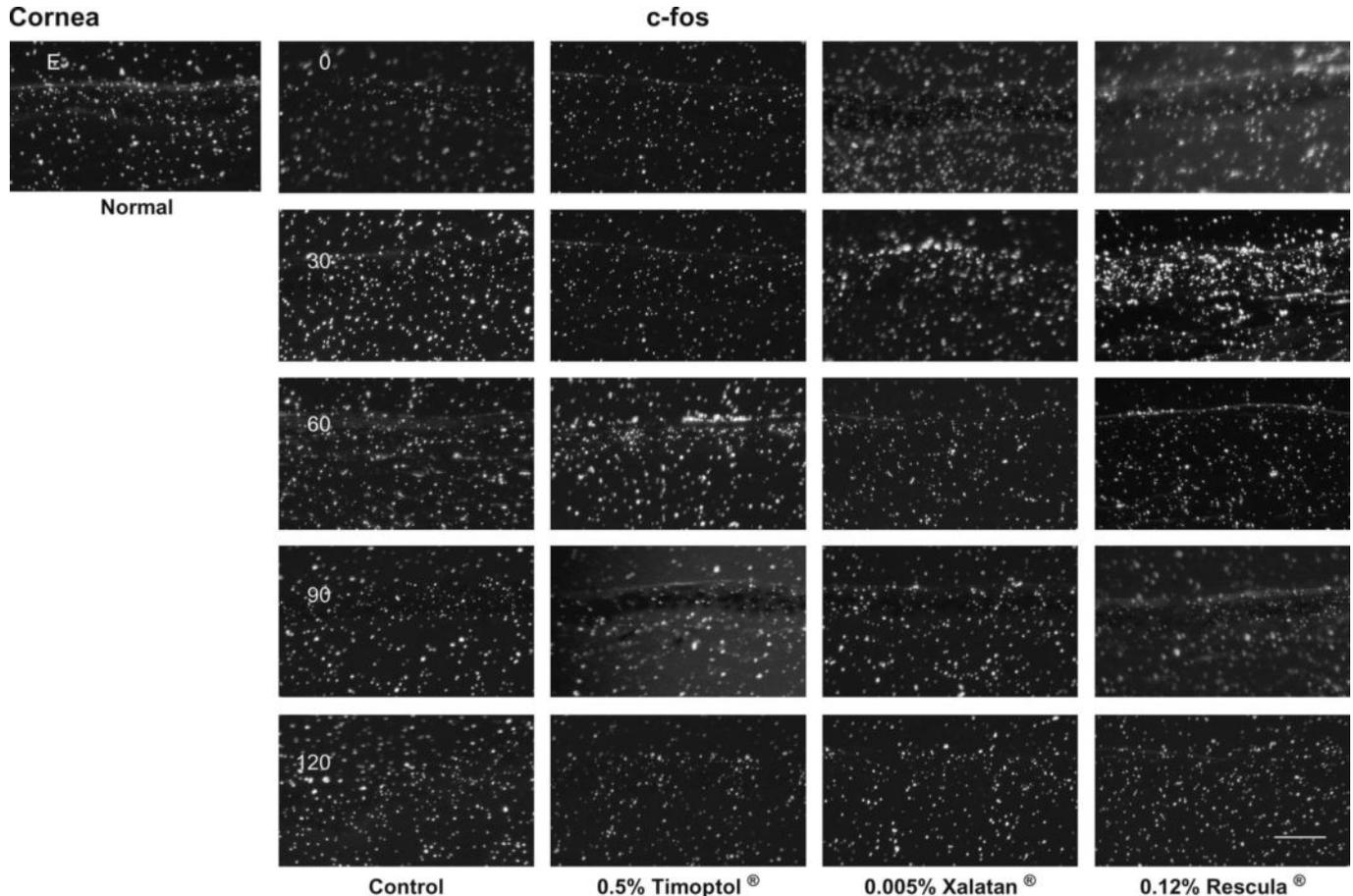
FIGURE 1. Dark-field photomicrographs showing expression of c-fos mRNA in the corneal epithelium after instillation of antiglaucoma eye drops. Thirty minutes to 1 hour after applying antiglaucoma medications, c-fos mRNA was detected in the corneal epithelium. Bar = 100 mm. E, epithelium.

expressed in many tissues. In contrast, COX-2, which is normally expressed at low levels, is potently induced by proinflammatory agents.[17–19]

Previously, we showed that mRNA encoding AP-1 components and COX-2 is transiently upregulated in healing corneal epithelium after epithelial debridement.[20–22] In this study, we examined the expression pattern of AP-1 and COX-2 in rat corneal epithelium treated with antiglaucoma eye drops and BAK preservatives.

MATERIALS AND METHODS

Investigational Agents

All experiments were performed in accordance with the ARVO Resolution on the Use of Animals in Research. Adult male Wistar rats (n = 88) were used. In 1 eye of each rat, the following were instilled: antiglaucomatous eye drops (0.5% timolol, Timoptol; 0.005% latanoprost, Xalatan; or 0.12% isopropyl unoprostone, Rescula), the chemical constituents (active ingredients) of Xalatan (0.005% latanoprost) and Rescula (0.12% unoprostone), or BAK preservative at

a concentration of 0.005%, 0.01%, or 0.02%. The affected eyes were allowed to heal for 15, 30, 60, 90, 120, and 180 minutes after treatment and then were enucleated. Fellow eyes served as controls. Eyes from untreated rats were also enucleated.

In Situ Hybridization for mRNA of AP-1 Components

In situ hybridization was carried out by using ^{35}S-radiolabeled oligonucleotide probes as previously reported.[20,23] Probes for detection of c-fos and c-jun mRNA were complementary to the nucleotides spanning amino acids 1 to 15 of rat c-Fos protein,[24] the last 20 amino acids of the predicted c-Jun,[25] or 1421 to 1460 of rat COX-2 mRNA[26] sequences. A computer-assisted homology search revealed no identical sequences in any genes in the database. The eyes were embedded in optimal cutting temperature (OCT) compound and quickly frozen. Cryosections (5 mm) were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 20 minutes, rinsed 3 times in 2× SSC, and dehydrated through a graded ethanol series. Hybridization was performed as described previously[15] by incubating sections
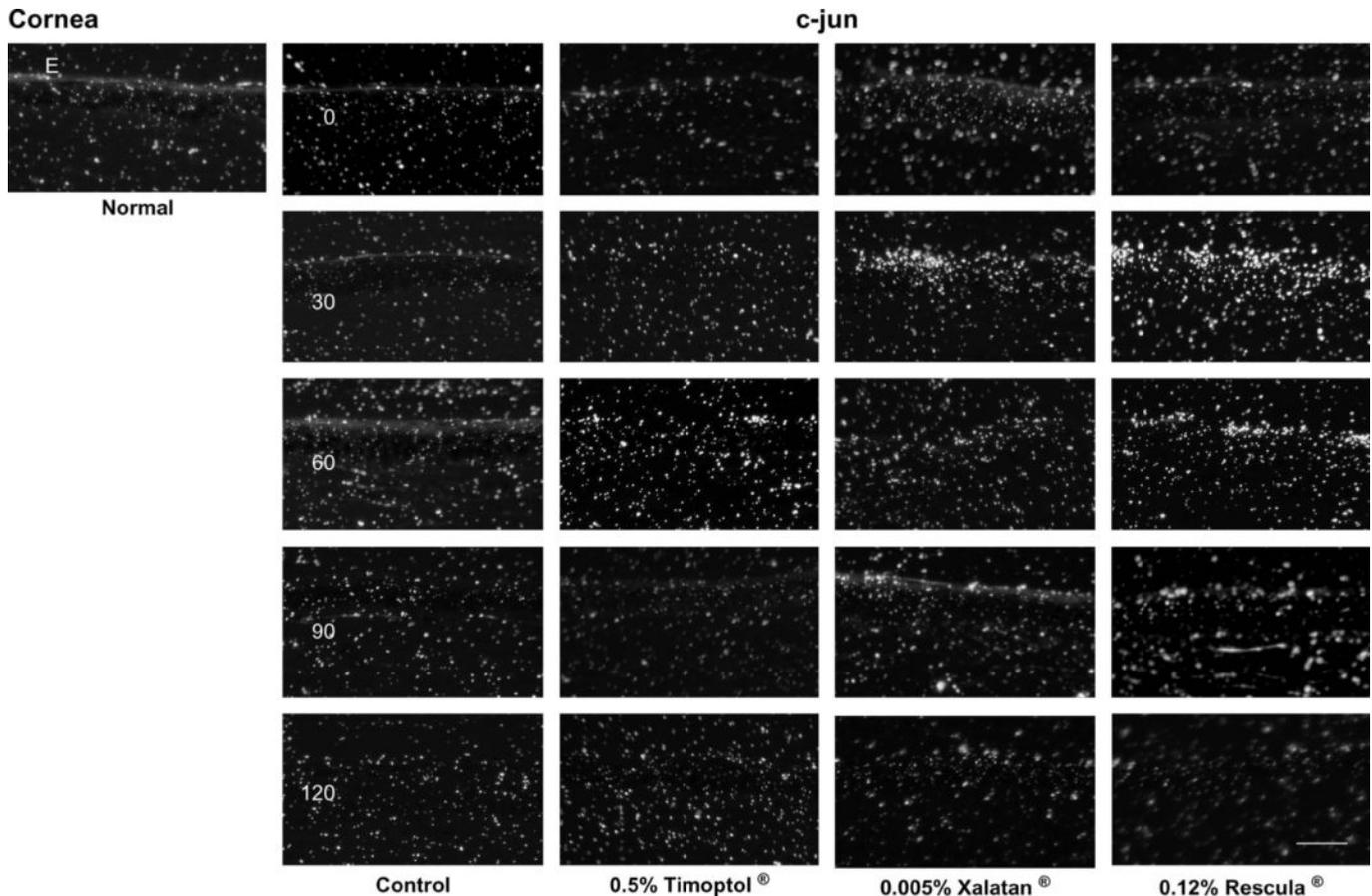
FIGURE 2. Dark-field photomicrographs showing expression of c-jun mRNA in the corneal epithelium after instillation of antiglaucoma eye drops. Thirty minutes to 1 hour after applying antiglaucoma medications, c-jun mRNA was detected in the corneal epithelium. Bar = 100 mm. E, epithelium.

with hybridization buffer containing each probe ($0.5–1.0 \times 10^6$ cpm/slide) for 16 hours at 37°C. After hybridization, sections were rinsed in $1\times$ SSC at 55°C for 4×15 minutes. The sections were dehydrated and coated with Ilford G-5 emulsion diluted 1:1 with water. The sections were dried in a level position for 30 minutes and exposed in tightly sealed dark boxes at 4°C for 8 weeks. After being developed in D-19 (Kodak, Rochester, NY) developer, fixed with photographic fixer, and washed with tap water, the sections were counterstained with 1% neutral red (sodium acetate buffer).

Incubation of sections with a mixture of each 35S-labeled probe and 100-fold excess of respective cold oligonucleotide resulted in no detectable signals.

Immunostaining for AP-1 Component Proteins

Immunohistochemistry for c-Fos and COX-2 proteins was carried out as previously reported.[20–22] In brief, the eyes were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 hours. Cryosections were incubated with primary antibodies diluted (1:200; c-Fos; Oncogene Science, Cambridge, MA; and 1:200; COX-2, Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C for 24 hours. After washing in

PBS, sections were incubated with biotinylated anti–rabbit immunoglobulin G (IgG) or anti–goat IgG (1:250; Vectastain ABC kit; Vector, Burlingame, CA) at room temperature for 1 hour and then with avidin-biotin peroxidase complex (Vectastain ABC kit; Vector) at room temperature for 1 hour. Peroxidase reaction product in tissue sections was visualized by using diaminobenzidine. After counterstaining with methyl green, sections were dehydrated and mounted in balsam. Sections incubated with normal rabbit or goat serum (1:200) and processed in the same way as described above showed no immunostaining in the corneal epithelium.

RESULTS

In Situ Hybridization

Expression of c-fos, c-jun, and COX-2 was minimally observed in uninjured rat corneal epithelium (Figs. 1–3). Thirty minutes to 1 hour after applying antiglaucoma medications, signals for c-fos and c-jun mRNA were detected in the corneal epithelium (Figs. 1, 2). Ninety minutes after applying 0.005% Xalatan, 0.12% Rescula, or these chemical
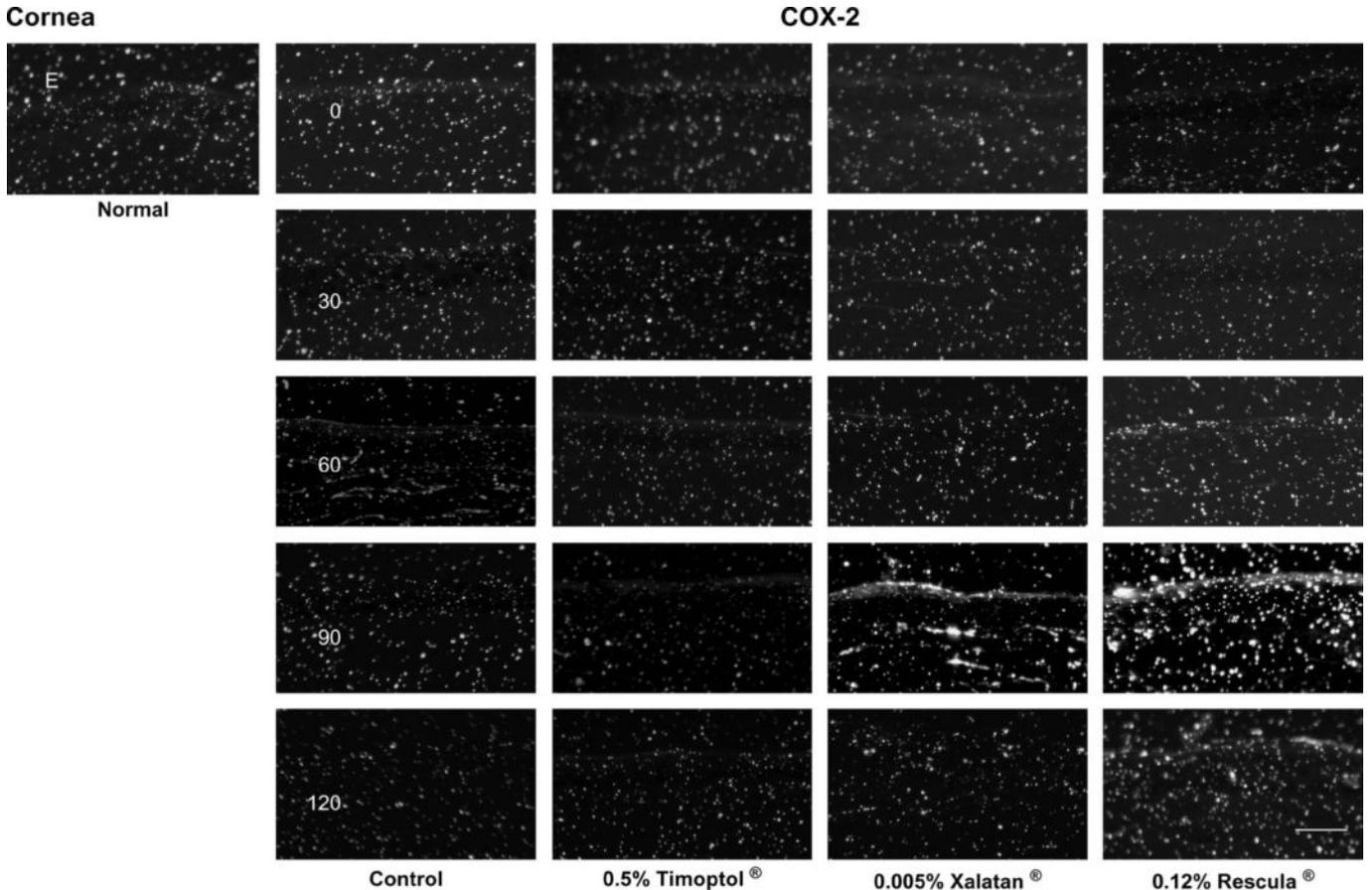
Cornea

COX-2

FIGURE 3. Dark-field photomicrographs showing expression of COX-2 mRNA in the corneal epithelium after instillation of antiglaucoma eye drops. Ninety minutes after applying 0.005% Xalatan and 0.12% Rescula, but not 0.5% Timoptol, COX-2 was detected in corneal epithelium. Bar = 100 mm. E, epithelium.

constituents, COX-2 was detected in corneal and conjunctival epithelium (Figs. 3–5). Expression of c-fos and c-jun seemed more marked with prostaglandins than with Timoptol. Thirty minutes to 1 hour after instillation of 0.02% BAK preservative, signals for c-fos and c-jun mRNA were detected in the corneal and conjunctival epithelium (Fig. 6). COX-2 was not induced by applying 0.5% Timoptol or BAK preservative

(Figs. 3, 6). Signals for this mRNA were not affected by applying BAK preservative at concentrations of 0.005% or 0.01% (Fig. 6).

Immunohistochemistry

No immunoreaction for c-Fos and COX-2 was detected in the healthy corneal epithelium (Figs. 7, 8). c-Fos protein
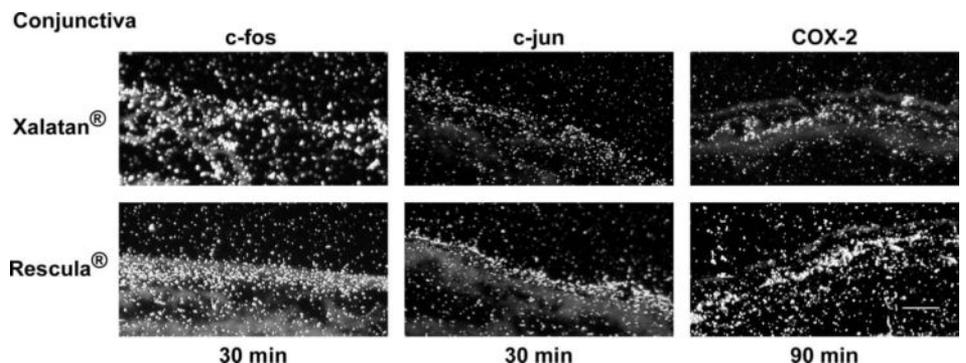

FIGURE 4. Dark-field photomicrographs showing expression of c-fos, c-jun, and COX-2 mRNA in the conjunctival epithelium after instillation of antiglaucoma eye drops. Bar = 100 mm.
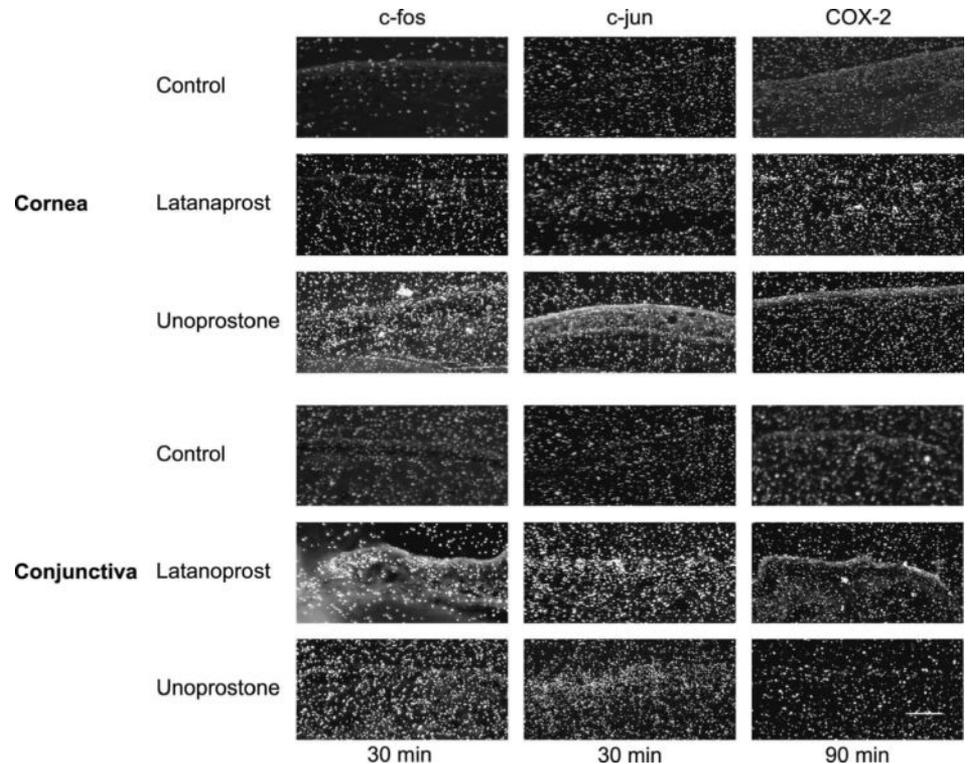
FIGURE 5. Dark-field photomicrographs showing expression of c-fos, c-jun, and COX-2 mRNA in the corneal and conjunctival epithelium after instillation of chemical constituents (active ingredients) 0.005% latanoprost and 0.12% unoprostone. c-fos, c-jun, and COX-2 mRNA was detected in the corneal and conjunctival epithelium after instillation. Bar = 100 mm.

was detected in corneal and conjunctival epithelium 120 minutes after applying antiglaucoma medications (Figs. 7, 9). COX-2 protein was detected in corneal and conjunctival epithelium 120 minutes after instillation of 0.005% Xalatan, 0.12% Rescula, and their chemical constituents (Figs. 8, 9). c-Fos protein was also detected in the conjunctival epithelium 120 minutes after instillation of 0.02% BAK preservative (Fig. 10). COX-2 protein was not detected in corneal and conjunctival epithelium after instillation of BAK preservative.

DISCUSSION

Corneal and conjunctival epithelial cells are transcriptionally activated transiently at an early phase after instillation of antiglaucoma eye drops and BAK preservative.

Expression of AP-1 was more marked after instillation of PGs than with timolol; 0.005% Xalatan and 0.12% Rescula but not 0.5% Timoptol induced expression of COX-2 protein in corneal and conjunctival epithelium. However, to study whether chemical constituents or preservatives cause upregulation of AP-1 components and COX-2, we examined the expression pattern of AP-1 and COX-2 after topical application of these ingredients separately. AP-1 and COX-2 were detected in corneal and conjunctival epithelium after instillation of 0.005% latanoprost, 0.12% unoprostone, and BAK preservative at a concentration of 0.02% but not 0.005% and 0.01%. BAK at a concentration of 0.02% has a toxic effect on the corneal epithelium, causing an increase in permeability to fluorescein,[27,28] and BAK 0.01%–0.3% increases AP-1

activity in organ cultured bovine cornea in a dose-dependent manner.[29] These findings suggest that high concentrations of BAK are detrimental to corneal and conjunctival epithelium, and glaucoma medications with low levels of or no BAK are consequently more benign to the ocular surface.

Increased numbers of inflammatory cells and fibroblasts have been described in the conjunctiva and Tenon capsule after long-term topical antiglaucoma medication. Indeed, antiglaucoma eye drops containing BAK stimulated an exaggerated myofibroblast response.[30–33] Moreover, BAK was found to be irreversibly toxic to cultured human Tenon capsule fibroblasts at clinically used concentrations. Fibroblast proliferation was higher after treatment with antiglaucoma eye drops with BAK preparation, indicating that the tissue was able to recuperate from the initial injury.[34] Long-term preoperative therapy has been reported to have a deleterious effect on surgical outcome by altering postoperative wound healing.[35,36]

In humans, the volume of tear fluid accumulated in the conjunctival sacs is $\sim$7 mL, and tear production is $\sim$1.2 mL/min.[37] However, dilution of antiglaucoma eye drops and BAK by tear fluid is greatly reduced in patients with dry eye. Therefore, there is potentially a greater risk of adverse events caused by these drugs in patients with dry eye.

AP-1 is rapidly induced in cells on exposure to a wide variety of extracellular stimuli. $PGF_{2\alpha}$ has been shown to increase protein kinase C (PKC) activity and PKC translocation in luteal cells.[38–40] PKC is involved in $PGF_{2\alpha}$-induced activation of the Raf/mitogen-activated protein kinase (MEK)/ extracellular signal-regulated kinase (ERK) signaling cascade
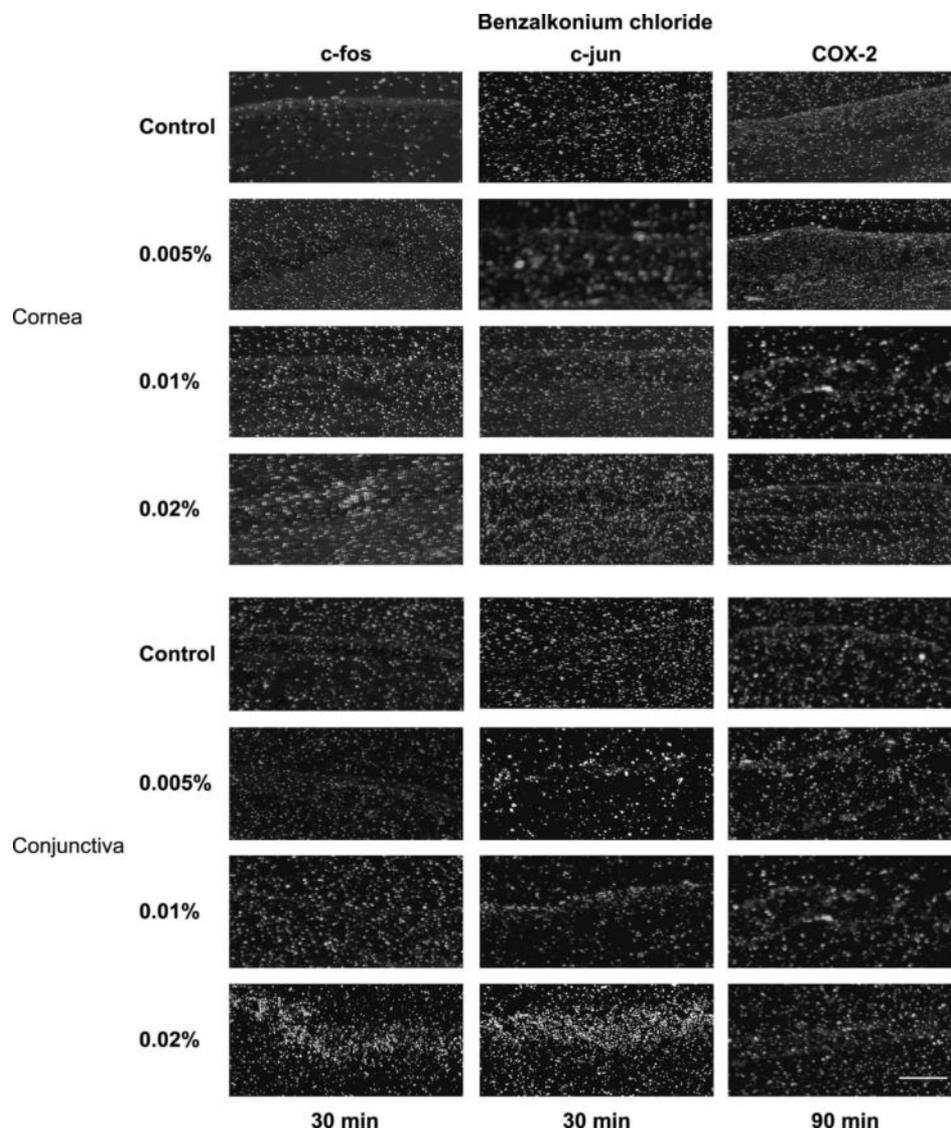
FIGURE 6. Dark-field photomicrographs showing expression of c-fos, c-jun, and COX-2 mRNA in the corneal and conjunctival epithelium after instillation of BAK preservative. Thirty minutes to 1 hour after instillation of 0.02% BAK preservative, signals for c-fos and c-jun but not COX-2 mRNA were detected in the corneal and conjunctival epithelium. Signals for mRNA were not affected by applying 0.005% or 0.01% BAK preservative. Bar = 100 mm.

in luteal cells, because the response to $PGF_{2\alpha}$ was mimicked by PKC activator phorbol 12-myristate 13-acetate (PMA).[41] $PGF_{2\alpha}$ and PMA stimulated c-fos and c-jun mRNA expression in primary cultured bovine luteal cells.[42]

In vitro studies have shown that latanoprost may increase PGE_2 release in iris and ciliary muscles.[43,44] COX-2 is strongly induced by proinflammatory agents.[17–19] Induction of COX-2 expression by PGs, in particular with respect to the cAMP-elevating PGE_2, has been extensively studied.[45–48] In the case of $PGF_{2\alpha}$, COX-2 induction has been observed in corpus luteum of different species.[49,50] COX-2 might be involved in inflammatory response in the corneal epithelium after instillation of 0.005% Xalatan, 0.12% Rescula, or their chemical constituents.

We previously showed that mRNA encoding AP-1 components and COX-2 is transiently upregulated in healing the corneal epithelium after epithelial debridement and ethanol exposure.[20–22,51,52] The expression pattern after antiglaucoma eye drops was similar to that after ethanol exposure. Such events in the epithelium are believed to be unfavorable in maintenance of tissue homeostasis. Therefore, upregulation of these components on exposure to antiglaucoma eye drops or their constituents including preservatives might suggest undesirable stimuli to the cells in the epithelium.

In the final analysis, all aspects of the potential benefits and detriments caused by antiglaucoma eye drops (and their preservatives) on corneal and conjunctival epithelium should be taken into consideration when prescribing these agents.
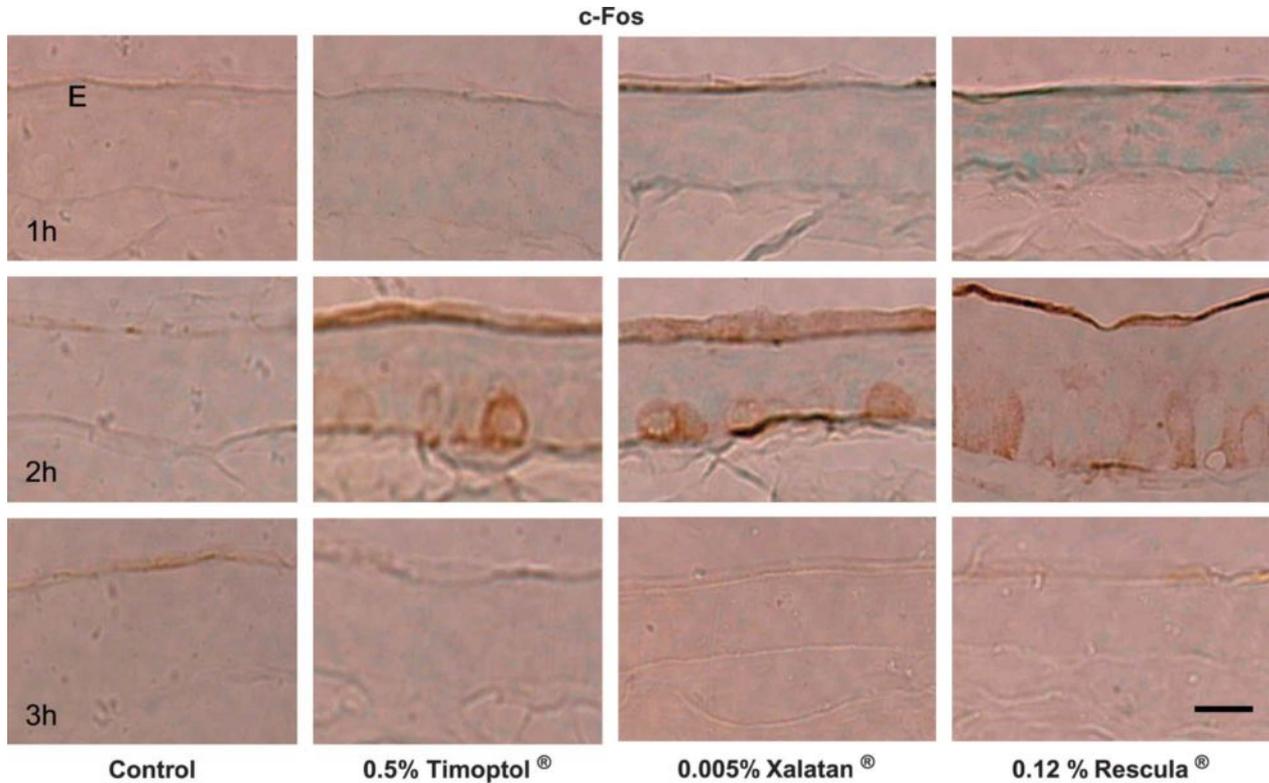
c-Fos

FIGURE 7. Protein expression of c-Fos in corneal epithelium after instillation of antiglaucoma eye drops. No immunoreaction for c-Fos was detected in control epithelium. c-Fos protein was detected in the corneal epithelium 120 minutes after applying antiglaucoma medications. Bar = 100 mm. E, epithelium.
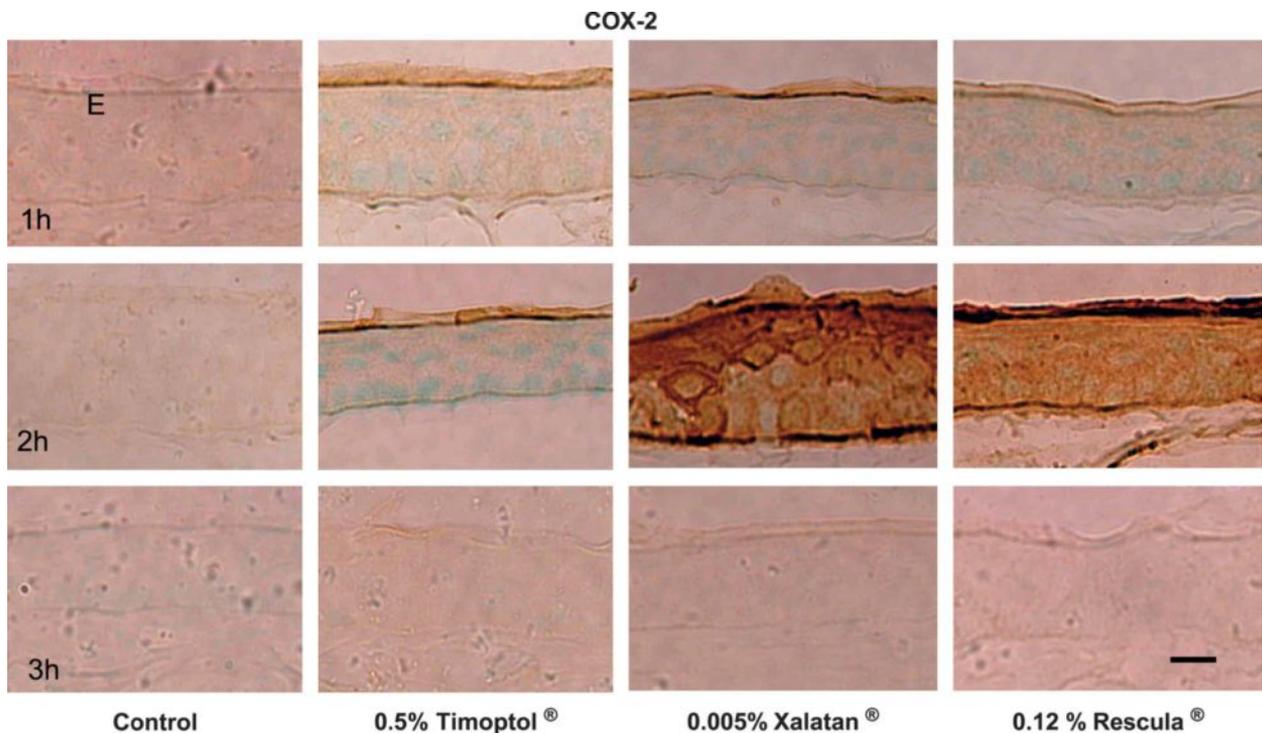
COX-2

FIGURE 8. Protein expression of COX-2 in corneal epithelium after instillation of antiglaucoma eye drops. No immunoreaction for COX-2 was detected in control epithelium. COX-2 protein was detected in the corneal epithelium 120 minutes after applying 0.005% Xalatan and 0.12% Rescula but not 0.5% Timoptol. Bar = 100 mm. E, epithelium.

FIGURE 9. Protein expression of c-Fos and COX-2 in corneal epithelium after instillation of chemical constituents 0.005% latanoprost and 0.12% unoprostone. No immunoreaction for c-Fos and COX-2 was detected in control epithelium. c-Fos and COX-2 proteins were detected in the corneal epithelium 120 minutes after applying 0.005% Xalatan and 0.12% Rescula but not 0.5% Timoptol. Bar = 100 mm. E, epithelium.
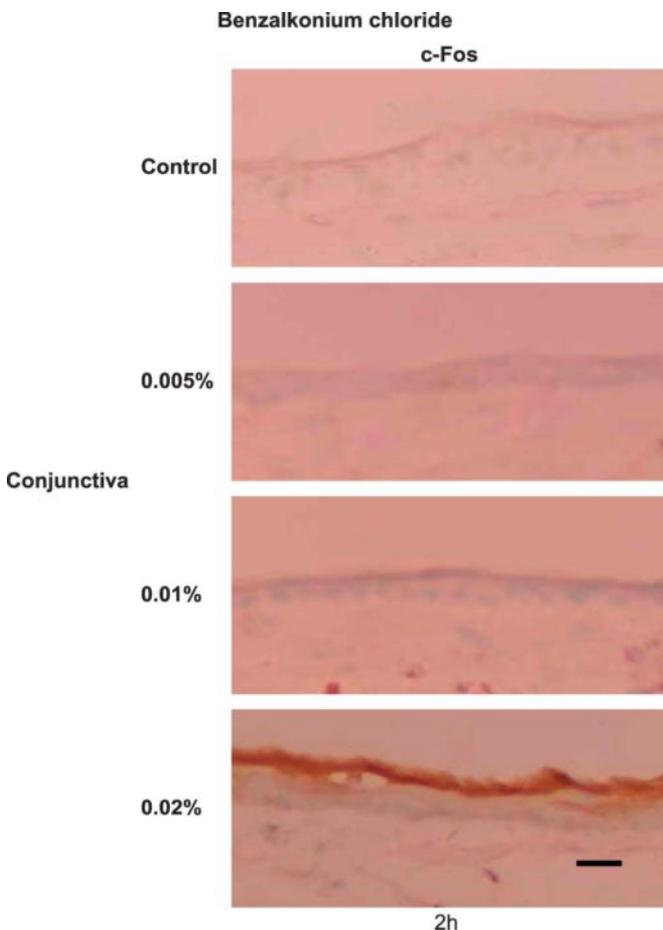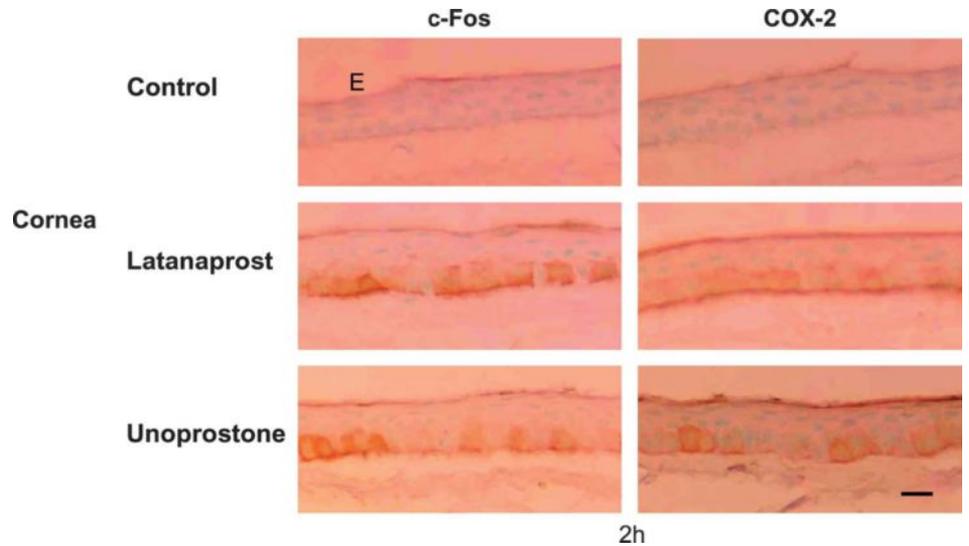

FIGURE 10. Protein expression of c-Fos in the conjunctival epithelium after instillation of BAK preservative. c-Fos protein was detected in the conjunctival epithelium 120 minutes after applying 0.02% BAK preservative. Bar = 100 mm.

REFERENCES

1. Wilson FM. Adverse external ocular effects of topical ophthalmic medications. *Surv Ophthalmol*. 1979;24:57–88.
2. Sherwood MB, Grierson I, Millar L, et al. Long-term morphologic effects of antiglaucomatous drugs on the conjunctiva and Tenon's capsule in glaucoma patients. *Ophthalmology.* 1989;96:327–335.
3. Herreras JM, Pastor JC, Calonge M, et al. Ocular surface alteration after long-term treatment with an antiglaucomatous drug. *Ophthalmology.* 1992;99:1082–1088.
4. Ishibashi T, Yokoi N, Kinoshita S. Comparison of the short-term effects on the human corneal surface of topical timolol maleate with and without benzalkonium chloride. *J Glaucoma*. 2003;12:486–490.
5. Berdy GJ, Abelson MB, Smith LM, et al. Preservative-free artificial tear preparations. Assessment of corneal epithelial toxic effects. *Arch Ophthalmol*. 1992;110:528–532.
6. Pisella PJ, Fillacier K, Elena PP, et al. Comparison of the effect of preserved and unpreserved formulation of timolol on the ocular surface of albino rabbits. *Ophthalmic Res*. 2000;32:3–8.
7. Noecker R. Effects of common ophthalmic preservatives on ocular health. *Adv Ther*. 2001;32:205–215.
8. Burstein NL. Preservative cytotoxic threshold for benzalkonium chloride and chlorhexidine digluconate in cat and rabbit corneas. *Invest Ophthalmol Vis Sci*. 1980;19:308–313.
9. Mietz H, Niesen U, Krieglstein GK. The effect of preservatives and antiglaucomatous medication on the histopathology of the conjunctiva. *Graefe's Arch Clin Exp Ophthalmol*. 1994;232:561–565.
10. De Saint Jean M, Debbasch C, Brignole F, et al. Toxicity of preserved and unpreserved antiglaucoma topical drugs in an in vitro model of conjunctival cells. *Curr Eye Res*. 2000;20:85–94.
11. Halazonetis TD, Georgopoulos K, Greenberg M, et al. C-Jun dimerizes with itself and with c-Fos, forming complexes of different DNA binding affinities. *Cell*. 1988;55:917–924.
12. Nakabeppu Y, Nathans D. The basic region of Fos mediates specific DNA binding. *EMBO J*. 1989;8:3833–3841.
13. Sheng M, McFadden K, Greenberg ME. Membrane depolarization and calcium induce c-fos transcription via phosphorylation of transcription factor CREB. *Neuron*. 1990;4:571–582.
14. Schule R, Rangarajan P, Kliewer S, et al. Functional antagonism between oncoprotein c-jun and the glucocorticoid receptor. *Cell*. 1990;62:1217–1226.
15. Yang-Yen J-F, Chambard JC, Sun Y-L, et al. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. *Cell*. 1990;62:1205–1215.

16. Greenberg ME, Ziff EB. Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. *Nature*. 1984;331:433–438.
17. Dubois RN, Abramson SB, Crofford L, et al. Cyclooxygenase in biology and disease. *FASEB J*. 1988;12:1063–1073.
18. Williams CS, Mann M, Dubois RN. The role of cyclooxgenases in inflammation, cancer, and development. *Oncogene*. 1999;18:7908–7916.
19. Kuwano T, Nakao S, Yamamoto H, et al. Cyclooxygenase 2 is a key enzyme for inflammatory cytokine-induced angiogenesis. *FASEB J*. 2004; 18:300–310.
20. Okada Y, Saika S, Hashizume N, et al. Expression of fos family and jun family proto-oncogenes during corneal epithelial wound healing. *Curr Eye Res*. 1996;15:824–832.
21. Okada Y, Saika S, Shira K, et al. Immunolocalization of proto-oncogene products in keratocytes after epithelial ablation, alkali burn and penetrating injury of the cornea in rats. *Graefes Arch Clin Exp Ophthalmol*. 1998;236:853–858.
22. Miyamoto T, Saika S, Okada Y, et al. Expression of cyclooxygenase-2 in corneal cells after photorefractive keratectomy and laser in situ keratimileusis in rabbits. *J Cataract Refract Surg*. 2004;30:2612–2617.
23. Senba E, Umemoto S, Kawai Y, et al. Differential expression of fos family and jun family proto-oncogene mRNAs in the rat hypothalamo–pituitary–adrenal axis after immobilization stress. *Brain Res Mol Brain Res*. 1994;24:283–294.
24. Curran T, Gordon MB, Rubino KL, et al. Isolation and characterization of the c-fos (rat) cDNA and analysis of post translational modification in vivo. *Oncogene*. 1984;2:79–84.
25. Angel P, Allegretto EA, Okino ST, et al. Oncogene jun encodes a sequence-specific trans-activator similar to AP-1. *Nature*. 1988;332:166–171.
26. Yamagata K, Andreasson KI, Kaufmann WE, et al. Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. *Neuron*. 1993;11:371–386.
27. Burstein NL. Preservative alteration of corneal permeability in humans and rabbits. *Invest Ophthalmol Vis Sci*. 1984;25:1453–1457.
28. Ramselaar JAM, Boot JP, Haeringen NJ, et al. Corneal epithelial permeability after instillation of ophthalmic solutions containing local anaesthetics and preservatives. *Curr Eye Res*. 1988;7:947–950.
29. Xu K-P, Li X-F, Yu F-SX. Corneal organ culture model for assessing epithelial responses to surfactants. *Toxicol Sci*. 2000;58:306–314.
30. Sherwood MB, Grieson I, Millar L, et al. Long-term morphological effects of antiglaucoma drugs on the conjunctiva and Tenon's capsule in glaucomatous patients. *Ophthalmology*. 1989;96:327–335.
31. Lavin MJ, Wormald RPL, Migdal CS, et al. The influence of prior therapy on the success of trabeculectomy. *Arch Ophthalmol*. 1990;108:1543–1555.
32. Young TL, Higginbotham R, Zou X, et al. Effects of topical glaucoma drugs on fistulized rabbit conjunctiva. *Ophthalmology*. 1990;97:1423–1427.
33. Nuzzi R, Vercelli A, Finazzo C, et al. Conjunctiva and subconjunctival tissue in primary open-angle glaucoma after long-term topical treatment: an immunohistochemical and ultrastructural study. *Graefes Arch Clin Exp Ophthalmol*. 1995;233:154–162.
34. Williams DE, Nguyen KD, Shapourifar-Tehrani S, et al. Effects of timolol, betaxolol, and levobunol of human Tenon's fibroblasts in tissue culture. *Invest Ophthalmol Vis Sci*. 1992;33:2233–2241.
35. Brandt JD, Wittpenn JR, Katz LJ, et al. Conjunctival impression cytology in patients with glaucoma using long-term topical medication. *Am J Ophthalmol*. 1991;112:297–301.
36. Broadway D, Grierson I, Hitchings R. Adverse effects of topical antiglaucomatous medications on the conjunctiva. *Br J Ophthalmol*. 1993; 77:590–596.
37. Mishima S, Gasset A, Klyce SD Jr, et al. Determination of tear volume and tear flow. *Invest Ophthalmol*. 1966;5:264–276.
38. Dowd JP, Alila HW, Hansel W. Phorbol ester receptors in bovine luteal cells: relationship to protein kinase C. *Mol Cell Endocrinol*. 1990;61: 199–206.
39. Abayasekera DR, Jones PM, Persaud SJ, et al. Prostaglandin $F_{2\alpha}$ activates protein kinase C in human ovarian cells. *Mol Cell Endocrinol*. 1993;91: 51–57.
40. Salli U, Supancic S, Stormshak F. Phosphorylation of myristoylated alanine-rich C kinase substrate (MARCKS) protein is associated with bovine luteal oxytocin exocytosis. *Biol Reprod*. 2000;63:12–20.
41. McGuire WJ, Juengel JI, Hawes BE, et al. Mitogenic signaling via G-protein–coupled receptors. *Endocr Rev*. 1997;17:698–714.
42. Chen D, Fong HW, Davis JS. Induction of c-fos and c-jun messenger ribonucleic acid expression by prostaglandin $F_{2\alpha}$ is mediated by a protein kinase C-dependent extracellular signal-regulated kinase mitogen-activated protein kinase pathway in bovine luteal cells. *Endocrinology*. 2001; 142:887–895.
43. Yousufzai SY, Ye Z, Abdel-Latif AA. Prostaglandin $F_{2\alpha}$ and its analogs induce release of endogenous prostaglandins in iris and ciliary muscles isolated from cat and other mammalian species. *Exp Eye Res*. 1996;63: 305–310.
44. Kashiwagi K, Kanai N, Tsuchida T, et al. Comparison between isopropyl unoprostone and latanoprost by prostagrandin E_2 induction, affinity to prostaglandin transporter, and intraocular metabolism. *Exp Eye Res*. 2002; 74:41–49.
45. Minghetti L, Polazzi E, Nicolini A, et al. Up-regulation of cyclooxygenase-2 expression in cultured microglia by prostaglandin E_2, cyclic AMP and non-steroidal anti-inflammatory drugs. *Eur J Neurosci*. 1997; 9:934–940.
46. Hinz B, Brune K, Pahl A. Cyclooxygenase-2 expression in lipopolysaccharide-stimulated human monocytes is modulated by cyclic AMP, prostaglandin E_2 and nonsteroidal anti-inflammatory drugs. *Biochem Biophys Res Commun*. 2000;278:790–796.
47. Hinz B, Brune K, Pahl A. Prostaglandin E_2 up-regulates cyclooxygenase-2 expression on lipopolysaccharide-stimulated RAW 264.7 macrophages. *Biochem Biophys Res Commun*. 2000;272:744–748.
48. Maldve RE, Kim Y, Muga SJ, et al. Prostaglandin E_2 regulation of cyclooxygenase expression in keratinocytes is mediated via cyclic nucleotide-linked prostaglandin receptors. *J Lipid Res*. 2000;41: 873–881.
49. Tsai SJ, Wiltbank MC. Prostaglandin $F_{2\alpha}$ induces expression of prostaglandin G/H synthase-2 in the ovine corpus luteum: a potential positive feedback loop during luteolysis. *Biol Reprod*. 1997;57:1016–1022.
50. Wu YL, Wiltbank MC. Transcriptional regulation of cyclooxygenase-2 gene in ovine large luteal cells. *Biol Reprod*. 2001;65:1565–1572.
51. Miyamoto T, Saika S, Ueyama T, et al. Cyclooxygenase 2 expression in rat corneas after ethanol exposure. *J Cataract Refract Surg*. 2006;32:1736–1740.
52. Okada Y, Saika S, Miyamoto T, et al. AP-1 expression in ethanol-treated corneal epithelium in vivo. *Ophthalmic Res*. 2007;39:84–91.