Induction of FGF-2 Synthesis by IL-1β in Aqueous Humor through PI3-Kinase and p38 in Rabbit Corneal Endothelium

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PURPOSE. To determine whether the elevated level of interleukin (IL)-1β in aqueous humor after transcorneal freezing up-regulates FGF-2 synthesis in rabbit corneal endothelium through PI3-kinase and p38 pathways.

METHODS. Transcorneal freezing was performed in New Zealand White rabbits to induce an injury-mediated inflammation. The concentration of IL-1β was measured, and the expression of FGF-2, p38, and Akt underwent Western blot analysis. Intracellular location of FGF-2 and actin cytoskeleton was determined by immunofluorescence staining.

RESULTS. Massive infiltration of polymorphonuclear leukocytes (PMNs) to the corneal endothelium was observed after freezing, and IL-1β concentration in the aqueous humor was elevated in a time-dependent manner after freezing. Similarly, FGF-2 expression was increased in a time-dependent manner. When corneal endothelium was stained with anti-FGF-2 antibody, the nuclear location of FGF-2 was observed primarily in the cornea after cryotreatment, whereas FGF-2 in normal corneal endothelium was localized at the plasma membrane. Treatment of the ex vivo corneal tissue with IL-1β upregulated FGF-2 and facilitated its nuclear location in corneal endothelium. Transcorneal freezing disrupted the actin cytoskeleton at the cortex, and cell shapes were altered from cobblestone morphology to irregular shape. Topical treatment with LY294002 and SB203580 on the cornea after cryotreatment blocked the phosphorylation of Akt and p38, respectively, in the corneal endothelium. These inhibitors also reduced FGF-2 levels and partially blocked morphologic changes after freezing.

CONCLUSIONS. These data suggest that after transcorneal freezing, IL-1β released by PMNs into the aqueous humor stimulates FGF-2 synthesis in corneal endothelium via PI3-kinase and p38. (Invest Ophthalmol Vis Sci. 2010;51:822–829) DOI:10.1167/iovs.09-4240

The retrocorneal fibrous membrane (RCFM), first described by Fuchs in 1901,1 has been observed in various clinical conditions associated with disease and damage to the corneal endothelium.2–4 The presence of RCFM (or posterior collagenous layer) posterior to the Descemet’s membrane is thought to represent an end-stage disease process of the corneal endothelium, resulting in functional alteration of the corneal endothelium and leading to corneal opacity and blindness. An in vitro model to elucidate the molecular mechanism of RCFM formation led us to the finding that activated polymorphonuclear leukocytes (PMNs) were able to transform the type IV collagen-synthesizing polygonal endothelial cells to type I collagen-synthesizing fibroblastic cells.5–7 Of the several proteins released by the activated PMNs, a 17-kDa protein band that caused endothelial mesenchymal transformation (EMT) of corneal endothelial cells (CECs) was identified, with the use of ProteinChip array technology, as interleukin 1β (IL-1β).7,8

The major proinflammatory cytokine IL-1β plays an important role in acute and chronic inflammatory diseases9–12 and a crucial role in the regulation of inflammation and wound healing on the ocular surface.13–16 Numerous studies have reported that interleukin 1α (IL-1α) and IL-1β both orchestrate the inflammatory process by inducing the production and release of secondary cytokines; IL-1β stimulates the expression of a variety of genes necessary for the wound repair processes.17–20 Both IL-1α and IL-1β markedly stimulate the synthesis and release of fibroblast growth factor 2 (FGF-2) in a variety of cell types.21–23 Similarly, CECs produce all isoforms of FGF-2 in response to IL-1β stimulation; IL-1β activates PI3-kinase, the enzyme activity of which was greatly stimulated after a 5-minute exposure to IL-1β. Such an early and rapid activation of PI3-kinase greatly enhanced FGF-2 production in CECs; pretreatment with LY294002 completely blocked the induction activity of IL-1β.8,24 The information obtained from our in vitro study indicates that FGF-2 production in response to IL-1β stimulation is an early event necessary for endothelial to mesenchymal transformation of CECs.25–29 An animal model of RCFM was used to validate the in vitro findings by investigating whether injury-mediated acute inflammation elevates the level of proinflammatory cytokine (IL-1β in this case) in aqueous humor and whether IL-1β is able to facilitate FGF-2 production in corneal endothelium in vivo. For this task, the established experimental protocols to produce RCFM in rabbit corneas were slightly modified30; rabbits received one cycle of transcorneal freeze injury, the dosage of which was far below that needed to cause RCFM production in rabbit corneas. We measured the concentration of IL-1β in aqueous humor and determined FGF-2 production by corneal endothelium after the cryotreatment.

In the present study, we demonstrated that after transcorneal freezing, IL-1β released by PMNs into aqueous humor stimulates the production of all isoforms of FGF-2 in corneal endothelium through PI3-kinase and p38 pathways. The IL-1β-
Induced FGF-2 subsequently alters the actin cytoskeleton and cell shapes, phenotypes observed during the EMT process.

**Materials and Methods**

**Materials**

New Zealand White rabbits, each weighing 4 to 5 pounds, were used in this study. All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Anti–Akt, phospho-Akt (Thr508), p38, and phospho-p38 antibodies were purchased from Cell Signaling Technology (Danvers, MA). SB203580, LY294002, anti–β-actin antibody, and peroxidase-conjugated secondary antibodies were obtained from Sigma-Aldrich (St. Louis, MO). Anti–FGF-2 antibody was purchased from Upstate (Charlottesville, VA). Rhodamine-phalloidin was from Molecular Probes (Eugene, OR), and fluorescein isothiocyanate-conjugated (FITC) or rhodamine-conjugated secondary antibodies were from Chemicon (Temecula, CA).

**Transcorneal Freezing Procedure**

This procedure was performed as described previously. In brief, each rabbit was anesthetized with a subcutaneous injection of ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (10 mg/kg). Tetracaine solution (0.5%) was instilled to the eyes, and a lid speculum was inserted. Transcorneal freezing was performed with a surgical cryostat unit (Cryogenic CR 300 Unit; Mira, Inc., Uxbridge, MA) using nitrous oxide and a probe temperature of ~80°C. The 2.5-mm probe was first applied to the center of cornea for 30 seconds and then removed for 30 seconds. The superior, inferior, temporal, and nasal corneal quadrants were successively frozen in a similar manner. The procedure was repeated. In this study, rabbits received only one cycle of transcorneal freeze injury, the dosage of which was far below that needed to cause RCFM production in rabbit corneas. The right eye of transcorneal freezing injury, the dosage of which was far below that needed to cause RCFM production in rabbit corneas. The right eye of each rabbit was treated with cryothermy, and the left eye was used as control. Neomycin/polyoxylin antibiotic ointment was instilled to prevent bacterial infection immediately after completion of the freezing procedure, and buprenorphine hydrochloride (Buprenex; Reckitt & Colman Pharmaceutical Inc., Richmond, VA) was injected subcutaneously every 12 hours for pain relief. In some experiments, pharmacologic inhibitors LY294002 (PI3-kinase inhibitor) and SB203580 (p38 inhibitor) were topically applied. These inhibitors were mixed with a 2.5% hydroxypropyl methylcellulose ointment (Gonak; Akorn Pharmaceuticals Inc., Gurnee, IL) and were used at a concentration of 200 µL each sample was added to the same volume of anti–IL-1β antibody-conjugated beads in a 96-well filter plate and were incubated at room temperature for 30 minutes. After a series of washes to remove the unbound proteins, 25 µL biotinylated IL-1β antibody, which will detect a different epitope from the bead-conjugated IL-1β antibody, was added to each well and incubated for 30 minutes. After another washing, 50 µL streptavidin-phycocerythrin was added to each well. Thereafter, 125 µL assay buffer was added to each well, and the well contents were analyzed (Bio-Plex Manager 2.0 software; Bio-Rad Laboratories, Inc.). The unknown IL-1β concentration was determined by finding the concentration on the standard curve derived from various concentrations of IL-1β standards in the assay. The cytokine assay allowed the quantitative measurement of multiple cytokines in a small sample volume, comparable to traditional ELISA.

**Immunohistochemistry Using Endothelium-Descemet’s Membrane Complex**

After aqueous sampling, rabbits were euthanatized by intravenous injection of sodium pentobarbital. Immediately thereafter, the corneas were removed and fixed for 10 minutes in 4% paraformaldehyde in PBS. The whole Descemet’s membrane with corneal endothelium was carefully peeled from the cornea using a blunt-tip surgical spatula and was placed on the slides (Super Plus; Fisher Scientific) endothelium side up. After washing, the corneal endothelium was permeabilized for 30 minutes with 1% Triton X-100 (Sigma-Aldrich) in PBS at room temperature, and nonspecific IgG binding was blocked with 4% bovine serum albumin in PBS for 1 hour at room temperature. Mouse monoclonal anti-human FGF-2 (Calbiochem, Cambridge, MA) was used as the primary antibody. The slides were incubated with primary antibody (1:200) for 2 hours at room temperature in a moist chamber and then washed with PBS. Next they were incubated with FITC-conjugated secondary antibody (1:200) for 2 hours. After extensive washing, the slides were mounted in a drop of mount medium (Vectashield; Vector Laboratories Inc., Burlingame, CA). Antibody labeling was examined with a fluorescence microscope. To evaluate morphologic changes, corneal endothelium was immunostained with rhodamine-phalloidin (1:200) at 48 hours after transcorneal freezing.

**Protein Preparation and Immunoblot Analysis**

The whole corneal endothelium-Descemet’s membrane complex was lysed with RIPA lysis and extraction buffer. Concentration of the resultant lysates was assessed with the Bradford protein assay system (Bio-Rad Laboratories, Inc.). The proteins were separated by SDS-PAGE using the discontinuous Tris-glycine buffer systems. Proteins separated by SDS-PAGE were transferred to a 0.22-µm nitrocellulose membrane (Whatman Inc., Florham Park, NJ), and nonspecific binding sites of nitrocellulose membrane were blocked by 5% nonfat milk in PBS containing 0.1% Tween-20. The incubations were performed with primary antibodies (1:2500 dilution) and peroxidase-conjugated secondary antibody (1:5000 dilution). Membranes were treated with enhanced chemiluminescence (ECL) reagent (Amersham Biosciences Corp., Piscataway, NJ) and exposed to ECL film. The relative density of the polypeptide bands detected on ECL film was determined using commercial software (Gel-doc; Bio-Rad Laboratories, Inc.).

**Ex Vivo Incubation of Rabbit Cornea**

Rabbit eyes were purchased from Pel Freeze Biologicals (Rogers, AR). Corneas were removed from the eyes, and the whole corneas were placed endothelial side up in individual wells of a 24-well tissue culture plate. Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen-Gibco, Grand Island, NY) without serum was used for ex vivo incubation.
Corneal tissue was treated with IL-1β/H9252 (5 ng/mL) for 24 hours in the absence of induced injury to evaluate the effects of IL-1β on the corneal endothelium. After 24-hour ex vivo incubation, the endothelium-Descemet’s membrane complex was carefully stripped and used for immunohistochemical staining and Western blot analysis, as described earlier.

**RESULTS**

**Corneal Opacities and Histopathology after Transcorneal Freezing**

External photographs were taken at 6, 12, 24, 30, 36, and 48 hours after cryotreatment to evaluate the effect of transcorneal freezing on corneal clarity. At 6 hours after freezing, the rabbit corneas showed mild corneal opacity. The corneal hazy continued to increase until 24 hours after cryotreatment and was markedly decreased at 48 hours after freeze injury (Fig. 1A). Hematoxylin and eosin staining of the corneal endothelium-Descemet’s membrane complex showed that the corneal endothelium was seriously damaged by freezing at 24 hours after cryotreatment. The wounded area completely lost its corneal endothelial cells and was clearly demarcated from the untreated area. At higher magnification, numerous PMNs were seen to infiltrate the corneal endothelium at the wound area and the wound border. DAPI staining clearly contrasted the polymorphonuclear shape of PMN nuclei (arrowhead) and the corneal endothelial cell nuclei (arrow). Scale bars, 100 μm.

**Measurement of Aqueous Concentration of IL-1β**

Because the corneal endothelium-Descemet’s membrane complex showed a marked infiltration of PMNs after freezing, the aqueous concentration of IL-1β was expected to increase. Therefore, we measured the IL-1β concentration in aqueous humor collected from 6 hours to 48 hours after transcorneal freezing. No IL-1β was detected in normal aqueous humor, whereas IL-1β was detected 6 hours after freezing. The concentration gradually increased, reaching the maximum level at 24 hours after cryotreatment. The IL-1β concentration then rapidly decreased; no IL-1β was detected at 36 hours after freeze-injury (Fig. 2).

**FGF-2 Production and Its Intracellular Location in Response to IL-1β Stimulation**

Our in vitro study demonstrated that IL-1β greatly induced FGF-2 production in CECs. Therefore, we investigated whether the aqueous IL-1β was able to induce FGF-2 produc-
the maximum level at 24 hours after freezing, and afterward the concentration rapidly decreased.

We previously reported that FGF-2 induced a cell shape change in CECs from a polygonal to a fibroblastic morphology through the reorganization of actin cytoskeleton. We, therefore, determined whether FGF-2 overproduction induced by cryotreatment modulated the structure of the actin cytoskeleton and subsequently the cell shape of the corneal endothelium in an in vivo system. The rabbit eye was subjected to cryoinjury, and the eye was enucleated 48 hours after freeze injury. Corneal endothelium-Descemet’s membrane complex was carefully stripped at 6, 12, 24, 30, 36, and 48 hours after cryotreatment and then was lysed with RIPA buffer. FGF-2 in the purified subcellular fraction was analyzed by immunoblotting; the untreated corneal endothelium-Descemet’s membrane complex contained all isoforms of FGF-2 at a detectable level, whereas the amount of FGF-2 obtained from the cryotreated tissues gradually increased in a time-dependent manner, reaching the maximum level 30 hours after freeze injury. The FGF-2 level was then also decreased in a time-dependent manner (Fig. 3A). Regardless of the time after freeze injury, the 18-kDa isoform of FGF-2 was the major form.

We then determined the intracellular location of FGF-2; in the absence of freeze injury, the corneal endothelium demonstrated a diffuse staining pattern of FGF-2 at the plasma membrane. On the other hand, heavy nuclear staining and diffuse ECM staining were observed in the corneal endothelium 24 hours after freeze-injury (Fig. 3B). Finally, we confirmed the in vivo finding on the inductive activity of injury-triggered IL-1β on FGF-2 production using an ex vivo study. Rabbit corneas were treated with IL-1β (5 ng/mL) for 24 hours in the absence of induced injury. Corneas maintained in the absence of IL-1β showed significant amounts of all isoforms of FGF-2, whereas there was a great increase of all isoforms of FGF-2 in the IL-1β-treated cornea (Fig. 4A) compared with that of the basal level. When the subcellular location of FGF-2 was analyzed, corneal endothelium stimulated with IL-1β demonstrated strong nuclear staining (Fig. 4B), suggesting that all isoforms of FGF-2 are localized in the nuclei on IL-1β stimulation. This finding is consistent with our previous findings that showed the freshly expressed 18-kDa FGF-2 was translocated into the nuclei before secretion to the extracellular space.31

Morphologic Change of Corneal Endothelium after Transcorneal Freezing

We previously reported that FGF-2 induced a cell shape change in CECs from a polygonal to a fibroblastic morphology through the reorganization of actin cytoskeleton.29,32 We, therefore, determined whether FGF-2 overproduction induced by cryotreatment modulated the structure of the actin cytoskeleton and subsequently the cell shape of the corneal endothelium in an in vivo system. The rabbit eye was subjected to cryoinjury, and the eye was enucleated 48 hours after freeze injury. Corneal endothelium-Descemet’s membrane was peeled from the cornea and immunostained with rhodamine-phalloidin. The cobblestone cell shape was greatly altered to a large and irregular shape, and actin cytoskeleton at the cortex was markedly disrupted (Fig. 5). Stress fiber formation was also observed in some cells that lost the adherens junctions. On the other hand, normal corneal endothelium showed a cobblestone cell shape, actin cytoskeleton was well organized at the cortex, and the adherens junctions were well maintained (Fig. 5).

Involvement of PI3-Kinase and p38 in the Inductive Activity of IL-1β on FGF-2 Production

As shown in Figure 1B and Figure 2, the aqueous IL-1β concentration was influenced by the degree of the inflammatory cell infiltration. We attempted to examine whether the release of IL-1β by PMNs into the aqueous humor was directed by signal transduction. To determine the optimum concentration of the inhibitors, we tested three different concentrations of each inhibitor to elucidate whether these inhibitors reach the corneal endothelium through the corneal epithelium and the stroma and to show the inhibitory effects on the FGF-2 synthesis of corneal endothelium. Both inhibitors inhibited FGF-2 production from the concentrations of 200 μM, and, even in higher concentrations, the effects were similar (data not shown). When the rabbit eyes were treated with PI3-kinase

![Figure 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932966/ figure2).

**Figure 2.** Aqueous concentration of IL-1β after cryotreatment. Aqueous humor was aspirated from the anterior chamber using a 0.1-ml syringe at 6, 12, 24, 30, 36, and 48 hours after freezing. The IL-1β concentration in aqueous humor was measured with a cytokine assay kit. IL-1β was detected at 6 hours after cryotreatment, and the concentration gradually increased. IL-1β concentration in aqueous humor reached the maximum level at 24 hours after freezing, and afterward the concentration rapidly decreased.

![Figure 3](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932966/ figure3).

**Figure 3.** FGF-2 production and its intracellular location in corneal endothelium after transcorneal freezing. (A) The whole corneal endothelium-Descemet’s membrane complex was carefully stripped at 6, 12, 24, 30, 36, and 48 hours after cryotreatment and then was lysed with RIPA buffer. FGF-2 in the purified subcellular fraction was analyzed by immunoblotting. The untreated corneal endothelium-Descemet’s membrane complex contained all isoforms of FGF-2 at a detectable level, whereas the amount of FGF-2 obtained from the cryotreated tissues gradually increased in a time-dependent manner until reaching the maximum level at 30 hours after freezing injury. Afterward, the level of FGF-2 gradually decreased. (B) The intracellular location of FGF-2 was determined by immunohistochemistry. The corneal endothelium of normal cornea demonstrated a diffuse staining pattern of FGF-2 at the plasma membrane, whereas the corneal endothelium 24 hours after cryoinjury showed heavy nuclear staining and diffuse ECM staining.
FGF-2, whereas corneas treated with IL-1β showed a significant amount of all isoforms of FGF-2, whereas corneas treated with IL-1β revealed a great increase of all isoforms of FGF-2 when compared with that of the basal level. (B) Subcellular location of FGF-2 was analyzed by immunohistochemistry. The corneal endothelium stimulated with IL-1β showed strong nuclear staining.

**Figure 4.** FGF-2 production and its intracellular location in response to IL-1β in an ex vivo study. (A) Rabbit corneas were treated with IL-1β (5 ng/mL) for 24 hours in the absence of induced injury, and the amount of FGF-2 was analyzed by immunoblotting. Corneas maintained in the absence of IL-1β showed a significant amount of all isoforms of FGF-2, whereas corneas treated with IL-1β revealed a great increase of all isoforms of FGF-2 when compared with that of the basal level. (B) Subcellular location of FGF-2 was analyzed by immunohistochemistry. The corneal endothelium stimulated with IL-1β showed strong nuclear staining.

inhibitor or p38 inhibitor before and after cryotreatment, neither LY294002 nor SB203580 blocked the release of IL-1β into the aqueous humor (Fig. 6A). Our in vitro study demonstrated that corneal endothelial cells produced FGF-2 in response to IL-1β stimulation through PI3-kinase and p38 pathways.8,24 We, therefore, determined whether PI3-kinase and p38 pathways were also involved in the inductive activity of IL-1β on FGF-2 production in the in vivo condition. Figure 6B shows that both inhibitors decreased the elevated FGF-2 production mediated by the cryoinjury, albeit at a partial level. To confirm the in vitro data of signal transduction of the inductive activity of IL-1β on FGF-2 production, we compared the phosphorylation of Akt at the Thr308 residue and the phosphorylation of p38 in cryotreated corneal endothelium with or without the specific inhibitors. Figures 6C and 6D show that LY294002 and SB203580, respectively, blocked the PI3-kinase and p38 activity in the corneal endothelium obtained from the cryoinjured corneas. The findings confirm our in vitro data and indicate that the inductive activity of IL-1β on FGF-2 is triggered by PI3-kinase and p38 pathways in the in vivo system. Finally, we determined whether these inhibitors reduced the degree of morphologic change of corneal endothelium after cryotherapy through the pathway inhibiting FGF-2 production. When corneal endothelium was stained with F-actin to elucidate the morphologic changes, the morphologic changes and the disruption of actin cytoskeleton triggered by cryotreatment were partially blocked by topical treatment of both inhibitors (Fig. 7).

**DISCUSSION**

Transcorneal freezing has been used to produce corneal wounds for the experimental study of various corneal cell functions.33-34 We developed an RCFM animal model in our previous study using transcorneal freezing.30 In the present study, we used a far smaller dosage of transcorneal freezing injury to investigate the relationship between injury-mediated inflammation and the subsequent cellular event triggered by inflammation. Our in vitro study clearly indicates that IL-1β plays a pivotal role in the EMT observed in nonregenerative wound-healing processes.6,24 Among the broad spectrum of EMT processes, the present study focused on the early events that occur during the injury-mediated inflammatory response and attempted the in vivo validation. In this study, we showed that transcorneal freezing induced massive PMN infiltration of the corneal endothelium and elevated the levels of aqueous concentration of IL-1β in a time-dependent manner. The aqueous concentration of IL-1β was detected at 6 hours after cryoinjury and reached the maximum level 24 hours after cryothermy. The increase of IL-1β concentration in the aqueous humor was followed by the increase of FGF-2 production in corneal endothelium; the amount of FGF-2 was gradually increased in a time-dependent manner and reached the maximum level at 30 hours after freezing injury; thus it is likely that the aqueous IL-1β probably stimulates FGF-2 production in corneal endothelium, as demonstrated in our in vitro studies.6,24 Nonetheless, it should be noted that transcorneal freezing also damages the corneal epithelium and corneal stroma and that this damage may trigger the influx of PMNs and the subsequent release of IL-1β by the infiltrated PMNs. It is not known whether the PMNs that infiltrate the epithelium and stroma release IL-1β and whether such IL-1β plays a role in the upregulation of FGF-2 in CECs in addition to the aqueous IL-1β. We further confirmed the inductive activity of IL-1β on the FGF-2 production of corneal endothelium using an ex vivo study. Subcellular location of the FGF-2 induced by cryoinjury also confirmed our in vitro study in which the freshly expressed FGF-2 is located in the nuclei.31

**Figure 5.** Morphologic change in corneal endothelium after transcorneal freezing. The rabbit eye that was subjected to cryoinjury was enucleated 48 hours after freeze-injury. Corneal endothelium-Descemet’s membrane complex was stripped and immunostained with rhodamine-phalloidin so that morphologic changes of the corneal endothelium could be investigated. Normal corneal endothelium showed a cobblestone shape, actin cytoskeleton was well organized at the corneal junctions, and actin cytoskeleton at the cortex was remarkably disrupted. Stress fiber formation was also observed in some cells that lost the adherens junctions.
Our in vitro study demonstrates that CECs produce FGF-2 in response to IL-1β through the PI3-kinase and p38 pathways. To determine whether the release of IL-1β by PMNs into the aqueous humor was directed by signal transduction, rabbit eyes were simultaneously treated with PI3-kinase inhibitor or p38 inhibitor before and after cryotreatment. Neither LY294002 nor SB203580 blocked the release of IL-1β into the aqueous humor. In our in vitro study, corneal endothelial cells produced FGF-2 in response to IL-1β stimulation through PI3-kinase and p38 pathway. We, therefore, determined whether PI3-kinase and p38 pathways were also involved in the inductive activity of IL-1β on FGF-2 production in vivo conditions. Both inhibitors reduced the elevated FGF-2 production mediated by the cryoinjury, albeit at a partial level. (A) To confirm the in vitro data of signal transduction of the inductive activity of IL-1β on FGF-2 production, phosphorylation of Akt at Thr308 residue and phosphorylation of p38 were compared in cryotreated corneal endothelium with or without the specific inhibitors. LY294002 and SB203580, respectively, blocked the PI3-kinase and p38 activity in the corneal endothelium obtained from the cryoinjured corneas. C, normal; LY, LY294002; SB, SB203580.

**Figure 6.** Involvement of PI3-kinase and p38 in the inductive activity of IL-1β on FGF-2 production. (A) To determine whether the release of IL-1β by PMNs into the aqueous humor was directed by signal transduction, rabbit eyes were simultaneously treated with PI3-kinase inhibitor or p38 inhibitor before and after cryotreatment. Neither LY294002 nor SB203580 blocked the release of IL-1β into the aqueous humor. In our in vitro study, corneal endothelial cells produced FGF-2 in response to IL-1β stimulation through PI3-kinase and p38 pathway. We, therefore, determined whether PI3-kinase and p38 pathways were also involved in the inductive activity of IL-1β on FGF-2 production in in vivo conditions. Both inhibitors reduced the elevated FGF-2 production mediated by the cryoinjury, albeit at a partial level. (C, D) To confirm the in vitro data of signal transduction of the inductive activity of IL-1β on FGF-2 production, phosphorylation of Akt at Thr308 residue and phosphorylation of p38 were compared in cryotreated corneal endothelium with or without the specific inhibitors. LY294002 and SB203580, respectively, blocked the PI3-kinase and p38 activity in the corneal endothelium obtained from the cryoinjured corneas. C, normal; LY, LY294002; SB, SB203580.
in the inductive pathway of IL-1β on FGF-2 production, PI3-kinase and p38 can be used as therapeutic targets to intervene in the production of FGF-2, the direct mediator of EMT. In clinical ocular diseases such as pseudoexfoliation syndrome and syphilitic interstitial keratitis or during the corneal wound-healing process after freezing or mechanical injury, CECs showed fibroblastlike transformation. These small molecular pharmacologic inhibitors may be used as biological agents to intervene not only in the inductive activity of IL-1β on FGF-2 but in the cellular activity of FGF-2 as they control EMT. Thus, these specific inhibitors may be used to control the whole spectrum of nonphysiological wound-healing processes, not only in RCFM but in all types of fibrosis.

References

FIGURE 7. Effects of pharmacologic inhibitors on morphologic changes of corneal endothelium after cryoinjury. To determine whether PI3-kinase inhibitor and p38 inhibitor reduced the degree of morphologic changes in corneal endothelium after cryoinjury, corneal endothelium was stained with F-actin 48 hours after freezing. The morphologic change and the disruption of actin cytoskeleton triggered by cryotreatment were partially blocked by topical treatment of both inhibitors. LY, LY294002; SB, SB203580.


