Effect of the Rho Kinase Inhibitor Y-27632 on Corneal Endothelial Wound Healing

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PURPOSE. The purpose of this study was to investigate the feasibility of using Rho-associated kinase (ROCK) inhibitor eye drops for treating severe corneal endothelial damage due to surgical invasion.

METHODS. A rabbit corneal endothelial damage model was created by mechanically scraping half the area of the corneal endothelium of eighteen eyes of Japanese white rabbits. A selective ROCK inhibitor, Y-27632 (10 mM), was applied topically for 2 weeks, and then the anterior segment was evaluated by slitlamp microscopy. The corneal endothelium was evaluated by phalloidin staining and immunohistochemical analysis. We then conducted pilot clinical research and applied Y-27632 eye drops topically to three patients who exhibited severe corneal edema due to corneal endothelial damage.

RESULTS. In the corneal endothelial damage rabbit model, more Ki67-positive cells were detected in Y-27632-treated eyes than in control eyes. Five of six corneas became transparent in Y-27632-treated eyes, whereas zero of six corneas became transparent in the control eyes (P < 0.01). Actin fibers were distributed at the cell cortex in the eyes treated with Y-27632, whereas actin distribution was partially disrupted, and stress fibers were observed in control eyes. N-cadherin and Na+/K+-ATPase were expressed in almost all cells in Y-27632-treated eyes, but expression decreased in control eyes. Preliminary human cases confirmed that ROCK inhibitor eye drops were considerably effective for treatment of corneal edema associated with cataract surgery.

CONCLUSIONS. ROCK inhibitor may be developed as an eye drop for treating acute corneal endothelial damage to prevent progression of bullous keratopathy. (University Hospital Medical Information Network Clinical Trial Registry no. UMIN000003625; www.umin.ac.jp/ctr)

Keywords: bullous keratopathy, corneal endothelial cells, PBK, ROCK inhibitor

In the United States, pseudophakic bullous keratopathy (PBK) has been the most frequent indicator for corneal transplantation from 1980 to 2005 and has accounted for 28.4% of the corneal transplantations from 2001 to 2005.1 More recently, the Eye Bank Association of America reported that 14,153 corneas (21.3%) were provided for treatment of Fuchs’ endothelial corneal dystrophy and 8244 corneas (12.4%) were provided for post-cataract surgery edema among a total of 66,305 corneas in 2013.2 In Asian countries, as in Western countries, cataract surgery is a leading cause of demand for corneal transplantation.1,3–5 For instance, PBK and aphakic bullous keratopathy (ABK) accounted for 23.4% of corneal transplantations in Singapore from 1991 to 2006,6 44.4% in Japan from 1999 to 2001,7 and 25.5% in India from 1987 to 1995.8

The corneal endothelium is critical for maintaining homeostatic corneal transparency, which it accomplishes by pump and barrier functions. However, the proliferative ability of corneal endothelial cells (CECs) is severely limited, so any trauma to the corneal endothelium decreases corneal endothelial cell density due to compensatory migration and spreading of the remaining cells. When corneal endothelial cell density decreases to a critical level (500–1000 cells/mm2), the cornea exhibits haziness, with edema, due to decompensation of the corneal endothelial function. No pharmaceutical intervention is available to reverse this corneal endothelial damage, and the only therapeutic choice is still corneal transplantation.10

However, we recently showed that a Rho-associated kinase (ROCK) inhibitor can be useful in treating corneal endothelial dysfunction. In 2009, we demonstrated that the selective ROCK inhibitor Y-27632 showed unique effects on cultured CECs, including (1) promotion of proliferation, (2) enhancement of cell adhesion, and (3) suppression of apoptosis.11 Our subsequent studies revealed that administration of ROCK inhibitor in the form of eye drops promotes CEC proliferation in rabbit and monkey in vivo models.12,13 In addition to animal experiments, we conducted a clinical trial of ROCK inhibitor eye drops in combination with a 2-mm–diameter transcorneal freezing procedure for treatment of human corneal endothelial dysfunction patients.13,14 In that clinical trial, we showed that Y-27632 eye drops effectively reduced corneal edema in...
patients with early stage Fuchs’ endothelial corneal dystrophy, who exhibited central edema with relatively healthy corneal endothelium remaining at the peripheral area. Those findings led us to hypothesize that a ROCK inhibitor could be an effective pharmaceutical treatment for those patients whose corneal endothelium was severely damaged by cataract surgery despite having a certain number of relatively healthy CECs.

In the present study, we tested the feasibility of using ROCK-inhibitor eye drops for treating severe corneal endothelial damage due to cataract surgery. We showed that ROCK inhibitor eye drops promote wound healing of severe corneal endothelial damage in a rabbit model by enhancing proliferation of the remaining CECs. We also performed a pilot clinical research study and showed that ROCK inhibitor eye drops were considerably effective in patients who exhibited corneal edema after cataract surgery.

**MATERIALS AND METHODS**

**Animal Experiment Approval**

In all experiments, animals were housed and treated in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Rabbit experiments were performed at Doshisha University (Kyoto, Japan) according to the protocol approved by the University’s Animal Care and Use Committee (approval no. A15012).

**Fluorescence Staining**

Rabbit corneal specimens were fixed in 4% formaldehyde and incubated in 1% bovine serum albumin for 30 minutes at room temperature to block nonspecific binding. The effect of the ROCK inhibitor on cell proliferation was evaluated by Ki67 staining using anti-mouse Ki67 antibody diluted 1:200 (Sigma-Aldrich Corp., St. Louis, MO, USA). Alexa Fluor 488-conjugated goat anti-mouse immunoglobulin G (IgG; 1:1000 dilution; Life Technologies Corp., Carlsbad, CA, USA) was used as a secondary antibody. Reconstructed corneal endothelium was investigated by conducting immunohistochemical analyses of N-cadherin (1:300 dilution; BD Biosciences, San Jose, CA, USA) and Na⁺/K⁺-ATPase (1:300 dilution; Upstate Biotechnology, Lake Placid, NY, USA). Alexa Fluor 488-conjugated goat anti-mouse (Life Technologies) was used as a secondary antibody. Reconstructed corneal endothelium was investigated by conducting immunohistochemical analyses of N-cadherin (1:300 dilution; BD Biosciences, San Jose, CA, USA) and Na⁺/K⁺-ATPase (1:300 dilution; Upstate Biotechnology, Lake Placid, NY, USA). Alexa Fluor 488-conjugated goat anti-mouse (Life Technologies) was used as a secondary antibody. Reconstructed corneal endothelium was investigated by conducting immunohistochemical analyses of N-cadherin (1:300 dilution; BD Biosciences, San Jose, CA, USA) and Na⁺/K⁺-ATPase (1:300 dilution; Upstate Biotechnology, Lake Placid, NY, USA).

**Rabbit Corneal Endothelial Damage Model**

A rabbit corneal endothelial damage model was created to mimic surgical trauma by removing the lenses of 18 eyes of 9 Japanese white rabbits, using a series 20000 Legacy surgical system (Alcon, Inc., Fort Worth, TX, USA), under general anesthesia; this procedure deepened the anterior chamber. One week after lens removal, half of the area of the corneal endothelium was mechanically scraped from Descemet’s membrane with a 20-gauge silicone needle (Soft tapered needle; Inami and Co., Ltd., Tokyo, Japan) (Figs. 1A, 1B). The scraped area was confirmed by 0.04% trypan blue staining. One eye of each rabbit was used for Y-27632 treatment, and the contralateral eye served as the control. The corneal endothelium of both eyes was damaged to reduce the number of
rabbits required for the study and for correct evaluation of the effect, because wound healing varied depending on the individual rabbit. This procedure was confirmed not to induce complete blindness or any severe general adverse effects. The experimenter who created the endothelial damage model was blinded to subsequent treatment with Y27632 or vehicle.

**Administration of Y-27632 Eye Drops in the Corneal Endothelial Damage Model**

Y27632 (10 mM) diluted in phosphate-buffered saline (PBS; 50 μL) was applied topically four times daily to nine eyes of the corneal endothelial damage model, whereas PBS was applied four times daily to nine eyes as a control. The effect of Y-27632 eye drops on proliferation of the corneal endothelium after 2 days was evaluated by staining with Ki67 (n = 3 samples). Corneal transparency was assessed with slit-lamp microscopy for 14 days (n = 6). Corneal thickness was evaluated by using a Pentacam system (Oculus; Optikgeräte GmbH, Wetzlar, Germany). Corneal endothelium was evaluated by contact specular microscopy (scanning slit specular microscope; Konan Medical, Nishinomiya, Japan).

**Organ Culture**

For rabbit cornea organ culture experiments, twenty rabbit eyes were purchased from the Funakoshi Corp. (Tokyo, Japan). Half the area of the corneal endothelium from ten corneas was mechanically scraped without removing Descemet's membrane. Then, five of these corneas were incubated at 37°C for 48 hours with Dulbecco's modified Eagle's medium (DMEM) supplemented with Y27632 (10 μM), while five corneas were incubated with DMEM alone as control. The proliferative ability of the corneal stroma was evaluated by mechanically removing half the area of the corneal endothelium of ten corneas along with Descemet's membrane. A total of five corneas were incubated at 37°C for 48 hours with DMEM supplemented with Y27632 (10 μM), while three corneas were incubated with DMEM alone as control. After 48 hours, the corneas were stained with Ki67 and phalloidin. The area of corneal endothelium or Descemet's membrane removed was confirmed by 0.04% trypan blue staining in preliminary experiments.

**Pilot Human Clinical Trial of ROCK Inhibitor Eye Drops**

The pilot human clinical trial performed in this study was conducted in accordance with tenets of Declaration of Helsinki. This study was performed according to a protocol approved by the Institutional Review Board of Kyoto Prefectural University of Medicine (approval number C-626-2). Clinical trial registration was obtained from University Hospital Medical Information Network, Clinical Trial Registry no. UMIN00003625; www.umin.ac.jp/ctr). Prior to this, a phase 1 clinical study of Y27632 eye drops involving ten healthy volunteers was conducted (approval number C-626-1), which confirmed that 10 mM Y27632 applied six times a day for 7 days caused no systemic or local side effects. After proper informed consent was obtained, three eyes of three patients were enrolled in this study, which ran from October 2012.

All three patients had undergone cataract surgery, and severe corneal edema had subsequently developed due to corneal endothelial damage. In two patients, more than half of Descemet's membrane area was detached accidentally and was removed during phacoemulsification (cases 1 and 2). In 1 patient, an iris cyst was observed with low corneal endothelial cell density due to an old corneal trauma, and one third to one half of the corneal endothelial area was damaged caused by dissection of the iris cyst during cataract surgery (case 3). For all patients, 1 mM of Y27632 was administered in the form of an eye drop six times daily for 4 months, followed by four times daily for 2 additional months.

**Statistical Analysis**

Statistical significance (P value) of differences in the mean values of the two-sample comparison was determined with Student's t-test. Values shown in the graphs represent means ± SEM.

**RESULTS**

**Effect of Y27632 on Cell Proliferation During Wound Healing in a Rabbit Model**

Administration of 10 mM Y27632 in the form of eye drops to the corneal endothelial damage model was followed 48 hours later by evaluation of the Ki67-positive cells in the wounded area at the center of cornea. The control eye showed approximately 13% of the cell population of Ki67-positive corneas, whereas the Y27632-treated eyes showed approximately 65% Ki67-positive cells (Figs. 1C, 1D). Actin staining showed that Y27632-treated eyes had an endothelial phenotype in which actin was distributed at the cell cortex, whereas control eyes showed irregular patterns of actin fibers.

**Effect of Y-27632 on Wound Healing in a Rabbit Model**

Representative slit-lamp microscopy demonstrated that control eyes exhibited stromal edema due to corneal endothelial dysfunction, especially where the corneal endothelium had initially been removed. On the other hand, eyes treated with Y-27632 eye drops exhibited clear corneas after 2 weeks. (Fig. 1E). Slitlamp microscopy revealed no adverse effects in the corneal epithelium or the stroma. Scheimpflug images and corneal thickness maps obtained with a Pentacam HR also demonstrated that control eyes showed substantial corneal edema while Y27632 treated eyes showed less corneal edema (Figs. 1F, 1G).

We also evaluated the regenerated corneal endothelium by contact specular microscopy, which demonstrated that control eyes showed blurred corneal endothelial images from the border (center of the cornea) to the damaged area. However, the regenerated corneal endothelium observed in Y27632 treated eyes formed a hexagonal monolayer, although the corneal endothelial cell density tended to be lower in the damaged area than in undamaged areas (Fig. 2A). Cell density was almost the same in the undamaged area of eyes treated with Y27632 as in the control eyes, implying that enhanced wound healing by Y27632 occurs mainly by promoted cell proliferation rather than by migration (Fig. 2B). Phalloidin staining showed that corneal endothelium at the border area in the eyes treated with Y27632 had a hexagonal and monolayer morphology and that actin fibers were distributed at the cell cortex. However, control eyes showed partial disruption of the cortical actin and contained stress fibers. N-cadherin (a marker of adheren junctons) and Na+/K+-ATPase (a marker of pump function) were expressed in almost all cells in the Y27632-treated eyes, but expression was decreased in control eyes, suggesting that the ROCK inhibitor enhanced functional recovery as well as morphological recovery (Fig. 2C). No Ki67 expression was observed in Y27632-treated eyes or control eyes after 2 weeks, suggesting that corneal endothelial...
cell proliferation was suppressed, even following treatment with Y-27632, when the remaining CECs covered the damaged area and cell-cell contact was established (Fig. 2C).

**Effect of Y-27632 on Wound Healing in an Organ Culture Model**

We conducted organ culture experiments to determine whether the ROCK inhibitor enhances cell proliferation and migration onto the corneal stroma, as occurs onto Descemet’s membrane. Half of the corneal endothelium (Fig. 1B) was removed with or without Descemet’s membrane. The numbers of Ki67-positive cells were counted 48 hours after organ culture in the absence or presence of Y-27632. In eyes where the corneal endothelium was removed without Descemet’s membrane, as in the in vivo model, 12.8% of the cells observed at the border site in the controls were Ki67-positive cells, whereas 41.7% were Ki67-positive cells in the Y-27632 treated eyes (Fig. 3B). In the eyes where the corneal endothelium was removed with Descemet’s membrane, 11.8% of the control cells were Ki67 positive, while 47.5% of the Y-27632-treated cells were Ki67 positive (Fig. 3C). The corneal endothelium that regenerated onto the corneal stroma had hexagonal cells in a monolayer in the eyes where the Descemet’s membrane was removed, similar to the eyes where the Descemet’s membrane was left intact (Fig. 3A).

**Pilot Human Clinical Research of ROCK Inhibitor Eye Drops for Treatment of Acute Corneal Endothelial Damage due to Cataract Surgery**

We conducted pilot clinical research to determine whether ROCK inhibitor eye drops could rescue the acute corneal endothelial damage due to cataract surgery. An 84-year-old female (case 1) treated with cataract surgery had undergone phacoemulsification performed by her previous physician. During the surgery, Descemet’s membrane had spontaneously detached from the upper incision tunnel, and more than two thirds was aspirated. A foldable IOL was implanted in the capsular bag. She was referred to the cornea clinic of Kyoto Prefectural University of Medicine due to severe corneal edema caused by the loss of corneal endothelium (Fig. 4A). Visual acuity in the right eye was counting fingers. Contact specular microscopy revealed no corneal endothelium at the corneal center owing to edema, but the lower peripheral cornea remained clear and contained relatively healthy-looking endothelial cells at a cell density of 2653 cells/mm² (Fig. 4C). The patient was treated with 1 mM Y-27632 eye drops six times daily for 4 months and four times for 2 months. At 2 weeks, the cornea had recovered its clarity, and the patient’s visual acuity had improved to 20/20 at 3 months (Fig. 4A). Contact specular microscopy showed that the corneal endothelium was regenerated directly onto stroma at 3 months where Descemet’s membrane had been removed (Fig. 4B, Descemet’s...
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A 71-year-old male (case 3) was diagnosed with an iris cyst that was attached to the iris. The patient was from an ocular trauma that occurred approximately 50 years previously. The initial visual acuity was 20/25 (3 months). The central corneal thickness was reduced to 611 μm after surgery and to 503 μm at 3 months. The central corneal endothelial cell density was approximately 500 cells/mm² and was not observable after cataract surgery due to corneal edema but was observed again after therapy with ROCK inhibitor eye drops at a density of approximately 500 cells/mm².

**FIGURE 3.** Effect of Y-27632 on wound healing in an organ culture model. (A) Half of the area of corneal endothelium samples from 10 corneas was mechanically scraped without Descemet’s membrane removal. Then, five corneas were incubated with DMEM supplemented with Y-27632 (10 μM) for 48 hours, while three corneas were incubated with DMEM as controls. Likewise, half of the area of the corneal endothelium of 10 corneas was mechanically removed along with Descemet’s membrane. Then, five corneas were incubated with DMEM supplemented with Y-27632 (10 μM) for 48 hours, while three corneas were incubated with DMEM as control. After 48 hours, Ki67 and phalloidin staining were performed. Nuclei were stained with DAPI. Scale bar: 50 μm. (B) Effect of Y-27632 on proliferation of CECs was evaluated by Ki67 staining after 2 days. Ki67-positive cells were counted at the wound edge (n = 3). Results are shown of Ki67 analysis of organ-cultured cornea in which the corneal endothelium was mechanically scraped with remaining Descemet’s membrane intact. (C) Results are shown of Ki67 analysis of organ cultured cornea in which corneal endothelium was mechanically scraped along with removal of Descemet’s membrane. *P < 0.01. All experiments were performed in duplicate.

removal line is indicated as a white dotted line). Although the regenerated corneal endothelium had a lower cell density than the undamaged area, the cell density of the undamaged area (2732 cells/mm²) did not decrease after cataract surgery (Fig. 4C), suggesting that wound healing occurred by a combination of proliferation and migration and not just by migration.

Case 2 is similar to case 1: an 84-year-old female underwent phacoemulsification, and more than two thirds of Descemet’s membrane was spontaneously detached from the upper incision tunnel and was aspirated. This patient also recovered corneal clarity after administration of 1 mM Y-27632 eye drops six times daily for 4 months and four times for 2 months.

A 71-year-old male (case 3) was diagnosed with an iris cyst due to an ocular trauma that had occurred approximately 50 years previously (Fig. 4D). The iris cyst was attached to the corneal endothelium and corneal endothelial cell density was 508 cells/mm² (Fig. 4E). The patient felt a visual disturbance due to cataract progression, so the cystic iris was dissected from the cornea and standard phacoemulsification was performed following IOL implantation. After the surgery, corneal edema was observed due to corneal endothelial damage, with a visual acuity of 20/63 (Fig. 4D). The patient was treated with the 1 mM Y-27632 eye drops four times daily for 3 months, and the cornea became clear, with a visual acuity of 20/25 (3 months). The central corneal thickness was reduced to 611 μm after surgery and to 503 μm at 3 months. The central corneal endothelial cell density was approximately 500 cells/mm² and was not observable after cataract surgery due to corneal edema but was observed again after therapy with ROCK inhibitor eye drops at a density of approximately 500 cells/mm².

**DISCUSSION**

ROCK was originally discovered as a target of the small GTP-binding protein RhoA. The Rho-binding domain within the coiled-coil region of ROCK was identified, but multiple contact points where several molecules can activate or inhibit ROCK were subsequently demonstrated. As ROCK mediates various cellular events, such as cell shape, motility, adhesion, and proliferation, ROCK signaling activation is involved in numerous diseases. Therefore, ROCK attracted research interest as a potential therapeutic target for vascular disease, cancer, neuronal degenerative disease, asthma, and glaucoma. Several pharmaceutical companies have started clinical trials to develop ROCK inhibitors as next-generation therapeutic agents. Indeed, fasudil was approved for the treatment of cerebral vasospasm in Japan and China in 1995 and ripasudil was approved for the treatment of glaucoma in Japan in 2014.

Earlier researchers reported that inactivation of Rho by C3 blocks G1/S progression in 3T3 fibroblasts, suggesting that activation of Rho facilitates cell-cycle progression, although the cellular responses regulated by ROCK are now known to be cell-type-dependent. In CECs, we demonstrated that inhibition of ROCK signaling promotes cell proliferation. We also showed that a ROCK inhibitor works via phosphatidylinositol 3-kinase signaling that subsequently regulates two proteins of the G1 phase of the cell cycle. ROCK inhibitor upregulates cyclin D and downregulates p27kip1 (p27), and then both activities are subsequently demonstrated. As ROCK mediates various cellular events, such as cell shape, motility, adhesion, and proliferation, ROCK signaling activation is involved in numerous diseases.

We also used a partial corneal endothelial damage model of rabbits and monkeys in previous studies and showed that when the central corneal endothelium was damaged by transcorneal freezing, ROCK inhibitor administration in the form of eye drops enhanced cell proliferation and wound healing of the damaged corneal endothelium. However, the limitation of this experimental model was that the permeability of the pharmaceutical agents to the corneal endothelium was artificially enhanced, because the corneal epithelium was removed as well as the endothelium by transcorneal freezing. In the current study, we mechanically removed half of the area of the corneal endothelium to mimic acute mechanical trauma occurring during cataract surgery. In agreement with the findings from the previous transcorneal freezing model, the ROCK inhibitor eye drops administered in the current model enhanced CEC proliferation and facilitated regeneration of the transparent cornea.

The other question in line with clinical settings is whether a ROCK inhibitor promotes cell proliferation of the remaining...
CECs onto a bare corneal stroma, because Descemet’s membrane can detach spontaneously and be removed during cataract surgery in a certain number of cases. Detachment of Descemet’s membrane can further impair the healing of wounded corneal endothelium, as Descemet’s membrane is a basement membrane of the corneal endothelium. We answered this question by using organ culture experiments, which demonstrated that the ROCK inhibitor promoted proliferation and regeneration of the remaining CECs to almost the same level on Descemet’s membrane and the stroma under our ex vivo experimental conditions. Coincidently, the ROCK inhibitor showed effectiveness in two patients whose Descemet’s membrane were removed during surgery. In those cases, the corneal endothelium was regenerated onto a bare corneal stroma, suggesting that the ROCK inhibitor can be applied to corneal endothelial damage occurring in cases where Descemet’s membrane is accidentally removed.

Corneal transplantation is the only definitive treatment for PBK, although conjunctival flap,27 phototherapeutic keratotomy,28 collagen cross-linking,29 therapeutic contact lens,30 and topical hyperosmotic agents31 have been used to reduce pain. No clinically practical medical therapy has been developed, although pharmaceutical agents such as EGF,32 PDGF,33 FGF-2,34 and small interfering RNA (siRNA) of

![Figure 4](image-url)
connexin 43 have been shown to promote the proliferative capacity of CECs. Here, we showed clinical cases where a ROCK inhibitor seemed to be effective in wound healing of corneal endothelium and in avoidance of development of PBK.

This study had a number of limitations: the cases are very preliminary, no vehicle control cases were included, and patients without treatment were not followed. Nevertheless, this is the first report to show that pharmaceutical agents can be effective in the treatment of post-cataract surgical corneal edema, and the findings encourage us to conduct a randomized clinical trial. Recently, ripasudil, a selective ROCK inhibitor, was approved in Japan for the treatment of glaucoma and ocular hypertension.22 Several preclinical and clinical research studies have shown that ROCK inhibitors do not have severe adverse side effects when applied locally and systemically, suggesting that ROCK inhibitors can be applied as eye drops. Hence, future studies to evaluate the specific form of ROCK inhibitor among numerous kinds of agents that possess the highest potency for enhancement of cell proliferation of CECs will enable promising development of eye drops for the treatment of corneal endothelial damage.

The number of corneal transplantations for PBK in the United States has shown a decline since the late 1990s, perhaps because (1) cataract surgery techniques and devices have developed very rapidly (e.g., phacoemulsification, viscoelastic, and biocompatible intraocular lenses), and (2) most surgeons have completed the learning curve for phacoemulsification. Indeed, the complication rate of PBK was estimated at up to 0.3% of cataract surgeries in 1994,49 and a recent review showed that endothelial cell loss during cataract surgery is <100 cells/mm². However, although data indicating the prevalence of PBK in developing countries are not well elucidated, physicians should still remain aware of PBK/ABK, given that cataract accounts for 51% of the global causes of blindness according to the World Health Organization, and numerous cataract patients are waiting for surgery. If pharmaceutical agents such as ROCK inhibitors can be applied to reduce post-surgical corneal edema, the incidence of PBK/ABK will be minimized.

In summary, we have demonstrated that ROCK inhibitor eye drops promote wound healing by enhancing cell proliferation of CECs in a rabbit model. We also show in preliminary human cases that post cataract surgical corneal edema can be treated with ROCK inhibitor eye drops. These data encourage us to develop ROCK inhibitor eye drops for acute corneal endothelial damage, such as that occurring after invasive ocular surgery and surgery for high risk cases, in order to reduce the incidence of PBK/ABK.

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SK holds a patent, no. 5657252, for Rho-associated kinase (ROCK) inhibitor for corneal endothelium. NO and NK are listed as inventors on the patent.

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