Effect of H-7 on Secondary Cataract After Phacoemulsification in the Live Rabbit Eye

Baohe Tian, Gregg A. Heatley, Mark S. Filla, and Paul L. Kaufman

Abstract

Purpose: This study is aimed to determine if the serine-threonine kinase inhibitor H-7 inhibits secondary cataract after phacoemulsification in the live rabbit eye.

Methods: Eighteen rabbits underwent extracapsular lens extraction by phacoemulsification in 1 eye. The eye was treated with intravitreal H-7 (300 or 1,200 μM ; $n = 6$ or 5) or balanced salt solution (BSS) ($n = 7$) immediately after the surgery and twice weekly for 10 weeks. Each eye received slit lamp biomicroscopy once a week, during which posterior capsule opacification (PCO) was evaluated. The eye was then enucleated and the lens capsule was prepared, fixed, and imaged. PCO was evaluated again on the isolated lens capsule under a phase microscope. Soemmering's ring area (SRA) and the entire lens capsule area were measured from capsule images on a computer and the percentage of SRA (PSRA) in the entire capsule area was calculated. Wet weight of the capsule (WW) was determined on a balance.

Results: No significant difference in PCO was observed in any comparison. No significant differences in SRA, PSRA, and WW were observed between the 300 μM H-7-treated eye and the BSS-treated eye. However, SRA, PSRA, and WW in the 1,200 μM H-7-treated eye were significantly smaller than those in the BSS-treated eye [28.3 ± 16.2 vs. $61.4 \pm 8.86 \text{ mm}^2$ ($P = 0.001$), $33\% \pm 20\%$ vs. $65\% \pm 15\%$ ($P = 0.01$), and 65.6 ± 27.9 vs. $127.0 \pm 37.3 \text{ mg}$ ($P = 0.01$)].

Conclusions: Intravitreal H-7 (1,200 μM) significantly inhibits Soemmering's ring formation in the live rabbit eye, suggesting that agents that inhibit the actomyosin system in cells may prevent secondary cataract after phacoemulsification.

Introduction

SECONDARY CATARACT is the most common complication of extracapsular cataract extraction by phacoemulsification and intraocular lens (IOL) implantation. It not only affects postoperative vision [e.g., posterior capsule opacification (PCO)], but also induces adhesions of anterior and posterior capsules to each other and to the IOL haptics [e.g., Soemmering's ring (SR)]. Generally, secondary cataract is caused by proliferation, migration, and epithelial-to-mesenchymal transition (EMT) of residual lens epithelial cells (LECs) after surgery.¹ Although improved IOL design and surgical technique have reduced the incidence of PCO,^{2,3} laser or surgical posterior capsulotomy is still sometimes necessary, and is not free from the risk of complications. Most importantly, laser or surgical posterior capsulotomy may not be feasible for the next generation of accommodating IOLs that need an intact posterior capsule to function.^{4,5} Additionally, despite reduced proliferation or migration of residual LECs in the central area of the

capsule by improved IOL design, even the best IOLs probably will not prevent SR formation in the peripheral area of the capsular bag. The SR-induced adhesions of the anterior and posterior capsules could substantially limit the movement of the postoperative capsule and accommodating IOLs and, in turn, prevent the IOLs from functioning.^{4,5} Therefore, it is still necessary to prevent secondary cataract after surgery with pharmaceutical agents.

It is well known that the actomyosin system plays major roles in cell proliferation, migration, and EMT. Down-regulation of actomyosin dynamics can affect all the secondary cataract-related cellular processes via inhibiting cell division, contractility and motility and the activation of several EMT-associated target genes.⁶⁻⁹ Integrin antagonists (e.g., RGD peptide or salmosin), which affect cellular contractility by inhibiting focal adhesions,^{10,11} reduced PCO formation in the rabbit eye,^{12,13} suggesting that the inhibition of cytoskeleton-associated proteins may prevent PCO. However, it remains to be determined if agents that directly

inhibit actomyosin-driven contractility will prevent secondary cataract after surgery in living animals.

A number of serine-threonine kinase inhibitors affect actomyosin dynamics. H-7 (1-(5-isoquinoliny-sulfonyl)-2-methylpiperazine), which inhibits several protein kinases including myosin light chain kinase (MLCK), Rho kinase, and protein kinases A and C, dramatically reduces actomyosin-driven contractility, leading to cellular relaxation, deterioration of the microfilaments and perturbation of their membrane anchorage, and loss of stress fibers and focal contacts in various types of cultured cells.^{14–16} Probably through these cytoskeletal modulations and related mechanisms, H-7 inhibits proliferation, migration, and EMT of several nontumor and tumor cell lines,^{17–19} affects collagenase production and matrix metalloproteinase release in cultured human keratinocytes and airway smooth muscle cells,^{20,21} and blocks wound healing in organ-cultured rat corneas.²² Based on these findings, we hypothesize that H-7 might reduce the incidence and/or severity of secondary cataract after lens surgery. In this study, we investigated the inhibitory effect of H-7 on secondary cataract formation after phacoemulsification without IOL implantation in the live rabbit eye.

Methods

Eighteen albino rabbits of both sexes, weighing 3–5 kg, were studied per the University of Wisconsin and NIH Guidelines for Animal Use and the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research. The protocol was approved by the Institutional Animal Care and Use Committee of the UW. No rabbits had undergone any ocular or nonocular procedures and all rabbit eyes were free of anterior chamber (AC) cells and flare by slit lamp biomicroscopy before the study.

Extracapsular lens extraction

Anesthesia was induced in rabbits by pretreatment with ketamine (35 mg/kg, intramuscularly [i.m.]), xylazine (4 mg/kg, i.m.), and buprenorphine (0.03 mg/kg, subcutaneously [s.q.]), and maintained with endotracheal 3% isoflurane in O₂ after animals were intubated. All rabbits underwent extracapsular lens extraction in 1 eye per standard clinical phacoemulsification techniques with some modifications. The pupil of each eye was fully dilated with 1% cyclopentolate, 10% phenylephrine, and 1% tropicamide before the surgery and the maximal pupillary dilation was maintained by pretreatment with 0.03% topical flurbiprofen and continuous administration of intracameral epinephrine through irrigation during the surgery. Balanced salt solution (BSS) was used as the irrigation solution [0.5 mL of epinephrine (1:1,000) and 0.5 mL of heparin (10,000 USP units/mL) in 500 mL BSS]. After performing a peripheral corneal paracentesis and deepening the AC with viscoelastic, a standard 2.8-mm keratome was used to create a shelved keratotomy at the sclerocorneal limbus and a bent needle and Utrata forceps were used to create a 3–4 mm anterior capsulotomy. Hydrodissection with BSS was used to separate capsule from peripheral cortex, and phacoemulsification was performed using an Alcon Surgical Series 20000 Legacy Phaco-Emulsifier Aspirator (flow rate: 36 mL/min; vacuum pressure: 460 mmHg; ultrasound power: 60%). After the lens nucleus and major cortical material were removed from the capsular bag, automated irrigation/aspiration of the capsular

bag was performed using a silicone-sleeve polishing cannula (flow rate: 20 mL/min; vacuum pressure: 100 mmHg). During the irrigation/aspiration, considerable care was taken to remove all observable lens cortical material from the capsular bag and to polish the capsule as completely as possible. No IOL was implanted into the eye (see Discussion section). At the completion of the procedures as above, the corneal incision was closed with 10-0 nylon sutures. H-7 solution or vehicle was then administered intravitreally (see the next paragraph), with the surgeon masked to the treatment. Thereafter, gentamycin (20 mg), methylprednisolone acetate (Depo-medrol; 20 mg), and dexamethasone sodium phosphate (1 mg) were administered subconjunctivally, followed by application of polymyxin/neomycin antibiotic ointment in the conjunctival *cul-de-sac*. Carprofen (2 mg/kg, s.q.) was administered once daily for 5 days beginning at 2 days before the surgery, and buprenorphine (0.01 mg/kg) was administered (s.q.) once daily for 1 or 2 days after surgery. Following surgery, 1 or 2 drops of 1% tropicamide and 10% phenylephrine were applied to the eye once daily for 2 weeks.

H-7 or vehicle administration

H-7 was obtained from Sigma (St. Louis, MO). H-7 solution or vehicle (BSS) was prepared freshly and filtered through a 0.22- μ m syringe-end filter (e.g., Millex®-GV) before use. The rabbit eye was treated intravitreally with 50 μ L of 9 mM (6 eyes) or 36 mM (5 eyes) H-7 or BSS (7 eyes) immediately after surgery and twice weekly for 10 weeks (8 weeks for 2 eyes in the BSS group) under anesthesia (5–10 mg/kg ketamine and 1.5–4 mg/kg xylazine, i.m.). H-7 concentration in the vitreous following the injection with 50 μ L of 9 or 36 mM H-7 was ~300 or 1,200 μ M, assuming a rabbit vitreous volume of ~1.5 mL²³ and rapid complete diffusion equilibrium. For the intravitreal injection, a 30-gauge ½-inch-long needle was inserted directly into the vitreous through the pars plana (2.5–4 mm from the limbus) at the 10 to 11 and 1 to 2 o'clock areas. Before injections, the conjunctival sac was disinfected with 5% povidone iodine followed by a rinse with sterile BSS. Polymyxin/neomycin antibiotic ointment was applied topically after each injection. To avoid potential interference with H-7's effects on residual LECs, no corticosteroids or nonsteroidal anti-inflammation drugs were administered to the animals during the 10-week intravitreal treatment period beyond the initial 3 days following the lens surgery as described above.

Slit lamp biomicroscopy

All rabbits received slit lamp examination once weekly. The cornea status and intraocular inflammation (AC flare and cells) were evaluated per standard clinical methods. PCO formation in the posterior capsule area within the capsulorhexis was evaluated on a 4-point scale: 0 (clear; no visible opacification on the midperipheral or central posterior capsule), 1 (mild; opacification in the midperipheral area only), 2 (moderate; sparse opacification on both the midperipheral and central capsules), and 3 (dense; diffuse and thick opacification in the entire posterior capsule area within the capsulorhexis) (Fig. 1).²⁴

Evaluation of lens capsular bags

After 10 weeks' treatment and observation, animals were sacrificed by overdose of pentobarbital sodium. Eyes were

FIG. 1. Definitions of the areas of the lens capsular bag.

enucleated and lens capsules were prepared by carefully dissecting the cornea and iris, cutting the anterior sclera in the coronal plane 2 mm posterior to the limbus and removing the vitreous under an operation microscope. The zonules were not touched. The lens capsular bag with attached anterior sclera and ciliary body was flattened in a glass culture dish, fixed with 2.5% glutaraldehyde after carefully recovering the circular shape of the anterior sclera and ciliary body ring and fully extending the posterior capsule, and imaged using a Zeiss Axiovert 200M phase microscope, an Axiocam HRm CCD camera, and Axiovision ver. 4.6.3 imaging software running the MosaiX module (Carl Zeiss Microimaging, Thornwood, NY). SR area (SRA) and the entire capsule area (the area measured along the capsule equator) of each capsular bag were measured in each image using the Axiovision software. The percentage of SRA (PSRA) in the entire capsule area was calculated. PCO was evaluated again per the isolated capsular bag under the microscope using the same 4-point scale as in the slit lamp biomicroscopy. Wet weight of the net capsule (WW) was determined on a balance (CAHN Instruments, Cerritos, CA) after the fixed capsular bag was dissected from its zonular attachments and freed from the anterior sclera and ciliary body.²⁵ Prior to weighing, fixative solution on the capsule surface and in the weighing dish was soaked up by an ophthalmic surgical sponge.

Data analysis

Comparisons of PCO score, SRA, PSRA, and WW between the BSS group and each of the 2 H-7 groups were made by the 2-tailed Mann-Whitney *U* test and the 2-tailed unpaired *t*-test.

Results

Slit lamp biomicroscopy

Following phacoemulsification, there were usually different degrees of cloudiness and edema around the corneal incision. The edema typically affected 10%–40% and, occasionally, 50%–80% of the corneal surface. However, the corneal cloudiness and edema of all eyes gradually decreased and completely disappeared within ~4 weeks.

Generally, the phacoemulsification and injection regimen induced moderate intraocular inflammation in the rabbit eye. The inflammation remained during the 10-week period with some fluctuations. On the first day after surgery, moderate AC flare and trace to 3⁺ AC cells were seen similarly in the H-7-treated eye and the BSS-treated eye. During the 10-week treatment, the AC flare was slightly more apparent in the H-7-treated eye (moderate flare) than in the BSS-treated eye (faint flare), and AC cells (trace or 1⁺) were seen more fre-

quently in the H-7-treated eye, especially in the 1,200 μ M H-7-treated eye, than in the BSS-treated eye.

Posterior capsule opacification

PCO started to form in all rabbit eyes during the first week after surgery and usually reached its maximum within 2 or 3 weeks, as observed by slit lamp biomicroscopy. Average final PCO scores of the BSS-treated eye, the 300 μ M H-7-treated eye, and the 1,200 μ M H-7-treated eye by biomicroscopy were 2.0 ± 0.58 , 2.2 ± 0.41 , and 2.4 ± 0.55 , respectively. PCO of the isolated capsular bag from the operated eyes of all groups was less apparent than that in their corresponding live eyes. Average PCO scores of the aforementioned 3 groups by tissue microscopy were 1.4 ± 0.54 , 1.3 ± 0.52 , and 1.8 ± 0.84 , respectively. No statistically significant difference was observed between any 2 groups in either evaluation. Morphologically, the PCO in the live eye appeared to be mainly composed of "white amorphous materials" that might primarily represent the deposition of the increased aqueous humor protein indicated by the AC flare; the PCO on the isolated capsule was free of the "white amorphous material" and composed primarily of proliferative tissues, scattered cells, and/or fibrosis (Fig. 2), which suggested that the deposited aqueous humor protein on the capsule might be dissolved or washed away during the capsule preparation and/or fixation process. Wrinkles were also seen on some isolated capsules, especially on the capsules of some 1,200 μ M H-7-treated eyes (Fig. 2C, D), perhaps representing inflammatory fibrosis-induced changes in posterior capsule contractility. However, some wrinkles on the capsular bag with less SR formation were clearly capsule folding that occurred during tissue preparation (Fig. 2D).

Soemmering's ring

Grossly, in the 7 eyes of the BSS group, 4 had marked (full or near full) SR and 3 had moderate SR; in the 6 eyes of the 300 μ M H-7 group, 3 had marked SR and 3 had moderate SR; in the 5 eyes of the 1,200 μ M H-7 group, 2 had moderate SR, 2 had mild SR, and 1 had minimal SR (Fig. 2). Distributions of SRA, PSRA, and WW in the different groups are shown in Fig. 3. No significant difference was observed between the 300 μ M H-7-treated eye and the BSS-treated eye in all 3 parameters. However, differences in SRA, PSRA, and WW between the 1,200 μ M H-7-treated eye and the BSS-treated eye were statistically significant by the 2-tailed Mann-Whitney *U* test ($P = 0.005$, $P = 0.018$, and $P = 0.018$, respectively) or the 2-tailed unpaired *t*-test [SRA: 28.3 ± 16.2 vs. 61.4 ± 8.86 mm² (mean $\pm$ standard deviation; $P = 0.001$); PSRA: $33\% \pm 20\%$ vs. $65\% \pm 15\%$ ($P = 0.01$); WW: 65.6 ± 27.9 vs. 127 ± 37.3 mg ($P = 0.01$)].

Discussion

Rabbits are widely used as an animal model for the prevention of secondary cataract after surgery not only because the secondary cataract in the rabbit eye reflects the condition in the human eye, but also because of the short time required for secondary cataract formation in this animal model.²⁵ Additional rationales for selecting the rabbit as animal model in the present study include the rabbit's large eyes and low cost. As (1) an intracapsular sustained release system for H-7 was not available, (2) long-term multiple topical administrations

FIG. 2. Posterior capsule opacification (PCO) and Soemmering's ring (SR) of isolated capsular bags. The *arrow* in each panel indicates the capsulorhexis. (A) A capsular bag from the balanced salt solution (BSS) group, showing moderate PCO [SR extending to the central area and minor midperipheral proliferative tissues (*arrowhead*)] and marked SR (full SR); (B) a capsular bag from the 300 μ M H-7 group, showing moderate PCO (SR extending to the midperipheral posterior capsule, and proliferative tissues, scattered cells, and wrinkles in the central and midperipheral areas) and moderate SR (partial SR formation in all 4 quadrants); (C) a capsular bag from the 1,200 μ M H-7 group, showing dense PCO (apparent proliferations, fibrosis, and wrinkles in the entire posterior capsule) and mild SR (apparent SR formation in only 1 quadrant); (D) a capsular bag from the 1,200 μ M H-7 group, showing moderate PCO (scattered cells and wrinkles/capsule folding in the central and/or midperipheral areas) and minimal SR (no apparent SR visible).

of a high dose of H-7 are drug-consumptive and thus very expensive and might be toxic to the cornea, and (3) frequent intracameral injections of the drug were intolerable for the cornea, H-7 was delivered into the rabbit eye by twice-weekly intravitreal injections in this study. Intravitreal injection is commonly used in studies of posterior segment diseases. A previous study indicated that H-7 in the vitreous could effectively diffuse into the AC.²⁶ As intravitreal injection did not directly mechanically invade the AC, it only induced mild inflammation in the anterior segment as evidenced in the BSS-treated eyes.

PCO is generally caused by proliferation and EMT of residual equatorial LECs that have migrated from the periphery of the capsular bag to the center of the posterior capsule and thus can directly affect postoperative vision. In the present study, no significant difference in PCO was observed between the H-7-treated eye and the BSS-treated eye. As inflammation stimulates wound healing that shares many features with PCO formation,^{27,28} it may also stimulate migration and proliferation of residual LECs to the central area of the posterior capsule, which could attenuate H-7's inhib-

itory effects. Additionally, capsular deposition of the observed aqueous humor protein (AC flare) and cells may also contribute to the early PCO formation in the rabbit eye. Therefore, the slightly denser PCO in the 1,200 μ M H-7-treated eye than in the BSS-treated eye may be not due to any direct effect of H-7 on the capsule, but rather due to the drug-induced intraocular inflammation (see below). A preliminary study has shown that H-7 substantially inhibits PCO formation in cultured human lens capsules,²⁹ supporting this speculation.

SR is the major part of secondary cataract following surgery in the rabbit eye. Although SR usually does not affect the postoperative vision, the SR-induced adhesions of the lens capsule might affect the function of future accommodating IOLs.^{4,5} Additionally, SR is a direct precursor to PCO^{30,31} and, eventually, will extend to the central area of the posterior capsule. Therefore, prevention of SR is as important as that of PCO, especially for future accommodating IOL implantation. To prevent the possible inhibitory effect of IOLs on LEC migration from complicating the experimental results, IOLs were not implanted following phacoemulsification in this

FIG. 3. Distributions of SR area (SRA) (A), percentage of SRA (PSRA) (B), and wet weight of the capsule (WW) (C) in different groups. No significant difference was observed between the 300 μ M H-7-treated eye and the BSS-treated eye in all 3 parameters. The differences in SRA, PSRA, and WW between the 1,200 μ M H-7-treated eye and the BSS-treated eye were statistically significant by the 2-tailed Mann-Whitney *U* test ($P = 0.005$ in A; $P = 0.018$ in B; $P = 0.018$ in C) or the 2-tailed unpaired *t*-test [A: 28.3 ± 16.2 vs. 61.4 ± 8.86 mm² ($P = 0.001$); B: $33\% \pm 20\%$ vs. $65\% \pm 15\%$ ($P = 0.01$); C: 65.6 ± 27.9 vs. 127 ± 37.3 mg ($P = 0.01$)].

study. As residual lens cortex and equatorial LECs are responsible for SR formation,^{30,31} SR's size and mass represent the speed of the regeneration/proliferation of these residual lens materials within the capsular bag. SRA/PSRA and WW are indications of the SR size and mass or density. The smaller values of SRA, PSRA, and WW in the 1,200 μ M H-7-treated eye compared with the BSS-treated eye indicate that 1,200 μ M intravitreal H-7 significantly inhibits SR formation in the rabbit.

In previous studies, 300 μ M H-7 maximally inhibits the actomyosin-driven contractility and perturbs the dynamics of the actin cytoskeleton and associated proteins in various cultured cells.^{14–16} Because 1,200 μ M intravitreal H-7 has shown a similar effect on the pupil's response to pilocarpine as 300 μ M intracameral H-7 in monkeys,²⁶ the peak concentration of H-7 in the AC following each treatment of the 1,200 μ M intravitreal H-7 could be $\sim$ 300 μ M in the rabbit eye. Additionally, the effective PCO-inhibition concentration of H-7 in the cultured human lens capsule is also 300 μ M.²⁹ Taken together, this suggests that H-7's inhibitory effects on the actomyosin-driven contractility and the actin cytoskeleton and associated proteins may be involved in the mechanism of the drug-induced SR inhibition. It is thought that differentiation of residual equatorial LECs into lens fiber cells plays an important role in SR formation³² and that cytoskeletal remodeling, an essential component of both fiber cell elongation and migration, is likely regulated by MLCK and Rho kinase.³³ Additionally, transforming growth factor β is widely implicated in secondary cataract formation after surgery⁴ and serine threonine kinases, including MLCK, Rho kinase, and protein kinases C and A, play important roles in transforming growth factor β signaling.^{19,34,35} As a broad-spectrum serine-threonine kinase inhibitor, H-7 may reduce SR formation by its multiple inhibitory effects on those protein kinases. Further studies are needed to clarify this issue.

In the present study, the observed AC flare and cells in the H-7-treated eye represent responses of the eye to all the interventions including the lens surgery, the intravitreal injection, and the H-7 treatment. Therefore, the response to H-7 alone may be relatively slight. As H-7 might separate cell-cell junctions in the ciliary epithelium,^{14–16} the increased AC flare in the H-7-treated eye compared with the BSS-treated eye could be primarily due to the drug-induced breakdown of the blood-aqueous barrier (BAB). The high dose of H-7 required for the intravitreal injection in the proof-of-concept study may be the cause of the BAB breakdown and can be avoided in future studies. Long-term sustained release of a low dose of H-7 or an analog directly into the capsular bag may not only enhance the drug's effect on secondary cataract, but also reduce the drug-induced inflammation and BAB breakdown. Additionally, as the human eye has a much stronger BAB than the rabbit eye,^{36,37} the H-7-induced BAB breakdown and the consequent increase in aqueous humor protein may not occur in the human to the same degree as in the rabbit. Indeed, no AC flare or cells have been seen following H-7 treatments, including intravitreal injection to a final intravitreal H-7 concentration of 1,200 μ M, in the eyes of nonhuman primates.²⁶ Our previous studies of H-7's effects on the cornea, ciliary body, and retina in monkeys have shown that 300 μ M H-7 in the AC or the vitreous achieved by intracameral or intravitreal administration did not affect the structures of the cornea and ciliary body or the retinal

vascular permeability or electrophysiology.^{38,39} These findings suggest that the effective secondary cataract-inhibition concentration of H-7 will not produce adverse effects on adjacent ocular tissues. Although clinically apparent corneal edema occurred after surgery in some eyes in the study, it appeared in both the treated and control eyes and disappeared in all eyes within 3–5 weeks, indicating that the corneal edema was due to the surgery rather than to the drug treatment. Further studies of H-7 (or analogs such as specific MLCK or rho kinase inhibitors) or agents affecting the actomyosin system by different mechanisms, coated on IOLs or other intracapsular devices, in the live nonhuman primate eye may clarify whether these cytoskeleton-modulating compounds are viable candidates as secondary cataract inhibitors.

Acknowledgments

This study was supported by grants from NIH (R21 EY017612, R01 EY02698, and P30 EY016665) and Research to Prevent Blindness. The authors thank Beth Hennes, Jared McDonald, Julie Kiland, Shelley Wilker, and Kim Maurer for their excellent assistance in rabbit surgeries, and John Peterson for his excellent biomicroscopic photography.

Author Disclosure Statement

No competing financial interests exist.

References

- Wormstone, I.M., Wang, L., and Liu, C.S. Posterior capsule opacification. *Exp. Eye Res.* 88:257–269, 2009.
- Spalton, D.J. Posterior capsular opacification after cataract surgery. *Eye* 13 (Pt 3b):489–492, 1999.
- Nishi, O., Nishi, K., and Sakanishi, K. Inhibition of migrating lens epithelial cells at the capsular bend created by the rectangular optic edge of a posterior chamber intraocular lens. *Ophthalmic Surg. Lasers* 29:587–594, 1998.
- McDonald, J.P., Croft, M.A., Vinje, E., et al. Sarfarazi elliptical accommodating intraocular lens (EAIOL) in rhesus monkey eyes *in vitro* and *vivo*. *Invest. Ophthalmol. Vis. Sci.* 44:E-Abstracts 256, 2003.
- McDonald, J.P., Croft, M.A., Ben-nun, J., Heatley, G., and Kaufman, P.L. Nulens Experimental accommodating IOL in the primate eye. *Invest. Ophthalmol. Vis. Sci.* 48: E-Abstract 3143, 2007.
- Mitchison, T.J., and Cramer, L.P. Actin-based cell motility and cell locomotion. *Cell* 84:371–379, 1996.
- Rosenblatt, J., Cramer, L.P., Baum, B., and McGee, K.M. Myosin II-dependent cortical movement is required for centrosome separation and positioning during mitotic spindle assembly. *Cell* 117:361–372, 2004.
- Wormstone, I.M., Tamiya, S., Anderson, I., and Duncan, G. TGF-beta2-induced matrix modification and cell transdifferentiation in the human lens capsular bag. *Invest. Ophthalmol. Vis. Sci.* 43:2301–2308, 2002.
- Vicente-Manzanares, M., Zareno, J., Whitmore, L., Choi, C.K., and Horwitz, A.F. Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells. *J. Cell. Biol.* 176:573–580, 2007.
- Novak, I.L., Slepchenko, B.M., Mogilner, A., and Loew, L.M. Cooperativity between cell contractility and adhesion. *Phys. Rev. Lett.* 93:268109, 2004.
- Dekkers, B.G., Bos, I.S., Gosens, R., et al. The integrin-blocking peptide RGDS inhibits airway smooth muscle remodeling in a guinea pig model of allergic asthma. *Am. J. Respir. Crit. Care Med.* 181:556–565, 2010.
- Nishi, O., Nishi, K., Mano, C., et al. Inhibition of migrating lens epithelial cells by blocking the adhesion molecule integrin: a preliminary report. *J. Cataract Refract. Surg.* 23:860–865, 1997.
- Kim, J.T., Lee, D.H., Chung, K.H., et al. Inhibitory effects of salmosin, a disintegrin, on posterior capsular opacification *in vitro* and *in vivo*. *Exp. Eye Res.* 74:585–594, 2002.
- Liu, X., Cai, S., Glasser, A., et al. Effects of H-7 on cultured human trabecular meshwork cells. *Mol. Vis.* 7:145–153, 2001.
- Tian, B., Kaufman, P.L., Volberg, T., Gabelt, B.T., and Geiger, B. H-7 disrupts the actin cytoskeleton and increases outflow facility. *Arch. Ophthalmol.* 116:633–643, 1998.
- Volberg, T., Geiger, B., Citi, S., and Bershadsky, A.D. Effect of protein kinase inhibitor H-7 on the contractility, integrity, and membrane anchorage of the microfilament system. *Cell Motil. Cytoskel.* 29:321–338, 1994.
- Shabana, A.H., Florescu-Zorila, S., Lecolle, S., Goldberg, M., and Forest, N. H-7 stimulates desmosome formation and inhibits growth in KB oral carcinoma cells. *Microsc. Res. Tech.* 43:233–241, 1998.
- Tang, X., and Zhang, C. Activation of protein kinases A and C promoted proliferation of chicken primordial germ cells. *Anim. Reprod. Sci.* 101:295–303, 2007.
- Bakin, A.V., Rinehart, C., Tomlinson, A.K., and Arteaga, C.L. p38 mitogen-activated protein kinase is required for TGFbeta-mediated fibroblastic transdifferentiation and cell migration. *J. Cell Sci.* 115:3193–3206, 2002.
- Sudbeck, B.D., Parks, W.C., Welgus, H.G., and Pentland, A.P. Collagen-stimulated induction of keratinocyte collagenase is mediated via tyrosine kinase and protein kinase C activities. *J. Biol. Chem.* 269:30022–30029, 1994.
- Foda, H.D., George, S., Rollo, E., et al. Regulation of gelatinases in human airway smooth muscle cells: mechanism of progelatinase A activation. *Am. J. Physiol.* 277:174–182, 1999.
- Hirakata, A., Gupta, A.G., and Proia, A.D. Effect of protein kinase C inhibitors and activators on corneal re-epithelialization in the rat. *Invest. Ophthalmol. Vis. Sci.* 34:216–221, 1993.
- Friedrich, S., Cheng, Y.L., and Saville, B. Drug distribution in the vitreous humor of the human eye: the effects of intravitreal injection position and volume. *Curr. Eye Res.* 16: 663–669, 1997.
- Inan, U.U., Oztürk, F., Kaynak, S., et al. Prevention of posterior capsule opacification by retinoic acid and mitomycin. *Graefes Arch. Clin. Exp. Ophthalmol.* 239:693–697, 2001.
- Wallentin, N., Lundgren, B., Holmén, J.B., and Lundberg, C. Development of posterior capsule opacification in the rabbit. *Ophthalmic Res.* 34:14–22, 2002.
- Tian, B., Millar, C., Kaufman, P.L., et al. H-7 effects on the iris and ciliary muscle in monkeys. *Arch. Ophthalmol.* 116: 1070–1077, 1998.
- Zaja-Milatovic, S., and Richmond, A. CXC chemokines and their receptors: a case for a significant biological role in cutaneous wound healing. *Histol. Histopathol.* 23:1399–1407, 2008.
- Suzuki, T., Sano, Y., and Kinoshita, S. Conjunctival inflammation induces Langerhans cell migration into the cornea. *Curr. Eye Res.* 21:550–553, 2000.
- Tian, B., Jeyalakshmi, S., Heatley, G.A., et al. Will cytoskeletal drugs prevent posterior capsule opacification (PCO)? *Invest. Ophthalmol. Vis. Sci.* 49:E-Abstract 2773, 2008.
- Chew, J., Werner, L., Stevens, S., et al. Evaluation of the effects of hydrodissection with antimitotics using a rabbit model of Soemmering's ring formation. *Clin. Experiment Ophthalmol.* 34:449–456, 2006.

31. Werner, L., Apple, D.J., and Pandey, S.K. Postoperative proliferation of anterior and equatorial lens epithelial cells. In: Buratto, L., Osher, R.H., and Masket, S., eds. *Cataract Surgery in Complicated Cases*. Thorofare: SLACK Incorporated; 2000; pp. 399–426.
32. Kappelhof, J.P., Vrensen, G.F., Vester, C.A., et al. The ring of Soemmerring in the rabbit: a scanning electron microscopic study. *Graefes Arch. Clin. Exp. Ophthalmol.* 223:111–120, 1985.
33. Robinson, M.L. Lens fiber cell differentiation. In: Dartt, D.A., Besharse, J.C., and Dana, R. eds. *Encyclopedia of the Eye*. Amsterdam: Elsevier; 2010; pp. 543–550.
34. Rosado, E., Schwartz, Z., Sylvia, V.L., et al. Transforming growth factor-beta1 regulation of growth zone chondrocytes is mediated by multiple interacting pathways. *Biochim. Biophys. Acta* 1590:1–15, 2002.
35. Meyer-ter-Vehn, T., Sieprath, S., Katzenberger, B., et al. Contractility as a prerequisite for TGF-beta-induced myofibroblast transdifferentiation in human tenon fibroblasts. *Invest. Ophthalmol. Vis. Sci.* 47:4895–4904, 2006.
36. Bito, L.Z. Species differences in the responses of the eye to irritation and trauma: a hypothesis of divergence in ocular defense mechanisms, and the choice of experimental animals for eye research. *Exp. Eye Res.* 39:807–829, 1984.
37. Klein, E.M., and Bito, L.Z. Species variations in the pathophysiologic responses of vertebrate eyes to a chemical irritant, nitrogen mustard. *Invest. Ophthalmol. Vis. Sci.* 24:184–191, 1983.
38. Kiland, J.A., Miller, C.L., Kim, C.B., et al. Effect of H-7 and Lat-B on retinal physiology. *Curr. Eye Res.* 31:441–455, 2006.
39. Tian, B., Sabanay, I., Peterson, J.A., et al. Acute effects of H-7 on ciliary epithelium and corneal endothelium in monkey eyes. *Curr. Eye Res.* 22:109–120, 2001.

Received: June 10, 2010

Accepted: August 25, 2010

Address correspondence to:

Dr. Baohe Tian

Department of Ophthalmology and Visual Sciences

University of Wisconsin-Madison

600 Highland Ave., CSC F4/319

Madison, WI 53792-3220

E-mail: btian@wisc.edu