Effects of Cholesterol-Lowering Statins on the Aqueous Humor Outflow Pathway

Julia Song, Pei-Feng Deng, Sandra S. Stinnett, David L. Epstein, and P. Vasantha Rao

PURPOSE. To investigate the effects of cholesterol-lowering statin drugs on trabecular meshwork cellular properties and aqueous humor outflow.

METHODS. Primary cell cultures of porcine trabecular meshwork (PTM) and ciliary body (PCB) were treated with either lovastatin or compactin, to determine the effects of statins on cell shape, actin cytoskeletal organization, and cell–extracellular matrix interactions (focal adhesions) by immunofluorescence staining. Changes in myosin light-chain (MLC) phosphorylation were evaluated by Western blot analysis. Changes in Rho GTPase content of membrane fractions from lovastatin-treated PTM cells were assessed by Western blot analysis. A constant-flow, organ-culture perfusion system was used to measure the effects of statins on aqueous humor outflow facility in the anterior segments of porcine eyes.

RESULTS. PTM and PCB cells treated with lovastatin or compactin exhibited dramatic changes in cell shape and cytoskeletal organization within 24 hours, consisting of cell rounding, actin depolymerization, and decreased focal adhesions. These effects were found to be reversible on supplementation with geranylgeranyl pyrophosphate. Both lovastatin and compactin decreased MLC phosphorylation in PTM and PCB cells. PTM cells treated with lovastatin exhibited marked decreases in membrane-bound Rho GTPase. In addition, perfusion of organ-cultured porcine eye anterior segments with 100 μM lovastatin for 96 hours caused a significant increase in aqueous humor outflow facility (110%) compared with control eyes, in a reversible manner.

CONCLUSIONS. This study demonstrates that the statin drugs lovastatin and compactin induce changes in cell shape and actin cytoskeletal organization and decrease MLC phosphorylation in PTM and PCB cells, all of which are events that are likely to lead to cellular and tissue relaxation. In addition, these effects of the statins appear to be mediated by inhibition of isoprenylation of the small GTP-binding proteins such as Rho GTPase. An important finding is that statins exert an ocular hypotensive response in an organ-culture perfusion model, indicating the potential for this class of drugs in glaucoma therapy. (Invest Ophthalmol Vis Sci. 2005;46:2424–2432) DOI: 10.1167/iovs.04-0776

To provide more specific medical treatment for glaucoma, a blinding disease, we need a better understanding of the cellular mechanisms that control aqueous humor outflow. Increased intraocular pressure (IOP) is a major risk factor for primary open-angle glaucoma (POAG), and lowering the IOP is the only current treatment available for the management of POAG. The conventional route of aqueous humor outflow through trabecular meshwork (TM) and Schlemm’s canal (SC) is generally thought to be the major pathway for the drainage of aqueous humor from the eye. Impaired drainage through the conventional outflow pathway is believed to be responsible for the increased IOP in POAG. Therefore, it would seem both necessary and critical to understand the normal regulation of aqueous humor outflow through TM and SC.

Our recent studies and those from other laboratories have demonstrated that the morphologic integrity of TM and SC may influence aqueous outflow through the conventional pathway. Perfusion of various pharmacological agents, particularly compounds known to affect actin cytoskeletal organization and cell–cell junctions, increase aqueous outflow and decrease IOP in both in vivo and in vitro systems. Selective inhibitors of the Rho/Rho kinase pathway, protein kinase C (PKC) or myosin light-chain kinase (MLCK), have all been documented to influence aqueous outflow facility. In many of these studies, the increased outflow facility correlated well with observed changes noted in TM cell shape, decreased myosin light-chain (MLC) phosphorylation, and altered actomyosin cytoskeletal organization. Collectively, these studies have revealed the importance of cellular tension (contraction and relaxation) and the state of the cell–cell junctions, as well as cell–extracellular matrix (ECM) interactions in the modulation of aqueous outflow through TM tissue and that the activity of Rho/Rho kinase pathway may be an important intracellular “switch” regulating these effects on outflow function of TM cells.

Statin drugs are widely used medications for the treatment of hypercholesterolemia and the prevention of coronary artery disease and stroke. Statins have also been demonstrated to be beneficial for many other human diseases, including macular degeneration and multiple sclerosis. Statins are selective inhibitors of HMG-CoA reductase, which decrease the biosynthesis of cholesterol and thereby reduce serum cholesterol levels in humans. However, statins not only decrease cholesterol biosynthesis, they also reduce the levels of various intermediary products of cholesterol biosynthesis. For example, statins decrease the synthesis of isoprenoids, including farnesyl pyrophosphate and geranylgeranyl pyrophosphate. Isoprenoids play an important role in basic cell biology by regulating the activities of various cellular proteins, including small GTP-binding proteins, heterotrimeric G-proteins, and laminins. By decreasing the production of isoprenoids, statins may have other beneficial effects in addition to lowering cholesterol.
actin cytoskeletal organization, and cell–cell and cell–ECM interactions, are covalently modified by isoprenylation at the C-terminal end of the protein.27,29,37–39 This enzymatically regulated posttranslational modification is essential for the activities and functionality of the Rho GTPases in vital cellular processes.22,29

In a preliminary human study, patients who were administered statin drugs had a reduced risk of glaucoma.10 To understand the possible mechanistic basis of these observations and evaluate the role of the Rho/Rho kinase signaling pathway in aqueous humor outflow function through the TM, we investigated the effects of statins on aqueous humor outflow facility. Using lovastatin and compactin, we demonstrated that statins induce changes in porcine TM and CB cell morphology, decrease MLC phosphorylation, and alter actomyosin cytoskeletal organization, similar to the effects of Rho kinase inhibitors.12 All the observed cellular changes induced by statins were associated with increased aqueous outflow facility in an organ-cultured porcine eye anterior segment perfusion model.

MATERIALS AND METHODS

Lovastatin (Mevacor) was generously provided as a gift by Merck Pharmaceuticals (Rahway, NJ). Horseradish peroxidase (HRP), rhodamine-phalloidin, vinculin monoclonal antibody, compactin, and geranylgeranyl pyrophosphate were purchased from Sigma-Aldrich (St. Louis, MO); cell culture medium and fetal bovine serum (FBS) from Invitrogen-Gibco Corp. (Carlsbad, CA); phosphospecific MLC polyclonal antibody from Cell Signaling Technology, Inc. (Beverly, MA); monoclonal anti-RhoA GTPase antibody from Upstate Cell Signaling Solutions (Lake Placid, NY); and Enhanced chemiluminescence (ECL) detection reagents from Amersham Pharmacia Biotech (Piscataway, NJ). All chemicals were of analytical grade.

Cell Cultures

Primary cell cultures of TM and CB were harvested from freshly obtained porcine eyes by collagenase digestion, as described previously by Rao et al.12 For isolating CB cells, the whole ciliary body was thoroughly chopped into small pieces and digested with collagenase IV (2 mg/mL). Digestion was performed for 2 hours in the presence of 0.2 mg/mL porcine albumin in medium-199, and at 37°C with continuous agitation. At the end of the collagenase digestion, the tissue sample was centrifuged (2800 rpm for 10 minutes), suspended in cell culture medium, and plated on 2% gelatin-coated plastic Petri dishes. Cells derived from this procedure were used as CB cells. Both TM and CB cells were cultured at 37°C under 5% CO2, 95% air in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% FBS and penicillin (100 U/mL) and streptomycin (100 μg/mL). All experiments were conducted with confluent cell cultures. Different batches of cells passaged between three and six times were used.

Cytoskeletal Staining

Both PTM and PCB cells were grown to confluence on 2% gelatin-coated, glass coverslips. Cells were treated with lovastatin (1–100 μM) or compactin (15 and 30 μM) for 24 hours in the presence of 10% FBS. Changes in cell shape were recorded with a phase-contrast microscope (IM 35; Carl Zeiss Meditec, Dublin, CA). After treatment with lovastatin or compactin, cells were fixed with 3.7% formaldehyde, as described previously.12 and actin and focal adhesions were stained with rhodamine-phalloidin and primary antibody raised against vinculin in combination with TRITC-conjugated secondary antibody, respectively. Representative fluorescence micrographs were recorded using a fluorescence microscope (Axioplan-II; Carl Zeiss Meditec).

MLC Phosphorylation

To determine the effects of statins on MLC phosphorylation in PTM and PCB primary cell cultures, cells were grown to confluence in the presence of 10% FBS and treated with either with lovastatin (15 and 30 μM) or compactin (25 and 50 μM) for 24 hours. The status of MLC phosphorylation in PTM and PCB cells was determined by urea-glyceral gel electrophoresis and Western blot analysis, according to the procedure of Garcia et al.12 as described previously,12 using phosphospecific anti-MLC (Thr18/Ser19) polyclonal antibody.

Cell Viability and Toxicity

To evaluate the effects of lovastatin and compactin on the viability of PTM cells, cells treated with these compounds (15–100 μM) for 96 hours were subsequently incubated with fluorescein diacetate and propidium iodide, as described in our previous studies.12 Viable cells and dead or damaged cells, which stained green and red, respectively, were evaluated by fluorescence microscope.

Isoprenoid Supplementation Studies

After the PTM and PCB cells were initially treated with lovastatin (30 μM) in 10% serum-containing medium for 24 hours, 10 μM geranylgeranyl pyrophosphate was added to the lovastatin-containing medium and maintained in supplemented medium for 24 hours. The cells were monitored for reversal of lovastatin-induced morphologic changes and fixed and stained for actin and focal adhesions, as described earlier. Similarly, in another set of experiments, we also tested the effects of geranylgeranyl pyrophosphate (20 μM) supplementation on PTM cells treated with 100 μM lovastatin for 96 hours, as described earlier.

Analysis of Membrane-Bound Rho GTPase

To determine the effects of lovastatin on membrane localization of Rho GTPase in TM cells, PTM cells were treated with lovastatin (30 μM) for 24 hours at 37°C in DMEM containing 10% serum. Cell pellets were fractionated to derive membrane fractions by the method of Orford et al.12 Briefly, cells were homogenized in hypotonic buffer containing 10 mM Tris buffer (pH 7.4), 0.2 mM MgCl2, 5 mM N-ethylmaleimide, 2.0 mM Na2VO4, 10 mM NaF, 60 mM phenylmethylsulfonyl fluoride (PMSF), 0.4 mM iodoacetamide, 0.3 μM aprotinin, 5.0 μM pepstatin, and 4.0 μM leupeptin. Cell lysates were centrifuged at 800g for 15 minutes, and the supernatants were further centrifuged at 100,000g for 1 hour at 4°C using a tabletop ultracentrifuge (Beckman-Coulter, Fullerton, CA). The pellets were suspended in the hypotonic buffer containing 5 M urea, 2 M thiourea and 2% CHAPS (3-[3-cholamidopropyl]dimethyl-ammonio-2-hydroxy-1-propanesulfonate) and used as the membrane fraction. Protein content of membrane fractions was determined with a protein assay reagent (Bio-Rad, Hercules, CA).

Equal amounts of protein (50 μg) from membrane fractions of lovastatin-treated and control samples were separated by 12.5% SDS-PAGE gels, followed by electrophoretic transfer of resolved proteins to nitrocellulose filters. Filters were then probed with anti-RhoA monoclonal antibody, and immunoblots were developed with ECL reagents.

Perfusion Studies

Porcine eyes (obtained fresh from a local abattoir) were placed in Perfusion Studies

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the anterior segments of the test eyes were transferred into, and perfused with, medium containing lovastatin (100 μM) for 96 hours. The contralateral fellow eyes were perfused with medium containing vehicle alone.

Data were recorded continuously at 1-minute intervals over 5 days. The outflow facility at the initial 20-hour perfusion before exposure of eyes to drug was taken as baseline facility. The percentage of change in the aqueous outflow facility from the baseline values was computed for both, drug- and sham-perfused eyes. At each 12-hour interval, the significance of differences in median percentage of change in outflow facility was assessed with the Wilcoxon signed ranks test.

In another set of perfusion experiments, we perfused the organ cultured eyes, first with 100 μM lovastatin for 72 hours. Then, the pumps were stopped and the anterior chambers were exchanged with medium, supplemented with 10 μM geranylgeranyl pyrophosphate without lovastatin. Perfusion was continued for another 48 hours with medium supplemented with geranylgeranyl pyrophosphate, and the changes in aqueous outflow were monitored.

PTM and PCB Morphology
At the end of the perfusion period (4 days), four pairs of sham control and drug-treated fellow eyes were fixed for histologic examination with 2.5% glutaraldehyde and 2% formaldehyde at room temperature. Tissue quadrants obtained from drug-treated and control eyes were fixed in 1.0% osmium tetroxide in 0.1 M sodium cacodylate buffer and then stained with 1% uranyl acetate. Finally, sections obtained by microtomy (70 nm) were stained sequentially with KMnO4 and Sato stain and photographed by a light and electron microscope (Jem-1200 EX; JOEL, Tokyo, Japan), as described previously.12

RESULTS

Effects of Statins on Cell Morphology and Cytoskeletal Organization in PTM and PCB cells

Both PTM and PCB cells treated for 24 hours with either lovastatin or compactin (15 and 30 μM) exhibited morphologic changes. These included cell rounding, cell–cell separation, and cell elongation and detachment (Fig. 1). Both lovastatin and compactin exhibited similar effects on PTM (Fig. 1A) and PCB (Fig. 1B) cells. These and subsequent experiments were repeated on at least three separate occasions.

Similarly, both PTM and PCB cells treated with lovastatin (15 and 30 μM) for 24 hours revealed dose-dependent, marked reductions in actin stress fiber and focal adhesion staining (Fig. 2). Compactin (15 and 30 μM) was also found to exert similar effects on both types of cells (data not shown). In addition to these experiments, we have also tested the dose-response effect ofLovastatin on cell morphology and actin cytoskeletal organization with concentrations ranging from 1, 10, 25, 50, and 100 μM. A clear dose-response effect was observed on both cell morphology and actin cytoskeletal organization starting from 1 μMLovastatin (data not shown).

Effects of Lovastatin and Compactin on MLC Phosphorylation in PTM and PCB Cells

To correlate lovastatin- and compactin-induced changes in cell morphology and actomyosin organization with the status of MLC phosphorylation, both PTM and PCB cells were treated withlovastatin and compactin, followed by evaluation of MLC phosphorylation by Western blot analysis. Figures 3A and 3B show the effects ofLovastatin (15 and 30 μM for 24 hours) on MLC phosphorylation in PTM and PCB cells, respectively, and as can be seen from the images, lovastatin reduced MLC phosphorylation in a dose-dependent manner. Compactin exhibited a similar effect on PTM cells (at both 25 and 50 μM with 24-hour treatments; Fig. 3C). These results were confirmed in two independent experiments.

Reversibility of Statin-Induced Changes in TM and CB Cells

Both lovastatin- and compactin-induced changes in PTM and PCB cell morphology and actomyosin organization at drug concentrations ranging from 15 to 100 μM were found to be completely reversible within 24 hours of washout of drug from the cell culture medium. In addition, both PTM and PCB cells did not reveal any noticeable cell toxicity on treatment with these drugs (15–100 μM) for 96 hours. Cells treated with 100 μMlovastatin for 96 hours were 100% viable, as assessed by

FIGURE 1. Lovastatin-induced morphologic changes in PTM (A) and PCB (B) cells. Cells were grown to confluence on glass coverslips in DMEM containing 10% FBS and treated with 50 μM of either lovastatin or compactin. Within 24 hours of drug administration, both cell types showed obvious changes in cell shape, including cell–cell separation, cell elongation, and cell rounding, compared with control cells.
fluorescein diacetate staining, and those cells that detached from the surface were also found to be viable (data not shown).

Reversal of Lovastatin-Induced Morphologic and Cytoskeletal Changes by Isoprenoid Supplementation of PTM Cells

Because statins are known to affect the synthesis of isoprenoids as well as cholesterol,26 we explored whether isoprenoid supplementation alone can influence the lovastatin-induced effects on cell morphology and actin cytoskeletal organization. PTM cells that had been treated initially with 30 μM lovastatin for 24 hours were supplemented with 10 μM geranylgeranyl pyrophosphate for 24 hours. During the supplementation phase, cells were maintained in the presence of lovastatin. Addition of geranylgeranyl pyrophosphate to the cell culture medium completely reversed the lovastatin-induced morphologic and cytoskeletal changes within 24 hours of supplementation (Fig. 4). A similar response was recorded in PCB cells supplemented with geranylgeranyl pyrophosphate (data not shown). These results were confirmed in two additional independent experiments. Cells treated with 10 μM geranylgeranyl pyrophosphate alone showed no difference compared with untreated controls cells in either cell morphology or cytoskeletal organization. In addition, TM cells treated with lovastatin alone (100 μM) for 96 hours revealed changes similar to those observed in a 24-hour treatment with this compound, but with many more cells exhibiting rounded cell morphology. Also, these changes were completely reversible with supplementation of 20 μM geranylgeranyl pyrophosphate for 48 to 72 hours (data not shown).

Lovastatin-Induced Changes in Localization of Rho GTPase to the TM Cell Membrane Fraction

Statins inhibit the synthesis of isoprenoid lipid intermediates, which are required for Rho GTPase activity.27,29 Rho GTPases are posttranslationally modified by a geranylgeranyl-transferase-mediated attachment of geranylgeranyl lipid group at the carboxyl terminal of the protein. This modification is essential for the membrane localization and GTPase activity of Rho GTPases.28,29 Therefore, we determined the content of Rho GTPase in membrane fractions isolated from lovastatin-treated TM cells by Western blot analysis. These data revealed a marked reduction in Rho GTPase levels in lovastatin-treated TM cells (30 μM for 24 hours) compared with untreated controls (Fig. 5) suggesting that lovastatin-mediated decreases in levels of geranylgeranyl isoprenoid cause impaired membrane localization of Rho GTPase.
Effects of Lovastatin on Outflow Facility

Organ-cultured porcine eye anterior segments (n = 8) perfused with lovastatin (100 μM) at a constant flow rate demonstrated a time-dependent increase in aqueous outflow facility (Table 1, Fig. 6). This increase in facility was progressive, starting from 3% at 12 hours after drug perfusion to 150% at 96 hours afterward. Sham-treated eyes exhibited a much lower increase in facility, starting with no change from the baseline facility (3%) at 12 hours and reaching 36% at 96 hours of perfusion. Although there was a large variance in each sample (Table 1), the difference in aqueous outflow facility between sham- and lovastatin-treated samples (n = 8) was statistically significant at 72 (P < 0.039) and 96 (P < 0.016) hours after drug perfusion.

In a second series of perfusions, we tested reversibility of lovastatin-induced changes in outflow facility with geranylgeranyl supplementation. For this, three pairs of cultured anterior segments of porcine eye were first perfused with 100 μM lovastatin for 72 hours after the initial 24 hours of baseline outflow recording. As in the previous perfusion experiment with lovastatin (Fig. 6), outflow facility increased steadily from the baseline facility. This increased facility in outflow started to decline steadily with perfusion of medium containing geranylgeranyl pyrophosphate (10 μM) indicating the reversible...
nature of lovastatin-induced changes in outflow facility (data not shown).

Histology of Aqueous Outflow Pathway in Lovastatin-Perfused, Organ-Cultured Eye Anterior Segments

Histologic examination of lovastatin-perfused porcine eyes (n = 4) by light microscopy revealed only subtle differences between sham- and lovastatin-perfused eyes. Three of four drug-perfused samples revealed distended TM with larger spaces between the TM beams compared with the compact TM in sham-perfused samples. Figure 7 shows a representative image of light microscope based histologic changes in a drug-perfused specimen.

To determine lovastatin-induced changes in TM cell morphology in TM beams, we performed electron microscope-based histologic analysis in drug- and sham-perfused eyes. In both these samples, we could not locate the aqueous plexi area with certainty. However, we did not find differences in TM cell morphology between sham- and drug-perfused samples (Fig. 8). We also did not detect any noticeable differences in corneal endothelium and stroma, between sham- and drug-perfused specimens, based on light microscopic analysis (data not shown).

DISCUSSION

In this study, we sought to determine the effects of statin drugs on the function of the aqueous humor outflow pathway. Statins are selective inhibitors of HMG-CoA reductase and are widely used in the clinical setting for cholesterol reduction and the treatment of myocardial infarction. The results of our study demonstrate that lovastatin increases aqueous outflow facility in the organ-cultured anterior eye segment perfusion model. This effect of the statins was associated with induced changes in cell shape, actomyosin cytoskeletal reorganization, and impaired membrane localization of Rho GTPase, as well as decreased MLC phosphorylation in both PTM and PCB cells in vitro.

TM tissue is known to possess smooth-muscle-like properties, and the contractile characteristics of TM and CB are thought to influence aqueous outflow through the TM. Further, based on several perfusion studies, induced changes in TM cell morphology, intercellular junctions, and focal adhesions correlated with observed changes in aqueous humor outflow facility through the TM.

The small GTPase Rho is believed to have an important role in the regulation of actin cytoskeletal organization and formation of intercellular junctions and focal adhesions in many cell types. Activated Rho GTPase, in turn, influences myosin II-mediated cellular contraction, through the sequential activation of Rho-kinase, which serves as the downstream effector of Rho GTPase. It is noteworthy that the statins have been shown to affect directly the activity of Rho GTPase while inhibiting cholesterol biosynthesis.

Both PTM and PCB primary cell cultures exhibited dramatic changes in cell morphology on treatment with lovastatin and compactin (Fig. 1), and this effect on cell morphology was also associated with an observed decrease in actin stress fiber and focal adhesion staining (Fig. 2). These morphologic and cytoskeletal changes were found to be completely reversible after supplementation of cell culture media with geranylgeranyl pyrophosphate (Fig. 4). This effect of geranylgeranyl pyrophosphate supplementation implicates the potential involvement of Rho GTPases activity in the observed lovastatin-induced changes in cell morphology and cytoskeletal integrity. In support of this finding, we also observed reduced levels of Rho GTPase in membrane fractions from lovastatin-treated PTM cells (Fig. 5). Thus, the geranylgeranyl supplementation data and the changes observed in Rho GTPase activity in our cell culture model suggest a role for Rho GTPases in regulating aqueous humor outflow facility.

<table>
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<th>Drug Perfusion Time (h)</th>
<th>Control</th>
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Drug effects are expressed as the percentage change in outflow facility (compared with baseline values) over 96 hours, in drug- versus sham-perfused paired controls. Data are expressed as the mean ± SE, with the median percentage of change in parentheses. n = 8.

* Wilcoxon signed rank test.
† At hour 96, n = 7.
Pase distribution in the membrane fraction of lovastatin-treated PTM cells collectively demonstrate the involvement of Rho GTPases in statin-induced effects on PTM cell morphology and contractile properties.

In addition to inducing changes in cell morphology and cytoskeletal integrity, we observed that lovastatin and compacitin also substantially reduced MLC phosphorylation levels in both TM and CB cells (Fig. 3). The phosphorylation status of MLC is a key determinant of cellular contraction, and decreased MLC phosphorylation implies cellular relaxation.49–51,55 TM and CB tissues both express Rho GTPase and Rho kinase.12,15,49,56 In earlier studies, these two tissues have been reported to exhibit distinct contractile responses when treated with external factors or agonists of G-protein coupled receptors.10 In this study, however, both the TM and the CB exhibited similar responses in cell morphology, cytoskeletal organization, and MLC phosphorylation.

As has been reported previously in a study of Rho kinase inhibitors,12 we found a strong relationship between the decreased MLC phosphorylation induced by statins and the observed increase in aqueous outflow facility in response to perfusion with lovastatin. Perfusion of organ-cultured porcine eye anterior segments. Perfusion with lovastatin (100 µM) for 96 hours resulted in a significant increase in aqueous outflow facility (150% increase from the initial baseline value) compared with sham-treated control eyes (35% increase from baseline value). After the initial baseline outflow facility was established, anterior segments were perfused with lovastatin for 96 hours, with outflow facility being monitored on a continuous basis. Changes in outflow facility of lovastatin-treated eyes are expressed as the percentage change over the initial 20-hour baseline outflow facility. Data were analyzed by Wilcoxon signed rank test, using the median percentage of change (see Table 1). The percentage of change in outflow facility was based on the mean results from eight independent samples.

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eye anterior segments with lovastatin resulted in increased aqueous humor outflow facility (Fig. 6). We also confirmed that increased aqueous humor outflow facility is reversed by supplementation with lovastatin with geranylgeranyl pyrophosphate. Whereas in the cell culture system, both TM and CB cells exhibited dramatic changes in cell morphology in a reversible manner (Fig. 1), we did not find alterations in TM cell morphology in lovastatin-perfused eyes (Fig. 8). However, the TM facing the anterior chamber exhibited widened space between the trabecular beams (Fig. 7), indicating possible influence on the geometry of TM. In this study, we used the organ-cultured anterior segment perfusion model instead of whole-eye perfusion, because statins typically require at least 18 to 24 hours to demonstrate detectable effects on cell morphology. To reduce the isoprenoid levels below normal, we had to perfuse the samples with statins for more than 24 hours.

Statins are widely used in clinical practice and exhibit widely different potencies for lowering cholesterol levels. Therefore, in the future, it is important to compare and contrast the effects of different classes of statins on aqueous outflow facility and IOP. Because the use of statins generally results in minimal adverse effects, their potential use for lowering IOP is obvious. It should be noted, however, that the concentrations of statins used in this study were much higher (100-fold) than the serum levels of statins in patients who are treated with statins. The results of our study may provide additional support to the recently reported beneficial effect of statins in decreasing glaucoma risk in patients. Alternatively, the latter finding relate more importantly to possible neuroprotective mechanisms, rather than IOP lowering from systemic statin administration. It is noteworthy that statins have been observed to increase retinal ganglion cell axonal growth through inhibition of Rho GTPase and Rho kinase. In addition, inhibitors of Rho kinase have also been reported to stimulate axonal growth in retinal ganglion cells.

In conclusion, this study demonstrates significantly increased aqueous outflow facility with lovastatin in an organ cultured porcine anterior eye segment perfusion model. This effect of statins on aqueous outflow facility appears to be associated with relaxation of TM and CB muscle induced by decreased MLC phosphorylation. The statins appear to exert these changes by affecting the activity of regulatory proteins involved in actomyosin cytoskeletal integrity, such as the Rho GTPases. Although this is an experimental in vitro study and the levels of statins used in this study far exceed those noted in the serum of patients who are on statin regimens, our findings suggest that these compounds could lend themselves to a novel treatment for glaucoma, since the statins are used widely in clinical practice with minimal adverse effects on a long-term basis. Further studies involving both living animals and human subjects seem well justified, to evaluate the therapeutic potential of statins in the treatment of glaucoma.

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References


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