



Review

The role of the actomyosin system in regulating trabecular fluid outflow

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ABSTRACT

Abnormally high resistance to aqueous humor drainage via the trabecular meshwork and Schlemm's canal is highly correlated with the development of primary open-angle glaucoma. Contractility of the actomyosin system in the trabecular cells or inner wall endothelium of Schlemm's canal is an important factor in the regulation of outflow resistance. Cytoskeletal agents, affecting F-actin integrity or actomyosin contractility, or gene therapies, employing overexpression of caldesmon or Rho-A inhibition, can decrease outflow resistance in the drainage pathway. In this review, we discuss the mechanisms underlying these and similar effects on trabecular outflow resistance in living animals and/or in cultured ocular anterior segments from enucleated animal or human eyes.

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1. Introduction

The trabecular meshwork (TM) consists of arrays of collagen beams covered by endothelial-like cells, with loose extracellular matrix (ECM) occupying the spaces between the cells of the adjacent beams. The outermost juxtacanalicular (JXT) or cribriform region has no collagenous beams, but rather several cell layers immersed in a loose web of ECM fibrils. The adjacent Schlemm's canal is a continuous endothelium-lined channel that drains aqueous humor to the general venous circulation (Lütjen-Drecoll et al., 1981; Rohen et al., 1981). TM structure and experimental flow studies suggest that flow resistance is maximal in the JXT region and/or the inner wall of Schlemm's canal, although the exact location of the major resistance barrier is not clear (Johnson, 2006). Glaucoma is a progressive optic neuropathy commonly associated with elevated intraocular pressure (IOP) consequent to abnormally high resistance to aqueous humor drainage via the TM and Schlemm's canal. Glaucomatous eyes exhibit fewer TM cells and abnormally appearing JXT ECM compared to the eyes of age-matched normal individuals (Lütjen-Drecoll et al., 1981; Rohen et al., 1981), suggesting that cells and ECM in the JXT region may be critical in resistance regulation.

The actomyosin system, composed of actin microfilaments and associated proteins, is present in essentially all cells, and is highly

organized in TM and Schlemm's canal cells. There are numerous microfilament-based structures in cells along the trabecular outflow pathway. These structures primarily include focal contacts, adherens cell–cell junctions, and bundles of microfilaments (Geiger et al., 1995). Filamentous actin is the major component of microfilaments, but other actin-associated proteins modulate its organization. Additionally, a physiologically contracted state of the JXT-Schlemm's canal region is required to maintain the microfilament-related structures in the outflow pathway. Microfilaments are involved in a variety of cellular processes from cell adhesion and motility to organelle traffic to adhesion-mediated signal transduction. Therefore, dynamics of the actomyosin system play important roles in changes in cell shape, volume, contractility, and adhesion to neighboring cells and to the ECM. These changes in TM and/or Schlemm's canal cells, which could affect trabecular outflow resistance by altering the dimensions or direction of flow pathways and the amount and composition of the ECM, can be modulated directly by actin-disrupting agents or indirectly by inhibition of specific protein kinase(s) or cellular contractility through administration of protein kinase inhibitors or gene therapy. In this review, we discuss the effects of pharmacological and genetic perturbation of the actomyosin system in TM and Schlemm's canal cells on trabecular outflow resistance.

2. Microfilament disruptors

Latrunculins, marine macrolides that are specific and potent actin-disrupting agents, sequester monomeric G-actin, leading to massive disassembly of filamentous actin. Addition of latrunculin A

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or B (LAT-A, LAT-B) causes destruction of actin bundles and associated proteins in a wide variety of cell types, including human TM cells (Cai et al., 2000; Epstein et al., 1999). This effect is manifested by cell rounding and retraction of the lamellipodium, and accompanied by an apparent “arborization” of the cells. In living monkeys, LAT-A or -B administered intracamerally or topically induces major increases in outflow facility (up to 2- to 4-fold; Peterson et al., 1999, 2000). Single or multiple topical treatments with LAT-A and/or -B also significantly decrease IOP in the monkey eye (Okka et al., 2004; Peterson et al., 1999, 2000). In organ cultures of enucleated porcine eyes or postmortem human eyes, LAT-B significantly increases outflow facility by 60–70% (Epstein et al., 1999; Ethier et al., 2006). Morphological studies indicate that the LAT-B-induced decrease in outflow resistance is associated with cytoskeleton disruption-related structural changes in the TM. Electron microscopy of live monkey eyes has revealed massive “ballooning” of the JXT region following LAT-B treatment, leading to a substantial expansion of the space between the inner wall of Schlemm’s canal and the trabecular collagen beams without observable separations between inner wall cells (see Fig. 1; Sabanay et al., 2006). However, in postmortem human eyes, the facility increase is accompanied by increased openings between inner wall cells (more border or paracellular pores) with only very modest rarefaction of the JXT tissue and separation of the inner wall of Schlemm’s canal from JXT tissue (Ethier et al., 2006). This difference could be attributed to different models used in the two studies. Nevertheless, the facility increase and the extent of inner wall separation from the JXT region in the postmortem human eye are both qualitatively similar to that in the live monkey eye, although the magnitude of the facility increase and morphologic changes are much less in the former than in the latter (Sabanay et al., 2006; Ethier et al., 2006).

3. Protein kinase inhibitors

The serine–threonine kinase inhibitor H-7, which affects a broad spectrum of protein kinases including myosin light chain kinase (MLCK), Rho kinase, and protein kinase C (PKC), inhibits actomyosin-driven contractility dramatically. This leads to cellular relaxation, deterioration of the actomyosin system and perturbation of its membrane anchorage, and loss of stress fibers and focal contacts in many types of cultured cells (Bershadsky et al., 1996; Epstein et al., 1999; Liu et al., 2001; Tian et al., 1998; Volberg et al., 1994). H-7, administered intracamerally or topically to living monkey eyes, doubles outflow facility and decreases IOP (Tian et al., 1998, 2004) by a mechanism independent of the ciliary muscle (Tian et al., 1999a). In cultured anterior segments of porcine, human or monkey eyes, H-7 also significantly increases outflow facility (Bahler et al., 2004; Epstein et al., 1999; Hu et al., 2006). Morphological studies in the live monkey eye indicate that the H-7-induced increase in outflow facility is associated with cellular relaxation and drainage-surface expansion of the TM and Schlemm’s canal, accompanied by loss of ECM. The inner wall cells of Schlemm’s canal become highly extended, yet cell–cell junctions are maintained (Fig. 2; Sabanay et al., 2000, 2004). The morphological changes in the TM of live monkey eyes are consistent with functional changes in isolated bovine TM strips, where the TM pre-contracted by carbachol was relaxed by H-7 (Thieme et al., 1999; Wiederholt et al., 2000). In postmortem cultured anterior segments of human eyes, H-7 causes a partial loss of the endothelial lining of Schlemm’s canal (Bahler et al., 2004).

The specific target kinases affected by H-7 are not well defined, because the non-selective PKC inhibitor staurosporine, the specific PKC inhibitors chelerythrine and GF109203X, and the specific MLCK inhibitor ML-7 similarly increase outflow facility in living monkeys or cultured porcine anterior segments (Tian et al., 1999b, 2000; Khurana et al., 2003). More recently, evidence has shown

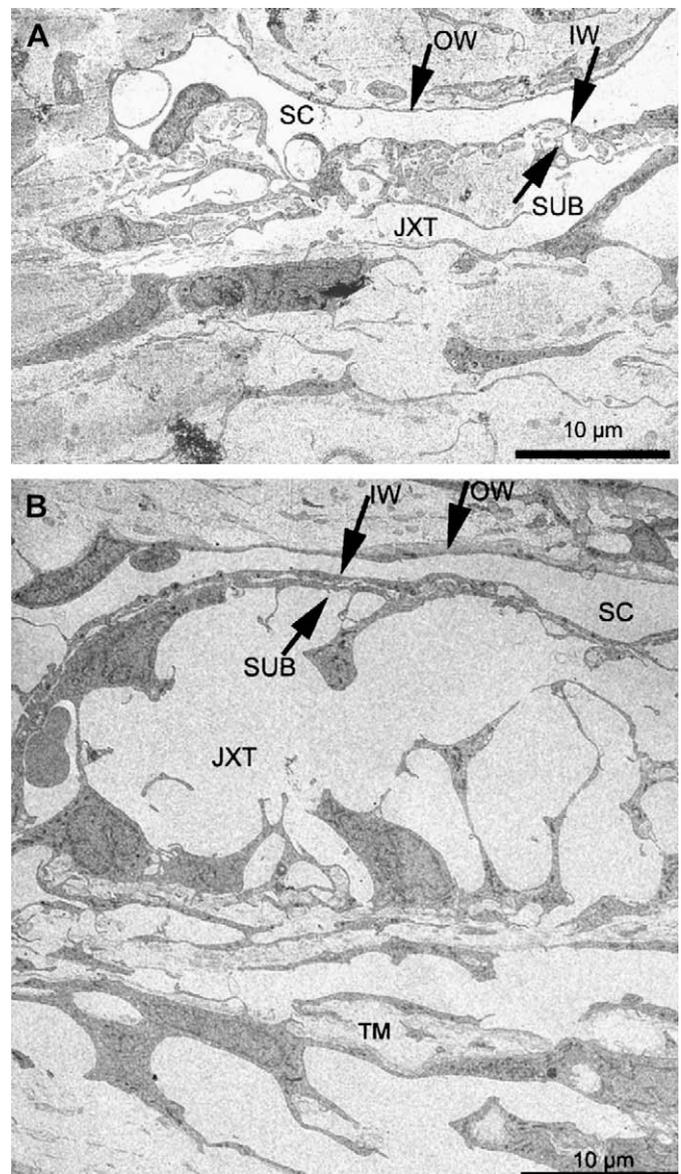


Fig. 1. Transmission electron microscopy of the trabecular meshwork (TM) following LAT-B treatment. IW, inner wall; JXT, juxtacanalicular region; OW, outer wall; SC, Schlemm’s canal; SUB, sub-canalicular cells. Panel A shows normal JXT region and its circumjacent structures; Panel B indicates the massive “ballooning” of the JXT region and the retention of close contact between IW and SUB (compare to (A)). The figure is modified from Sabanay et al. (2006).

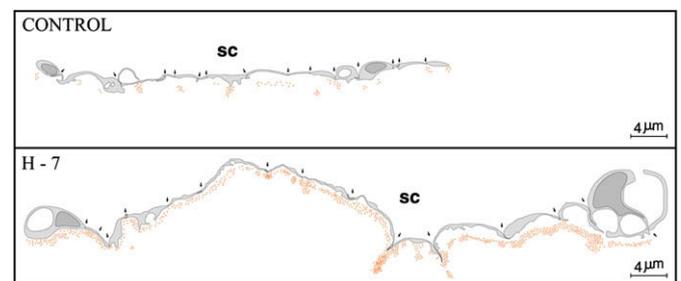


Fig. 2. Schematic drawing depicting 15-cell stretches (cell–cell junctions marked by arrows) along Schlemm’s canal (SC) and distribution of perfused gold particles that crossed the juxtacanalicular area of control and H-7 treated eyes. Location of individual gold particles represented by small dots. The figure is modified from Sabanay et al. (2000).

that inhibition of Rho-associated coiled coil-forming protein kinase (ROCK; Rho kinase) may play a key role in regulating trabecular outflow. Rho kinase is a serine–threonine protein kinase that mainly promotes myosin II activity by inhibiting myosin light chain phosphatase as well as by phosphorylating the myosin regulatory light chain. This, in turn, induces the assembly of contractile actomyosin bundles that generate strong tensile forces (Wettschureck and Offermanns, 2002). A specific Rho kinase inhibitor, Y-27632, induces reversible changes in cell shape and decreases in actin stress fibers, focal adhesions, and protein phosphotyrosine staining in human TM cells and Schlemm's canal cells (Honjo et al., 2001a; Rao et al., 2001). In isolated bovine TM strips, Y-27632 completely blocks Ca^{2+} -independent phorbol myristate acetate or endothelin-1-induced contraction (Renieri et al., 2008; Rosenthal et al., 2005; Thieme et al., 2000). As expected, Rho kinase inhibitors, such as Y-27632, Y-39983, HA-1077, H-1152 and INS117548, increase outflow facility and/or decrease IOP in living rabbits, enucleated porcine eyes and/or living monkeys similar to H-7 (Honjo et al., 2001a, 2001b; Peterson et al., 2008; Rao et al., 2001, 2005b; Tian and Kaufman, 2005; Tokushige et al., 2007; Waki et al., 2001). A recent morphological study in bovine eyes indicates that, with Y-27632, the inner wall of Schlemm's canal and the JXT connective tissue are significantly distended compared to control eyes, with discernible separation between the inner wall and JXT connective tissue. The average percent effective filtration length of the inner wall of Schlemm's canal (filtration length/total length \times 100) is 3-fold larger in Y-27632-treated eyes than in controls. A significant positive correlation is found between the average percent effective filtration length of the inner wall and the average percent separation length (separation length/total length \times 100; separation length = length exhibiting separations between the JXT connective tissue and inner wall), suggesting that the structural correlate to the increase in outflow facility after Y-27632 is physical separation between the JXT connective tissue and inner wall of Schlemm's canal (Lu et al., 2008).

These findings indicate that inhibition of myosin light chain phosphorylation and consequent cellular relaxation are likely the key mechanisms by which protein kinase inhibitors increase outflow facility. The target kinase(s) may include MLCK (Volberg et al., 1994), Rho kinase or ROCK (Thieme et al., 2000; Tian and Kaufman, 2005) and/or PKC (Khurana et al., 2003).

4. Exoenzyme C3 transferase and nonmuscle caldesmon

TM relaxation can also be induced by modulating proteins, such as caldesmon, that negatively regulate actin–myosin interactions. When caldesmon is overexpressed, actin becomes uncoupled from myosin. Additionally, exoenzyme C3 transferase may also affect actin–myosin interactions by inhibiting Rho-GTP at the beginning of the Rho activation cascade, thereby blocking the whole Rho cascade. Within these pathways and among the end products are numerous targets not only for pharmacologic but also for gene therapy development.

Recently, adenovirus-delivered exoenzyme C3 transferase (C3-toxin) cDNA and nonmuscle caldesmon cDNA have been successfully expressed in cultured human TM cells (Grosheva et al., 2006; Liu et al., 2005). Outflow facility in organ-cultured human or monkey eyes has been dramatically increased following overexpression of these genes (Gabelt et al., 2006; Liu et al., 2005). Specific inhibition of Rho-kinase activity in the TM by dominant negative Rho expression increases outflow facility in organ cultured human anterior segments (Rao et al., 2005a).

5. Microtubule inhibitors

The microtubule system is composed of microtubules and associated proteins, and is one of the three major systems (the

microfilament system, the microtubule system and the intermediate filament system) in the cytoskeleton network. Apparently, dynamics of the microtubule system may modulate outflow resistance since microtubule inhibitors increase outflow facility. Microtubules are not intrinsically contractile, but are important for directional cell motility and, driven by specific microtubule motor proteins for cytoplasmic trafficking of vesicles and organelles. Associated proteins that bind to microtubules can affect the latter's stability and potentially attach the latter to other cytoskeletal filaments (e.g., microfilaments). Microtubule function could affect outflow pathway events through direct cellular mechanical effects (e.g., tensegrity; Chen and Ingber, 1999), influences on ECM or cell membrane turnover (via vesicle movement), or through secondary signaling (e.g., leading to activation of the actin cytoskeleton).

Ethacrynic acid (ECA), a sulfhydryl-reactive diuretic drug, inhibits microtubule assembly *in vitro*, and induces a rapid decrease in phosphotyrosine levels of focal adhesion kinase and a more subtle decrease in paxillin phosphorylation. Dephosphorylation of these proteins, which is detected before the onset of retraction, stress fiber disruption, or complete disruption of focal adhesions, disrupts signaling pathways that normally maintain the stability of the actin microfilaments and cellular adhesions, indicating a close relationship between the microtubule system and the actomyosin system. This action leads both to cell shape change *in culture* (Erickson-Lamy et al., 1992; O'Brien et al., 1997), and to facility changes *in vivo* (Epstein et al., 1987; Liang et al., 1992). Recent evidence has shown that several new derivatives of ECA significantly decrease IOP in cats and monkeys (Shimazaki et al., 2004, 2007). These ECA derivatives are more potent than ECA in terms of inducing cell shape alterations and decreasing actin stress fiber content in human TM cells (Rao et al., 2005c), suggesting that microtubule disruption may reduce outflow resistance at least partially through perturbation of the actomyosin system.

6. Clinical relevance

At present, the only effective approach available to treat glaucoma is to reduce IOP. Hypotension medications used clinically include aqueous humor secretory inhibitors (e.g., beta-adrenergic receptor antagonists, α_2 -adrenergic agonists, and carbonic anhydrase inhibitors), uveoscleral-outflow enhancers (e.g., prostaglandin analogues), cholinergic drugs that affect trabecular outflow indirectly by contracting the ciliary muscle and deforming the TM, and epinephrine drugs that work on both the TM (inducing changes in cell shape through a beta-adrenergic receptor-cAMP/PKA-mediated cellular relaxation) and the uveoscleral (mediating endogenously synthesized prostaglandins) outflow routes. Secretory suppression may affect supplies of oxygen and nutrients to the non-vascularized cornea, lens and TM. Prostaglandin analogues do not substantially improve trabecular outflow. Cholinergic drug effects on the pupil and accommodation limit their clinical use. Epinephrine-like drugs are no longer used clinically because of their local and systemic side effects. Thus, there are no TM-selective outflow enhancers in current clinical use.

Numerous studies indicate that dynamics of the TM cytoskeleton may be involved in the regulation of aqueous humor outflow. Reduction of outflow resistance induced by cytoskeletal agents acting directly on the TM/Schlemm's canal of glaucomatous eyes may mimic the normal physiological function. However, cytoskeletal drugs could, in principle, have detrimental effects on other anterior segment tissues, especially the cornea. Lower drug concentrations in larger volumes could minimize corneal toxicity without significantly sacrificing the drug's effect on the TM following topical administration. This speculation is supported by several studies: intracameral doses of ECA reduce IOP in glaucoma patients without inducing corneal or anterior segment side effects (Melamed et al., 1992);

a maximal resistance-reducing intracameral dose of H-7 produces general relaxation and expansion in the TM/Schlemm's canal but no visible changes in the corneal endothelium or ciliary epithelium (Sabanay et al., 2000; Tian et al., 2001); and multiple doses of 0.01% topical LAT-B, which decreases IOP similar to a single dose of 0.02% LAT-B, did not increase corneal thickness as did the latter (Okka et al., 2004; Peterson et al., 2000). However, the potential cornea toxicity is still an obstacle to the use of higher concentrations of the drugs topically for a greater outflow facility increase.

To overcome this problem, novel methods of drug delivery need to be developed. Receptors might be different in different cell types or ECM, so understanding better the bio-molecular differences between cornea and TM, the different molecular targets or mechanisms for different actin-disrupting agents, and a pro-drug, gene therapy or other site-activated approach, could facilitate development of TM-selective "drugs" that reduce outflow resistance without affecting other ocular tissues.

7. Conclusions

This review has provided evidence supporting the crucial role of the actomyosin system in TM cells and endothelium of Schlemm's canal in regulating trabecular fluid outflow. Relaxation of the TM and/or inner wall of Schlemm's canal, which may be induced by pharmacological or genetic perturbation of the actin cytoskeleton, is the major structural change responsible for the increase in trabecular outflow facility. Cytoskeletal drugs and/or cytoskeleton-modulating gene therapies may have potential to open a new avenue in glaucoma treatments.

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