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Open Access Journal of Ophthalmology
ISSN: 2578-465X

Rho Kinase Inhibitors for Glaucoma and Optic Neuropathy Treatment: Enzyme Activity, Cell Effects, IOP-Lowering and Neuroprotection Perspectives

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Review Article

Volume 7 Issue 1

Received Date: December 18, 2021

Published Date: January 31, 2022

DOI: 10.23880/oajo-16000233

Abstract

Rho kinases, (Rho-Associated Coiled-Coil Kinases; ROCKs) are ubiquitous enzymes found inside all cells of the body. Consequently, ROCKs are involved in numerous cellular functions such as cell contraction, cell migration and proliferation. As such, their over-expression / activation has been implicated in diseases ranging from diabetes, erectile dysfunction, hypertension, asthma, cancer, cardiovascular hypertrophy, inflammatory diseases, pulmonary hypertension and so on. In the eye, ROCKs are involved in the development of glaucoma, Fuchs corneal dystrophy, diabetic retinopathy and age-related macular degeneration. Inhibitors of ROCKs have proven useful drugs to treat the afore-mentioned disorders, and in particular they are effective intraocular pressure lowering agents with added neuroprotective activities. This review addresses the latter aspect of ROCK physiology, pharmacology and pathology, and the clinical utility of ROCK inhibitors towards preservation of eyesight from the ravages of glaucomatous optic neuropathy.

Keywords: Eye Diseases; Glaucoma; Glaucomatous Optic Neuropathy; Intraocular Pressure; Retinal Ganglion Cells; Neurodegeneration; Neuroprotection

Abbreviations: ANC: Anterior Chamber; AQH: Aqueous Humor; ECM: Extracellular Matrix; GON: Glaucomatous Optic Neuropathy; ICFP: Intracranial Fluid Pressure; IOP: Intraocular Pressure; JCT: Juxtacanicular Tissue; LC: Lamina Cribosa; NTG: Normotensive Glaucoma; OHT: Ocular Hypertension; ONH: Optic Nerve Head; PACG: Primary Angle-Closure Glaucoma; POAG: Primary Open-Angle Glaucoma; RGC: Retinal Ganglion Cells; ROCK: Rho Kinase; SC: Schlemm's Canal; TM: Trabecular Meshwork.

Introduction

Optic nerve damage leading to visual field loss is caused by several different forms of glaucoma, an optic neuropathy

in which the death of retinal ganglion cells (RGCs) and their axons are end-stage characteristic features [1-4]. While the exact factors involved and the natural history development of glaucomatous optic neuropathy (GON) are incompletely understood, accumulating evidence points to a multifactorial assault on various elements of the eye-brain axis [2-4]. This encompasses both structural and functional components. Fundamentally, it appears that chronically elevated intraocular pressure (IOP) is a principal culprit and trigger in the onset of primary open-angle glaucoma (POAG) [1-6], while primary angle-closure glaucoma (PACG) results due to sudden acutely rising IOP. Even though, ocular hypertension (OHT) is a major risk factor for POAG, advancing / old age, low intracranial fluid pressure (ICFP), family history/African or Hispanic ancestry, IOP spikes [7], low retinal blood flow [8] and defects in axonal transport mechanisms within the optic nerve [9] also contribute to the visual impairment and eventual blindness in glaucoma patients [1-5]. Since glaucomatous optic nerve damage and visual field defects occur asymptomatically, they generally progress slowly over decades and the patient remains oblivious to that damage that is occurring to their RGCs and their axons within the optic nerve and various brain structures [1-5,10,11-17]. Hence, early detection, diagnosis and treatment of different forms of glaucoma is critical to restricting retinal/brain damage, and thus for preserving eyesight of those that suffer from GON [18,19]. It is predicted that by 2040, there will be >112 million patients on our planet afflicted with glaucoma [20]. Thus, it is imperative that novel suitable treatment modalities be found and approved for effective, efficient and optimal medical management of glaucomas [21-23].

Pathological Aspects of OHT Associated with POAG

As is well documented, the anterior chamber (ANC) of the eye contains aqueous humor (AQH) which is formed and released by non-pigmented ciliary epithelial cells of the ciliary body[24] (Figure 1). The AQH provides oxygen and nutrients to the avascular tissues/cells lining the ANC and removes metabolic waste from the ANC as it flows towards the drainage pathway, the trabecular meshwork (TM) and the Schlemm's canal (SC) [25-29] (Figure 1). The TM comprises arrays of beams of connective tissue possessing a core of elastic and collagen fibers which are covered by monolayers of endothelial-like TM, with loose extracellular matrix (ECM) laid down between neighboring beams [26,27,29]. Recent work has identified several different type of cells making up the TM which suggests multiple functionalities [30,31]. The cribriform region is the outermost non-lamellated juxtacanalicular tissue (JCT) which is made up of a loose formless ECM web. This outermost cribriform layer is continuous with the endothelial lining of SC which drains the AQH into the venous circulation [28,32].

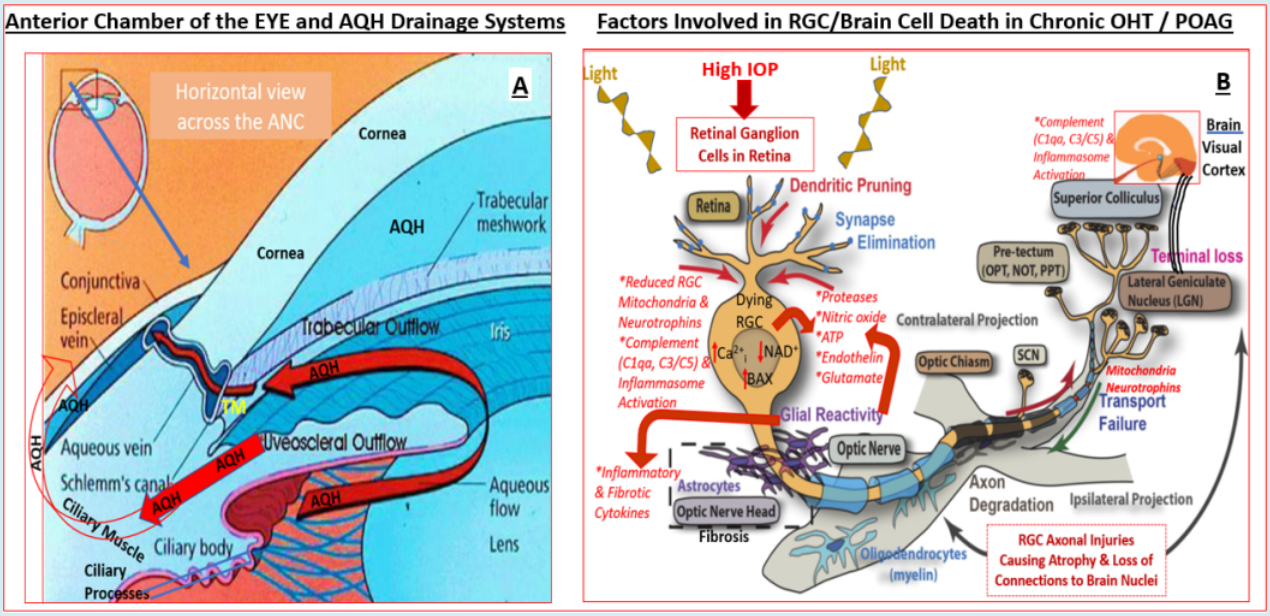


Figure 1: The AQH production and egress from ANC of eye, and the factors that kill RGCs /RGC axons in chronic OHT/POAG.

The aging process, coupled with other local pathological factors in POAG such as aberrant release of damaging cytokines like tissue growth factor-β (TGF-β) [33,34] and connective tissue growth factor cause deposition of excess collagen and fibronectin [35,36] in and around the TM thereby obstructing the efficient drainage of the AQH from

the ANC. Normally, the TM actively produces and secretes proteases that digest the accumulated extracellular matrix (ECM) and the TM cells phagocytose and eliminate the resultant components of ECM. However, age-related stiffness [26,37] and senescence of TM cells [38] and oxidative stress [39-41] and mitochondrial damage [42,43] causes a diminution in ECM clearance [44] and clogging of the TM/SC structures, with resultant accumulation of excess AQH in the AC and an increased IOP. Such chronically elevated IOP propagates and mechanically stretches, distorts [45] and damages the vulnerable tissues at the back of the eye at the optic nerve head (ONH) [46-48] and lamina cribosa (LC) [11,49,50] regions where the RGC axons gather before leaving the eyeball as part of the optic nerve connecting the retina to the brain. The ensuing local inflammation [51-53], vasoconstriction of retinal blood vessels [8] and RGC axonal constriction [9,54-56], and some vascular leakage leads to release of cytokines [51], chemokines, proteases [57] and other substances like endothelin [56,58] (Figure 1). Additionally, immune cells migrate to the retina and activated microglia [18,59-61] release copious amounts of nitric oxide [46,47] and other damaging factors [56,58] and deposit complement components in the retina [61] and brain structures associated with visual perception [18,62]. These events and agents conspire to cause retinal ischemia, restrict growth factor and mitochondrial transport from the brain to the RGCs which ultimately cause RGC axon atrophy and RGC apoptosis [18,63-65] (Figure 1). If left untreated this leads to peripheral vision loss and can eventually result in blindness [1-5,14].

Current and Future Treatment Modalities for OHT/POAG

OHT appears to be a major root cause of visual impairment in POAG and many other forms of glaucoma including normotensive glaucoma (NTG) [66,67]. Several clinical trials have demonstrated the benefits of lowering IOP to slow down the progression of visual damage and loss of vision [68-72]. Indeed, strong evidence supports the concept that lowering of IOP whether by topical drugs [14, 68-72,73] or after surgical intervention [74,75] results in both structural and functional improvements. Thus, the removal of excess AQH from the ANC of the eye reverses LC displacement [73], reduces retinal nerve fiber layer thinning [76], and prevents RGC loss [75] coupled with enhancement of visual fields in the OHT/glaucoma patients. Thus, for every 1mmHg reduction in IOP there is a 10-13% reduction in the progression of GON [1-5,14,66-70].

As a result of the afore-mentioned positive effects of IOP-lowering, many drugs, AQH drainage shunts, and various surgical techniques to relieve the pressure in the eye have been developed and approved by health agencies worldwide. Thus, topical ocularly administered drugs to reduce AQH production (e.g. beta-blockers: timolol, betaxolol; carbonic anhydrase inhibitors: dorzolamide, brinzolamide; alpha-adrenergic agonists: brimonidine and apraclonidine) [1,3,24], to enhance AQH outflow from the ANC via the TM/SC (e.g. muscarinic agonists: pilocarpine; rho kinase [ROCK] inhibitors: ripasudil, netarsudil; FP-receptor prostaglandin agonists (latanoprost, travoprost, tafluprost [76-78]; EP2-receptor agonists: omidenepag isopropyl [79-81]) , and drugs to promote drainage of AQH via the uveoscleral pathway (e.g. FP-receptor prostaglandin agonists: latanoprost, travoprost, tafluprost [75,76]; EP2-receptor agonists: omidenepag isopropyl [79-81]), and various conjugates (e.g. latanoprostene bunod [82]) and combination products [83,84] are prescribed to lower and control IOP [1-4] (Table 1). ROCK inhibitors (e.g. ripasudil [85-87]; netarsudil [88,89]; and others) that promote AQH outflow via the TM/SC conventional pathway and also by reducing episcleral venous pressure will be discussed in more detail below. Many other classes of investigational compounds with ocular hypotensive activity have also been studied in various animal models of acute and chronic OHT and POAG [3,90-92] (Table 2).

Brand Name of Drug & Year of Clinical Introduction/ FDA or EMEA or Japan Approval (where known)	Generic Name & Drug Type. (IOP reduction achieved in OHT/POAG patients)	Dosage Type (%, w/v)	Topical Ocular Dosing Frequency	Mechanism of Action to Lower IOP	Some Side-Effects/ Adverse reactions
Approved Enhancers of Conventional Outflow (TM/SC Pathway) of AQH to Lower IOP					
Isopto carpine (1974); Pilopine	Pilocarpine (muscarinic receptor agonist)	1%, 2%, 4% ; 4% Gel	1 drop 2-4-times daily; single application of gel across the eye	Enhances conventional (TM) outflow of AQH	Brow-ache, miosis; accommodative change, eye irritation, eye pain, blurred vision, and/or visual impairment, potential tachycardia.

Isopto Carbachol	Carbachol (muscarinic receptor agonist)	1.5, 3% solution	1-2 drops up to 3-times daily	Enhances conventional (TM) outflow of AQH	Brow-ache, miosis, accommodative change, eye irritation, eye pain, blurred vision, and/or visual impairment, potential tachycardia.
Glanatec (2014 Japan)	Ripasudil (Rho kinase [ROCK] inhibitor) (3.5-4.5 mmHg IOP reduction)	0.4% solution		Enhances conventional (TM) outflow of AQH	Conjunctival hyperemia, allergic conjunctivitis, blepharitis, punctate keratitis
Rhopressa (2017)	Netarsudil (Rho kinase [ROCK] inhibitor) (5 mmHg IOP reduction)	0.02% solution	1 drop daily	Enhances conventional (TM) outflow of AQH; also decreases episcleral veinous pressure	Conjunctival hyperemia, corneal verticillata, instillation site pain, and conjunctival hemorrhage.
Approved Inhibitors of AQH Production to Lower IOP					
Timoptic (1978) Timoptic-XE Gel	Timolol (beta-adrenoceptor antagonist);	0.25%, 0.5% solution or gel-forming solution	1 drop 1-2-times daily	Reduces production of AQH from CB	Signs and symptoms of ocular irritation, (e.g. burning, stinging, itching, tearing, redness), conjunctivitis, blepharitis, keratitis, dry eyes, decreased corneal sensitivity, blurred vision, corneal erosion. Visual disturbance, including refractive changes
Betoptic (1985)	Betaxolol (beta-1-selective adrenoceptor antagonist);	0.25% suspension; 0.5% solution	1 drop 2-times daily; 1-2 drops twice daily	Reduces production of AQH from CB	Transient ocular discomfort, Decreased corneal sensitivity, erythema, itching sensation, corneal punctate keratitis, anisocoria, blurred vision, foreign body sensation, tearing, dryness of eyes, inflammation, discharge, ocular pain, decreased visual acuity, crusty lashes and photophobia; Bradycardia, heart block; Pulmonary distress characterized by dyspnoea, bronchospasm, thickened bronchial secretions, asthma and respiratory failure; Insomnia, dizziness, vertigo, headaches, depression, lethargy
Alphagan (1996)	Brimonidine (2-6 mmHg IOP reduction)	0.15%, 0.2% solution	1 drop 3-times daily	Reduces production of AQH from CB and enhances UVS AQH outflow	Allergic conjunctivitis, conjunctival hyperemia, and eye pruritis; local ocular hypersensitivity; blurred vision, burning sensation of eyes, drowsiness, eye headache, stinging of eyes, foreign body sensation

Iopidine (1987)	Apraclonidine	0.5% solution	1-2 drops 3-times daily	Reduces production of AQH from CB	Hyperemia (redness), itching, tearing of the eye, Blurred vision or change in vision, chest pain, clumsiness or unsteadiness, depression, dizziness, eye discharge, irritation, or pain, irregular heartbeat
Trusopt (1994)	Dorzolamide (carbonic anhydrase inhibitor;	2% solution	1 drop 3-times daily	Reduces AQH generation by the CB	Transient bitter taste and superficial punctate keratitis, eye irritation, burning, stinging, and ocular discomfort; blurred vision, excessive tearing, dry eyes, and increased sensitivity to light,
Azopt (1998)	Brinzolamide (carbonic anhydrase inhibitor;	1% suspension	1 drop 3-times daily	Reduces AQH generation by the CB	Temporary blurred vision, bitter/ sour/unusual taste, dry eyes, temporary discomfort, itching, redness of the eye, foreign body sensation, eye discharge, and headache.
Approved Stimulators of UVS Outflow of AQH to Lower IOP					
Xalatan (1996)	Latanoprost (FP-prostaglandin receptor-selective agonist;	0.005% solution	1 drop at bedtime	Enhances AQH outflow via the UVS pathway and some via TM/SC pathway	Blurred vision, burning, stinging, itching, hyperemia, foreign body sensation, changes in eyelash number/color/length/thickness, iridial darkening, (pigmentation), periocular skin darkening, deepening of eyelid sulcus (loss of periorbital fat), dry eye, eyelid crusting and discomfort, increased sensitivity to light.
Travatan (2001)	Travoprost (FP-prostaglandin receptor-selective agonist;	0.004% solution	1 drop at bedtime	Enhances AQH outflow via the UVS pathway and some via TM/SC pathway	Blurred vision, burning, stinging, itching, hyperemia, foreign body sensation, changes in eyelash number/color/length/thickness, iridial darkening, (pigmentation), periocular skin darkening, deepening of eyelid sulcus (loss of periorbital fat), dry eye, eyelid crusting and discomfort, increased sensitivity to light.
Lumigan (2001)	Bimatoprost (FP-prostaglandin receptor-selective agonist;	0.03% solution	1 drop at bedtime	Enhances AQH outflow via the UVS pathway and some via TM/SC pathway	Increased conjunctival hyperemia, darkening of eyelids, increased thickening and number of eyelashes, dry eye, eye irritation, eye itching. Hirsutism (a condition of hair growth on parts of the body normally without hair).

Taflotan (2008 Japan) Zioptan (2012 USA)	Tafluprost (FP-prostaglandin receptor-selective agonist;	0.0015% solution	1 drop at bedtime	Enhances AQH outflow via the UVS pathway and some via TM/SC pathway	Ocular surface burning, stinging, irritation, hyperemia, foreign body sensation, dry eyes, watering eyes, iridial darkening, periocular skin darkening, abnormal eyelash growth, and increased sensitivity to light.
Rescula (2000)	Unoprostone (FP-prostaglandin receptor agonist;	0.15% solution	1 drop twice daily	Enhances AQH outflow via the UVS pathway and some via TM/SC pathway	Eye burning, stinging, dry eyes, itching, increased length of eyelashes, and injection; iridial darkening, blepharitis, cataract, conjunctivitis, corneal lesion, discharge from the eye, eye hemorrhage, eye pain, keratitis, irritation, and photophobia.
Eybelis (2018 Japan)	Omidenepag Isopropyl (EP2-receptor selective non-prostaglandin agonist;	0.002% solution	1 drop daily	Enhances AQH outflow via the UVS pathway and via TM/SC pathway	Transient conjunctival hyperemia, corneal thickening.
Some Approved Combination Products for Lowering IOP					
Cosopt (1998)	Dorzolamide + Timolol	2% + 0.5%	1 drop 2-times daily	Reduce AQH production from CB	Combination of side-effects from both drugs
Combigan (2007)	Brimonidine + Timolol	0.2% + 0.5%	1 drop every 12 hrs	Reduce AQH production from CB	Combination of side-effects from both drugs
Simbrinza (2013)	Brinzolamide + Brimonidine	1% + 0.2%	1 drop 3-times daily	Reduce AQH production from CB	Combination of side-effects from both drugs
Roclatan (2019)	Netarsudil + Latanoprost	0.02% + 0.005%	1 drop daily	Enhancement of AQH outflow via TM/SC and UVS pathways	Combination of side-effects from both drugs
Xalacom	Latanoprost + Timolol	0.005% + 0.5%	1 drop daily	Enhancement of AQH outflow and by inhibiting AQH production	Combination of side-effects from both drugs
Duotrav	Travoprost + Timolol	0.004% + 0.5%	1 drop daily	Enhancement of AQH outflow and by inhibiting AQH production	Combination of side-effects from both drugs
Ganfort	Bimatoprost + Timolol	0.03% + 0.5%	1 drop daily	Enhancement of AQH outflow and by inhibiting AQH production	Combination of side-effects from both drugs

Taflotan + Timolol	Taflotan + Timolol (>13 mmHg IOP reduction; 40% lowering)	0.0015% + 0.5%	1 drop daily	Enhancement of AQH outflow and by inhibiting AQH production	Combination of side-effects from both drugs
Other Products for Lowering IOP					
Vyzulta (2017)	Latanoprostene Bunod (conjugate of latanoprost and an NO-donor agent)	0.024% solution	1 drop at bedtime	Enhances AQH outflow via the UVS pathway and via TM/SC pathway	Eye discomfort / irritation, hyperemia, temporary blurred vision, increase in eyelash number/ length/thickness and darkening of the eyelashes/eyelids and iris.
Durysta Implant (2020)	Intracamerally injected sustained-delivery biodegradable polymer containing bimatoprost	Not applicable	Once implanted into the ANC of the eye (intracameral injection), the drug elutes off the implant over 6-months.	Enhances AQH outflow via the UVS pathway and via TM/SC pathway	Conjunctival hyperemia, foreign body sensation, eye pain, photophobia, conjunctival hemorrhage dry eye, eye irritation increased IOP, corneal endothelial cell loss, vision blurred, iritis, headache.

Table 1: Approved ocular hypotensive drugs to treat chronic OHT and POAG with highlighted ROCK inhibitors.

Compound Classes	Drug Candidates	Reported or Potential Mode(s) Of Action
Conventional Outflow (via TM pathway) Promotors		
Inhibitors of chloride transport	Ticrynafen; Ethacrynic acid; Indacrinone	Inhibition of Na+-K+-Cl--transporter activity in the TM changes cell shape & volume and thus AQH efflux is increased
Kinase inhibitors	Chelerythrine; Staurosporin;	Modification of actomyosin contractility that leads to changes in actin cytoskeleton of TM and this leads to AQH efflux; direct relaxation of the TM may also be involved
	LIM-K inhibitors (e.g. LX7101);	
	Myosin-II ATPase inhibitor: Blebbistatin.	
	Src kinase inhibitor	
Rho Kinase (ROCK) Inhibitors	Fasudil; Y-27632; AMA0076; ITRI-E-212	Modification of actomyosin contractility that leads to changes in actin cytoskeleton of TM and this leads to AQH efflux; direct relaxation of the TM may also be involved.
Marine macrolids	Latrunculins A and B; Bumetanide; Swinholide	Promote sequestration of actin monomers and dimers in TM; cause cell TM shape change and thus AH efflux
Guanylate cyclase activators	Natriuretic peptides and constrained cyclic peptides: ANP; CNP; TAK-6	Type-A and type-B receptor activation leads to cGMP production, TM relaxation and AQH efflux via TM.
NO Donors	Sodium nitroprusside; Hydralazine; 3-morpholinosyndnonimine; (S)-nitrosoacetylpenicillamine; NCX-125	NO activates intracellular soluble guanylate cyclase to increase cGMP production, TM relaxation and AQH efflux via TM.

Soluble guanylate cyclase activators	IWP-953; MGV354;	These compounds directly activate intracellular soluble guanylate cyclase to increase cGMP production, TM relaxation and AQH efflux via TM.
k-opioid receptor agonists	Bremazocine; Dynorphin	Release natriuretic peptides and thus raise cGMP in TM leading to its relaxation & thus AQH efflux
Cannabinoid receptor agonists	WIN55212-2; CP55940; SR141716A	Receptor stimulation opens BKC-channels and relaxes TM which then causes AQH efflux via TM and SC
Serotonin-2 receptor antagonists	ketanserin	Local production of MMPs; ECM degradation; stimulation of AQH efflux via TM/SC
Autotaxin/ Lysophosphatidic acid inhibitors	Aiprenon	Promotion of AQH egress from TM/SC pathway
Uveoslceral Outflow promotors (via CM bundles and sclera)		
EP2- and EP4- PG-receptor agonists	AL-6598 ; Butaprost ; ONO-AE1-259-01; PF-04217329 ; PF-04475270	Receptor activation increases cAMP that relaxes CM & TM; EP_2 agonists also cause release of MMPs that breakdown ECM ("clog") around CM bundles and within sclera thus causing UVS outflow of AQH
Serotonin-2 (5HT-2) receptor agonists	(R)-DOI; a-methyl-5HT; AL-34662	Contraction / relaxation of CM and TM by activation of $5HT_2$ receptors. May also release MMPs and/or PGs or other local mediators that promote CM remodeling and thus promote UVS outflow
Bradykinin B_2-receptor agonists	Bradykinin; FR-190997; BKA278	B_2-receptor activation causes PI hydrolysis production of IPs and DAG; cause PG release and release of MMPs that digest ECM and this promote UVS outflow in cynomolgus monkey; conventional outflow also stimulated in isolated bovine /porcine anterior eye segments
Dual pharmacophore PGs	FP/EP3 receptor agonist (ONO-954)	Promote UVSC outflow
Inflow inhibitors (reduce AQH production)		
Chloride channels inhibitors	5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB)	Ion flux of CP NPE cells causes reduction of AQH formation
Dopamine receptor agonists	PD128907; CHF1035; CHF1024; SDZ GLC-756; (S)-(-)-3-hydroxyphenyl)-N-n-propylpiperidine (3-PPP)	Inhibit release of NE & prevent AQH production; may also release natriuretic peptides
Na^+-K^+-ATPase inhibitors	Ouabain; Digoxin analogs	Ciliary process Na^+-K^+-ATPase inhibited leading to inhibition of AQH production
Aquaporin Inhibitors	Various aromatic sulfonamides and dihydrobenzofurans	Inhibit release of NE & prevent AQH production
Other IOP-lowering agents		

Mas receptor stimulator Angiotensin-II receptor antagonists	DIZE via ACE-2 activation	Prevent ECM (including TGFβ) accumulation (outflow stimulation ?)
	CS-088	Various mechanisms of action; not robust IOP-lowering
Ca2+-channel inhibitors	Lomerazine; Nivaldipine; Nifedipine; Nimodipine; Verapamil; Brovincamine; Iganidipine	Enhance retinal blood flow; some may lower IOP; work well in normal tension glaucoma patients
Alpha-adrenergic receptor antagonists	Oxymetazoline; 5-methylurapidil; Ketanserin	Work mostly via outflow mechanism but this needs to be defined
ATP-sensitive K^+-channel activators	Cromakalim; Levocromakalim; CKPL1	Purported MOA involving episcleral veinous pressure modulation

Table 2: Recently discovered ocular hypotensive agents and their potential modes of action in animal models of OHT / POAG.

Of the approved drugs to treat these diseases, FP-receptor prostaglandin agonists and recently approved (in Japan) EP2-receptor agonist (omidenepag isopropyl) represent first-line treatment modalities. When all drug classes fail to lower and/or control IOP, surgery [74,75,93,94] and implantation of AQH microshunt devices [95,96] with or without topical ocular medications are required to remove excess AQH from the ANC to lower and control eye pressure. Since patients who continue to lose vision also include those with normal IOPs (normotensive glaucoma; NTG), further reduction of IOP is usually needed. In future, in addition to reducing IOP, patients with all forms of glaucoma will require drugs and other combinatorial neuroprotective / cytoprotective agents and rejuvenating treatments that can reduce RGC/RGC axons loss and thus protect the retina-brain axis components to preserve eyesight [4,21-23,64,97,98].

Why are ROCK Inhibitors Important for OHT/Glaucoma Treatment?

Due to the important and beneficial roles of AQH perfusing through the AC of the eye, drugs or treatment options which promote AQH egress via the TM/SC system to lower IOP are preferred over those that prevent production of AQH. Until recently, the only approved ocular hypotensive drugs that specifically helped remove excess AQH from the ANC of the eye via the conventional TM/SC pathway are pilocarpine and to a lesser extent brimonidine. Due to the local ocular side-effects of both drugs (i.e. brow-ache, lens accommodative issue, eye allergy, etc.), new drugs that are TM/SC AQH drainage promoters have been actively sought. ROCK inhibitors such as fasudil, HMN-1152, Y-27632, Y-39983, ripasudil, netarsudil, AMA-0076, ITRI-E-(S)4046, (Tables 1 & 2) are such drugs, of which only ripasudil and netarsudil have been approved for OHT/POAG treatment thus far.

ROCKs belong to a family of serine-threonine kinase enzymes. ROCK-1 (ROCK-β) and ROCK-2 (ROCK-α), sometimes also labeled ROCK-I and ROCK-II, have been discovered and they share a 65% amino acid sequences in common and 92% homology within their functional active enzymic region. Each ROCK sub-type is composed of 5 domains, a catalytic kinase domain at the N-terminus, followed by a central coiled-coil domain containing a rho-binding domain and a C-terminal pleckstrin-homology domain including an internal cysteine-rich domain [88,99]. While all ROCKs primarily reside in the cytoplasm, they also migrate to the nucleus and membrane depending on the extra- and intra-cellular signaling (Figure 2). ROCK-1 appears preferentially expressed in kidney, liver, spleen and testis, whereas brain, heart, lung, skeletal muscle and the eye appear enriched in ROCK-2. ROCKs phosphorylate different substrates including LIM kinase, myosin light chain (MLC) and MLC phosphatase which regulate actin filament arrangement and contractility [86-88,99] (Figure. 2). Due to the relative ubiquity of ROCKs in cells and tissues of the body they are involved in many functions / dysfunctions [88,99]. In the eye, TM and ciliary muscle contain relatively high expression of ROCKs, and these enzymes appear to be higher in the ONH of the glaucomatous eye than in normal eyes of patients which has implications for neurodegeneration within the retina [100].

Many studies have shown that ROCK inhibitors (Figure 3) that actively compete for the catalytic ATP-binding site (Figure 4; Table 3) [101-103] dramatically inhibit actomyosin-driven cell contractility [104-107], thereby causing relaxation of the cells (Figure 4), disruption of filamentous actin and loss of focal contacts within microfilaments (Figure 5) [89,104,105]. Additionally, inhibitors of ROCK block MLC phosphorylation, promote myosin II activity, increase the actin depolymerization and decrease cell-ECM adherence (Figure 5) [89,104-107]. Consequently, microtubule bundles are assembled thus generating strong tensile forces, which then induce ECM remodeling within TM/SC. Thus, ROCK inhibitors cause cell relaxation, expand ECM spaces thereby increasing the paracellular fluid flow across inner wall of Schlemm's canal and JCTs and thus promoting AQH outflow via the TM (Figure 6) [107-111]. Conversely,

substances that activate Rho GTPases such as endothelin-1, sphingosine-1-phosphate, TGF-β2 and lysophosphatidic acid (LPA), decrease the AQH outflow from TM/SC [107-111]. In enhancing TM/SC-based outflow of AQH from the ANC of the eye in normotensive and ocular hypertensive rats, rabbits, Cynomolgus monkey (and human eyes), ROCK inhibitors lower IOP for over several hours in numerous species (Figures 7 & 8) [89,102,103,107-111]. A prominent new feature of one particular ROCK inhibitor, netarsudil, is the inherent norepinephrine transporter (NET) inhibitory activity and its reduction of episcleral venous pressure, which contribute to its overall ocular hypertensive actions [88,89]. A fixed-dose combination of netarsudil and the FP-receptor agonist latanoprost yields additive IOP-lowering in animals and OHT/POAG patients [112,113].

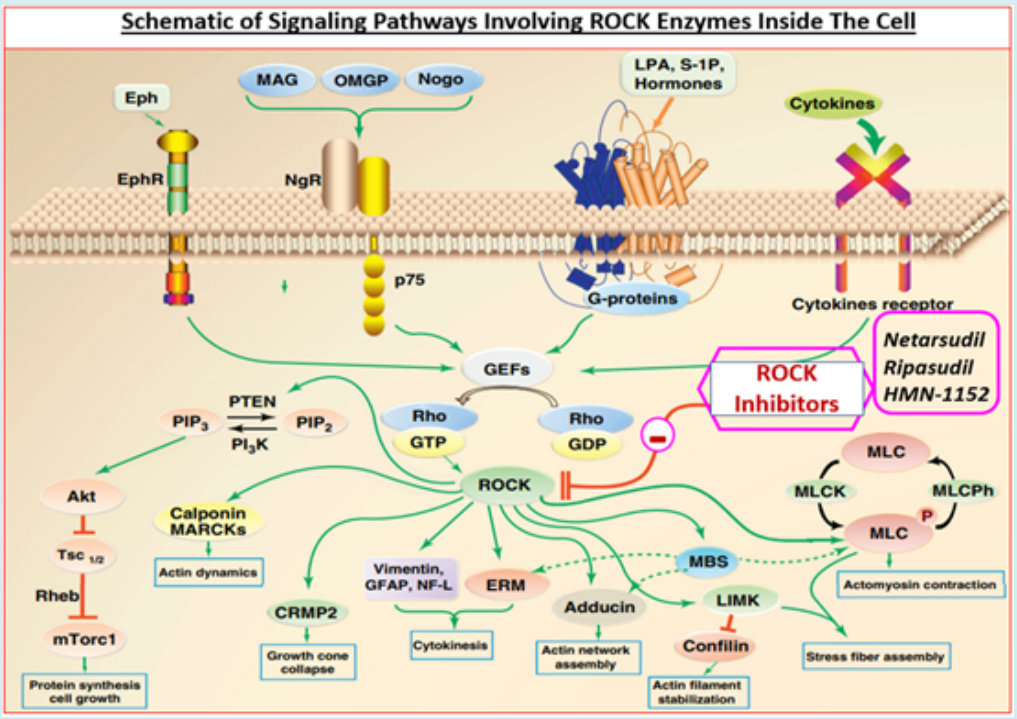


Figure 2: Activation and inhibition of ROCKsand the consequential effects on cell function.

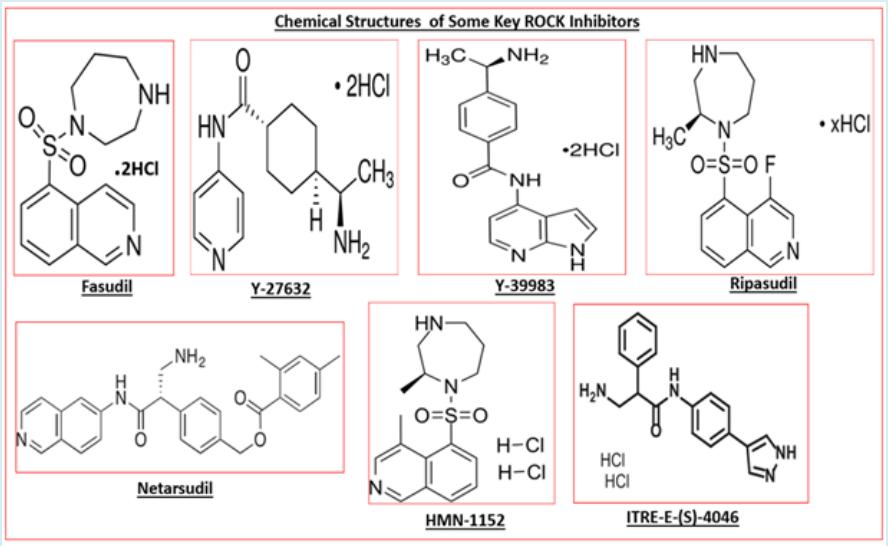


Figure 3: First and second generation of ROCK inhibitors that lower IOP in animals (and some in human subjects).

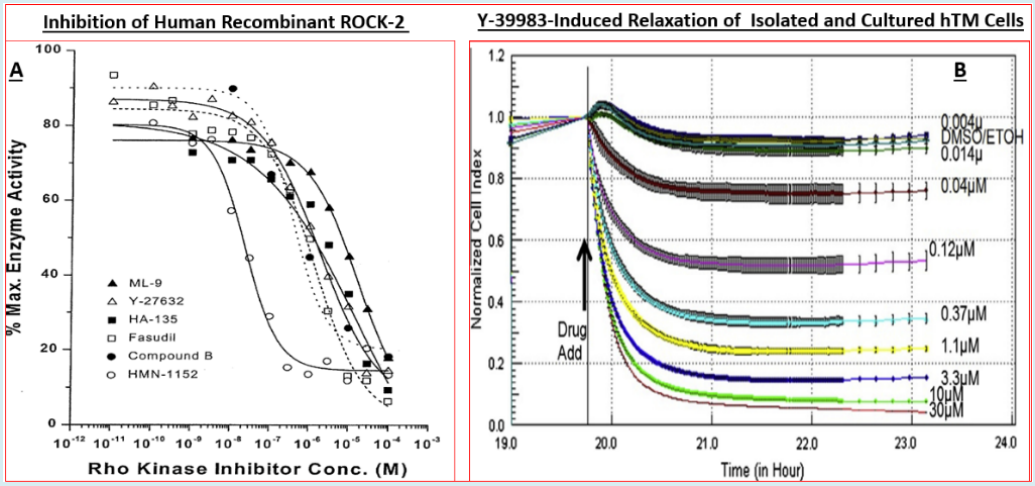


Figure 4: ROCK inhibitors competing for ATP bindingsite of ROCK-2 and cell-based assay of Y-39983 inhibitory activity.

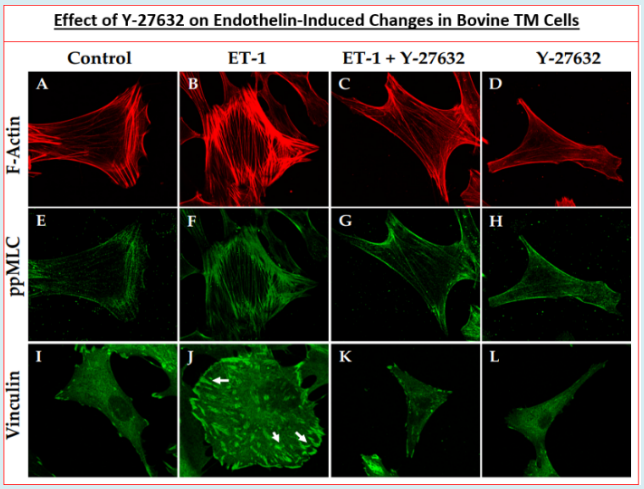


Figure 5: ROCK inhibitor effects on various cellular morphological markers.

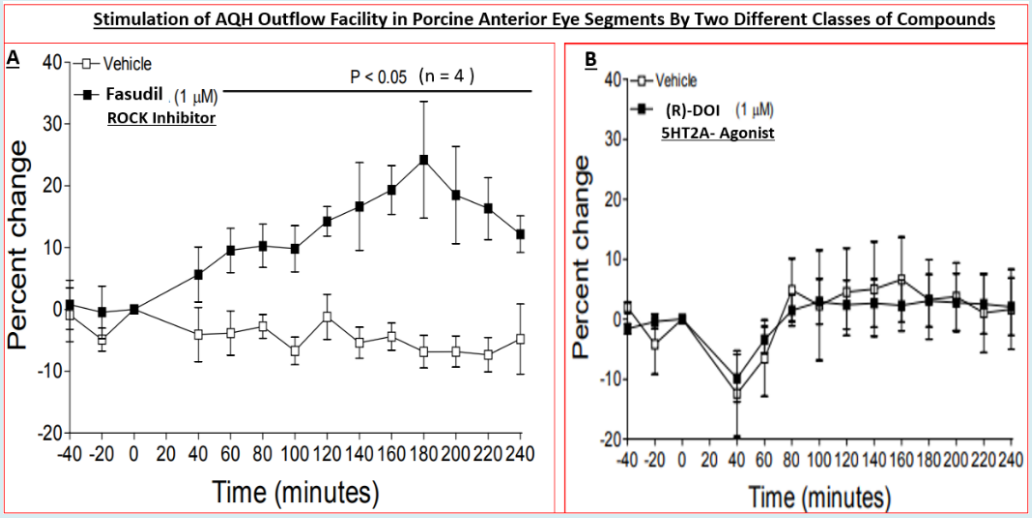


Figure 6: Effects of a ROCK inhibitor and a serotonin-2 receptor agonist on outflow from ANC of perfused porcine eyes in vitro.

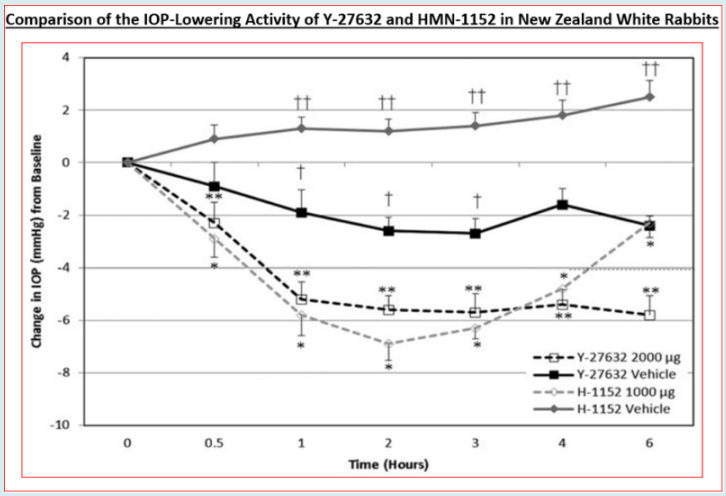


Figure 7: IOP-lowering effects of two different ROCK inhibitors in rabbit eyes.

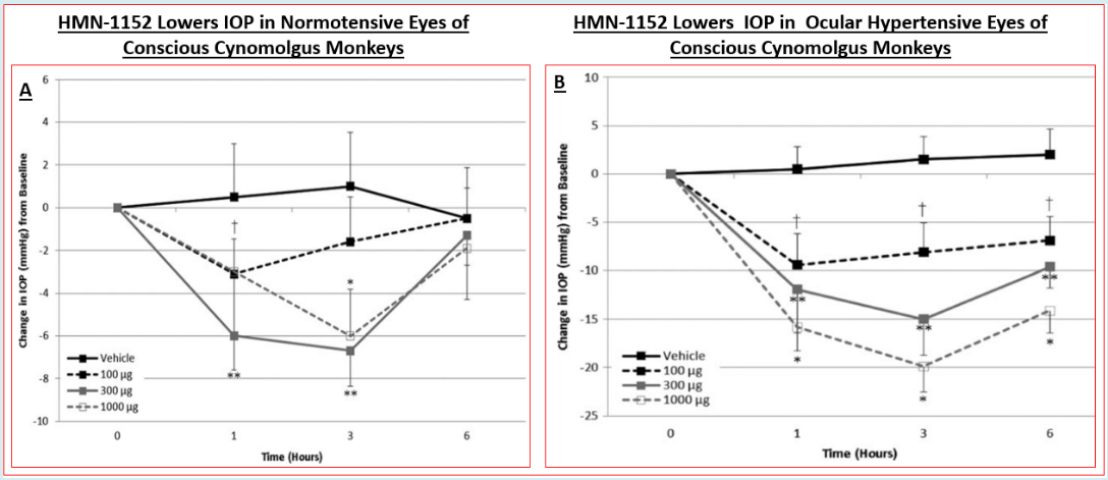
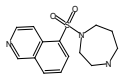
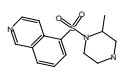


Figure 8: Ocular hypotensive activity of a high-potency ROCK inhibitor in monkey eyes.

Compound	[γ-^{33}P]-ATP-Based Assay (IC_{50}, nM)	IMAP Fluorescence Polarization-Based Assay (IC_{50}, nM)
 **Fasudil**	1690 ± 185 nM (N = 10)	291 ± 43 nM (N = 9)
 **H-7**	2341 ± 395 nM (N = 5)	913 ± 644 nM (N = 3)

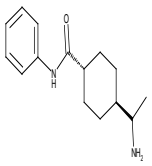
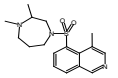
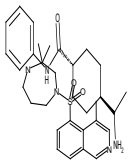
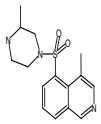
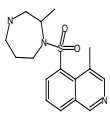
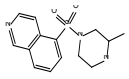
 **Y-27632**	2802 ± 865 nM (N = 3)	797 ± 206 nM (N = 3)
 **A**	3463 ± 1800 nM (N = 4)	270 ± 113 nM (N = 3)
 **B**	485 ± 207 nM (N = 3)	108 ± 53 nM (N = 2)
 **C**	1512 ± 704 nM (N = 4)	2007 ± 85 nM (N = 3)
 **D**	2625 ± 307 nM (N = 4)	2390 ± 1260 nM (N = 3)
 **HMN-1152**	47 ± 14 nM (N = 4)	30 ± 14 (N = 4)
 **HA-135**	6702 ± 900 nM (N = 2)	3900 ± 2100 (N = 2)

Table 3: ROCK-2 Inhibition Constants (IC50) Obtained from Two Different Assay Systems in vitro.

Data in the above table are mean ± SEM; N = the number of independent assays conducted using each type of assay system. Compounds A-D represents new ROCK inhibitors. Literature ROCK-2 inhibition constant (IC50) data for other ROCK inhibitors are: ripasudil = 9.0 nM; netarsudil= 1 nM; AMA0076= 2.3 nM; ITRI-E-(S)-4046 = 3.2 nM; Y-39983 = 3.6

nM. Table 3 illustrates the relative potencies and selectivities of various ROCK inhibitors for ROCK-1, ROCK-2 and other protein kinases. Note that potency is inversely related to IC50 values.

The other reasons that ROCK inhibitors represent important new drugs for ocular disease treatments involve their ability to reduce fibrosis induced by pro-fibrotic endogenous substances released by stressed TM cells such as TGF-β2 (Figure 9) [89,114,115], relax blood vessels to enhance local blood flow at the back of the eye [116-118], including the ONH, thereby overcoming ischemia/hypoxia that prevails in POAG/normotensive glaucoma [NTG] and in other forms of glaucoma [1-4,7,71,72]. Additionally, ROCK inhibitors exert neuroprotective activities by protecting (and preserving) RGCs from neuro-excitants like glutamate receptor agonists and other agents like endothelin that cause Ca2+-overloading of retinal neurons [119-121] (Figure 10). RGC axonal regeneration due to neurite extension by ROCK inhibitors such as Y-27632 and Y-39983 has also been reported [118,121-125]. Consequently, blockers of the active sites of ROCKs are very useful addition to the clinicians' armamentarium in the quest to find new generation of outflow-promoting drugs for OHT/glaucoma treatment for the preservation of eyesight that exert their beneficial effects via multiple mechanisms of action that are mediated via the actomyosin components [104,105,126] and potentially by dampening the microglial activity within the retina, optic nerve and the brain centers involved in transmission and decoding of visual signals [127] (Figure 11).

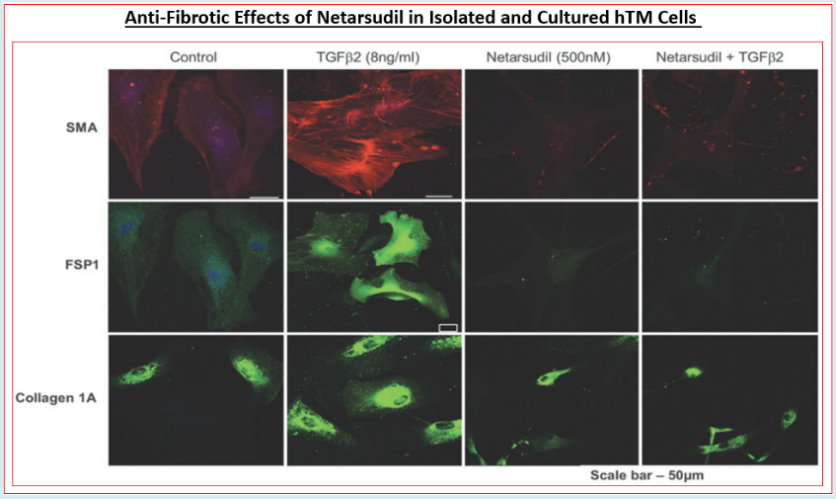


Figure 9: Inhibition of pro-fibrotic actions of TGF-β by a potent ROCK inhibitor in vitro.

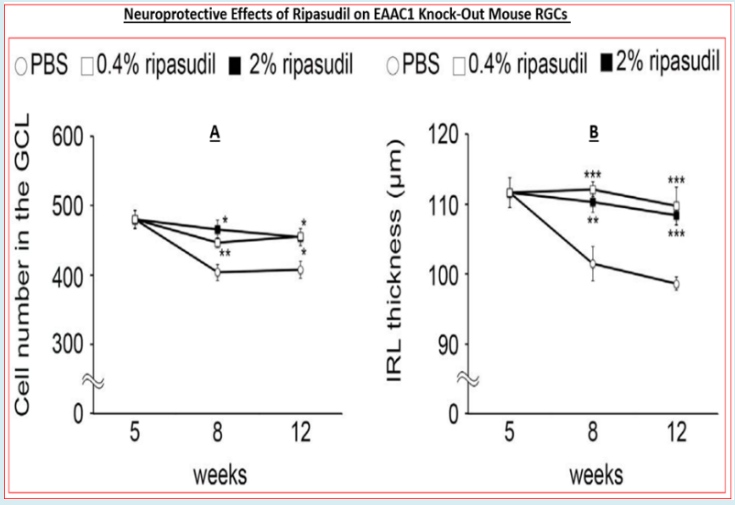


Figure 10: Inhibition of ROCK prevents RGC cell death and preserves retinal thickness in vivo.

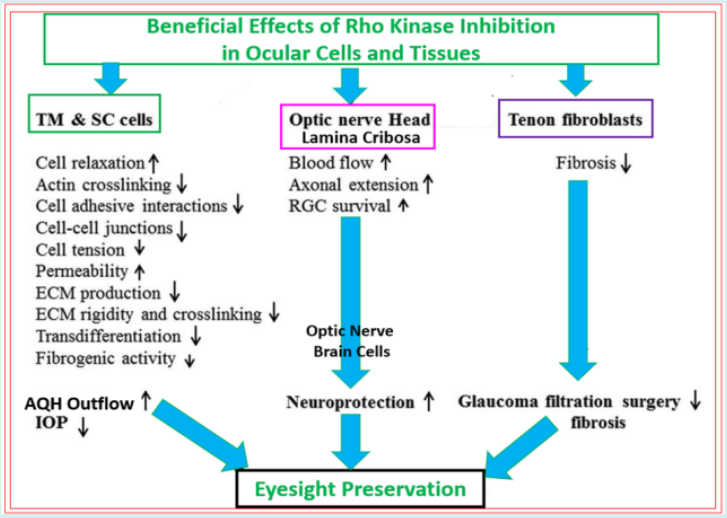


Figure 11: ROCK inhibition causes IOP reduction, protect RGCs, reduces fibrosis and vision loss.

Some Selected Studies Characterizing Inhibitors of ROCKs

The human kinome is reported to contain over 500 different kinase enzymes grouped in families that catalyze phosphorylation of various lipids, carbohydrates and proteins, including other enzymes. Unsurprisingly, ROCKs are cornerstones of cellular signaling and communication, and their up-regulation is implicated in many diverse diseases/disorders ranging from systemic hypertension, cardiovascular hypertrophy, diabetes mellitus, pulmonary hypertension, renal disease/vasospasm, asthma, cancer, erectile dysfunction, hyper-proliferative diseases, inflammatory diseases, and glaucoma [99,106,128-135]. These findings prompted discovery and characterization of many classes of ROCK inhibitors (Figure 3) [86,89,106,108,116,136,137] and their eventual introduction into clinical practice for the medical treatments of the latter maladies.

Specifically, nanomolar affinity/potency compounds that competitively compete for the active ATP-binding site of ROCK-1 and ROCK-2 have been reported that potently block these enzymes in vitro / ex vivo in a concentration-dependent manner (Figures 4A, 4B, 5 & 6) (Table 3) [89,101-116] and in vivo in animal models of disease to exert their biological effects such as potently and efficaciously lowering IOP and enhancing retinal blood flow in a dose-dependent manner (Figures.7, 8 & 10) [87,89,102,103,108-118]. All ROCK inhibitors traverse the cell membranes to engage with the cytoplasmic ROCKs and prevent down-stream signaling thereby relaxing cells/tissues, (e.g. isolated TM cells) (Figure 4B) [89,102-105], preventing cell migration/proliferation, reducing focal adhesions and phosphorylation of myosin light-chain kinase (Figure 5) [89,102-105], preventing pro-fibrotic actions (Figure 9) [89,114,115] and thus promoting fluid drainage from the ANC of the eye (Figure 6A) [137] where another class of compound failed to induce such an effect (Figurer 6B). In ocular physiology/pathology, ROCK-2 seems more important that ROCK-1, but both isoforms of ROCK were elevated in the ONH of glaucoma patients [100]. Thus, most researchers have focused on inhibitors of ROCK-2, although due to the high homology between these isoforms, most such inhibitors also carry weaker ROCK-1 inhibitory activity as well [89,102,103], and most also show some degree of selectivity for ROCKs relative to other kinases (Table 4) [89].

Functional activity of various ROCK inhibitors at recombinant human ROCK-2, using both radiometric (using [γ-^{33}P]-ATP) and fluorescence polarization assays has been determined (Figure 4A) (Table 3) [89,101,136,137]. Data from the literature shows that whilst the absolute inhibitory potency values for various ROCK inhibitors differ (Table 3), due to the use of human recombinant kinases from different sources and disparate cell-based assays (Figures 4B, 5 & 6), the relative rank order of potencies are fairly consistent among many laboratories [89,101,136,137]. Despite such differences, the in vitro potencies are well translated in the IOP-lowering models of OHT/POAG in vivo whether ocular hypotensive activity is studied in rabbits or non-human primates [87,89,103,107,108]. Thus for instance, the higher affinity and potency of HMN-1152 relative to Y-27632 (Table 3) is borne out by its greater IOP-reducing activity after topical ocular dosing in both species (Figure 7) (Table 5). Indeed, the usefulness of the rabbit- and/or monkey-based in vivo testing models of OHT/POAG have permitted optimization and selection of future drug candidates [102,103]. Furthermore, the ability of at least

ripasudil [85-87] and netarsudil [88,106,113,138] to lower and control IOP in human subjects has been demonstrated in numerous clinical trials, and the mechanism of action to reduce IOP confirmed to be by enhancing AQH drainage via the conventional TM/SC outflow pathway [139]. Due to this robust outflow promoting effect of netarsudil and ripasudil, fixed-dose combination products of each drug [112], with additive ocular hypotensive actions, have been also approved by many health authorities of several different countries. Whether the anti-fibrotic, neuroprotective and axonal regenerating efficacies of these ROCK inhibitors observed in vitro and in animal models noted above can be translated to the human RGC, RGC axonal and thalamic nuclei/visual cortical degenerative conditions characteristic of POAG/NTG patients remains to be determined [140].

In Vitro Potency OF Rho-Associated Protein Kinase Inhibitors (nM)								
Compound	**ROCK1 K_i**	**ROCK2 K_i**	**PKA K_i**	**PKCT K_i**	**MRCKA K_i**	**CAM2A K_i**	**PTM IC_{50}**	**HTM IC_{50}**
Netarsudil	1	1	5	92	129	5,3 12	79	16
Netarsudil-MI	0.2	0.2	1	27	7	13,689	2	3
AR-12286	2	2	69	9,322	28	5,855	924	818
Y27632	22	41	21,006	413	485	16,863	9,970	1,738
Fasudil	76	47	216	3,162	5,983	3,162	10,060	3,942

Values represent the average of ≥3 replicate experiments.CAM2A, calcium/calmodulin-dependent protei n kinase 2A; HTM, human trabecular meshwork ; IC_{50}, half maximal inhibitory concentration; K_1, inhibition constant; MRCKA, myotonic dystrophy kinase-related CDC42-binding kinase A; PKA, protein kinase A; PKCT, protei n kinase C, theta; PTM, porcine trabecular meshwork; ROCK1, Rho-associated protei n kinase I ; ROCK2, Rho-associated protein kinase 2.

Table 4: The relative potency and selectivities of various ROCK inhibitors determined in vitro.
Note that potency is inversely related to IC_{50} or K_1 values.

IOP Reduction By Various ROCK Inhibitors in OHT Monkey Eyes				
%Max. IOP Reduction in Ocular Hypertensive Monkey Eyes				
Compound	**Topical Ocular Dose (µg)**	**1 Hour Post Dosing**	**3 Hour Post Dosing**	**6 Hour Post Dosing**
HMN-1152 (ROCK II IC_{50}=47 nM)	100 µg	28	25	19
	300 µg	29	30	21
	1mg	42	51	36
Fasudil (ROCK II IC_{50}=1690 n M)	500 µg	33	28	16
H-7 (ROCK II IC_{50}=2341 n M)	1 mg	31	21	12
Y-27632 (ROCK II IC_{50}=2802 n M)	300 µg	15	14	8
	l mg	24	36	32
HA-135 (ROCK II IC_{50}= 6702 n M)	500 µg	19	15	1
Cmpd D (ROCK II IC_{50}=2625 nM)	500 µg	23	20	20
ML-9 (ROCK II IC_{50} = 12,000 nM)	300 µg	7	6	5

Table 5: Relative efficacies of ROCK inhibitors in lowering IOP.

Conclusions

This mini-review has endeavored to present current knowledge of the role of ROCKs in various pathological conditions, and the utility of inhibitors of ROCK-1 and ROCK-2 to ameliorate such human ocular diseases as ocular hypertension, POAG and NTG. Due to the multiple beneficial actions of ROCK inhibitors in ocular physiology ranging from the front of the eye to the retina and beyond (Figure 11), they represent uniquely valuable new drugs in the tool chest of ophthalmic clinicians to help them treat the ever-increasing number of global patients with chronic OHT and various forms of glaucoma. The TM/SC cell, RGC and RGC axon protecting actions of ROCK inhibitors observed in vitro and in animals, coupled with their ocular hypotensive activity demonstrated in animals and humans, and

enhancement of retinal blood flow in several species clearly differentiates them from other classes of drugs used to treat OHT and glaucoma. However, whether the neuroprotective, neurite and axonal elongation and blood-flow enhancing actions of ROCK inhibitors can unequivocally be shown in a reproducible manner in multiple clinical trials remains to be investigated. The exaggerated hyperemic activity associated with most if not all ROCK inhibitors may limit their topical ocular utility but it is too early to predict that aspect since they only have a recent approval history and much more data need to be generated and shared. We hope that suitable formulations and other forms of drug delivery, alone or in combination with other agents, can be discovered and deployed to reduce the ocular surface redness that ROCK inhibitors cause without impacting their beneficial actions in terms of helping to preserve vision on a long-term basis.

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