REVIEW ARTICLE

Rho-kinase Inhibitors in Ocular Diseases: A Translational Research Journey

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Abstract

Aim: This review summarizes current data on Rho-kinase (ROCK) inhibitors use in ocular diseases, primarily glaucoma.

Background: Translational research over the last decade culminating in the development of ROCK inhibitors has provided a much-needed shot in the arm to glaucoma pharmacopeia. ROCK pathway is intricately involved in cytoskeletal modulation with action on cell morphology, cell motility, cell adhesion, cell apoptosis, and smooth muscle contraction. This cytoskeletal modulation property has been utilized to modify trabecular meshwork (TM) resistance, resulting in the discovery of ROCK inhibitors to increase trabecular outflow.

Review results: Multicentric trials on ROCK inhibitors for antiglaucoma medications are summarized. The focus is on linking pharmacological action to the clinical utility of these drugs. While the Rho Kinase Elevated intraocular Pressure (IOP) Treatment (ROCKET) trials compared monotherapy with ROCK inhibitor netarsudil vs timolol, MERCURY trials compared a fixed dose combination of latanoprost and ROCK inhibitor netarsudil [fixed combination netarsudil-latanoprost (FCNL)] vs monotherapy with either and bimatoprost-timolol combination. While ROCKET trials showed ROCK inhibitors to be non-inferior to timolol, MERCURY trials showed FCNL achieving a much greater IOP reduction than monotherapy with either. Conjunctival hyperemia was the most common side effect reported with ROCK inhibitor use.

Conclusion: Moderate efficacy of ROCK inhibitors with a common side effect of conjunctival hyperemia, makes it an adjunctive antiglaucoma drug of choice and not a first-line therapy

Clinical significance: ROCK inhibitors' action on diseased TM is more physiological compared to available antiglaucoma medications that either reduce aqueous secretion or enhance uveoscleral outflow. The property of ROCK inhibition to stabilize the endothelium of both retinal vasculature and cornea has opened a new chapter in the treatment of diabetic retinopathy and corneal decompensation.

Keywords: Antiglaucoma drugs, Corneal regeneration, Diabetic retinopathy, Fixed dose combination, Glaucoma, Magnetic Resonance Imaging and Rectal Cancer European Equivalence trial, Open-angle glaucoma, Rho Kinase Elevated IOP treatment trial, Rho-kinase inhibitors, Systematic review.

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Background

Glaucoma is the leading cause of irreversible blindness globally with an estimated prevalence of 80 million people affected by 2020. The disease burden is expected to increase to 112 million by 2040. Asia, by virtue of its population would account for 60% global burden of the disease.[1] Treatment guidelines for glaucoma management center on medical, laser, or surgical modalities with medical management remaining the cornerstone. Prostaglandin analogs (PGA), remain the first-line antiglaucoma drugs, however, they are known to be ineffective in more than 20% of patients.[2] Most surveys of glaucoma management report almost 50% of patients requiring polypharmacy for IOP control at presentation with about one-third needing add-on therapy over the 1st year of single-drug treatment.[3,4] The common add-on therapy for PGA remains β-blockers, carbonic anhydrase inhibitors, and α-2 agonists, all of them penalizing the innocent ciliary body to reduce aqueous secretion. ROCK inhibitors on the other hand with a more physiological action on diseased TM are welcome adjuncts.

The concept of ROCK inhibitors use for glaucoma started with the finding that TM cells actively regulate their outflow resistance.[5] Systematic molecular research, based on this finding led David Epstein to launch the Trabecular Meshwork Study Club in 1993, leading to the discovery of cytochalasin in 1997, latrunculin in 1998, followed by current ROCK inhibitor ripasudil and netarsudil in 2014 and 2019, respectively. ROCK inhibitors are a recent entry in the foray of glaucoma pharmacopeia and have evolved into effective, physiological agents for IOP control.

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Mechanism of Action

Rho proteins (Rho A, B, C) are small G proteins that get activated by cytokines like endothelin, angiotensin II, thrombin, transforming growth factor beta (TGF-β), or integrins to guanosine triphosphate form.[5,6] ROCK is the downstream regulator of such activated Rho proteins. It is a serine-threonine-specific protein kinase that exists in two isoforms namely ROCK 1 and ROCK 2, both being present in TM.[7] The genetic loci of these two isoforms have been identified on

chromosome 18 (18q11.1) and 2 (2p24).[8] ROCK triggers actin-myosin interactions, focal adhesions, matrix reorganization, neuronal cytokinesis, formation of stress fibers, and cell stiffening.[9] Inhibitors of ROCK by action on actinomycin-driven cellular contraction, reduce extracellular matrix proteins production, disrupting both actin stress fibers and focal adhesions. This cytoskeleton modulation of TM relaxes stiffened TM beams and is primarily responsible for the increased outflow of aqueous.[10] At Schlemm's canal (SC) level ROCK inhibitors improve endothelial permeability, and widen the circumference and endothelial pores of SC, in addition to reducing focal adhesions with juxtacanalicular meshwork.[5,7,11]

Ripasudil/Ripatec [0.4% Twice a Day (BD)]

Extensively studied in the Oriental race, the drug has been approved for open-angle glaucoma (OAG) and ocular hypertension in Japan since 2014. The drug is moderately effective resulting in a 3–7 mm Hg reduction of IOP with peak onset within 2 hours.[12] Prescribed twice daily, the drug has nocturnal efficacy, which gives it an edge over β blockers and carbonic anhydrase inhibitors, the latter having negligible effect at night.[13,14]

The effect seems to be sustained with long-term studies of 1–2 years duration reporting no long-term drift.[12,15] Side effects are primarily ocular, with no systemic side effects reported to date. Conjunctival hyperemia is ubiquitous and occurs in 55–96% of cases.[12,15] The transient hyperemia is noninflammatory and is a consequence of vasodilating effect of the drug. Blepharitis and allergic conjunctivitis in 20% of cases along with a transient reduction in central corneal thickness in 9% have been other reported side effects.[12,15] The drug has additive effects with both timolol and prostaglandins.[16] In vitro studies also report pilocarpine reduces the ROCK effect of Ripasudil on smooth muscle actin (SMA) expression.[17]

Netarsudil [Rhopressa 0.02% Once a Day (OD)]

Netarsudil combines the effect of both ROCK inhibition along with norepinephrine transporter inhibition, the latter reducing episcleral venous pressure and aqueous production. Available as an ester prodrug, netarsudil is converted by corneal esterase into its active form.[18]

The drug has been studied by four ROCKET trials of which ROCKET-3 was abandoned. ROCKET-1 and 2, were double-masked, randomized, multicentre, parallel-group studies, conducted across 37 centers in the United States (US) for 1167 patients. ROCKET-1 duration was for 3 months and ROCKET-2 for 12 months.[19]

Review Results

ROCKET-1 trial—This trial investigated the safety and efficacy of OD 0.02% netarsudil compared to BD 0.5% timolol, for 411 patients. Netarsudil with an IOP reduction of 15–22% was non-inferior to 17–22% with timolol.[19]

ROCKET-2 trial—An additional arm of patients was added to the two arms of ROCKET-1, for whom 0.02% netarsudil was prescribed BD. Netarsudil in this frequency was non-inferior to timolol, with slightly enhanced IOP reduction.[19,20] Transient conjunctival hyperemia at 50–53% was the most common side effect at 59% with BD dose vs 53% with OD use. The BD regimen however resulted in more complications with conjunctival perilimbal microhemorrhages at 17%, corneal verticillata (corneal deposits) in 15% vs 15 and 9% in the OD dose arm.[19,20] The unusual complication of corneal verticillata initiated a Corneal Observation Study, which reported a 26% incidence of cornea verticillata over a 12-month follow-up, with no clinical impact on visual function.[21]

ROCKET-4 trial—This study analyzed the hypotensive effect of netarsudil over unmedicated IOP ranging from 21 to 29 mm Hg, in 186 patients. Pressure control was checked by office diurnal over a 6-month period. Netarsudil met the criteria for non-inferiority to timolol, with its effect being sustained over 6 months.[22]

Roclatan/PG324 (Fixed Combination Netarsudil 0.02% with Latanoprost 0.05%—FCNL)

This fixed drug combination (FDC) combines the trabecular outflow-enhancing property of ROCK inhibitor with the uveoscleral outflow-enhancing effect of PGA.[23] The drug was granted Food and Drug Administration approval in 2019,[24,25] and has undergone two trials, namely MERCURY I and MERCURY II, with MERCURY III being underway.

MERCURY I trial—A double-masked, randomized, parallel-group phase 3 trial, enrolling 718 patients of OAG or ocular hypertension across 56 centers in the US. The study had three arms, wherein the first arm, FDC Roclatan (netarsudil 0.02% with latanoprost 0.05%) was given once at night, the second arm was given netarsudil 0.02% once at night, and in the third arm latanoprost 0.05% was given once at night. Office diurnal at 3 and 12 months, reported superiority of Roclatan over individual ingredients by an additional 1.8–3.0 mm Hg.[3,23] A consistent mean diurnal IOP of <18 mm Hg was maintained in 82% patients on FCNL vs 54% in netarsudil arm and 69% in latanoprost arm. Adverse events noted were conjunctival hyperemia in 53%, conjunctival hemorrhages in 11%, and cornea verticillata in 5%.

MERCURY II trial—this was a 90-day efficacy trial of FCNL Roclatan compared to its individual constituents as monotherapy. This trial corroborated MERCURY I findings of additional benefit on IOP control, with the FCNL group achieving a mean office diurnal IOP of <15 mm Hg in 42% vs 16 and 18% in netarsudil and latanoprost arm, respectively.[3,25]

MERCURY III trial—this trial is underway and compares FCNL with a fixed combination of bimatoprost 0.03% with timolol 0.5%.[3]

SNJ 1656 (Y-39983) Senju Pharma

This ROCK inhibitor has been used in concentrations of 0.003–0.1% in both once and BD dosing. Phase II trials have reported a 3 mm Hg reduction in IOP with axonal regeneration in the crushed optic nerve in a cat model.[24]

The available topical ROCK inhibitors are detailed in Fig. 1 and Table 1.

Discussion

Expanded Uses of ROCK Inhibitors in Glaucoma

Effect on Optic Nerve Head Circulation and Axonal Apoptosis

Immunohistochemical assays have identified Rho A, ROCK-1, and ROCK-2 in TM, ciliary muscle, and optic nerve head. *In vitro* study on human eyes found increased expression of Rho A in optic nerve heads of glaucoma patients compared to age-matched normal.[26] ROCK has been found to mediate vasoconstrictor effect on optic nerve head arteries and glutamate-induced excitotoxicity at the axonal level, with Rho inhibitors reversing the same.[7,27] This could pave the way for retinal ganglion cell survival and improved blood flow to the optic nerve head by ROCK inhibitors[28]

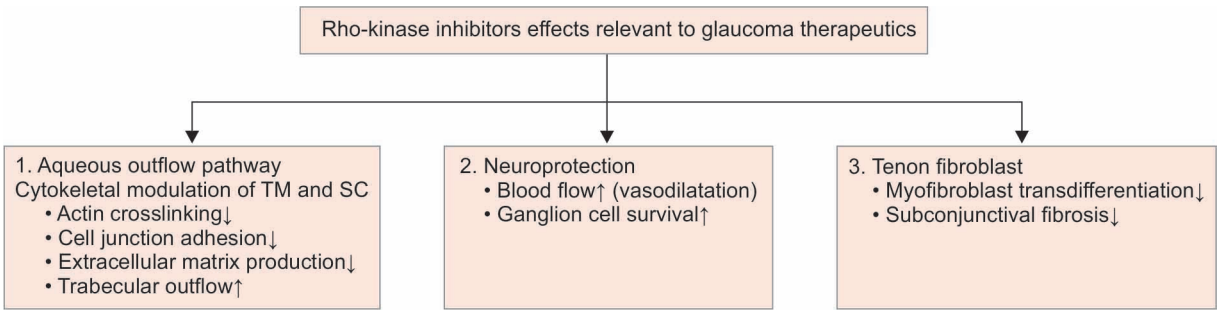

Fig. 1: ROCK inhibitors effects relevant to glaucoma therapeutics

Table 1: Currently available ROCK inhibitors

ROCK inhibitor	*Clinical uses*	*Clinical trials*	*Manufacturer and date of approval*
Fasudil (HA 1077)	For cerebral vasospasm		2011 in Japan and China (Kowa Co)
Ripasudil (K -115) or Glanatec - 0.4% BD	Approved for OAG and ocular hypertension	Ripasudil observation study	2014 Japan (Kowa Co Ltd, Nagoya, Aichi)
Netarsudil or Rhopressa (AR 122286), amino isoquinoline amide) 0.02% HS		ROCKET 1, 2, 4	2016 USA(Aerie Pharma)
Roclatan (Netarsudil 0.02% and latanoprost 0.05%) HS		MERCURY 1, 2,3	2019 USA (Aerie Pharma)
Ripasudil or Ripatec 0.4% BD			2020 India (Ajanta Pharma Ltd)

Effect on Tenon Fibroblast and Bleb Scarring

Rho kinase (ROCK) is involved in human tenon fibroblast transdifferentiation to myofibroblast, the event being triggered by inflammatory cytokines like TGF β and measurable by αSMA expression.[13] *In vitro* studies show ROCK inhibitors to reduce this SMA expression induction.[29] This reduction in transdifferentiation of Tenon's fibroblasts into myofibroblasts, has the potential to reduce filtering bleb scarring. *In vitro* studies on TGF β activated peripapillary scleral fibroblasts pretreated by ROCK inhibitors, confirm reduction in myoblast transdifferentiation.[30] Scleral fibroblast proliferation induced in mouse glaucoma model has been reduced by pretreatment with subconjunctival ROCK inhibitors. *In vivo* studies extrapolating this employed Ripasudil drops in eyes scheduled for glaucoma filtration surgery, and documented enhanced bleb longevity.[31]

Effect in Secondary Glaucoma

Accumulation of extracellular matrix in TM is seen in glaucoma subsequent to steroid use and uveitis.[24] ROCK inhibitors reduce inflammatory cell infiltration and cytokine production in uveitic animal glaucoma models.[31,32]

Expanded Ocular Indications for ROCK Inhibitors Use

Corneal Regeneration

Cellular apoptosis mediated by actin cytoskeleton contractile force is regulated by ROCK.[6] Experimental and human studies document ROCK inhibitors to reduce apoptosis and promote corneal endothelial cell (CEC) adhesion and proliferation.[33]

Cell-based therapy for corneal endothelial recovery using cultured allogenic CEC is benefitted from ROCK inhibitor use as evidenced by experimental studies on rabbits and monkeys.[34,35] Human trials using intracameral CEC-ROCK inhibitor combination have reported success in patients with corneal decompensation post cataract surgery and Fuchs's dystrophy.[36,37]

Corneal neovascularization postchemical burns is a common cause of corneal blindness. Rock inhibitors have been effectively utilized to regress corneal neovascularization, and promote epithelial defect healing in the alkali burn mouse model and in the paired box 6 knockout mouse model, the action being mediated by limbal stem cell cloning action on adherence and reactive oxygen scavenging.[38]

Retinal Disease

Diabetic retinopathy—molecular research on retinal microvasculature and vitreous hyalocyte has confirmed the activation of ROCK pathway in diabetic retinal microvasculature.[39] ROCK pathway has been implicated at multiple aspects of diabetic pathophysiology from initial retinal microvascular damage and hyperpermeability, contraction of proliferative vitreoretinal membrane, retinal fibrosis, and generation of inflammatory mediator's to neuronal damage.[40] Leukocyte adhesion to capillary endothelium coupled with loss of tight junctions mediated by intercellular adhesion molecule 1 (ICAM-1) and leucocyte β 2 integrins, the initial event in diabetic retinopathy is regulated by ROCK pathway.[41] Experimental studies in diabetic rat model document intravitreal injection of ROCK inhibitors, to reduce ICAM expression, leucocyte adhesion and help in halting process of diabetic retinopathy.[42]

At the vascular level ROCKs inactivate endothelial nitric oxide synthase (eNOS), which is responsible for the generation of nitric oxide (NO), a potent vasodilator and antiapoptotic agent.[37,43] ROCK inhibitors by blocking this effect on e NOS, enhance NO generation and therefore protect retinal vessel endothelium.[39] Understanding of these molecular level events has led to the term "ROCK-activated endothelial cells," being a key event in diabetic retinopathy.[40]

ROCK signaling is known to upregulate vascular endothelial growth factor (VEGF) and experimental studies show that the use of ROCK inhibitors reduces this VEGF-induced angiogenesis,

restores pericyte coverage of vessels, and reestablish tight junctions. This normalization of retinal vasculature reduces ischemia and transudation, effects utilized in neovascularization and macular edema.[41,42] High glucose levels in the retina microenvironment upregulate inflammatory signaling pathways like activated B cells (p-NF-κB), inducible NOS (iNOS), VEGF, and monocyte chemo-attractant protein 1 (MCP1/CCL2), the same is attenuated by ROCK inhibitors.[44] This anti-inflammatory ability of ROCK makes this drug a better option than anti-VEGF therapy for the diabetic retina.

Normalization of the blood-retinal barrier by ROCK inhibitors has been utilized to treat diabetic macular edema recalcitrant to anti-VEGF therapy and has an edge over intravitreal steroids by not inducing cataract or glaucoma.[45] The cytoskeleton modulation effect of ROCK inhibitors, has also been explored as a therapeutic option to prevent cicatricial contraction in proliferative vitreoretinopathy. In hypertensive retinopathy ROCK inhibitor-induced vasodilatation has therapeutic implications in reducing retinal vascular complications..[46]

Age-related macular degeneration (ARMD)—macrophage polarization and subretinal fibrosis in ARMD regulated by fibrotic drivers like TGF β utilize ROCK signaling. [47] ROCK being the downstream effector of these fibrotic drivers is amenable to ROCK inhibition, which has been used to block TGF β induced contraction by retinal pigment epithelium. [40,48]

Optic Nerve Neuroprotection

Neuroprotection as a reality has been envisioned for long and Rock inhibitors offer a ray of hope in this direction. Abnormal activation of ROCK has been identified as a key event in the pathogenesis of ischemia/reperfusion (I/R)—induced retinal injury initiates inflammatory cascade starting with transendothelial migration of leukocytes, an event mediated by ROCKs.[26] Translational research on these findings successfully employing ROCK inhibitors (K115 and fasudil) in rodents have been shown to delay retinal ganglion cell death through Nox1 downregulation and attenuate I/R-induced apoptosis by inducible iNOS expression.[26,49] Experimental studies have further discovered retinal ganglion cell and nerve regeneration along with reduction of reactive gliosis with ROCK inhibitor use.[27] It has been suggested that this effect may partly be due to inhibiting N methyl D aspartate-induced neurotoxicity.[39]

Clinical Significance

The last decade of molecular research of different ocular sites of ROCK activity has suggested multiple roles for ROCK inhibitor use. The use of these drugs in glaucoma, corneal injury, diabetic retinopathy, macular degeneration, and optic nerve regeneration heralds a new era of the use of molecular targeting drugs in ophthalmology. In the field of glaucoma, ROCK inhibitors have multiple actions in improving trabecular outflow, enhancing ocular blood flow at the optic nerve head, and promoting RGC survival. In addition, it has the potential to improve filtering bleb longevity by reducing bleb scarring. Their moderate efficacy however with the very often seen side effect of conjunctival hyperemia, makes it an adjunctive anti-glaucoma drug of choice and not a first-line therapy.

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