Recent Advances

Latest Update in Glaucoma: The first approvals for Rho Kinase inhibitors

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Abstract

Glaucoma, the leading cause of irreversible blindness, blinds causes irreversible blindness in 4.0 million people worldwide. In India the estimated number of glaucoma patients is 12 million which is one fifth of the global morbidity of glaucoma.1 Elevation of intraocular pressure (IOP) is the only proven modifiable risk factor for most forms of glaucoma. Different etiological mechanisms have been proposed for increased IOP. Aqueous humor (AH) drainage from conventional or trabecular and uveoscleral pathway is a proven cause in most glaucoma patients and especially so in primary open angle glaucoma. IOP reduction slows vision loss and remains the mainstay of treatment for all types of glaucoma. Rho kinase is a serine/threonine protein kinase. It is a downstream effector of Rho GTPase, a Ras superfamily GTPase. it has a role in the conventional path of aqueous drainage. Rho Kinase (ROCK) inhibitors Ripasudil, K-115 and Netarsudil, AR-13503 have recently been approved for ophthalmological use in therapy of glaucoma as IOP-lowering agents. This is a brief overview of the Rho Kinase (ROCK) inhibitors.

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Glaucoma causes irreversible blindness in 4.0 million people worldwide and is the leading cause of irreversible blindness globally. Elevation of intraocular pressure (IOP) is the only proven modifiable risk factor for most forms of glaucoma.[1] Different etiological mechanisms have been proposed for increased IOP. Aqueous humor (AH) drainage from conventional or trabecular and uveoscleral pathway is a proven cause in most glaucoma patients and especially so in primary open angle glaucoma. Intra-ocular-pressure reduction slows vision loss and remains the mainstay of treatment for all types of glaucoma.

Rho Kinase (ROCK) inhibitors Ripasudil, K-115 and Netarsudil, AR-13503 have recently been approved for ophthalmological use in therapy of glaucoma as IOP-lowering agents in Japan and the United States of America. A Rho kinase/norepinephrine transporter inhibitor will soon complete Phase 3 clinical development.[2,3] Additionally, combination therapy using Rho kinase inhibitors in combination with timolol or latanoprost is being exploited to harness additive efficacy to lower IOP.

Rho kinase, a serine/threonine protein kinase, is instrumental in the regulating and modulating cell shape and size by changes on the cytoskeleton. Rho kinase is a downstream effectors of Rho GTPase. Rho GTPases belong to Ras superfamily—which contains monomeric GTP-binding proteins. The roles of RhoA, Rac1 and CDC42 have been elucidated in the regulation of actin dynamics and various actin- associated cellular activities.[4,5] The intracellular GTPases act as molecular switches –

i) an active conformation with GTP-binding and

ii) inactive conformation with GDP-binding.

Guanine-nucleotide-exchange-factors (GEFs), GTPase-activating-proteins (GAPs) and guanine-nucleotide-dissociation-inhibitors (GDIs) control and modulate the transformation of GDP and GTP binding.[4] These GEFs and GAPs are activated by receptors in the cell membrane.[6,7]

RhoA has many downstream effectors like the best studied coiled-coil serine/threonine kinase, also called Rho kinase, which has two isoforms--ROCK1 (ROKb/P160) and ROCK2 (ROKa). Rho kinase genes are located on chromosome 2[ROCK2 (ROKa)] and 18 [for ROCK1 (ROKb/P160)].[8,9,10]

N-terminal serine/threonine kinase domain is attached to a coiled-coil region with the Rho-binding domain (RBD) and a pleckstrin homology domain with a C terminus cysteine-rich domain.[9] 65 percent homology exists between ROCK1 (ROKb/P160) and ROCK2 (ROKa). While many cellular effects are common to both isoforms some isoform-specific activities have also been described.[10]

Rho kinase is activated by Rho GTPase and then it phosphorylates various intracellular substrates characterized substrates include myosin light chain (MLC), myosin phosphatase substrate 1 (MYPT1, the regulatory subunit of myosin phosphatase), LIM kinase, CP1-17, calponin and the ERM (ezrin, radixin and moesin) proteins.[8,10] Rho kinase, acting through substrates, regulates actin cytoskeletal dynamics, actomyosin contraction, cell adhesion, cell stiffness, cell morphology and ECM reorganization. While cellular contraction can be regulated via both calcium-dependent and independent means involving myosin light chain kinase and myosin phosphatase, respectively, Rho kinase has been demonstrated to regulate cellular contraction in smooth muscle tissues mainly through modulating myosin II activity in a calcium-independent manner.[5,10]

Its action on calcium-independent regulation of smooth muscle contraction is well known. Alan Hall, in his classical treatise, demonstrated that Rho kinase pathway regulated the actin cytoskeleton and coordinated different cellular responses like shape and adhesion.[5] Cytoskeletal dynamics,

actomyosin contractility, cell adhesion, cell stiffening, cell morphology and extracellular matrix reorganization are all mediated by that Rho kinase pathway. Aqueous Humor outflow occurs through the trabecular pathway and the uveoscleral pathway. The trabecular path consists of, trabecular meshwork, juxtacanalicular tissue, Schlemm's canal and the aqueous drains into episcleral veins. The cytoskeletal and extracellular matrix changes affect the aqueous outflow through the conventional or trabecular pathway. Physiological evidence now suggests a relationship between Rho kinase functionality and AH outflow in elevated as well as normal intra-ocular pressure. Rho kinase inhibitors are shown to alter cell shape of the cells in the trabecular meshwork. The enhanced transport through the conventional pathway lowers the IOP.[11] Additional changes in the permeability of the episcleral vessels adds to the effect in normotensive intra-ocular-pressure compared to the other drugs which act better in elevated pressures only.

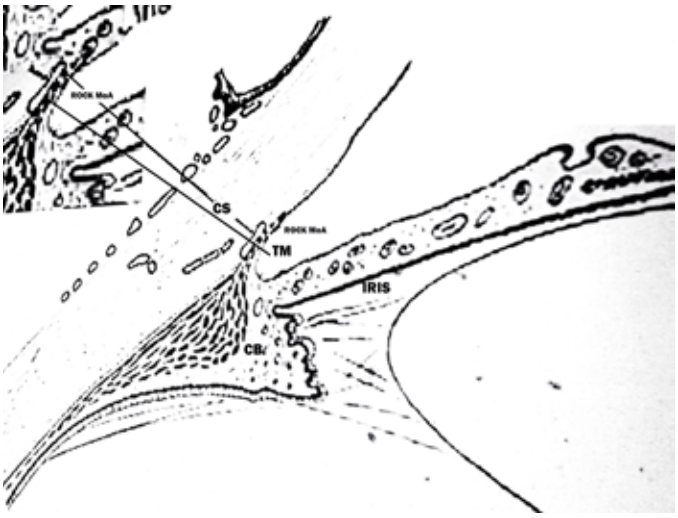

Figure 1: *TM- Trabecular Meshwork, CS- Canal of Schlemm, ROCK MoA- Rho Kinase Inhibitors Mechanism of Action Site, CB- Ciliary Body*

The Rho GTPase/Rho kinase signaling pathway is an identified mechanism integrating inputs from various external signals /factors to generate intracellular products to regulate cellular changes affect AH outflow through the trabecular pathway. This work is providing information about other related molecular pathways and targets for pharmacological manipulation of IOP. Studies to elucidate Rho/Rho kinase signaling and the effect of external cues in the healthy AH outflow pathway and dysregulation of this pathway (eg in aging and glaucoma) are still required but the work from animal models has shed a lot of light on the working of the pathway. Apart from lowering IOP, Rho kinase inhibitors also provide neuroprotection by reduced retinal ganglion cell apoptosis and anti-fibrotic activity to slow or prevent tissue scarring following glaucoma filtration surgery. Rho kinase inhibitors inhibited TGF-β2, lysophosphatidic acid (LPA) and RhoA-induced cell transdifferentiation of the trabecular meshwork cells to myofibroblastic cells.[12]

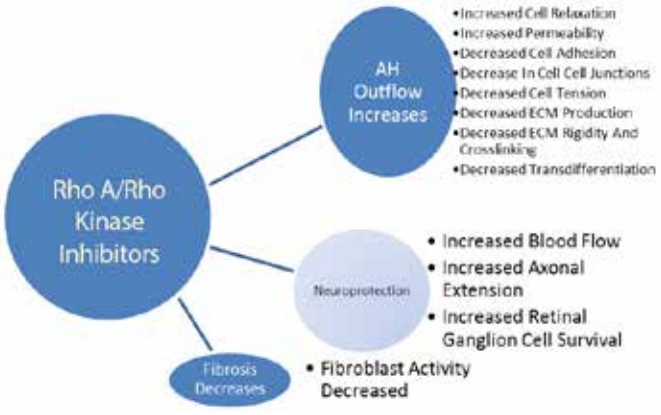

Figure 2: *Rho Kinase Inhibitors Actions*

Several studies to evaluate ocular hypotensive effects of different Rho kinase inhibitors in humans have been undertaken. Some of the Rho kinase inhibitors evaluated for clinical safety and efficacy in humans are K-115 (ripasudil), fasudil, AR-13324 (netarsudil), AMA0076,SNJ-1656 and AR-12286. Ocular hypotensive effects have been demonstrated in these agents. Ripasudil (Kowa) has advanced to phase 3 clinical studies in Japan and netarsudil (Aerie) to phase3 study in USA. In Japan, Ripasudil has been approved recently for treatment of ocular hypertension and glaucoma.13 Additive efficacy in combination with beta blocker timolol (Aqueous Humour production blocker) and prostaglandin F2α analog latanoprost (uveoscleral outflow enhancer) has been reported. In phase 3 trials 0.4% Ripasudil administered topically twice a day was reported to reduce mean diurnal IOP by 2.9 mmHg in glaucoma patients when used as monotherapy. Additive efficacy demonstrates that the Rho kinase mechanism of lowering IOP by increasing trabecular outflow can be used concurrently with other approved classes of glaucoma drugs. The results of phase 3 clinical trials of Rho kinase/norepinephrine transporter inhibitor Netarsudil (Aerie Pharmaceuticals) are awaited. In phase 2 clinical trials in elevated IOP patients 0.02% Netarsudil instilled topically once-daily reduced mean diurnal IOP ranging from 5.7 to 6.8 mmHg.[14] 0.02% Netarsudil demonstrated same IOP lowering effect at both lower and higher baseline IOPs in contrast to the gold standard Latanoprost which shows better efficacy at higher baseline pressures. A Phase 1 study in healthy subjects with low baseline IOPs (14-20 mm of Hg) 0.02% Netarsudil in once daily dose reduced mean diurnal IOP from 16.2 mmHg to 11.3 mmHg after 8 days of treatment.[15] Thus the drug is being looked at with interest for the ocular hypertensive patients following the results of the analysis of follow up of the cohort of OHTS patients and also for normotensive glaucoma. It has been postulated that substantial IOP reductions in lower baseline IOPs could be due to Netarsudil's action of lowering episcleral venous pressure reported in rabbits.[16] Episcleral venous pressure is reported to account for more than half of measured IOP in normotensive patients. Hence the action could be used as an additive effect in most forms of glaucoma.

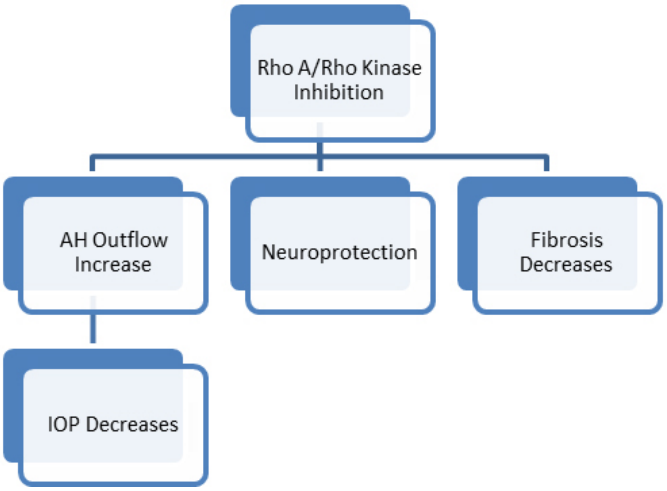

Figure 3: *Main Actions of Rho Kinase Pathway Inhibition*

Aerie Pharmaceuticals has reported that PG324-a fixed combination of 0.005% Latanoprost and 0.02% Netarsudil in once-daily instillation gave IOP reductions 1.6-3.2 mm of Hg greater than latanoprost alone.[15] It is currently available as Rocklatan (netarsudil and latanoprost ophthalmic solution), a fixed dose combination of the Rho kinase inhibitor netarsudil (Rhopressa) and the prostaglandin F2α analogue latanoprost (Xalatan). It reduces elevated intraocular pressure (IOP) in open-angle glaucoma and ocular hypertension. It is prescribed as a once daily instillation. Cause changes to the eyelashes, including increased length, thickness and number of lashes. Eyelash changes are usually reversible upon discontinuation of treatment. The most common adverse reaction reported was conjunctival hyperemia (eye redness) though instillation site pain, corneal verticillata and conjunctival hemorrhage may also occur.[17,18]

Rho kinase inhibitors may also be used as neuroprotective agents. Rho kinase signaling is involved in different central nervous system (CNS) disorders.[19] Rho GTPase signaling pathway is involved in axonal outgrowth inhibition by Nogo, myelin-associated glycoprotein (MAG) and oligodendrocyte-myelin glycoprotein (OMgp). 19 Rho GTPase and Rho kinase influence neuronal cell death by affecting activity of several molecules involved in cell survival and death like PTEN (Phosphatase and Tensin homolog) and microtubule-associated proteins.

Goldhagen et al demonstrated elevated levels of RhoA and Rho kinase at the optic nerve head and optic nerve head thereby elucidating role of Rho GTPase signaling in optic nerve damage due to glaucoma. The rat optic nerve crush model of retinal ganglion cell apoptosis and axonal degeneration demonstrated similar findings. Apoptosis and axonal degeneration were associated with elevated levels of RhoA, RhoA activation, caspase-3 and ROCK2 in the retinal ganglion cell layer.[20]

Experimental studies have shown that inhibition of the Rho/Rho kinase signaling pathway resulted in suppression of neuronal damage in different CNS disease models. It also increased axonal extension and increased neuronal survival.[19] C3 exoenzyme induced RhoA inhibition and shRNA mediated RhoA suppression increased axonal outgrowth and retina ganglion cell survival in different animal glaucomatous damage models. Rho kinase inhibitors like Fasudil (HA1007), Ripasudil (K-115), Y-39983 (SJN-1656) and Y-27632 exhibited neuroprotection, enhanced axonal outgrowth and RGC survival in animal models. Fasudil and Y-39983 demonstrated increased blood flow to the optic nerve head in rabbits. Rho GTPase and Rho kinase inhibitors are therefore potential neuroprotective agents21 awaiting a studies to demonstrate neuroprotective effect in human patients.

Asymptomatic transient conjunctival hyperemia is the most common adverse event reported with use of different Rho kinase inhibitors. Conjunctival hyperemia expected from Rho kinase inhibitors because they relax smooth muscle cells and dilate blood vessels.

The Rho GTPase/Rho kinase signaling pathway is increasingly being postulated as an important mechanism in integrating inputs from external factors and generating outputs as well as cellular effects regulating aqueous humour outflow through the trabecular pathway. Ripasudil is the first Rho kinase inhibitor approved for the treatment of patients with glaucoma. Rho kinase/norepinephrine transporter inhibitor will soon complete Phase 3 clinical development. Additionally, combinatorial therapy using Rho kinase inhibitors in combination with timolol or latanoprost is being exploited to harness additive efficacy to lower IOP. Importantly, as this work continues, it is providing important and novel insights into other related molecular pathways and targets for pharmacological manipulation of IOP. Most significantly, additional studies are required to understand how Rho/Rho kinase signaling is regulated by external factors in the healthy AH outflow pathway, as well as to identify the basis for dysregulation of this pathway with aging and ultimately in glaucomatous eyes.

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