Emerging role of Rho-Kinase Inhibitors – Review

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Abstract :

This review article targets to provide information regarding the role of ROCK and its inhibitors in glaucoma, corneal diseases, and retinal pathologies. Rho-associated protein kinase (ROCK) is a well-characterized effector of Rho GTPase, a small GTP-binding protein. The Rho/ROCK signaling pathways contribute to a wide range of fundamental cellular events, such as cell adhesion, motility, proliferation, differentiation, and apoptosis. We found strong evidence demonstrating that inhibition of Rho kinase considerably decreases IOP, increases healing of the corneal endothelium, and decreases progression of diabetic retinopathy. The main side effect of ROCK inhibitors is conjunctival hyperemia that is often present in more than half of the patients in certain formulations. Other properties such as neuro protection (enhancing optic nerve blood flow and promoting axonal regeneration), anti-fibrotic activity, and endothelial cell proliferation may enhance the visual prognosis and surgical results in glaucoma.

The aim is to provide authoritative and cutting-edge reviews of topical state-of-the-art basic research that is expected to have broad clinical impact in the next few years.

Keywords : Glaucoma; Intraocular Pressure; Aqueous humor; Trabecular Meshwork, ROCK; Rho Kinase Inhibitors.

Introduction

The Rho kinase (ROCK) signaling pathway is involved in several cellular events that include cell proliferation and cytoskeleton modulation leading to cell adhesion. The ROCK pathway in the human eye has been hypothesized to play important roles in corneal endothelial cell physiology and pathologic states. In addition, ROCK signaling has been identified as an important regulator of trabecular meshwork (TM) outflow, which is altered in glaucomatous eyes. These roles in corneal and glaucomatous disease states have led to the growing interest in the development of drugs selectively targeting this pathway (ROCK inhibitors).

A search for Rho kinase inhibitors led to the discovery of several molecules of therapeutic interest, leaving us today with new ocular hypotensive agents approved for clinical use: ripasudil in Japan and netarsudil in the United States. These represent members of the first new class of clinically useful ocular hypotensive agents since the US Food and Drug Administration approval of latanoprost in 1996. The development of Rho kinase inhibitors as a class of medications to lower IOP in patients with glaucoma and ocular hypertension represents a triumph in translational research.

Few studies began a focus on the role of cell mechanics in the aqueous humor outflow pathways and the role of Rho kinase in this process.They were able to show that cytoskeletally-active agents such as latrunculin (that depolymerizes f-actin) and H7 (a protein kinase inhibitor that affects rho kinase) significantly decreased aqueous humor outflow resistance.[1-4]

Rho kinase inhibitors are effective alone or when combined with other known ocular hypotensive medications. They also offer the possibility of neuroprotective activity, a favorable impact on ocular blood flow, and even an antifibrotic effect that may prove useful in conventional glaucoma surgery. Local adverse effects, however, including conjunctival hyperemia, subconjunctival hemorrhages, and cornea verticillata, are common.

Development of Rho kinase inhibitors targeted to the cells of the outflow pathway and the retina may allow these agents to have even greater clinical impact. The aim of this review is to discuss the idea underlying the development of Rho kinase inhibitors as a therapy to lower IOP and to summarize the results of the clinical studies reported to date. The neuroprotective and vasoactive properties of Rho kinase inhibitors, as well as the antifibrotic properties, of these agents are reviewed in the context of their possible role in the medical and surgical treatment of glaucoma. The use of Rho Kinase (ROCK) inhibitors as therapeutic agents in ophthalmology has been a topic of discussion for several years, particularly in the realm of glaucoma, Fuchs' endothelial dystrophy, and diabetic retinopathy.

Rho kinase Signaling Pathway :

Rho kinase is a downstream effector of the RhoA protein, a

small GTPase. GTPases alternate between two conformations: a Guanosine Triphosphate (GTP)-bound active conformation and a Guanosine Diphosphate (GDP)-bound inactive conformation.

This GTPase activation regulation is controlled by Guanine nucleotide Exchange Factors (GEFs), GTPase Activating Proteins (GAPs), and Guanine nucleotide Dissociation Inhibitors (GDIs) .[5, 6, 7] After activation of RhoA, the coiledtail serine/threonine kinase, the downstream effector Rho kinase, becomes active. The Rho kinase has two isoforms, ROCK1 (ROKβ) and ROCK 2 (ROKα). Although both isoforms share similar effects, they also fulfill some isoform-specific roles. Rho kinase phosphorylates various intracellular substrates, including the two seen in Figure 1,

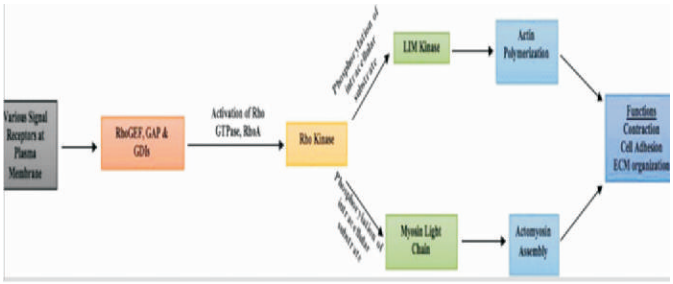


Figure 1 : RhoGEF: Rho Guanine nucleotide Exchange Factor; GAP: GTPase Activating Proteins; GDI: Guanine nucleotide Disassociation Inhibitor; RhoA: Ras Homolog Gene Family Member A; ECM: Extracellular Matrix; GTPase: Guanosine triphosphatase

the myosin light chain, and the LIM kinase.[6]

These substrates then interact to control actomyosin contractility, membrane permeability, cellular adhesion, cell stiffening, cell morphological changes, extracellular matrix organization, as well as DNA synthesis .[5, 8]

The Rho/ROCK signaling pathways contribute to a wide range of fundamental cellular events, such as cell adhesion, motility, proliferation, differentiation, and apoptosis. The role of ROCK in the control of a wide spectrum of biological events has made it a subject of intensive investigation as an important therapeutic target in a wide range of diseases, including vascular disease, cancer, neuronal degenerative disease, asthma, and glaucoma.

Drugs : Ripasudil 0.4% (available in India) , Netarsudil 0.02% (Available in India) , SNJ-1656 and AR-12286 (under trial) and fixed-dose combination (FDC) of netarsudil with latanoprost.

Ripasudil :

Ripasudil, also known as K-115 from various clinical trials, is an ophthalmic solution used as a treatment of glaucoma. It has the chemical formula of C15H18FN3O2S and has the International Union of Pure and Applied Chemistry (IUPAC),name being 4-fluoro-5-(((2S)-2-methyl-1,4-diazepan-1-yl)sulfonyl) isoquinoline .[9]

This new drug was shown to lower IOP within two hours of instillation of the drop solution, and was proven to do so consistently over a period of a full year .[9, 10] The most commonly seen adverse effect of Ripasudil is conjunctival hyperemia. This is a dose-dependent side effect and is seen in the majority of patients treated with Ripasudil. Conjunctival hemorrhage was also seen in treated patients, however, this side effect showed no dose dependency.[11]

Ripasudil (K-115): Ripasudil hydrochloride hydrate, (0.4% or Ripasudil) a fluorinated analog of Fasudil, is a small molecule ROCK inhibitor developed and introduced by Kowa Company, Ltd (Naka-ku, Nagoya, Japan) for treatment of glaucoma and OHT in 2014.[12]

Phase 1 and Phase 2 clinical trials as well as a 24-hour time-course study established Ripasudil 0.4% BID as a clinically useful concentration and dosing frequency for the treatment of glaucoma and OHT.

A phase II clinical trial was conducted during year 2013, in Japan, that determined the optimal dosage of ripasudil, K-115. A group of individuals with open angle glaucoma were assigned to four different groups: a placebo, 0.1% ripasudil, 0.2% ripasudil, and 0.4% ripasudil. The results of this study showed that over an eight-week period, there was a decrease in baseline IOP in all groups using ripasudil. It also showed that as concentration of dosage increased, IOP decreased. After comparing the results of the trial, researchers concluded that the optimal dosage, based on dose-response alone, was the 0.4% dose, which had a reduction in baseline IOP of -4.5 mm Hg, two hours after the last instillation of the drop. However, this study also showed that there may be a direct correlation between increased dosage and increased cases of conjunctival hyperemia. The reported cases of conjunctival hyperemia were 13.0%, 43.4%, 57.4%, and 65.3% in the placebo, 0.1% ripasudil, 0.2% ripasudil, and 0.4% ripasudil groups, respectively [10]. In Japan, a ripasudil drop solution was approved at a 0.4% concentration, as a twice daily treatment, to be used to treat glaucoma .[12]

In 2014, ripasudil, a ROCK inhibitor, gained approval in Japan to be specifically used for treatment of ocular hypertension and glaucoma . As recently as December 18th, 2017, Rhopressa, a Rho kinase inhibiting drug consisting of Netarsudil, gained Food and Drug Administration (FDA) approval; the first of its kind to do so in the United States of America .[13] Netarsudil (AR-13324) is not only a ROCK inhibitor but also norepinephrine

transporter inhibitor leading to additional benefits in glaucoma.

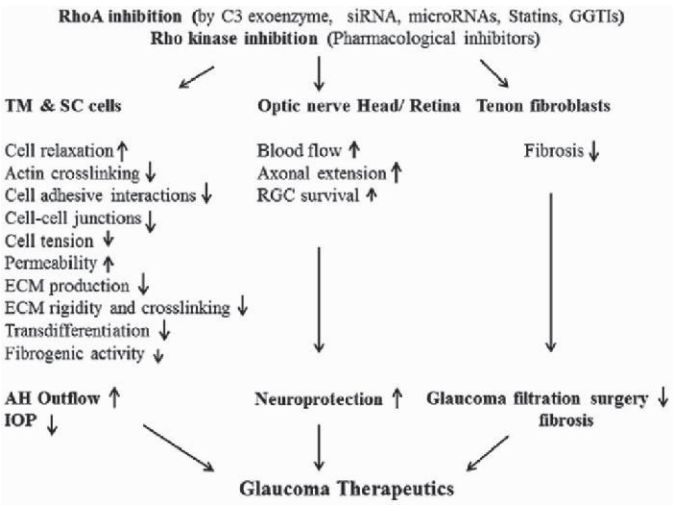
A non-comparative, year-long, open-label study reported IOP reduction of 2.6–3.7 mmHg from baseline with POAG or OHT patients after monotherapy.[14]

Side effects of Ripasudil included dose-dependent conjunctival hyperemia and non-dose dependent conjunctival hemorrhage. Nonocular side effects were rare and generally not severe: constipation (0.6%), headache (0.1%), dizziness (0.1%), nausea (0.1%), and others.

The Rho Kinase Elevated IOP Treatment trials 1 and 2 (ROCKET-1 and ROCKET-2), two phase three clinical trials, investigated safety and effectiveness relating to netarsudil and timolol in a sample of patients with elevated IOP. In a double-masked, randomized non-inferiority clinical trial, Netarsudil once a day (q.d.), produced significant lowering from baseline IOP, which was non-inferior to timolol (ROCKET-1). Netarsudil twice a day (b.i.d.) showed non-inferiority to timolol as well (ROCKET-2) .[15] In the United States, a netarsudil drop was approved in a 0.02% concentration, as a one drop q.d. treatment to lower IOP for treatment of glaucoma .[16]

Application of Rho Kinase Inhibitors in Ophthalmology Glaucoma :

Glaucoma is classified as a progressive form of optic neuropathy. The most predominant risk factor with either type is elevated IOP. In open angle glaucoma, it is proposed that this elevation in IOP is due to the clogging of AH drainage canal, through the Trabecular Meshwork (TM).[17] Although the physiological mechanism for this impairment is not entirely known, it is proposed that the best therapeutic remedy for open angle glaucoma is lowering the IOP by enhancing the outflow of AH, as shown in



Rho kinase inhibitors have been tested and proven to alter cell shape in the trabecular meshwork, allowing for enhanced AH outflow and the lowering of IOP .[18, 19]

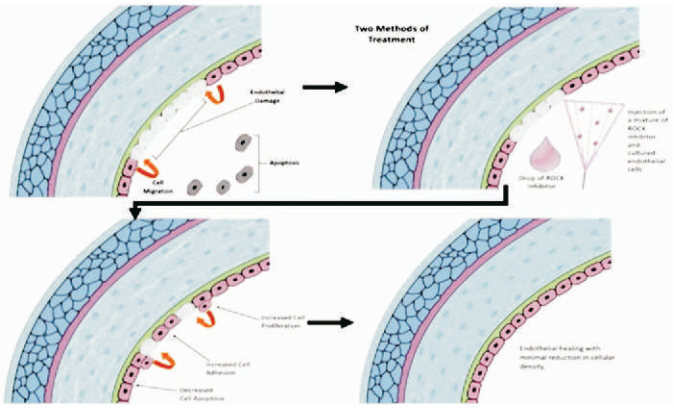
Mechanism :

Rho kinase inhibitors help lower IOP by increasing AH outflow, reducing AH production, and decreasing episcleral venous pressure (EVP).[20] This is done in two different ways, which involves Rho kinase pathway inhibition and, as with Netarsudil, norepinephrine transport inhibition.

Many ROCK-inhibiting drugs chemically include a norepinephrine transport inhibitor. This norepinephrine transport inhibitor helps reduce AH production and decreases EVP, which according to the modified Goldman equation, have a direct relationship with IOP.[21, 22] Norepinephrine transport inhibition lowers AH production by vasoconstriction, reducing blood flow to ciliary processes. A study showed that AH production may be reduced by 20% to 23% by the norepinephrine transport inhibitor .[22] This inhibitor affects the EVP via vasoconstriction, similar to the way brimonidine, a well-known vasoconstrictor, has been shown to lower EVP in animals .[23] The reduction in EVP accounts for more than a third of the reduction in IOP, as shown in a study using Dutch Belted rabbits .[24]

Mechanism of Action of ROCK Inhibitor in Corneal Endothelium Healing :

Due to the wide range of cellular responses controlled by Rho kinase signaling pathway, it is hypothesized that ROCK inhibitors could play a part in both increasing cell adhesion and proliferation in the corneal endothelium .[25] This would allow for the preservation of corneal endothelial cells and the slowing of apoptosis. For this reason, the use of Rho kinase inhibitors may also help with acute corneal endothelial damage, that can potentially occur in cataract surgery.[26] Successful clinical trials have been performed to show the positive effects of Rho kinase inhibitors on the corneal endothelium. [26, 27]



Role in vitreoretinal pathologies :

ROCK signalling pathway in the pathogenesis of DMO has increased interest in this field. Activation of the Rho kinase pathway also has a direct correlation with microvascular endothelial damage via inactivation of the nitric oxide synthase. Inhibition of nitric oxide levels prevents vasodilation and allows for apoptosis, which increases the leukocyte-induced damage. Leukocyte stasis plays a role in the microvascular complications in DR.[28] ROCK pathway has been reported to regulate certain adhesion molecules in vascular endothelial cells.[29]

ROCK inhibitors can be beneficial for patients with symptoms of DR, by reducing the adhesion of leukocytes and increasing nitric oxide levels. They also prevent RGC apoptosis.[30] ROCK inhibitors might represent a new treatment strategy in early stages of DR which usually is only observed with no ophthalmic therapeutic intervention. Intravitreal implants to deliver these ROCK inhibitors are also being studied. In the later stages of DR, retinal neovascularization and epiretinal fibrovascular membranes are formed, the contraction of which can cause tractional retinal detachment. ROCK inhibition has effectively prevented contraction of these membranes in animal model.[31] ROCK inhibitors have also been studied as therapeutic agents for diabetic macular edema [32] and retinal ischemia.

Adverse Effects of ROCK Inhibitors :

Although ROCK inhibitors showed a promising safety profile, they have both local and systemic adverse events. ROCK inhibitors induce conjunctival hyperemia and sub conjunctival hemorrhage due to their vasodilatory effect. The latter may increase the clearance of concomitantly administered topical drugs thereby reducing their intended ocular effects.[33] Other local effects include: blepharitis, ocular irritation, increased lacrimation, and blurred vision. On the systemic level, they may cause blood pressure reduction and an associated increase in heart rate.[34] Strategies allowing reduced systemic exposure as a soft drug approach have been applied to develop ROCK inhibitors for localized applications.[35] In addition, systemic ROCK inhibition was found to induce a reversible reduction in lymphocyte counts in few individuals.[36]

Conclusion:

ROCK inhibitors appear to be a promising new drug with a special mechanism of action. They can be considered as second line of treatment or as adjuvants. Along with the IOP lowering action, it increases ocular blood flow and prevents RGC death. ROCK inhibitor can consequently possibly be considered as first line of remedy in NTG. It is valuable in patients in whom IOP is not under control with maximum medical therapy, which is a common scenario in developing countries like ours.

Ripasudil may be taken into consideration as the initial drug while restarting antiglaucoma medications in post-trabeculectomy patients due to its antifibroblastic activity. ROCK inhibitors have shown promising results in secondary glaucomas as well. Its additional uses such as corneal endothelial protection and role in DR and macular edema are helpful in patients with glaucoma with these diseases. Conjunctival hyperemia being reported in a signficant number of patients might limit its use. Reassuring the patient prior to starting the drug regarding this possible side effect might go a long way in improving compliance. Additional clinical trials investigating the reviewed treatment options of Rho kinase inhibitors are necessary to further validate previous findings on the topic. Nonetheless, it is clear that Rho kinase inhibitors have the potential to be another potent therapeutic option for several chronic diseases in ophthalmology.

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