ejpmr, 2020,7(8), 318-330

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

RHO KINASE INHIBITORS: THE MULTIFACETED DRUG IN OPHTHALMOLOGY

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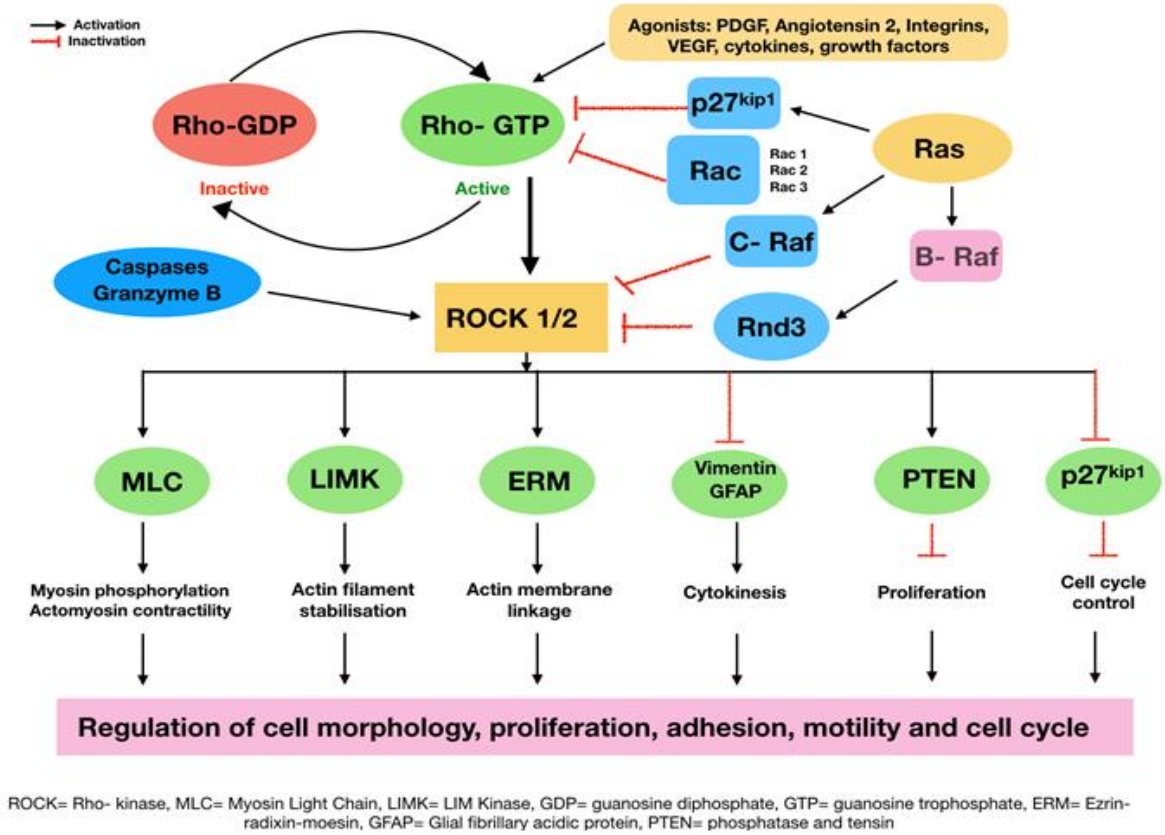
Article Received on 09/06/2020 **Article Revised on 29/06/2020** **Article Accepted on 19/07/2020**

ABSTRACT

The evolution of Rho Kinase (ROCK) inhibitors as therapeutic agents in ophthalmology has been seen as a positive approach for containing and managing various diseases. In this review, a detailed and comprehensive overview of the published literature on the use of Rho kinase inhibitors for various ophthalmological purposes has been provided by us. A systematic review of many databases has been performed to identify ample literature on ROCK inhibitors. This research found strong evidence establishing that inhibition of Rho kinase markedly decreases IOP, increases healing of the corneal endothelium, stalls the progression of diabetic retinopathy, has a significant role in axial myopia and various other Vitreo-retinal diseases. It is quite evident that Rho kinase inhibitors have the ability to be another effective therapeutic alternative for a variety of chronic diseases in ophthalmology.

KEYWORDS: Rho kinase, Rho kinase inhibitors, ROCK, Y-27632, Ripasudil (K-115), Netarsudil.



ROCK= Rho- kinase, MLC= Myosin Light Chain, LIMK= LIM Kinase, GDP= guanosine diphosphate, GTP= guanosine trophosphate, ERM= Ezrin-radixin-moesin, GFAP= Glial fibrillary acidic protein, PTEN= phosphatase and tensin

Fig. 1: ROCK pathway.

METHODOLOGY

We searched PubMed, Science Direct and Journal of Ophthalmology for literature published in English between 1995 and March 2020, incorporating the general search term "Rho kinase inhibitors" with connecting terms relevant to subheadings — e.g., "open angle glaucoma", "retinal venous occlusion", "intraocular pressure", "diabetic retinopathy", "corneal endothelium", "axial myopia", "ARMD", "history", and various other terms. References from identified studies have been reviewed and included if deemed appropriate, valid and scientifically important. If referenced in a selected English paper, we contemplated papers in other languages too. We preferentially selected papers that have been published in the last 10 years, but we have included relevant older references.

INTRODUCTION

Rho kinase is a serine / threonine protein kinase. It acts on the cytoskeleton and thus regulate cell morphology. It has a down streaming effect on Rho GTPase. It actively controls the calcium-independent contraction of smooth muscle.[1] They have also been related to the regulation of force of actomyosin contraction, cytoskeletal mechanics, cell adhesion, reorganization of extracellular matrix, and cell size and shape. Study on effects on Rho kinase dates back to late 1990s, and continues till date.[2] The majority of focus of the studies has been on the role of Rho kinase inhibitor (ROCK) on Intraocular Pressure (IOP). Fewer researches have also been done to study the effect of Rho kinase inhibitors on diabetic retinopathy and corneal endothelium. More work is currently ongoing on the various therapeutic choices, dosages and formulations for Rho kinase inhibitors. Alan Hall[2] explained the connection between the Rho pathway and the role of actin cytoskeleton. From 2010 onwards, researches are performed to explore the potential usage of Rho kinase inhibitors for various diseases, like diabetic retinopathy and endothelial corneal damage.[3] As information was attained from these studies, additional clinical trials were performed to establish the right type, dose and period of usage of Rho kinase inhibitors.

Rho kinase (ROCK) (fig. 1)

The Rho family consists of RhoA, RhoB and RhoC. These are a family of G-proteins that are specifically activated by binding to GTP (guanosine triphosphate) and inactivated by binding to GDP (guanosine diphosphate). The following cytokines, including endothelin-1 (ET-1), thrombin, angiotensin II, lysophosphatidic acid, and transforming growth factor (TGF)-b, or integrin help in their activation.[4] They influence cell composition, orientation, replication, cytokinesis, adhesion, polarity, apoptosis, neurite elongation and smooth muscle contraction.

There is a 65% similarity and 87% identicality in kinase domain between ROCK1 and ROCK2,[5] with their genes located on chromosome 18 (18q11.1) and chromosome 2 (2p24), respectively.

Hence, they can activate similar targets with little differences in their effect.[6] Rho binds to Rho kinase only in the active guanosine triphosphate-bound form. However, arachidonic acid, sphingosyl-phosphorylcholine and apoptosis are the other activators of Rho kinase.

Rho Kinase inhibitors

Rho kinase inhibitors have a variety of implications. ROCK inhibitors resist axonal degeneration. They facilitate axon regeneration. Ripasudil (K-115) and netarsudil (AR-13324) are the two Rho kinase inhibitors currently approved for management of glaucoma. Other known ROCK inhibitors include SAR-407899, RKI-1447, Y-27632, Wf-536, AMA-0076, Y-39983, SB-772077-B, GSK-269962A and H-1152. All the ROCK inhibitors act on ROCK1 ands ROCK2. KD-025 is the only known ROCK2- specific inhibitor.

Rho Kinase Inhibitors effect on lowering resistance of aqueous outflow

Kaufman et al, in 1977[7] enhanced the fact that an actin depolymerizing agent, cytochalasin B, plays an important role in controlling resistance of aqueous humor outflow by manipulating the cytoskeleton. The decrease in the outflow resistance was due to increase in the pore density of Schlemm's canal cells and also the breaks between cells.[8] The aqueous humor passes through the juxtacanalicular connective tissue (JCT). It then enters the pores which are spaced widely in the inner wall endothelium of Schlemm's canal (SC). Thus, the outflow resistance is increased markedly by this nonuniform flow.[9] The stiffness of Schlemm's canal cell is decreased, which raises the quantity of these pores. As a consequence, the resistance of aqueous outflow is diminished.

Kaufman and Geiger showed that latrunculin-A and latrunculin-B[10,11] which are actin depolymerizing agents increased the outflow facility. They increase the pore density in the inner wall endothelium and also separate the endothelium of the inner wall from the JCT.[12]

H-7 aids in blocking the phosphorylation activity of Rho kinase.[13] They, thus inhibit the contraction of the cell and induce relaxation. It thus reversibly decreases resistance of the outflow.

Y-27632 and fasudil were the one of the initial specific Rho kinase inhibitors which were considered for the impact on outflow.[14] The cytoskeleton of cells of Schlemm's canal and trabecular meshwork were highly affected by them. They decreased the amount of stress fibers of actin. Reductions in outflow resistance and 65% of IOP were seen.[15] However, unconventional outflow was unaffected.

MLC kinase remarkably lowered the outflow resistance without any repercussions on unconventional flow.[16] Further, H-1152 reduced MLC phosphorylation in the trabecular meshwork.

Trabecular meshwork cells express both ROCK1 and ROCK2. As a consequence, the affirmation of cytoskeletal control of resistance of outflow is high. Moreover, the likelihood for control to be altered by Rho kinase inhibitors is present.[17]

RhoA and ROCKs are present in cultured human SC cells, TM cells and bovine ciliary muscles. It has been analyzed by immunoblot analysis.[14] Nakajima et al.[18] used the RT-PCR analysis to testify the presence of ROCK1 and 2 in trabecular meshwork and ciliary muscles. By using immuno-histochemical analysis. Goldhagen et al. found the presence of RhoA, ROCK1 and ROCK2 in the human aqueous outflow pathway inclusive of SC, TM and JCT.[19] It is hypothesised that in glaucomatous eyes, expression of the Rho/ROCK pathway is increased in the tissue responsible for the outflow.

This can also be clarified why several medicines used to regulate glaucoma can influence the expression of Rho / Rho kinase in the outflow pathway.

Mechanisms of action of Rho-kinase inhibitor in glaucoma treatment

(a) Improving aqueous humor outflow

Rho-kinase inhibitors can improve aqueous outflow via relaxation of the trabecular meshwork tissue[20,14] as discussed above. In rabbits, when the topical or intravitreal injection of Rho-kinase inhibitors are administered, it has been seen that there is a reduction of IOP within 30 minutes.[21]

(b) Improving optic nerve perfusion

Altered optic nerve perfusion is considered to participate in pathophysiology of few varieties of glaucoma, specifically normal tension glaucoma, due to either vasospasm or altered hemodynamics.[22,23] Evidence have shown that Rho-kinase has a significant contribution in cardiovascular and cerebral diseases. They have shown benefit in the treatment of cardiac vasospasm.[24] RhoA, ROCK1 and ROCK2 are normally expressed on human optic nerve head (ONH). Increased amount of Rho GTPase and extracellular matrix proteins are found in cultured glaucomatous ONH astrocytes. In glaucoma eyes of the human, RhoA expression in the ONH is increased significantly in comparison to the normal subjects. This points towards a probable association of RhoA/ROCK pathway in causing damage to the optic nerve head in glaucoma. In vascular smooth muscles, Rho/ROCK pathway is expressed, and ROCK inhibitors relax various vascular smooth muscles, which increase ocular and retinal blood flow by inducing vasodilatation and, hence, providing neuroprotective action. Hence, ROCK inhibitors may improve the blood flow rate in the optic nerve head. Thus, Rho / ROCK signaling could be a successful goal for managing optic neuropathy in glaucoma.

(c) Retinal ganglion cell (RGC) protection

Studies conducted in animals have demonstrated that Rho-kinase is involved in the neurotoxicity. If Rho-kinase is inhibited in the cell body, it will prove to be not only neuroprotective but will also help in overcoming growth inhibition.[25,26] An improvement in the survival of retinal ganglion cells with Rho-kinase inhibition is seen in animals.[27] RhoA antagonist C3 when injected intraocularly, has been documented to improve axonal regeneration as well as RGC survival following optic nerve axotomy in rats. Inactivation of ROCK signaling leads to neuroprotection of retinal neuronal cells involved in ischemic or reperfusion injuries. Retinal ischemia / reperfusion inflammation contributes to destruction of neuronal cells in the inner retinal membranes, like RGCs and amacrine cells, and temporary retinal ischemia-induced neuronal apoptosis occurs via the reperfusion process rather than ischemia. During reperfusion, injury is stimulated by penetration of leukocytes through neural tissue by endothelial vascular cells. The Rho / Rho kinase system leads to the extravasation of leukocytes by controlling the leukocyte cytoskeleton and the tight junctions of endothelial cells. ROCK inhibitors attenuate ischemia / reperfusion mediated retinal cell apoptosis in the inner retinal layers (including RGCs) by reducing the Bax / Bcl-2 mRNA ratio and caspase-3 and iNOS expression and by controlling leukocyte infiltration in the neural tissue.

(d) Promoting RGC axon regeneration

The Rho / ROCK pathway has been predominantly identified with inhibitory signalling for nerve elongation, and inactivation of the Rho / ROCK pathway can facilitate regeneration. After the injury to mammalian CNS, there may be failure of axon regeneration. It is because of the restricted supply of neurotrophic factors (NTFs) that facilitate neuron survival and regeneration of axon and the existence of myelin-and scar-derived inhibitory molecules such as Nogo-A, oligodendrocyte-myelin glycoprotein (Omgp), chondroitin sulphate proteoglycan (CSPG), myelin-associated glycoprotein (MAG), ephrins and semaphorins. On activation, they can alter actin dynamics and induce a collapse of growth cone. Rho and ROCK inhibition has been shown to improve the regeneration of RGC axon. The intraocular transmission of C3-exoenzyme, an inactivator of Rho GTPase, has been shown to facilitate the regeneration of RGC axons in the optic nerve following a micro-crush lesion.

(e) As an anti-fibrotic agent in glaucoma surgery

Subconjunctival scarring of the filtering bleb site which is one of the reasons for the failure of glaucoma surgery is primarily caused by the distribution, movement and contraction of tenon fibroblasts (TFs). The important phase in the healing of wounds and the development of scars is the conversion of fibroblasts to myofibroblasts. It is in concordance with the expression of α-smooth muscle actin (α-SMA). The increase in α-SMA expression signals the active fibroblasts and improves the production of ECM proteins, growth factors and integrins. ROCK inhibitors can prevent cell proliferation, invasion, and cytokinesis. Thus the Rho / ROCK pathway has important roles in the control of wound healing of the filtra-

tion canal. ROCK inhibitors have been documented to reduce or block LPA-induced and TGF-β-induced α-SMA expressions in TFs, indicating that ROCK inhibitors act as potent anti-scarring agent by preventing trans-differentiation of TFs into myofibroblasts.

Rho Kinase Inhibitors used in glaucoma

SNJ-1656

In animal studiess[21], it has been found that topical administration of SNJ-1656 leads to an improvised outflow and facility with reduction in IOP. Conjunctival hyperemia was found in all patients but, in most instances, resolved within 24 hours of single instillation.

AR-12286

AR-12286 is both stable and active in affecting the morphology of trabecular meshwork cells. It was seen that 0.5% concentration for 8 days, when used, significantly lowered IOP with an average maximum decrease of 7 mmHg.[28] However, side effects like conjunctival hyperemia, increased lacrimation, ocular irritation and blurred vision were found. AR-12286 was withdrawn for use in glaucoma because of short period of effect.[29]

Ripasudil (K-115)

Ripasudil was advocated in Japan in September 2014, for the treatment of glaucoma and ocular hypertension. Ripasudil 0.4% BID may be used for the treatment. The medication has been shown to reduce IOP within two hours of instilling the solution and has been proven to do so reliably over a five-year cycle.[30] Most common associated side effects are conjunctival hyperemia, blepharitis, and allergic conjunctivitis.

Netarsudil (AR-13324)

Netarsudil belongs to the class of amino-isoquinoline amide inhibitors of Rho kinase. Norepinephrine transfer inhibition decreases the output of AH through vasoconstriction, raising the distribution of blood through ciliary processes. In the United States, this was licensed for use in the care of glaucoma at the end of 2017. Netarsudil shall have a longer period of operation than AR-12286. This is slightly different from other Rho kinase inhibitors. It decreases IOP in animals by reducing tolerance to outflow, but also increases aqueous humor development in monkeys[31] and episcleral venous pressure in rabbits and humans[32] too.

Conjunctival hyperemia, increased lacrimation and subconjunctival hemorrhage (are the commonly seen side effects.

Y-27632

Y-27632 has been successful in suppressing fibroproliferation and scar forming in glaucoma surgery done on a rabbit model.[33] ROCK inhibitors also have the ability to be anti-scarring agents following glaucoma filtering surgery. Y-27632 reportedly promotes integrin adhesion in cultured THP-1 monocytes. This enhanced cell adhesion to the ECM can be due to changes in the cell structure and transfer of focal adhesions to the cell periphery.

ROCKLATAN

It is a combination of netarsudil (0.02%) with latanoprost (0.005%) and gained FDA approval in June 2019.[34] Rocklatan is the first anti-glaucoma drop which lowers IOP using all three known mechanisms i.e. increasing AH outflow (both pathways), reducing AH production and reducing episcleral venous pressure.

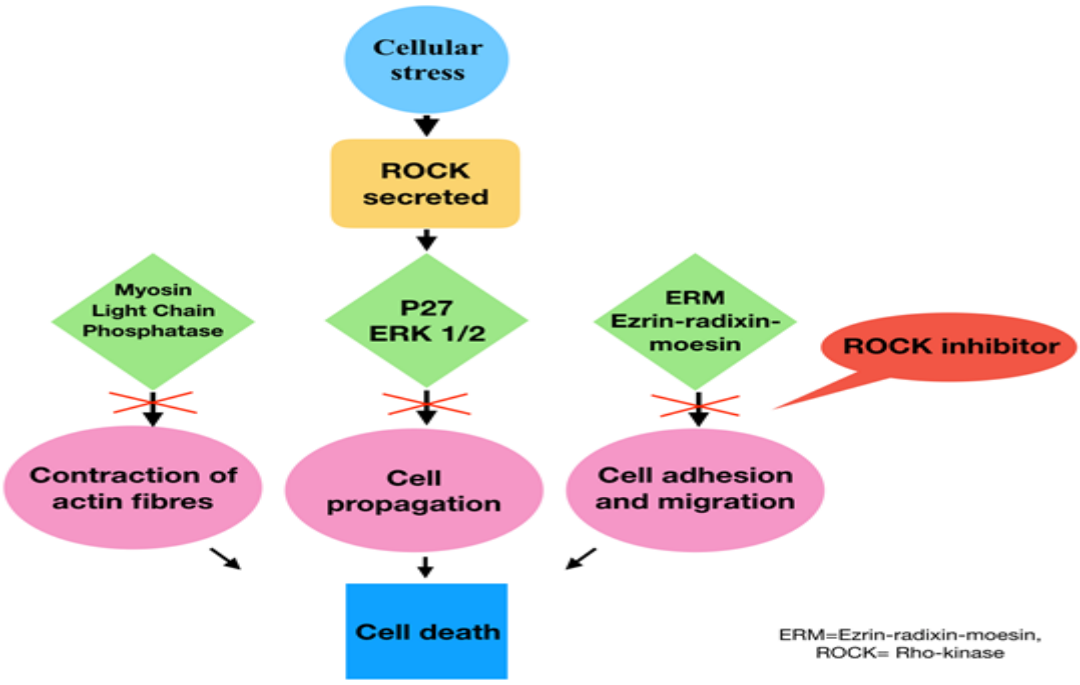


Fig. 2: Rock helping in healing of corneal endothelium.

ROCKLATAN (netarsudil 0.02% and latanoprost 0.005%) was evaluated in 2 randomized and controlled clinical trials[35] where patients with open-angle glaucoma and ocular hypertension were taken into consideration. A comparison between the IOP lowering effect of ROCKLATAN dosed once daily to individually administered netarsudil 0.02% once daily and latanoprost 0.005% once daily was done in patients with IOP < 36 mmHg. The average IOP lowering effect of ROCKLATAN was found to be 1 to 3 mmHg greater than monotherapy. IOP reductions were maintained continuously for 12 months.

At present we do not have any selective Rho-kinase inhibitor for glaucoma treatment. This is important because if we increase the concentration of any non-specific Rho-kinase inhibitor, it may lead to many adverse implications. It is however difficult to say about the exact results of enhanced amount of non-specific Rho-kinase inhibitors, but a drug to impact only trabecular meshwork might will be a better option.

Healing of corneal endothelium (fig. 2)

Corneal endothelial cell (CEC) have a poor regenerative ability because of their restricted proliferative capacity. Therefore, for compensating for the loss after any corneal damage, there is migration and spreading of the residual CECs.

The significance of ROCK signaling is widely recognized in various cellular conditions and pathologies. Yet the function of ROCK signaling differs based on cell morphology. It has been seen that use of Y-27632, increased CEC proliferation, adhesion to the substrate, as well as inhibited CEC apoptosis.[36] Such results demonstrated that the application of a ROCK inhibitor could substantially enhance the potential to cultivate CECs which may be used in tissue engineering therapies.

(a) Cell Division

ROCK inhibitor increased the amount of cyclic D and inhibited phosphorylation of p27kip1 by activating the phosphatidylinositol 3-kinase signaling.[37] Cyclin D and p27 are G1/S progression regulators. Successive studies have also shown that ROCK inhibitor Y-27632 has improved cell proliferation.[38] Two ROCK inhibitors, Y-27632 and Y-39983, improved wound healing levels and improved the corneal transparency. The amount of Ki67-positive proliferating cells was also improved, indicating that proliferation of CEC is facilitated by ROCK inhibition both in vitro and in vivo.[39]

(b) Increased Cell Adhesion

Though cellular adhesion is the main mechanism in healing of the corneal endothelium; another process of corneal endothelial repair is injecting the cultured endothelial cells in an anterior chamber and inhibiting Rho kinase. The cellular adhesion enables the efficient distribution of these cells to the inside of the cornea. In addition, ROCK inhibitors require increased cell adhesion due to enhanced actin-myosin contractility. There is also a stronger possibility for corneal endothelial wound repair through infusion of cultured endothelial cells and Rho kinase inhibitors.[38]

(c) Slowing of Cellular Apoptosis

ROCK activation is known to be a key regulator in the apoptosis cycle.[40] Lack of interaction with extracellular matrix and adjacent cells causes temporary apoptotic cell blebbing of the membrane through actomyosin contraction by MLC phosphorylation.

Caspase-3-mediated cleavage and activation of ROCK1 was shown[41] to be crucial in inducing MLC phosphorylation. It eventually led to membrane blebbing in NIH3T3 cells which were treated with TNF-α. Same process has been also seen in various other specific forms of apoptotic stimuli.[42]

Some studies indicate that activation of ROCK signaling is not that important for earlier apoptosis processes as for the execution phase.[43]

ROCK-dependent re-arrangement of actin cytoskeleton leads to congregation of the death-inducing signaling complex. TNF-α mediated apoptosis is attenuated by the inhibition of ROCK.

The members of the Rho subfamily thus play an important role in converting GDP-bound inactive models to GTP-bound active models.[44]

(d) Tissue engineering

Tissue engineering has been speculated as modern treatment that may supplement existing corneal transplantation utilizing donor corneas. Two potential approaches are accessible in transplanting cultured CECs to the recipient corneas.[45] One method is to make a cultured CEC layer and implant it in the same manner as DSEK or DMEK. And the second technique uses the cultured CECs which are injected into the anterior chamber in the manner of a cell suspension. Cell injection has many benefits over sheet transplantation, including simpler transplantation methods, decreased invasiveness for patients, better storage of cell supply, and the nullifying of use of artificial substrate. ROCK inhibitor therapy improves the conformity of CECs to the substrate.[36] In a study[46], cultured CECs in the form of a cell suspension integrated with a ROCK inhibitor were injected into two animal models with corneal endothelial dysfunction (rabbit and cynomolgus monkey). The corneal endothelium was regenerated and a transparent cornea was restored.

Fuch's endothelial corneal dystrophy (FECD)

Research on the usage of ROCK inhibitors in FECD therapy remain scarce, and randomized controlled studies are also required before using this eye drop as a standard therapeutic alternative. Case studies reported the effects of the topically applied ROCK inhibitor in the early stag-

es of FECD to suppress endothelial apoptosis and improve cell proliferation. Interestingly, this approach seems to be effective in later phases of the disorder, where a one-week therapy regimen culminated in substantial and continuous vision recovery (6/18 to 6/6) over a six-year span.

Recently, the technique of Descemet stripping only (DSO) has shown potential in certain cases of FECD.[47] In this process, the central endothelium is extracted and, ensuring that the peripheral endothelial cell density is > 1000mm^2, the remaining endothelial cells migrate from the periphery and fill the empty space centrally (with related decrease in peripheral endothelial cell density), preserving corneal transparency and removing the need for donor transplantation, graft rejection and prolonged steroid use. This DSO approach has been paired with the topical usage of a ROCK inhibitor (ripasudil) to speed up regeneration and improve endothelial cell density relative to DSO alone. ROCK Inhibitors are used in cultured endothelial cells obtained from a donor or a patient. The drug allows the formation of endothelial cells (in vitro) which are then inserted into the anterior chamber of the recipient.[49]

Moloney et al.[50] mentioned a number of FD patients undergoing DSO. Even after removal of Descemet, there was no corneal resolution for 2 months. Hence, a ROCK inhibitor was used as a salvage technique. In the first non-responder, Y-27632 was topically administered initiallty but later was substituted by another topical formulation of ripasudil. This cleared the cornea within 10 days. Likewise, two other respondents and an adult individual with a tiny edematous patch were issued ripasudil, both of which culminated in a translucent cornea within 14 days. Previous studies[51] with topical ripasudil caused transient guttae-like results in humans, most possibly attributed to protrusion along intercellular borders triggered by decreased actomyosin contractility in CECs. Transient morphological shifts in CECs such as indistinct cell borders with pseudo-guttae have been reported with non-contact specular microscopy in healthy subjects following Ripasudil administration. All these changes were temporary. Before the next administration, CEC morphology came back to normal. However, by reducing rejection, decreased corticosteroid utilization and theoretically less associated side effects, long-term medical treatment is reduced, given that CECs do not decompensate. DSOs with topical rhokinase inhibitors can reduce the expense of visual recovery for both individual patients and society as a whole.

Acute corneal endothelial damage

The treatment for the corneal endothelial damage, particularly in cataract surgery, is another possible indicator for ROCK inhibitor administration. Corneal decompensation after cataract surgery is responsible for 20–40% of corneal transplantation conducted in Asian countries.[52] The amount of CECs sufficient for redistribution, decides how the cornea remains translucent or sustains corneal decompensation. Inadequate CEC is responsible for the corneal haze. ROCK inhibitor eye drops may potentially facilitate the dissemination of remaining CECs after the corneal endothelial injury. They can thus maximize the amount of CECs eligible for treatment, and reduce the chance of corneal decompensation.[53] In Japan[54], three people undergoing cataract surgery experienced extreme corneal edema and corneal haziness and were at high risk of eventual decompensation. They were both diagnosed with a reduction in Rho kinase inhibitor owing to these factors. Under these factors, they were all treated with a Rho kinase inhibitor drop. Corneal clarity was restored within one to two months, indicating an improvement in corneal endothelial cell density in all three cases. In rabbits[55], it's found that ripasudil has close impact on wound healing corneal endothelium. Therefore, ripasudil as a medication in these type of cases tends to be a potential approach towards the treatment.

Axial myopia

The chief structural alteration in pathological myopia is the unrestricted elongation of eyeball, especially in axial length. The elongation is also seen in animal models of myopia, like chicks and guinea pigs.[56] The axial elongation is in accordant with remodeling of sclera and changes in biological properties. Myopes have a composition of increased extracellular matrix (ECM), mostly due to reduced collagen and glycosaminoglycan (GAG), accompanied by scleral thinning. This further leads to reduction in the resistance of sclera in response to the usual IOPs.[57] Recent studies have indicated that scleral cells have secretory roles along with contractile capacities, which is significant in evaluating scleral matrix tension, tissue expansion and contraction.[58]

Throughout the development of myopia, the tension and pressure on the sclera is increased while the axial elongation continued, culminating in the subsequent thinning of the scleral tissue.[59] Previous experiments have demonstrated an improved production of a-SMA in scleral cells which are under pressure in mice and cat models.[60] Nevertheless, the relation of this tension and pressure on sclera and promotion of the separation of fibroblasts to myofibroblasts is uncertain.

Role of Rho kinase in axial myopia

Once exogenous mechanical tension comes into action, cell-matrix adhesion complexes relay this stimulus to cytoskeleton. It then stimulates RhoA from GDP-to GTP-bound molecules and causes downstream molecular changes.[61] Transcription factors linked to myocardin, like MRTF-A (MKL1) and MRTF-B (MKL2), are commonly distributed in many tissues.[62] This mechanical force then activates Rho GTPases, polymerises actin-cytoskeleton into stress fibers, enabling translocation of nucleus of MRTFs.[63] Previous studies have indicated that, in human lung and cases of cardiac fibrosis translocation of MRTF-A to the nucleus leads to differentiation of myofibroblast.[64] RhoA / ROCK activation has been

found to regulate MRTF-A controlled gene expression.[65]

It has therefore been established that RhoA / ROCK2 consists of an intrinsic mechano-transduction pathway capable of transducing and converting mechanical stimuli into a signal which promotes expression of scleral a-SMA in a myopia model of mammalian.[66]

Rho kinase inhibitor and axial myopia

The researchers have noticed that intravitreal administration of the Rho kinase inhibitor to the chicken model of form-deprivation myopia greatly suppresses axial lengthening.[67] This result confirmed for the first time that Rho kinase is involved in axial lengthening, i.e. that Rho kinase is involved in the development of axial myopia, and that Rho kinase inhibitors are highly useful for prophylaxis or axial myopia therapy. The axial length and average transverse diameter of the Y27632 treatment group and the hydroxyfasudil treatment group were significantly reduced relative to those of the control group. However, there is still scarcity of research in the area of use of Rho kinase inhibitors in myopia and further study is required.

Diabetic retinopathy

Proliferative diabetic retinopathy (PDR) causes neovascularization, vitreous hemorrhage, pre-retinal fibrovascular proliferation, and tractional retinal detachment.

Panretinal photocoagulation (PRP) and various other vitreoretinal procedures stay the prominent treatment approaches for advanced DR. Vascular endothelial growth factor (VEGF) has an important contribution in causing DME along with neovascularization in PDR. Anti-VEGF antibodies are used to enhance the treatment of DR. Inspite of that, at this stage we require a novel substitution and adjunctive treatment, owing to the immense physical and economic effect of new medications on patients.

(a) ROCK in Microvascular Complications in DR

Microvascular complications such as hyperpermeability, angiogenesis, microthrombosis, and inflammation are found in DR pathogenesis. Retinal leukocyte stasis (leukostasis) may be seen at early non-proliferative stages of DR, which is brought about by adhesion molecules, leukocyte β2 integrins (CD18/CD11a and CD18/CD11b) and intercellular adhesion molecule-1 (ICAM-1).[68] ROCK pathways have been documented to control the expression and role of ICAM-1 in endothelial cells and may be triggered in vascular cells by serum from patients with diabetic retinopathy.[69] In addition, a research with streptozotocin-induced diabetic model verified the activation of the Rho / ROCK pathway in retinal microvessels.[70] In comparison, intravitreal fasudil substantially decreased ICAM-1 production, leukocyte adhesion, and the amount of compromised endothelial cells in diabetic rat retinas. Thus, ROCK inhibition can reflect a promising technique for treating initial stages of diabetic retinopathy.

(b) *ROCK in Angiogenesis*

VEGF plays a major role in hyperpermeability and angiogenesis in cases of diabetic retinopathy. Y27632 causes the inhibition of ROCK by preventing the endothelial hyperpermeability caused by VEGF. However, the role of ROCK in cases related to TNF-α is discorded.[71] ROCK signaling could also upregulate VEGF in diabetic retina. Y27632 prevents VEGF-induced angiogenesis in the oxygen-induced retinopathy (OIR) model[72] while fasudil inhibits angiogenesis in corneal and OIR models.[73] In comparison, the analysis with the ROCK inhibitor H-1152 demonstrated enhanced VEGF-induced angiogenesis in the OIR model and an in vitro ERK1/2 activation pattern.[74]

(c) *ROCK in Diabetic Macular Edema*

The Rho / ROCK system is associated with significant degradation of protein and BRB destruction. Using the combination of bevacizumab and fasudil intravitreal injection in eyes with severe DME that were immune to prevailing anti-VEGF therapy, was successful. It suggested that ROCK inhibition is fundamentally distinct from anti-VEGF therapy.[75]

(d) ROCK and Proliferative Membrane

Inhibition of ROCK effectively inhibited the α—SMA structure and prevented proliferative membrane contraction in the rabbit model. ROCK inhibition approximately fully removes collagen gel contraction which is induced by PDR vitreous and controlled by MLC phosphorylation suppression.[76]

(e) *Retinal Ischemia and ROCK Inhibition*

Retinal ischaemia and microthrombosis have no reliable cure. Recent paper showed that while inhibiting preretinal angiogenesis, ripasudil inhibition of ROCK may induce intraretinal vascularization which leads to diminished area of hypoxia in the OIR model.[73] In addition, ripasudil therapy may enhance retinal vascular perfusion and induce pericyte coverage. Fasudil has also been proven to enhance ischemia in severe ischemic stroke patients.[77] Earlier it was stated that when ROCK is inhibited, it can induce dilation of the retinal vessel which, in effect, can lead to the improvement of ischemia. Recent studies in cats have shown that ripasudil injection if given intravitreally may notably increase the blood flow and velocity in the retina.[78] Hence, in cases of retinal vascular disorder, ROCK inhibition can act as a novel therapeutic approach for retinal ischemia.

Retinal Vein Occlusion

It is caused by narrowing of retinal veins owing to elevated blood pressure and arteriosclerosis. Patients with RVO usually present with retinal edema and non-perfusion regions involving impairment of vision function. Retinal edema and non-perfusion areas have been improved by the use of anti-VEGF (vascular endothelial

growth factor) compounds. Nonetheless, despite the use of anti-VEGF antibodies, recurrences of macular edema and development of non-perfusion areas have been seen in some patients. In addition, anti-VEGF also adds to the economic burden as it is needed to be administered repeatedly due to its nature of symptomatic relief only.

Rho kinase in RVO

Retinal edema develops from decreased permeability of the retinal vessels, leukostasis as well as deterioration of BRB which is triggered by increased VEGF.[79] Under the ischemic conditions, VEGF readily triggers ROCK in endothelial cells.[80] VEGF-induced angiogenesis is prevented by the ROCK inhibitors by inhibiting the proliferation as well as the migration of vascular endothelial cells. In the brain microvascular endothelial cells, frequency of occludin expression is inhibited by ROCK inhibitors following oxygen-glucose deficiency.[81] Ripasudil is found to reduce edema by maintaining the integrity of the tight junction in the retina.

In RVO patients, the nonperfusion zone is highly significant. Its development depends on amount of capillary reduction alongwith with hypoperfusion. Once ROCK is activated, smooth muscle nerve cells are relaxed, which decreases blood flow.[82] In cats, Ripasudil is found to improve retinal blood flow in regular circumstances.[78] Ripasudil prevents both the development of a nonperfusion region as well as decrease of blood flow through retinal vein occlusion, indicating ROCK inhibitors improve the flow of blood due to vasodilatory action, and enhance eNOS transmission.[83] The retinal capillary non-perfusion region of the ripasudil-treated population was shown to be low and that it blocked a reduction in flow of blood. Thus, the results revealed that by increasing retinal blood flow, ripasudil prevents the progression of the symptoms of RVO.

ROCK 1 and 2 are omnipresent in body[84], particularly throughout retina. Under diseased situation, when ROCK is activated, it causes numerous modifications, like vascular smooth muscle relaxation particularly leukocyte induction.[85] Thus, in the RVO model, ROCK activation can be correlated with oedema and nonperfusion region development. It was found that ROCK 1 and 2 were normally coherent and abnormal ROCK movement occurred in RVO. When ROCK is activated, it decreases the blood supply through contraction of the vascular smooth muscle of the brain.[86] ROCK increases nitric oxide and improves vascular permeability by causing a breakup of tight junctions. These results revealed that modulation of ROCK behavior reduced vasoconstriction and avoided hyperpermeability and edema by raising blood flow in the RVO murine model.[87]

To summarise, we can say that ripasudil has a bright future as a therapeutic agent for retinal edema as well as in RVO for the cases with capillary non-perfusion.

Age-Related Macular Degeneration and ROCK

(a) Macrophage Polarization and Aging

Two forms of AMRD are seen. The dry ARMD that eventually contributes to macular atrophy. The wet AMRD is marked by choroidal neovascularisation and leakage. Recently, Zandi et al. found that ROCK2 signaling is involved in macrophage polarization which increases with age. A selective inhibition of ROCK2 was seen to strengthened this pathology.[88]

(b) Subretinal Fibrosis

The choroidal neovascularisation membrane (CNVM) caused by wet AMRD ultimately induces fibrosis, which may contribute to permanent vision impairment.[89] There has actually been no suitable cure for the fibrosis caused by CNVM. AMA0428, a ROCK inhibitor has been certified in successfully reducing the fibrosis in the CNVM mouse model.[90] The Rho / ROCK pathway gives a downstream signal for the fibrotic disease agents like TGF-β.[91] However, the mechanism by which ROCK inhibition blocks the sub retinal fibrosis which is related to TGF-β is unclear.

Proliferative vitreoretinopathy and ROCK

In PVR, there is development of membranes inside the hyaloid and retina on all retinal surfaces which eventually contracts after retinal reattachment surgery.[92,93] TGF-*β*2 and PDGF are the cytokines which play a major role in PVR pathogenesis.[94] According to some recent studies, ROCK pathway is implicated in PVR pathogenesis. Relevance of ROCK has been seen in TGF-β-induced retinal pigment epithelium gel contraction has been documented.[95] ROCK inhibition blocking the TRD development was also documented in one of the studies[96] and also that ROCK inhibitors can help prevent and improve PVR other than vitrectomy surgery.

CONCLUSION

There is growing evidence showing Rho kinase inhibitors as valuable therapeutic modalities for the treatment of glaucoma, corneal disease, axial myopia, vireo-retinal cases and several other ophthalmological conditions (Table 1). The need of the hour is to further investigate the strength, dosage and route of administration of the various ROCK inhibitors. It is also necessary to carry out trials to identify receptor specific ROCK inhibitors, so that in future, these drugs can be used; as they not only curtail the side-effects of various other medications and procedures but are also economically beneficial.

Table 1: Summary of use of ROCK inhibitors in ophthalmology.	
Glaucoma	Improving aqueous outflow by relaxing trabecular meshwork tissue
	Improving optic nerve perfusion
	Protecting retinal ganglion cells
	Promotion of RGC axon regeneration
	As an anti-fibrotic agent in glaucoma surgery
Healing of corneal endothelium	Improves cell proliferation
	Increases cell adhesion
	Slows cellular apoptosis
	Used in tissue engineering
Axial myopia	Suppresses axial lengthening
Diabetic retinopathy	Inhibition of microvascular complications
	Prevents VEGF induced angiogenesis
	Decreases macular edema
	Prevention of proliferative membrane contraction
	Increases retinal blood velocity and flow
Retinal Vein Occlusion	Reduces vasoconstriction
	Decreases hyper-permeability and edema by increasing blood flow
Age related macular degeneration	Decreases macrophage polarising
	Decreases sub-retinal fibrosis
Proliferative vireo-retinopathy	Prevents tractional retinal detachment probability

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