Review Article

Rho Kinase Inhibitors - Role in Glaucoma and Cornea Practice

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Abstract

The rho-kinase pathway has been implicated in the pathogenesis of various ocular disorders like glaucoma, Fuchs corneal dystrophy, diabetic retinopathy, diabetic macular edema, age-related macular degeneration (AMD), ROP. ROCK molecules and the ROCK pathway regulate multiple physiological functions, such as cell contraction, migration, proliferation, angiogenesis, chemotaxis, neural protection and vasodilatation, mainly via reorganization of the cytoskeleton. Corneal endothelium increases endothelial cell proliferation and adhesion and decreases endothelium to mesenchyme transformation. Various studies have demonstrated their safety and efficacy for ophthalmic use. This review highlights the use of ROCK inhibitors in glaucoma and corneal diseases.

Keywords: Cornea, Glaucoma, ROCK inhibitor, Rho kinase.

Introduction

Rho kinase (ROCK) is a serine-threonine protein kinase family member with a small molecular weight (160 kDa). ROCK-I (ROKβ) and ROCKII (ROKα) are the two isoforms described in humans. Both share similar amino acid identities, contain an N-terminal kinase domain and an identical ATP-binding pocket. ROCK molecules are ubiquitously expressed in all cell tissues and organs, although the degree of expression varies.[1]

Rho-kinase, forms a Rho/Rho-kinase complex on interaction with GTP-bound Rho proteins. ROCK molecules and the ROCK pathway regulate a wide spectrum of fundamental cellular events, including multiple physiological functions, such as cell contraction, migration, proliferation, angiogenesis, chemotaxis, neural protection and vasodilatation, mainly via reorganization of the cytoskeleton. Increased activity of ROCK pathway results in the breakdown of endothelial cell barrier, vascular hyperpermeability, and dephosphorylation of endothelial nitric oxide synthase leading to apoptosis and vasoconstriction.[1–3] Upregulation of the ROCK pathway has been implicated in the pathogenesis of various ocular disorders, such as ocular hypertension (OHT) and glaucoma, corneal neovascularisation, diabetic retinopathy, diabetic macular edema and age-related macular degeneration (AMD), ROP, which has made them potential therapeutic targets.[1,4,5]

Two ROCK inhibitors have gained approval for ocular use till date. In 2014, Ripasudil, a ROCK inhibitor was approved in Japan as a second line treatment of ocular hypertension and glaucoma.[6] In December 2017 US-FDA approved Rhopressa, a Rho kinase-inhibiting drug consisting of Netarsudil for treatment of ocular hypertension and glaucoma.[7]

Methods

A literature review was performed using various electronic databases, including PubMed, Science Direct, using keywords "Rho kinase inhibitor", "glaucoma", "cornea", "corneal endothelium", and various others. Multiple articles were reviewed for relevance. All articles deemed relevant were included in this review. In this review we have focussed mainly on use of ROCK inhibitors in cornea and glaucoma.

ROCK inhibitors and glaucoma

Glaucoma is a multifactorial disease causing progressive optic neuropathy and is a major cause of irreversible blindness.

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The most predominant risk factor for optic nerve damage is elevated IOP. Aqueous outflow occurs mainly through conventional pathway (80%) i.e. trabecular meshwork, schlemm's canal to episcleral vein. Rest of aqueous is drained through uveoscleral pathway. Medical management of glaucoma mainly targets for reduction in IOP. Novel treatment modalities are focusing on increasing blood flow to the retina and optic nerve head, retinal ganglion cell preservation, as well as lowering the intraocular pressure (IOP).[8]

Rho kinase inhibitors help to lower IOP by various mechanisms. They increase aqueous outflow by altering cell shape in the trabecular meshwork calcium-independent pathway, increase permeability in Trabecular Meshwork and Schlemm's canal, reduce aqueous production, and decrease episcleral venous pressure (EVP). ROCK inhibitors induce reorganisation of the extracellular matrix and weaken cell binding to the extracellular matrix, resulting in wider empty spaces in trabecular meshwork and schlemm's canal. In addition they lead to neuroprotection by increasing blood flow to optic nerve head and retina.[3,8–10] They also cause wound modulation by decreasing fibrosis which increase success rate after trabeculectomy.[11]

Ota *et al.* studied molecular pharmacology of ROCK inhibitors (Ripasudil and Y27632) by elucidating their efficiency on aqueous outflow in 2D or 3D cultures of human trabecular meshwork (HTM) prepared in the presence of TGFβ2. ROCK inhibitors greatly reduced the TGFβ2-induced increase in transendothelial electrical resistance and ECM expression and made HTM less compact.[11]

Ripasudil hydrochloride hydrate drop solution is approved at 0.4% concentration for use in OHT and glaucoma as a twice-daily treatment.[6] A phase 2 study demonstrated that the mean IOP reduction in patients with POAG or OHT was 4.5 and 3.1 mm Hg at 2 and 8 hours after the instillation of 0.4% ripasudil.[12] In addition, a phase 3 study demonstrated the additive IOP reduction of 2.9 mm Hg in combination with timolol and 3.2 mm Hg in combination with latanoprost after 2 hours of administration.[13] Tanihara *et al.* conducted a multicentric, prospective study in 388 patients with primary open-angle glaucoma, OHT or exfoliation glaucoma and reported that 1 year of the administration of 0.4% ripasudil is safe and effective as a monotherapy or as part of an additive therapy. The most frequently observed adverse events were conjunctival hyperemia (74.6%), blepharitis (20.6%) and allergic conjunctivitis (17.2%). Most of the conjunctival hyperemia findings were mild and resolved spontaneously. All the adverse events either resolved spontaneously or with treatment after the discontinuation of the drug.[14]

Netarsudil *(AR-13,324)* acts as ROCK and norepinephrine transport inhibitors. Vasoconstriction due to norepinephrine transport inhibition reduces blood flow to the ciliary processes and inhibits production of aqueous. It also causes a reduction in episcleral venous pressure.[10] It reduces IOP within two hours of instillation and sustains this decrease for 24-hours. A phase 2 study demonstrated that the mean reduction of IOP in patients with glaucoma after once-daily dosing of 0.02% netarsudil was 5.7mm Hg.[15]

In the United States, netarsudil drop is approved in a 0.02% concentration, once daily, in the evening, for treatment in open-angle glaucoma or ocular hypertension. The most common side effect of topical netarsudil is conjunctival hyperaemia, seen in more than half of the patients. Other reported side effects are corneal verticillata (approximately 20%), instillation site pain, conjunctival hemorrhages, and reticular epithelial edema.[16, 17]

The Rho kinase-elevated IOP treatment (ROCKET) trials compared the efficacy and safety of once-daily netarsudil (0.02%) with twice-daily timolol maleate (0.5%). 839 patients were included in each group. Mean IOP reduction from baseline was 4.8 mmHg in netarsudil group and 5.0 mmHg in timolol group. They concluded that the IOP-lowering efficacy of netarsudil was non-inferior to that of timolol.[18]

PG324 (Rocklatan) an FDA approved fixed-dose combination of Netarsudil (0.02%) and latanoprost (0.005%) was shown to have significant reduction in IOP and was superior to both Netarsudil and latanoprost alone.[19]

Sun *et al.*, discovered *compound 12b*, derivative of 3, 4-dihydrobenzo[f] [1, 4]oxazepin-5(2H)-one as a new class of ROCK inhibitors. The mean IOP-lowering effect in a rabbit ocular normotensive model was 34.3% after 1-hour of instillation. No obvious hyperemia was observed which they attributed to considerable selectivity of the compound to ROCK I and II.[20]

Other Rock inhibitors like HA-1077 (Fasudil), Y-27632, H-1152 or H-1152P (Dimethylfasudil), INS-115644, AR-12286 (Verosudil), AMA0076 having potential to manage glaucoma by reduction in IOP and neuroprotection are under investigation.[21]

ROCK inhibitors and cornea

The innermost layer of cornea, the endothelium, has very limited proliferation capacity. Increased endothelial cell loss in corneal endothelial disease states (such as Fuchs' endothelial corneal dystrophy (FECD) and post-surgery) is initially compensated by migration and spread of the surrounding endothelial cells, leading to decreased endothelial cell density. Endothelial cells undergo a transition to mesenchymal cells with the acquisition of fibroblast-like contractile properties. These compensatory mechanisms help to keep the corneal transparency until endothelial reserve reduces below 500 cells/mm^2.[1, 22]

ROCK inhibitors can be delivered via topical route or an anterior chamber injection with cultured endothelial cells. ROCK inhibitors lead to increase in cyclin D levels and suppression of the phosphorylation of cyclin-dependent kinase inhibitor 1B, p27/kip1, which further leads to an increased proliferation rate.[22] They further enhance acto-myosin contractility which leads to increased cellular adhesion.[23] Rho kinase act on actin cytoskeleton and allows for cellular contraction, membrane blebbing, nuclear disintegration and

causes apoptosis. ROCK inhibitors slow this process within a day of use.[24] They inhibit the transformation of corneal keratocytes and endothelial cells to myofibroblasts and fibroblasts. ROCK inhibitors suppress the overproduction of ROS and inflammatory cytokines such as vascular endothelial growth factor (VEGF) and tumour necrosis factor (TNF), matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9) thereby, protect the cornea from the invasion of neovessels.[25]

ROCK inhibitors also promote the healing of corneal epithelial defects by reducing inflammatory cell infiltration, improving cell-cell adhesion and cell– matrix interactions.[26]

Fuchs endothelial corneal dystrophy (FECD) is one of the common indications for corneal transplantation. This condition leads to progressive loss of corneal endothelial cells and excrescences of descemet membrane, termed guttae.

Desmetorrhexis without endothelial transplant has shown variable and inconsistent results. Macsai and Shiloach conducted a prospective study using ripasudil as an adjuvant to descemet stripping only (DSO) and showed significantly faster recovery and higher endothelial cell density after one year in the DSO + ripasudil group compared to the DSO only group.[27] In another prospective interventional case series, cornea cleared in 95% of eyes after four weeks on average when DSO was supplemented with ripasudil.[28, 29]

A study was conducted in 11 patients diagnosed with bullous keratopathy with no detectable corneal endothelial cells (CECs). The cause of bullous keratopathy was Fuchs's endothelial corneal dystrophy (7 eyes), argon-laser iridotomy–induced bullous keratopathy (2 eyes), pseudoexfoliation syndrome–related bullous keratopathy (1 eye), and intraocular surgery–related bullous keratopathy (1 eye). The mean corneal thickness at baseline was 743 µm. A total of 1×10^6 human donor CECs supplemented with a ROCK inhibitor Y-27632 (final volume, 300 µL) were injected into the anterior chamber and patients were given prone position for 3 hours. All the eyes demonstrated CEC density of more than 500 cells per square millimeter at 24 weeks of injection. 10 out of 11 eyes attained corneal thickness of less than 630 µm and an improvement in best corrected visual acuity of two lines or more was recorded in 9 out of 11 treated eyes.[30]

An experimental study, was conducted by Schlötzer-Schrehardt *et al.* in which they took endothelial cell-Descemet membrane lamellae from FECD patients (n=450) undergoing Descemet membrane endothelial keratoplasty, normal research-grade donor corneas (n=30) after scraping off central endothelial cells, normal donor corneas (n=20) without endothelial injury, and immortalized cell lines (n=3) generated from FECD patients. They dissected descemet membrane lamellae into halves and incubated for 24 to 72 hours in a storage medium with or without a single dose of 30 µM ripasudil. They noticed significant upregulation of genes and proteins related to cell cycle progression, cell-matrix adhesion and migration, and endothelial barrier and pump function up to 72 hours in ripasudil-stimulated lamellae. Classical markers of endothelial-to-mesenchyme transition were downregulated in both FECD and normal specimens compared to specimens not stimulated with ripasudil.[31]

In a recent clinical study, Okumura *et al.* noticed that the use of topical ROCK inhibitor (Y-27632) could recover corneal transparency in patients with corneal endothelial dysfunction in primate model and human clinical case series. Corneal endothelial cell density, percentage of ZO-1 and Na^+/K^+-ATPase positive cells in the regenerated area was significantly higher in the Y-27632 group compared with the controls at 4 weeks.[25]

Kim *et al.* showed that novel ROCK inhibitors like sovesudil and PHP-0961, have capacity to regenerate human corneal endothelial cells by enhancing cell proliferation and adhesion between the cells.[32]

In Acute corneal trauma during cataract surgeries, ROCK inhibitors allows for increased healing and migration of corneal endothelial cells to cover the afflicted area leading to recovery of corneal transparency and increase in corneal endothelial cell density.[33] Ripasudil reduced DNA damage, increased the density of endothelial cells, and protected the integrity and function of endothelium in crosslinking-Induced corneal endothelium dysfunction.[34]

Apart from cornea and glaucoma, ROCK inhibitors have been shown to be effective in various retinal diseases like diabetic retinopathy, diabetic macular edema, proliferative vitreoretinopathy, ROP, ARMD and ischemic optic neuropathies.[1,4,5] Intravitreal injection of ROCK inhibitor in cases of diabetic retinopathy and macular edema reduces adhesion of leukocytes to microvascular structures, slow down endothelial damage in the retinal microvascular vessels and increase nitric oxide level.[5]

Conclusion

ROCK inhibitors are emerging as an effective modality for medical management of glaucoma and corneal endothelial dysfunction. Various studies have shown increased success rates of glaucoma and corneal surgeries with adjuvant use of ROCK inhibitors. Further research requires the development of newer drugs with more potent action and fewer side effects.

Conflicts of interest

There is no conflict of interest to disclose.

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