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REVIEW

RHO-KINASE INHIBITORS – A NEW BROAD-SPECTRUM TREATMENT IN OPHTHALMIC DISEASES. A REVIEW

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Abstract

Rho-kinases, also called coiled-coil containing protein kinases, are proteins that belong to the guanine triphosphate-ase family. Rho-kinase (ROCK) inhibition represents a relatively new therapeutic target for many diseases as cardiovascular diseases, neuronal degeneration, asthma, cancer. Due to their involvement in multiple biological processes, these compounds are increasingly addressed in the last ten years for clinical trials related to ophthalmic pathologies like glaucoma, endothelial dysfunction and vitreoretinal diseases. ROCK inhibition pathway seems to play important roles in: promoting endothelial cell proliferation and wound healing, regulation of trabecular meshwork outflow, limiting diabetes-induced microvascular damage, inhibiting the development of neovascular choroidal membranes in wet age related macular degeneration. This review of literature explores the mechanisms of action and effects of ROCK inhibitors in a variety of ocular pathologies as corneal pathologies, glaucoma, diabetic retinopathy, age related macular degeneration; also listing clinical trials (completed or ongoing) on ROCK inhibitors administered in patients with those particular disease.

Rezumat

Rho-kinaza, o protein-kinază cu structura helicoidală, este o proteină care aparține familiei guanin-trifosfataze. Inhibarea rho-kinazei (ROCK) reprezintă o țintă terapeutică relativ nouă pentru multe afecțiuni precum bolile cardiovasculare, degenerescența neuronală, astmul, cancerul. Datorită implicării lor în multiple procese biologice, acești compuși sunt abordați în ultimii zece ani în studii clinice legate de patologii oftalmice precum glaucomul, disfuncția corneană endotelială și bolile vitreoretiniene. Calea de inhibare a ROCK pare să joace roluri importante în: promovarea proliferării celulelor endoteliale și a vindecării plăgilor, reglarea fluxului din rețeaua trabeculară, limitarea leziunilor microvasculare induse de diabet, inhibarea dezvoltării membranelor coroidiene neovasculare în degenerescența maculară legată de vârsta. Acest articol face o revizuire a literaturii și explorează mecanismele de acțiune și efectele inhibitorilor ROCK într-o varietate de patologii oculare, cum ar fi patologiile corneei, glaucomul, retinopatia diabetică, degenerescența maculară legată de vârstă, enumerând, de asemenea, studiile clinice, finalizate sau în curs.

Keywords: rho-kinase inhibitors, ophthalmology, cornea, glaucoma, diabetic retinopathy, age related macular degeneration

Introduction

Rho-kinase (ROCK) inhibition represents a relatively new therapeutic target for many diseases as cardiovascular diseases [62], neuronal degeneration [45, 67], asthma [105] and cancer [57]. Due to their involvement in multiple biological processes, these compounds are increasingly addressed in the last ten years for clinical trials related to ophthalmic pathologies as glaucoma, endothelial dysfunction and vitreoretinal diseases. It occurs that ROCK inhibitors can influence the pathological processes in many ocular structures which increased the interest in producing novel drugs that act on corneal epithelium or endothelium, trabecular meshwork, retinal vessels and cells [65, 66]. As a result, selective inhibitors of specific signalling pathways have been extensively investigated as a potential novel therapy for all these illnesses [54, 59, 81].

ROCK and ROCK inhibitors

Rho-kinases, also called coiled-coil containing protein kinases, are proteins that belong to the guanine triphosphate-ase (GTPase) family; these molecules are important in a wide variety of biological processes and pathways regarding migration, proliferation, adhesion and contraction [4]. The interaction with these cellular processes is mediated through the GTP state, respectively the switch between an active GTP-bound state and an inactive GTP-bound state [13, 16]. GTB-bound state activates ROCK and these are also known as effector proteins [18]. Among ROCK proteins two isoforms are

encountered in humans: ROCK1 and ROCK2. They are members of serine/threonine AGC kinases family and are ubiquitously expressed in all organs, but predominating in some, depending on cell type (ROCK-1 found in non-neuronal tissue as spleen, lung, kidney and liver, and ROCK-2 found in brain, muscle and heart) [29, 82].

Multiple evidences demonstrated that over-expression of ROCK proteins is involved in the pathogenesis of numerous diseases. Inhibition of ROCK showed beneficial effects in animal and human disease models [27]. Among all organs, however, the effect at the ocular level seems to be among the most diverse, influencing multiple ocular structures, thus being able to represent new therapeutic lines for some of the most frequent ophthalmological pathologies.

ROCK inhibitors effect on cornea

Corneal wound healing seems to be influenced by multiple molecules, among which ATP and lipid mediator lysophosphatidic acid (LPA) were demonstrated to be released from the injured cells to enhance epithelial cell migration and wound healing in the cornea [97, 98, 100, 102]. The Rho family regulate actin cytoskeleton, cell migration and proliferation, regulation of cell polarity and gene transcription [28, 42, 103]. ROCK family pathways lead to increase contractility and decrease adhesion [5, 30].

Table I

Clinical trials on ROCK inhibitors administered in patients with corneal endothelial cells pathologies

Trial	Status	Pathologies	Rock inhibitors preparations	Evidence
Moloney *et al*. [63]	Completed	Patients with Fuchs dystrophy, who underwent central Descemetorhexis not exceeding 4 mm	Two preparations of ROCK inhibitor were obtained for trial: Y-27632 at 10 mM and ripasudil hydrochloride hydrate (Glanatec® ophthalmic solution 0.4%).	In Fuchs dystrophy with visual degradation due to central guttae, Descemetorhexis without grafting is a viable procedure for visual rehabilitation.
Moloney *et al*. [64]	Completed	Descemet stripping only With Topical Ripasudil for Fuchs Endothelial Dystrophy	Ripasudil 0.4% was applied topically from day 1 post-operatively at a dose of 6 times *per* day until corneal clearance.	This treatment option is emerging as a reliable intervention for select patients with Fuchs' Endothelial Corneal Dystrophy (FECD) with an acceptable safety profile.
Moruyama *et al*. [55]	Completed	To investigate the time-dependent change of corneal endothelial cell (CEC) morphology and density (CECD) in patients with glaucoma post instillation of rho-associated protein kinase inhibitor ripasudil (Rip) 0.4% eye drops	Ripasudil (Rip) 0.4% eye drops	Transient morphological changes, revealed that CEC morphology gradually recovered to normal within 6 hours.
Macsai *et al*. [53]	Completed	Fuchs corneal dystrophy and Descemet only streaming	Topical ripasudil 0.4% (Glanatec®) 4 times *per* day for 2 months	DSO with topical rho kinase inhibitors may be an alternative treatment for patients with FD and a peripheral ECD greater than 1000 cells/mm^2.
Alkharashi *et al*. [116]	Completed	Cataract surgery/ Phacoemulsification	Ripasudil (Glanatec® ophthalmic solution 0.4%)	The use of ROCK inhibitors might prevent postoperative significant visual deterioration.
Devers Eye Institute Portland, Oregon, United States	Recruiting	Ripasudil for Enhanced Corneal Clearing Following Descemet Membrane Endothelial Keratoplasty in Fuchs' Dystrophy	Ripasudil hydrochloride hydrate (Glanatec® ophthalmic solution 0.4%)	The goal of this study is to test the potential benefits of Ripasudil therapy administered after Descemet Membrane Endothelial Keratoplasty (DMEK) surgery.
Massachusetts Eye and Ear Infirmary Boston, United States	Completed January 2021	Fuchs endothelial dystrophy and cataract to under combined cataract surgery with Descemetorhexis Without Endothelial Keratoplasty	Netarsudil 0.02% ophthalmic solution	to be published

Endothelial cells are the key element in the proper function of the cornea. Their inability to mitosis and their vital function in maintaining corneal transparency have led to numerous clinical trials involving the discovery of possible regenerative therapies for endothelial cell loss [46, 71-73, 90, 91].

Fuchs Endothelial Dystrophy and iatrogenic loss of the endothelial cells are some of the most important

causes for corneal transplantation, an intervention that is not available in many countries [14, 21, 84].

The endothelial cells of the cornea control the cornea hydration, maintaining corneal transparency. The corneal endothelial cell (CEC) density in normal individuals ranges between 2000 and 3000 cells/mm^2, but in pathologic states it can drop under 1000 cells/mm^2 and result in corneal oedema. In state of endothelial cells loss, the remaining cells compensate by migration and spreading. Rho-kinase, by decreasing adhesion and promoting contractility, inhibits this process [59]. There is evidence of expression of ROCK-1 and ROCK-2 in corneal endothelial cells [59]. ROCK inhibitors play a role in cell cycle and apoptosis [47, 68]. They also seem to promote endothelial cell proliferation and wound healing [25, 70, 72]. In the cornea endothelium apoptotic stimuli are activated by ROCK. ROCK inhibition supresses subsequent cell death [70]. ROCK inhibitors molecules reversibly change the cytoskeleton by the loss of actin stress fibbers, focal cells adhesions and intercellular tight junctions, making possible the migration and proliferation of corneal endothelial cells [59, 66, 78].

Molecules of ROCK inhibitors were extensively researched *in vitro* and *in vivo* animal models. Y-27632 and H-1152 exhibited significant stimulatory effect on CEC migration and proliferation *in vitro* [59]. Monolayers of confluent CECs treated with Y-27632 showed a complete collapse of actin cyto-skeletal architecture with markedly reduced actin stress fibbers, tight junctions, and cell adhesions [60]. In the corneal endothelial damage rabbit model, more Ki67-positive cells were detected in Y-27632-treated eyes than in control eyes. Actin fibbers were distributed at the cell cortex in the eyes treated with Y-27632, whereas actin distribution was partially disrupted, and stress fibbers were observed in control eyes. N-cadherin and Na^+/K^+-ATPase were expressed in almost all cells in Y-27632-treated eyes, but expression decreased in control eyes [74]. Y-27632 and Y-39983 employ both cyclin D and p27 *via* PI 3-kinase signalling to promote CEC proliferation [75].

To date, no approved pharmacologic therapy exists for corneal endothelial cells dysfunction in humans. In addition to experimental corneal studies in animals, two ROCK inhibitors molecules have also been administered to humans in cases of pathological (Fuchs endothelial dystrophy) or iatrogenic (post phacoemulsification) corneal endothelial damage. Those molecules are Ripasudil and Netarsudil, approved for glaucoma treatment. To date, ongoing clinical trials are using topical treatment with ROCK inhibitors after descemetorexis without endothelial keratoplasty (DWEK), see Table I (ROCK inhibitors administered in patients with corneal endothelial cells pathologies).

ROCK inhibitors and glaucoma

The most important risk factor that seems to be able to change the evolution of glaucoma is the intraocular pressure, and to date, most treatments have been designed to reduce this pressure, either by decreasing the secretion rate of aqueous humour or by increasing the outflow rate of aqueous humour through the uveoscleral tract [52, 94]. In addition to these treatments, ROCK inhibitors, through the changes they make on the cells at the trabecular meshwork level (decrease the resistance of this network to the outflow of aqueous humour), further increases the rate of excretion of aqueous humour through the Schlemm's channel, by the conventional pathway [33, 52, 76, 94].

An important role that ROCK inhibitors play in glaucomatous pathology is neuroprotective. By relaxing the smooth muscles of the retinal vascular bed, the blood supply from the retina and the optic nerve increases. It also decreases the degree of apoptosis of retinal ganglion cells and promotes regeneration of their axons [23, 95].

Currently, rho-kinase inhibitors are administered following filtering surgical treatment for glaucoma. Post-trabeculectomy ROCK inhibitors have been shown to have a beneficial effect due to their properties of inhibiting fibroblast proliferation, migration and cell adhesion, thus promoting wound healing and preventing scar formation in the filtering bleb [12, 35].

The first rho-kinase medication approved to treat glaucoma was Ripasudil hydrochloride hydrate 0.4%. This fasudil derivative was first approved in Japan in 2014 under the trade name Glanatec®, previously called K-115 [22]. This drug appears to play an important role in attenuating the evolution of glaucoma through its hitherto known mechanisms of action: it increases the outflow of aqueous humour by altering conventional pathways, on one hand, and neuroprotection, on the other [10, 49, 93].

Ripasudil 0.4% is currently used in patients with POAG, PACG (primary angle closure glaucoma), uveitic glaucoma, steroid-induced glaucoma, exfoliative glaucoma or in patients with ocular hypertension (OHT) and appears to have a beneficial effect in patients with increase in intraocular pressure (IOP) secondary to uveitis [48, 56, 58, 101]. It is also administered post-trabeculectomy because a reduction in the incidence of scarring has been observed [19, 24].

The treatment can be given either in monotherapy or as adjunctive therapy in combination with a prostaglandin analogue (which increases uveoscleral outflow) or a β-blocker (which decreases the rate of aqueous humour production), further decreasing IOP. The recommended dose for Ripasudil 0.4% is one drop twice a day in the long term for patients with glaucoma or intraocular hypertension. According to specialized studies to date it has been observed that the drug administered alone decreases IOP by about 3.5 mmHg (2.6 mmHg -

3.7 mmHg), administered in combination with a prostaglandin analogue decreases the additional IOP by approximately 3.2 mmHg and when supplemented with a beta-blocker treatment decreases the IOP by approximately 2.9 mmHg in addition [20, 36-39, 92]. The side effects of Ripasudil documented in the literature were: blepharitis, allergic conjunctivitis, conjunctival hyperaemia and punctate keratitis, transient symptoms that appear to have been dose dependent. Systemic side effects of the drug are rare and often minor (fatigue, headache, dizziness, constipation, nausea, etc.) [20, 36-39, 48, 92].

Another ROCK inhibitor was approved in the United States in 2017 under the trade name Rhopressa® [34]. The active substance identified in the drug is Netarsudil, formerly known as AR-13324, which has both a role as a ROCK inhibitor and a norepinephrine transporter inhibitor, thus having an important effect on the pathogenesis of glaucoma [15, 34].

To date, treatment with Netarsudil has been shown to be effective in open-angle glaucoma and OHT, as it decreases aqueous humour production, reduces episcleral vein pressure and increases aqueous humour outflow through conventional pathways. Used after trabeculectomy filter surgery, this drug decreases the incidence of scarring in the bleb [26, 50].

In clinical trials to date, the conclusion was that single-dose Netarsudil 0.02% alone has similar effects on IOP as (do) two drugs such as prostaglandin analogues (once daily) or beta-blockers (twice daily) [8, 9, 11, 41, 43, 85, 96]. The existence of a fixed combination of Latanoprost 0.005% and Netarsudil 0.02% under the trade name Rocklatan® (PG324), given once daily, has led to increased treatment compliance and a further decrease in IOP (approximately 3.5 mmHg), compared to the administration of a prostaglandin analogue alone [9, 11, 96].

Patients taking Netarsudil may experience the following side effects: palpebral erythema, blurred vision, conjunctival hyperaemia, subconjunctival haemorrhage, hyper-lacrimation and *cornea verticillata* [8, 9, 11, 26, 41, 43, 85, 96].

Currently, other ROCK inhibitors have been discovered, but they are still in clinical trials to treat primary or secondary glaucomatous pathology or intraocular hypertension. New agents that are still under study are: SNJ-1656 (formerly known as Y-39983), AR-12286, PHP-201 (also called AMA-0076) and ATS-907. They have been shown to have a beneficial effect on statistically significant IOP-decreasing ocular pathology (Table II) [86, 99, 106].

Table II

Clinical trials on ROCK inhibitors administered in glaucoma patients

Trial	Status	Pathologies	Rock inhibitors preparations	Evidence
Inazaki *et al*. [37]	Completed	Adults with open-angle glaucoma inadequately controlled with maximum medical therapy.	Ripasudil Ophthalmic Solution 0.4%	Treatment with ripasudil decreased IOP in patients with poorly controlled glaucoma with maximal medical therapy and it was well-tolerated.
Inazaki *et al*. [36]	Completed	Adults with open-angle glaucoma inadequately controlled with maximum medical therapy.	Ripasudil Ophthalmic Solution 0.4%	Treatment with ripasudil decreased IOP in patients with poorly controlled glaucoma with maximal medical therapy and it was well-tolerated.
Futakuchi *et al*. [20]	Completed	Adults with uveitic glaucoma, exfoliation glaucoma and steroid-induced glaucoma.	Ripasudil Ophthalmic Solution 0.4%	Ripasudil administration resulted in a statistically significant decrease in IOP, with no statistically significant side effects. These findings suggest that treatment with ripasudil is a safe and effective therapeutic modality in secondary glaucoma.
Tanihara *et al*. [92]	Completed	Adults with open-angle glaucoma or ocular hypertension.	Ripasudil Ophthalmic Solution 0.4%	Administration of 0.4% ripasudil decreased IOP levels, while at the same time having no statistically significant side effects when administered either as monotherapy or as additive therapy.
Inoue *et al*. [38]	Completed	Adults with open-angle glaucoma.	Ripasudil Ophthalmic Solution 0.4%	Switching from a PG analogue to PG/timolol fixed combination eye drops or adding ripasudil to PG analogue therapy were equally safe and effective in reducing IOP.
Asrani *et al*. [8]	Completed	Adults with open-angle glaucoma or ocular hypertension.	Fixed-dose combination (FDC) of netarsudil and latanoprost *versus* monotherapy with netarsudil or latanoprost.	Once daily administration of netarsudil/ latanoprost FDC achieved a statistically significant decrease in IOP and was superior to monotherapy of netarsudil or latanoprost.

Trial	Status	Pathologies	Rock inhibitors preparations	Evidence
Brubaker *et al*. [11]	Completed	Adults with open-angle glaucoma or ocular hypertension.	Fixed-dose combination (FDC) of netarsudil and latanoprost *versus* monotherapy with netarsudil or latanoprost.	Results at 12 months revealed superior efficacy for netarsudil/latanoprost FDC compared with the individual components, netarsudil and latanoprost.
Asrani *et al*. [9]	Completed	Adults with open-angle glaucoma or ocular hypertension.	Fixed-dose combination (FDC) of netarsudil and latanoprost *versus* monotherapy with netarsudil or latanoprost.	The results reveal superior efficacy for netarsudil/latanoprost FDC compared to the individual components, netarsudil and latanoprost, in order to decrease IOP in glaucoma patients.
Walters *et al*. [96]	Completed	Adults with open-angle glaucoma or ocular hypertension.	Fixed-dose combination (FDC) of netarsudil and latanoprost *versus* monotherapy with netarsudil or latanoprost.	Results at 3 months revealed superior efficacy for netarsudil/latanoprost FDC compared with the individual components, netarsudil and latanoprost and at the same time greater adherence to treatment.
Kahook *et al*. [41]	Completed	Adults with open-angle glaucoma or ocular hypertension.	Netarsudil Ophthalmic Solution 0.02%	The results at 12 months revealed that the administration of Netarsudil had a statistically significant effect in lowering the IOP and at the same time was well tolerated by most patients.
Singh *et al*. [85]	Completed	Adults with open-angle glaucoma or ocular hypertension.	Netarsudil Ophthalmic Solution 0.02%	Once daily administration of netarsudil 0.02% decreased IOP similarly to patients receiving timolol 0.5% twice daily.
Khouri *et al*. [43]	Completed	Adults with open-angle glaucoma or ocular hypertension.	Netarsudil Ophthalmic Solution 0.02%	Once daily administration of netarsudil 0.02% decreased IOP similarly to patients receiving timolol 0.5% twice daily.
New York Glaucoma Research Institute [86, 99]	Active, not recruiting	A Study to Assess the Effect of Rho-Kinase Inhibitor AR-12286 Ophthalmic Solution 0.5% and 0.7% in Patients With Uncontrolled Advanced Glaucoma With Prior Failed Trabeculectomy or Tube Shunt	AR-12286 Ophthalmic Solution 0.5% and 0.7%	To evaluate the ocular hypotensive efficacy of the rho-kinase Inhibitor (AR-12286 0.5% and 0.7%) ophthalmic solutions in glaucoma patients with failed prior glaucoma filtering surgery and uncontrolled IOP who are facing further surgical intervention. Patients will be treated for 6 months in this initial trial. To evaluate the efficacy of AR-12286 in enabling treated patients to delay or avoid the necessity of further surgical intervention.
New York Glaucoma Research Institute [86, 99]	Active, not recruiting	A Compassionate Case Study to Assess the Hypotensive Efficacy of Rho-Kinase Inhibitor AR-12286 Ophthalmic Solution 0.5% and 0.7% in Glaucoma Patients With Uncontrolled Intraocular Pressure to Avoid Surgical Intervention	AR-12286 Ophthalmic Solution 0.5% and 0.7%	To evaluate the ocular hypotensive efficacy of the rho-kinase Inhibitor (AR-12286 0.5% and 0.7%) ophthalmic solutions in open-angle glaucoma patients with uncontrolled IOP who are facing surgical intervention. Patients will be treated for 6 months in this initial trial. To evaluate the efficacy of AR-12286 in enabling treated patients to delay or avoid the necessity of surgical intervention.
New York Glaucoma Research Institute, United States [86, 99]	Active, not recruiting	A Study to Assess the Effect of Rho-Kinase Inhibitor AR-12286 Ophthalmic Solution 0.5% and 0.7% in Patients With Chronic Angle-closure Glaucoma (ROCK-CACG)	AR-12286 Ophthalmic Solution 0.5% and 0.7%	To evaluate the ocular hypotensive efficacy of Rho kinase Inhibitor (AR-12286 0.5% and 0.7%) ophthalmic solutions in patients diagnosed with chronic angle-closure glaucoma treated for 6 months.

Trial	Status	Pathologies	Rock inhibitors preparations	Evidence
Glaucoma Associates of New York, Robert Ritch, MD, LLC. [86, 99]	Completed	A Prospective Study to Assess the Hypotensive Efficacy of Rho-Kinase Inhibitor AR-12286 Ophthalmic Solution 0.5% and 0.7% in Patients with Exfoliation Syndrome and Ocular Hypertension or Glaucoma (ROCK)	AR-12286 Ophthalmic Solution 0.5% and 0.7%	This drug is currently being tested in patients with primary open-angle glaucoma (POAG), but not yet in glaucoma in exfoliation syndrome. Because of the mechanism of glaucoma in XFS and the mechanism of action of rho-kinase inhibitors, there is reason to think it would be more effective in eyes with XFS and glaucoma than in primary open-angle glaucoma (ordinary glaucoma).
United Medical Research Institute Inglewood, California, United States Aerie Pharmaceuticals [96]	Completed	AR-12286 Fixed Dose Combination to Lower Elevated Intraocular Pressure	AR-12286 Ophthalmic Solution 0.5% and 0.7%	This is a double-masked, randomized, controlled study assessing the safety and ocular hypotensive efficacy of two AR 12286/travoprost fixed-dose combination products compared to Travatan® Z in patients with elevated intraocular pressure

ROCK inhibitors and diabetic retinopathy

At present, the mechanisms by which diabetic retinopathy occurs are not fully understood. The pathogenesis is multifactorial and involves micro-vascular dysfunction in response to ischemia. Damage to endothelial cells and loss of vessel integrity will cause saccular dilation of capillaries and will cause thrombosis, occlusion and degeneration of the capillaries and the appearance of the intra-retinal haemorrhages [17, 88]. Hyperglycaemia causes ROCK activation which stimulates phosphorylation of myosin regulatory light chain (MLC) [51]. The ROCK pathway activation promotes leukocyte adhesion to the retinal capillaries, thus promoting endothelial damage due to binding to adhesion molecules like ICAM 1 (intercellular adhesion molecule-1) and $ß_2$-integrins [40, 87]. This leukocyte-induced endothelial damage represents a critical mechanism for diabetic retinopathy development, inducing inflammation and disruption of blood-retinal barrier. These finding reveals that ROCK pathway activation is also involved in diabetic macular oedema through blood-retinal barrier disruption [80, 83]. Additional to leukocyte induced endothelial damage, ROCK signalling also induces direct endothelial damage by inactivating endothelial nitric oxide synthase (eNOS) [6, 65]. eNOS generates nitric oxide which is a strong vasodilatator and antiapoptotic factor [79]. ROCK pathway is also involved in the pathogenesis of diabetic angiogenesis, VEGF-induced angiogenesis respectively, by stimulating the migration of the endothelial cells and retinal neovascularization [31].

ROCK inhibitors were demonstrated to increase eNOS expression in retinal endothelial cells and limit the leukocyte adhesion [6, 7]. Consequently, ROCK inhibitors seem to limit diabetes-induced microvascular damage [44]. Arita *et al*. demonstrated the antiangiogenic properties of ROCK inhibitors, due to inhibition of VEGF induced capillary endothelial cell migration and proliferation.

Table III

Clinical trials on ROCK inhibitors administered in patients with diabetic retinopathy

Trial	Status	Pathologies	Rock inhibitors preparations	Evidence
Nourinia, Ramin *et al*. [69]	Completed	This prospective, interventional case series included 15 eyes of 15 patients with diabetic macular oedema unresponsive to previous intravitreal bevacizumab (IVB) injections.	Intravitreal injection of 0.025 mg Fasudil and 1.25 mg Bevacizumab	Visual acuity and central macular thickness improved statistically significant following injection. No adverse event was observed during the study period. Intravitreal ROCK inhibitors seem to entail structural and visual benefits in eyes with diabetic macular oedema refractory to IVB monotherapy.
Ahmadieh H *et al*. [1]	Completed	Prospective interventional case series, patients with persistent diabetic macular oedema with macular laser photocoagulation and multiple intravitreal bevacizumab injections.	Intravitreal injection of 0.025 mg/0.05 mL Fasudil and intravitreal injection of 1.25 mg/0.05 mL Bevacizumab	Visual acuity and central macular thickness improved statistically significant following injection. Combined intravitreal Bevacizumab and Fasudil injection may have promising effects in severe cases of diabetic macular oedema resistant to current therapeutic modalities.

Trial	Status	Pathologies	Rock inhibitors preparations	Evidence
Minami Y *et al*. [61]	Completed	Retrospective study in subjects with glaucoma or ocular hypertension and diabetic retinopathy with diabetic macular oedema present before the administration of Ripasudil	Ripasudil Ophthalmic Solution 0.4%, administered topically	One month after ripasudil therapy, the mean foveal thickness decreased significantly. There was no significant difference in visual acuity.
Ahmadieh H *et al*. [2]	Completed	Prospective randomised clinical trial with subjects presenting centre-involving diabetic macular oedema	Intravitreal bevacizumab (IVB) 1.25 mg plus intravitreal Fasudil (IVF) 50 µM/L *versus* intravitreal bevacizumab (IVB) 1.25 mg.	Mean best corrected visual acuity was significantly improved in both groups at month 3 ($P < 0.001$), but it persisted up to month 6 only in the IVB/IVF group. Central macular thickness was significantly reduced in both groups at month 3 ($p = 0.006$, $p < 0.001$), but this reduction sustained only in the IVB/IVF group up to month 6 ($p < 0.001$).

Recently, several ROCK inhibitors (fasudil, ripasudil) were administered by intravitreal injection, in conjunction with anti-VEGF factors, for persistent diabetic macular oedema (Table III) [1, 61, 69].

ROCK inhibitors and age related macular degeneration

Age related macular degeneration (AMD) represents the main cause for low vision in developed countries. There are two clinical forms of AMD, the dry form and the wet form, the las one being characterized by the presence of neovascular membrane of the choroid. In AMD, ROCK pathway is involved in neovascularisation. Age determines an increase signalling of ROCK2; this molecule determines macrophage polarization [104]. It seems that macrophages play an important role in the development of neovascular choroidal membranes in wet AMD [104]. Experimental evidence demonstrates a potential role of ROCK inhibitors in neovascular AMD. In mouse models of neovascular AMD, ROCK inhibitors reduced sub-retinal fibrosis, but the exact mechanism is not completely understood [32]. To date there are no studies in humans with wet AMD.

Conclusions

It seems that ROCK inhibitors represent an important pipeline for emerging treatments in ophthalmic pathologies. It is quite remarkable that these molecules can influence pathologies related to cornea, trabecular meshwork, retina and choroid. ROCK inhibitors were FDA approved for treatment of glaucoma and shown good results in many clinical trials. Their usage in cornea and retinal pathologies is under clinical research, with particular good outcomes reported in endothelial disease.

Conflict of interest

The authors declare no conflict of interest.

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