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The Future of Glaucoma Treatment Ripasudil

Zafer F. Ismaiel , Mahmoud A. Abdel Hamed , Samah M. Fawzy , Nada E. Amer
Department of ophthalmology, Ain Shams University, college of medicine, Cairo, Egypt

ABSTRACT
Glaucoma is a leading cause for worldwide blindness and is characterized by progressive optic nerve damage. The etiology of glaucoma is unknown, but elevated intraocular pressure (IOP) and advanced age have been identified as risk factors. IOP reduction is the only known treatment for glaucoma. Recently, drugs that inhibit Rho associated protein kinase (ROCK) have been studied in animals and people for their ability to lower IOP and potentially treat POAG. ROCK inhibitors lower IOP through a trabecular mechanism and may represent a new therapeutic approach for the treatment of glaucoma. Ripasudil is the first Rho-kinase inhibitor ophthalmic solution developed for the treatment of glaucoma and ocular hypertension in Japan 2014. ROCK inhibition not only reduces intraocular pressure (IOP) but also increases ocular blood flow.

Keywords: Glaucoma, Optic nerve, K-115, Ripasudil, Rho kinase, ROCK inhibitor, Trabecular meshwork, Ocular blood flow.

INTRODUCTION

Glaucoma is an optic neuropathy in which at least one eye has accelerated ganglion cell death characterized by excavated cupping appearance of the optic nerve with progressive thinning of retinal nerve fiber layer tissue and corresponding subsequent visual field loss [1]. Glaucoma is the second leading cause of preventable blindness worldwide. Despite availability of medical and surgical treatment many patients with glaucoma currently continue to lose vision [2]. Clinically, it is well accepted that the major risk factor for glaucoma is elevated intraocular pressure (IOP).

Despite of use many drugs to modify the course of the disease, none of the current medications for POAG is able to reduce the IOP by more than 25%–30%. Also, some glaucoma patients show disease progression despite of the therapeutics [3].

Ripasudil hydrochloride hydrate (K-115), a specific Rho-associated coiled-coil containing protein kinase (ROCK) inhibitor, is ophthalmic solution developed for the treatment of glaucoma and ocular hypertension in Japan. Topical administration of K-115 decreased intraocular pressure (IOP) and increased outflow facility [4] .Rho kinase (ROCK) inhibitors are a novel potential class of glaucoma therapeutics with multiple compounds currently in Phase II and III US Food and Drug Administration trials in the United States. These selective agents work by relaxing the trabecular meshwork through inhibition of the actin cytoskeleton contractile tone of smooth muscle. This new mechanism aims to increase outflow facility in TM [5].

Considerable evidence has shown that TM cells are highly contractile and play an active role in aqueous humor dynamics. It has been shown that TM tissues possess smooth muscle cell-like properties. The contraction and relaxation properties of TM cells are regulated by several enzymes, which have become experimental therapeutic targets for lowering IOP. Cellular properties of the trabecular meshwork are critical for conventional outflow [6].

There are high levels of RhoA in TM cells that induce a contractile morphology, increased actin fibers, increased focal cell to cell adhesions, increased levels of phosphorylated myosin light chain (MLC) and increased extracellular matrix protein production. These changes will decrease aqueous humor drainage because of cellular and morphological changes in the TM cells. The ciliary muscle (CM) also plays an important role in the conventional route. As contraction of the CM leads to increased trabecular meshwork pore size and increased aqueous drainage [7].

Moreover, a significant number of patients presenting with glaucoma continue to lose vision despite responding well to therapies that lower eye pressure non-IOP-dependent, so enhancement of optic nerve blood supply and neuroprotection are potential treatment strategies for glaucoma. Significantly elevated levels of RhoA have been detected by immunostaining in the optic nerve head of glaucomatous eyes compared with age-matched controls, reinforcing the association of Rho proteins and glaucoma pathophysiology [8].

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MECHANISM OF ACTION

Mechanism to decrease IOP

Rho GTPase, participates in signaling pathways leading to the formation of actin stress fibers and focal adhesions [9]. Rho GTPase is activated in response to growth factors, mechanical stretching, cytokines and extracellular matrix. Rho plays a critical role in a multiple of cellular processes associated with cytoskeletal rearrangements. These include cell morphology, cell motility, cytokinesis, apoptosis and, smooth muscle contraction [10]. Multiple Rho target molecules have been identified as downstream Rho effectors, including Rho-associated coil-forming protein serine/threonine kinases ROCK [11]. Stimulating the Rho pathway, enhances phosphorylation of the myosin light chain, thus increasing the contractility of those fibers [12].

Activation of the Rho GTPase pathway in the eye causes cellular and molecular changes within cells in the TM that results in contraction of smooth muscle like cells and diminished aqueous humor drainage. Multiple studies support this molecular understanding of conventional outflow showed that ROCK inhibition induces cell rounding, and cell-cell detachment, and decreases actin stress fibers and focal adhesion staining [13]. In addition to TM relaxation and morphological changes, ROCK inhibition increased outflow in human anterior segments. Increased outflow has been shown to decrease IOP, making Rho GTPase signaling a target for glaucoma therapy [14].

There are two known ROCK isoforms: ROCK-I and ROCK-II. Although both ROCK-I and ROCK-II are expressed in the CM and TM of humans, the TM exhibits greater expression levels of both kinases. These differences are consistent with the proposed mechanism of action of ROCK inhibitors, whereby inhibition of ROCKs cause increased aqueous outflow, leading to a decrease in IOP [15]. Additional factors as local concentration of ROCK inhibitor and differences in activation/expression of other Rho GTPase pathway signaling molecules may also contribute to IOP reduction. Besides Rho GTPases and ROCKs, other downstream targets in this pathway play an important role in IOP regulation. For example, ROCKs regulate myosin light chain phosphatase (MLCP), MLC, LIM domain kinase (LIMK) and CPI-17 [16].

Mechanism to act as Neuroprotective

Neuroprotection strategies include preventing or slowing the death of RGCs and/or enhancing blood flow to the optic nerve, thereby reducing the development of glaucomatous optic neuropathy [17]. Studies done to investigate the effect of K-115, a novel Rho kinase (ROCK) inhibitor, on retinal ganglion cell (RGC) survival in an optic nerve crush (NC) model.K-115, a ROCK inhibitor, can prolong RGC cell survival by suppressing oxidative stress through pathways involving the Nox family[18]. The NADPH oxidases NOX family are proteins that transfer electrons across biological membranes. Previous studies have shown that oxidative stress is involved in RGC death after axonal injury. In order to evaluate the efficacy of K-115, a comparison done between the density of RGCs in mice treated with either ROCK inhibitors such as K-115 and fasudil 7 days. They found that the neuroprotective effects of K-115 and fasudil after NC were similar, but that the specificity of the effect of K-115 was 2 to 18 times higher than that of fasudil [19].

Additionally, K-115 dramatically suppressed oxidative stress, including ROS (free radicals) production by the RGCs themselves. In glaucoma ,it has been reported that increases in oxidative stress markers can be found in a patient's aqueous humor and plasma[20].Researches done using an experimental glaucoma model, strongly suggests that glaucoma should also be considered as a chronic neurodegenerative disease associated with oxidative stress[17].

K-115 significantly suppressed NC induced oxidative stress by inhibiting ROS production in RGCs. These results strongly suggest that the prevention of oxidative stress in the mitochondria or nucleus should be regarded as candidates for the treatment of glaucoma. Furthermore, suppression of Rho activity also has the potential to be a new neuro protective treatment for glaucoma [17].

PHARMACOLOGY OF RIPASUDIL

Pharmacodynamics

Ripasudil is a highly selective and potent ROCK inhibitor. It was originally developed from fasudil, as both compounds share the same core structure of 5-(1, 4-diazepan-1sulfonyl) isoquinoline . Fasudil was already recognized as a potent Rho-kinase inhibitor, but after exploring the chemical derivatives of

fasudil, developers observed that the incorporation of a fluorine atom at C4 position of isoquinoline and the attachment of a methyl group to the C2 position of 1, 4-diazepane dramatically improved the pharmacological action. In short, ripasudil showed much more potent and selective Rho-kinase inhibitory activity than fasudil [21]. The IOP in both rabbits and monkeys was significantly ($p < 0.05$) reduced in a dose dependent manner by topical instillation of ripasudil, at concentrations of 0.0625–0.5 % (in rabbits) and 0.1–0.4 % (in monkeys). In monkeys, maximum reduction of IOP versus vehicle was observed at 2 h, and was significantly greater with ripasudil 0.4 % than with latanoprost 0.005 %. The maximum reduction effect observed with latanoprost occurred at 4 h. The significant reduction in IOP observed in the monkeys receiving ripasudil 0.4 % continued until 6 h after instillation. Ripasudil has high intraocular permeability [22].

In healthy human volunteers single instillations of ripasudil 0.05–0.8 % were associated with reductions from baseline in IOP in a dose dependent manner, with the maximum reduction reached after 2 h; ripasudil 0.4 and 0.8 % were both associated with steady, significantly ($p < 0.05$) reduced IOPs [23]. In-vitro and in-vivo data indicate that ripasudil attenuates retinal neovascularization and reduces the areas of avascular retina; it may thus have potential in retinal neovascular diseases such as diabetic retinopathy. Preliminary, preclinical data indicate that ripasudil may also be of use in neuroprotective treatment for glaucoma [24].

Pharmacokinetics

Ripasudil achieves a half-life of 0.49 to 0.73 hours in humans and is predominantly excreted in the urine. After 7 days of twice-daily ripasudil 0.4 % treatment in 8 healthy male volunteers, the maximum plasma concentration 0.622 ng/ml was reached after 0.083 h. The corresponding main metabolite (M1) pharmacokinetic values were 1.465 ng/mL, 0.500 h and 4.761 ng·h/mL, respectively. The metabolite M2 occurred at negligible levels. Ripasudil has a plasma protein binding rate of 55.4–59.8 % . Renal clearance was 7.112 L/h for ripasudil and 17.516 L/h for M1, and the elimination half-life was 0.455 and 2.189 h, respectively [25].

Ripasudil therapeutic trials in POAG

Phase I clinical trials was to evaluate the efficacy and safety of the selective Rho kinase inhibitor K-115 as a candidate drug for the treatment of glaucoma. Randomized, placebo-controlled, double-masked, group comparison clinical trial done.. This study revealed that K-115 is a promising drug for lowering IOP in healthy adult eyes, with tolerable adverse events during at least short-term administration[26].

Ripasudil was associated with a significant, dosage dependent reduction in IOP in a randomized, double-masked, placebo-controlled, multicentre, prospective phase II study. On the basis of the results from this study, ripasudil 0.4 % was selected as the optimal dosage. Results also showed that IOP could be controlled over a 24h period with twice daily dosing [21].

In a phase III Ripasudil (K-115) combined with other anti-glaucoma drugs, 2 studies done to show the additive effect on IOP if ripasudil combined with timolol or combined with latanoprost. Results of the studies found additive IOP lowering effects of ripasudil in combination with timolol and in combination with latanoprost [27].

ADDITIONAL MEDICAL USES

Ripasudil on activation of human conjunctival fibroblasts

On 2016, Ripasudil has been shown to prevent excessive scarring after glaucoma filtration surgery by attenuating the activation of conjunctival fibroblasts. Ripasudil may be of therapeutic utility, preventing excessive scarring after glaucoma filtration surgery.

However the study done on ripasudil was subjected to several limitation as the researchers used only two lines of primary human subconjunctival fibroblasts to evaluate the antifibrotic effect of ripasudil. Further studies using more lines of conjunctival fibroblasts and other ocular fibroblasts, such as tenon's fibroblasts or scleral fibroblasts, will therefore be required to verify the effects. Also other fibrogenic factors, such as connective tissue growth factor or fibroblast growth factor, should be investigated in future studies. Ripasudil may be useful for inhibiting excessive post-surgical wound healing accompanied by scarring and fibrosis specially after glaucoma filtration surgery [28].

Effect of Ripasudil on corneal endothelial wound healing

In March 2016 Ripasudil was shown to promote corneal endothelial cell (CEC) proliferation in cultured human cells as well as wound healing and endothelium regeneration in a rabbit wound model. Experimenters believed that these characteristics could prevent or improve the CEC density drop associated with cataract surgery or corneal trauma. This would prevent an array of symptoms including general haziness, edema of the cornea, or keratopathy, and would generally improve the recovery of a post-operation patient [29].

Ripasudil effect on Retinal Angiogenesis and Hypoxia

Rho-associated coiled-coil–containing protein kinase (ROCK) is a downstream effector of the small GTP-binding protein RhoA. ROCK is known to be activated by various cytokines including VEGF [30]. Studies have demonstrated the role of ROCK activation in the pathogenesis of diabetic retinopathy such as retinal endothelial injury and proliferative membrane contraction in vitro and in vivo [31]. Furthermore, ROCK inhibition blocked VEGF-induced angiogenesis in a corneal model. Recently, ROCK demonstrated as a key molecule in exudative AMD [32]. Clinical observations on DR patients, intravitreal ROCK inhibitor fasudil combined with anti-VEGF agents also improved diabetic macular edema, which was refractory to anti- VEGF therapy [33].

From these results, ROCK is expected to be a novel therapeutic target for retinal diseases. A novel, potent and selective ROCK inhibitor, ripasudil hydrochloride hydrate (K-115), has been developed [34], the enzyme inhibitory effect of ripasudil is approximately 5 to 10 times higher than previous ROCK inhibitors such as fasudil. It is recently reported that ripasudil has neuroprotective effects for retinal ganglion cells after systemic administration [35].

Recently, the therapeutic potential of ROCK inhibitors have been investigated in the treatment of retinal diseases.[31] A ROCK inhibitor, fasudil, has already been shown to improve ischemia in patients with acute ischemic stroke. ***Nagaoka et al***. [36] previously showed ROCK inhibition could cause retinal vessel dilation. This dilated effect on retinal vessels could also contribute to improvement of ischemia. Further investigation is necessary to explore the therapeutic potential of ripasudil eye drop as having a neuroprotective effect on ischemic neovascular diseases.

CONCLUSION

Ripasudil (K-115) is a newly approved glaucoma treatment that is able to achieve potent IOP-lowering effects via its action as a selective Rho kinase inhibitor. K-115 is the first Rho-kinase inhibitor ophthalmic solution developed for the treatment of glaucoma and ocular hypertension in Japan 2014.

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