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Towards axonal regeneration and neuroprotection in glaucoma: Rho kinase inhibitors as promising therapeutics

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ABSTRACT

Due to a prolonged life expectancy worldwide, the incidence of age-related neurodegenerative disorders such as glaucoma is increasing. Glaucoma is the second cause of blindness, resulting from a slow and progressive loss of retinal ganglion cells (RGCs) and their axons. Up to now, intraocular pressure (IOP) reduction is the only treatment modality by which ophthalmologists attempt to control disease progression. However, not all patients benefit from this therapy, and the pathophysiology of glaucoma is not always associated with an elevated IOP. These limitations, together with the multifactorial etiology of glaucoma, urge the pressing medical need for novel and alternative treatment strategies. Such new therapies should focus on preventing or retarding RGC death, but also on repair of injured axons, to ultimately preserve or improve structural and functional connectivity. In this respect, Rho-associated coiled-coil forming protein kinase (ROCK) inhibitors hold a promising potential to become very prominent drugs for future glaucoma treatment. Their field of action in the eye does not seem to be restricted to IOP reduction by targeting the trabecular meshwork or improving filtration surgery outcome. Indeed, over the past years, important progress has been made in elucidating their ability to improve ocular blood flow, to prevent RGC death/increase RGC survival and to retard axonal degeneration or induce proper axonal regeneration. Within this review, we aim to highlight the currently known capacity of ROCK inhibition to promote neuroprotection and regeneration in several *in vitro*, *ex vivo* and *in vivo* experimental glaucoma models.

Contents

Abbreviations: AAV, adeno-associated viral; AH, aqueous humor; ALS, amyotrophic lateral sclerosis; Cdk5, cyclin dependent kinase 5; CNTF, ciliary neurotrophic factor; CRMP-2, collapsin response mediator protein-2; CSPGs, chondroitin sulfate proteoglycans; Dpi, day post injection; ECM, extracellular matrix; ERM, ezrin/radixin/moesin; GAP, growth associated protein; GFAP, Glial fibrillary acidic protein; IOP, intraocular pressure; IPL, inner plexiform layer; MAG, myelin-associated glycoprotein; MAPs, microtubule-associated proteins; MLC, myosin light chain; MS, multiple sclerosis; NMDA, *N*-mehtyl-D-aspartate; NTG, normal tension glaucoma; OMgp, oligodendorcyte myelin glycoprotein; ONC, optic nerve crush; ONH, optic nerve head; pSTAT3, phosphorylated STAT3; PTEN, phosphatase tensin homolog; RGCs, retinal ganglion cells; Rho, associated coiled-coil protein kinase ROCK; TM, trabecular meshwork.

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1. Introduction

The term glaucoma refers to a wide range of optic neuropathies associated with degeneration of retinal ganglion cells (RGCs) and their respective axons, leading to slow progressive visual field loss (Foster et al., 2002). If not adequately treated, most types of glaucoma progress without obvious symptoms towards gradual loss of visual function or even blindness (Friedman et al., 2004). Nowadays, it is considered as the second leading cause of blindness throughout the world (Resnikoff et al., 2008). This neurodegenerative disease mostly appears after the 4th decade of life, and its frequency significantly increases with age. In 2010, at least 60.5 million people suffered from glaucoma worldwide. This number is expected to increase up to 80 million by 2020 (Quigley and Broman, 2006). Risk factors for glaucoma not only include age, but also family history (Wolfs et al., 1998), gender (Rudnicka et al., 2006), ethnic background (Racette et al., 2003), severe myopia (Mitchell et al., 1999), disturbed cerebrospinal fluid pressure (Fleischman and Allingham, 2013), vascular disorders (Bonomi et al., 2000) and importantly, an increased intraocular pressure (IOP) (Boland and Quigley, 2007).

With the currently available treatment modalities, glaucomatous visual field deterioration cannot be prevented, yet lowering the IOP can slow down glaucoma disease progression (Van Veldhuisen et al., 2000; Kass et al., 2002; Anastassiadis et al., 2011). Therefore glaucoma treatment is mainly directed towards lowering IOP (Van Veldhuisen et al., 2000; Kass et al., 2002). A sustained reduction of IOP can be achieved with topical therapy, laser therapy or surgical interventions (Quigley, 2011). Nevertheless, signs of progression can be seen in many patients despite well-controlled IOP. Therefore, the development of neuroprotective strategies may be of high value in the future treatment of this multifactorial neurodegenerative disorder. Until now, only two potential neuroprotective agents have been investigated in clinical trials for glaucoma, *i.e.* memantine and brimonidine.

Over the past few years, many studies have highlighted the important role of the Rho and Rho-associated coiled-coil protein kinase (ROCK) pathway in the pathogenesis and treatment of glaucoma. Modulation of this pathway seems to be involved in the regulation of IOP *via* the trabecular meshwork (TM) and may also serve as a potent anti-scarring agent after glaucoma surgery. More importantly, the amount of studies reporting ROCK inhibition as a very appealing therapeutic approach that confers neuroprotection and axonal regeneration, substantially increased over the last decade. Therefore, the purpose of this review is to summarize the current knowledge on the upcoming neuroprotective and axon regenerative potential of ROCK inhibitors, which may be of great importance in the development of a novel neuroprotective/regenerative strategy for glaucoma therapy.

2. Glaucoma pathophysiology

The exact mechanisms of how glaucoma develops are still not well-known, although the initiation and propagation of this disease is thought to be situated around the optic nerve head (ONH). Yet, two principal hypotheses, the mechanical and ischemic/vascular theories have been described (Fechtner and Weinreb, 1994; Flammer et al., 2002b; Flammer and Mozaffarieh, 2007) (Fig. 1). The classical mechanical theory suggests that the development of glaucoma is a direct consequence of an increased IOP causing damage to the ONH (Yan et al., 1994). The IOP reflects the balance between aqueous humor (AH) production and outflow through either the conventional pathway *via* the TM, or the unconventional pathway *via* the uveoscleral route (Goel et al., 2010). IOP elevation above 21 mmHg due to impaired TM function, is considered as the most important measurable risk factor for the development of glaucoma (Weinreb and Khaw, 2004) and believed to induce damage to the RGC axons by tissue deformation at the level of the lamina cribrosa, thereby leading to ONH cupping. When RGC axons exit the eye at the ONH, the lamina cribrosa provides the only support and protection in an otherwise firm scleral shell, making it the most vulnerable site of the retina/optic nerve to mechanical stress (Sigal and Ethier, 2009). Cupping of the ONH in response to elevated IOP causes compression of RGC axons, leading to a disruption of the axoplasmic transport between the retina and the brain, which is essential for proper RGC survival (Flammer et al., 2002b). Additionally, glial cells at the ONH shift their production of extracellular matrix (ECM) components and increase their secretion of matrix metalloproteinases, thereby reducing mechanical support to nerve fibers, making them even more vulnerable to elevated IOP (Hernandez, 2000; Kirwan et al., 2004; Dahlmann-Noor et al., 2010; Quill et al., 2011; Akhter et al., 2013; De Groef et al., 2014).

Although generally accepted for a long time, the mechanical theory fails to explain several features correlated with the appearance of glaucoma. Many patients (25–30%) suffer from glaucomatous symptoms without the appearance of an increased IOP (normal tension glaucoma (NTG)) and pressure reduction does not avoid glaucomatous damage in all patients with initial ocular hypertension. On the other hand, some patients with ocular hypertension show no damage to the optic nerve (Chauhan and Drance, 1992; Martinez-Bello et al., 2000; Agarwal et al., 2009). These observations indicate that multiple other factors, unrelated to IOP, play an important role in the development of glaucoma. Increased incidence of glaucoma, especially NTG, is observed in patients with systemic vascular disorders like hypotension (Hayreh et al., 1994; Hayreh, 1999), cardiovascular disease (Hayreh, 1999), vasospastic disorders (Broadway and Drance, 1998), migraine (Wang et al., 1997), diabetes (Mitchell et al., 1997) and cerebral ischemia (Stroman et al., 1995). Therefore, the vascular theory considers a disturbed blood supply to the retina as the primary cause of optic atrophy (Flammer et al., 2002b). Insufficient vascular autoregulation to adapt ocular perfusion may result in a decreased retinal blood supply, causing ischemic damage (Morgan, 2004). This then leads to an impaired perfusion of the ONH, causing ischemic injury to the retina. Notably, the mechanical and vascular theory are closely interlinked, because excavation of the ONH at the lamina cribrosa as a direct consequence of elevated IOP, also leads to kinking of the retinal blood vessels (Flammer, 1994; Flammer et al., 2002b; Flammer and Mozaffarieh, 2007; Chang and Goldberg, 2012).