

The Emerging Role of Statins in Glaucoma Pathological Mechanisms and Therapeutics

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Abstract

Statins inhibit the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and hence have a profound effect in lowering serum cholesterol. Their predominant clinical use to date is in primary and secondary prevention of cardiovascular disease. However recently interest has developed regarding the so-called “pleiotropic” effects of statins—these drugs have significant anti-fibrotic, anti-inflammatory, and immunomodulatory properties. Such effects of statins have already been shown to be beneficial in modulating the pathological mechanisms involved in pulmonary fibrosis, renal disease, non-ischaemic cardiac failure, and tissue scarring. Many of these actions are mediated by inhibition of the Rho kinase pathway. Epidemiological studies suggest that patients who take statins have a lower risk of developing glaucoma, and lower rates of glaucoma progression. Here, we review what is known about the pleiotropic effect of statins to date, and examine how these effects may modulate the molecular mechanisms involved in glaucoma pathogenesis.

Keywords

Statin, Glaucoma, Rho Kinase, Optic Nerve, Trabecular Meshwork

1. Introduction

Recent population-based studies suggest that the use of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (commonly known as statins) may reduce the risk of glaucoma development and progression [1] [2]. Statins are already established as a first-line treatment in cardiovascular disease due to their cholesterol lowering effect. However, statins also possess important pleiotropic immunomodulatory and anti-inflammatory capabilities, which have resulted in an expansion of their indications for usage [3]. They appear to have a

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neuroprotective effect in several diseases of the central nervous system including Alzheimer's disease, and ischaemic stroke [3]. They also have an antifibrotic action—as seen in models of pulmonary and renal fibrosis [4]-[6]. The mechanism of action of statins in these disease processes is still the subject of much debate. Numerous molecular pathways seem to be involved, including the Rho-kinase pathway and modulation of the actin cytoskeleton [7]-[9]. We hypothesise that statins may also be protective in glaucoma. Glaucoma is a progressive optic neuropathy, with a known fibrotic and neurodegenerative component [10]. The purpose of this article is to review the emerging role of statins in primary open angle glaucoma (POAG). In order to understand the potential mechanisms of action of statins in glaucoma, it is beneficial to first review the aetiology of the disease process and current treatment options.

2. POAG

Glaucoma is the second leading cause of irreversible, but potentially preventable vision loss worldwide, affecting an estimated 60.5 million people [11]-[13]. It refers to a spectrum of clinical conditions distinguished by a progressive optic neuropathy and associated visual field loss, with raised intraocular pressure (IOP) being one of the primary risk factors [14] [15]. Two principal theories have been described for the pathophysiological mechanism for primary open angle glaucoma (POAG), but these are not mutually exclusive. The vascular theory proposes that optic neuropathy results from a compromise to the microvasculature resulting in ischaemic damage to the optic nerve head. This may be as a consequence of the raised IOP or other factors which influence ocular blood flow [16]. The mechanical theory suggests that raised IOP alters the architecture of the lamina cribrosa (LC), compromising the retinal ganglion cell (RGC) axons as they pass through this structure. The LC is a specialised series of plates of connective tissue which acts as a support for axons as they traverse the corneoscleral shell.

The normal intraocular pressure (range 11 - 21 mmHg) is dependent upon a delicate balance of aqueous humour production, inflow and outflow [17]. Aqueous humour is produced by the ciliary body, and provides nourishment to the avascular cornea and lens (Figure 1). The main drainage pathway for the aqueous from the anterior chamber is the pressure-dependent conventional outflow pathway—through the trabecular meshwork (TM).

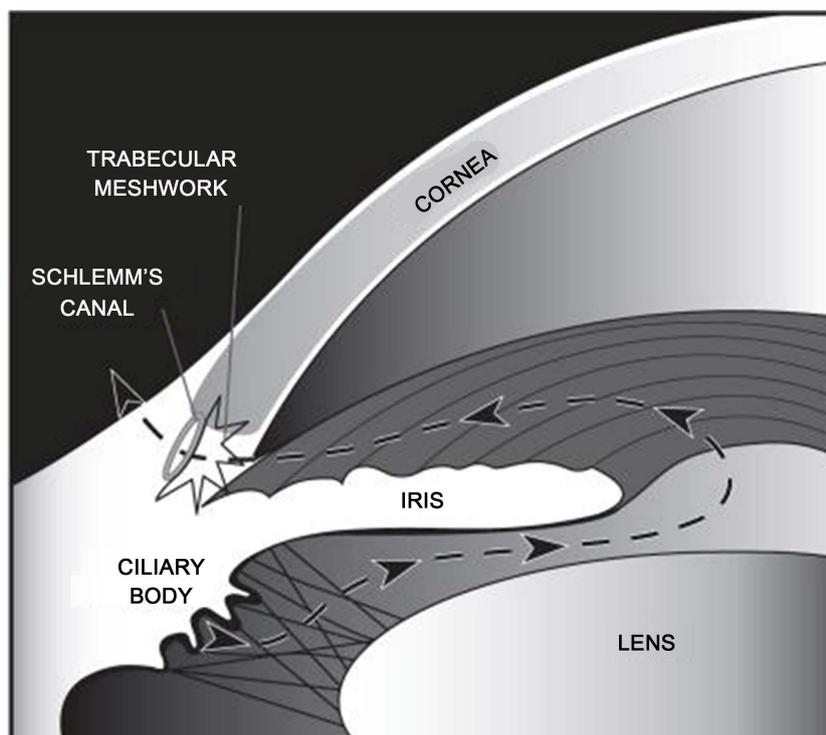


Figure 1. The aqueous outflow pathway (Goel, M., et al., *Aqueous humor dynamics: a review*. Open Ophthalmol J, 2010, 4, pp. 52-59).

This normally accounts for up to 90% of aqueous outflow. A framework of lattice-like collagen beams forms the TM, with extracellular matrix (ECM) residing in the paracellular spaces between the beams [18]. Aqueous humour drains through the TM, then the juxtacanalicular connective tissue (JCT) region, and then into Schlemm's canal (SC), from where it is returned to the systemic circulation via the episcleral veins. In order to enter SC, aqueous must diffuse through the paracellular spaces between the endothelial cells of SC, or through giant vacuoles which form as outpouchings into SC. The unconventional, or uveoscleral outflow pathway normally accounts for the remaining ~10% of aqueous outflow. However, with increasing age, up to 50% of aqueous outflow can occur by the uveoscleral pathway [18].

Extracellular matrix remodelling is at the heart of glaucomatous pathophysiology, and has been shown to be a feature of glaucomatous damage both at the TM where it impairs aqueous drainage, and at the optic nerve head—particularly the LC [19] [20]. Importantly in the LC, remodelling affects its biomechanical properties—leading to increased tissue stiffness, fibrosis, and posterior bowing of this structure [14] [16] [21] [22]. The resultant mechanical compression of the RGC in this area induces axoplasmic stasis contributing to cell death [10] [23]. Two important types of cell that populate the LC include astrocytes (glial fibrillary acidic protein (GFAP) positive) and LC cells (GFAP negative) [24]. Results of previous research in our laboratory support the profibrotic nature of LC cells when exposed to glaucomatous stimuli, suggesting they play an integral role in glaucomatous ECM remodelling [25]-[27].

Present day glaucoma therapies aim only to reduce the IOP—the only modifiable risk factor for glaucoma progression. Most agents aim to reduce aqueous production (beta-blockers, carbonic anhydrase inhibitors, alpha-agonists), and some increase outflow via the uveoscleral pathway (prostaglandin analogues). However glaucoma may progress despite apparently normal IOP, suggesting the need for novel treatments that may modulate the inflammatory and fibrotic aspects of glaucoma pathophysiology. Our own research group has investigated the role of anti-connective tissue growth factor (CTGF) as a potential anti-fibrotic agent in glaucoma. We have shown that anti-CTGF is effective in blocking extracellular matrix production in human TM and LC cells [28]. Very promising novel agents are inhibitors of the Rho pathway, which is integral to the regulation of aqueous outflow and matrix composition [29] [30]. Various Rho kinase inhibitors are already in phase 2 and 3 clinical trials, with conjunctival hyperaemia being one of the main adverse effects [31]. Clinical trials of combination therapies of Rho kinase inhibitors with prostaglandin analogues are also underway.

3. The HMG CoA Pathway

Statins are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase—the crucial rate limiting step in hepatic cholesterol biosynthesis. Independently of the cholesterol lowering effect, the inhibition of HMG-CoA leads to reduced synthesis of isoprenoid intermediaries—farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) (**Figure 2**) [32]. These precursors of cholesterol biosynthesis are essential for post-translational modification and prenylation of certain small GTPase proteins, include the Ras and Rho superfamilies [33]. Prenylation of these proteins facilitates their intracellular trafficking and covalent attachment to the lipid membrane, which is often essential for biological function. These small GTPases cycle between an inactive GDP bound form found in the cytosol in association with guanine dissociation inhibitor (Rho GDI), and an active GTP bound form usually associated with the cell membrane (**Figure 3**). When Rho proteins are released from GDIs, they can insert into the cell membrane where they are activated by guanine nucleotide exchange factors (GEFs), and this initiates interaction with membrane effector proteins such as Rho kinase (ROCK). The Rho family of proteins is implicated in many key intracellular events and signalling pathways, including regulation of the actin cytoskeleton, cell adhesion, cell to cell interaction, and cell-cycle progression. This topic is comprehensively reviewed by BurrIDGE and colleagues [34]. It is thought that inhibition of the Rho pathway by statins is one of the major mechanisms via which statins affect cell physiology.

4. Statins

4.1. Chemical Structure, Pharmacokinetic, and Pharmacodynamics Properties

While all statins share a common mechanism of action, they differ in the chemical structures, pharmacokinetic profiles, and lipid-modifying efficacy. Lovastatin, pravastatin, and simvastatin are fungal-derived, whereas atorvastatin, cerivastatin, fluvastatin, pravastatin, pitavastatin, and rosuvastatin are fully synthetic compounds [35]. All statins have a similar chemical structure composed of the following: an analogue of the target enzyme

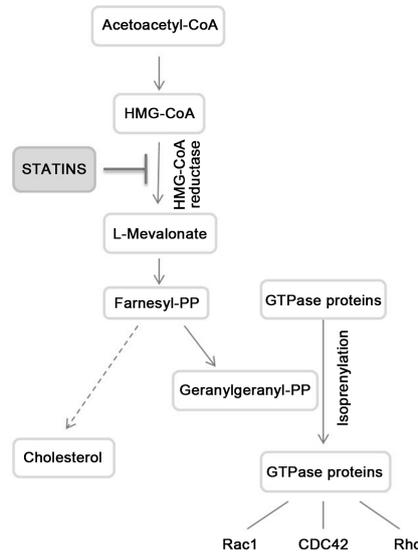


Figure 2. The HMG CoA pathway—inhibition by statins. Inhibition of HMG-CoA by statins inhibits the rate-limiting step in cholesterol biosynthesis, as well as reducing the production of isoprenoid intermediaries. The latter limits the isoprenylation of small GTPase proteins such as Rho, Rac1, and CDC42, and results in modulation of various cellular functions.

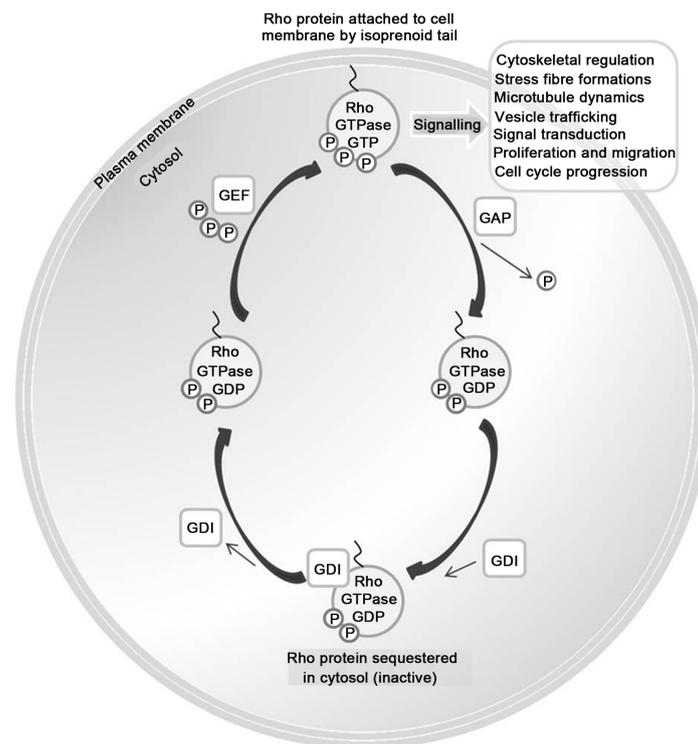


Figure 3. The Rho cycle. GEF: Guanine Exchange Factor. GAP: GTPase-Activating Protein. GDI: Guanine Dissociation Inhibitor. GEF proteins promote the exchange of GDP for GTP on Rho proteins. Binding of GTP facilitates a conformational change in Rho proteins leading to their interaction with effector molecules, and initiation of downstream signalling. GAPs facilitate GTP hydrolysis—so the Rho protein is left bound to GDP. In this state GDIs bind the inactive Rho protein. GDIs prevent membrane translocation of Rho proteins, so they are sequestered in the cytosol.

substrate HMG-CoA; a photophobic ring structure that is involved in binding of the statin to the enzyme; and side groups of the rings that define the solubility properties of the drug [35]. Through examination of this structure the mechanism of action of statins becomes evident—competitive inhibition of HMG-CoA through mimicry of the enzymes substrate.

Much of the pharmacokinetic properties of statins stem from the structure of the side groups—atorvastatin, fluvastatin, lovastatin, and simvastatin are relatively lipophilic compounds, whereas pravastatin and rosuvastatin are more hydrophilic [35]. The absorption of all statins is rapid, and peak plasma concentration is reached within 4 hours after administration [36]–[38]. All statins show great hepatoselectivity with respect to inhibition of HMG-CoA reductase, largely because of efficient first-pass uptake. The bioavailability of statins varies, from 5% for lovastatin, to 60% for pitavastatin [38] [39]. Statins are predominantly metabolised by the cytochrome P₄₅₀ family of enzymes (CYP450) [40]. The main route for elimination for the majority of statins is via the bile after metabolism by the liver [41]. Statins are highly efficacious at lowering serum low-density lipoprotein (LDL), and can also increase serum high-density lipoprotein (HDL) by varying degrees [42] [43]. The widespread use of statins is now testament to their safety and efficacy. Generally statins are well tolerated with serious adverse effects being very rare [44]. The most serious adverse effect is myopathy, which may progress to rhabdomyolysis. The incidence of myopathy is dose-related, with an approximate incidence of 1 in 1000 patients treated [45]. The risk is increased when statins are used alongside other agents that share common metabolic pathways.

4.2. Application of Statins

The clinical application of statins is rapidly expanding. While prevention of cardiovascular disease is still the primary indication for statins, recent research in the fields of pulmonary and renal disease strongly suggests that statins would also be of benefit in these diseases. Lowering of serum cholesterol is considered to be the main mechanism responsible for the widely recognised beneficial effect of statins in cardiovascular disease [46]–[48]. As well as being of benefit in atherosclerosis and hypercholesterolaemia, statins also improve survival in non-ischaemic heart failure—which is generally unrelated to atherosclerosis [49]. This variant of heart failure has a prominent inflammatory component, and is associated with neurohormonal imbalance—for example activation of the renin-angiotensin-aldosterone and sympathetic system by the low-output state. In vascular cells, angiotensin-II is a key stimulant of reactive oxygen species (ROS) production [50]. Statins are effective in modifying these maladaptive responses, reducing angiotensin-II-induced release of ROS, and improving cardiac function in non-ischaemic heart failure [50]–[52]. Recent clinical trials have also demonstrated the benefit of statins as stroke prophylactic agents—reducing the sequelae of stroke by 25% to 35% [53] [54].

The beneficial effect of statins in pulmonary diseases such as chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) has recently come to light. Observational studies suggest that patients with COPD who take statins have reduced hospitalisation and mortality rates from COPD exacerbations [55] [56]. The prominent inflammatory and fibrotic components in these lung pathologies bear similarity to the molecular mechanisms observed in the glaucomatous trabecular meshwork and lamina cribrosa. These pathological mechanisms—including abnormal matrix remodelling secondary to enhanced matrix metalloprotease action, increased release of pro-inflammatory cytokines (including TGF- β 1, IL-6 and IL-8), dysregulated apoptosis, increased reactive oxygen species—are modulated by statins in *in vitro* models of pulmonary disease [57]–[60].

A recent meta-analysis showed that statins are effective in reducing progression in chronic kidney disease [61]. In renal diseases such as renal interstitial fibrosis and diabetic nephropathy, statins have been shown to reduce fibrosis. Statins are capable of inhibiting TGF- β expression and collagen production [62], but may also reduce fibrosis via a TGF- β independent mechanism [63]. Importantly, recent laboratory evidence shows that statins are effective in inhibiting the pressure-induced fibrotic response of rat renal tubular cells—rosuvastatin reduced the expression of CTGF, TGF- β , fibronectin, and SMAD3 in cells cultured in a high pressure environment [4]. The anti-inflammatory and anti-fibrotic effect of statins was also observed in a model of diabetic nephropathy—rosuvastatin reverses the angiotensin-II-induced upregulation of TGF- β 1, fibronectin, and collagen-IV [64]. These studies are very relevant to the field of glaucoma—where differential expression of genes involved in fibrosis and inflammation occurs in the setting of high intraocular pressure [19].

The anti-fibrotic effect of statins observed in pulmonary and renal disease has prompted innovative research in the area of anti-scarring therapeutics. Treatment of experimental wounds to rabbit ears with local statin injections resulted in reduced hypertrophic scar formation [65], where mRNA analysis of the tissue revealed signifi-

cantly lower CTGF expression. Prevention of intra-abdominal scarring and adhesion formation is another emerging therapeutic target. Intra-peritoneal administration of losartan and atorvastatin in mice resulted in reduction of intra-peritoneal bands to a minimum [66]. Statin-containing cellulose film (statofilm) has also been shown to be superior to the established Seprafilm™ in preventing post-operative intraperitoneal adhesion in a rat model, opening up new avenues for future clinical application [67].

5. Statins and Glaucoma

5.1. Epidemiological Evidence

There are conflicting findings in the literature regarding whether statins are beneficial in patients with POAG. Stein *et al* conducted a retrospective longitudinal cohort analysis which found that statin use was associated with a significant reduction in the risk of POAG [1]. A dose response effect was also observed—suggesting that longer duration of statin exposure was associated with great reduction in the risk of developing glaucoma or requiring medical treatment for glaucoma. McGwin and colleagues carried out a case control study in male patients which showed that persons who had been prescribed statins for at least 2 years had a 40% reduced odds of developing glaucoma [68]. An interesting study by Leung and co-workers prospectively examined rates of visual field progression in patients who were taking statins versus those who were not. They found that statins were associated with visual field stabilisation in patients with normal tension glaucoma (NTG) [2]. The optic nerve and retinal nerve fibre layer in glaucoma suspects was examined by De Castro and colleagues using the confocal scanning laser ophthalmoscope—and findings suggested that glaucomatous progression was slowed in patients who were taking a statin medication [69]. In contrast to this, other researchers have found no support for the beneficial effect of statins in glaucoma [70] [71].

By what molecular mechanisms might statins be potentially beneficial in glaucoma? This question opens up many intriguing avenues regarding the pleiotropic effects of statins, and few studies have directly addressed this dilemma to date. Evidence from both human and animal studies has shown that statins have prominent anti-fibrotic and immune-modulating actions in several organ systems including the eye (Table 1), which may have useful effects in glaucoma. Let us review the known pleiotropic effects of statins and see how these may apply to glaucoma.

5.2. Rho Inhibition

Probably the most important pleiotropic effect of statins is the inhibition of small GTPases, including the Rho family of proteins [77]. The Rho pathway is responsible for various integral intracellular processes and for the interaction between cells and their environment. Three subfamilies constitute the Rho superfamily—Rho, Rac, and Cdc42. Regulation of the actin cytoskeleton, microtubule dynamics, vesicle trafficking, cell polarity and cell-cycle progression are all under the control of the Rho proteins (Figure 4). This topic is comprehensively reviewed by Burridge *et al.* [34]. The human trabecular meshwork expresses many components of the Rho pathway, suggesting that this is a key player in regulating the contractile tone and cellular morphology of the aqueous outflow pathway [30] [78]. The Rho proteins contain a lipophilic isoprenoid group, which permits their attachment to the cell membrane and is generally essential for biological function. By inhibiting the conversion of HMG-CoA to L-mevalonic acid, statins inhibit the synthesis of isoprenoid intermediaries, including FFP and GGPP. Hence proper subcellular localisation and trafficking of these GTPase proteins is inhibited, with significant functional consequences. Importantly, post-translationally immature forms of G-proteins may maintain partial function [79] [80], and interfere with the activity of mature membrane-anchored proteins.

By inhibiting Rho and its downstream effector proteins including Rho kinase (ROCK), statins are likely to affect the contractile properties of the conventional outflow pathway. Cells of the conventional pathway possess a contractile tone which is regulated through Rho signaling [81]. ROCK phosphorylates and inhibits the myosin-binding subunit of myosin light chain (MLC) phosphatase. This action increases MLC phosphorylation and myosin contractility, hence driving the formation of stress fibres and focal adhesion [34]. Early work on specific inhibitors of the Rho pathway has shown that Rho inhibition results in relaxation of the contractile tone of cells of the aqueous outflow pathway *in vitro* and *ex vivo* [78] [82] [83]. This increases aqueous outflow and reduces intraocular pressure. Indeed Rho kinase inhibitors have been shown to be potent agents in lowering intraocular pressure, and are undergoing phase 2 and 3 clinical trials [31] [84]. This effect of Rho inhibition in lowering the

Table 1. Summary of statin-mediated pharmacological effects on pathological mechanisms in glaucoma.

Summary of statin mediated pharmacological effects on pathological mechanisms in glaucoma			
Glaucoma mechanism	Study type/tissue	Statin effect	Reference
Fibrosis	Rat renal tubular cells (<i>in vitro</i>)	Inhibit pressure-induced fibrotic response (decreased CTGF, TGF- β 1, fibronectin, Smad, phospho-Smad3)	[4]
	Rat renal tubular cells Human airway fibroblasts (<i>in vitro</i>)	Reduce TGF- β induced expression of fibronectin, CTGF, α -SMA	[4]-[6]
	Human airway fibroblasts (<i>in vitro</i>)	Reduced collagen gel contraction	[6]
	Murine model, rat model (<i>in vivo</i>)	Reduced post-operative adhesions (via downregulation of TGF- β 1, tPA, PAI-1)	[66] [67]
	Murine model (<i>in vivo</i>)	Protect against tubulointestinal fibrosis injury via upregulation of eNOS/HSP70, and downregulation of Cav-1	[72]
	Rabbit model, skin (<i>in vivo</i>)	Reduced hypertrophic scar formation (reduced CTGF expression)	[65]
	Rat model, vascular smooth muscle cells (<i>in vitro</i> , <i>in vivo</i>)	Inhibit Ang-II-induced SMAD activation and related fibrosis	[64]
Matrix remodelling	Murine model (<i>in vivo</i>)	Reduced post-infarct tissue remodelling	[74]
	Human mesangial cells (<i>in vitro</i>)	Inhibit Ang-II-induced downregulation of MMP-2. Inhibit Ang-II-induced upregulation of TIMP-2 and MMP-9. Reduce TGF- β , Fibronectin, Col-IV	[64]
	Primary human TM cells	Suppress SPARC expression	[74]
Cytokine production	Human vascular smooth muscle cells (<i>in vitro</i>)	Reduced basal and Ang-II-induced IL-8 production	[58]
	Primary bronchial epithelial cells (<i>in vitro</i>)	Reduced IL-17-induced upregulation of IL-8, IL-6, VEGF. Reduced TGF- β 1-induced upregulation of IL-6, MMP-2, MMP-9	[57]
Endothelial function	Rat primary astrocytes, microglia, macrophages (<i>in vitro</i>)	Inhibit iNOS, TNF- α , IL-1beta, IL-6	[75]
	Human model (<i>in vivo</i>)	Improved cardiac endothelial function Decreased serum s-ICAM1, CRP, vWF	[51]
Oxidant response	Human model (<i>in vivo</i>)	Promote systemic anti-oxidant effects, via inhibition of myeloperoxidase-derived and nitric oxide-derived oxidants	[60]
Apoptosis	Murine model (<i>in vivo</i>)	Decreased myocyte apoptosis post-infarct	[73]
Ischaemia	Murine model (<i>in vivo</i>)	Improved retinal ganglion cell survival post ischaemic injury	[76]

TGF: Transforming growth factor; CTGF: Connective tissue growth factor; IL: Interleukin; eNOS: Endothelial nitric oxide synthase; iNOS: Inducible nitric oxide synthase; HSP70: Heat shock protein-70; Cav-1: Caveolin-1; tPA: Tissue plasminogen activator; PAI-1: Plasminogen activator inhibitor-1; Ang-II: Angiotensin-II; S-ICAM1: Soluble intercellular adhesion molecule-1; vWF: Von Willibrand factor; CRP: C-reactive protein; TNF: Tumour necrosis factor; TM: Trabecular meshwork.

contractile tone of smooth muscle structures has already proven beneficial in other pathologies—including sub-arachnoid haemorrhage—where Rho inhibitors have been shown to reduce the incidence of cerebral vasospasm [85]. More recent studies in this field have demonstrated the potential beneficial effect of combination therapy with a statin plus a Rho kinase inhibitor [86].

Rho inhibitors also affect the actin cytoskeleton, and cellular morphology of the aqueous outflow pathway. *In vitro* work has shown decreases in actin stress fibres and focal adhesions in cultured porcine and human TM cells [83]. Treatment of monkey Schlemm's canal (SC) cells with a Rho inhibitor increases the number of giant vacuoles within cell, and decreases the expression of certain cytoskeletal proteins (ZO-1 and claudin-5) [87]. This leads to morphological changes—cell rounding and detachment of cells from each other, as well as wider paracellular spaces [78] [88]. In cultured cells of the SC, Rho inhibition results in increased permeation *in vitro*, which facilitates aqueous drainage [88]. *Ex vivo* perfusion experiments, where porcine, monkey, cow and cadaver eyes are perfused with an aqueous humour substitute, have shown that perfusion with a Rho inhibitor increases the conventional outflow facility [78] [83] [88] [89]. To our knowledge, no such experiments have been

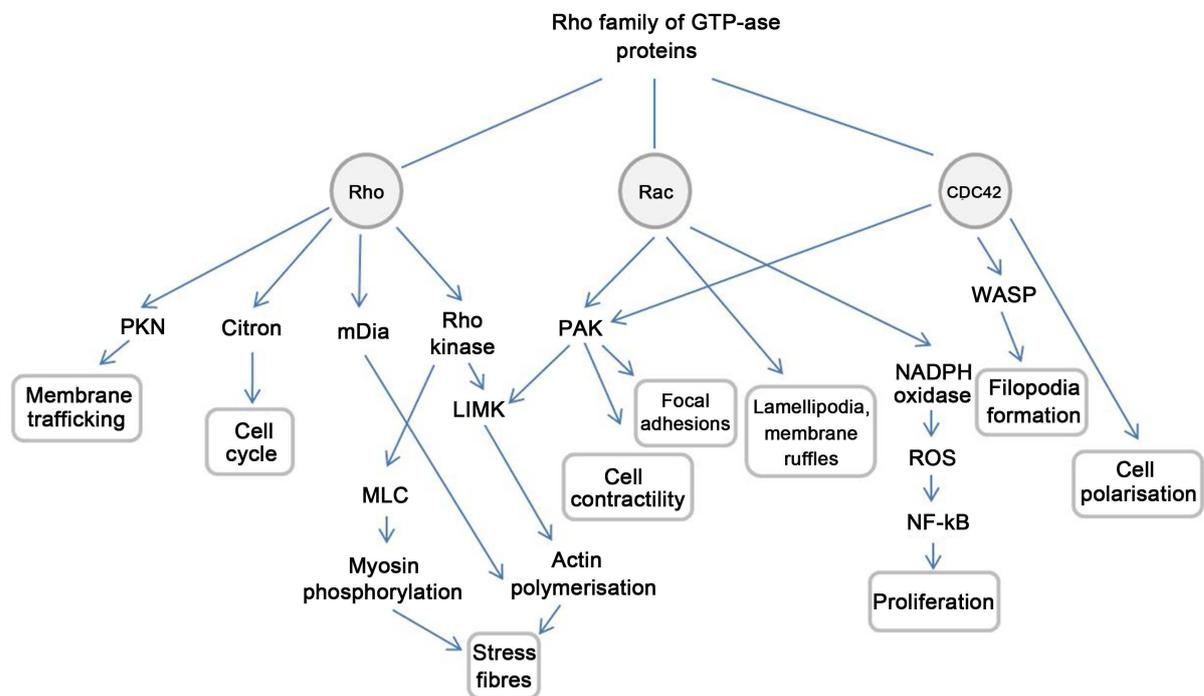


Figure 4. The Rho signalling pathway. PKN: Protein kinase N; mDia: Mammalian diaphanous protein; MLC: Myosin light chain; LIMK: LIM kinase; PAK: p21-activated kinase; NADPH: Nicotinamide adenine dinucleotide phosphate; ROS: Reactive oxygen species; NF- κ B: Nuclear factor kappa-light-chain-enhancer of activated B cells; WASP: Wiskott-Aldrich syndrome protein. This diagram illustrates the major effector pathways for the Rho family of proteins (Rho, Rac and CDC-42) which are relevant to glaucoma pathogenesis.

carried out to date with statins, however considering the Rho inhibiting action of statins, it is likely that they have similar results. There is certainly potential for further studies to ascertain the effect of statins themselves on the cell morphology and contractile tone of the aqueous outflow pathway.

5.3. Rac and Reactive Oxygen Species (ROS)

One of the 3 key members of the Rho family is Rac1—this important GTPase protein is responsible for cytoskeletal remodelling—specifically the formation of lamellipodia and membrane ruffles [90]. Lamellipodia are actin-rich cellular protrusions, essential for cell migration, and play an important role in the invasion and metastases of cancer cells [91]. Furthermore, Rac1 binds to p67^{phox} which leads to activation of the NADPH oxidase system and generation of ROS [92]. The presence of high concentrations of ROS can overwhelm the cell's natural defence mechanisms and lead to programmed cell death. However the role of ROS in cell physiology is more complex than that and more recent studies have shown that in some scenarios, ROS (in small doses) promote cell survival—contrary to the traditional view that they are solely destructive molecules [93] [94]. ROS have also been shown to act as signalling molecules in their own right [95]. In smooth muscle and heart cells, it has been shown that by inhibiting the prenylation and subsequent activation of Rac1, statins inactivate NADPH oxidase and hence reduce angiotensin-II-induced ROS production [50] [96]. Our own research group has previously demonstrated evidence of oxidative stress and mitochondrial dysfunction in lamina cribrosa cells from the optic nerve heads of glaucoma donors, compared to normal donors [97]. Furthermore, our group has shown that up to 50% of POAG patients have a pathogenic mitochondrial DNA mutation, which may lead to mitochondrial respiratory dysfunction and subsequent predisposition to oxidative stress in TM, LC and RGC [98]. Increased levels of ROS have been found in the aqueous humour of glaucoma patients [99] [100]. Glutathione is a tripeptide found in the eye and other tissues, and is a key element of the protective mechanism of the eye against oxidative stress [101]. Altered glutathione levels have been reported in the aqueous humour of glaucoma patients [102], and abnormally low levels of glutathione have even been demonstrated in the serum of glaucoma patients [101]. ROS affect the cellularity of the trabecular meshwork, and may cause endothelial dysfunction—

which would contribute to impaired aqueous outflow and higher IOP [103]. Mitochondrial dysfunction in LC and RG cells allows the build-up of ROS, and may lead to increased susceptibility to cell death and impaired repair mechanisms [97] [104]. By reducing the production of ROS in ocular tissues, statins may help to reduce ROS-induced damage and glaucoma progression.

5.4. Endothelium-Derived Nitric Oxide

In the eye, endothelium-derived nitric oxide (NO) is an important regulator of blood flow to the choroid, optic nerve and retina [105]. Since NO is derived from the endothelium, endothelial dysfunction impairs ocular haemodynamics by reducing the production of NO and increasing the production of ROS. Endothelial dysfunction and abnormalities of the L-arginine/nitric oxide system in the vascular tree have been observed in a variety of ocular pathologies, including glaucoma [106]-[108]. Furthermore, studies in humans using laser Doppler flowmetry have demonstrated that inhibition of NO synthase leads to reduced choroidal and optic nerve head blood flow [105].

Statins influence NO levels by a number of mechanisms. Firstly—the cholesterol lowering effect. Raised serum LDL-cholesterol causes endothelial dysfunction, downregulated endothelial nitric oxide synthase (eNOS) expression, decreased receptor-mediated NO release [109], and increased ROS production which decreases NO bioavailability [110]. By reducing LDL-cholesterol, statins therefore improve endothelial function and facilitate NO production. Statins also affect NO production through cholesterol independent mechanisms. Statins increase eNOS gene expression by prolonging eNOS mRNA half-life, via a mechanism owing to inhibition of RhoA [111]. Furthermore, statins reduce the bio-availability of the membrane protein Caveolin-1. eNOS binds to Caveolin-1 in caveolae, which results in reduced NO production [112]. Genome wide association studies have suggested Caveolin-1 and -2 mutations in glaucoma [113]. Finally, statins activate the phosphatidylinositol 3-kinase (PI3K)/protein kinase Akt pathway. Akt is an important kinase involved in a number of key cellular functions including cell survival and growth, but it also serves to phosphorylate and activate eNOS—hence facilitating NO production. Statins therefore improve endothelial and vascular function via numerous cholesterol-dependent and -independent mechanisms. Such evidence suggests that statins would be of benefit in improving ocular blood flow in pathologies with compromised vasculature—including glaucoma. By improving endothelial function, statins may also mediate improved function of the endothelium-lined Schlemm's canal, which has altered biomechanical behaviour in glaucoma [114].

5.5. The Immune System

Immunomodulation is a well-known pleiotropic effect of statins. This has been shown to be beneficial in several pathologies where the immune system is upregulated, including multiple sclerosis and systemic lupus erythematosus [115]. Antigen presentation requires endocytosis of the antigen, internal processing, and presentation of major histocompatibility complex (MHC) II molecules at the cell surface. MHC II has been shown to be upregulated in a mouse model of glaucoma [116]. Furthermore, in a rat model of glaucoma microglial activation in the optic nerve correlated with the degree of axonal damage, and both MHC I and II were persistently upregulated in the microglia of optic nerves in the rat glaucoma model [117]. Gene expression of cytokines, including IL-6, have been shown to be upregulated in rat optic nerve heads subjected to raised intraocular pressure [118]. Many of the immunomodulatory effects of statins occur via the inhibition of small GTPase proteins [50] [77]. Antigen presentation and MHC II expression is related to changes in the actin cytoskeleton—which is controlled by the Rho pathway. Statins also decrease macrophage expression of tumour necrosis factor and interleukin-1 β [75].

6. Effect of Statins on Ocular Tissues

Novel work by Villarreal *et al.* at the Massachusetts Eye and Ear Infirmary is one of the few to examine the molecular mechanisms involved in ocular tissues treated with statins [74]. In this project, human trabecular meshwork cells were treated with lovastatin *in vitro*, and SPARC mRNA and protein expression were determined. SPARC is a matricellular protein—and a critical mediator of aqueous outflow and IOP [119]-[121]. It is associated with increased tissue fibrosis and aberrant tissue remodelling, and it has been shown to be upregulated in TM cells following TGF- β 2 treatment [122]. Statin treatment suppressed SPARC expression in TM cells—supporting the potential therapeutic role for statins in glaucoma.

The Rho signalling pathway has also been examined in TM cells treated with statins. Stubbs *et al.* showed that lovastatin increases the accumulation of unprenylated forms of RhoA and RhoB in human TM cells [123]. This may disrupt the Rho-dependent regulation of TM cell cytoskeletal organisation. Treatment of human TM cells with lovastatin resulted in disruption of filamentous actin organisation, an effect which was reversed by the supplementation of the cell cultures with GGPP.

Krempler *et al.* conducted novel research into the effect of statins on retinal ganglion cell survival and visual function following acute retinal ischaemia/reperfusion in mice [76]. Treatment of the animals with statins after the ischaemic insult resulted not only in histological evidence of improved retinal ganglion cell survival, but also improved visual function as measured behaviourally (by visual acuity and contrast sensitivity). This technique of producing acute global ischaemia represents a model of the changes observed after central retinal artery occlusion, as well as acute angle closure glaucoma [124]. This work hence supports the role for statins as a useful pharmacological tool in various ocular pathologies associated with ischaemic damage.

7. Concluding Remarks

The beneficial effect of statins extends far beyond cholesterol-lowering. Their pleiotropic effects include anti-fibrotic, anti-inflammatory, and immunomodulatory action, many of which are mediated by inhibition of the Rho pathway. These effects are evident in a multitude of human tissues, and statins as pleiotropic agents are already being studied in the fields of cardiovascular, pulmonary, and renal disease. Now, statins have caught the eye of glaucoma scholars as an intriguing new therapeutic agent, opening up many new avenues for molecular and clinical research.

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