·Basic Research·

# Neuroprotective effect of systemic and/or intravitreal rosuvastatin administration in rat glaucoma model

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## Abstract

**• AIM: To evaluate the neuroprotective effect of rosuvastatin, in a rat experimental glaucoma model.**

**• METHODS: Ocular hypertension was induced in right eyes of Long-Evans rats ($n$=30) by cauterization of three episcleral veins. Left eyes were defined as controls. Rats were divided into five groups: oral rosuvastatin, intravitreal rosuvastatin, oral +intravitreal rosuvastatin, intravitreal sham and glaucoma without intervention. Rats were sacrificed at day 14. Retinal ganglion cell (RGC) number was assessed by histopathological analysis. Terminal deoxynucleotidyl transferase-mediated dUTP -nick end -labeling (TUNEL) staining and the expression of glial fibrillary acidic protein (GFAP) in RGC layer was also examined.**

**• RESULTS: A significant intraocular pressure (IOP) elevation was seen ($P$=0.002). Elevated IOP resulted in a significant decrease in number of RGCs in group 5 (70.33 ±8.2 cells/mm$^2$) when compared with controls (92.50 ±13.72 cells/mm$^2$; $P$=0.03). The RGC number in group 1 (92.4±7.3 cells/mm$^2$) was significantly higher than group 5 ($P$=0.03). The numbers of RGC in groups 2, 3 (57.3±8.2 cells/mm$^2$, 60.5±12.9 cells/mm$^2$) were comparable with that of group 5 ($P$=0.18 and $P$=0.31). The apoptosis rates with TUNEL staining were also parallel to RGC number. Animals with experimentally induced glaucoma showed an increase in retinal GFAP immunoreactivity.**

**• CONCLUSION: Decrease in RGC loss and apoptosis suggest the neuroprotective potential of oral rosuvastatin treatment in a rat model of ocular hypertension. However intravitreal rosuvastatin showed a contrary effect and further studies are required.**

• **KEYWORDS:** rat glaucoma model; retinal ganglion cell number; rosuvastatin; neuroprotection
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## INTRODUCTION

Statins, also known as 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors, are commonly used as cholesterol-lowering drugs in patients with hyperlipidemia. Current literature shows the efficacy of statins in reduction of the incidence of cerebrovascular and cardiovascular events, uncommitted of their effect on cholesterol levels [1-4]. During the past decade, evidence has emerged that statins also have neuroprotective effects in patients with several diseases of the central nervous system, including Alzheimer's disease (AD), Parkinson disease (PD), multiple sclerosis (MS), and ischemic stroke [5]. Statins upregulate endothelial nitric oxide synthase and inhibit inducible nitric oxide synthase, which may have neuroprotective effects [6-7]. Statins may also attenuate the inflammatory cytokine responses that accompany cerebral ischemia and possess antioxidant properties that may ameliorate ischemic oxidative stresses in the brain [7-9]. The preservation of endothelial nitric oxide synthase activity in cerebral vasculature may be important in maintaining blood flow and limiting neuronal loss.

In glaucoma, retinal ganglion cell (RGC) death and atrophy of optic nerve lead to progressive vision loss and visual dysfunction. Elevated intraocular pressure (IOP) is a major risk factor for glaucoma. Many patients, however, continue to lose vision despite adequate IOP control. This evidence suggests that other mechanisms than IOP also contribute to disease progression.

The results of the studies investigating the beneficial effect of statins in glaucoma patients are inconsistent [10-11]. While some studies suggest that statins may have protective effect [12-13], some do not support this effect [10-11]. Stein *et al* [14] evaluated

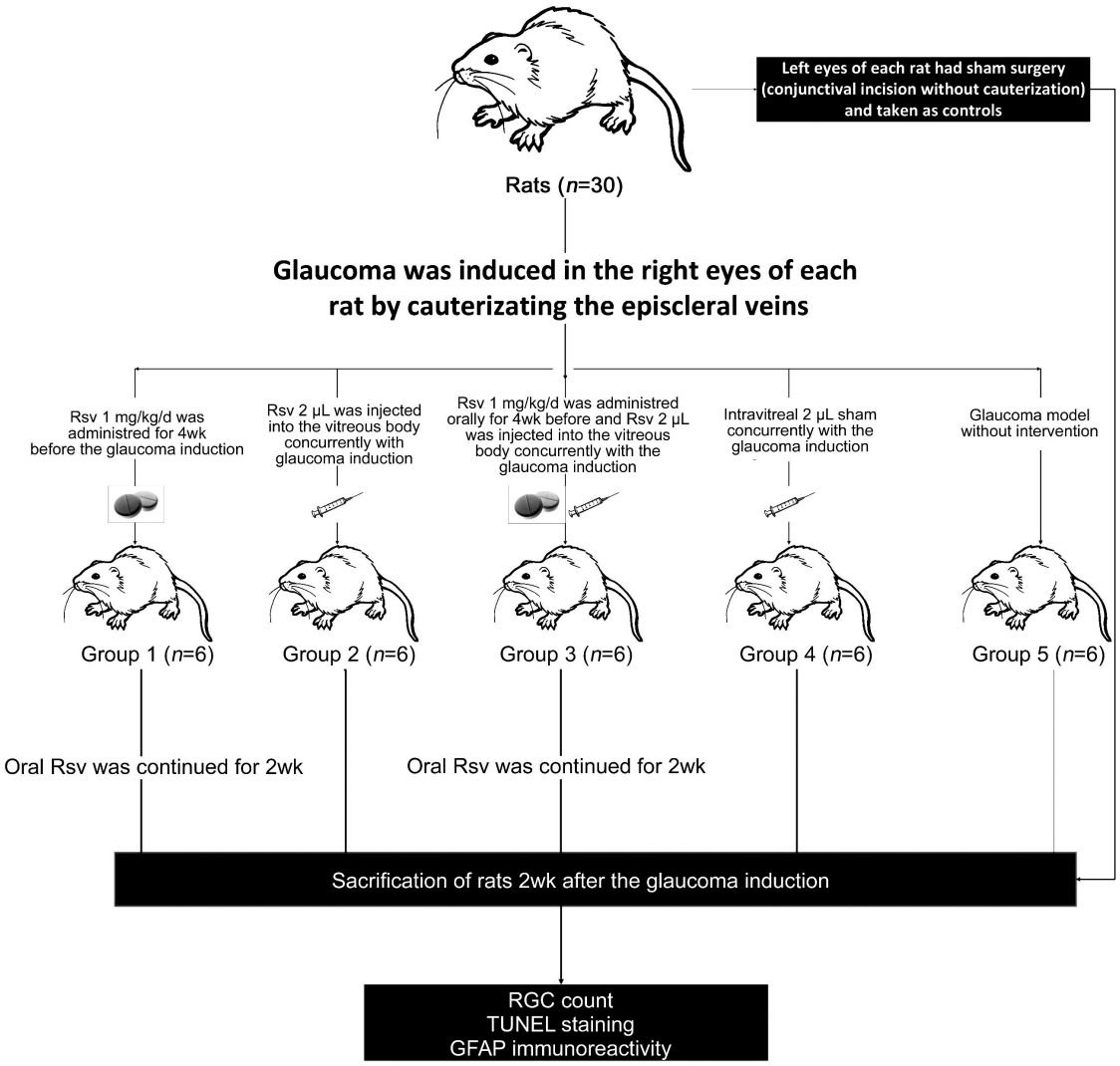


**Figure 1 Flowchart diagram of the study** Rsv: Rosuvastatin.

the relationship between open angle glaucoma (OAG) and statin use and found a significant reduction in the risk of OAG after statin usage among persons with hyperlipidemia. However a prospective study which is interventional might provide additional extents into the role of statins in the prevention of early OAG.

In our study, we evaluated the neuroprotective effect of systemic and/or intravitreal rosuvastatin administration in a rat experimental glaucoma model.

## MATERIALS AND METHODS

**Animals** Sixty eyes of 30 adult male Long-Evans rats (290-330 g) were included in this study. There were 5 groups consisted of 6 animals per group; group 1 (oral 1 mg/kg/d rosuvastatin), group 2 (intravitreal 2 μL/30 μmol/L rosuvastatin), group 3 (combined oral 1 mg/kg/d + intravitreal 2 μL/30 μmol/L rosuvastatin), group 4 (intravitreal 2 μL sham with glaucoma model) and group 5 (glaucoma without intervention) (Figure 1). Glaucoma was induced in the right eye of each animal by cauterizating three episcleral veins, as described by Shareef *et al* [15]. The left eye of each animal had sham surgery (conjunctival incision without cauterization) and served as the control eyes. Animals were kept with a 12-hour light/dark cycle with standard food and water provided. The research followed the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research; and the research was approved by the institutional review board. All efforts were made to minimize the number of animals used and their suffering.

**Methods**

**Drug administration and intravitreal injection** The 1 mg/kg/d rosuvastatin was administered in the form of crushed tablets (Crestor; AstraZeneca) suspended in sterile water *via* oral gavage in groups 1 and 3. After 4wk of oral treatment experimental glaucoma was induced. Administration of oral rosuvastatin was also continued after the induction of glaucoma for 2wk.

For intraocular injections, animals were anesthetized by intramuscular (IM) injection of ketamine hydrochloride, 50 mg/kg and xylazine hydrochloride, 0.5 mg/kg. Liquid volume in the vitreous was considered to be approximately 80 μL in average. Two microliters of rosuvastatin (final concentration in the eye: 30 μmol/L; dissolved in 15% dimethylsulfoxide; Sigma, Taufkirchen, Germany) were injected into the vitreous body posterior to the ora serrata by

using 30-gauge syringe (Becton, Dickinson & Co. Ltd., Dogheda, Ireland) and experimental glaucoma induced at the same session. Two microliters of 15% dimethylsulfoxide were injected into the vitreous body posterior to the ora serrata and experimental glaucoma was induced in the sham with glaucoma group.

**Experimental glaucoma induced by occlusion of episcleral veins** Glaucoma was induced by cauterization of the three episcleral veins in the right eye of each animal. The rats were anesthetized by IM injection of ketamine hydrochloride, 50 mg/kg and xylazine hydrochloride, 0.5 mg/kg. Before the procedure, IOP was checked with a tonometer (Tono-Pen; Medtronic Solan, FL, USA). A small conjunctival incision was made in each quadrant at the limbus, and the extraocular muscles were isolated. Four major limbal draining veins were identified based on deep location under the rectus muscles, relative immobility, larger caliber, and branching pattern. Two dorsal episcleral veins under the superior rectus muscle and one temporal episcleral vein under the lateral rectus muscle were cauterized using a surgical microscope (Olympus, Tokyo, Japan) and a cautery (Bovie Co., FL, USA)[16]. After surgery, chloramphenicol eye drops and oxytetracycline ointment were applied to the eyes. Only the eyes that did not suffer scleral burns with subsequent necrosis or any complications from the surgery were used. The left eye of each animal had sham surgery (conjunctival incision without cauterization) and served as control.

**Intraocular pressure measurement** IOP was measured using a tonometer (Tono-Pen; Medtronic Solan, FL, USA) after IM injection of ketamine hydrochloride, 50 mg/kg and xylazine hydrochloride, 0.5 mg/kg in both eyes. IOP measured three times holding the probe perpendicular to the central cornea and the measurements were averaged. IOPs were checked at 1st day, 1st week and 2nd week.

**Evaluation of retinal ganglion cell densities** Enucleated globes were fixed in 10% buffered formalin. The globes were cut into two halves with horizontal sections from optic nerve to cornea. Each half was processed and embedded in paraffin blocks. Tissue sections of 3 microns were cut from representative formalin-fixed and paraffin-embedded tissue blocks. Sections were de-parafinized in xylene and rehydrated. Each sample was stained with haematoxylin-eosin. The pathologist (Gocun PU) counting the RGCs was blind to the experimental procedures. Four visual fields were sampled from the posterior portion of each retina using a 40× objective (Olympus, BX51, Japan). Cell counts in the RGC layer were performed at this magnification by using a graduated graticule measuring 0.25 $mm^2$. The RGC numbers were also quantified in each visual field, and the total count for the four sampled fields was expressed in $mm^2$. If the cells appeared in the RGC layer and had large, round cell bodies, they were categorized as RGCs. Counts were made horizontally along the full length of the visual streak from the center of the optic nerve head, extending out towards the far retinal periphery. Care was taken to remain in the central area of the visual streak. As counts got on along the axis, retinal areas above and below the central regions were inspected to procure that the fields with highest cell densities were always picked out for counting[17].

**TUNEL staining** Apoptotic cells were detected by TdT-dUTP terminal nick end-labelling (TUNEL) in each half of retinas. TUNEL was performed as previously defined using the ApopTag Peroxidase In Situ Apoptosis detection kit (S7110, Millipore, Inc., Temecula, CA, USA), following the manufacturer's instructions. Peroxidase substrate 3, 3-diaminobenzidine was used to stain for apoptotic cells. Methyl green (0.5%) was used as a nuclear stain. TUNEL positive cells were observed under a light microscope (Olympus, BX51, Japan). The numbers of TUNEL-positive cells in the RGC layer per retina were counted in three or more sections. The mean cell counts of these sections were used to set the proportion of cells undergoing apoptosis for each layer of each particular eye[17].

**Glial fibrillary acidic protein immunoreactivity** Glial fibrillary acidic protein (GFAP) immunoreactivity in the retina was evaluated after glaucoma induction in right eyes and control left eyes. GFAP immunostaining was performed as previously described using the following the GFAP antibody-Astrocyte Marker 100 μL (ab4648, ABCAM, Cambridge, UK) manufacturer's instructions. GFAP expression was viewed under the light microscope (Olympus, BX51, Japan). GFAP expression was assessed by using extent and severity scale (severity; absence of stain, mild, moderate, severe and extent; focal, diffuse). Pathologist evaluating RGC densities, TUNEL staining and GFAP immunoreactivity was blind to all study groups.

**Statistical Analysis** Statistical significance of the differences in IOP between before and after the episcleral vein cauterization procedure was determined by the Wilcoxon test. The differences' statistical significance in RGC number and proportion of cells undergoing apoptosis in RGC layer between treatment groups and fellow control eyes were determined by Kruskal Wallis test. Chi-square test was used to compare GFAP expression between treatment groups and fellow control eyes. The results of the left eyes were compared with the rights eyes of the same groups, but not between the right eyes in other groups. Statistical analyses were performed with SPSS 18.0 for windows (SPSS Inc, Chicago, Illinois, USA). The level of statistical significance was set at $P<0.05$.

## RESULTS

**Intraocular Pressure** Animals used in the present study had elevated IOP throughout the experiment (Figure 2). IOP values

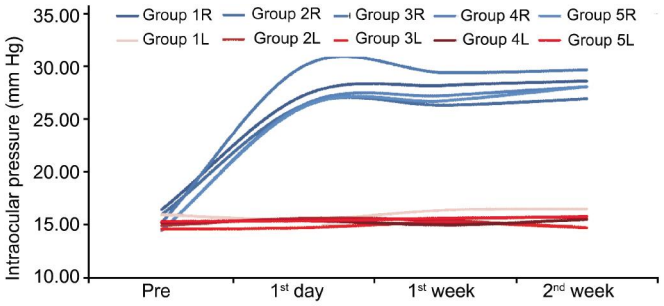


**Figure 2 Elevated IOP in rat eyes that underwent unilateral cauterization of three episcleral veins (operated) compared with the opposite left eyes (control)** IOP was measured on the indicated times in eyes treated with oral rosuvastatin (group 1), intravitreal rosuvastatin (group 2), oral and intravitreal rosuvastatin (group 3), intravitreal sham (group 4) and glaucoma without intervention (group 5). The difference in mean IOP between cauterized eyes and control eyes was significant at all time points after surgery ($P$<0.05). All values are mean±SD ($n$=6). R: Right eye; L: Left eye.

in eyes before the induction of glaucoma [15.31±1.30 mm Hg (12.50-17.75)] were similar to those reported in the literature. One day after induction of glaucoma, a significant increase in IOP was observed in the ipsilateral eye [28.73±2.45 mm Hg (25.50-34.00), $P$=0.002]. Two weeks after the induction of glaucoma, IOP was elevated 1.9 fold in the ipsilateral eye [28.08±0.92 mm Hg (27.50-30.00), $P$=0.002], compared to contralateral eye [14.70±1.45 mm Hg (14.20-15.75)].

Oral rosuvastatin treatment had no effect on IOP. In glaucomatous animals, oral rosuvastatin had no effect on IOP in either ipsilateral (28.20±1.40 mm Hg) or contralateral (14.70±1.60 mm Hg) eyes. Intravitreal sham injection had also no effect on IOP. Intravitreal rosuvastatin injection had also no effect on IOP. IOP values of group 2 were comparable with that of group 5 (26.95±0.81 mm Hg, 28.08±2.82 mm Hg respectively, $P$=0.40).

**Evaluation of Retinal Ganglion Cell Densities and TUNEL Staining** Light microscopic examination of Group 5 revealed decreased number of RGC, together with significant morphologic alterations and apoptosis (Figures 3-6). In group 5, the number of RGC appeared to be significantly reduced, and the rate of cells undergoing apoptosis was found to be significantly greater when compared with the control eyes [70.33±8.2 cells/mm$^2$ (44-93), 92.50±13.72 cells/mm$^2$ (72-106); $P$=0.03 and 5.6% (4.5-7.9), 0; $P$=0.001, respectively]. In group 1, the number of RGC and the rate of cells undergoing apoptosis in the RGC layer was detected to be similar compared with the control eyes [92.4±7.3 cells/mm$^2$ (75-104), 94.83±9.7 (84-109); $P$=0.90 and 0.9% (0-1.3), 0; $P$=0.3, respectively].

When a comparison regarding the number of RGC and the proportion of cells undergoing apoptosis in the RGC layer was made between groups 1 and 5, increased rate of cells

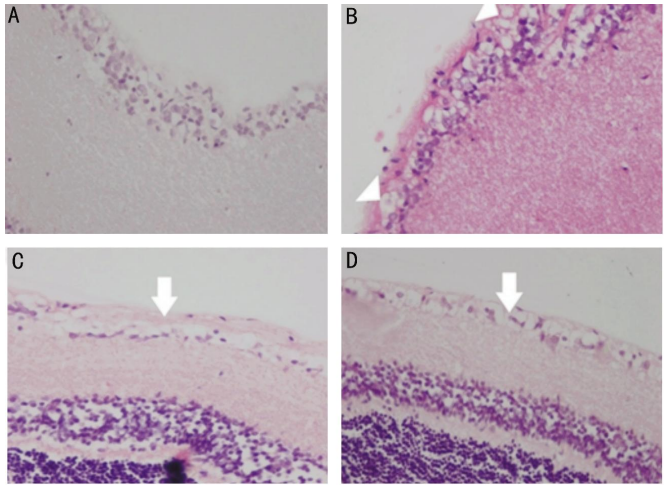


**Figure 3 Effect of rosuvastatin treatment on RGC number** Retinal photomicrographs were obtained at 2wk in control left eye (A), after oral rosuvastatin treatment (B), intravitreal rosuvastatin treatment (C) and glaucoma without intervention (D). Disruption of retinal architecture, cell loss in retinal ganglion cell (RGC) layer (arrow) were remarkable (HE ×200) in glaucoma without intervention and intravitreal rosuvastatin groups. Vacuolization of retinal ganglion cell (RGC) was seen (arrow head). Retinal architecture in RGC layer was relatively to be well-preserved (HE×200) in oral rosuvastatin group.

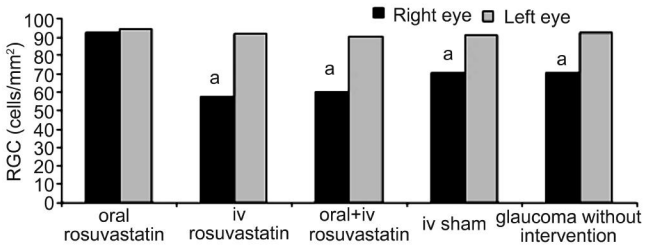


**Figure 4 The mean number of RGC in retina** The mean numbers of RGC at 2wk after IOP elevation were analyzed. Statistical significance was evaluated by Kruskal Wallis test ($n$=6). $^aP$ <0.05 compared to fellow control eyes. RGC: Retinal ganglion cell; iv: Intravitreal.

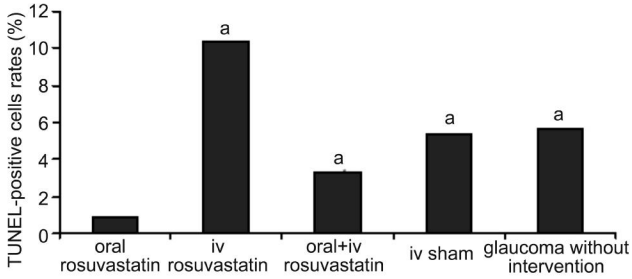


**Figure 5 The rate of TUNEL-positive cells in the retina** Cross sections from the retina at 2wk after IOP elevation were analyzed. The rate of TUNEL-positive cell in the ganglion cell layer are shown. Statistical significance was evaluated by Kruskal Wallis test. ($n$ =6). $^aP$<0.05 compared to fellow control eyes. RGC: Retinal ganglion cell; iv: Intravitreal.

undergoing apoptosis and statistically significant cell loss were detected in group 5 ($P$ =0.03, $P$ =0.001, respectively; Table 1). Oral rosuvastatin treatment significantly reduced

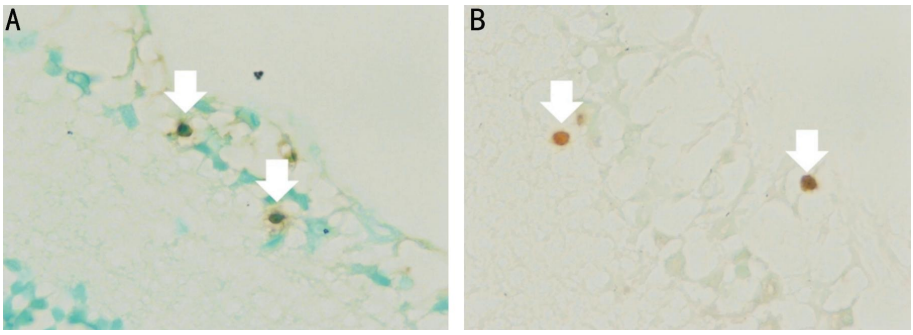


**Figure 6 Effect of rosuvastatin treatment on TUNEL−positive cells in the retina** Retinal photomicrographs were obtained at 2wk in control glaucoma without intervention (A), after intravitreal rosuvastatin (B). No TUNEL-positive cells are detected in retinal tissue sections obtained from control left eyes. After episcleral vein cauterization and elevation of IOP, TUNEL-positive cells were present only in the GCL (arrows). Oral rosuvastatin treatment reduced retinal cell apoptosis induced by ocular hypertension in rats.

**Table 1 Comparison of total number of cells and the apoptosis rate of RGC layer in groups 1 and 5**

| Parameters | Group 1 (oral rosuvastatin) | Group 5 (glaucoma without intervention) | *P* |
|---|---|---|---|
| RGC (cell/mm²) | 92.4±7.3 (75-104) | 70.33±8.2 (44-93) | 0.03[a] |
| Apoptosis (%) | 0.9±0.2 (0-1.3) | 5.6±1.4 (4.5-7.9) | 0.001[a] |

[a]*P* <0.05, Kruskal Wallis test. RGC: Retinal ganglion cell.

**Table 2 Comparison of total number of cells and the apoptosis rate of RGC layer in groups 2 and 5**

| Parameters | Group 2 (iv rosuvastatin) | Group 5 (glaucoma without intervention) | *P* |
|---|---|---|---|
| RGC (cell/mm²) | 57.33±8.23 (47-66) | 70.33±18.30 (44-93) | 0.18 |
| Apoptosis (%) | 10.4±2.2 (8.5-12.4) | 5.6±1.4 (4.5-7.9) | 0.001[a] |

[a]*P*<0.05, Kruskal Wallis test. RGC: Retinal ganglion cell; iv: Intravitreal.

**Table 3 Comparison of total number of cells and the apoptosis rate of RGC layer in groups 3 and 5**

| Parameters | Group 3 (oral+iv rosuvastatin) | Group 5 (glaucoma without intervention) | *P* |
|---|---|---|---|
| RGC (cell/mm²) | 60.50±12.90 (50-78) | 70.33±18.30 (44-93) | 0.31 |
| Apoptosis (%) | 3.3±0.9 (2.0-3.8) | 5.6±1.4 (4.5-7.9) | 0.15 |

Kruskal Wallis test. RGC: Retinal ganglion cell; iv: Intravitreal.

the RGC loss and TUNEL-positive cells in the ganglion cell layer.

However, in groups 2 and 3 , the number of RGC was found to be lower and the proportion of cells undergoing apoptosis was found to be higher in the RGC layer compared with that of their controls [57.3±8.2 cells/mm$^2$ (47-66), 60.5±12.9 cells/mm$^2$ (50-78) and 10.4% (8.5-12.4), 3.3% (2-3.8); respectively]. The distribution of the number of RGC and rate of cells undergoing apoptosis in the RGC layer of all groups can be seen in Figures 3-6.

Furthermore, when a comparison was made between groups 2 and 5, the proportion of cells undergoing apoptosis was higher in group 2, and the difference was statistically significant ($P$=0.001). But, there was no significant difference regarding the number of RGC between the two groups ($P$= 0.18; Table 2).

When a comparison was made between groups 3 and 5, the number of RGC and proportion of cells undergoing apoptosis in the RGC layer were also comparable ($P$ =0.31, $P$ =0.15; respectively, Table 3).

In cross section, no TUNEL-positive cells were found in the fellow control eyes. After induction of ocular hypertension, positive TUNEL reaction was markedly increased. The TUNEL-positive cells were specifically located in the RGC layer. Oral rosuvastatin treatment significantly reduced TUNEL-positive cells in the RGC layer.

**Expression of Glial Fibrillary Acidic Protein** After the induction of experimental glaucoma by occlusion of episcleral veins, GFAP immunostaining was performed to evaluate the glial cell activation in response to retinal stress or injury caused by IOP elevation and the effects of rosuvastatin administration. In the retina of control fellow eyes, GFAP immunoreactivity was limited to astrocytes and to the end feet of Müller cells at the inner limiting membrane. Animals with experimentally induced glaucoma showed an increase in retinal GFAP immunoreactivity that extended to the outer nuclear layer ($P$=0.03) (Figure 7). Oral rosuvastatin treatment caused decrease in GFAP expression compared with that of untreated cauterized eyes but it was not statistically significant ($P$=0.2).

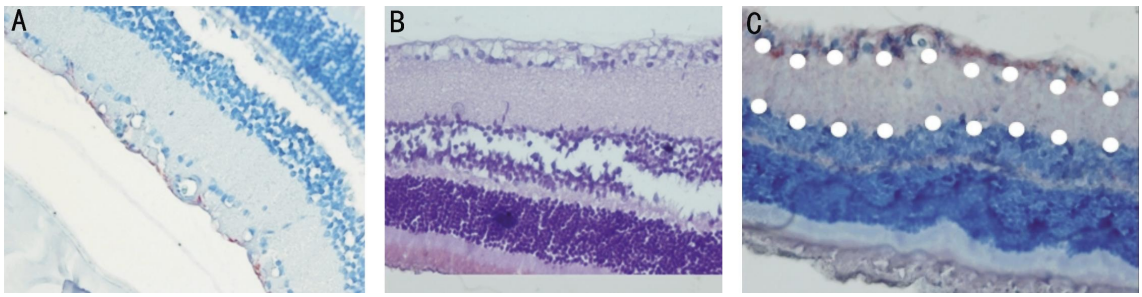


**Figure 7 Effect of oral rosuvastatin treatment on GFAP immunoreactivity in the retina** A: In the normal retina, GFAP immunoreactivity was limited to astrocytes and to the end feet of Müller cells at the inner limiting membrane; B: Oral rosuvastatin treatment reduced GFAP expression compared with untreated cauterized eyes ($P$=0.2); C: After episcleral vein cauterization and elevation of IOP (group 5), GFAP immunoreactivity was increased ($P$=0.03) (GFAP immunreactivity can be seen with intracytoplasmic staining as a ground glass appearance in diffuse extension pattern between the white dots. Statistical significance was evaluated by Chi-square test ($n$=6).

## DISCUSSION

In the present study, we examined the effects of the rosuvastatin on RGC loss, apoptosis and glial activation in experimental glaucoma model. This is the first study examining the role of rosuvastatin in an animal model of glaucoma. Two weeks after the induction of glaucoma, we found that oral rosuvastatin exerted a significant neuroprotective effect on RGC density, resulting in reduced loss of RGC and proportion of cells undergoing apoptosis but intravitreal administrated rosuvastatin showed a contrary effect.

Among the several members of the statin family, rosuvastatin is one of the most potent statins and is able to form multiple polar bonds with the HMG-CoA reductase enzyme [18]. It is also relatively hydrophilic and displays a potential protective effect in cerebral ischemia in mice[19], if administered for 10d before the ischemic insult.

Cauterization of 2 or more extraocular veins of the rat has been described to increase IOP [15]. The mean IOP increase correlates with the number of cauterized veins. Most researchers typically occluded three veins in their studies. This procedure induces apoptotic death of RGC, thinning of the nerve fiber layer, cupping of the optic disc, and degeneration of the optic nerve[20-23].

In our experiment, rosuvastatin had no effect on IOP. The absence of decreased IOP in oral rosuvastatin-treated animals supports the idea that instead of reducing IOP, it has a direct neuroprotective action on RGCs.

In our study, percentage of RGC loss is in general agreement with those reported by others using similar models [20-23]. We observed 2.5% loss in RGCs in glaucomatous eyes of orally treated animals; compared to 23.9% loss in control eyes (untreated), 37.6% loss in intravitreally treated eyes and 33.1% loss in orally+intravitreally treated eyes.

Furthermore, selective TUNEL-positive cells were found in the RGC layer in the experimental glaucoma model. Control eyes had no TUNEL-positive cells and 2wk after cauterization, TUNEL-positive cells were significantly increased. However, we observed 0.9% TUNEL-positive cells in RGCs in glaucomatous eyes of orally treated animals; compared to 10.4% TUNEL-positive cells in control glaucoma ($P$ =0.001). TUNEL-positive cells decreased indicating that apoptosis of RGCs was decreased by treatment.

To evaluate retinal damage due to ocular hypertension, expression of GFAP may also been monitored. GFAP is a form of intermediate filament, which is present in Müller cells and astrocytes in the normal retina. When retinal damage occurs, GFAP expression is primarily elevated in Müller cells and astrocytes. The classic hallmark of glial cell activation is increased expression of GFAP[24-25]. Hypertrophic morphology and increased GFAP immunostaining in retinal astrocytes and Müller cells in glaucomatous eyes indicate that activation of retinal macroglial cells has occurred in the glaucomatous retina [24-25]. In our study, after the induction of IOP elevation, cauterized eyes showed a significant increase in GFAP expression 2wk after the injury. Before IOP elevation, GFAP immunoreactivity might be confined to astrocytes and the end feet of Müller cells at the inner limiting membrane, but after IOP elevation, it might extend to the outer nuclear layer. Increased GFAP expression might show glial cell activation after the IOP elevation. However, in the current study, GFAP immunoreactivity was found to be decreased in eyes of animals received oral rosuvastatin treatment compared to those of untreated cauterized eyes (group 5).

Kretz *et al* [26] demonstrated that intravitreal simvastatin injection induced heat shock protein expression in axotomized RGCs, protected neurons from apoptotic death and enhanced RGC survival early and even delayed after optic nerve injury. However, we have not been able to demonstrate the benefical effects of intravitreal rosuvastatin in our study. On the contrary, we have found a significant association between the intravitreal rosuvastatin injection and RGC loss. Rosuvastatin exhibited specificity for uptake into the liver in rats [27] as the liver is the target organ, this may translate into an advantageous clinical characteristic.

Rosuvastatin is administered as the active form however, simvastatin is a prodrug which is administered as inactive forms and activated in liver with enzimatic reaction. According to the current literature, rosuvastatin has lower metabolization rate, higher water solubility and longer half life (19h versus 3h) compared to simvastatin. While lipophilicity results in efficient hepatic shunting, the same property will result in ready passage through nonhepatic cell membranes [28]. These might explain why intravitreal rosuvastatin decreased RGC survival in our study whereas some reports suggest that intravitreal simvastatin might be neuroprotective.

Sicard *et al* [29] evaluated influence of rosuvastatin on the NAD (P)H oxidase activity in the retina and electroretinographic response of genetically hypertensive rats. They concluded that; rosuvastatin therapy could decrease production of retinal superoxide anion through the inhibition of NAD (P)H oxidase activity, independently of a reduction in plasma and tissue cholesterol levels, but might not be sufficient to restore retinal function.

There are conflicting findings in the literature regarding whether statins may be beneficial in patients with glaucoma. A case-control study by McGwin *et al* [12] demonstrated that individuals prescribed statins for >24mo had 40% reduced odds of developing OAG. A prospective study by Leung *et al* [13] demonstrated that statin use was associated with visual field stabilization over 3y among patients with normal tension glaucoma. De Castro *et al* [30] showed that statin use slowed glaucomatous changes to the optic nerve and nerve fiber layer on confocal scanning laser ophthalmoscopy. In a recent retrospective longitudinal cohort analysis by Stein *et al* [14] statin use was associated with a significant reduction in the risk of OAG among persons with hyperlipidemia. A large case-control study by Owen *et al* [10] using information from a primary care database in the United Kingdom, found no relationship between statin use and OAG.

It is clear that statins exert neuroprotective effects in experimental animals and *in vitro* conditions, while clinical trials suggest that statins may also have beneficial effects in neurodegenerative conditions in humans. Acute stroke, AD, PD, MS and glaucoma are different conditions, and the evidence for statins to inhibit disease specific pathogenic processes is inconclusive.

Statins exert both peripheral and central effects which might be protective under diverse neuropathogenic conditions. Particularly, their ability to improve blood flow, to modulate the immune response and to reduce oxidative damage may lead to neuroprotection. Although some statins can cross the blood-brain and blood-retina barrier, it is unclear to what extent they affect metabolism and signalling in the brain and vitreous, and particularly at what dosage they start influencing isoprenoid production. This may account for why promising experimental *in vivo* findings have so far translated into disappointing clinical results. We suggest that the pharmacology of statins in the brain and vitreous should be further investigated to eliminate this uncertainty.

Decrease in RGC loss and apoptosis suggest the neuroprotective potential of oral rosuvastatin treatment in a rat model of ocular hypertension. Since intravitreal drug injection is one of the most popular treatment approaches in ophthalmology currently, we wanted to compare this approach with systemical administration of rosuvastatin. However we did not find any significant neuroprotective effect in intravitreal injection group. In the current study, intravitreal rosuvastatin showed a contrary effect and further studies will shed light on intravitreal rosuvastatin pharmacokinetics, intravitreal dosage of rosuvastatin.

In conclusion, we demonstrated that oral rosuvastatin has a neuroprotective effect in a rat model of glaucoma. However intravitreal administration of rosuvastatin had an inverse effect. Because statins are safely and widely used to treat hyperlipidemia, clinical administration of oral rosuvastatin for the purpose of pharmacologic neuroprotection may be a new and beneficial therapy for patients with glaucoma in the future.

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**REFERENCES**

1 Cholesterol Treatment Trialists' (CTT) Collaboration, Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J, Collins R. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010;376(9753):1670-1681.

2 Stroke Prevetion by Aggressive Reduction in Cholestrol Levels (SPARCL) Investigators, Karam JG, Loney-Hutchinson L, McFarlane SI. High-dose atorvastatin after stroke or transient ischemic attack: The Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) Investigators. *J Cardiometab Syndr* 2008;3(1):68-69.

3 Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JM, Wun CC, Davis BR, Braunwald E. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *N Engl J Med* 1996;335(14):1001-1009.

4 Kent DM. Stroke-an equal opportunity for the initiation of statin therapy. *N Engl J Med* 2006;355(6):613-615.

5 Schmeer C, Kretz A, Isenmann S. Statin-mediated protective effects in the central nervous system: general mechanisms and putative role of stress proteins. *Restor Neurol Neurosci* 2006;24(2):79-95.

6 Zacco A, Togo J, Spence K, Ellis A, Lloyd D, Furlong S, Piser T. 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors protect cortical neurons from excitotoxicity. *J Neurosci* 2003;23 (35): 11104-11111.

7 Vaughan CJ, Delanty N. Neuroprotective properties of statins in cerebral ischemia and stroke. *Stroke* 1999;30(9):1969-1973.

8 Honjo M, Tanihara H, Nishijima K, Kiryu J, Honda Y, Yue BY, Sawamura T. Statin inhibits leukocyte-endothelial interaction and prevents neuronal death induced by ischemia-reperfusion injury in the rat retina. *Arch Ophthalmol* 2002;120(12):1707-1713.

9 Cimino M, Gelosa P, Gianella A, Nobili E, Tremoli E, Sironi L. Statins: multiple mechanisms of action in the ischemic brain. *Neuroscientist* 2007; 13(13):208-213.

10 Owen CG, Carey IM, Shah S, de Wilde S, Wormald R, Whincup PH, Cook DG. Hypotensive medication, statins, and the risk of glaucoma. *Invest Ophthalmol Vis Sci* 2010;51(7):3524-3530.

11 Iskedjian M, Walker JH, Desjardins O, Robin AL, Covert DW, Bergamini MV, Einarson TR. Effect of selected antihypertensives, antidiabetics, statins and diuretics on adjunctive medical treatment of glaucoma: a population based study. *Curr Med Res Opin* 2009;25 (8): 1879-1888.

12 McGwin G Jr, McNeal S, Owsley C, Girkin C, Epstein D, Lee PP. Statins and other cholesterol-lowering medications and the presence of glaucoma. *Arch Ophthalmol* 2004;122(6):822-826.

13 Leung DY, Li FC, Kwong YY, Tham CC, Chi SC, Lam DS. Simvastatin and disease stabilization in normal tension glaucoma: a cohort study. *Ophthalmology* 2010;117(3):471-476.

14 Stein JD, Newman-Casey PA, Talwar N, Nan B, Richards JE, Musch DC. The relationship between statin use and open-angle glaucoma. *Ophthalmology* 2012;119(10):2074-2081.

15 Shareef SR, Garcia-Valenzuela E, Salierno A, Walsh J, Sharma SC. Chronic ocular hypertension following episcleral venous occlusion in rats. *Exp Eye Res* 1995;61(3):379-382.

16 Park HY, Kim JH, Park CK. Alterations of the synapse of the inner retinal layers after chronic intraocular pressure elevation in glaucoma animal model. *Mol Brain* 2014;7:53.

17 Aktas Z, Gurelik G, Akyurek N, Onol M, Hasanreisoglu B. Neuroprotective effect of topically applied brimonidine tartrate 0.2% in endothelin-1-induced optic nerve ischaemia model. *Clin Experiment Ophthalmol* 2007;35(6):527-534.

18 Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science* 2001;292(5519):1160-1164.

19 Laufs U, Gertz K, Dirnagl U, Bohm M, Nickenig G, Endres M. Rosuvastatin, a new HMG-CoA reductase inhibitor, upregulates endothelial nitric oxide synthase and protects from ischemic stroke in mice. *Brain Res* 2002;942(1-2):23-30.

20 Neufeld AH, Sawada A, Becker B. Inhibition of nitric-oxide synthase 2 by aminoguanidine provides neuroprotection of retinal ganglion cells in a rat model of chronic glaucoma. *Proc Natl Acad Sci U S A* 1999;96(17): 9944-9948.

21 Mittag TW, Danias J, Pohorenec G, Yuan HM, Burakgazi E, Chalmers-Redman R, Podos SM, Tatton WG. Retinal damage after 3 to 4 months of elevated intraocular pressure in a rat glaucoma model. *Invest Ophthalmol Vis Sci* 2000;41(11):3451-3459.

22 Garcia-Valenzuela E, Shareef S, Walsh J, Sharma SC. Programmed cell death of retinal ganglion cells during experimental glaucoma. *Exp Eye Res* 1995;61(1):33-44.

23 Urcola JH, Hernandez M, Vecino E. Three experimental glaucoma models in rats: comparison of the effects of intraocular pressure elevation on retinal ganglion cell size and death. *Exp Eye Res* 2006;83 (2): 429-437.

24 Wang X, Tay SS, Ng YK. An immunohistochemical study of neuronal and glial cell reactions in retinae of rats with experimental glaucoma. *Exp Brain Res* 2000;132(4):476-484.

25 Wang L, Cioffi GA, Cull G, Dong J, Fortune B. Immunohistologic evidence for retinal glial cell changes in human glaucoma. *Invest Ophthalmol Vis Sci* 2002;43(4):1088-1094.

26 Kretz A, Schmeer C, Tausch S, Isenmann S. Simvastatin promotes heat shock protein 27 expression and Akt activation in the rat retina and protects axotomized retinal ganglion cells *in vivo*. *Neurobiol Dis* 2006;21 (2):421-430.

27 Nezasa K, Higaki K, Matsumura T, Inazawa K, Hasegawa H, Nakano M, Koike M. Liver-specific distribution of rosuvastatin in rats: comparison with pravastatin and simvastatin. *Drug Metab Dispos* 2002;30 (11): 1158-1163.

28 Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundam Clin Pharmacol* 2005;19 (1): 117-125.

29 Sicard P, Acar N, Gregoire S, Lauzier B, Bron AM, Creuzot-Garcher C, Bretillon L, Vergely C, Rochette L. Influence of rosuvastatin on the NAD (P)H oxidase activity in the retina and electroretinographic response of spontaneously hypertensive rats. *Br J Pharmacol* 2007;151(7):979-986.

30 De Castro DK, Punjabi OS, Bostrom AG, Stamper RL, Lietman TM, Ray K, Lin SC. Effect of statin drugs and aspirin on progression in open-angle glaucoma suspects using confocal scanning laser ophthalmoscopy. *Clin Experiment Ophthalmol* 2007;35(6):506-513.