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Original Article

Prevention of retinal ganglion cell swelling by systemic brimonidine in a rat experimental glaucoma model

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Abstract

Background: The objective of this study was to evaluate the neuroprotective effect of brimonidine on retinal ganglion cells in rats with elevated intraocular pressure and to characterize the subpopulation of cells that can be rescued, as well as assess the effect of this drug on retinal ganglion cell soma size.

Methods: Episcleral vein cauterization was used to increase intraocular pressure for 5 weeks on left eyes, considering right eyes as intrinsic controls in all cases. All the animals were then given weekly intraperitoneal injections, the experimental group receiving brimonidine, and the control group were administered only phosphate-buffered saline. Surviving retinal ganglion cells were quantified and their area and distribution measured by retrograde labelling with fluorogold.

Results: Brimonidine administered systemically has a neuroprotective effect on retinal ganglion cells, which is unrelated to its capacity to lower intraocular pressure. It prevents the increase of cell size that is associated with stages prior to cell death. This phenomenon is particularly evident in the zones of the retina most susceptible to the damage caused by glaucoma (middle and periphery).

Conclusion: This effect of preventing retinal ganglion cell swelling can be considered as a new marker to study neuroprotection from antiglaucomatous drugs in the early stages of neurodegeneration in glaucoma.

Key words: brimonidine, glaucoma, neuroprotection, retinal ganglion cell.

Introduction

Primary open-angle glaucoma is a slowly progressive optic neuropathy involving structural changes to the optic nerve that lead to degeneration of retinal ganglion cells (RGCs) that is clinically correlated to loss of visual field. Death of RGCs, a hallmark of glaucoma, occurs in two phases. During the first phase there is an alteration in the trophic responsiveness of RGCs, and it is at this point that the principal risk factor for glaucoma progression, elevated intraocular pressure (IOP), can be identified.[1] According to mechanical and vascular theories, this elevated IOP triggers the apoptotic cascade in RGCs.[1–4] The production of free radicals, together with the neurotoxicity of nitric oxide and glutamate amplify the initial effects of the lesion, favouring the advance and progression of glaucoma.[3,5–7] This then leads to the secondary phase that is postulated to favour ongoing glaucoma damage, even when the initial insult triggered by elevated IOP is brought under control. These factors are also considered to be responsible for the increased probability of progression of lesions in patients with pre-existing severe glaucoma damage.[3]

Glaucoma neuroprotection is a strategy to save RGCs that are damaged or undergoing cell death,

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and at the same time favouring healthy cells, protecting them from a hostile environment. This type of strategy is especially important as up to 17% of glaucoma patients do not evolve well despite their IOP having been successfully reduced and brought under control.[3]

Brimonidine (BMD), a highly selective agonist of α2-adrenergic receptors, has been shown to exhibit a neuroprotective effect in glaucoma. This effect is independent of its ocular hypotensive effects when administered topically, as similar therapeutic effects are observed when it is applied intraperitoneally (i.p.), but in this latter route of administration there is no associated reduction in IOP.[8,9] BMD fulfils three out of four established criteria to be considered a neuroprotective agent: (i) there are specific receptors for it in the retina;[10] (ii) it increases RGC survival;[8,11–13] and (iii) it reaches the retina and vitreous in neuroprotective concentrations.[14] Nevertheless, clinical trials to evaluate the neuroportective effect of this drug in glaucoma and related optic neuropathies have not yet been carried out. It is precisely for this reason that experimental models of glaucoma are of great value, as they permit a direct comparison of the effect of different treatments on RGCs.

One phenomenon that precedes cell death, already reported in neurons, is increased cell size,[15] and this has been reported to occur in glaucoma,[8,9,11,12] as well as in the early stages of damage to peripheral RGCs.[1,16–18] Previous studies by our group have analysed the degree of RGC neuroprotection mediated by BMD 3 months after IOP elevation. However, we had not identified whether systemic BMD was acting on any specific RGC subtype, or on more vulnerable regions of the retina, in the episcleral vein cauterization (EVC) glaucoma model.[2] Furthermore, we wanted to check whether RGC soma size decreases after an initial swelling phenomenon or this enlargement of the cells is prevented from the initial periods of intraocular hypertension. Thus, in the present study, we have analysed RGC density and soma size in central, middle and peripheral zones of the retina in an early period of experimental glaucoma generated by EVC (5 weeks), in order to associate changes in the RGC soma size to the degeneration of RGCs because of the experimental glaucoma and to assess any modulation of this phenomenon by BMD.

Methods

Animals

We employed 10 female Sprague–Dawley rats, weighing between 250 and 300 g. The animals were given free access to food and water, and kept at a stable temperature of 21°C and with a 12-h light–dark cycle. The guidelines in the ARVO statement concerning the care and treatment of animals in ophthalmic research were adhered to.

Episcleral vein cauterization

Intraocular pressure was elevated in the 10 rats by means of the cauterization of three episcleral veins (EVC) of the left eyes, considering the right eyes of all animals to be the controls. We followed the methods described in previous articles.[9,18]

IOP measurement in awake animals

Intraocular pressure was measured weekly using electronic indentation tonometry (TonoPen XL, Mentor, Norwell, MA, USA). Animals were kept awake at the time of the procedure, which was always carried out at the same time of the day to avoid natural circadian changes in IOP, following the method described previously by Hernández *et al.*[9] and Urcola *et al.*[18] Measurements of IOP were repeated five times for each eye. The IOP readings were accepted if the confidence interval was greater than or equal to 95%. The values of the IOP measurements were then averaged, and results were expressed as mean IOP ± SEM (standard error of the mean). Results from statistical analysis are represented as: $P \leq 0.05$, $P \leq 0.01$ and $P < 0.001$ significant differences with respect to each unoperated control eye (right eye) (calculated using the Student's *t*-test).

Treatment with BMD

Intraocular pressure was monitored following surgery. Once IOP had permanently stabilized at a higher level (4th day), the 10 rats employed were divided into two groups: (i) the first group ($n = 5$) was treated with i.p. injections of phosphate-buffered saline (PBS) as a placebo; and (ii) the second group ($n = 5$) received i.p. injections of BMD (Alphagan, 0.2%, Allergan Laboratories, Madrid, Spain; 1 mg/kg). Both groups were treated once a week for 5 weeks.

Retrograde labelling of RGCs

Retinal ganglion cells were retrogradely labelled with fluorogold (Flurochrome Inc., Engelwood, CO, USA) dissolved at 3% in a solution containing 0.9% NaCl and 0.1% dimethyl sulfoxide, 1 day before tissue fixation, that is, the animal remained alive for 24 h to permit labelling of the entire RGC population. We injected 20 μL of the fluorogold solution at a distance of 4 mm from the optic disc, using

a 30-G needle. Animals were anaesthetized again 24 h later and perfused with 4% paraformaldehyde in 0.1 mol/L PBS (pH 7.4). The eyes were enucleated and the lens and vitreous removed. The optic cups were post fixed in the same fixative for 2 h. Subsequently, the retinas were dissected out and mounted on a glass slide, leaving the layer of the RGCs facing upwards to facilitate subsequent analysis. The tissue was covered in PBS/glycerol (1:1) to prevent dehydration.[9,18]

Image capture

Images were taken with an epifluorescence microscope (Axioskop 2; Zeiss, Jena, Germany) coupled to a digital camera (Coolsnap, RS Photometrics, Tucson, AZ, USA). A total of 24 fields were captured systemically for each retina using a 20× lens (0.08 mm^2). Images were captured using the optic disc as a reference for the different retinal zones (centre, middle and periphery). For the present study, only nine fields of the temporal-dorsal quadrant from each retina were considered, those corresponding to the central, middle and peripheral zones of the temporal, dorsal and temporal-dorsal regions (Fig. 1). We decided to analyse cell morphology and density based on the data obtained from the temporal-dorsal quadrant, given the fact that in our previous studies there were no significant differences in RGC density and distribution between different quadrants of the retina (dorsal, medial, nasal and temporal) in the EVC model of experimental glaucoma in the rat.

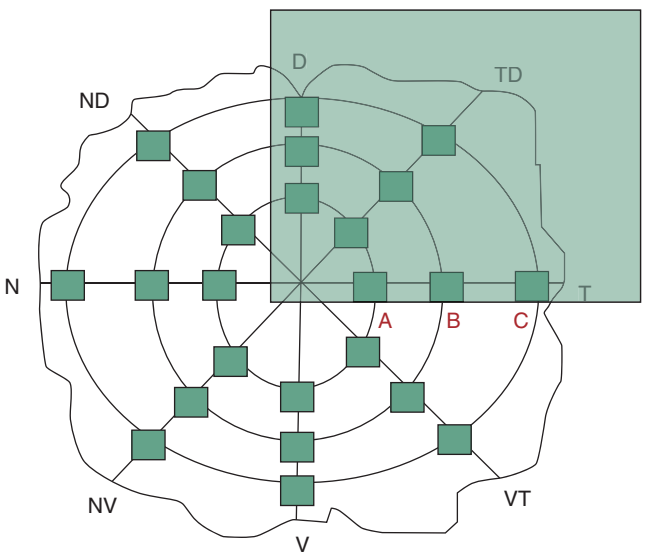

Figure 1. Scheme of retinal topography in terms of central (A), middle (B) and peripheral (C) zones, proximity to the optic nerve and temporal (T), temporal-dorsal (TD), nasal (N), nasal-dorsal (ND), nasal-temporal (NT), dorsal (D) and ventral (V) orientations. Retinal ganglion cells were only counted in areas from the temporal-dorsal quadrant, included in the square.

The number and size of the RGCs in each retinal zone was compared between glaucoma eyes (left) and control eyes (right), as reported elsewhere.[9,16–18]

Quantitative and qualitative analysis of RGCs

We counted the number of RGCs in each field, as well as measuring the lengths of the major and minor axes and areas of their somas. Analysis of these parameters was carried out using a digital tablet linked to a computer screen (Easypen, Genius), in combination with image analysis software (Scion Image; Scion, Frederick, MD, USA), as reported elsewhere.[9,18] Briefly, the soma of each of the counted RGCs was manually measured on the computer screen and the values obtained were transferred to a spreadsheet for subsequent statistical analysis of the data. We chose to manually count and measure cells, as this allowed us to assess the RGC somas with precision. In order to identify possible RGC subpopulations that were more sensitive to the neuroprotective effects of BMD, we calculated average values of RGC density and soma area for each of the different retinal zones.

Statistical analysis was performed using SPSS software (SPSS Sciences, Chicago, IL, USA). Density and soma area were expressed as mean ± standard deviation. The mean values for the different retinas, as well as for the different zones were compared using a one-way ANOVA test, followed by the Scheffé test and single variable analysis. The minimum value of differences considered significant was set at $P = 0.05$.

Results

Effect of BMD on IOP

The mean basal IOP in the right eyes of the control group was 20.7 ± 0.1 mmHg, and the mean IOP in the right eyes of the BMD-treated group was 20.1 ± 0.3 mmHg. This difference was not statistically different ($P = 0.078$). The average IOP in EVC PBS-treated eyes was 29.2, and the mean increase in IOP was 8.4 ± 0.5 mmHg ($P < 0.0001$) or 41% with respect to corresponding right eye. The mean IOP in left eyes subjected to EVC, but treated with BMD was 28.9 ± 0.3 mmHg, with an average increase of 8.9 ± 0.1 mmHg (or 44%), with respect to the corresponding right eye ($P < 0.0001$). We did not find any statistically significant differences between the IOP of left eyes subjected to EVC in the two groups (those treated with BMD or PBS) ($P = 0.61$). Indeed, we found a stable increase in

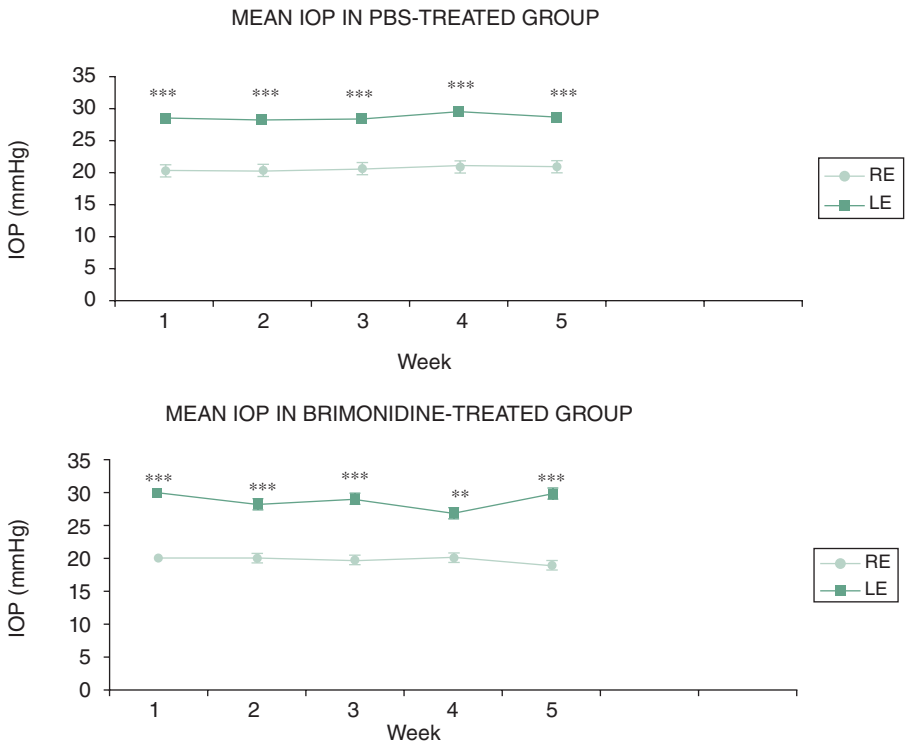

Figure 2. Intraocular pressure (IOP) in control and glaucoma eyes that received weekly intraperitoneal injections of PBS (upper graph) or brimonidine (lower graph). Right eye, control; left eye, episcleral vein cauterization; results from statistical analysis are represented as: $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ significant differences with respect to each control eye (right eye). IOP, intraocular pressure; LE, left eye; PBS, phosphate buffer solution; RE, right eye.

IOP following EVC, from the fifth day of treatment approximately. This effect was not modified by treatment with BMD (Fig. 2).

Effect of BMD on RGC survival

Episcleral vein cauterization followed by PBS treatment induced mortality in 32 ± 7.6% ($P < 0.001$) of RGCs with respect to control retinas. Mortality was higher in middle and peripheral (38 ± 8.5%, $P < 0.001$ and 37 ± 10%, $P < 0.001$, respectively), than in central zones (23 ± 8.7%, $P < 0.01$) (Figs 3,4; Table 1).

The mean RGC density in eyes that underwent EVC followed by i.p. BMD treatment was 3569 ± 143 cells/mm^2. In central zones, RGC density reached 3721 ± 20 cells/mm^2, and in middle and peripheral zones it was 3550 ± 24 cells/mm^2 and 3437 ± 31 cells/mm^2, respectively. On the other hand, the mean RGC density in unoperated eyes (right eyes) with weekly treatment with i.p. BMD was 3600 ± 143 cells/mm^2. In central zones RGC density was 3750 ± 25 cells/mm^2, and in middle and peripheral zones, 3600 ± 63 cells/mm^2 and 3450 ± 50 cells/mm^2, respectively. We did not find statistically significant differences between these two groups, and attribute this to the treatment with BMD inducing an RGC survival rate of 99 ± 1.4% ($P = 0.99$).

Effect of BMD on RGC soma size

In unoperated eyes with PBS treatment, the mean global area of RGC somas, was 117.6 ± 1.2 μm^2. More specifically, the mean areas in the different zones were 111.1 ± 1.6 (central), 116.7 ± 1.8 (middle) and 125.01 ± 1.9 (peripheral) μm^2.

In eyes subjected to EVC followed by PBS treatment, the overall mean area of the somas was 148.4 ± 0.1 μm^2, with a mean increase in area of 31 ± 0.1 μm^2 ($P < 0.001$) (+26 ± 1.7%) with respect to the control group. More specifically, the mean values in the different zones were: 121.35 ± 0.7 (central), 161.5 ± 1.1 (middle) and 162.3 ± 1.1 (peripheral) μm^2, and the corresponding mean increases were: 10.3 ± 0.1 μm^2 ($P < 0.01$) (central), 44.8 ± 0.1 μm^2 ($P < 0.001$) (middle) and 37.3 ± 0.1 μm^2 ($P < 0.001$) (periphery).

In the BMD-treated group, the mean soma area in unoperated eyes was 106.9.6 ± 3.6 μm^2, with the values by zone being 101.2 ± 6.5 (central), 107.7 ± 5.1 (middle) and 111.2 ± 7.1 (peripheral) μm^2. We did not find any statistically significant differences in soma size, when comparing different zones of the retina, between unoperated and glaucoma eyes within this BMD-treated group. However, in this group the overall average obtained for RGCs from all the zones analysed was statistically smaller in those eyes that underwent EVC than in unoperated

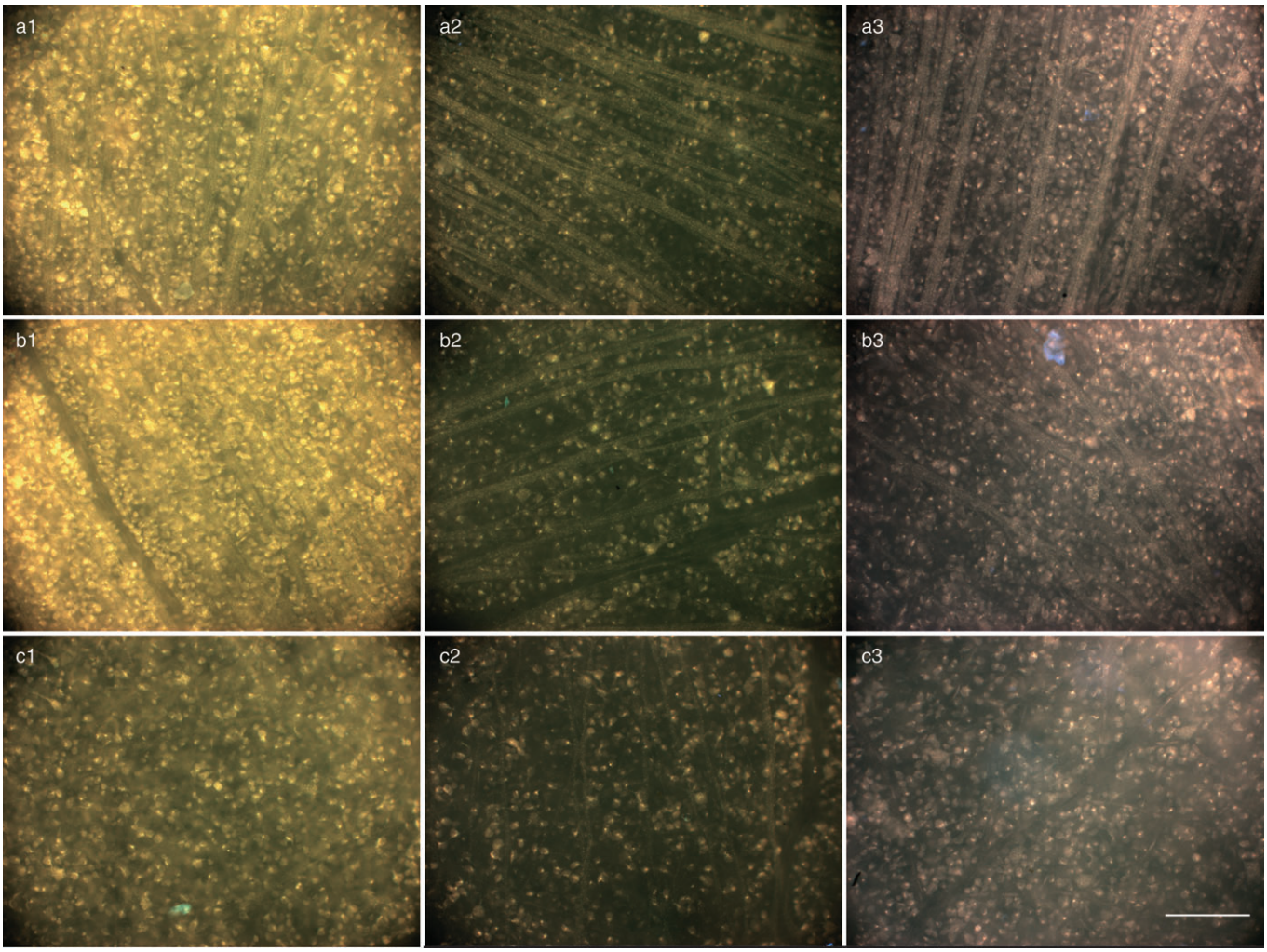

Figure 3. Fluorogold- (3%) labelled retinal ganglion cells in peripheral (c1, c2, c3), middle (b1, b2, b3) and central (a1, a2, a3) zones of unoperated eyes with weekly intraperitoneal injections of phosphate buffer solution (a), episcleral vein cauterization eyes with weekly injections of phosphate buffer solution (b) and episcleral vein cauterization eyes following weekly injections of phosphate buffer solution for 5 weeks (c). Scale bar, 100 μm.

eyes (106.9 ± 3.6 μm² *vs.* 91.3 ± 3.6 μm², $P = 0.009$). Moreover, we did find significant differences between the means of RGC soma area for the glaucoma eyes that received placebo treatment (PBS) *versus* those which received BMD treatment. In the latter, mean soma size was smaller, with a mean difference in soma area of 38.6 ± 2.7% ($P < 0.001$) (Figs 3,4; Table 2).

Multiple variable analysis of soma area as a function of location on the retina (centre, middle or periphery), to examine the possible relationship between variables that are dependent on the eye (control or glaucoma) and treatment, did not detect any association between treatment and the preservation of retinal soma size in the central zone ($P = 0.727$). In contrast, this analysis did attribute the observed lack of increase in retinal mean soma size to the dependent variable of BMD treatment in the middle and peripheral zones ($P < 0.001$).

Discussion

Effect of BMD treatment on IOP

Brimonidine is a highly selective agonist of α-2 adrenoceptors, which has been approved for the treatment of primary open-angle glaucoma. It has been shown to penetrate the choroid-retina[14] when administered topically or systemically. Topical administration achieves optimal concentrations of BMD in the aqueous, ciliary body and iris where, following activation of α-2 receptors, aqueous production decreases and uveoscleral outflow increases.[19] It is precisely this

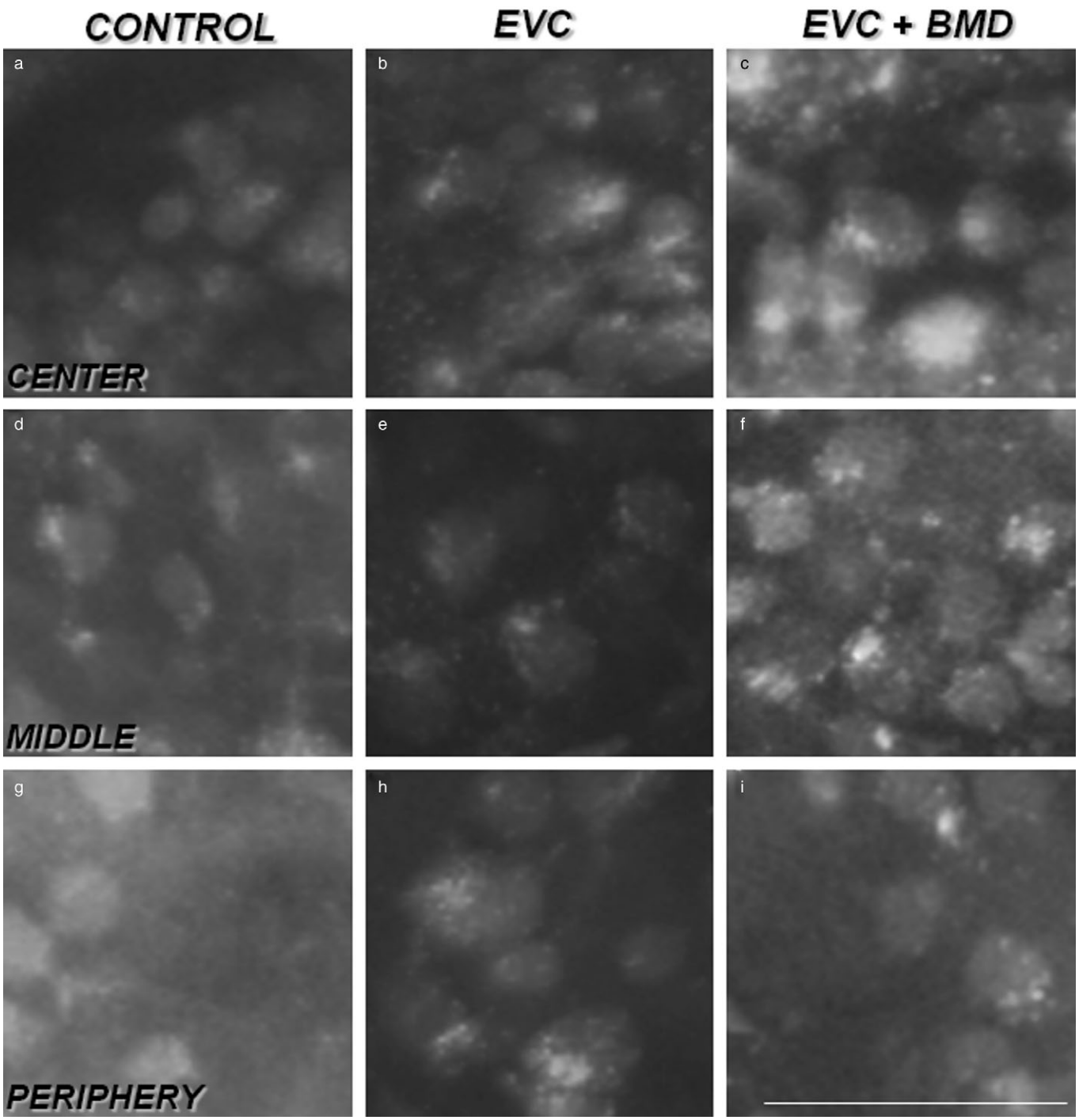

Figure 4. Fluorogold- (3%) labelled RGCs in peripheral, middle and central zones of normotensive eyes (control) (a, d, g, respectively) and EVC eyes (glaucoma) (b, e, h) and eyes from animals treated with i.p brimonidine for 5 weeks following EVC (c, f, i). Scale bar, 100 μm. BMD, brimonidine; EVC, episcleral vein cauterization; i.p., intraperitoneal; RGC, retinal ganglion cells.

enhanced access to target organs that produce and excrete the aqueous that underlies the hypotensive effect of BMD when applied topically. By contrast, earlier studies by our group and others[8,9] have not found IOP alterations following i.p. administration of BMD. Thus, systemic administration of this drug, which does not produce the hypotensive effect achieved by topical application,

Table 1. RGC density as a function of the zone, eye and treatment (cells/mm^2)

	Centre	Middle	Periphery	Total average
Control + PBS (RE)	3652 ± 139	3918 ± 185	3011 ± 160	3527 ± 269
EVC (LE)	2827 ± 207**	2445 ± 213***	1906 ± 170***	2392 ± 207***
BMD (RE)	3750 ± 25	3600 ± 63	3450 ± 50	3600 ± 143
BMD + EVC (LE)	3721 ± 20	3550 ± 24	3437 ± 31	3569 ± 143

Results from statistical analysis are represented as: *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ significant differences with respect to each control unoperated eye (right eye). BMD, brimonidine; EVC, episcleral vein cauterization; i.p., intraperitoneal; LE, left eye; RE, right eye; RGC, retinal ganglion cells.

Table 2. RGC mean soma size as a function of the zone, eye and treatment (μm^2)

	Centre	Middle	Periphery	Total average
Control + PBS (RE)	111.1 ± 1.6	116.7 ± 1.8	125 ± 1.9	117.6 ± 1.2
EVC (LE)	121.3 ± 1.7**	161.5 ± 1.8***	162.3 ± 1.2***	148.4 ± 0.8***
BMD (RE)	101.2 ± 6.5	107.7 ± 5.1	111.2 ± 7.1	106.9 ± 3.6
BMD + EVC (LE)	91.4 ± 8.2	95.4 ± 4.7	88.8 ± 4.7	91.3 ± 3.6**

Results from statistical analysis are represented as: *$P < 0.05$ **$P < 0.01$ ***$P < 0.001$ significant differences with respect to each control unoperated eye (right eye). BMD, brimonidine; EVC, episcleral vein cauterization; i.p., intraperitoneal; LE, left eye; RE, right eye; RGC, retinal ganglion cells.

allows us to evaluate the neuroprotective effect of BMD on RGCs independently.

Effect of BMD treatment on RGC survival

In our experimental glaucoma model, 32 ± 7.6% RGC mortality was induced in comparison with control eyes ($P < 0.001$), with this rate being higher in the middle and peripheral of the retina, than in the central zone. Thus, these zones appear to be more susceptible to primary damage induced by increased IOP. This is consistent with previous studies by our group, which found a greater extent of RGC death in middle and peripheral than central zones in various different experimental glaucoma models when the retinas were maintained under conditions of elevated IOP for 24 weeks.[18] Our new data, from the present study, reveals that these differences are even more evident after shorter periods of glaucomatous damage (5 weeks), indicating where the earliest signs of neurodegeneration, triggered by the initial IOP-dependent insult, can be observed.

Brimonidine administered intraperitoneally resulted in complete survival of RGCs following increased IOP because of EVC. This illustrates the way in which i.p. administration, which does not activate the hypotensive effects of topical administration, allows us to examine the well documented RGC neuroprotective effect of BMD[8,9] independent of the hypotensive effect associated with topical administration.

Effect of BMD treatment on RGC soma size

Episcleral vein cauterization induced a 26 ± 1.05% increase in the mean area of RGC somata; this increase was larger in middle and peripheral (38 ± 0.78% and 29.6 ± 0.54%, respectively) than in central zones (9 ± 0.5%). It is then conceivable that RGCs increase their soma size upon entering apoptosis, induced by the initial increase in IOP. Further, in the same way that, in terms of mortality, RGCs exhibit greater susceptibility to this type of damage in middle and peripheral zones, this pattern is reflected in the increased size of RGC somata.

Notably, BMD treatment in eyes subjected to EVC was also associated with a reduction in RGC soma size that was more pronounced in middle and peripheral zones than in the centre (soma area reductions of 42.2 ± 0.63%, 43.2 ± 0.78% and 24.8 ± 1.3%, respectively). Furthermore, mean RGC soma area averaged over the zones was found to be lower in those eyes submitted to EVC than in control right eyes after treatment with BMD. However, these differences did not appear when comparing mean soma area in different zones of the retina. This phenomenon seems to reinforce the concept of BMD having a neuroprotective effect, as it prevents the death of those RGCs in which apoptosis has not begun, although neurodegeneration of these cells cannot be prevented by BMD once the cascade of apoptosis has been triggered. For this reason, in those eyes that underwent EVC and were treated with BMD, mean soma area was smaller when the apoptotic cascade

has been triggered by increased IOP, as those RGCs that experience the increase in soma size associated with the earliest periods of apoptosis cannot be rescued, and those still vulnerable but not affected by initial neurodegeneration remain protected by systemic BMD.

Because BMD treatment was not associated with a hypotensive effect on IOP, we have been able to evaluate its neuroprotective effect, which was more pronounced in the more susceptible cells, located in the middle and peripheral zones of the retina in the temporal-dorsal quadrant.

Retinal ganglion cell death in glaucoma is mainly mediated by apoptosis, because of excessive oxidative stress and glutamate-mediated excitotoxicity, activating NMDA receptors and initiating uptake of Ca^{2+} that, in turn, leads to an ionic imbalance and cell death, and this has been associated with swelling of RGC somata and dendrites.[4–8,20] The mechanism of action of BMD could be mediated by the direct activation of α2-adrenergic receptors on RGCs,[10,11,21,22] antiapoptotic pathways, or inhibition of glutamate-mediated excitotoxicity. It has been shown that i.p. injection of BMD causes upregulation of two important early antiapoptotic genes, bcl2 at the RNA level and bcl-xl at the protein level, in rat retina, which would explain the early neuroprotection and prevention of RGC swelling afforded by this drug.[23] Alpha-2 adrenoreceptor agonists can also induce hyperpolarization through modulation of NMDA receptors. This mechanism involves a direct effect on calcium signalling at a cytosolic level, as well as an inhibition of L-type voltage-dependent calcium channels in the internal plexiform layer, resulting in a reduction in the levels of intracellular calcium in presynaptic cells and of glutamate, inducing hyperpolarization and reducing glutamate-mediated excitotoxicity.[4–7,24] The consequences of these effects at the different independent levels of IOP would involve greater resistance by the more susceptible RGCs to glaucoma damage and maintenance of original cell size by inhibition of the swelling phenomenon associated with excitotoxic neurodegeneration of RGCs. Supporting this idea, some other glaucoma drugs such as latanoprost, which do not activate adrenergic receptors, do not reduce the size of RGC somata.[9]

This study has allowed us to identify the regions of the retina most vulnerable to glaucomatous damage, revealing that middle and peripheral zones are the most affected with a sharp decrease in RGC density. These are also the zones where swelling of the mean soma size can be detected at an earlier stage, and, notably, also those most susceptible to the soma size-dependent neuroprotective effect of systemic BMD. Examining RGC soma size may thus represent a novel criterion for the screening of drugs with potential neuroprotective effects for glaucoma.

Acknowledgements

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References

1. García-Valenzuela E, Shareef S, Walsh J, Sharma SC. Programmed cell death of retinal ganglion cells during experimental glaucoma. *Exp Eye Res* 1995; **61**: 33–44.
2. Shareef SR, García-Valenzuela E, Salierno A, Walsh J, Sharma SC. Chronic ocular hypertension following episcleral venous occlusion in rats. *Exp Eye Res* 1995; **61**: 379–82.
3. Yoles E, Schwartz M. Potencial neuroprotective therapy for glaucomatous optic neuropathy. *Surv Ophthalmol* 1998; **42**: 367–72.
4. Agarwal R, Gupta SK, Agarwal P, Saxena R, Agrawal SS. Current concepts in the pathophysiology of glaucoma. *Indian J Ophthalmol* 2009; **57**: 257–66.
5. Hartwick ATE, Zhang X, Chauhan BC, Baldridge WH. Functional assessment of glutamate clearance mechanisms in a chronic rat glaucoma model using retinal ganglion cell calcium imaging. *J Neurochem* 2005; **94**: 794–807.
6. Wansley S, Gabelt BT, Dahl DB *et al*. Vitreous glutamate concentration and axon loss in monkeys with experimental glaucoma. *Arch Ophthalmol* 2005; **123**: 64–70.
7. Casson RJ. Possible role of excitotoxicity in the pathogenesis of glaucoma. *Clin Experiment Ophthalmol* 2006; **34**: 54–63.
8. Ahmed FA, Hegazy K, Chaudhary P, Sharma SC. Neuroprotective effect of alpha(2) agonist (brimonidine) on adult rat retinal ganglion cells after increase intraocular pressure. *Brain Res* 2001; **913**: 133–9.
9. Hernández M, Urcola JH, Vecino E. Retinal ganglion cell neuroprotection in a rat model of glaucoma following brimonidine, latanoprost or combined treatments. *Exp Eye Res* 2008; **10**: 798–806.
10. Kalapesi FB, Coroneo MT, Hill MA. Human ganglion cells express the alpha-2 adrenergic receptor: relevance to neuroprotection. *Br J Ophthalmol* 2005; **89**: 758–63.
11. Wheeler LA, Gil D, Woldemussie E. Role of α2-adrenergic receptors in neuroprotection and glaucoma. *Surv Ophthalmol* 2001; **45**: 290–4.
12. Woldemussie E, Ruiz G, Wijono M, Wheeler LA. Neuroprotection of retinal ganglion cells by brimonidine in rats with laser-induced chronic ocular hypertension. *Invest Ophthalmol Vis Sci* 2001; **42**: 2849–55.

13. Lafuente MP, Villegas-Pérez MP, Mayor S, Aguilera ME, Miralles del Imperial J, Vidal-Sanz M. Neuroprotective effects of brimonidine against transient ischemic-induced retinal ganglion cell death. A close response in vivo study. *Exp Eye Res* 2002; **74**: 181–9.
14. Acheampong A, Shackleton M, John B, Burke J, Wheeler L, Tang-Liu D. Distribution of brimonidine into anterior and posterior tissues of monkey, rabbit, and rat eyes. *Drug Metab Dispos* 2002; **30**: 421–9.
15. Inglefield JR, Schwartz-Bloom RD. Activation of excitatory amino acid receptors in the rat hippocampal slice increases intracellular Cl and cell volume. *J Neurochem* 1998; **71**: 1396–404.
16. Ruíz-Ederra J, García M, Martín F *et al*. Comparación de tres métodos de inducción del incremento crónico de la presión intraocular en el cerdo (glaucoma experimental). *Arch Soc Esp Oftalmol* 2005; **80**: 571–80.
17. Ruíz-Ederra J, García M, Hernández M *et al*. The pig eye as a novel model of glaucoma. *Exp Eye Res* 2005; **81**: 561–9.
18. Urcola JH, Hernández 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**: 429–37.
19. Toris CB, Gleason ML, Camras CB, Yablonski ME. Effects of brimonidine on aqueous humor dynamics in human eyes. *Arch Ophthalmol* 1995; **113**: 1514–7.
20. Olney JW. Glutamate-induced retinal degeneration in neonatal mice. Electron microscopy of the acutely evolving lesion. *J Neuropathol Exp Neurol* 1969; **28**: 455–74.
21. Dong CY, Guo Y, Wheeler L, Hare WA. Alpha2 adrenergic receptor–mediated modulation of cytosolic Ca^{++} signals at the inner plexiform layer of the rat retina. *Invest Ophthalmol Vis Sci* 2007; **48**: 1410–5.
22. Wheeler LA, Woldemussie E, Lai R. Role of alpha-2 adrenergic receptor agonists in neuroprotection. *Eur J Ophthalmol* 2003; **11**: 30–5.
23. Lai RK, Chun T, Hasson D, Lee S, Mehrbod F, Wheeler L. Alpha-2 adrenoceptor agonist protects retinal function after acute retinal ischemic injury in the rat. *Vis Neurosci* 2002; **19**: 175–85.
24. Dong CJ, Guo Y, Agey P, Wheeler L, Hare WA. Alpha2 adrenergic modulation of NMDA receptor function as a major mechanism of RGC protection in experimental glaucoma and retinal excitotoxicity. *Invest Ophthalmol Vis Sci* 2008; **49**: 4515–22.