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Brimonidine's neuroprotective effects against transient ischaemia-induced retinal ganglion cell death

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PURPOSE. *Brimonidine is a lowering pressure agent currently used in glaucoma. This chronic degenerative condition is characterised by neuronal death, and an agent which offers neuroprotection may slow down or impede the progression of neuronal cell death.*
METHODS. *The effects of brimonidine (BMD) on the short- and long-term survival of retinal ganglion cells (RGCs) after transient retinal ischaemia are reported here using a rat model. The fluorescent tracer Fluorogold (FG) was applied to both superior colliculi to retrogradely label RGCs. A ninety-minute period of ischaemia was induced and densities of surviving RGCs were estimated over time by counting FG-labelled RGCs in 12 standard regions of each retina.*
RESULTS. *Seven days after inducing transient ischaemia, there was loss of approximately half of the RGC population. Topical pre-treatment with 0.1% or 0.5% BMD prevented ischaemia-induced RGC death.*
CONCLUSIONS. *These results indicate that optimal neuroprotective effects against the early loss of RGCs are seen with 0.1% or 0.5% BMD. Ischaemia-induced RGC loss continued between day 7 and day 21 in the vehicle treated groups and amounted to approximately 25% of the RGC population. Topical pre-treatment with 0.1% or 0.5% BMD was also effective in reducing the slow loss of RGCs.* Eur J Ophthalmol 2001; 11 (Suppl 2): S36-S40

KEY WORDS. *α2-selective adrenergic agonists, Neuroprotection, Neuronal degeneration*

INTRODUCTION

The concept of neuroprotection (i.e. prevention of neuronal death) has emerged from recent work indicating that injury-induced neuronal death may be prevented or slowed by certain drugs. Neuroprotective agents are particularly attractive for use in chronic neurodegenerative diseases in which treatment of the causal mechanism is not a feasible approach. One disease that might benefit from neuroprotective therapy is glaucoma, a chronic neurodegenerative disease that is one of the leading causes of blindness in developed countries (1). Glaucoma is characterised by degeneration of retinal ganglion cells (RGCs) which leads to progressive loss of the visual field. Patients with glaucoma often have an abnormally elevated intraocular pressure (IOP), and current therapy is largely based on the use of IOP-lowering drugs. A high IOP is thought to lead to axotomy-like injuries to optic nerve axons and thus to RGC degeneration (2, 3). This degeneration may take the form of apoptosis, which leads to slow loss of RGCs and thus progressive loss

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of the visual field (4–6).

In some patients, RGC and visual field degeneration occurs in the absence of elevated IOP. In this ("low-tension") type of glaucoma, it has been suggested that RGC degeneration may be a result of ischaemia. Indeed, transient ischaemia of the retina also leads to progressive loss of RGCs (7, 8) and may take the form of apoptosis (9, 10).

Although the main current therapy for glaucoma consists of IOP-lowering drugs, efforts are being directed toward the development of drugs that may halt or slow the progressive degeneration of RGCs. A number of recent studies have shown that neuronal death induced by a variety of injuries can be prevented experimentally with the use of different neuroprotective agents (11–14). In both these axotomy or ischaemia-induced injuries, there is a rapid first phase of cell death followed by a slower secondary phase that lasts for a considerable time (3, 7, 8).

Recent studies have shown that α_2-selective adrenergic agonists, currently used in ophthalmology because of their ability to lower IOP (15, 16), also have potent neuroprotective effects against transient ischaemia-induced RGC death (17, 18). This paper summarises recent investigations into the neuroprotective effects of topical brimonidine that document short and long-lasting neuroprotection against transient ischaemia-induced RGC loss.

METHODS

Adult Sprague-Dawley rats (200–250 g) were used in these studies. Animals were treated according to institutional guidelines and European Union regulations for the use of animals in research. Surgical manipulations were carried out under general anaesthesia. Rats had free access to water and food and were kept in cages in temperature- and light-controlled rooms.

Retrograde labelling of RGCs

Rats were anaesthetized with an intraperitoneal injection of a mixture of ketamine (75 mg/kg) (Ketolar®, Parke-Davies, S.L. Barcelona, Spain) and xylazine (10 mg/kg) (Rompún®, Bayer, S.A. Barcelona, Spain) in sterile saline. Both superior colliculi were exposed and a small pledget of gelatine sponge (Espongostan® Film, Ferrosan A/S, Denmark) soaked in a solution of 3% Fluorogold (FG) in saline containing 10% dimethyl sulphoxide was applied over their surface. This procedure results in the retrograde labelling of most RGCs within 7 days (7, 18). Furthermore, FG-labelling of the RGC population persists for up to 4 weeks after tracer application (7).

At the end of the procedure, a steroid-antibiotic ointment containing neomycin and dexamethasone (Fludronef®, Iquinosa, Madrid, Spain) was applied over the ocular surface to prevent corneal desiccation during recovery from anaesthesia, and animals were returned to their cages and allowed to recover.

Transient ischemia of the retina

Induction of transient ischemia of the left retina has been described previously (17).

Drug application

BMD, an alpha-2 selective adrenergic agonist also known as AGN 190342, or UK-14,304 (19) was provided by Allergan Inc. (Irvine, CA, USA) for these studies. Topical application of BMD on the left eye one hour before induction of retinal ischaemia has been described previously (17, 18). Animals were divided into different groups depending on the concentration of BMD used: 0.1% or 0.5%. The animals were processed 7 or 21 days after ischaemia.

Tissue processing and analysis of retinas

The retinal tissue processing and analysis has been described previously (17). Twelve standard rectangular areas (0.36 x 0.24 mm) of each left and right retina were photographed. The number of labelled RGCs in the 12 photographs were counted, divided by the area of the picture, and pooled to estimate a mean RGC density per retina. Statistical analysis of the differences between groups of retinas was done using non-parametric ANOVA tests (Statistix® V1.0 for Windows 95). Differences were considered significant when p was less than 0.05.

RESULTS

Short-term effects of BMD applied topically

To determine if topical application of BMD had neuroprotective effects, we analysed groups of rats 7 days after treatment with vehicle alone, or vehicle containing 0.1% or 0.5% BMD.

In rats treated with vehicle, RGC densities 7 days after ischaemia had decreased to almost half of the original RGC population (Fig. 1). In rats treated with 0.1 or 0.5% BMD, the densities of FG-labelled RGCs 7 days after ischaemia were significantly greater than those obtained in the saline-treated animals, and comparable to those obtained in their contralateral non-ischaemic fellow retinas (Fig. 1). Thus, at 7 days, 0.1% or 0.5% BMD induced optimal survival of RGCs.

Long-term effects of BMD applied topically

To determine if topical application of BMD had persistent neuroprotective effects, we analysed two groups of rats 21 days after treatment with saline alone or saline containing 0.5% BMD. In the saline-treated groups, the densities of FG-labelled RGCs decreased significantly between 7 and 21 days after ischaemia (Fig. 1). Pre-treatment with 0.5% BMD considerably diminished RGC loss (Fig. 1), suggesting that topically applied BMD had long lasting neuroprotective effects.

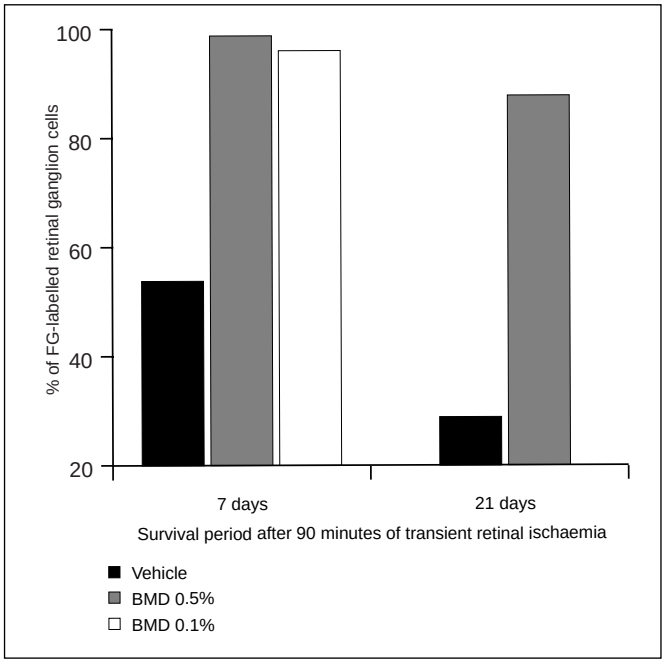

Fig. 1 - *Topically applied brimonidine (BMD) neuroprotects from ischemia-induced RGC death by 7 days, and diminishes the loss of RGCs that follows between 7 and 21days. Densities of FG-labelled RGCs (expressed as percentages of the densities found in their right nonischemic retinas) in the experimental retinas of groups of adult Sprague-Dawley rats 7 and 21 days after 90 minutes of transient ischemia induced by ligature of the left ophthalmic vessels. Pre-treatment, 1 hour before ischemia with two 5 µl drops of saline alone (vehicle) resulted 7 and 21 days later in the loss of 46, and 71%, respectively, of the RGC population. Topical pre-treatment with two 5 µl drops of saline containing 0.1% or 0.5% BMD resulted 7 days later in the rescue of most of the RGC population destined to die and 21 days later in the rescue of a high proportion of RGCs. (n=6 for all groups). Data from Table V of Reference 18.*

DISCUSSION

Short-term neuroprotection

In the present study we show the neuroprotective effects of BMD 7 days after ischaemia. These results are consistent with electroretinographic studies (20) that have assessed the effects of topical BMD on the retina after pressure-induced retinal ischaemia. Previous studies suggest that it is likely that the neuroprotective effects observed with topical BMD are mediated through α_2-receptors (18, 21, 22).

Alpha-2 selective adrenergic agonists have been shown to protect RGCs against several types of retinal insults, including partial optic nerve crush injury (21), pressure-induced acute retinal ischaemia (20, 22), and chronic elevation of IOP induced by episcleral vein photocoagulation (23). Our previous (17, 18) and present studies also show that alpha-2 adrenergic agonists have potent neuroprotective effects on RGCs against transient ischaemia induced by ligature of the ophthalmic vessels.

Several hypotheses as to how alpha-2 adrenergic agonists exert their neuroprotective effects have been proposed. These include: up-regulation of endogenous production of trophic factors, reduction or counteraction of excessive glutamate release, and an anti-apoptotic effect at the mitochondrial level. It is likely that all three mechanisms act in synergy to induce the neuroprotective response.

It has been suggested that BMD exerts its neuroprotective effect through increased levels of basic fi-

broblast growth factor (bFGF) in the retina (24). Although the exact mechanism by which bFGF protects neurons from insults such as hypoxia is not known, it has been suggested that bFGF may act as a free radical scavenger (25).

After retinal ischaemia, excessive glutamate release is involved in RGC death (26) through the activation of N-methyl-D-aspartate (NMDA) receptors, which are present in most RGCs (27). In BMD-treated rats, the levels of glutamate in the vitreous were comparable to those of nonischaemic control eyes (22). This suggests that BMD can prevent excessive release of glutamate into the vitreous after ischaemia (22). Thus, the neuroprotective effects of α_2 agonists, such as BMD could be explained by their capacity to reduce glutamate release during hypoxia. Indeed, α_2-adrenergic receptors have been observed in the RGC layer of the retina (24, 28, 29), and activation of α_2-adrenoreceptors leads to a decrease in neurotransmitter release (30).

Long-term neuroprotection

Ninety minutes of transient ischaemia of the retina leads to the death of approximately half of the original RGC population within 1 week, and induces additional RGC loss between day 7 and day 21. The protracted loss of RGCs was substantially prevented by 0.5% BMD. Ischaemia-induced neuronal death occurs by necrosis and apoptosis in many regions of the central nervous system (31). Indeed, ischaemia-induced RGC death can be reduced with antiapoptotic agents (9, 14). It is also possible that alpha-2 adrenergic agonists might have an anti-apoptotic effect on the early events that lead to delayed ischaemia-induced RGC death.

CONCLUSIONS

In glaucoma, visual field loss due to RGC death may develop over time in a number of patients, despite treatment to maintain their IOP below risk levels. For a neurodegenerative condition such as glaucoma, therapeutic strategies aiming at blocking or slowing injury-triggered cell death may be of benefit.

In ischaemia-induced retinal damage, neuroprotection involves limitation or prevention of injury-induced RGC death. In this study BMD offered consistent neuroprotection in the rat model of ischaemia-induced retinal damage. Our present findings are in agreement with previous studies (17, 18) showing a potent neuroprotective effect of alpha-2 adrenergic agonists against transient ischaemia-induced RGC death in the adult rat retina.

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