

Alpha-2 adrenergic receptor agonists are neuroprotective in experimental models of glaucoma

L.A. WHEELER and E. WOLDEMUSIE

Department of Biological Sciences, Allergan, Inc., Irvine, California CA - USA

PURPOSE. *The glaucomas are characterized by chronic progressive ganglion cell loss over many years. A drug with neuroprotective activity should increase the resistance of retinal ganglion cells (RGC) to chronic stress or injury and therefore enhance survival. Brimonidine is a highly selective and potent alpha-2 adrenergic receptor agonist, which lowers intra-ocular pressure (IOP) and is neuroprotective. Immunohistochemistry data have shown that the specific receptor targets, the alpha-2 receptors, are located in the inner retina.*

METHODS. *Brimonidine 0.1 mg/kg given intraperitoneally promoted RGC survival compared with vehicle using the optic nerve crush model even when administered up to 24 hours before injury. Using the chronic ocular hypertensive rat model, brimonidine 1 mg/kg/day (with osmotic pump) significantly prevented the loss of RGCs when compared with vehicle or timolol. This ability was due to the neuroprotective action of brimonidine, since it did not affect IOP. In addition, brimonidine 0.1 mg/day reached concentrations in the retina of Sprague-Dawley rats within 30 minutes of injection, which was sufficient to activate the alpha-2 receptor (≥ 2 nM) and maintained these concentrations for 6 hours.*

CONCLUSIONS. *Having demonstrated that:*

- a. the specific receptor target of brimonidine is located in the retina, which is important for optic neuroprotection,*
 - b. the agent shows neuroprotective ability in animal models,*
 - c. pharmacological concentrations of the drug can be reached in the retina,*
- clinical trial has been initiated to determine whether brimonidine is neuroprotective in patients with nonarteritic ischaemic optic neuropathy. Eur J Ophthalmol 2001; 11 (Suppl 2): S30-S35*

KEY WORDS. *Alpha-2 adrenoreceptor agonist, Brimonidine, Glaucoma, Neuroprotection, Neuroprotective signalling pathways*

INTRODUCTION

The glaucomas are a family of diseases of the eye that can be characterised by a progressive retinal ganglion cell (RGC) loss. Elevated intra-ocular optical pressure (IOP) is one of several primary risk factors (1). In healthy eyes, the RGCs and neighbouring cells maintain a balance between intrinsic cell survival signals and neurotoxic signals that result in cell death. However, in

glaucomatous eyes, stress or neurotoxic factors may tip the balance towards the loss of RGC cells through an apoptotic mechanism (1–6). Neuroprotection of the optic nerve through enhancing RGC survival (by enhancing survival pathways) and/or prevention of RGC death (by interfering with neurotoxic signals) are important therapeutic approaches to enhance the ability of these cells to resist stress and survive (7).

Highly selective alpha-2 adrenergic receptor agonists

represent a class of agents that not only lower IOP and have shown neuroprotective activity in the laboratory. Alpha-2 adrenergic receptor agonists have previously been demonstrated to be neuroprotective in animal models of stroke involving cerebral ischaemia (8, 9). Brimonidine has previously been shown to enhance survival of retinal neurons after many types of injuries and insults in various animal models, including calibrated optic nerve crush (10, 11) and chronic ocular hypertension (12, 13) (Tab. I). Brimonidine may act through stimulating growth factor production (e.g. basic fibroblast growth factor) as well as survival pathways (i.e. intracellular kinases and survival genes e.g. bcl-2) and inhibiting cell death pathways (14, 15). The studies mentioned above suggest that activation of the alpha-2 receptor and its signalling pathways offer the potential to protect RGCs from injury. Current and novel neuroprotective agents need to be evaluated in models to determine their potential usefulness before testing in human clinical trials. Important criteria to evaluate neuroprotective potential are:

- Target receptor should be present in the retina and/or optic nerve
- Interaction of the drug with the receptor must enhance neuronal survival or decrease injury
- The drug must achieve pharmacological concentrations at the retina after clinical dosing

Data are presented here that further support the consistent neuroprotection potential of brimonidine in models of retinal and optic nerve injury, and describe how brimonidine meets the above criteria that provide the basis for human clinical trials.

TABLE I - ANIMAL MODELS IN WHICH BRIMONIDINE HAS SHOWN NEUROPROTECTIVE ACTIVITY

Model
• Optic nerve crush (10, 11)
• Ocular hypertensive rat (12, 13, 32)
• Pressure induced ischaemia (25, 26)
• Vascular ischaemia (27)
• SOD-1 over-expressing mice (28)
• Light-induced photoreceptor damage (29, 30)
• Photoreceptor degeneration (31)

METHODS

Localization of alpha-2 receptors in the retina

Immunohistochemistry for the alpha-2A receptor was done as a representative of the alpha-2 receptor class. Paraformaldehyde fixed, frozen sections of rat retinal samples were used. Following overnight incubation at 4°C with antibodies for the alpha-2A subtype (supplied by Dr. J. Regan, University of Arizona, USA), tissues were washed several times and incubated with secondary antibody followed by Vectastain® ABC for colour development. They were counter-stained with methyl green. In control samples, primary antibody was omitted.

Optic nerve crush

Sprague-Dawley rats were anaesthetized with a mixture of ketamine, acepromazine and xylazine. Calibrated optic nerve injury was applied as described previously (11). Brimonidine (n=8) (0.1 mg/kg) was applied by intra-peritoneal (IP) injection, at 24 or 14 hours before optic nerve injury or immediately after injury (time 0). Control animals (n=6) received vehicle at each time point. Retinal ganglion cell degeneration was evaluated 12 days later by retrograde labelling at a cut end of the optic nerve (2-3 mm from the globe) with rhodamine-labelled dextran (11, 16). Fluorescently labelled ganglion cells were counted in eight designated regions.

Chronic ocular hypertension

IOP was increased by laser photocoagulation of episcleral and limbal veins on days 0 and 8. Drugs were applied by osmotic pump implanted subcutaneously on the back at the time of first laser treatment. Brimonidine and timolol were given at 1 and 2 mg/kg/day, respectively. Control animals received vehicle. Animals were treated for 3 weeks. IOP was measured weekly with TONO-PEN. As in the optic nerve crush model, ganglion cell damage or survival was evaluated by retrograde transport of rhodamine-labelled dextran at the end of the experiment.

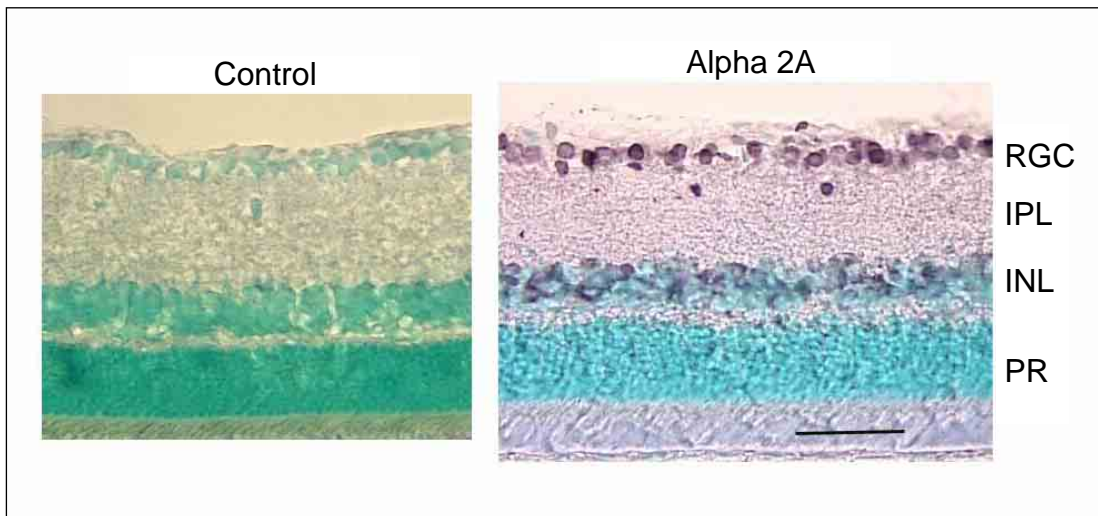


Fig. 1 - Immunoreactivity of the alpha 2A adrenergic receptors (right panel) is localized mainly in the somas of the cells in the ganglion cell layer and some cells in the inner nuclear cell layer. Left panel shows control staining where primary antibody was omitted. RGC, ganglion cells; IPL, inner plexiform layer; INL, inner nuclear layer; PR, photoreceptors. Scale bar = 50 μ m.

RESULTS

Localization of alpha-2 receptors in the retina

Following incubation with antibodies directed against the alpha-2A receptor subtype, the ganglion cell layer was stained suggesting that the specific target of brimonidine, is located in the inner retina (Fig. 1). Some cells in the inner nuclear layer, possibly amacrine cells, were also immunopositive.

Optic nerve crush

By using the optic nerve crush model, it was found that brimonidine (0.1 mg/kg) injected IP at the time of injury (time 0) or 14 hours before injury resulted in RGC survival of two-fold compared with those animals who received vehicle (Tab. II). A significantly greater number of surviving cells was also observed when brimonidine was administered 24 hours before injury. After injection of 0.1 mg/kg, brimonidine reached a peak concentration of 35 nM in the retina after 30 minutes and stayed above 2 nM for 6 hours. Alpha-2 receptors can be activated by brimonidine of ≥ 2 nM concentration (17).

Chronic ocular hypertension

Laser treatment of the episcleral and limbal veins increased the IOP two-fold. This resulted in a subsequent loss of RGCs. After three weeks of elevated

IOP, there was a 34 ± 3 % decrease in RGCs in the vehicle-treated rats (Tab. III) compared to a loss of only 16 ± 3 % when the rats were treated with brimonidine (1 mg/kg/day). Rats treated with timolol 2 mg/kg/day lost 32 ± 3 % of RGCs, this was comparable with loss of RGCs seen in vehicle-treated rats.

TABLE II - SURVIVAL OF RETINAL GANGLION CELLS AFTER OPTIC NERVE INJURY

Treatment	RGC survival ratio (treated/control)
Vehicle	1
Brimonidine 0.1 mg/kg at time 0* \ddagger	(2.2 \pm 0.1)
Brimonidine 14 hours before injury	(1.9 \pm 0.3)

*Intraperitoneal administration of brimonidine at the time of optic nerve injury

\ddagger p < 0.01 vs vehicle. Values are expressed as ratio of treated over control. Results are mean \pm S.E. from 6 to 8 animals

TABLE III - NEUROPROTECTIVE POTENTIAL OF BRIMONIDINE IN THE CHRONIC OCULAR HYPERTENSIVE RAT AFTER ELEVATION OF IOP

Treatment	RGC loss (%)
Control	0
Vehicle	34 \pm 3
Brimonidine (1 mg/kg/day)	16 \pm 3
Timolol (2 mg/kg/day)	32 \pm 3

Results are mean \pm S.E. from 10 animals

DISCUSSION

Receptor specificity in the retina is important for the success of a drug as a neuroprotective agent. Such specificity can minimize the potential for side effects (18). The immunohistochemistry data presented here confirm that alpha-2 receptors are present in the rat retina in locations that are important for optic neuroprotection. Radioligand binding studies have shown previously that alpha-2 receptors are present in the human retina (19). Further support has been provided with functional studies in which alpha-2 receptor antagonists blocked the retinal neuroprotection induced by agonists such as brimonidine (11).

Optic nerve crush is an axonal injury where approximately 50% of the RGCs are killed or injured at the time of crush. Another 80 to 90% of the ganglion cells degenerate by Day 12. This second slow phase of cell death is thought to be a secondary neuronal degeneration (11). This is a process that may be important in glaucoma. Using the optic nerve crush model, brimonidine exhibited neuroprotective ability when given at the time of injury as well as when given up to 24 hours before injury had taken place. Ongoing research suggests that brimonidine may provide neuroprotection by enhancing RGC survival, perhaps through up-regulation of an intracellular signalling pathway that promotes neuronal survival (15, 20, 21). The chronic ocular hypertensive model mimics a more gradual or chronic death of the RGCs as seen in the glaucomas (22, 23). Neither brimonidine nor timolol had any significant effect on IOP (< 10%). Ganglion cell loss in rats treated with timolol (a beta-adrenergic receptor antagonist that lowers IOP) was similar to that treated with vehicle. Brimonidine slowed RGC loss compared with vehicle or timolol. The protective effect of brimonidine on RGCs in this model may be due to its neuroprotective ability since IOP was not significantly lowered.

The studies described above support the potential of brimonidine as a therapeutic neuroprotective agent. The specific target, the alpha-2 receptor, is present in the retina and activation of this receptor results in the activation of neuroprotective signalling pathways.

Furthermore, brimonidine was found to reach pharmacological concentrations in the retina after dosing (17). In this study, brimonidine reached a peak concentration of 35 nM and then remained above 2 nM

for 6 hours. Previous studies have showed that a concentration of brimonidine 2 nM is needed to activate alpha-2 adrenergic receptors, while a concentration of approximately 2000 nM is needed to activate alpha-1 adrenergic receptors (17). Thus, if brimonidine is present at greater than 2 nM and less than 2000 nM in the human retina following topical application, then it has the ability to maintain neuroprotective function at the alpha-2 receptor while minimizing side effects. A recent study in patients receiving 0.2% brimonidine (Alphagan®) twice daily for 4–14 days before undergoing elective pars plana vitrectomy demonstrated that vitreous levels of brimonidine were in the pharmacological range needed to activate alpha-2 receptors but below that needed to activate alpha-1 receptors (24).

Brimonidine has demonstrated potential utility for neuroprotection in laboratory experiments meets the first three criteria for a retinal/optic nerve neuroprotective agent. Therefore, Allergan has initiated clinical trials testing of brimonidine in a randomized clinical trial in patients with nonarteritic ischaemic optic neuropathy. In nonarteritic ischaemic optic neuropathy, the axons of retinal ganglion cells are injured. The neuroprotective potential of brimonidine can therefore be assessed in this group of patients as a proof of principle study.

Reprint requests to:
Larry A. Wheeler, MD
Department of Biological Sciences
Allergan, Inc.
2525 Dupont Drive
Irvine, CA 92612, USA
Wheeler_Larry@allergan.com

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