Experimental and Clinical Evidence for Brimonidine as an Optic Nerve and Retinal Neuroprotective Agent

An Evidence-Based Review

Meredith Saylor, BA; Linda K. McLoon, PhD; Andrew R. Harrison, MD; Michael S. Lee, MD

Objective: To review the available evidence for the neuroprotective qualities of brimonidine tartrate in optic nerve and retinal injury.

Methods: References for this study were obtained by running a search of the PubMed database using keywords brimonidine, neuroprotection, ischemic optic neuropathy, and α2-adrenergic agonists. References focusing on ocular hypertension were excluded.

Results: Forty-eight articles addressing 1 of 4 criteria for neuroprotection were included. The literature confirms that brimonidine therapy meets the first 3 criteria for neuroprotection: receptors on its target tissues, adequate penetration into the vitreous and retina at pharmacologic levels, and induction of intracellular changes that enhance neuronal resistance to insults or interrupt apoptosis in animal models. Brimonidine did not meet the final neuroprotective criterion of success in humans.

Conclusions: Experimental evidence has demonstrated that brimonidine is a potential neuroprotective agent. However, to date, clinical trials have failed to translate into similar efficacy in humans.


The main objective of neuroprotective treatments for optic neuropathies is to increase retinal ganglion cell survival.1 To be deemed neuroprotective, an agent must meet the following 4 criteria: (1) receptors on its target tissues such as the optic nerve or retina, (2) adequate penetration into the vitreous and retina at pharmacologic levels, (3) induction of intracellular changes that enhance neuronal resistance to insult or interrupt programmed cell death mechanisms (apoptosis) in animal models, and (4) demonstration of similar efficacy in clinical trials.2-5

Brimonidine tartrate (Alphagan; Allergan, Inc, Irvine, California) is a highly selective α2-adrenergic agonist with weak α1 activity.10 Several animal studies11-15 demonstrated the presence of α2 receptors in the retina or optic nerve head, laying the foundation for the potential neuroprotective role of bri-

Author Affiliations:
Departments of Ophthalmology (Ms Saylor and Drs McLoon, Harrison, and Lee), Otolaryngology (Dr Harrison), and Neurology and Neurosurgery (Dr Lee), University of Minnesota, Minneapolis.


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Investigations revealed that a minimum concentration of brimonidine must adequately penetrate through the anterior neural layer and amacrine cells in rats. Rabbit eyes express 3 subtypes of $\alpha_2$ receptors, as do monkeys and humans. Radioligand-binding studies similarly documented the existence of $\alpha_2$ receptors in porcine and bovine retinas, however, studies have yet to prove the presence of these receptors in the human optic nerve head.

When $\alpha_2$-receptor agonists clonidine and xylazine are administered, they specifically increase the activation of the extracellular-regulated kinase pathway in Muller cells in the retina and inhibit cyclic adenosine monophosphate production. Endogenous ligands modulate dopamine-containing neurons in the retina via these $\alpha_2$ receptors. Findings from Hadjicostantinou et al and other evidence demonstrate that these $\alpha_2$ receptors are able to bind endogenous ligands and specific $\alpha_2$-receptor agonists and antagonists and that this binding exerts measurable effects in the treated retinas.

Treating the retina with $\alpha_2$-adrenoreceptor agonists results in the attenuation of decreased compound action potential after optic nerve injury. Other experiments demonstrated that $\alpha_2$ antagonists such as rauwolscine and yohimbine hydrochloride specifically impede the neuroprotective effects of $\alpha_2$ agonists in models of optic nerve and photoreceptor injury, respectively. This finding suggests that the receptors are present within the retina and that the activation of such receptors alters several signaling pathways in the retina. In addition, the activation of these receptors seems to have a role in neuroprotective activity after optic nerve or retinal injury.

**ADEQUATE PENETRATION INTO THE VITREOUS AND RETINA AT PHARMACOLOGIC LEVELS**

Brimonidine must adequately penetrate through the anterior structural barriers of the eye, including the cornea, conjunctiva, and sclera, to reach the posterior vitreous and retina at pharmacologic doses. Preclinical investigations revealed that a minimum concentration of 2nM brimonidine is needed to activate $\alpha_2$ receptors, which equates to 0.88 ng/mL. Animal findings demonstrated that the aqueous concentrations of brimonidine in albino and pigmented rabbits after topical administration ranges between 0.03 and 2.2 ng/mL during a 6-hour period after topical administration. After single and multiple dosing, topically applied brimonidine distributes into the posterior segment of monkey and rabbit eyes at concentrations sufficient to activate $\alpha_2$ receptors. Specifically, after multiple topical administrations of brimonidine tartrate (0.5% [5 mg/mL]) in monkey and rabbit eyes, the dose-normalized concentrations in the vitreous were 82 nM and 172 nM, respectively. Intraperitoneal injection of brimonidine tartrate (0.5 mg/kg) in rats revealed parallel results; peak vitreal humor concentration was 22 nM, and peak neuroretinal concentration reached 138 nM. Each of these concentrations exceeds the 2-nM concentration required for $\alpha_2$-receptor activation within the target tissues.

Similarly, findings in humans showed that topical instillation of brimonidine tartrate (0.2%) 2 to 3 times daily for 4 to 14 days before testing yields brimonidine concentrations in the vitreous well above the level necessary to activate $\alpha_2$ receptors in the retina. Twelve hours after the last dosing, the mean (SD) brimonidine concentration among 5 patients with pseudophakia was 14.9 (8.1) nM. The overall mean brimonidine concentration in this study was 185 nM. Results of animal studies suggest that brimonidine binds to melanin and maintains its peak concentration in the ciliary body for 6 hours after a single dose. This may explain the significant vitreal concentrations detected at 10 to 14 hours after the last dose in humans. These findings indicate that topically applied brimonidine reaches adequate intraocular concentrations for neuroprotection to be possible.

**ENHANCEMENT OF NEURONAL RESISTANCE TO INSULT AND INTERRUPTION OF APOPTOSIS**

Experimental models suggest that brimonidine confers neuroprotection in several types of ocular injury, including ischemia-induced injury, optic nerve compression or optic nerve crush injury, photoreceptor degeneration, and ocular hypertension and glaucoma. We will exclude discussion of glaucomatous or ocular hypertension–induced injury, as it is difficult to extrapolate the neuroprotective effects of brimonidine treatment independent of its role in reducing intraocular pressure.

Several experimental animal models demonstrated the neuroprotective effects of topically and systemically administered brimonidine in reducing the effects of optic nerve injury, as measured by decreased apoptosis or enhanced retinal ganglion cell survival. Topical application of brimonidine 1 hour before partial optic nerve crush or ischemic optic nerve injury in rats is effective in decreasing apoptosis in retinal ganglion cells, as indicated by a large decrease in terminal deoxynucleotidyl transferase–mediated biotin–deoxyuridine triphosphate nick-end labeling (TUNEL) staining. After ischemic injury caused by 60- or 90-minute ligation of the ophthalmic artery, systemic administration of brimonidine rescued 26% to 33% of the retina ganglion cell population, while topical administration prevented up to 55% of retinal ganglion cell loss, allowing almost 100% survival of the retinal ganglion cells. The effect of brimonidine treatment was shown to be dose dependent and $\alpha_2$-adrenergic receptor specific.

Using the ophthalmic vessel ligation method of optic nerve ischemia, brimonidine was administered to rats 1 hour before 90-minute ligation. This single dose of topical or systemic brimonidine rescued 42% of retinal ganglion cells 7 days after the induction of ischemia. This neuroprotective effect persisted at 21 days, suggesting that brimonidine treatment could be neuroprotective during the second phase of apoptosis seen in these types of optic nerve injury. These findings were confirmed and extended. In another study, topical pretreatment with brimonidine 1 hour before transient ligation of the ophthalmic vessels in rats significantly protected against ischemia-induced damage of the inner and outer nuclear layers morphometrically and functionally after 3 months. In addition, this brimonidine treatment prevented degeneration of retinotectal projections. Eight-four per-
percent of the volume of retinotectal projections was preserved in the brimonidine-treated rats compared with 50% in the rats that received ischemic optic nerve injury and no drug treatment.30

The long-term neuroprotective activity of brimonidine was examined when topically administered before and after ischemic insult produced by a photocogulopathy method.30 These data support that pretreatment topically with brimonidine 7 days before photocogulation-induced optic nerve ischemia in rats can prevent long-term (5 months) neuronal and axonal loss. A limited and variable degree of ganglion cell rescue resulted from treatment with topical brimonidine for 14 days, starting after the ischemic insult. This contrasts findings by Lafuente et al,28 who found that intraperitoneal brimonidine administered 4, 24, or 72 hours after the onset of transient ischemia and reperfusion had no effect on retinal ganglion cell survival. Differences in these 2 studies could be because of differing methods of producing ischemia, various routes of brimonidine administration, varying time between reperfusion and the initiation of treatment, or differences in the duration of treatment after injury.

In a model of endothelin 1 (ET-1)–induced chronic ischemia, topical brimonidine tartrate had a primary effect on vasoconstriction and decreased ocular blood flow.1 Endothelin 1 acts via the ETₐ and ETₐ receptors, which are present in the retina, optic nerve, and optic nerve head of rabbits and humans.37 Therefore, there is ample evidence that after optic nerve injury produced by several methods brimonidine treatment has the ability to be neuroprotective to the injured ganglion cells.

The exact mechanism of the role of brimonidine in neuroprotection remains unclear. Several studies proposed possible mechanisms for the ability of the drug to prolong cellular survival and function and attenuate apoptosis. For example, degeneration of optic nerves in rats leads to elevated intraocular levels of excitotoxins, including aspartate and glutamate.38 Activation of the α₂-adrenergic receptors by brimonidine reduced levels of intravitreal glutamate secondary to ischemia in the rat.24 Several studies23,39–41 demonstrated that intravitreal administration of brimonidine in rats results in elevated levels of neurotrophic factors, including brain-derived neurotrophic factor and fibroblast growth factor. In vitro and after intravitreal injection, these factors are important in preserving retinal ganglion cells after various forms of injury.42 Activation of α₂-selective adrenergic agonists by brimonidine also has been shown to upregulate intrinsic cell survival signaling pathways and antiapoptotic genes such as Bcl-2 and BCL-Xₐ.4 The neuroprotective properties of brimonidine are likely multifactorial.

EFFICACY OF NEUROPROTECTION IN CLINICAL TRIALS

Despite the accumulation of medical literature regarding the various aspects of nonarteritic anterior ischemic optic neuropathy (NAION) and Leber hereditary optic neuropathy in humans, little is understood about these diseases. NAION has no effective treatment; therefore, the potential of brimonidine for treatment of this ischemic injury is exciting. Even in the absence of controlled clinical trials, physicians prescribed brimonidine and α agonists as treatment for NAION. This perhaps stems from the substantial experimental evidence demonstrating the efficacy of brimonidine as a neuroprotective agent in animal models of ischemia. However, the results of clinical investigations suggest that brimonidine treatment has failed to meet the fourth and final criteria for neuroprotection, namely, similar efficacy in human clinical trials.

Two studies examined the effectiveness of brimonidine as a treatment for NAION in humans, one retrospective43 and the other prospective.44 Both studies examined the potential effectiveness of brimonidine treatment given, on average, 1 week after the NAION attack and examined alterations in visual acuity as their primary endpoint. Neither study demonstrated neuroprotective efficacy of brimonidine as found in experimental animal models. Fazzzone et al45 performed a retrospective study of 31 patients with NAION evaluated within 3 weeks of the onset of visual loss. Fourteen patients received topical brimonidine tartrate up to 4 times daily within 14 days of the onset of visual loss, while the other 17 patients were demographically matched to the treatment group and served as control subjects. No positive effects of brimonidine treatment were seen in this small group of patients. The authors reported a trend toward worse outcomes at 8 weeks’ follow-up in the brimonidine-treated group for visual fields, color vision, and visual acuity testing. However, no statistical differences were seen, suggesting that the power of the study was low because of few patients and the lack of control over which patients received brimonidine treatment and which did not.

A double-masked randomized placebo-controlled trial assessing the efficacy and tolerability of brimonidine tartrate (0.2%) for the treatment of NAION was undertaken.44 Unlike in the study by Fazzzone et al,43 the results did not indicate any negative effects of brimonidine use in patients with NAION. There seemed to be a slight nonsignificant improvement in visual fields for the treatment group compared with the control group. No serious adverse effects or events were noted. Ultimately, the results were inconclusive, and a statistically significant advantage for the patients receiving brimonidine was not demonstrated. After unmasking the data, the investigators chose to halt the trial because of poor recruitment.44 Again, treatment was begun within the first week after visual loss but not immediately after the first sign of NAION.

Studies involving other optic neuropathies such as Leber hereditary optic neuropathy reported similar unsuccessful results in clinical trials. Topical treatment with brimonidine purite (0.15%) 4 times daily to the unaffected eye for up to 2 years did not prevent second eye involvement in 9 patients with recently documented monocular vision loss from Leber hereditary optic neuropathy.45 A recent prospective placebo-controlled, double-masked, randomized clinical trial of 17 patients with retinal dystrophies also found no statistically significant results in favor of brimonidine treatment; however, a nonsignificant trend was found, suggesting slower progression of visual field loss in eyes treated with topical brimonidine (0.2%).46
Topical brimonidine exhibited a positive effect on reducing collateral damage caused by laser photoagulation for choroidal neovascularization in a small human study.47 Contrary to the previously discussed trials, the patients received brimonidine before laser treatment and for 1 month after laser treatment. This is consistent with previous findings of ischemia in rats showing that brimonidine treatment is most efficacious before the injury.30 Nevertheless, clinical shortcomings such as small sample size and inconclusive results warrant further research.

WHY CLINICAL MODELS MAY HAVE FAILED

Several differences exist between the experimental and clinical models, as well as unique challenges when working with human subjects. This may explain the failure of clinical results, despite the successes of diverse animal models in demonstrating the neuroprotective effects of brimonidine treatment. For instance, earlier treatment in the clinical trials may be required. The most notable successes in experimental models involved groups in which treatment with brimonidine preceded the optic nerve injury.30,37 However, the annual incidence of NAION is 5 cases per 100,000. Among subjects who experience an attack of NAION, 15% to 25% will experience an attack in the fellow eye within the next 5 years. Practically speaking, this precludes a large-scale trial using brimonidine before the ischemic injury. Other data in animal models showed that posttreatment with brimonidine yields mild to moderate effects on ganglion cell survival.30 These findings suggest that a window of time exists between the initial phases of injury and the final phases of cell death. During this time frame, posttreatment with brimonidine may represent other avenues for enhancing the effectiveness of treatment.

Another explanation for discrepancies may relate to differences between species. For example, there are multiple types of α2-adrenergic receptors. Rabbit eyes possess all 3 subtypes (α2A, α2B, and α2C) of α-adrenergic receptors, but immunofluorescence labeling indicates that the anterior segment of the eye possesses only α2B and α2C receptors.16 However, all 3 subtypes have been cloned from humans. In fact, radioligand-labeling investigations reveal that α2C-adrenergic receptors are the most prominent subtype in the iris, neurosensory retina, human ciliary body, and retinal pigment epithelium–choriocapillaries.18 Nevertheless, α2C-adrenergic receptors of animals and humans have different molecular and structural characteristics; variations in the concentrations of the receptors on the target tissues of animals vs humans also exist. Furthermore, there are differences between species in the microcirculation of the optic nerve head in animals and humans.30 Each of these differences serves as a challenge when attempting to translate animal model successes into effective clinical applications.

CONCLUSIONS

The literature confirms that brimonidine meets the following 3 criteria for neuroprotection: (1) receptors on its target tissues such as the optic nerve or retina, (2) adequate penetration into the vitreous and retina at pharmacologic levels, and (3) induction of intracellular changes that enhance neuronal resistance to insults or interrupt programmed cell death mechanisms (apoptosis) in animal models. However, the achievements in animal models regarding the neuroprotective effects of brimonidine in treating ischemic optic nerve injury have not translated into effective clinical applications. The fourth criterion, demonstration of similar efficacy in clinical trials, has yet to be met.

Ultimately, these differing data and the lack of efficacy in clinical applications warrant additional studies. Potential focuses for these studies include the following: the cellular and molecular differences between ischemia-induced optic nerve injury in animals vs humans, the specific cellular and molecular mechanisms underlying the neuroprotective activity of brimonidine, a decrease in the time between disease onset and treatment, and perhaps new modes of delivery of neuroprotective agents and neurotrophic factors.

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Correspondence: Michael S. Lee, MD, Department of Ophthalmology, University of Minnesota, 420 Delaware St SE, Mayo Mail Code 493, Minneapolis, MN 55455 (mikelee@umn.edu).

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