Preclinical Evaluation of Brimonidine

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Abstract. Preclinical studies of brimonidine show that it is a potent α_2-adrenoceptor agonist that is 1000-fold more selective for the α_2- vs. the α_1-adrenoceptor, and is 7–12-fold more α_2-selective than clonidine and 23- to 32-fold more α_2-selective than apraclonidine (*p*-aminoclonidine). Brimonidine decreased intraocular pressure (IOP) in various animal models but, unlike apraclonidine, brimonidine was not mydriatic. The site and pharmacology of the IOP response depends on the animal species. In rabbits, the IOP response to brimonidine is mediated by an ocular α_2-adrenoceptor while in monkeys, a central nervous system (CNS) 'imidazoline' receptor appears to be involved. Brimonidine decreased IOP by suppressing the rate of aqueous humor flow and enhancing uveoscleral outflow. Topical brimonidine resulted in posterior segment drug levels adequate to activate α_2-adrenoceptors, but was not vasoconstrictive in a model designed to assess the vasoactivity of the human retinal microvasculature. Brimonidine protected the rat optic nerve from secondary damage following mechanical injury to the optic nerve and was nontoxic in an array of experiments designed to evaluate ocular and organ toxicity. Taken together, the high α_2-adrenoceptor selectivity, ocular hypotensive efficacy, retinal bioavailability and neuroprotective properties make brimonidine an important addition to the field of antiglaucoma agents. (**Surv Ophthalmol 41 [Suppl 1]:** S9–S18, 1996)

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Alpha-adrenoceptor agonists have played a significant role in the medical management of glaucoma. Epinephrine, a nonselective adrenoceptor agonist, has been the mainstay of glaucoma therapy for decades.[53] Clonidine, the first α_2-adrenoceptor agonist to be marketed for the treatment of glaucoma, has been available for over 15 years in some countries. It is a highly effective IOP-lowering agent, but its use has been greatly limited by significant systemic side effects, which include sedation and systemic hypotension. The systemic side effects that are observed with clonidine appear to be directly related to its ability to penetrate into the central nervous system.[26–28,34]

Apraclonidine, also known as *p*-aminoclonidine and Iopidine®, is a hydrophilic analog of clonidine. It is the first α_2-adrenoceptor agonist approved for the treatment of intraocular pressure (IOP) elevation associated with anterior segment laser surgery, and for short-term therapy to delay surgery in patients with uncontrolled IOP receiving maximally tolerated medical therapy.[37] It is also an effective IOP-lowering agent. Apraclonidine has fewer of the central nervous system effects associated with clonidine; however, apraclonidine has a high affinity for the α_1-adrenoceptor, which results in ocular side effects such as mydriasis,[18,21] conjunctival blanching,[1,21,49] ciliary vasoconstriction,[23,24,58] eyelid retraction[21] and reduction in conjunctival oxygen tension.[50] Long-term use of apraclonidine has been associated with a high incidence of ocular allergies,[16,48] which may be related to its oxidative lability and the reactivity of hapten-forming intermediates.[43]

Brimonidine, also known as UK-14,304 and AGN 190342, is a less lipophilic analog of clonidine,[19] and, like apraclonidine, provides clinically significant lowering of IOP in humans. Brimonidine's molecular structure, however, differs from that of apraclonidine and this gives brimonidine higher α_2-adrenoceptor selectivity and a lower potential for hapten formation via oxidative metabolism.[43]

This review will focus on the receptor and ocular pharmacology of brimonidine, its mode of ocular hypotensive action, and posterior segment effects. Comparisons will be made to clonidine and apra-

clonidine where data are available. Comparisons of clonidine and brimonidine or clonidine and apraclonidine are available in the literature, but not among all three agents. In addition, binding constants can be altered by a number of variables. The following studies compared all three agents under identical conditions to better evaluate similarities and differences.

Brimonidine

RECEPTOR PHARMACOLOGY

Brimonidine's α_2-adrenoceptor selectivity is well known. Hundreds of scientific articles have been published in which brimonidine was used as a reference α_2-adrenoceptor agonist. It is considered a standard reference compound because of its high α_2-adrenoceptor selectivity.[17] Brimonidine's receptor selectivity and affinity were compared with apraclonidine and clonidine in radioligand binding and tissue bath bioassays.[10,11,15,61] Radioligand binding assays were conducted with [^{3}H]prazosin and [^{3}H]rauwolscine to label α_1- and α_2-adrenoceptors in the human cerebral cortex and the human colonic cell line (HT-29), respectively. Tissue bath bioassays were used to measure α_1-adrenoceptor activation in the isolated rabbit iris dilator muscle and α_2-adrenoceptor stimulation in the isolated rabbit vas deferens. Stimulation of the α_1-receptors in the rabbit iris dilator muscle causes mydriasis.[33] Stimulation of the prejunctional α_2-receptors in the rabbit vas deferens inhibits the electrically-induced contractile response.[35]

The combined data from these assays show that brimonidine is 23–32-fold more α_2-adrenoceptor selective than apraclonidine and is 7–12-fold more selective than clonidine (means are depicted in Fig. 1). The affinity of brimonidine was highest (1–2 nM) for the α_2 receptor and lowest (1850–2650 nM) for the α_1 receptor. These results suggest that compared to clonidine and apraclonidine, brimonidine would be the least likely to produce α_1-adrenoceptor-mediated side effects, such as mydriasis and ocular vasoconstriction.

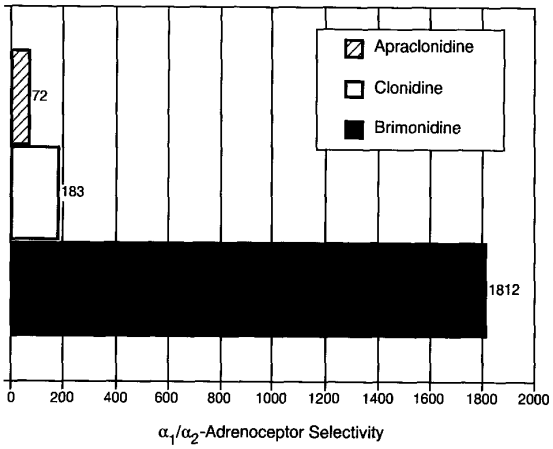

Fig. 1. Mean α_2- vs α_1-adrenoceptor selectivity or brimonidine, clonidine and apraclonidine in radioligand binding and tissue bath functional assays.[10,11,14,60] Data are derived from ratios of α_1 and α_2 affinity constraints (Ki) in binding assays and EC_{50}'s in functional assays. In binding assays, α_1-adrenoceptors in the human cerebral cortex and α_2-adrenoceptors in the human colonic (HT-29) cell line were labeled with [^{3}H]prazosin and [^{3}H]rauwolscine, respectively. In functional assays, α_1-adrenoceptor activity was determined by contraction of the rabbit iris dilator muscle; α_2 activity was measured by inhibition of electrically-induced contractions in the rabbit vas deferens. Brimonidine was 28 times more α_2-selective than apraclonidine and 10 times more selective than clonidine.

OCULAR PHARMACOLOGY

The ocular hypotensive effect of brimonidine has been demonstrated in animal models including normotensive and ocular hypertensive rabbits and monkeys and in cats. In some of the following studies, brimonidine concentrations are expressed as the tartrate salt, as in the clinical studies. In others, the concentration of brimonidine is expressed as the base, which is 66% of the salt.

Normotensive Rabbits: Single-Drop Studies

In normotensive rabbits, brimonidine concentrations ranging from 0.0001% to 0.5% applied in single drop studies, resulted in concentration-related IOP lowering.[9,14] Brimonidine 0.1% was at the top of the concentration response curve, and produced a peak ocular hypotensive response of 5.1 ± 0.6 mm Hg (mean ± sem). A contralateral IOP-lowering response occurred at concentrations of 0.1% or greater. The concentration response curves for brimonidine, clonidine and apraclonidine show that brimonidine is at least 100-fold more potent in decreasing IOP than apraclonidine.[15] At equivalent concentrations (0.1%), brimonidine had a longer duration of ocular hypotensive activity than apraclonidine (Fig. 2A). The marginal ocular hypotensive response to apraclonidine in the rabbit is due, in part, to the unique sensitivity of this species to α_2-adrenoceptor stimulation. In rabbits, α_1-adrenoceptor stimulation increases IOP while α_2-adrenoceptor stimulation decreases IOP.[12,29,44] Apraclonidine has high affinity for α_1-adrenoceptors and consequently has a lesser ocular hypotensive response.

Normotensive Rabbits: Chronic Administration

The chronic administration of brimonidine in

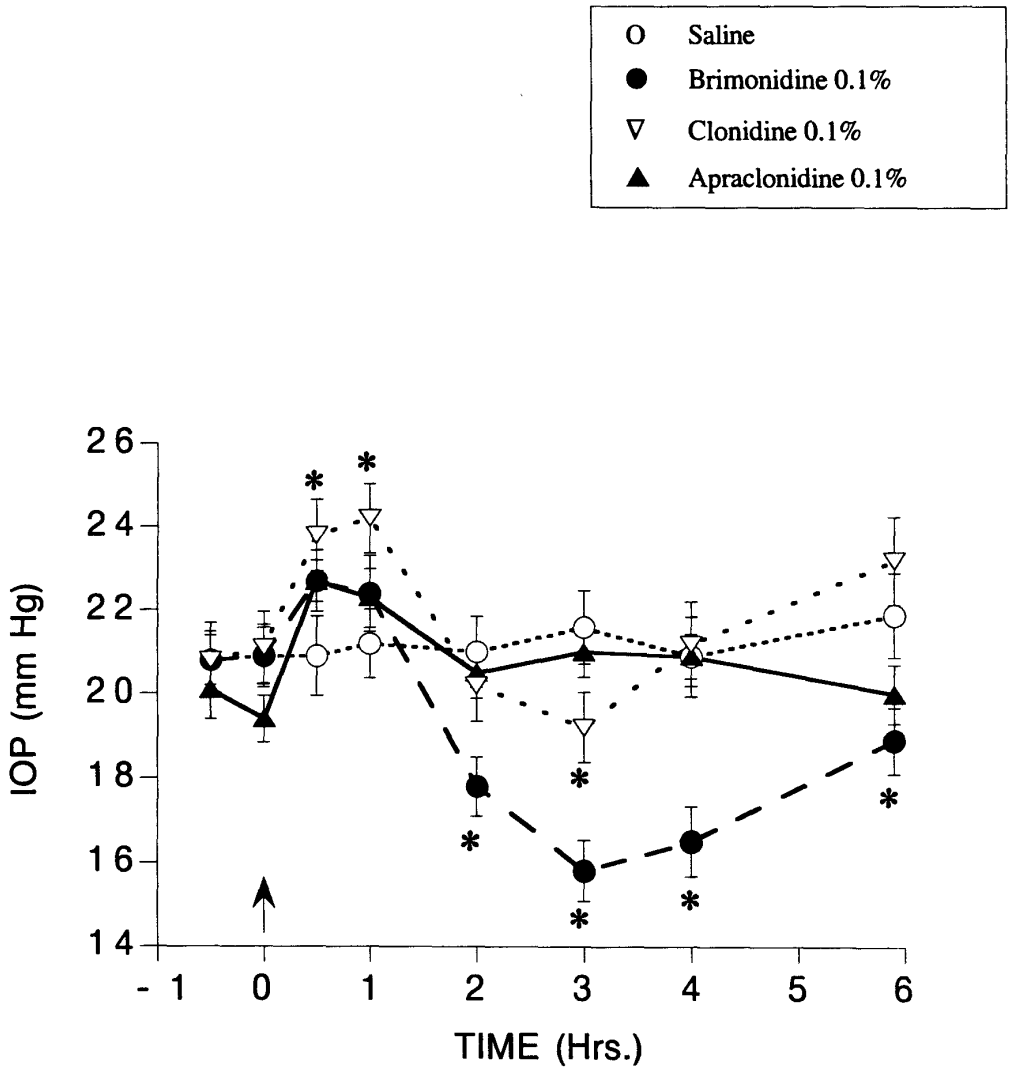

Fig. 2A. The IOP responses to saline (○; n = 16), and 0.1% concentrations of brimonidine (●; n = 28), clonidine (∇; n = 21) and apraclonidine (▲; n = 10) in conscious normotensive New Zealand white (albino) rabbits. Data are expressed as mean ± sem. Drugs were applied unilaterally as a single 50 μl eyedrop. Intraocular pressure was measured noninvasively with a 30R model Digilab pneumatonometer. Twenty-five microliters of an anesthetic (proparacaine) was topically applied before IOP measurements to minimize ocular discomfort due to tonometry. Two baseline measurements were made prior to instillation of the drugs, followed by periodic measurements up to 6 hours post-instillation. Asterisks indicate a significant difference from saline control; $p < 0.05$, unpaired Student's t test. Brimonidine was more effective at lowering IOP than clonidine or apraclonidine.

normotensive rabbits does not result in a loss of the ocular hypotensive response over time. The ocular hypotensive response to concentrations ranging from 0.08–0.8% at the end of 6 months of twice-daily dosing was equivalent to or greater than the initial response.[12] The maximum IOP decreases for the 0.08%, 0.2%, 0.5% and 0.8% concentrations were 3.9 ± 0.4, 5.1 ± 0.4, 6.9 ± 0.3, and 7.1 ± 0.4 mm Hg, respectively. Maintenance of the ocular hypotensive response is supported by biochemical evidence, which shows that α_2-receptor-linked inhibition of cyclic adenosine monophosphate (cAMP) in rabbit iris/ciliary body does not undergo receptor desensitization with the chronic administration of brimonidine. Taken together, these results show that tolerance does not develop to the chronic administration of brimonidine in rabbits.[12]

Ocular Hypertensive Rabbits

In addition to its ocular hypotensive effect in nor-

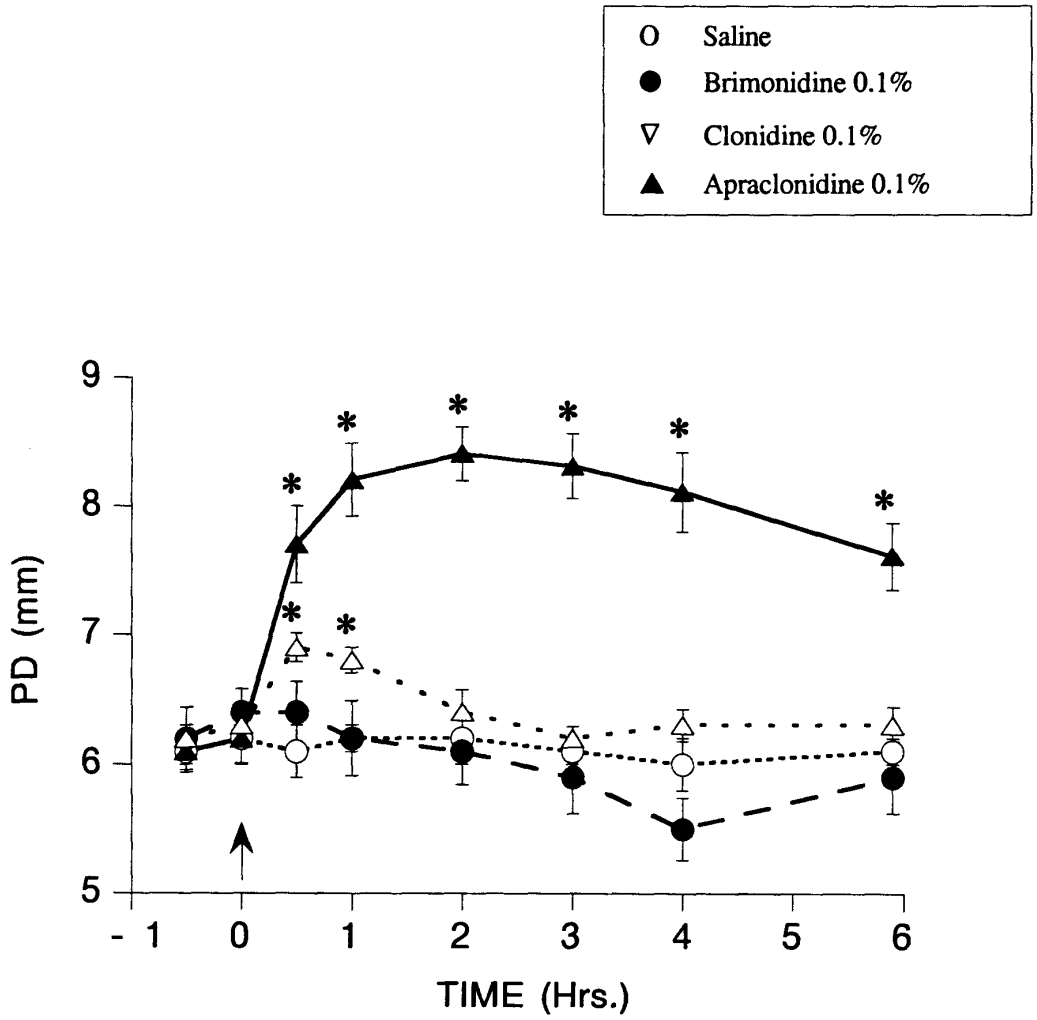

Fig. 2B. The pupil diameter responses to saline (○; n = 16), and 0.1% concentrations of brimonidine (●; n = 28), clonidine (∇; n = 21) and apraclonidine (▲; n = 10) in conscious normotensive New Zealand White (albino) rabbits. Data are expressed as mean ± sem. Drugs were applied unilaterally as a single 50 μl eyedrop. Pupil diameter was measured noninvasively with a millimeter ruler. Two baseline measurements were made prior to instillation of the drugs, followed by periodic measurements up to 6 hours post-instillation. Asterisks indicate a significant difference from saline control; $p < 0.05$, unpaired Student's t test. Apraclonidine produced a profound and long-lasting mydriatic effect. Clonidine increased pupil size transiently and brimonidine was without effect.

motensive rabbits, brimonidine was effective in a rabbit model of ocular hypertension.[14] Brimonidine 0.1% suppressed the rise in IOP induced by 60 mL/kg of water loading administered orally for more than 5 hours.

Normotensive Monkeys

The IOP response to unilaterally-applied brimonidine was investigated in normotensive capuchin and cynomolgus monkeys.

In capuchin monkeys,[14] a single application of 0.1% and 1.0% brimonidine decreased IOP bilaterally. The maximum IOP drop with the 0.1% concentration was 2.5 ± 0.6 mm Hg and with the 1.0% concentration, it was 4.1 ± 0.8 mm Hg. In ketamine-sedated cynomolgus monkeys, brimonidine 0.3% decreased IOP by a maximum of 4.9 ± 2 mm Hg, brimonidine 0.5% decreased IOP by 9.9 ± 1.2 mm Hg, and brimonidine 1.0% decreased it by 7.3 ± 1.5 mm Hg.[52]

In ketamine-sedated cynomolgus monkeys,[25] brimonidine 0.1%, 1%, and 2% suppressed IOP by a maximum of 12 mm Hg. In conscious cynomolgus monkeys, brimonidine 0.03%, 0.1% and 0.3% de-

creased IOP by a maximum of 3.5 ± 0.8 mm Hg, 6.3 ± 1.4 mm Hg and 9.3 ± 1.3 mm Hg, respectively.[9] The IOP response in monkeys was without evidence of the transient initial rise, which contrasts with the rabbit and underlines a species difference to α_2-adrenoceptor agonists.[9]

Ocular Hypertensive Monkeys

Brimonidine was evaluated in monkeys made unilaterally ocular hypertensive by argon laser photocoagulation of the trabecular meshwork. This experimental model is characterized by features commonly seen in human glaucoma.[46]

In one study,[52] one drop of 0.5% brimonidine reduced IOP in ketamine-sedated monkeys for 24 hours. The maximum IOP decrease was 22.3 ± 3.2 mm Hg, which occurred 4 hours after dosing. In the same study, 0.5% brimonidine was administered twice daily for 5 days, with a maximum reduction in IOP of approximately 15 mm Hg. No tolerance of the ocular hypotensive response was noted.

In another study comparing the concentration-response relationship in conscious monkeys,[15] the ranked order of potency for decreasing IOP in the hypertensive eye was brimonidine = clonidine > apraclonidine. Brimonidine was 10-fold more potent as an ocular hypotensive agent than apraclonidine in this animal model and produced a maximum IOP reduction of 12.3 ± 0.8 mm Hg with the 0.3% concentration. Clonidine and apraclonidine lowered IOP by 11.8 ± 2 mm Hg (0.3% concentration) and 8.5 ± 2 mm Hg (3% concentration), respectively.

Cats

The IOP response to brimonidine was also investigated in cats.[14] Unilateral dosing with the 0.1% concentration produced a delayed ocular hypotensive response starting at 3 hours post-dose. A maximum hypotensive effect of 6.9 ± 1.1 mm Hg was seen at 5 hours. A contralateral IOP response of 5.2 ± 1.4 mm Hg occurred at 1 hour. This cat IOP profile was similar to that of the rabbit.

PUPIL SIZE

The effect of brimonidine on pupil size was evaluated in rabbits, monkeys and cats.

Rabbits

Brimonidine had very little effect on pupil size in rabbits. Concentration response curves in rabbits show that the ranked order of potency for eliciting a mydriatic effect was apraclonidine (high effect) >> clonidine > brimonidine (no effect).[15] The pupillary responses to brimonidine, clonidine and apraclonidine at the 0.1% concentration in rabbits at 6 hours post-dosing are shown in Fig. 2B. Apraclonidine produced a profound and long-lasting mydriasis. Mydriasis is related to stimulation of α_1-adrenoceptors in the iris dilator muscle. Apraclonidine has high affinity for the α_1-receptors in this organ (see Receptor Pharmacology, *supra*). The low α_1-receptor affinity of brimonidine results in the absence of a mydriatic response at therapeutic concentrations.

Monkeys and Cats

In monkeys[9,25] and cats,[14] ocularly administered brimonidine produced miosis, which is mediated by prejunctional α_2-adrenoceptors on sympathetic nerves innervating the dilator muscle.[14] Withdrawal of sympathetic tone to the iris dilator muscle allows greater contribution of the iris sphincter to pupil size and, thus, miosis. The iris dilator muscle of higher mammals such as cats and monkeys is less sensitive to α_1-receptor stimulation than rabbits,[57] which may explain the relatively smaller mydriatic response to apraclonidine in monkeys[25] and man[1,49] compared to rabbits.

Vasoactivity

The vascular response to α_2-adrenoceptor agonists can vary according to species, tissue and location within a given vascular bed.[45] α_2-adrenoceptors can mediate vasoconstriction and play a role in the autoregulation of capillary pressure and tissue oxygen delivery.[22,40] On the other hand, brimonidine can produce vasodilation[5,7] via α_2-adrenoceptors on endothelial cells, which release endothelial-derived relaxing factor.

To assess the potential for vasoactivity in functional human retinal microvasculature, brimonidine, clonidine and apraclonidine were applied topically to retinal tissue transplanted into the hamster cheek pouch membrane, an immunologically tolerant tissue.[54] The hamster cheek pouch model is widely used to study the effects of drugs on human microvasculature.[30] The arteriolar caliber in the retinal xenografts was measured by intravital microscopy (microscopy in living systems).

Vessel caliber was unchanged by brimonidine at concentrations up to 10^{-5} M. A high concentration (10^{-4} M) reduced vessel caliber by 11.1 ± 4.2% at 1 minute, 1.4 ± 3.0% at 5 minutes, and 2.3 ± 2.6% at 10 minutes (n = 5). Clonidine evoked a marked concentration-dependent decrease in arteriolar caliber of up to 34.8 ± 6.3% with 10^{-4} M concentration (n = 6) at 1 minute. At the 10^{-7} M concentration, caliber size was decreased by 13.5 ± 3.7%. The effects for clonidine were similar at 5 minutes, and less at 10 minutes. Apraclonidine was the most potent compound in this model, producing a response

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