

DISTRIBUTION OF BRIMONIDINE INTO ANTERIOR AND POSTERIOR TISSUES OF MONKEY, RABBIT, AND RAT EYES

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ABSTRACT:

The objectives of the study were to evaluate the distribution of brimonidine (α_2 -adrenergic agonist) into anterior and posterior ocular tissues. Single or multiple doses of a 0.2 or 0.5% brimonidine tartrate solution were administered to one or both eyes of monkeys or to one eye of rabbits. Brimonidine was administered intraperitoneally to rats. After topical administration, [^{14}C]brimonidine was rapidly absorbed into the cornea and conjunctiva and distributed throughout the eye. [^{14}C]Radioactivity was higher and cleared more slowly in pigmented tissues (iris/ciliary body, choroid/retina, and optic nerve) than in nonpigmented tissues. Single and multiple dosing led to a similar drug distribution, with higher levels of brimonidine measured in pigmented tissues after multiple dosing. Most of the radioactivity extracted from ocular tissues represented unchanged brimonidine. In the rabbits and the monkey treated in

only one eye, levels of radioactivity in the untreated eye were low, consistent with the low systemic levels and rapid drug clearance. Posterior ocular tissue concentrations of radioactivity exceeded systemic blood concentrations. The vitreous humor brimonidine concentrations in monkeys treated topically with 0.2% brimonidine tartrate was 82 ± 45 nM. Vitreous levels in rabbits confirmed the penetration of brimonidine to the posterior segment. Similar concentrations of brimonidine (22 to 390 nM) were measured in the vitreous and retina of rats injected intraperitoneally with brimonidine. Both topically applied and systemically administered brimonidine reach the back of the eye at nanomolar concentrations sufficient to activate α_2 -adrenergic receptors. The brimonidine levels achieved at the retina are relevant for neuroprotection models.

Brimonidine (AGN 190342¹; Fig. 1) is a highly selective α_2 -adrenergic agonist approved for the treatment of open-angle glaucoma. Glaucoma represents a family of ocular diseases characterized by a progressive optic neuropathy and loss of retinal ganglion nerve cells (Adkins and Balfour, 1998). One of the primary risk factors for glaucoma is elevated intraocular pressure. When applied to the eye, brimonidine activates α_2 -adrenergic receptors, resulting in decreased aqueous humor production and increased uveoscleral outflow (Toris et al., 1995). These effects on aqueous humor dynamics lead to a reduction in intraocular pressure.

Laboratory studies with brimonidine suggest that activation of α_2 -receptors in the retina and/or optic nerve can promote the survival of retinal ganglion nerve cells (David, 1998). Studies show that intraperitoneal and topical administration of brimonidine promoted retinal ganglion cell survival after calibrated optic nerve compression and ischemia/reperfusion in animal models of neuronal injury (Wheeler et al., 1999, 2001; Yoles et al., 1999; Donello et al., 2001). Importantly, if the ocular instillation of brimonidine promotes retinal

ganglion cell survival in glaucomatous neuropathy, then a new therapeutic approach to glaucoma management may be indicated in which neuroprotection and intraocular pressure reduction are valued outcomes of the therapeutic regimen.

For a medication to protect the optic nerve, however, it must have access to the posterior portion of the eye. Consequently, if ocular instillation of brimonidine is to have a neuroprotective role in glaucoma, brimonidine must be present in the vicinity of retinal ganglion cells at concentrations sufficient for bioactivity. Brimonidine is a lipophilic drug with a pK_a of 7.4. Previous studies have reported the ocular penetration of brimonidine into the anterior portion of rabbit eyes after ocular dosing (Chien et al., 1990; Acheampong et al., 1995; Tang-Liu et al., 1996). Measurable brimonidine levels were recently reported in aqueous humor and vitreous humor of human eyes after topical dosing (Karamanos et al., 1999; Kent et al., 2001).

Delivery of ocular drugs to the posterior portion of the eye after topical dosing requires drug penetration through the anterior structural barriers of the cornea, conjunctiva, and sclera (Fig. 2). The posterior segment includes the posterior sclera, vitreous, retina, choroid, and optic nerve. Penetration across the cornea is proposed as the primary pathway by which drugs reach the aqueous humor and anterior segment after topical ocular administration, whereas the conjunctiva/sclera route of drug penetration is important for access to ciliary body and posterior tissues (Maurice and Mishima, 1984; Burstein and Anderson, 1985; Lee and Robinson, 1986; Grass and Robinson, 1988; Chien et al., 1990; Schoenwald, 1993). A previous *in vivo* ocular

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¹ Abbreviations used are: AGN 190342, 5-bromo-6-(2-imidazolidinylideneamino)quinoxaline; LSC, liquid scintillation counting; HPLC, high-pressure liquid chromatography; AUC, area under the concentration-time curve; Eq, equivalents.

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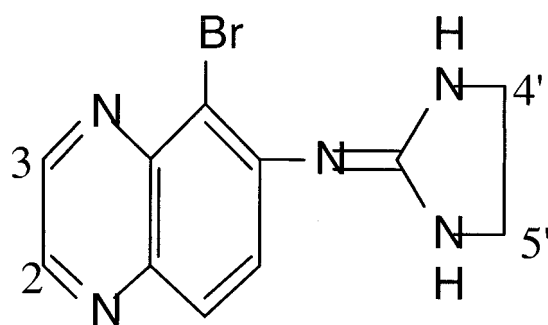


FIG. 1. Chemical structure of brimonidine.

penetration study of brimonidine in albino rabbits demonstrated that when brimonidine solution was applied within a cylindrical well in contact with the cornea surface, the rank order of tissue brimonidine concentrations was cornea > iris > aqueous humor > ciliary body > anterior sclera > conjunctiva > lens (Chien et al., 1990). The same study showed that when brimonidine was applied onto the conjunctiva, outside of the cylindrical well, the rank order of tissue concentrations was conjunctiva > cornea > anterior sclera > ciliary body > iris > aqueous humor > lens.

In the studies presented here, the absorption and distribution of brimonidine into anterior and posterior ocular tissues were investigated after topical application to the eyes of monkeys and rabbits and following intraperitoneal administration to rats. Brimonidine was observed to readily penetrate into the eye and to achieve concentrations in posterior portions of the eye sufficient to selectively activate α_2 -adrenergic receptors.

Materials and Methods

Animals. Cynomolgus monkeys (3.3–4.8 kg) were obtained from Shamrock Ltd. (Henfield, Sussex, UK). Monkeys were housed singly and maintained in a temperature- and humidity-controlled environment using a 12:12-h light/dark schedule. Food (a normal laboratory diet supplemented with bread and fresh fruit and/or vegetables) and water were available ad libitum. Monkeys were anesthetized with ketamine and pentobarbital and were deeply unconscious before sacrifice by exsanguination.

Female New Zealand pigmented rabbits (1.8–3.7 kg) were obtained from

Vista Rabbitry (Vista, CA) and Irish Farms (Norco, CA). Rabbits were individually housed and maintained in temperature-controlled rooms with a 12:12-h light/dark schedule. Food and water were available ad libitum. Rabbits were euthanized by an intravenous injection of Eutha-6 (Western Medical Supply Co., Arcadia, CA).

Male Sprague-Dawley rats (approximately 300 g) were obtained from Hazleton Biotechnologies (Vienna, VA). Rats were maintained in a temperature-controlled environment using a 12:12-h light/dark schedule. Food and water were available ad libitum. Rats were euthanized by intravenous injection of sodium pentobarbital.

The care and use of all animals was in accordance with the policies of the Internal Animal Care and Use Committee and the *Guide for the Care and Use of Laboratory Animals* (NIH, 1985) and Good Laboratory Practice regulations.

Drug. A 0.2 or 0.5% ophthalmic solution of brimonidine tartrate, fortified with radiolabeled drug, was used for topical drug treatments. Nonradiolabeled brimonidine tartrate was prepared by Allergan, Inc.; [^{14} C]brimonidine tartrate (the aromatic ring uniformly labeled) (53.8 mCi/mmol; radiochemical purity 95–98%) was obtained from Sigma Chemical Co. (St. Louis, MO). Solutions of 0.01 or 0.1% brimonidine tartrate were made in phosphate-buffered saline, pH 7.4, for intraperitoneal injections.

Experimental Protocols. Monkeys. A multiple-dose study was carried out using a 0.2% drug solution applied to the lower conjunctival cul-de-sac of both eyes of 4 male cynomolgus monkeys twice daily, every 12 h, for 5 days. Any spill was collected with a cotton-tipped applicator, and the radioactivity contained in the spill was measured to determine the actual dose administered. Animals were sacrificed at 2 h after the final dose. Immediately before sacrifice, tear and blood samples were collected. After sacrifice, samples of the upper and lower conjunctiva of the right eye were excised; the eyes were removed and rinsed with saline, and the eye rinse was saved for analysis. Aqueous and vitreous humor samples were separately collected with a syringe and needle. Eyes were then dissected, and samples of iris, ciliary body, lens, choroid/retina, and upper and lower sclera were collected.

In a single-dose study using a 0.5% concentration of drug, 35 μ l of 0.5% drug solution (29.6 μ Ci; 119 μ g of brimonidine) was applied to the lower conjunctival cul-de-sac of both eyes of male monkeys. One animal was sacrificed at each of six time points (0.5, 1, 2, 4, 8, and 24 h) after drug administration. An additional animal was used as a nontreated control. Immediately before sacrifice, tear and blood samples were collected. After sacrifice, tissue samples were collected as described for the study using the 0.2% concentration of drug.

In a multiple-dose study using a 0.5% concentration of drug, 35 μ l of 0.5% drug solution (8.4 μ Ci; 119 μ g of brimonidine) was applied twice daily (at 12-h intervals) to the lower conjunctival cul-de-sac of both eyes of male monkeys for 14 days. Two animals were sacrificed at 1 h, 1 day, 15 days, 60 days, and 90 days after the final dose on day 14. An additional animal received the drug treatment in only one eye; this animal was sacrificed at 1 h after the final dose on day 14. Tear and blood samples from all animals were collected during the study on days 1, 7, and 13, and immediately before sacrifice. Tissue samples were collected after sacrifice, as described for the other studies using monkeys.

Rabbits. In the single-dose study using rabbits, 35 μ l of 0.5% drug solution (16 μ Ci; 115 μ g of brimonidine) was applied to the lower conjunctival cul-de-sac of the left eye. Animals (four at each time point) were sacrificed after drug administration at 10 and 40 min, at 1.5, 3, and 6 h, and at 1, 15, 30, and 60 days. Two additional animals were used as untreated controls. Immediately before sacrifice, tear samples were collected from the treated and contralateral eyes, and a blood sample was obtained. Following sacrifice, aqueous samples were removed with tuberculin syringes; the bulbar conjunctivae were collected, and the eyes were removed and dissected. The cornea, sclera, iris, ciliary body, lens, choroid/retina, optic nerve, lens, and vitreous humor were collected.

In the multiple-dose study using rabbits, 35 μ l of 0.5% drug solution (2 μ Ci; 113 μ g of brimonidine) was applied to the lower conjunctival cul-de-sac of the left eye twice daily (at 6:30 AM and 4:30 PM) for 14 days. Animals (six at each time point) were sacrificed at 10, 20, and 40 min, at 1, 1.5, 2, 3, 6, and 12 h, 1 day, and at 15, 30, 60, and 90 days after the last dose. An additional three animals were used as untreated controls. Tear, blood, and tissue samples were obtained as described above.

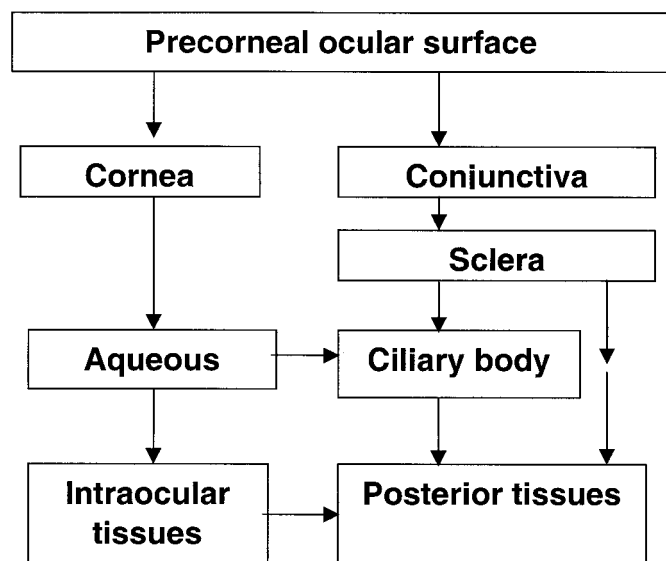


FIG. 2. Compartmentalized scheme of drug penetration across cornea and conjunctival/sclera into anterior and posterior tissues.

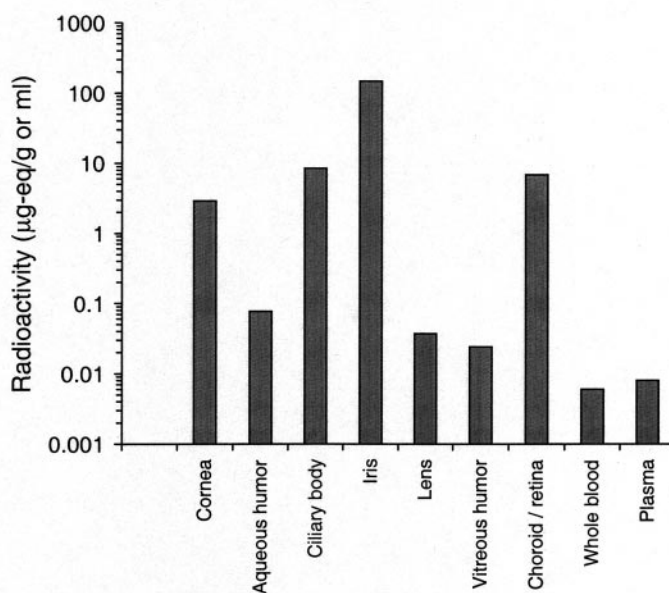


FIG. 3. Radioactivity in ocular and systemic tissues of cynomolgus monkeys after 5 days of topical 0.2% [^{14}C]brimonidine tartrate b.i.d. Concentrations of radioactivity were measured at 2 h after the last dose of drug. Mean values are shown ($n = 4$ eyes).

Rats. A 0.1- or 1.0-mg/ml brimonidine tartrate solution was injected intraperitoneally into Sprague-Dawley rats, achieving a final dose of approximately 0.5 or 5.0 mg/kg. Animals (three at each time point) were sacrificed at 10, 20, and 30 min and at 1, 2, 4, 6, and 24 h postdose. Before sacrifice, blood samples were obtained by cardiac puncture. Following sacrifice, retina, and vitreous humor samples were obtained.

Determination of Total Radioactivity. In the studies using monkeys, aliquots of eye rinse, aqueous humor, vitreous humor, and plasma were mixed with MI31 scintillant (Packard BioScience, Meriden, CT), and radioactivity was quantified by liquid scintillation counting (LSC). Lens, choroid/retina, and sclera samples were combusted using a Packard tissue oxidizer, and the radioactivity absorbed to Optisorb I (Fisons plc, Loughborough, UK) was mixed with Optisorb S scintillant and quantified by LSC. The combustion efficiency of the tissue oxidizer was determined by combustion of ^{14}C standards. The recovery of radioactivity was greater than 97%, and the tissue radioactivity was corrected for the combustion efficiency. Other tissue samples and Schirmer strips (tear samples) were extracted with methanol, and an aliquot of the extract was mixed with scintillant and quantified by LSC, allowing the calculation of total radioactivity contained in the extract. The residues after extraction were combusted, and the residual radioactivity was determined as described above. For extracted tissues, the total tissue radioactivity was the sum of the extracted radioactivity and the residual radioactivity.

In the studies using rabbits, the radioisotopic content of aqueous and vitreous samples and tear strips was quantified by LSC using Ready Solv HP scintillation cocktail (Beckman Coulter, Inc., Fullerton, CA). Blood samples were allowed to dry in a tissue combustion cone. These samples and other tissue samples (conjunctivae, sclera, uvea-sclera, cornea, iris, ciliary body, lens, choroid/retina, and optic nerve head) were combusted using a Packard tissue oxidizer. After combustion, $^{14}\text{CO}_2$ trapped by Carbosorb (Packard Bioscience) was quantified in a liquid scintillation counter. Combustion efficiency was approximately 99%.

Radioisotopic HPLC Analysis of Drug and Metabolites. In the multiple-dose study using rabbits and the studies using the 0.5% drug concentration in monkeys, radiolabeled components in extracts of specified samples were analyzed using HPLC. Briefly, aqueous humor samples and solid tissue samples were extracted with methanol and clarified by centrifugation. To increase extraction efficiencies, iris and ciliary body samples were alkalinized with 1 N NaOH before extraction; the resultant extracts were neutralized with 1 M HCl. Solvent extracts were dried by centrifugal evaporation or under N_2 at 30°C ,

and the residue was reconstituted in 200 to 300 μl of mobile phase for HPLC injection. Samples of iris and the ciliary body were alkalinized. Separation of brimonidine and drug-related substances was achieved on a Beckman Ultrasphere C_{18} column (Beckman Coulter, Inc., Fullerton, CA) using a methanol/acetonitrile/0.04 M sodium heptane-sulfonate, pH 3.4 (20:10:70 by volume) mobile phase at a flow rate of 1.0 ml/min, as previously described (Acheampong et al., 1995). The HPLC retention time of brimonidine was approximately 16 min. Metabolites IIIa, IV, and V had shorter retention times; the identity of these metabolites has been confirmed using liquid chromatography-mass spectrometry (Acheampong et al., 1996).

Gas Chromatography/Mass Spectrometry Analysis. Levels of brimonidine in plasma samples were determined using a validated gas chromatography/mass spectrometry method (Acheampong and Tang-Liu, 1995) by Oneida Research Services (Whitesboro, NY). This method was also used to determine the concentration of brimonidine in extracts of ocular samples obtained from rats after intraperitoneal administration of nonradiolabeled brimonidine tartrate.

Data Analysis. Using the specific activity of [^{14}C]brimonidine in the administered solution, tissue radioactivity was converted to microgram equivalents of the free base per gram of tissue (for ocular tissues, including aqueous humor and vitreous humor) or per milliliter of fluid (for plasma). For expression of radioactivity concentrations in nanomolar units, 1 $\mu\text{g/g}$ is equivalent to 3426 nM. Pharmacokinetic parameters were calculated for total radioactivity and for intact brimonidine. Area under the concentration-time curves (AUC) for total radioactivity and brimonidine were calculated using a linear trapezoidal method (Tang-Liu and Burke, 1988). The initial and terminal elimination rate constants (β) were calculated by regression analysis, and the corresponding half-lives were calculated by $\ln 2/\beta$. The maximum concentration (C_{max}) values for brimonidine (when gas chromatography/mass spectrometry data were available) or C_{max} values for radioactivity were also determined.

Results

Multiple Topical Dosing to Cynomolgus Monkeys (0.2% Dose). Concentrations of radioactivity were measured in ocular tissues after 5 days of application twice daily of a 0.2% brimonidine tartrate solution to both eyes of cynomolgus monkeys. Tissue samples were taken 2 h after the last dose was administered. Radioactivity penetrated to posterior ocular tissues, including the vitreous humor and

TABLE 1

Maximal concentrations of brimonidine achieved in vitreous humor at 1 to 2 h after topical or systemic administration

Brimonidine concentrations were derived from gas chromatography/mass spectrometry or radioactivity concentrations.

Study	<i>n</i> ^a	Brimonidine Tartrate Dose Administered	Vitreous Humor Brimonidine	Vitreous Humor Brimonidine Normalized to 0.2% Dose
<i>nM</i>				
Multiple doses, monkeys ^b	4	0.2%, ¹⁴ C label	82 ± 45	82 ± 45
Single dose, monkeys ^c	2	0.5%, ¹⁴ C label	38	15
Multiple doses, monkeys ^c	4	0.5%, ¹⁴ C label	240 ± 55	97 ± 22
	1		220 (treated eye)	88 (treated eye)
Multiple doses, monkey ^b		0.5% ¹⁴ C label		
	1		10 (untreated eye)	4 (untreated eye)
	4		170 ± 150 (treated eye)	68 ± 60 (treated eye)
Single dose, rabbits ^c		0.5% ¹⁴ C label		
	4		6.9 ± 2.6 (untreated eye)	2.8 ± 1.1 (untreated eye)
	6		420 ± 160 (treated eye)	170 ± 66 (treated eye)
Multiple doses, rabbits ^c		0.5% ¹⁴ C label		
	6		20 ± 7 (untreated eye)	7 ± 3 (untreated eye)
i.p. injection into rats ^c	6	0.5 mg/kg	22 ± 6	Not applicable
i.p. injection into rats ^c	6	5.0 mg/kg	390 ± 96	Not applicable

^a Number of eyes/time point.
^b Brimonidine concentrations at 1 or 2 h after the final dose.
^c Brimonidine concentrations were calculated based on *C*_{max} values.

optic nerve (Fig. 3). Attempts to separate the retina from the choroid were unsuccessful; therefore, tissue concentrations are presented for retina/choroid. Higher radioactivity was measured in pigmented ocular tissues (iris, ciliary body, and choroid/retina). To assess whether brimonidine concentrations in the posterior segment are sufficient to achieve α_2 -receptor-mediated activity, vitreous humor concentrations were measured (Table 1). The concentration of brimonidine in vitreous humor was 82 ± 45 nM.

Single Topical Dosing with Cynomolgus Monkeys (0.5% Dose). [¹⁴C]Brimonidine put into both eyes of cynomolgus monkeys rapidly penetrated into anterior and posterior ocular tissues. Less than 0.06% of the applied radioactivity (after correction for spillage) could be rinsed from the eyes 30 min and 1 h after drug administration. Radioactivity was found in all ocular tissues examined, including the vitreous humor (Fig. 4) and choroid/retina. No radioactivity was detected in ocular tissues from an untreated control animal. Table 2 summarizes the ocular pharmacokinetic parameter values for total radioactivity and intact brimonidine. Levels of radioactivity were highest in tear samples at 30 min and 1 h, but at all time points from 30 min to 24 h, the highest tissue concentration was found in the iris. High concentrations of radioactivity were also measured in the conjunctiva, sclera, and cornea. There was some systemic absorption, but plasma and blood levels of radioactivity were low compared with the levels achieved in ocular tissues.

HPLC analysis of tear samples and ocular extracts indicated that although at least three metabolites (MIIIa, MIV, and MV) were detected in ocular tissues, intact brimonidine was the major radioactive component at all times, accounting for 65 to 100% of total sample extract radioactivity. The apparent elimination half-life (*t*_{1/2}) of radioactivity and brimonidine from nonpigmented ocular tissues was quite rapid (Table 2). However, the apparent rate of elimination from pigmented ocular tissues (iris, ciliary body, and choroid/retina) was slow; the measured concentrations of radioactivity and brimonidine were relatively unchanged over the 24-h time course after dosing.

Multiple Topical Dosing to Cynomolgus Monkeys (0.5% Dose). After 2 weeks of ocular application twice daily of 0.5% [¹⁴C]brimonidine in cynomolgus monkeys, the concentrations of radioactivity measured in ocular tissues were generally higher than after a single dose (Table 3). This finding was most striking in pigmented tissues (iris, ciliary body, and choroid/retina) in which levels of radioactivity were up to 40-fold higher than after a single dose. The radioactivity

concentrations in ocular tissues were measurable up to 90 days after the last dose, as the lower limit of quantitation of radioactivity concentration was 1 ng-Eq/g or ml. The rank order of maximal tissue concentrations of radioactivity (μg-Eq/g) was iris (610) > lower bulbar conjunctiva (56.2) > ciliary body (32.7) > choroid/retina (29.3) > upper bulbar conjunctiva (29.1) > upper sclera (20.1) > lower sclera (17.7) > cornea (9.79) > lens (0.671) > aqueous humor (0.326) > vitreous humor (0.061). As in the single-dose study, systemic levels of radioactivity were relatively low. Steady-state levels of radioactivity were reached by day 7 of the study. Radioactivity was detected in the contralateral eye of the animal dosed in only one eye, presumably due to uptake from the systemic circulation, but the levels of radioactivity were 1 to 3 orders of magnitude less than in the treated eye (Fig. 4).

The vitreous humor concentrations are presented in Table 1 as non-normalized and dose-normalized. Because the clinical dose is 0.2% but the rabbit and monkey studies were conducted with 0.2% and 0.5% dose concentrations, the vitreous humor was normalized to the 0.2% clinical dose (Table 1). The normalized concentration for 0.5% dosing in monkeys was 97 nM, in good agreement with the vitreous humor concentration of 82 nM measured in the multiple-dose study using the 0.2% concentration of drug.

Unchanged brimonidine was the major radioactive component in all ocular tissue extracts (Table 4). In all tissues analyzed, the mean percentage of sample extract radioactivity identified as intact brimonidine ranged from 83 to 98% over all time points (1 h to 90 days) in the animals dosed in both eyes. Three metabolites were detected in monkey ocular tissues. Metabolites MIV and MV were previously characterized as quinoxalin-2-one and quinoxalin-3-one derivatives, whereas MIIIa was identified as a quinoxalin-2,3-dione derivative (Acheampong et al., 1995, 1996). Each of the three metabolites detected represented less than 10% of the radioactivity in the extracts compared with up to 37% of total radioactivity for rabbit ocular tissues in the current and previous studies (Acheampong et al., 1995). Unchanged brimonidine was the major radioactive component at all times and in all samples analyzed. The methanol extraction efficiency for radiolabeled substances in the cornea and conjunctiva at day 15 was approximately 50%. The extraction solvent for day 15 corneal and conjunctival tissue was not optimized for extraction of drug-related substances in the cornea and conjunctiva because these tissues contain a lipid epidermal layer and connective structures, respectively.

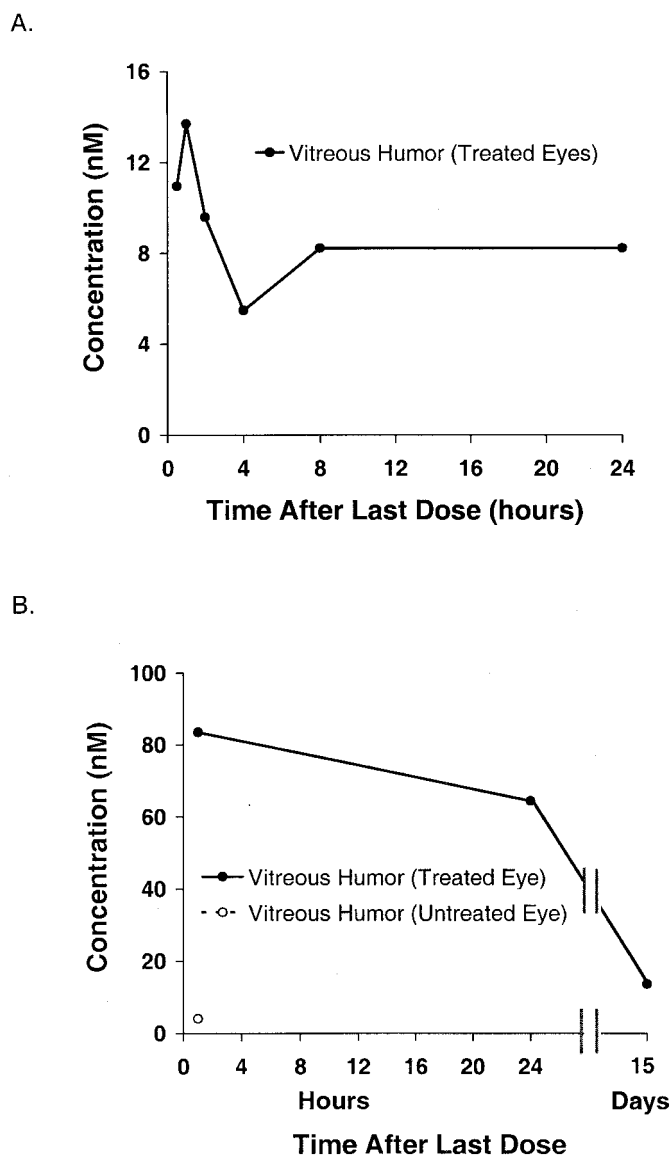


FIG. 4. Time course of radioactivity concentrations in vitreous humor of monkey eyes after topical dosing.

A, normalized concentrations of radioactivity in vitreous humor of cynomolgus monkeys after a single application of [^{14}C]brimonidine to both eyes ($n = 2$ eyes). B, normalized concentrations of radioactivity in vitreous humor of cynomolgus monkeys after 2 weeks of topical b.i.d. dosing ($n = 4$ treated eyes; $n = 1$ untreated eye). Mean values were derived from microgram equivalents of brimonidine per gram and normalized to a 0.2% dose.

Single Topical Dosing to Rabbits (0.5% Dose). Studies were carried out with larger sample sizes and more time points in rabbits than in monkeys, allowing more reliable calculations of rate constants and elimination half-lives, but the overall ocular drug pharmacokinetics in rabbits were similar to those in monkeys. Following a single topical application of [^{14}C]brimonidine to the left eyes of pigmented rabbits, radioactivity was rapidly absorbed by ocular surface tissues and distributed throughout the eye. Much lower levels of radioactivity were measured in the contralateral eye and in the systemic circulation (Table 5; Fig. 5A). In both rabbits and cynomolgus monkeys, the ocular tissue containing the highest concentration of radioactivity was the iris. However, radioactivity was also measured in posterior portions of the eye, including the vitreous humor, choroid/retina, and optic nerve head (Table 5). Significant concentrations of radioactivity

were found in the vitreous humor and the aqueous humor during the 24-h period after dosing (Fig. 5). Radioactivity was slowly cleared from many ocular tissues; terminal half-lives of radioactivity in the sclera, iris, ciliary body, choroid/retina, and optic nerve head were longer than 2 weeks.

Multiple Topical Dosing to Rabbits (0.5% Dose). A similar distribution of radioactivity in ocular tissues was obtained after administration of [^{14}C]brimonidine twice daily to rabbits for 2 weeks. As was observed in the studies using cynomolgus monkeys, however, significantly more radioactivity was found in the pigmented tissues (iris, ciliary body, and choroid/retina) after multiple dosing than after a single dose. The concentrations of radioactivity in these tissues were sustained at peak values from 10 min to approximately 24 h after the last dose. As in the single-dose study, concentrations of radioactivity in the treated eye were much greater than in the contralateral eye (Fig. 5B). Furthermore, concentrations of radioactivity in the posterior portion of the eye, measured from 10 min to 24 h after the last drug instillation, were 1 to 4 orders of magnitude greater than the concentration of radioactivity in the blood. The radioactivity concentrations in ocular tissues were measurable up to 90 days after the last dose, as the lower limit of quantitation of radioactivity concentration was 1 ng-Eq/g or ml. The rank order of tissue mean AUC (10 min-90 days) ($\mu\text{g-Eq} \cdot \text{day/g}$ or ml) of radioactivity was iris (2330) > ciliary body (1030) > choroid-retina (813) > sclera (283) > cornea (32.1) = tear (28.3) > conjunctiva (8.27) > optic nerve head (2.24) > vitreous humor (1.59) > lens (0.868) > aqueous humor (0.100) > plasma (0.00578) > blood (0.00339). The rank order of maximal tissue concentrations of radioactivity ($\mu\text{g-Eq/g}$ or ml) was iris (114) > ciliary body (63.9) > choroid/retina (20.8) > conjunctiva (8.39) > aqueous humor (0.842) > optic nerve head (0.412) > vitreous humor (0.124) = lens (0.120) > plasma (0.020) > blood (0.015). The concentration of brimonidine in vitreous, normalized to a 0.2% dose, was 170 nM (Table 1).

In the tissue extracts subjected to HPLC analysis (iris, ciliary body, conjunctiva, cornea, and aqueous humor), up to three metabolites were detected, corresponding to MIIIa, MIV, and MV. However, most of the radioactivity in each sample was identified as intact brimonidine. At 3 h after the last dose, metabolites represented 37, 25, 5, 5, <5, and <5% of the total radioactivity in the lower conjunctiva, upper conjunctiva, iris, ciliary body, cornea, and aqueous humor, respectively.

Intraperitoneal Dosing of Rats (0.5–5 mg/kg). Brimonidine concentrations in the vitreous humor, retina, and plasma of rats were determined by gas chromatography/mass spectrometric analysis of samples after a single intraperitoneal injection of 0.5 mg/kg or 5.0 mg/kg brimonidine tartrate (Fig. 6). With either dose administered, comparable concentrations of brimonidine were achieved in the retina and plasma. Although brimonidine concentrations in the vitreous humor seemed to be lower, the measured concentrations were nonetheless in the nanomolar range (Table 1). The maximal vitreous humor concentrations of brimonidine were 22 and 390 nM for the 0.5 and 5 mg/kg doses, respectively. A concentration of 138 nM brimonidine was achieved in the retina with the 0.5 mg/kg dose.

Discussion

These studies demonstrated that topically applied brimonidine widely distributes into the posterior segment of monkey and rabbit eyes after single and multiple dosing and that intraperitoneal administration of brimonidine also results in significant availability of brimonidine in the posterior segment of rat eyes. Furthermore, experiments with unilateral topical dosing showed high drug levels in the treated eye compared with the contralateral untreated eye and plasma,

TABLE 2

Summary of pharmacokinetic data after a single 0.5% ocular dose of [¹⁴C]brimonidine in cynomolgus monkeys (n = 2 eyes at each of six time points)

Terminal half-lives for ¹⁴C were: aqueous humor, 13.3 h; lens, 18.4 h. The terminal half-life for brimonidine in aqueous humor was 13.8 h. AUC was estimated over 24 h.

Tissue/Fluids	¹⁴ C				Brimonidine			
	<i>T</i> _{max}	<i>C</i> _{max}	<i>t</i> _{1/2}	AUC	<i>T</i> _{max}	<i>C</i> _{max}	<i>t</i> _{1/2}	AUC
	<i>h</i>	μg-Eq/g	<i>h</i>	μg-Eq · day/g	<i>h</i>	μg-Eq/g	<i>h</i>	μg-Eq · day/g
Tear	1.0	88.3	N.C.	203.0	1.0	78.6	N.C.	175.0
Lower bulbar conjunctiva	1.0	22.0	1.0	74.7	1.0	21.1	1.0	71.5
Upper bulbar conjunctiva	0.5	16.4	1.0	41.8	0.5	15.5	1.0	39.8
Cornea	0.5	8.19	1.1	48.7	0.5	7.26	1.1	46.3
Lower sclera	0.5	6.67	0.6	92.4	N.A.	N.A.	N.A.	N.A.
Upper sclera	0.5	4.43	N.C.	53.2	N.A.	N.A.	N.A.	N.A.
Aqueous humor	0.5	0.468	1.1	0.944	0.5	0.418	1.1	0.743
Iris	8.0	126.0	N.C.	1658.0	8.0	124.0	N.C.	1615.0
Ciliary body	24.0	2.12	N.C.	28.7	24.0	1.98	N.C.	26.8
Lens	4.0	0.246	N.C.	2.7	N.A.	N.A.	N.C.	N.A.
Vitreous humor	1.0	0.011	2.3	0.14	N.A.	N.A.	N.C.	N.A.
Choroid/retina	8.0	1.84	N.C.	26.5	N.A.	N.A.	N.C.	N.A.
Blood	1.0	0.008	N.C.	0.060	N.A.	N.A.	N.A.	N.A.
Plasma	4.0	0.006	N.C.	0.033	0.5	0.003	N.A.	0.005

N.C., not calculable; N.A., not analyzed.

TABLE 3

Summary of pharmacokinetic data after 14 days of 0.5% ocular doses of [¹⁴C]brimonidine given twice daily to cynomolgus monkeys (n = 4 eyes at each of five time points)

Terminal half-lives of radioactivity, determined between 15 and 90 days, were 33.3 days for iris and 44.2 days for vitreous humor. AUC was estimated over 90 days.

Tissues/Fluids	¹⁴ C			Brimonidine		
	<i>T</i> _{max}	<i>C</i> _{max}	AUC	<i>T</i> _{max}	<i>C</i> _{max}	AUC
	<i>h</i>	μg-Eq/g	μg-Eq · day/g	<i>h</i>	μg/g	μg · day/g
Tear	1.0	24.8	138.0	1.0	22.70	N.C.
Lower bulbar conjunctiva	1.0	56.2	542.0	1.0	53.50	438.0
Upper bulbar conjunctiva	1.0	29.1	438.0	1.0	26.80	217.0
Cornea	1.0	9.82	97.3	1.0	9.11	43.8
Lower sclera	24.0	21.4	439.0	N.A.	N.A.	N.A.
Upper sclera	1.0	20.1	255.0	N.A.	N.A.	N.A.
Aqueous humor	1.0	0.327	3.13	1.0	0.270	N.C.
Iris	24.0	610.0	10659.0	24.0	589.0	10378.0
Ciliary body	24.0	32.8	1341.0	24.0	32.0	1237.0
Lens	1.0	0.673	13.8	N.A.	N.A.	N.A.
Vitreous humor	1.0	0.071	0.96	N.A.	N.A.	N.A.
Choroid/retina	24.0	30.600	1499.0	N.A.	N.A.	N.A.
Blood	15 days	0.013	0.46	N.A.	N.A.	N.A.
Plasma	1.0	0.012	0.04	1.0	0.003	N.A.

N.C., not calculable; N.A., not analyzed.

suggesting that brimonidine penetrates into the posterior tissues by a local route and not by systemic absorption. The penetration of brimonidine to the anterior segment can occur through a combination of cornea and sclera pathways (Chien et al., 1990). Following topical application, significant access of brimonidine into the posterior segment may occur by the conjunctival/sclera pathway since brimonidine concentrations in conjunctiva and sclera were relatively higher than in the cornea and aqueous humor compartments (Table 2). This study examined maximum concentration ranges in the different tissues with doses of 0.2 and 0.5%. This study provided the maximum concentrations, relative to pharmacological activity (EC₅₀), in the anterior and posterior tissues following the clinical therapeutic dose of 0.2% and higher dose of 0.5%.

Following topical application, [¹⁴C]brimonidine was higher in pigmented ocular tissues, including the iris, ciliary body, and chord/retina. Significant amounts were also found in the vitreous and optic nerve head. The absorption, retention, and activity of drugs applied topically to the eye can be affected by binding with ocular melanin (Salminen et al., 1985; Zane et al., 1990), and brimonidine has been

shown to bind with high affinity, but reversibly, to ocular bovine melanin in vitro (Tang-Liu et al., 1992). In a previous pharmacokinetic study of the disposition of a single dose of brimonidine applied topically to the eye, the AUC (0–6 h) of brimonidine was 10-fold higher in the iris-ciliary body of pigmented rabbits than in the iris-ciliary body of albino rabbits (Acheampong et al., 1995), suggesting that the binding of brimonidine to ocular melanin affects the disposition of the drug. Our results in the present study extend these previous results and demonstrate that brimonidine is also higher in an additional pigmented ocular tissue, the choroid/retina. Measurements of tissue levels of drugs can overestimate the amount of drug available for receptor activation since most of it may be bound to melanin. Furthermore, drug binding to melanin has potential implications, such as the generation of a depot or slow-release site, which may explain the higher concentrations of drug in the vitreous humor of rabbits and monkeys following chronic dosing (Table 1). Long-term studies with 0.5 and 0.2% brimonidine have shown that chronic treatment with brimonidine is safe (Angelov et al., 1996), suggesting that there are no detrimental effects of brimonidine binding to melanin.

TABLE 4

Percentage of recovered radioactivity representing intact brimonidine and metabolites 1 h or 15 days after the final dose administered to cynomolgus monkeys in the multiple 0.5% dose study

Results were obtained by HPLC of extracts of ocular tissues and tear samples; data shown are means $\pm$ S.D. of values from four eyes (two animals).

Tissues/Fluids	Time After	Extraction	Brimonidine	MIIIa	MIV	MV
				%		
Tear (Schirmer strip)	1 h	87.4 $\pm$ 2.3	89.8 $\pm$ 5.4	4.4 $\pm$ 2.7	2.6 $\pm$ 2.1	3.2 $\pm$ 1.3
	15 days	78.5 $\pm$ 6.1	N.A.	N.A.	N.A.	N.A.
Lower bulbar conjunctiva	1 h	64.8 $\pm$ 13.0	93.8 $\pm$ 2.2	1.3 $\pm$ 0.5	1.6 $\pm$ 1.3	3.4 $\pm$ 2.1
	15 days	40.3 $\pm$ 3.3	91.1 $\pm$ 2.7	2.1 $\pm$ 1.1	2.7 $\pm$ 2.2	4.1 $\pm$ 2.3
Upper bulbar conjunctiva	1 h	82.7 $\pm$ 4.2	95.1 $\pm$ 0.5	1.4 $\pm$ 0.6	1.8 $\pm$ 1.1	1.7 $\pm$ 0.9
	15 days	50.6 $\pm$ 3.3	93.9 $\pm$ 1.6	1.3 $\pm$ 0.8	1.5 $\pm$ 1.0	3.5 $\pm$ 1.7
Cornea	1 h	82.1 $\pm$ 8.7	92.1 $\pm$ 1.6	1.5 $\pm$ 0.5	2.4 $\pm$ 0.7	4.1 $\pm$ 0.6
	15 days	53.1 $\pm$ 6.4	89.8 $\pm$ 5.2	1.6 $\pm$ 1.0	3.3 $\pm$ 1.8	5.4 $\pm$ 3.4
Aqueous humor	1 h	100.0 $\pm$ 0.0	82.7 $\pm$ 5.9	4.9 $\pm$ 3.3	7.0 $\pm$ 1.4	5.4 $\pm$ 2.2
	15 days	100.0 $\pm$ 0.0	N.A.	N.A.	N.A.	N.A.
Iris	1 h	92.6 $\pm$ 5.6	96.8 $\pm$ 0.5	0.8 $\pm$ 0.5	0.9 $\pm$ 0.4	1.5 $\pm$ 0.6
	15 days	97.6 $\pm$ 0.5	96.2 $\pm$ 0.5	1.4 $\pm$ 0.4	0.9 $\pm$ 0.3	1.6 $\pm$ 0.4
Ciliary body	1 h	88.8 $\pm$ 3.8	95.5 $\pm$ 1.0	0.5 $\pm$ 0.4	1.2 $\pm$ 0.3	2.9 $\pm$ 1.1
	15 days	92.3 $\pm$ 2.3	97.7 $\pm$ 1.7	0.3 $\pm$ 0.3	0.6 $\pm$ 0.5	1.5 $\pm$ 0.9

N.A., data not available; levels of radioactivity were too low for parent and metabolite profiling.

TABLE 5

Summary of pharmacokinetic data after a single ocular application of [^{14} C]brimonidine to one eye of pigmented rabbits ($n = 4$ eyes at each of nine time points)

Terminal half-lives of radioactivity were calculated over the time intervals of 1 to 15 days for aqueous humor, 15 to 30 days for conjunctiva, and 15 to 60 days for cornea, sclera, iris, ciliary body, vitreous humor, choroid/retina, and optic nerve head.

Tissues/Fluids	Treated Eye			Untreated Eye		
	$T_{\max}$	$C_{\max}$	$t_{1/2}^*$	$T_{\max}$	$C_{\max}$	$t_{1/2}^*$
	h	$\mu\text{g-Eq/g or ml}$	days	h	$\mu\text{g-Eq/g or ml}$	h
Tear	0.17	195.0	N.D.	24.0	0.00434	N.D.
Lower bulbar conjunctiva	0.17	12.9	9.17	1.5	0.0145	N.D.
Upper bulbar conjunctiva	0.17	12.9	8.04	1.5	0.011	N.D.
Cornea	0.17	11.5	13.3	1.5	0.00976	N.D.
Aqueous humor	1.5	1.26	3.06	3.0	0.000763	N.D.
Iris	1.5	53.8	17.3	6.0	0.332	10.9
Ciliary body	1.5	19.1	19.5	6.0	0.445	13.1
Lens	1.5	0.107	N.D.	1.5	0.000247	N.D.
Vitreous humor	0.17	0.0493	10.4	3.0	0.00201	N.D.
Choroid/retina	1.5	3.22	37.2	3.0	0.124	N.D.
Blood	1.5	0.00789	N.D.			

N.D., half-life was not determined due to insufficient data in the terminal phase.

In the ocular samples subjected to HPLC analysis (including the conjunctiva, iris, ciliary body, and aqueous humor from both monkeys and rabbits), 65 to 100% of the radioactivity represented intact brimonidine. The radioactive concentrations in the posterior tissues following topical dosing was previously thought to be too low for radio-chromatographic analysis using HPLC with a radiometric detector. Although the posterior eye samples were not subjected to HPLC analysis in the studies using topically applied brimonidine, it is very likely that the pattern of metabolic activity in the anterior and posterior tissues is such that a substantial portion of the radioactivity measured in the posterior tissue samples also represented intact brimonidine.

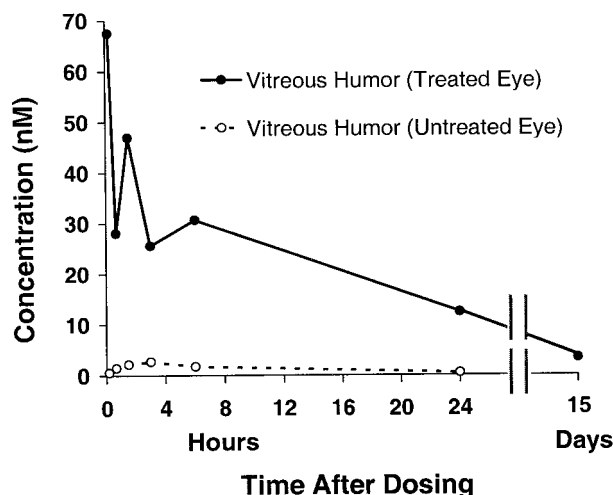
Drug levels in the vitreous humor represent the free-drug concentration available to the receptors in the neurosensory retina that is not bound to melanin and other extracellular tissue components and is a more reliable indicator of potential for drug effects as discussed earlier. Data from this study show that dose-normalized vitreous humor concentrations of brimonidine following multiple topical administration in the monkey (0.5% dose) and rabbit (0.5% dose) were 82 and 170 nM, respectively. Following intraperitoneal administration of 0.5 mg/kg in rats, the maximal vitreous humor concentration reached 22 nM, and the peak neuroretinal level was 138 nM. The EC_{50} for brimonidine to activate the α_2 -receptor in isolated assay

systems is 2 nM (Burke et al., 1996). Thus, the concentration of drug in the vitreous humor following topical or intraperitoneal administration exceeds the concentration shown to activate α_2 -receptors in these systems, and enough drug reaches the posterior pole to exert biological activity in the retina.

In humans, vitreous concentrations of brimonidine were recently reported to be greater than 2 nM, with a mean value of 185 nM, after topical ocular administration of 0.2% brimonidine tartrate 2 to 3 times daily for 1 to 2 weeks (Kent et al., 2001). When the vitreous humor concentrations are normalized to the clinical dose of 0.2% brimonidine tartrate, the dose-normalized $C_{\max}$ values were 82 and 170 nM for multiple dosing in monkeys and rabbits (Table 1), respectively, and seem to be within the range of human vitreous concentrations. Compared with the primate eye, the rabbit eye has been more extensively used to test ocular drug delivery into anterior segment (Maurice and Mishima, 1984; Lee and Robinson, 1986; Schoenwald, 1993). Nonetheless, there are similarities and differences in ocular anatomy between rabbit and primates. For example, the corneal drug permeability and corneal diameter are similar between rabbit and humans, whereas differences exist in tear turnover rate and spontaneous blinking rate (Maurice and Mishima, 1984; Schoenwald, 1993).

The presence of α_2 -adrenergic receptors has previously been demonstrated in the retina (Elena et al., 1989; Matsuo and Cynader, 1992).

A.



B.

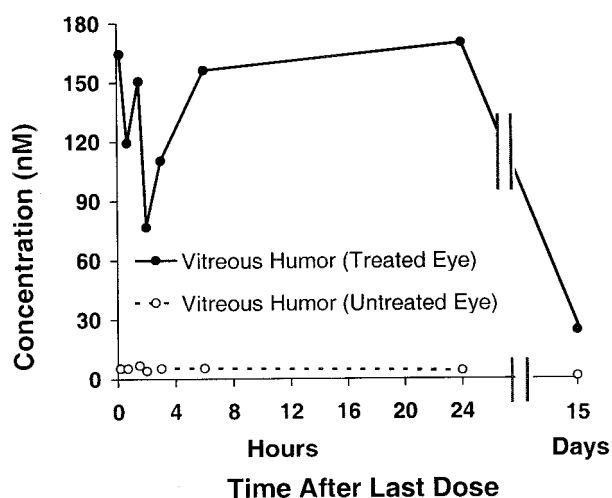
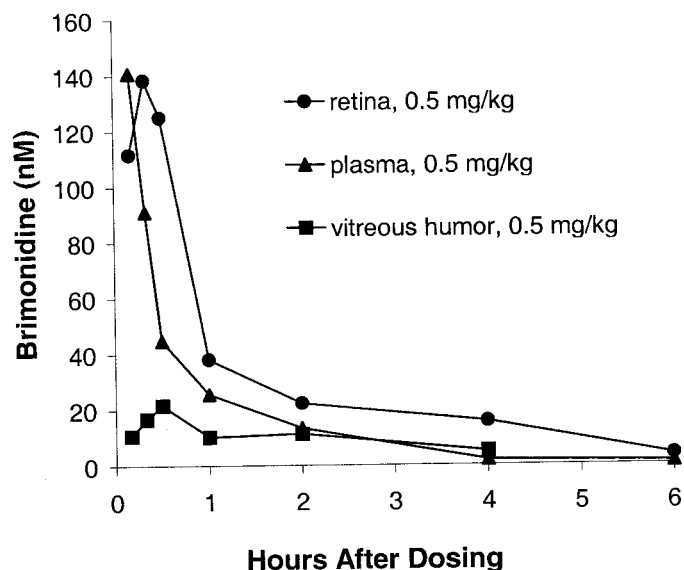


FIG. 5. Time course of radioactivity in vitreous humor of rabbit eyes after topical dosing.

A, normalized concentrations of radioactivity in vitreous humor of rabbits after a single application of [^{14}C]brimonidine to the left eye ($n = 4$ treated or untreated eyes). B, normalized concentrations of radioactivity in vitreous humor of rabbits after 2 weeks of topical b.i.d. dosing ($n = 5$ or 6 treated or untreated eyes). Mean values form microgram equivalents of brimonidine per gram and normalized to a 0.2% dose are shown.

Retinal α_2 -adrenergic receptor activation has been shown to enhance retinal ganglion cell survival after exposure to insults (Wheeler et al., 1999, 2001). Brimonidine has been shown to protect cultured rat retinal ganglion neurons from kainate-induced excitotoxicity (Lai et al., 1997). Furthermore, intraperitoneal administration of brimonidine has been demonstrated to enhance retinal ganglion cell survival after calibrated compression of the optic nerve or following ischemia/reperfusion in rat models of neuronal injury and ocular hypertension (Wheeler et al., 1999, 2001; Yoles et al., 1999). The neuroprotection produced by brimonidine was blocked by the α_2 -receptor antagonist rauwolscine, demonstrating the role of α_2 -receptor activation in the enhancement of neuronal survival. In these animal models of neuronal injury, brimonidine was administered intraperitoneally. The results also suggest that brimonidine applied topically to the eye would reach the retina in concentrations sufficient for biological activity. Because

A



B

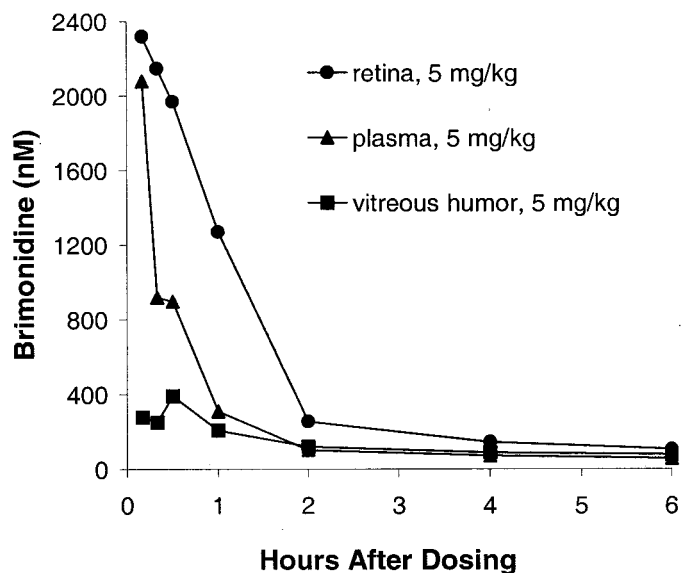


FIG. 6. Brimonidine concentration in posterior ocular tissues and plasma after intraperitoneal dosing of rats.

Brimonidine concentrations were measured at time points from 10 min to 24 h after a single intraperitoneal injection of 0.5 mg/kg brimonidine tartrate (A) or 5.0 mg/kg brimonidine tartrate (B). Mean values are shown ($n = 3$ to 6 eyes).

brimonidine is neuroprotective in cultured neurons and in animal models, it is likely that its neuroprotective action is at the level of the retina. Thus, topical application of brimonidine to the eye might be predicted to result in enhanced survival of retinal ganglion cells.

In conclusion, we have demonstrated the ocular pharmacokinetics of topically applied [^{14}C]brimonidine in monkeys and rabbits and systemically administered brimonidine and [^{14}C]brimonidine in rats. Brimonidine demonstrated good ocular distribution. Significant concentrations of radioactivity were measured in the posterior tissues of the eye. Low nanomolar concentrations of brimonidine, sufficient to selectively activate α_2 -adrenergic receptors, were available at the retina. In animal models, activation of α_2 -adrenergic receptors promotes the survival of retinal ganglion cells. Together these observa-

tions indicate that pharmacologically active brimonidine levels are achievable in the retina of neuroprotection models.

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