



Effect of Solution pH on Distribution of Ophthalmically Administered Brimonidine in Posterior Ocular Tissues in Pigmented Rabbits

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ABSTRACT

Introduction: Brimonidine bioavailability in the aqueous humor depends on the solution pH following topical administration. The purpose of this study was to investigate the effect of solution pH on brimonidine distribution in the posterior ocular tissues in pigmented rabbits.

Methods: The anterior retina/choroid, posterior retina/choroid, and vitreous body of pigmented rabbits were collected 0.67, 1.5, 3, 6, 12, 24, 168, and 360 h after the administration of a single topical dose of 0.2% brimonidine tartrate ophthalmic solution, pH 6.4 (Alphagan[®]; Allergan Inc., Irvine, CA, USA). Brimonidine concentrations in these tissues were quantified using liquid chromatography/tandem mass spectrometry. Pharmacokinetic parameters were determined using noncompartmental analysis, and the results were compared with tissues from eyes administered 0.1% brimonidine tartrate ophthalmic solution, pH 7.3 (Aiphagan[®]; Senju Pharmaceutical Co., Ltd., Osaka, Japan) in our previous study conducted using the same procedure.

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Results: Topically applied brimonidine was distributed rapidly into the posterior tissues of the eye after a single ophthalmic administration of the 0.2% ophthalmic solution. The areas under the curve from time 0 to 360 h following dosing with the 0.2% ophthalmic solution were 500,000, 14,300, and 28.7 ng h/g in the anterior and posterior retina/choroid, and vitreous body, respectively.

Conclusion: The differences in the areas under the curve between two ophthalmic solutions were less than the difference in drug concentrations between these two products in any tissues. This finding indicates that the change in the solution pH from 6.4 to 7.3 increases brimonidine bioavailability into the posterior ocular tissues similarly as into the aqueous humor.

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Keywords: Brimonidine; Distribution; Penetration route; Pharmacokinetics; Retina/choroid; Vitreous body

INTRODUCTION

Brimonidine tartrate, a highly selective α_2 -adrenergic agonist, is an intraocular pressure (IOP)-lowering drug. Brimonidine tartrate decreases the IOP by reducing the production of aqueous humor and increasing its outflow via the uveoscleral pathway [1]. It has been

reported that the bioavailability of brimonidine in the aqueous humor following ophthalmic administration is enhanced by increasing the solution pH, in accordance with the pH partition hypothesis [2–4]. Brimonidine is a base ($pK_a = 7.78$), and this change in bioavailability is assumed to be caused by the increase in non-ionized molecules, which have a higher membrane permeability than ionized molecules in a more alkaline pH. An ophthalmic formulation containing brimonidine tartrate as an active ingredient has been improved based on this characteristic. In this context, the distribution of brimonidine in the anterior parts of the eye following ophthalmic administration has been well investigated.

The distribution of brimonidine in the posterior parts of the eye after ophthalmic administration is as important as that in the anterior parts because brimonidine tartrate not only has IOP-lowering effects but also neuroprotective effects. In a randomized clinical trial, twice-daily treatment of eyes with 0.2% brimonidine tartrate ophthalmic solution, pH 6.4 (0.2% ophthalmic solution; Alphagan[®]; Allergan Inc., Irvine, CA, USA), preserved visual function better than did treatment with 0.5% timolol maleate ophthalmic solution, despite the similar IOP-lowering effect of both drugs [5]. Alpha₂-adrenergic receptors are expressed in the retina [6–8], and in a few animal studies these receptors have been shown to mediate the neuroprotective effect following activation [9, 10].

Several research groups have investigated the distribution of brimonidine in the posterior ocular tissues after ophthalmic administration [11–15]. However, unlike our body of knowledge on the distribution of brimonidine in the aqueous humor, it is still unclear how solution pH influences brimonidine bioavailability in the posterior parts of the eye following ophthalmic administration. Furthermore, there is no report of investigations on the effect of solution pH on any topically applied drugs.

Therefore, the purpose of this study was to investigate the effect of the solution pH on brimonidine bioavailability in the posterior ocular tissues following ophthalmic administration. In an earlier study, we determined

brimonidine concentrations in the posterior ocular tissues of pigmented rabbits after a single ophthalmic administration of 0.1% brimonidine tartrate ophthalmic solution, pH 7.3 (0.1% ophthalmic solution, Alphagan[®]; Senju Pharmaceutical Co., Ltd., Osaka, Japan) [15]. In the present study, brimonidine concentrations in the ocular tissues were investigated following the administration of 0.2% ophthalmic solution at pH 6.4 in a similar manner as in the previous study, and the bioavailability of brimonidine in the posterior ocular tissues was compared between these commercial ophthalmic solutions. The results of this investigation are significant in the context of improving the neuroprotective effect of the ophthalmic solution by modifying the pH of the solution.

METHODS

Animals

Forty male pigmented rabbits (Dutch) weighing 1.7–2.1 kg were obtained from Kitayama Labes Co. Ltd. (Nagano, Japan). All animals were housed individually in a temperature- and humidity-controlled facility on a 12/12-h light/dark cycle. Food and water were available ad libitum. All animal management in this study was performed in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the local Institutional Animal Care and Use Committees.

Drugs and Chemicals

Alphagan[®], a 0.2% ophthalmic solution of brimonidine tartrate at pH 6.4 (Allergan Inc.), was topically administered. Brimonidine tartrate and 5-chloro-6-(2-imidazolidinylideneamino) quinoxaline, internal standard (IS) for quantitation, were provided by Allergan Inc. All other reagents were of special grade or higher and were obtained commercially.

Topical Administration and Tissue Sampling

A 35- μ L drop of ophthalmic solution was topically applied to one eye of all rabbits. The rabbits were subsequently euthanized by an intravenous overdose of pentobarbital sodium at eight time points after administration of the solution (0.67, 1.5, 3, 6, 12, 24, 168, and 360 h). The eyes were then enucleated from each animal, frozen in a dry ice/acetone bath, and divided at the equator. The vitreous body, anterior retina/choroid, and posterior retina/choroid were collected, and all tissue samples were stored at -80°C until sample processing.

Tissue Sample Processing

All tissue samples were pretreated with solid-phase extraction methods before analysis. All weighed tissues were minced with methanol using scissors, followed by sonication and centrifugation (8000 or 10,000 g), and then the supernatants were collected and evaporated to dryness under nitrogen gas. Dried residues were dissolved in a mixture of methanol/pure water (1:1) and subsequently added to OASIS HLB μ Elution 96-well plates (Waters Corp., Milford, MA, USA) preconditioned with methanol and pure water. After rinsing with pure water, analytes were eluted using acetonitrile. Pretreatment of the vitreous body involved filtering the solutions before the solid-phase extraction.

Analytical Method

Brimonidine concentrations in the ocular tissue samples were determined using liquid chromatography/tandem mass spectrometry with an amide column. An ACQUITY ultraperformance liquid chromatography system (UPLC) and a Micromass Quattro Premier mass spectrometer (both Waters Corp.) were used for the analysis. Analytes were separated using an ACQUITY UPLC ethylene bridged hybrid (BEH) Amide column (2.1 \times 50 mm, 1.7 μm ; Waters Corp.) under gradient chromatography conditions. The injection volume was 10 μL , flow rate was

0.3 mL/min, and the mobile phase consisted of methanol/10 mM ammonium formate (2:3) and acetonitrile. Brimonidine and the IS were analyzed in the positive ionization mode with the multiple reaction monitoring transitions of 292.10 \rightarrow 212.20 and 248.20 \rightarrow 205.20, respectively. The lower limits of quantitation were 0.012 and 0.006 ng/tissue (retina/choroid and vitreous body, respectively).

Pharmacokinetics Analysis

Pharmacokinetic parameters after single topical administration were determined using non-compartmental analysis using Phoenix[®] WinNonlin[®] version 6.1 software (Certara LP, Princeton, NJ, USA). The following parameters were determined: the time to reach maximum concentration (T_{max}), the maximum concentration (C_{max}), the elimination half-life ($T_{1/2}$), and the area under the curve from time 0 to 360 h (AUC_{0-360}).

RESULTS

Topically applied brimonidine was distributed rapidly into all tissues after a single ophthalmic administration of 0.2% ophthalmic solution (Fig. 1). The highest brimonidine concentration was in the anterior retina/choroid, followed in

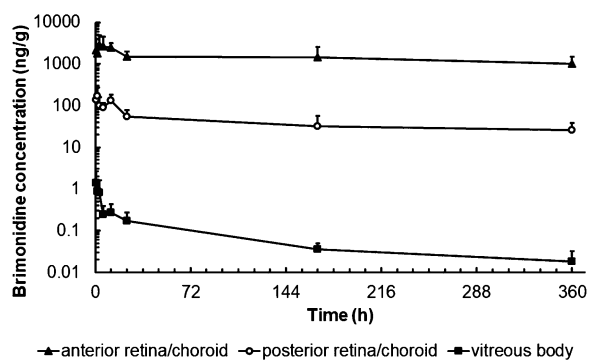


Fig. 1 Brimonidine concentration–time curves in posterior ocular tissues after a single topical administration of brimonidine tartrate ophthalmic solution in pigmented rabbits. Each pigmented rabbit was administered 35 μL of 0.2% brimonidine tartrate ophthalmic solution. Data are presented as the mean + standard deviation ($n = 5$)

decreasing amounts by the posterior retina/choroid and the vitreous body, in order.

In the anterior retina/choroid, the T_{max} was 3 h, C_{max} was 2820 ± 1940 ng/g (mean \pm standard deviation [SD]) $T_{1/2}$ was 600 h, and AUC_{0-360} was $500,000 \pm 90,000$ ng h/g (mean \pm standard error [SE]) (Table 1). In the posterior retina/choroid, the C_{max} and AUC_{0-360} were 173 ± 102 (SD) ng/g and $14,300 \pm 2100$ (SE) ng h/g, respectively, which were 16- and 35-fold lower than the values in the anterior retina/choroid, respectively. The T_{max} and $T_{1/2}$ in the posterior retina/choroid were 1.5 and 306 h, respectively, and the $T_{1/2}$ values were relatively similar to those of the anterior retina/choroid. In the vitreous body, the T_{max} , C_{max} , $T_{1/2}$, and AUC_{0-360} were 0.67 h, 1.42 ± 1.21 (SD) ng/g, 90.2 h, and 28.7 ± 3.9 (SE) ng h/g, respectively.

DISCUSSION

The solution pH influences the brimonidine bioavailability in the aqueous humor following ophthalmic administration. Dong et al. [3] showed that the concentration of brimonidine in the aqueous humor after topical administration was comparable between a 0.2% ophthalmic solution at pH 6.4 and a 0.15% ophthalmic solution at pH 7.3, thereby demonstrating that the bioavailability of topically applied brimonidine in the aqueous humor is improved by approximately 1.3-fold by increasing the pH of the formulation from 6.4 to 7.3. Here, we report, for the first time, the

effect of the solution pH on the distribution of brimonidine into the posterior ocular tissues following ophthalmic administration. We obtained brimonidine distribution into the posterior ocular tissues following topical administration of 0.2% brimonidine tartrate ophthalmic solution at pH 6.4, allowing us to compare these results with those obtained using a 0.1% ophthalmic solution at pH 7.3 in our previous study [15]; both studies were conducted under the same experimental conditions.

In all posterior ocular tissues, such as the anterior retina/choroid, posterior retina/choroid, and vitreous body, the AUC_{0-360} ratios following ophthalmic administration were < 2 -fold, which was the difference in the brimonidine concentrations of these two products. There was no difference between the two products that could affect the distribution of brimonidine—with the exception of pH. This result indicates that the bioavailability of brimonidine in the posterior ocular tissues was improved by increasing the pH of the ophthalmic solution, a result similar to that observed in the aqueous humor. The study by Dong et al. [3] indicated that there was a 1.5-fold difference in brimonidine concentration in the aqueous humor between the 0.2% ophthalmic solution at pH 6.4 and the 0.1% ophthalmic solution at pH 7.3. In the anterior retina/choroid, the AUC_{0-360} following administration of the 0.2% ophthalmic solution was 1.7-fold higher than that after administration of the 0.1% ophthalmic solution. This value was relatively similar to the estimated ratio in the

Table 1 Pharmacokinetics parameters in the posterior ocular tissues after single topical administration of 0.2% brimonidine tartrate ophthalmic solution in pigmented rabbits

Tissue	T_{max} (h)	C_{max} (ng/g)	$T_{1/2}$ (h)	AUC_{0-360} (ng h/g)
Anterior retina/choroid	3.0	2820 ± 1940	600	$500,000 \pm 90,000$
Posterior retina/choroid	1.5	173 ± 102	306	$14,300 \pm 2100$
Vitreous body	0.67	1.42 ± 1.21	90.2	28.7 ± 3.9

T_{max} Time to reach maximum concentration, C_{max} maximum concentration, presented as the mean \pm standard deviation ($n = 5$); $T_{1/2}$ elimination half-life. AUC_{0-360} area under the curve from time 0 to 360 h, presented as the mean \pm standard error ($n = 5$ eyes at each of eight time points)

aqueous humor. Furthermore, the AUC_{0-360} ratio in the posterior retina/choroid was 1.1, in contrast to that in the anterior retina/choroid. These results indicate a variation in the contribution ratios of the penetration routes between the anterior and posterior segments in the retina/choroid following ophthalmic administration.

Three possible local penetration routes into the posterior ocular tissues have been suggested after topical administration: (1) periocular and transposterior sclera, (2) transvitreal, and (3) uveal routes [16]. The periocular and transposterior sclera route is a pathway via the conjunctival cul-de-sac, periocular Tenon tissue, and posterior sclera. The transvitreal route is channeled via the cornea, aqueous humor, and vitreal body, while the uveal route involves the cornea, aqueous humor, and choroid. The first step in the process in all three routes involves penetration of either the cornea or conjunctiva, and it is surmised that the solution pH particularly influences this process. In vitro permeability studies have shown that the pH affects corneal penetration more than conjunctival penetration [17, 18]. In the present study, the AUC_{0-360} ratio of the two ophthalmic solutions in the anterior retina/choroid was comparable to the estimated ratio in the aqueous humor. This similarity indicates that penetration of the anterior retina/choroid by ophthalmically administered brimonidine may mainly occur through the uveal route, which involves the aqueous humor.

Some studies have indicated that the periocular and transposterior sclera route plays a main role in the penetration of topically applied drugs to the posterior parts of the retina/choroid [16, 19]. The first process in this route following ophthalmic administration is conjunctival penetration. In the in vitro studies described above, the effect of pH on the conjunctival penetration was less than that on corneal penetration, which is the first process of the uveal route [17, 18]. However, our results show that the difference in AUC_{0-360} between the two ophthalmic solutions in the posterior retina/choroid was less than that in anterior retina/choroid, which seem to be inconsistent with the in vitro results. However, this result

suggests that after ophthalmic administration in an in vivo model, some factors not associated with in vitro permeability experiments, but involved in brimonidine distribution, may be functional.

Our previous study demonstrated that the vitreal concentration could be a surrogate indicator of the concentration of free brimonidine in the posterior retina/choroid after ophthalmic dosing of 0.1% ophthalmic solution [15]. This result led us to conclude that brimonidine concentrations were correlated between these tissues. The data from the present experiment show similar AUC_{0-360} ratios between the 0.1 and 0.2% ophthalmic solutions in these tissues, indicating that the brimonidine concentration relationship between the two tissues, as previously reported, is also applicable when a different formulation is administered.

The finding of the current study regarding the effect of solution pH on brimonidine distribution is based on data obtained in the rabbit. A few studies have shown brimonidine concentrations in the vitreal body of human after topical administration of each ophthalmic product of brimonidine [12–14]. However, the effect of solution pH in humans remains uncertain because there are differences in study conditions among the published studies, such as the dosing duration and sampling time points. Further studies to reveal the effect of solution pH in human are needed for a more accurate prediction of the clinical neuroprotective effects of these products.

CONCLUSIONS

The results of the present study demonstrate that the bioavailability of brimonidine in the posterior ocular tissues was improved by increasing the pH of the ophthalmic solution. We believe that this finding supports the validity of using a brimonidine ophthalmic solution with a lower drug concentration and a higher pH to achieve the predicted neuroprotective effect concomitantly with the IOP-lowering effect. In addition, the AUC_{0-360} ratios of the two ophthalmic solutions were different between the anterior and posterior parts of the

retina/choroid, suggesting that different penetration routes may be the main contributors to the distribution of brimonidine in these tissues following ophthalmic administration.

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Compliance with Ethics Guidelines. All institutional and national guidelines for the care and use of laboratory animals were followed.

Data Availability. The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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