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Influence of pH and buffer concentration on the ocular bioavailability of ophthalmic AGN 191103 formulations in albino rabbits

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Abstract

We investigated the effect of formulation pH and buffer concentration on the ocular bioavailability of AGN 191103, a basic amine that lowers intraocular pressure. Our objective was to increase AGN 191103's ocular bioavailability enough to allow a clinically significant reduction in dose. Formulations contained ^{14}C-AGN 191103 (0.1–1% w/v), phosphate or borate buffer (0–30 mM), HCl to pH of 7.4–8.5 and benzalkonium chloride. The first experiment assessed the effect of pH and buffer concentration on the ocular bioavailability of 1% formulations; the second identified conditions of drug and buffer concentration and pH that would yield ocular concentrations comparable to that of a 1%, pH 7.2, 30 mM phosphate formulation known to be effective in vivo. Albino rabbits were given one 35-μl eyedrop, 6 h after which aqueous humors (AqH), corneas and iris-ciliary bodies (ICB) were collected. AGN 191103 is not metabolized by rabbit eyes, so samples were analyzed by liquid scintillation counting with or without combustion. Concentrations in AqH and cornea increased with increasing pH; this trend became more pronounced with increasing buffer concentration. As pH increased from 7.4 to 8.5: (1) corneal concentrations increased 94% and 300%, respectively, after unbuffered and 30 mM buffered formulation administration; (2) AqH concentrations increased 197% and 462%, respectively, after unbuffered and 30 mM buffered formulation administration; and (3) ICB concentrations did not significantly change, or increased 84%, respectively, after ubbuffered or 30 mM buffered formulation administration. Increasing buffer concentration did not affect tissue concentrations at pH 7.4, but significantly increased them at pH 8.5. A 0.2%, pH 8.2, 30 mM borate formulation elicited AqH concentrations comparable to those produced by the 1%, pH 7.2, 30 mM phosphate formulation. Our results indicate that increasing the formulation pH from 7.2 to 8.2 may allow a 5-fold reduction in the dosing concentration, which will markedly reduce the potential for systemic side effects. © 1997 Elsevier Science B.V.

Keywords: Ocular bioavailability; Formulation; pH; Buffer capacity; Albino rabbit

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1. Introduction

Less than 10% of the drug contained in an eyedrop is typically absorbed by the eye (Slovin and Robinson, 1993). The remainder is lost by drainage from the precorneal area either by spillage or by normal tear turnover, nonproductive drug absorption (mainly by conjunctiva) and binding of the drug to proteins and other components of tear fluid. Unabsorbed drug often ends up in the blood, where it can elicit undesirable systemic side effects.

One way to reduce the amount of drug in blood, and therefore the incidence and severity of systemic side effects, is to reduce the dose instilled. Merely reducing the dose may lower ocular as well as systemic concentrations, however, resulting in subtherapeutic ocular concentrations. If the fraction of dose absorbed by the eye can be increased by maximizing relevant formulation parameters, then the dose and therefore systemic concentrations can be reduced while maintaining therapeutic ocular concentrations.

α-Adrenergic agonists comprise a class of compounds showing great promise as effective treatments for glaucoma (Camras, 1995; Derick, 1995; Harris et al., 1995; Kaufman and Gabelt, 1995). These compounds lower intraocular pressure by decreasing the rate of aqueous humor production. Since these compounds may elicit unwanted systemic effects such as sedation and hypotension (Morrison, 1995), it is desirable to minimize the dose in order to minimize or avoid systemic complications. One α-adrenergic agonist in particular, AGN 191103, shows enviable potency in lowering intraocular pressure in rabbits and monkeys (unpublished data), but its use may be limited by dose-dependent sedation. AGN 191103 is a basic amine with a pK_a of 9.53 that has been formulated as a 1% solution buffered with 30 mM phosphate at pH 7.2. This pH is more than two pH units below the pK_a, however, and only 0.47% of the drug exists as the unprotonated species (Ansel, 1981). Only 0.74% of the drug is unprotonated at the physiological tear pH of 7.4, while 4.7% and 9.3% are unprotonated at pH 8.2 and 8.5, respectively. Since it is generally the neutral species that penetrates the cornea (Chien et al., 1990; Suhonen et al., 1991), we hypothesized that increasing the formulation pH, thereby increasing the fraction of AGN 191103 existing as the unprotonated species, would increase AGN 191103's ocular bioavailability. This in turn would allow a reduction in total AGN 191103 formulation concentration and, therefore, a reduction in systemic concentrations after ophthalmic dosing.

We optimized the formulation during two separate experiments in rabbits. First, we broadly assessed the effect of formulation pH and buffer capacity on drug concentrations in relevant ocular tissues. Second, we tested a narrower range of six formulations in order to determine which combination of formulation concentration, pH and buffer concentration would yield the same tissue concentrations as the 1%, pH 7.2, 30 mM phosphate solution.

2. Materials and methods

2.1. Chemicals and reagents

Clonidine hydrochloride, sodium borate decahydrate, sodium phosphate monohydrate, sodium phosphate dibasic, sodium chloride, sodium hydroxide, nitrogen, acetonitrile, acetic acid, triethylamine, heptanesulfonic acid, Beckman Ready Flow III® and Purina Certified Rabbit Chow® were procured from commercial distributors. Benzalkonium chloride (BAK) was purchased from E. Merck (Frankfurt, Germany). Eutha-6® sodium pentobarbital was supplied by Western Medical Supply (Arcadia, CA). All chemicals were reagent-grade or better; all solvents were HPLC-grade.

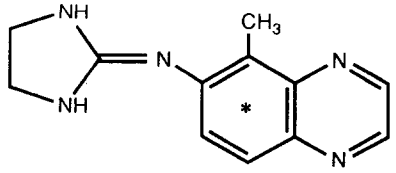
2.2. Formulations

^{14}C-AGN 191103 (239 μCi/mg; 98.7% radiochemically pure) was synthesized by Sigma (St. Louis, MO) (Scheme 1). Eleven nonradiolabeled prototype solutions were prepared at Allergan and contained AGN 191103 (0.1, 0.2, or 1% w/v), phosphate or borate buffer (0 to 30 mM), 0.0050% BAK, sodium chloride sufficient to produce an osmolality of 280–314 mOsm/kg, and

HCl sufficient to achieve pH of 7.2 to 8.5. The nonradiolabeled formulations were fortified with ^{14}C-AGN 191103 by adding 0.100 or 0.250 ml of a 1 mg/ml methanolic ^{14}C-AGN 191103 solution to separate glass tubes, evaporating to dryness with nitrogen and then reconstituting in 0.600 or 1.40 ml of a nonradiolabeled AGN 191103 formulation. These formulations were 0.1–1% (w/v) in total AGN 191103. Each 35 μl dose contained $\sim$1.5 μCi of radioactivity. All formulations were stored at ambient temperature until use.

2.3. Analysis of formulations

Each formulation was analyzed for total AGN 191103 concentration, radioactivity concentration and ^{14}C-AGN 191103 radiochemical purity. Total AGN 191103 concentrations were quantified using a validated reversed-phase HPLC method employing ultraviolet (UV) and radiometric detectors connected in tandem. Equipment used included the following: Waters WISP Model 712 Autosampler (Waters Associates, Milford, MA), Beckman Model 126 Gradient Pump System, Model 166 UV Detector and Model 171 Radiometric Detector (Beckman Instruments, Fullerton, CA) and Nelson PS/2 software (Nelson Analytical, Cupertino, CA). The column was Beckman 5 μm C_8, 4.6 mm $\times$ 25 cm and the isocratic mobile phase consisted of 200 ml of acetonitrile, 20 ml of acetic acid, 7 ml of triethylamine, 1.88 g of heptanesulfonic acid and glass-distilled deionized water sufficient to make 2000 ml of solution. Flow rate of mobile phase and Ready Flow III® scintillant was 1.7 and 4.0 ml/min, respectively. UV detection was at 254 nm and injection volume was 20 μl. Clonidine hydrochloride was used as internal standard.



^{14}C-AGN 191103
* denotes radiolabeled aromatic ring

Scheme 1. Structure of ^{14}C-AGN 191103.

Radiochemical purity was measured by monitoring the output from the radiometric HPLC detector during analysis of total AGN 191103, and was calculated by dividing the area under the ^{14}C-AGN 191103 peak by the total area under all peaks eluting within the chromatographic run time of 42 min.

Radioactivity concentrations of test articles were measured by liquid scintillation counting (LSC; Beckman Model LS 3801, Beckman Instruments, Fullerton, CA).

2.4. Animals

This study complied with all requirements of the United States Department of Agriculture (USDA) and all regulations issued by the USDA implementing the Animal Welfare Act, 9 CFR, Parts 1, 2, and 3. The animal procedures used have been approved by Allergan's Animal Care and Use Committee. Sixty female New Zealand albino rabbits purchased from Vista Rabbitry (Vista, CA) and weighing 2.0–3.5 kg were used.

2.5. Experimental

Formulations were evaluated during two experiments conducted in sequence, each of which measured ocular tissue concentrations 6 h after dosing. A previous study has indicated that 6 h is well past the t_{max} of $\sim$90 min and is in the terminal elimination phase (Small et al., 1992). The first experiment assessed the influence of pH and buffer concentration on the bioavailability of ophthalmic 1% AGN 191103 formulations and the second refined the results of the first by identifying conditions of AGN 191103 concentration, pH and buffer concentration that would yield ocular bioavailability comparable to that of a 1%, pH 7.2, 30 mM phosphate buffered formulation known to be effective in animals.

2.5.1. Effect of pH and buffer concentration at constant AGN 191103 concentration

Forty-two rabbits were divided into one group of 18 and four groups of six. The group of 18 was

administered the pH 7.9 solution buffered with 15 mM phosphate and the other four groups were given pH 7.4 or 8.5 formulations that were either unbuffered or contained 30 mM phosphate (pH 7.4) or borate (pH 8.5). All formulations contained 1% (w/v) AGN 191103. The left eye of each rabbit was dosed with one of the five formulations by gently pulling the lower eyelid away from the eye, pipetting 35 μl into the lower cul de sac and gently hand-holding the eyelids closed for $\sim$ 10 s. Rabbits were euthanized 6 h postdose by intravenous injection of pentobarbital, after which aqueous humor, cornea and iris-ciliary body were collected from dosed eyes. All tissues were stored at ≤ 4°C until analysis.

2.5.2. Effect of pH and AGN 191103 concentration at constant buffer concentration

Eighteen rabbits were divided into six groups of three. Each group received a single drop of one radiolabeled formulation containing 0.1 or 0.2% ^{14}C-AGN 191103 buffered with 30 mM phosphate or borate at pH 7.9, 8.2, or 8.5. Rabbits were dosed as described above, except that dosing was bilateral. At 6 h postdose rabbits were euthanized with pentobarbital, after which cornea and aqueous humor were collected from both eyes of each animal. All tissues were stored at ≤ 4°C until analysis.

2.6. Analysis of tissues

An aliquot of each aqueous humor sample was added to 10 ml of scintillation cocktail and analyzed by LSC. Corneas and iris-ciliary bodies were oxidized and the resulting radioactivity was also measured by LSC.

2.7. Data analysis

Tissue concentrations were normalized to dosing concentrations of 0.100, 0.200 or 1.00%. A previous study has shown that intact AGN 191103 comprises over 95% of ocular radioactivity after topical administration (Small et al., 1992); therefore, disintegrations per minute (dpm) in ocular tissues were converted to μg/g or ml. The mean and standard deviation (S.D.) of individual tissue concentrations were calculated within each formulation. The mean and S.D. of concentrations in each matrix were calculated within each formulation and compared between formulations. Concentrations in ocular tissues were assumed to be zero prior to dosing.

Data were statistically analyzed using the RS/1 programs RS/DISCOVER and ANOVA and the Macintosh program StatView II® (Abacus Concepts, Berkeley, CA). A value of $P < 0.05$, determined by an unpaired one-tailed t-test, was deemed statistically significant.

3. Results and discussion

3.1. Influence of pH and buffer concentration at constant AGN 191103 concentration

Formulation AGN 191103 concentrations ranged from 0.959 to 1.05% and the radioactivity in each 35 μl dose ranged from 1.15 to 1.33 μCi. Radiochemical purity in all formulations was $> 98\%$.

Table 1 lists drug concentrations in corneas, aqueous humors and iris-ciliary bodies 6 h after dosing. Fig. 1 depicts the effect of increasing pH and of buffer concentration. Consistent with our hypothesis, AGN 191103 concentrations in aqueous humor and cornea increased with increasing pH. The increase was especially apparent in aqueous humor. With unbuffered formulations, the 6 h aqueous humor concentrations (mean $\pm$ S.D.) tripled as the pH was raised from 7.4 to 8.5 (0.287 ± 0.104 to 0.852 ± 0.548 μg/ml; $P = 0.016$). At buffer concentrations of 30 mM, the increase was more than five-fold, from 0.345 ± 0.134 μg/ml at pH 7.4 to 1.94 ± 0.84 μg/ml at pH 8.5 ($P = 0.0005$). Increasing the pH from 7.4 to 8.5 doubled corneal concentrations after unbuffered formulation administration (2.10 ± 0.99 to 4.07 ± 2.48 μg/g; $P = 0.050$) and quadrupled them after administration of fomulations containing 30 mM buffer (2.41 ± 0.94 to 9.63 ± 4.60 μg/g; $P = 0.0019$).

Increasing the pH of the 30 mM formulation from 7.4 to 8.5 resulted in a doubling of iris-ciliary body concentrations (1.54 ± 0.82 to $2.84 \pm$

Table 1
AGN 191103 concentrations in the corneas, aqueous humors and iris ciliary bodies of albino rabbits 6 h after topical administration of 1% ^{14}C-AGN 191103 formulations that varied in pH and buffer concentration

Buffer (mM)	pH		
	7.4	7.9	8.5
Cornea (μg/g)			
0	2.10 ± 0.99 (6)	ND[a]	4.07 ± 2.48 (6)
15	ND[a]	2.81 ± 1.54 (18)	ND[a]
30	2.41 ± 0.94 (6)	ND[a]	9.63 ± 4.60 (6)
Aqueous humor (μg/ml)			
0	0.287 ± 0.0104 (6)	ND[a]	0.852 ± 0.548 (6)
15	ND[a]	0.436 ± 0.222 (17)	ND[a]
30	0.345 ± 0.134 (6)	ND[a]	1.94 ± 0.84 (6)
Iris ciliary body (μg/g)			
0	1.63 ± 0.70 (5)	ND[a]	1.24 ± 0.69 (5)
15	ND[a]	1.46 ± 0.71 (18)	ND[a]
30	1.54 ± 0.82 (6)	ND[a]	2.84 ± 1.34 (6)

Data are expressed as mean ± S.D. (N).
[a] Not determined.

1.34 μg/g; $P = 0.035$), as did increasing the buffer concentration of the pH 8.5 formulation from zero to 30 mM (1.24 ± 0.69 and 2.84 ± 1.34 μg/g, respectively; $P = 0.020$).

Unlike those in cornea and aqueous humor, concentrations in iris-ciliary body did not increase with increasing pH in unbuffered solutions. Although the reason for this was not investigated, it may reflect several anatomical or physiological influences. Firstly, it is known that the mass of drug that appears in aqueous humor after topical administration generally increases with increasing drug lipophilicity. However, the fraction of that drug that gets there via scleral absorption, as opposed to corneal absorption, decreases with increasing lipophilicity (Chien et al., 1990). This suggests that corneal and scleral absorption are affected to different magnitudes by changes in drug lipophilicity and ionization. Since the iris-ciliary body is anchored to the front ocular surface at the junction of the cornea and sclera, drug concentrations in the iris-ciliary body may be influenced by scleral penetration in a way that aqueous humor and corneal concentrations are not. Secondly, aqueous humor flows from the ciliary body past the iris to the aqueous humor and therefore drug that enters the aqueous humor via corneal absorption must travel against this flow in order to get to parts of the iris and the ciliary body. Since this is unlikely to happen, it is unlikely that iris-ciliary body concentrations accurately reflect aqueous humor drug concentrations, and therefore probably are not accurate indicators of corneal absorption. Thirdly, the iris-ciliary body is vascularized and rapid removal of drug by blood may create in these tissues a dynamic state

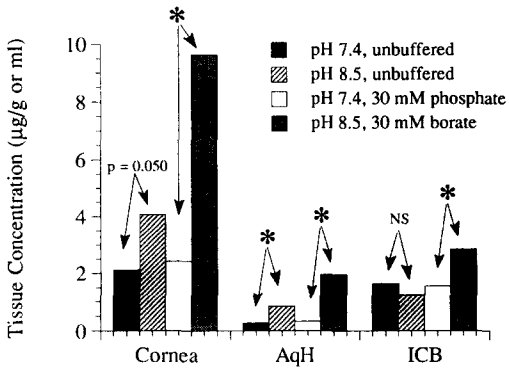


Fig. 1. Mean AGN 191103 concentrations in cornea, aqueous humor (AqH) and iris-ciliary body (ICB) after ophthalmic administration of unbuffered or buffered 1% solutions of pH 7.4 or 8.5 to albino rabbits. Asterisks and NS signify $P < 0.05$ and $P > 0.0.5$, respectively.

of flux that could attenuate any difference caused by formulation parameters. Regardless of the reason, the data indicate that the iris-ciliary body is less sensitive to changes in pH and buffer concentrations than are cornea and aqueous humor.

At pH 7.4 the buffer concentration had no discernable effect on AGN 191103 aqueous humor concentrations in any of the three tissues studied (Table 1). When the pH of the formulation was raised to 8.5, however, increased buffer concentration resulted in significantly higher drug concentrations in all three tissues. At pH 8.5, increasing the buffer concentration from zero to 30 mM more than doubled the concentrations in all three matrices (cornea from 4.07 ± 2.48 to 9.63 ± 4.60 μg/g, $P = 0.013$; aqueous humor from 0.852 ± 0.548 to 1.94 ± 0.84 μg/ml, $P = 0.012$; and iris-ciliary body from 1.24 ± 0.69 to 2.84 ± 1.34 μg/g, $P = 0.020$).

This makes sense intuitively. The buffer serves no real purpose at physiological pH, since the pH of the instilled drop and the ocular environment into which it is instilled is the same. When the formulation pH is higher than the ocular pH, however, higher buffer concentration may better resist the neutralization to physiological pH, thereby prolonging the mean residence time of the unionized species that is preferentially absorbed.

It appears from this study that increasing the pH to 8.5, while maintaining the buffer concentration of 30 mM currently used in the clinically tested 1% pH 7.2 formulation, would lead to clinically significant increases in the ocular bioavailability of AGN 191103. These increases may allow substantial reductions in dosing concentration while maintaining equivalent efficacy and significantly reducing systemic side effects. Assuming linear pharmacokinetics, comparison of these results with those obtained following dosing of albino rabbits with a 1%, pH 7.2, 30 mM phosphate formulation (unpublished data) suggest that dosing concentrations of 0.12% AGN 191103 at pH 8.5 and 1% AGN 191103 at pH 7.2 would result in an equivalent flux of drug into the aqueous humor. This would permit an estimated eight-fold reduction in formulation concentration and a commensurate reduction in systemic exposure without affecting ocular concentrations.

Table 2
AGN 191103 concentrations in cornea and aqueous humor 6 h after administration of ^{14}C-AGN 191103 formulations differing in concentration and pH

Formulation		Tissue concentration	
Concentration (%)	pH	Cornea (μg/g)	Aqueous humor (μg/ml)
0.100	7.9	0.356 ± 0.165	0.0304 ± 0.0089
0.100	8.2	0.445 ± 0.274	0.0740 ± 0.0376
0.100	8.5	0.830 ± 0.245	0.148 ± 0.053
0.200	7.9	1.19 ± 1.21	0.0763 ± 0.0357
0.200	8.2	1.24 ± 0.22	0.254 ± 0.141
0.200	8.5	2.15 ± 0.80	0.404 ± 0.226

Results are expressed as mean $\pm$ S.D. ($n = 6$).

3.2. Influence of pH and AGN 191103 concentration at constant buffer concentration

Results from this experiment are presented in Table 2. The 0.1% formulations ranged from 0.110 to 0.114% and 32.5 to 37.4 μCi/ml. The 0.2% formulations ranged from 0.211% to 0.216% and 37.2 to 37.8 μCi/ml. Radiochemical purity was 97.9%.

The 'target' aqueous humor concentration was 0.243 μg/ml, which was the mean AGN 191103 concentration in albino rabbits 6 h after administration of a single 35 μl eyedrop of the 1% pH 7.2 formulation buffered with 30 mM phosphate (unpublished data). The 0.2% pH 8.2 formulation yielded an aqueous humor concentration of 0.254 ± 0.141 μg/ml, which was nearly identical to the target concentration. This suggests that increasing the formulation pH to 8.2 will allow a five-fold reduction in the dosing concentration, which will markedly reduce the potential for systemic side effects. This five-fold reduction in dosing concentration is a little less than the eight-fold reduction predicted from the first experiment, but is still substantial and could prove to be quite clinically significant.

An interesting result of this study was that tissue concentrations were not dose-proportional at any of the three pHs. Within each pH, corneal and aqueous humor concentrations after administration of the 0.2% formulation were not 100% higher than those after administration of the 0.1%

formulation, but rather were 151% to 243% higher. Reducing the dosing concentration only five-fold, instead of eight-fold as predicted from the above experiments with 1% solutions, is consistent with this trend.

There are at least two plausible explanations for this nonlinearity. One is that higher AGN 191103 doses decrease intraocular clearance and/or volume of distribution. Since AGN 191103 lowers intraocular pressure by reducing the rate of aqueous humor production, a lower clearance may reflect slower washout by new aqueous humor, while a reduced volume of distribution may reflect reduced aqueous humor volume. Either of these phenomena would increase aqueous humor and corneal concentrations and would therefore explain the observed nonproportionality with dose. Another explanation consistent with the data is that AGN 191103 is enhancing its own absorption by increasing the formulation buffer capacity in the pH range of 7.5 to 8.5, thereby resisting the return to physiological pH and prolonging the mean residence time of the unionized species. The respective pK_as of AGN 191103 and boric acid are 9.5 and 9.23 (Perrin, 1974) and the respective concentrations of AGN 191103 and borate in the 1% pH 8.5 solution are approximately 44 and 30 μM. Given these similarities, the incremental buffer capacity contributed by the AGN 191103 in the 1% solution nearly doubles that of the borate alone (Perrin, 1974) and may contribute significantly to a slower return to physiological pH.

There are two other possible, but unlikely, explanations for the nonlinearity at higher AGN 191103 concentrations. One is that AGN 191103 enhances its own penetration by altering corneal integrity. This is improbable given that safety evaluation studies have shown that chronic administration has no observable effect on corneal structure. The other unlikely explanation is that AGN 191103 decreases tear flow, which increases its residence time in precorneal tear fluid. Lacrimination may be influenced by drugs and drug concentration (Conrad et al., 1978) but safety evaluation studies have indicated no tendency for AGN 191103 to alter flow and it is therefore improbable that decreased lacrimination caused the observed nonlinearity in this experiment.

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