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Increasing pH of ophthalmic AGN 191103 formulation increases ocular but not systemic bioavailability in albino rabbits

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Abstract

AGN 191103 is an α_2-adrenergic agonist that lowers intraocular pressure in animals, but produces dose-dependent sedation. The objective of this study was to test the hypothesis that a 0.2% pH 8.2 ophthalmic AGN 191103 formulation yields comparable ocular concentrations as, but lower systemic concentrations than, a 1% pH 7.2 formulation. Specifically, AGN 191103 concentrations in aqueous humor and plasma were measured for 24 h after acute administration of ^{14}C-AGN 191103 formulated as a 0.94% pH 7.2 or 0.24% pH 8.2 solution and for 8 h after $7\frac{1}{2}$ days of twice-daily administration of AGN 191103 formulated as a 0.2% pH 7.2 or 0.2% pH 8.2 solution. After acute administration of the 0.94% pH 7.2 or 0.24% pH 8.2 solution, there was no difference in aqueous humor $AUC_{0-t_{last}}$ (5879 ± 541 vs. 5697 ± 385 ng·h/ml, respectively; $p > 0.5$) or C_{max} (1310 ± 160 vs. 1630 ± 180 ng/ml, respectively; $p = 0.238$) in dosed eyes. Plasma $AUC_{0-t_{last}}$ was dose-proportionally 77% lower (3.37 ± 0.35 vs. 14.8 ± 3.3 ng·h/ml) and C_{max} was 75% lower (1.40 ± 0.16 vs. 5.59 ± 2.87 ng/ml) after 0.24% pH 8.2 administration than 0.94% pH 7.2 dosing. The ratio of aqueous humor $AUC_{0-\infty}$ to plasma $AUC_{0-\infty}$ was 333% higher after dosing of 0.24% pH 8.2 than of 0.94% pH 7.2 solution. After repeated twice-daily instillation of 0.2% pH 7.2 or 0.2% pH 8.2 solution, aqueous humor $AUC_{0-t_{last}}$ after pH 8.2 administration was 304% that following pH 7.2 dosing (1120 ± 140 vs. 368 ± 120 ng·h/ml; $p < 0.0005$). Plasma $AUC_{0-t_{last}}$ following pH 8.2 dosing did not differ significantly from that following pH 7.2 instillation (2.13 ± 0.23 vs. 2.70 ± 0.36 ng·h/ml; $p > 0.1$). The ratio of aqueous humor $AUC_{0-\infty}$ to plasma $AUC_{0-\infty}$ was 339% higher after dosing with 0.2% pH 8.2 than 0.2% pH 7.2 solution. These results indicate that in albino rabbits a 0.2% pH 8.2 AGN 191103 solution produces comparable aqueous humor concentrations as a 1% pH 7.2 formulation, but only one-fourth the plasma concentrations. © 1997 Elsevier Science B.V.

Keywords: Formulation pH; Ocular bioavailability; Rabbit; Safety margin; Systemic bioavailability

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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.

The incidence and severity of systemic side effects can be lessened by reducing the dose instilled. Merely reducing the dose may lower ocular as well as systemic concentrations, however, resulting in subtherapeutic ocular concentrations. If the ocular bioavailability can be increased by optimizing 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 treatments for glaucoma (Derick, 1995; Kaufman and Gabelt, 1995; Harris et al., 1995; Camras, 1995). 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 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 (unpublished data) that has been formulated as a 1% solution buffered with 30 mM phosphate at pH 7.2. Previous work involving AGN 191103 has focused on optimizing drug concentration, buffer concentration and formulation pH within acceptable parameters in order to maximize ocular concentrations (Small et al., 1996). This work has shown that ocular AGN 191103 concentrations after ophthalmic administration are formulation pH-dependent, increasing up to 5-fold as pH increases from 7.4 to 8.5. These investigations did not measure drug concentrations in systemic blood, however, nor did they assess the concentration–time course of drug in ocular tissues, relying instead on concentrations at a single time point in the terminal elimination phase to reflect the extent of drug absorption.

Our hypothesis for the present study was that formulation pH would significantly affect ocular bioavailability, but would not affect systemic concentrations. Our belief for the former stems directly from our previous work. Our belief for the latter is based on the fact that very little of an ophthalmic dose is absorbed by the eye, and much of the remainder ends up in systemic blood. Therefore, even a 5-fold increase in ocular bioavailability, say from 1 to 5%, may reduce systemic concentrations less than 5%.

We tested our hypothesis in two complementary experiments, both involving quantitation of the concentration–time course in relevant ocular tissues and blood after ophthalmic administration of AGN 191103. In one experiment, we gave a single dose of ^{14}C-AGN 191103 formulated as a 1% pH 7.2 or a 0.2% pH 8.2 solution, which our hypothesis predicted would yield comparable ocular AGN 191103 concentrations but dissimilar systemic concentrations. In another experiment, we administered multiple ophthalmic doses of AGN 191103 formulated as a 0.2% solution of pH 7.2 or 8.2, which our hypothesis predicted would produce comparable systemic concentrations but different ocular concentrations.

2. Materials and methods

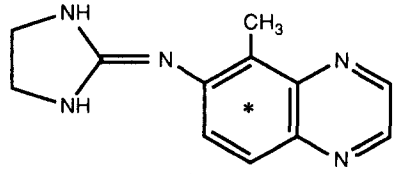
2.1. Chemicals and reagents

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 high-performance liquid chromatography (HPLC) grade.

2.2. Formulations

^{14}C-AGN 191103 (239 μCi/mg; 98.7% radiochemically pure) was synthesized by Sigma (St. Louis, MO). Three AGN 191103 solutions were prepared at Allergan: 1.0% (w/v) pH 7.2, 0.2% pH 7.2, and 0.2% pH 8.2. All contained 30 mM phosphate (pH 7.2) or borate (pH 8.2), 0.0050% BAK, sodium chloride sufficient to produce an osmolality of ~ 300 mOsm/kg and HCl sufficient to pH. The 1% pH 7.2 and a portion of the 0.2% pH 8.2 formulation were fortified with ^{14}C-AGN 191103 using previously described methods (Small et al., 1996), such that each 35-μl dose contained about 1.5 μCi of radioactivity. All formulations were stored at ambient temperature until use.



^{14}C-AGN 191103

* denotes labeled aromatic ring

2.3. Analysis of formulations

AGN 191103 concentrations, radioactivity concentrations, and radiochemical purity were quantified by reversed-phase HPLC and liquid scintillation counting as previously reported (Small et al., 1996).

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. Female New Zealand albino rabbits weighing 2.0–3.5 kg were purchased from Vista Rabbitry (Vista, CA).

2.5. Experimental

Formulations were evaluated during two experiments, each of which quantified the concentration–time profile of AGN 191103 in aqueous humor and plasma after ophthalmic administration. The first experiment measured aqueous humor and plasma concentrations after acute administration of ^{14}C-AGN 191103 formulated as a 1% pH 7.2 or 0.2% pH 8.2 solution. The second experiment quantified aqueous humor and plasma concentrations after multiple administration of AGN 191103 formulated as a 0.2% pH 7.2 or 0.2% pH 8.2 solution.

2.5.1. Single dose: 1% pH 7.2 vs. 0.2% pH 8.2

One hundred and thirteen rabbits were divided into groups of 52, 55 and six that remained untreated. Unilateral doses were administered by pipetting 35.0 μl of solution into the lower cul-de-sac of each rabbit's left eye. Fifty-five animals were given the 1% pH 7.2 solution, after which blood was collected from four animals at 10, 20 and 40 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 16 or 24 h. Fifty-eight animals were given the 0.2% pH 8.2 formulation, after which blood was sampled from five animals at 20 and 40 min 1, 1.5, 2, 3, 4, 6, 9, 12 or 24 h. Different sampling times and number of animals per time point resulted from an adjustment made between study arms. Blood was kept on ice until processing. Rabbits were euthanized by intravenous injection of pentobarbital immediately following blood collection, after which aqueous humors were aspirated from both eyes. Plasma was harvested by centrifugation of blood within 30 min of sampling. All plasma samples were stored at ≤ -20°C until analysis.

2.5.2. Multiple dose: 0.2% pH 7.2 vs. 0.2% pH 8.2

Fifty-three rabbits were divided into two groups of 25 and an untreated group of three. Unilateral doses were administered as described above. One group received the 0.2% pH 7.2 formulation and the other received the 0.2% pH 8.2

solution. Doses were administered twice-daily for 7 days, at approximately 07:00 and 15:00, and once at approximately 07:00 on day 8. Blood was collected from five animals per time point 45 min and 1.5, 3, 5 or 8 h after dosing and was kept on ice until processing. Rabbits were euthanized by intravenous injection of pentobarbital immediately following blood collection, after which aqueous humors were collected from dosed eyes. Plasma was harvested by centrifugation of blood within 30 min of sampling. All plasma samples were stored at ≤ -20°C until analysis.

2.6. Analysis of tissues

2.6.1. Aqueous humor

AGN 191103 concentrations in 50- or 200-μl aliquots were quantified by liquid scintillation counting (LSC) after administration of radiolabeled drug and by reversed-phase HPLC (Small et al., 1992) after instillation of nonradiolabeled material.

2.6.2. Plasma

AGN 191103 concentrations in samples taken through 6 h were quantified by a validated gas chromatography/mass spectrometry (GC/MS) method using D_4-AGN 191103 as an internal standard. Measurements of total radioactivity indicated that plasma concentrations beyond 6 h would be below the GC/MS assay's limit of quantitation (LOQ) of 0.0196 ng/ml. Plasma aliquots of 0.500 ml were extracted with ethyl acetate under basic conditions, followed by back-extraction into sodium acetate buffer and then another basic extraction into methylene chloride. The organic layer was removed and evaporated to dryness, derivatized with bis(3,5-trifluoromethyl) benzoyl chloride and then evaporated again. The residue was reconstituted in ethyl acetate and injected onto a Finnigan 9611 GC/MS (Cincinnati, OH) equipped with a Hewlett-Packard 7673A autoinjector (Avondale, PA) and a J and W Scientific DB5GC column (15 m × 0.25 mm i.d., 0.25 μm coating thickness, Krackeler Scientific, Albany, NY). The mass spectrometer was a Finnigan SQ 4600 and the data system was Finnigan INCOS. The derivatized AGN 191103 $[M]^-$ and D_4-AGN 191103 $[M]^-$ were monitored at m/z 707 and 711, respectively. AGN 191103 and D_4-AGN 191103 nearly coeluted at about 3.75 min.

2.7. Data analysis

Aqueous humor disintegrations per minute (dpm) were converted to ng/ml based on the specific activity in the dosing formulation and previous work indicating that intact AGN 191103 comprises over 95% of aqueous humor radioactivity (Small et al., 1992). Dpm were considered below the limit of quantitation (BLQ) if they were less than two standard deviations above the mean of untreated samples. The mean and standard error of the mean (S.E.M.) of individual plasma and aqueous humor concentrations were calculated within each group at each sampling time.

Pharmacokinetic parameters were calculated using noncompartmental methods (Gibaldi and Perrier, 1982). Maximum concentrations (C_{max}) and the times at which they occurred (t_{max}) were identified by inspection of composite curves. The terminal half-life ($t_{1/2}$) of the composite curve was calculated as $(\ln 2)/k$, where k is the absolute value of the slope of the terminal linear phase of ln concentration versus time. Areas under the concentration versus time and concentration × time versus time curves from zero to the last quantifiable sampling time ($AUC_{0-t_{last}}$ and $AUMC_{0-t_{last}}$, respectively) were assessed using a previously described method to calculate the mean, S.E.M., and degrees of freedom (df) of concentration–time curves generated from individual animals such that each animal contributes only one datum to a pool of data (Tang-Liu and Burke, 1988). AUC from the last quantifiable sampling time through infinity ($AUC_{t_{last}-\infty}$) was calculated as C_{last}/k, where C_{last} was the last measured concentration. AUMC from the last sampling time through infinity ($AUMC_{t_{last}-\infty}$) was calculated as $(t_{last}C_{last}/k) + C_{last}/k^2$. $AUC_{0-\infty}$ was defined as $AUC_{0-t_{last}} + AUC_{t_{last}-\infty}$ and $AUMC_{0-\infty}$ was defined as $AUMC_{0-t_{last}} + AUMC_{t_{last}-\infty}$. The mean residence time (MRT) was calculated as $AUMC_{0-\infty}/AUC_{0-\infty}$. Differences between groups were compared using Student's t-test, and were deemed statistically significant if $p < 0.05$.

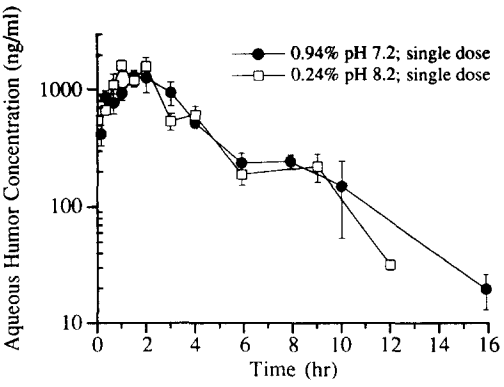


Fig. 1. Aqueous humor concentrations (mean ± S.E.M., $n = 4$ or 5) of AGN 191103 following administration of a single 35-μl eyedrop of a 0.94% pH 7.2 or 0.24% pH 8.2 solution to albino rabbits.

3. Results

3.1. 1% pH 7.2 vs. 0.2% pH 8.2

The 1% formulation was 0.938% (w/v) in total AGN 191103 and was 100% radiochemically pure; each 35-μl dose contained 328 μg of AGN 191103 and 2.63 μCi of radioactivity. The 0.2% pH 8.2 formulation was 0.244% in total AGN 191103 and was 99.4% radiochemically pure; each 35-μl dose contained 85.5 μg of AGN 191103 and 2.91 μCi of radioactivity. The limit of quantitation (LOQ) in aqueous humor, determined by LSC, was 3.42 ng/ml after 1% pH 7.2 administration and 17.4 ng/ml after 0.2% pH 8.2 administration. The LOQ in plasma, determined by GC/MS, was 0.0196 ng/ml after both treatments.

Aqueous humor concentrations after acute administration of 0.94% pH 7.2 and 0.24% pH 8.2 formulations are shown in Fig. 1. Pharmacokinetic parameters are summarized in Table 1. Aqueous humor concentrations were quantifiable at the first sampling time of 10 min (0.94% pH 7.2) or 20 min (0.24% pH 8.2) and were above the LOQ through 16 h (0.94% pH 7.2) or 12 h (0.24% pH 8.2) but not 24 h. Most aqueous humor concentrations fell below the LOQ in the contralateral eye.

There was no statistically significant difference between formulations in aqueous humor $AUC_{0-t_{last}}$ ($p > 0.5$) or C_{max} ($p = 0.238$) in dosed eyes. A comparison of aqueous humor $AUC_{0-t_{last}}$ to $AUC_{0-\infty}$ and of $AUMC_{0-t_{last}}$ to $AUMC_{0-\infty}$ indicates that nearly the entire concentration–time curve fell under the measured portion of the curve, leaving less than 2% of the $AUC_{0-\infty}$ and less than 5% of the $AUMC_{0-\infty}$ to be estimated by extrapolation. Aqueous humor t_{max} and MRT were also comparable between the two formulations.

Plasma concentrations after acute administration of 0.94% pH 7.2 and 0.24% pH 8.2 formulations are shown in Fig. 2. Pharmacokinetic

Table 1
Pharmacokinetic parameters of AGN 191103 in aqueous humor and plasma following administration of a single 35-μl eyedrop of a 0.94% pH 7.2 ($n = 4$) or 0.24% pH 8.2 ($n = 5$) solution to one eye of albino rabbits

	Aqueous humor		Plasma	
	0.94% pH 7.2	0.24% pH 8.2	0.94% pH 7.2	0.24% pH 8.2
C_{max} (ng/ml)	1310 ± 160	1630 ± 180	5.59 ± 2.87	1.40 ± 0.16
t_{max} (h)	1.5	1	2	0.67
k (h^{-1})	0.282	0.327	0.641	0.675
$t_{1/2}$ (h)	2.45	2.12	1.08	1.03
$AUC_{0-t_{last}}$ df	9.10	18.1	8.17	6.62
$AUC_{0-t_{last}}$ (ng·h/ml)	5879 ± 541	5697 ± 385.4	14.8 ± 3.3	3.37 ± 0.35
$AUC_{0-\infty}$ (ng·h/ml)	5949	5794	15.5	3.48
$AUMC_{0-t_{last}}$ (ng·h^2/ml)	23 183	18 933	36.8	6.10
$AUMC_{0-\infty}$ (ng·h^2/ml)	24 551	20 395	42.1	6.95
MRT (h)	4.13	3.52	2.72	2.00

C_{max} and $AUC_{0-t_{last}}$ are expressed as mean ± S.E.M..

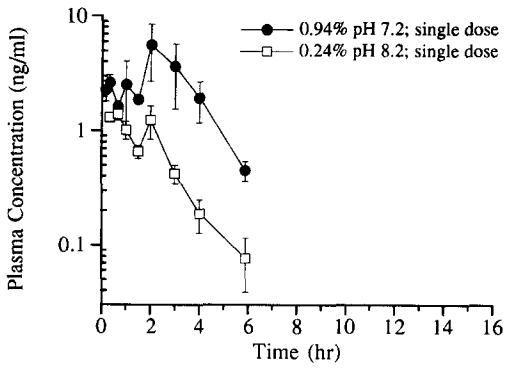


Fig. 2. Plasma concentrations (mean ± S.E.M., $n = 4$ or 5) of AGN 191103 following administration of a single 35-μl eye-drop of a 0.94% pH 7.2 or 0.24% pH 8.2 solution to one eye of albino rabbits.

parameters are summarized in Table 1. Plasma concentrations of AGN 191103 were quantifiable at the first sampling time of 10 min (0.94% pH 7.2) or 20 min (0.24% pH 8.2) and remained so in all animals through the last sampling time of 6 h. A single 1-h plasma concentration after 0.24% pH 8.2 administration was excessively high and was omitted as an outlier.

Plasma $AUC_{0-t_{last}}$ differed significantly between formulations ($p < 0.01$). A comparison of plasma $AUC_{0-t_{last}}$ to $AUC_{0-\infty}$ and of $AUMC_{0-t_{last}}$ to $AUMC_{0-\infty}$ indicates that most of the concentration–time curve fell under the measured portion of the curve, leaving less than 5% of the $AUC_{0-\infty}$ and less than 15% of the $AUMC_{0-\infty}$ to be estimated by extrapolation. Plasma C_{max} and AUCs indicate that the increased ocular bioavailability conferred by higher formulation pH was not accompanied by higher systemic bioavailability. The 74% reduction in formulation concentration from 0.938% to 0.244% produced respective 75 and 77% reductions in plasma C_{max} and $AUC_{0-\infty}$. Although the difference in plasma C_{max} values was not statistically significant between formulations ($p = 0.141$), this may be due to the high variability of concentrations measured 2 h after 0.94% pH 7.2 administration, rather than from a true lack of difference in maximum concentrations. The shorter t_{max} and MRT, and the plasma concentration–time profile shown in Fig. 2, are consistent with more rapid absorption of the higher pH formulation into systemic blood (Rowland and Tozer, 1980), but the proportional $AUC_{0-\infty}$ estimates indicate that the extent of systemic absorption was no different between formulations.

The ratio of aqueous humor $AUC_{0-\infty}$ to plasma $AUC_{0-\infty}$, a parameter indicative of safety margin (Olejnik, 1993), was 333% higher after pH 8.2 administration, illustrating that the higher pH formulation targeted AGN 191103 more efficiently to ocular tissues and supporting the hypothesis that increased ocular bioavailability was not accompanied by a concomitant increase in systemic bioavailability.

3.2. 0.2% pH 7.2 vs. 0.2% pH 8.2

Each 35-μl dose contained 70 μg of AGN 191103. The LOQs determined by HPLC in aqueous humor and by GC/MS in plasma were 10.0 and 0.0196 ng/ml, respectively.

Aqueous humor concentrations after multiple dosing with 0.2% pH 7.2 and 0.2% pH 8.2 formulations are shown in Fig. 3. Pharmacokinetic parameters are summarized in Table 2. Aqueous humor concentrations were quantifiable from the first sampling time of 45 min through 5 h (0.2% pH 7.2) or 3 h (0.2% pH 8.2). Concentrations 8 h after 0.2% pH 7.2 dosing and 5 h after 0.2% pH 8.2 administration were BLQ. Mean aqueous hu-

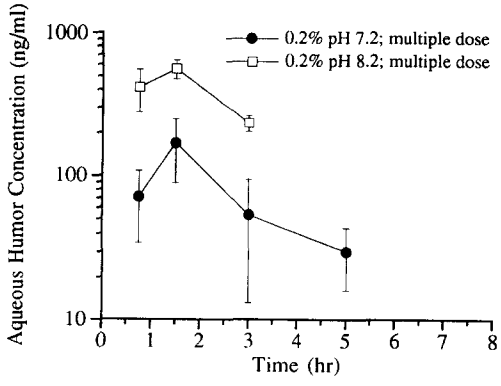


Fig. 3. Aqueous humor concentrations (mean ± S.E.M., $n = 5$) of AGN 191103 following twice-daily ophthalmic administration of a 0.2% pH 7.2 or 0.2% pH 8.2 solution to albino rabbits for $7\frac{1}{2}$ days.

Table 2
Pharmacokinetic parameters of AGN 191103 in aqueous humor and plasma following twice-daily ophthalmic administration of a 0.2% pH 7.2 or 0.2% pH 8.2 solution to both eyes of albino rabbits for $7\frac{1}{2}$ days ($n = 5$)

	Aqueous humor		Plasma	
	0.2% pH 7.2	0.2% pH 8.2	0.2% pH 7.2	0.2% pH 8.2
C_{max} (ng/ml)	170 ± 81	561 ± 85	1.05 ± 0.17	1.23 ± 0.20
t_{max} (h)	1.5	1.5	1.5	0.75
k (h^{-1})	0.485	0.578	0.388	0.429
$t_{1/2}$ (h)	1.43	1.20	1.79	1.62
t_{last} (h)	5	3	8	8
$AUC_{0-t_{last}}$ df	8.63	8.37	12.3	8.69
$AUC_{0-t_{last}}$ (ng·h/ml)	368 ± 120	1120 ± 140	2.70 ± 0.36	2.13 ± 0.23
$AUC_{0-\infty}$ (ng·h/ml)	430	1527	2.82	2.29
$AUMC_{0-t_{last}}$ (ng·h^2/ml)	758 ± 264	1710 ± 177	6.10 ± 0.81	3.83 ± 0.41
$AUMC_{0-\infty}$ (ng·h^2/ml)	1195	3634	7.42	5.42
MRT (h)	2.78	2.38	2.63	2.37

C_{max} and $AUC_{0-tlast}$ are expressed as mean ± SEM.

mor concentrations at 0.75, 1.5 and 3 h after pH 8.2 administration were 4.8-, 2.3- and 3.4-fold higher, respectively, than those after pH 7.2 instillation. Aqueous humor $AUC_{0-t_{last}}$ after pH 8.2 administration was over 2-fold higher than that following pH 7.2 dosing ($p < 0.0005$). A comparison of aqueous humor $AUC_{0-t_{last}}$ to $AUC_{0-\infty}$ indicates that most of the concentration–time curve fell under the measured portion of $AUC_{0-\infty}$, but that 14 and 27% of $AUC_{0-\infty}$ was estimated by extrapolation after pH 7.2 and 8.2 dosing, respectively. Likewise, 37 and 53% of $AUMC_{0-\infty}$ was extrapolated after pH 7.2 and 8.2 dosing, lending a degree of uncertainty to estimates of $AUMC_{0-\infty}$ and MRT.

Mean aqueous humor concentrations following pH 8.2 administration were not quantifiable after 3 h because three of the five concentrations were BLQ. Only two of the five pH 7.2 aqueous humor concentrations were BLQ at 3 h. Therefore, the 3-h pH 7.2 aqueous humor concentrations were reported as 30.0 ± 13.9 ng/ml, while the pH 8.2 concentrations were reported as BLQ. The difference in reported means at 3 h is therefore more an artifact of the method chosen to treat values BLQ than a reflection of a true difference. The lower aqueous humor concentrations measured 5 h after pH 8.2 administration are likely the consequence of concentrations being very near the LOQ after both formulations and are probably not as reliable as those concentrations measured at earlier sampling times.

Plasma concentrations after multiple dosing with 0.2% pH 7.2 and 0.2% pH 8.2 formulations are shown in Fig. 4. Pharmacokinetic parameters are summarized in Table 2. Plasma $AUC_{0-t_{last}}$ following pH 8.2 administration did not differ from that following pH 7.2 instillation ($p > 0.1$). Most of the concentration–time curve fell under the measured portion of the curve, leaving less than 7% of the $AUC_{0-\infty}$ and less than 30% of the

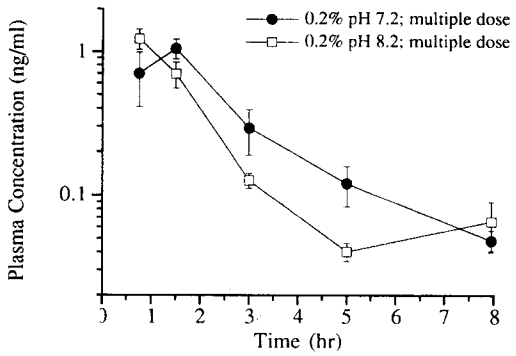


Fig. 4. Plasma concentrations (mean ± S.E.M., $n = 5$) of AGN 191103 following twice-daily ophthalmic administration of a 0.2% pH 7.2 or 0.2% pH 8.2 solution to both eyes of albino rabbits for $7\frac{1}{2}$ days.

$AUMC_{0-\infty}$ to be estimated by extrapolation. Therefore, MRT estimates of about 2.5 h are most likely reliable.

The ratio of aqueous humor $AUC_{0-\infty}$ to plasma $AUC_{0-\infty}$ was 339% higher after pH 8.2 administration, indicating that the higher pH formulation has a higher safety margin than the lower pH solution.

4. Discussion

We assume the following relative relationship between ocular and systemic bioavailabilities after administration of pH 7.2 and 8.2 formulations of equal concentration:

$$\frac{AUC_{AqH}^{pH\ 8.2}}{AUC_{AqH}^{pH\ 7.2}} = \frac{F_{AqH}^{pH\ 8.2}}{F_{AqH}^{pH\ 7.2}}$$

$$\frac{AUC_{plasma}^{pH\ 8.2}}{AUC_{plasma}^{pH\ 7.2}} = \frac{F_{plasma}^{pH\ 8.2} - F_{plasma}^{pH\ 8.2} F_{AqH}^{pH\ 8.2}}{F_{plasma}^{pH\ 7.2} - F_{AqH}^{pH\ 7.2}}$$

where $F_{AqH}^{pH\ 7.2}$ and $F_{AqH}^{pH\ 8.2}$ are ocular bioavailabilities after pH 7.2 and 8.2 administration, $F_{plasma}^{pH\ 7.2}$ and $F_{plasma}^{pH\ 8.2}$ would be the systemic bioavailabilities after pH 7.2 and 8.2 administration if ocular bioavailability were zero, and the term $F_{plasma}^{pH\ 8.2} F_{AqH}^{pH\ 8.2}$ reflects the fraction of applied drug that becomes unavailable for systemic absorption because of increased ocular bioavailability. If F_{plasma} is substantially greater than F_{AqH}, then the ratio of systemic concentrations reduces to:

$$\frac{AUC_{plasma}^{pH\ 8.2}}{AUC_{plasma}^{pH\ 7.2}} = \frac{F_{plasma}^{pH\ 8.2}}{F_{plasma}^{pH\ 7.2}}$$

If both $F_{plasma}^{pH\ 8.2}$ and $F_{plasma}^{pH\ 7.2}$ are high, then this fraction further reduces to approximately one, indicating minimal effect of increased ocular bioavailability on systemic bioavailability. Therefore, in cases of high systemic bioavailability, large increases in ocular bioavailability may cause only small changes in systemic concentrations. This prediction holds for many drugs, since for most ocular drugs the assumptions of high systemic bioavailability and low ocular bioavailability are valid (Lee and Robinson, 1986; Chang and Lee, 1987; Slovin and Robinson, 1993). This relationship is analogous to that existing between free and bound concentrations of highly protein bound drugs, in which a 1900% increase in free fraction from 0.1 to 2% will decrease bound concentrations only 1.90%, from 99.9 to 98.0%.

The results of this study are consistent with the above model, the pH-partition theory that predicts that unionized molecules penetrate biological membranes more readily than their charged counterparts (Brodie, 1964), in vitro evidence of a dependence of ocular penetration on formulation pH (Ashton et al., 1991; Richman et al., 1991), and previous work with AGN 191103 indicating that increasing the formulation pH to 8.2 would allow us to reduce the formulation concentration to 0.2% and maintain comparable aqueous humor AGN 191103 concentrations (Small et al., 1996). At equivalent formulation concentrations of AGN 191103 but different pH values, the pH-partitioning theory and this model predict that ocular concentrations will be higher with the higher pH formulation, but that the extent of systemic absorption will be comparable since it is already high at the lower pH. Likewise, these models predict that at a higher formulation pH there exists a lower formulation concentration such that ocular concentrations will be comparable but systemic concentrations will be different. The choice between these approaches depends on whether systemic concentrations limit the permitted dose. If a drug is very safe, then the goal would be to maximize ocular concentrations regardless of the resulting systemic exposure. If the drug elicits adverse systemic reactions, then the objective would be to minimize systemic concentrations while still maintaining sufficient ocular efficacy. Regardless of the approach chosen, the higher pH formulation will have a larger safety margin, since the ratio of ocular to systemic concentrations will be higher with the higher pH formulation.

The results of this study are consistent with previous reports, but the magnitude of improvement in the safety margin conferred by pH manipulation in the present study exceeds those previously reported. The largest in vivo improvement reported prior to the present work was found with timolol (Kyyronen and Urtti, 1990), in which rabbit aqueous humor concentrations increased 1–3-fold at 0.5 and 4 h post-dose as formulation pH increased from 6.2 to 7.5. Higher

pH also caused timolol to be absorbed faster systemically, leading to 2-fold higher plasma C_{max}, shorter systemic MRT and comparable aqueous humor C_{max}/plasma C_{max} ratios at 0.5 and 4 h in spite of higher aqueous humor C_{max} at higher pH values. However, plasma AUCs were the same, indicating that differences in plasma concentrations were due to different rates, and not different extents of absorption, and that the safety margin did improve with increasing pH. Some advantage of altering formulation pH has also been shown for pilocarpine (Sieg and Robinson, 1977), pyrilamine, cimetidine and histamine (Hui et al., 1984), although the magnitudes of improvement were lower. Although these increases could be explained by the pH-partitioning theory, glycerin, which does not dissociate, also doubled in aqueous humor concentration in the pH range 5–8 (Sieg and Robinson, 1977). The increase in glycerin bioavailability was attributed by the authors to decreased lacrimation at the higher pH, but this claim was later refuted (Conrad et al., 1978). Whether lacrimation rate played a role in this finding still is not clear, but the facts remain that the degree of ionization cannot explain the difference in aqueous humor glycerin concentrations, and that any other basis for this observation may also affect ocular absorption of ionizable compounds.

The increase in glycerin bioavailability illustrates a problem with explaining changes in ocular bioavailability, and that is the potential plethora of formulation parameters and physiological processes that may affect ocular concentrations. For example, increased absorption of basic amines at higher formulation pH is often attributed to a lower degree of drug ionization in precorneal tears. This is consistent with the observed results, but other factors that may contribute to this result are decreased lacrimation rate, decreased protein binding in the precorneal region, decreased absorption by the conjunctiva, and compromised ocular surface integrity at the higher pH. Any one of these factors may have as its root cause the formulation pH, the buffer chosen to maintain that pH, the preservative, or the drug itself, which may be less irritating in its unionized form. Because of a potential confluence of multiple effects, studies attempting to identify the underlying reason behind altered bioavailability must limit the number of formulation differences in any given comparison. Although the present study altered the buffer component as well as pH, previous work with phosphate-buffered formulations suggests that most or all or the increase observed in the present study was due to the pH change, and not the different buffers (Small et al., 1996).

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References

Ashton, P., Podder, S.K. and Lee, V.H.L., Formulation influence on conjunctival penetration of four beta blockers in the pigmented rabbit: a comparison with corneal penetration. *Pharm. Res.*, 8 (1991) 1166–1174.

Brodie, B.B., *Absorption and Distribution of Drugs*, Livingstone, Edinburgh, 1964.

Camras, C.B., Clinical applications of α_2-adrenergic agonists in ophthalmology. *J. Glaucoma*, 4 (1995) S30–S35.

Chang, S.C. and Lee, V.H.L., Nasal and conjunctival contributions to the systemic absorption of topical timolol in the pigmented rabbit: implications in the design of strategies to maximize the ratio of ocular to systemic absorption. *J. Ocul. Pharmacol.*, 3 (1987) 159–169.

Conrad, J.M., Reay, W.A., Polcyn, R.E. and Robinson, J.R., Influence of tonicity and pH on lacrimation and ocular drug bioavailability. *J. Parent. Drug Assoc.*, 32 (1978) 149–161.

Derick, R.J., Adrenergic agonist medications: basic mechanisms. *J. Glaucoma*, 4 (1995) S1–S7.

Gibaldi, M. and Perrier, D., *Pharmacokinetics*, 2nd edn., Dekker, New York, 1982.

Harris, A., Caldemeyer, K.S., Mansberger, S.L. and Martin, B.J., α_2-Adrenergic agonists' effects on ocular hemodynamics. *J. Glaucoma*, 4 (1995) S19–S23.

Hui, H.-W., Zeleznick, L. and Robinson, J.R., Ocular disposition of topically applied histamine, cimetidine, and pyrilamine in the albino rabbit. *Curr. Eye Res.*, 3 (1984) 321–330.

Kaufman, P.L. and Gabelt, B., α_2-Adrenergic agonist effects on aqueous humor dynamics. *J. Glaucoma*, 4 (1995) S8–S14.

Kyyronen, K. and Urtti, A., Effects of epinephrine pretreatment and solution pH on ocular and systemic absorption of ocularly applied timolol in rabbits. *J. Pharm. Sci.*, 79 (1990) 688–691.

Lee, V.H.L. and Robinson, J.R., Topical ocular drug delivery: recent developments and future challenges. *J. Ocul. Pharmacol.*, 2 (1986) 67–108.

Morrison, J.C., Side effects of α-adrenergic agonists. *J. Glaucoma*, 4 (1995) S36–S38.

Olejnik, O., Conventional systems in ophthalmic drug delivery. *Drugs Pharm. Sci.*, 58 (1993) 177–198.

Richman, J.B., Tang-Liu, D.D.-S., and Shackleton, M., Buffering capacity of pilocarpine alters the ocular uptake of levobunolol from combination formulations in vitro and in vivo. *Pharm. Res.*, 8 (1991) S289.

Rowland, M. and Tozer, T.N., *Clinical Pharmacokinetics: Concepts and Applications*, Lea and Febiger, Philadelphia, 1980.

Sieg, J.W. and Robinson, J.R., Vehicle effects on ocular drug bioavailability II: evaluation of pilocarpine. *J. Pharm. Sci.*, 66 (1977) 1222–1228.

Slovin, E.M. and Robinson, J. R., Bioadhesives in ocular drug delivery. In Edman, P. (Ed.), *Biopharmaceutics of Ocular Drug Delivery*, CRC Press, Boca Raton, FL, 1993, pp. 145–148.

Small, D., Dais, M., Zolezio, H. and Tang-Liu, D., Ocular disposition of AGN 191103 following topical dosing to albino rabbits. American Association of Pharmaceutical Scientists Annual Meeting and Exposition, San Antonio, TX, USA, 1992, p. S273.

Small, D., Dais, M., Wong, M. and Tang-Liu, D., Influence of pH and buffer concentration on the ocular bioavailability of ophthalmic AGN 191103 formulations in albino rabbits. *Int. J. Pharm.* 149 (1997) 195–201.

Tang-Liu, D.D.-S. and Burke, P.J., The effect of azone on ocular levobunolol absorption: calculating the area under the curve and its standard error using tissue sampling compartments. *Pharm. Res.*, 5 (1988) 238–241.