Formulation, *in Vitro* Dissolution, and Ocular Bioavailability of High- and Low-Melting Phenylephrine Oxazolidines

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Received January 31, 1993; accepted March 12, 1993

The *in vitro* dissolution and the relative ocular bioavailability of high- and low-melting phenylephrine oxazolidines (HMP and LMP) from a nonaqueous suspension (silicone fluid) were compared. Stability-indicating HPLC assays were developed for evaluation of the prototype formulations, in which a normal-phase HPLC method was necessary for analysis of PO, while a reverse-phase HPLC method was required for analysis of the primary degradation product, phenylephrine (PE), following its separation from the formulation using a short silica gel column. PO was formulated as an ophthalmic suspension in silicone fluid (20 cs) because of its property of undergoing rapid hydrolysis in aqueous media. An experimental test system for measuring the dissolution characteristics of a water-immiscible multiparticulate suspension was designed to obtain the dissolution profiles of suspensions of HMP and LMP. The dissolution rates, which were nearly identical for LMP and HMP, were obtained assuming a quasi-infinite reservoir. A reverse-phase HPLC assay with fluorescence detection was used for measuring the concentrations of PE in aqueous humor and corneal samples. Statistical analysis of the bioavailability data showed that suspensions containing HMP and LMP were equal in extent of absorption following a single topical application to the rabbit eye. The results correlated well with the *in vitro* dissolution rates of the suspensions of HMP and LMP.

KEY WORDS: phenylephrine; phenylephrine oxazolidine; high-performance liquid chromatography; ophthalmic formulation; suspension dissolution; ocular bioavailability.

INTRODUCTION

In a previous report (1), the differences in physicochemical properties observed between high- and low-melting phenylephrine oxazolidines (HMP and LMP) were characterized and attributed to crystal imperfections (1). Crystal imperfections have been found to have a major impact on pharmaceutical manufacturing as well as chemical reactivity and dissolution rate (2,3) and, as a result of the latter, bioavailability differences. A classical example is digoxin for which dissolution and bioavailability differ whenever its crystal properties are not well controlled (4). Grant and Chow have suggested that the presence of a high density of lattice imperfections could possibly be utilized to increase the bioavailability of a solid drug (3–8). The objective of this study is to evaluate *in vitro* dissolution of suspended particles of HMP and LMP in a nonaqueous vehicle and their relative ocular biovailability in the rabbit eye.

MATERIALS AND METHODS

Reagents and Chemicals

High- and low-melting phenylephrine oxazolidines were obtained from Sterling Organics (control Nos. 339-172B and 339-172C). The HPLC mobile phases were prepared using methanol, acetonitrile, hexane, isopropyl alcohol (all HPLC grade), and acetic acid (AR grade). Silica gel (60–200 mesh) was reagent grade and obtained from the J. T. Baker Chemical Co. (Phillipsburg, NJ). All other reagents used were either HPLC grade or AR grade.

Preliminary Ophthalmic Formulation

Stability-Indicating HPLC Assays. A stability-indicating normal-phase HPLC method was developed for quantitation of phenylephrine oxazolidine (PO) in a nonaqueous suspension. The HPLC system that was used consisted of a solvent delivery pump (Model LC-6A, Shimadzu Corp., Kyoto, Japan), an injection valve (Model 7164, Rheodyne, Cotati, CA) fitted with a 20-μl loop, a variable-wavelength UV-VIS detector (Model SPD-6AV, Shimadzu Corp., Kyoto, Japan), a fluorescence detector (model RF-535, Shimadzu Corp.), and an integrator (Model CR-601, Shimadzu Corp.). The separations were performed on a normal-phase column (Waters CN μ-Bondapak, 10 μm, 4.0 × 300 mm, Millipore Corp., Milford, MA) at 280 nm and 0.01 AUFS. The mobile phase consisted of a 95:5 ratio of hexane:isopropyl alcohol at a flow rate of 1.0 mL/min.

Phenylephrine (PE), a degradation product, was assayed by reverse-phase HPLC because of its significantly higher polarity. PE was separated from PO using a short silica gel column (15-mL Buchner glass funnel with frit, Corning Inc., catalog No. 36060, 40-60C, Corning, NY) and ethyl acetate/methanol (95:5) as a solvent. A quantity of 1.5 g of silica gel was carefully placed over the fritted glass of the column. In a separate beaker, a 1-mL sample of PO suspension was added to 10 mL of ethyl/acetate (95:5) and dissolved by sonication. The resulting solution was then carefully transferred over the silica gel. To ensure complete removal of PO, a total of 200 mL of ethyl acetate/methanol was eluted through the silica gel in increments of about 10 mL each and discarded. The silica gel was allowed to dry by use of vacuum suction until the gel particles could flow freely. In order to remove PE from the column for analysis, about 30 mL of methanol/1% acetic acid (1:9) was added in increments of 10 mL to the dry silica gel. The eluent containing PE was collected directly into a 50-mL volumetric flask. The eluting solvent was used to bring the volume up to 50 mL and a small sample volume was then injected onto the HPLC column for analysis.

The HPLC assay for PE in the eluent solution used a reverse phase C-18 column (Waters μ-Bondapak 10 μm, 4.6 × 300 mm). The operating conditions were as follows: flow rate, 1.0 mL/min; detection wavelength, 279 nm; sensitivity,

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0724-8741/93/1100-1627$07.00/0

0.01 AUFS; attenuation, 0; chart speed, 5 mm/min; mobile phase, methanol/1% acetic acid (1:9). A standard curve was constructed for each experiment for the conversion of peak areas to concentrations. A 1% suspension of PO was prepared which also contained 11.2 and 22.4% of PE in order to test the analytical recovery of the assay method.

Formulation Development. Since PO is rapidly hydrolyzed to PE in an aqueous environment, a nonaqueous vehicle, polydimethylsiloxane fluid (silicone fluid; Dow Corning 360 Medical Fluid, 20 cs, Dow Corning, Midland, MI), was selected as a vehicle. Other additives used in the formulations included one or more of the following: chlorobutanol, methylparaben and propylparaben as preservatives, and the non ionic surfactant, sorbitan trioleate (Arlacel 85, ICI United States, Inc., Wilmington, DE).

Silicone fluid was heated to 70°C to promote dissolution of the additives employed. The vehicle solution was then allowed to cool to room temperature, at which time micronized PO was added to a mortar along with a small volume of the vehicle, which was slowly added while triturating to ensure dispersion. Once the slurry showed no appearance of agglomeration, sufficient vehicle was added to complete the formulation volume.

Drug Release from Suspensions

An experimental test system for measuring the dissolution characteristics of the water-immiscible suspension was designed (Fig. 1) in order to compare drug release between HMP and LMP particles. A 250-mL Ehrlenmyer flask, clamped with a stainless-steel needle (18 G, 4 in. long) for use as sampling port, was filled up to the neck (3.0 cm in diameter) with 240 mL of dissolution medium and stirred using a 4-cm-long magnetic stirrer. A volume of 2.0 mL of suspension was carefully introduced at the top of the aqueous medium at time 0. The sample was withdrawn at specified time periods using a syringe and needle (25 G, 6 in.) 2 cm from the flask bottom (8 cm in diameter). The dissolution rates of HMP and LMP from silicone fluid were measured under the following conditions: dissolution medium, pH 7.4 isotonic phosphate buffer containing 0.01% EDTA; volume of medium, 240 mL; volume of suspension, 2.0 mL; concentration of PO suspension, 0.25%; temperature, ambient temperature (≈22°C); stirring speed, ≈120 rpm; sampling volume, 0.6 mL; and sampling interval, 2, 5, 9, 13, 18, 23, and 28 min. PO in each sample was assayed by measuring PE using reverse-phase HPLC methodology.

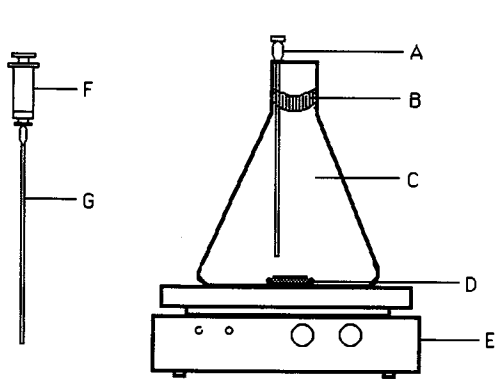

Fig. 1. Schematic representation of the dissolution system. A, sampling port (4 in., 18 G); B, suspension; C, dissolution medium; D, stirring bar; E, magnetic stirrer; F, sampling syringe; G, sampling needle (6 in., 25 G).

Relative Ocular Bioavailability Study

HPLC Assay of Phenylephrine in Ocular Tissues. PE concentrations in cornea and aqueous humor were determined by fluorescence detection using a C-8 column (Waters Novapak column, 150 × 3.9 mm, 4 μm) with a matching C-8 guard column. The extraction procedures for PE from ocular tissues were described elsewhere (9). The mobile phase was methanol:0.5% acetic acid: acetonitrile (5:91:4); a flow rate of 1.0 mL/min was used. Detection was obtained at I_{ex}/I_{em} = 265/316 nm at a sensitivity range of 4. Chart speed was 0.5 cm/min. Aqueous humor and corneal samples, devoid of drug, were used to establish a baseline free of interfering peaks. Linear standard curves were obtained and used to convert peak height to concentration.

Topical Ocular Dosing Method. New Zealand white rabbits of either sex, approximately 3 months old and ranging in weight from 3 to 5 lb, were selected for the study. Before starting each experiment, 24 rabbits were randomly divided into two equal treatment groups, weighed, and placed in individual restraining boxes. Each suspension was shaken well before dosing. Each rabbit was administered a volume of 25 μL of suspension to the right eye by slightly pulling away the lower eyelid from the globe and allowing the measured drop to fall onto the cornea and collect into the lower conjunctival sac. The eyelid was held for about 15 sec after instillation, then carefully returned to its normal position. At time intervals of 10, 20, 35, and 90 min following drug administration, the rabbits were sacrificed by a lethal injection of about 1 mL of a euthanasia solution (Beuthanasia-D Special, Schering-Plough Animal Health Corp., Kenilworth, NJ) diluted with an equal volume of normal saline to the marginal ear vein. Within 2–3 min, cornea and aqueous humor samples were removed and frozen immediately for future analysis.

RESULTS AND DISCUSSION

Ophthalmic Formulation

Stability-Indicating HPLC Assays. The concentration of PO was determined by HPLC from a single 20-μL injection, which allowed simultaneous quantitation of pivalaldehyde (a degradation product) and methyl- and propylparabens. PE was assayed by reverse-phase HPLC following its separation from the suspension. A typical sample chromatogram is presented in Fig. 2. All compounds were completely resolved from PO on the HPLC column. The detector response was linear for PE, PO, and pivalaldehyde over the concentration ranges of 1 to 100, 4 to 200, and 15 to 310 μg/mL, respectively. The ratios of peak areas of two standard solutions measured at 260 and 280 nm were 2.12 and 2.11, respectively, suggesting the absence of an interfering compound overlapping with the drug peak. The consistent recovery data recorded in Table I indicate that the assay was specific and precise. The excellent recovery of PE (11.2 and 22.4%) from 1% nonaqueous suspensions of PO is summarized in Table II.

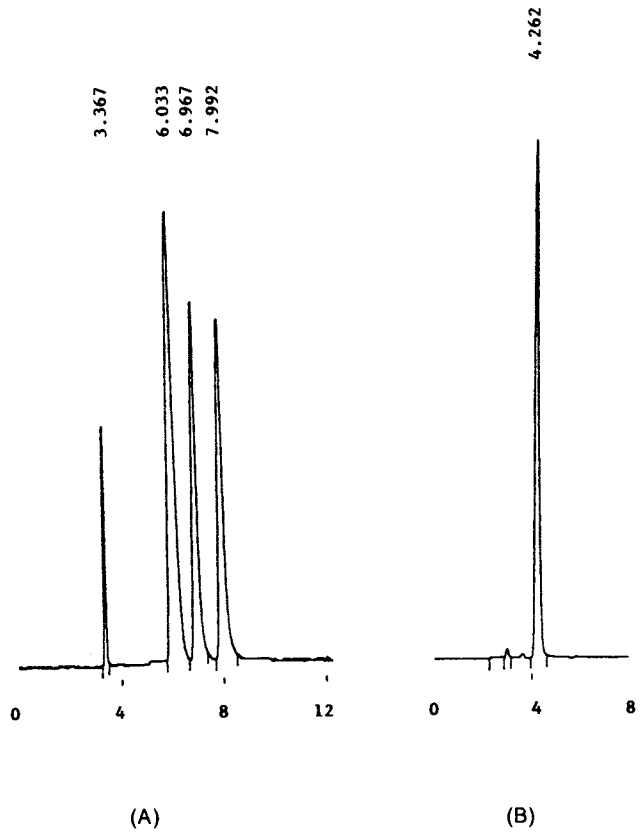

Fig. 2. HPLC chromatograms of the stability-indicating assay for phenylephrine oxazolidine (PO) in suspension. (A) Normal phase: 1, pivalaldehyde (t_R = 3.367 min.); 2, PO (t_R = 6.033 min.); 3, propylparaben (t_R = 6.967 min.); 4, methylparaben (t_R = 7.992 min.). (B) Reverse phase: PE (t_R = 4.262 min.).

Suspension Development. During the development of the formulation, it was found that the presence of Arlacel 85 in suspension catalyzed the chemical degradation of PO. With or without the addition of Arlacel 85, neither aggregation nor caking was observed; suspended drug particles could be easily dispersed upon shaking after 13 months of storage. Chlorobutanol and methyl- and propylparaben were soluble in the silicone vehicle at concentrations effective in inhibiting bacterial and mold growth (11), but because chlorobutanol has been shown readily to permeate standard polyethylene ophthalmic containers (10), glass packaging was used.

Table I. Recovery (R) of the Normal-Phase HPLC Assay for Phenylephrine Oxazolidine and Pivalaldehyde

	Phenylephrine oxazolidine at amount added of		Pivalaldehyde at amount added of	
	0.5%	1.0%	0.05%	0.1%
R1 (%)	99.92	99.79	104.17	97.69
R2 (%)	98.29	98.83	102.24	100.13
R3 (%)	99.00	99.65	102.05	99.78
Average (%)	99.79	99.42	102.82	99.20
SD (%)	0.812	0.519	1.173	1.32

Table II. Recovery (R) of the Reverse-Phase HPLC Assay for Phenylephrine in Suspension

	Amount added (%)[a]	
	11.2%	22.4%
R1 (%)	102.13	99.80
R2 (%)	101.58	99.05
R3 (%)	103.14	99.02
Average (%)	102.28	99.29
SD (%)	0.79	0.44

[a] Concentration is expressed as percentage degradation of PO.

An acceptable suspension contained 1.0% HMP, 0.25% chlorobutanol, 0.03% methylparaben, and 0.01% propylparaben.

Dissolution from Nonaqueous Suspension

Excluding petrolatum, nonaqueous vehicles for use in the eye have not been commercially successful (10). The dissolution and interfacial transfer of drug particles from nonaqueous media into tear fluid can be the rate-determining step in absorption (12,13). A simple, *in vitro* test system involving release of drug suspended in silicone fluid adjacent to an aqueous phase was designed as an interfacial transfer model in order to estimate the time required for the transfer step to occur.

The average dissolution profiles of HMP and LMP from suspensions are given in Fig. 3. The amount of PO released as a function of time was linear up to 50% released, assuming a quasi-infinite reservoir (R^2 = 0.999 for HMP and 0.997 for LMP). The average dissolution rates are 0.126 (±0.011; n = 3) mg min^{-1} for HMP and 0.130 (±0.007; n = 3) mg min^{-1} for LMP based on a zero-order release model. The dissolved amounts (mg) of PO were calculated from the measured amounts of PE through molar conversion. Unlike the results obtained from intrinsic dissolution testing, the difference in

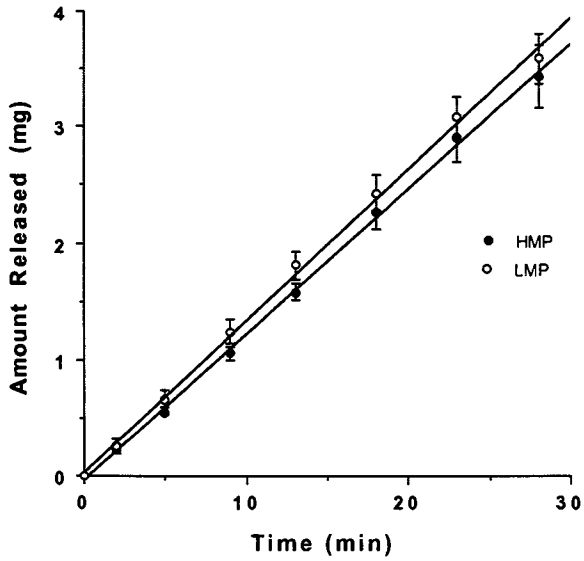

Fig. 3. Average dissolution profiles (±SD) of suspensions containing HMP and LMP.

dissolution rates of suspended particles in silicone fluid was statistically insignificant for LMP and HMP (t = 0.471).

Particulate dissolution models for disperse systems include the Noyes-Whitney, Hixon-Crowell's cube root, Niebergall-Goyan's square-root, and Higuchi-Hiestand models for diffusion-controlled dissolution of uniform size systems (14–16). Higuchi, Carstensen and Musa, and Brook also incorporated a size distribution function into the model to describe the dissolution of multisized drug particles for pharmaceutical suspensions (17,18). All of these models were developed for a single-phase system. An attempt was made to fit the present data to the cube-root, square-root, and ⅔-root models. It was found that only the Higuchi-Hiestand model provided a slightly better fit than the zero-order release model, with R^2 = 0.998 and $K_{1/3}$ = 0.058 for HMP and R^2 = 1.000 and $K_{1/3}$ = 0.061 for LMP.

The transfer of the drug from the suspended solid to the aqueous medium is complex and assumed to occur stepwise as follows. First, a small amount of drug dissolves in silicone fluid (about 0.25 mg/mL at RT). The dissolution includes detachment from the solid and diffusion through the silicone oil diffusion layer followed by partitioning into the aqueous phase. Simultaneously, drug particles (as they partially dissolve) fall to the oil–water interface at a rate influenced by the forces of sedimentation and stirring. For these particles, drug dissolution occurs at the interface and into the aqueous phase. When intrinsic dissolution rates were compared per unit area to drug release from an oil suspension, it was found that the average intrinsic dissolution was greater than the average dissolution from the suspension assuming 100% coverage of the particles at the interface (0.074 vs 0.018 mg cm^2 min^{-1} for LMP). Thus, the significant difference found in the intrinsic dissolution between HMP and LMP in the aqueous system was not observed in the present model. If factors such as temperature, agitation, natural convection, particle size, and concentration of suspension are controlled, the apparent dissolution rates of the suspensions will be dependent upon rate-limiting step(s) of the processes mentioned above. The fact that the Higuchi-Hiestand model provided the best fit suggests that the limiting rate in this model system is drug diffusion through the oil diffusion layer since PO is a lipophilic compound which may be encapsulated by a significant oil diffusion layer even when in contact with the aqueous phase at the interface.

Relative Ocular Bioavailability

In order to determine a potential difference in bioavailability between HMP and LMP, concentrations of PE in aqueous humor and cornea were measured following a single topical instillation of 1% PO suspensions (25 μL) of HMP and LMP.

Following topical instillation, aqueous humor and cornea were removed and assayed for PE at predetermined time intervals. The retention times for PE in aqueous humor and cornea were 5.95 and 5.66 min, respectively. The peaks were symmetrical and free of any interfering extractable components. Profiles of cornea and aqueous concentrations of PE are presented graphically in Figs. 4 and 5. Areas under the curves of each tissue concentration of drug (AUC) were obtained and compared (19). Table III summarizes the statistical treatment of the bioavailability data for HMP and LMP after topical application to the rabbit eye. A comparison of the AUC for either cornea or aqueous humor shows that suspensions containing HMP and LMP are approximately equal in extent of absorption, which agrees with the *in vitro* dissolution tests of the suspensions.

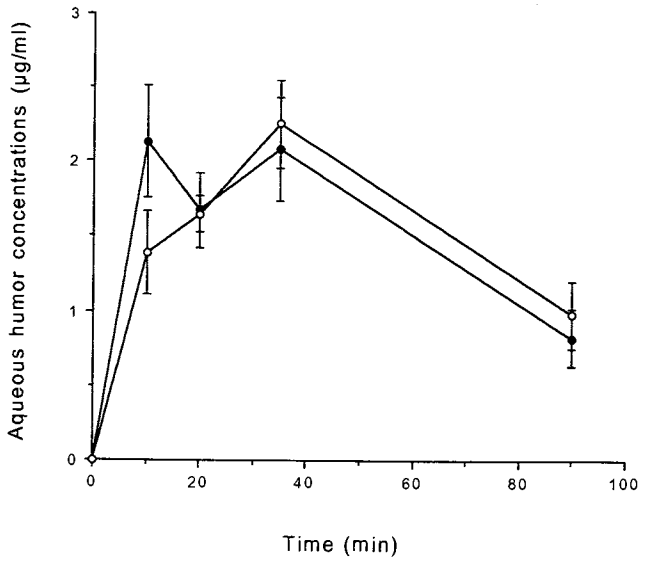

Fig. 4. Comparison of mean (±SE) phenylephrine concentration in aqueous humor at each time interval after topical administration to the rabbit eye of 1% of suspensions of high- and low-melting phenylephrine oxazolidines. HMP (●); LMP (○).

The most significant difference in the profiles is the higher concentration at 10 min for HMP compared to LMP in both cornea and aqueous humor samples. Although these differences are not statistically different at $P < 0.05$, a trend is evident ($P < 0.1$ and $P < 0.15$ for cornea and aqueous humor, respectively). No explanation can be given for the different peaks at 10 min for LMP and HMP either based on

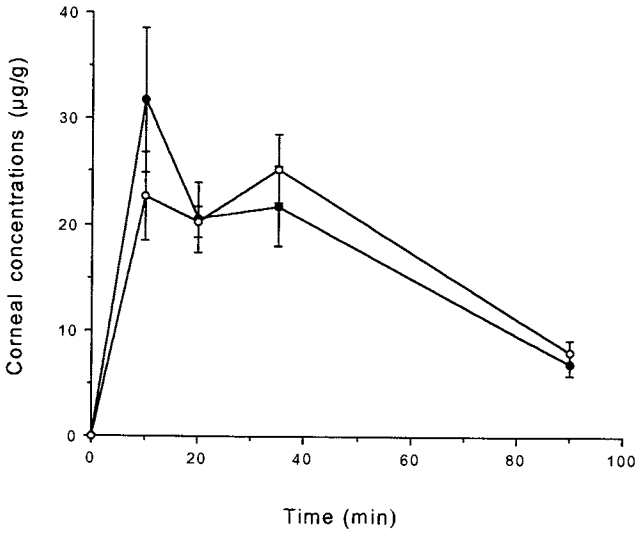

Fig. 5. Comparison of mean (±SE) phenylephrine concentration in cornea at each time interval after topical administration to the rabbit eye of 1% suspensions of high- and low-melting phenylephrine oxazolidines. HMP (●); LMP (○).

Table III. Statistical Analysis of Phenylephrine in the Aqueous Humor and Cornea Following Topical Instillation of 1% High- and Low-Melting Phenylephrine Oxazolidine (HMP and LMP) Suspensions

	Aqueous humor		Cornea	
	HMP	LMP	HMP	LMP
Group AUC				
$\mu g\ mL^{-1}\ min$	137.9	140.4		
$\mu g\ g^{-1}\ min$			1526.3	1583.5
Group SE(AUC)[a]	14.1	12.6	156.0	129.3
df[b]	37	38	40	32
95% CI[c]	110.2–165.5	115.7–165.0	1220.5–1832.1	1330.1–1836.9
t statistic				
$AUC_{difference}$[d]	2.5		57.2	
$S_{difference}$[d]	18.9		202.6	
df_{diff}[e]	74		71	
t value[d]	0.133		0.282	
95% CI_{diff}[f]	−34.5–39.5		−339.9–454.4	

[a] Standard error (SE) of the estimate of the group AUC.
[b] Approximate degrees of freedom for SE of the estimate of AUC.
[c] Ninety-five percent confidence interval for AUC.
[d] r value determined from $t = (AUC_{difference}/S_{difference}) = (AUC_H - AUC_L)/[SE(AUC_H)^2 + SE(AUC_L)^2]^{1/2}$.
[e] Approximate degrees of freedom.
[f] Ninety-five percent confidence interval for AUC differences.

solubility in silicone fluid, which represents about 5% of the total dose administered (LMP = 0.416 and HMP = 0.416 μg/mL at 37°C), or based upon dissolution of the suspended particles. However, a lesser proportion of the suspended particles may be contributing to the amount absorbed into the eye. If the time of dissolution is relatively slow compared to the retention time of the particles in the eye, then only a small proportion of drug reaching the cornea or aqueous humor would come from the particles.

Another peak was found at 40 min for both LMP and HMP in cornea as well as aqueous humor. The two peaks may be related to the initial rapid penetration of PO dissolved in the suspension vehicle, followed by the slow dissolution of the retained particles (rate limiting). Under these conditions, the suspended particles in the conjunctival sac could be releasing drug to the tears on a continuous basis for as long as they are retained in the conjunctival sac, whereas drug in solution would be expected to transfer to tears more rapidly. Normally, solutions are drained from the rabbit eye in about 3–5 min, whereas, suspended particles may remain for 15–30 min (10,12,13,20). Another possibility is that ocular and systemic absorption are both contributing to concentrations of PE in aqueous humor and cornea, however, our results do not substantiate either speculation.

Although differences exist in the initial concentrations for HMP and LMP when comparing a single dose, these differences (which are already very small) would be expected to become much less significant upon multiple dosing if they are an indication of different rates of absorption.

ACKNOWLEDGMENTS

This work was abstracted in part from a thesis submitted by Yihong Qiu to the Graduate College, The University of Iowa, and supported by Angelini Pharmaceuticals, Inc., River Edge, NJ.

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