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OCULAR ABSORPTION AND DISPOSITION OF PHENYLEPHRINE AND PHENYLEPHRINE OXAZOLIDINE

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ABSTRACT

The ocular bioavailability of phenylephrine oxazolidine (PO), a prodrug intended for rapid corneal penetration, was micronized and suspended in sesame oil (1 and 10 per cent) and compared in bioavailability to phenylephrine HCl (PE) dissolved (10 per cent) in a buffered (pH 5·75), viscous (30 centipoise) vehicle. Cornea and aqueous humor of New Zealand rabbits were measured over time following 10 μl instillation to the eye. Based upon AUC measurements, corneal and aqueous humor levels were approximately 6 and 8 times greater for 10 per cent PO versus 10 per cent PE, respectively. In addition, the ocular pharmacokinetic values were determined for PE applied in a constant concentration (1 per cent) to the cornea over 180 min to anesthetized rabbits. Cornea and aqueous humor were measured for drug content over time. Using moment analysis and an initial slope method, the absorption rate constant, k_a, the steady state volume of distribution in the eye, V_{SS}, and ocular clearance, Q_e, were calculated. Values obtained for PE were $4{\cdot}15 \times 10^{-5}$ min^{-1}, 0·423ml and 14·6 μl min^{-1}, respectively. The half-life for drug elimination ranged from 63–83 min depending on the tissue or route of administration.

KEY WORDS Phenylephrine Prodrug Rabbits Ocular Pharmacokinetics

INTRODUCTION

Recently, an oxazolidine prodrug of phenylephrine was synthesized, suspended in sesame oil, and tested for mydriatic activity against phenylephrine HCl.[1] The 1 per cent prodrug suspension was found equal in mydriatic activity in New Zealand rabbits to a 10 per cent viscous ageous solution of phenylephrine HCl with the exception that the time of maximum response occurred 60 min earlier with the prodrug.

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The one-tenth reduction in the dosage of the prodrug compared to phenylephrine HCl was a consequence of its rapid corneal penetration. This was attributed to the greater lipophilicity of the prodrug which was observed to be 3-fold greater than phenylephrine HCl in its log octanol/buffer (pH 7·4) distribution coefficient: 1·38[1] vs -1·67,[2] respectively.

The primary disadvantage of the prodrug is its rapid hydrolysis to phenylephrine. Over a pH range of 1–7·5, a hydrolysis half-life of 6–13 min was determined.[1] Therefore, the prodrug must be stored and administered from a non-aqueous vehicle. Nevertheless, a lower ophthalmic dose is considered a welcome advantage to clinicians using phenylephrine either in ocular surgery or routine clinical examinations for the purpose of lessening the risk of cardiovascular side-effects.

The purpose of this study was to determine the ocular pharmacokinetics of phenylephrine HCl and to compare its ocular bioavailability to that of the prodrug.

MATERIALS AND METHODS

Materials

The synthesis of oxazolidine prodrug of phenylephrine, 2-t-butyl-3-methyl-5-(m-hydroxphenyl)-1,3-oxazolidine, has been previously described.[1] Its identity was established from IR (KBr), NMR (CDCl), elemental analysis, and mass spectroscopy. A purity of 99 per cent was based upon results obtained from differential scanning calorimetry.[1,3] New Zealand white rabbits, 3–4 months old, of either sex, and weighing 1·4 to 1·8 Kg were purchased (Morrison Rabbitry, West Branch, IA) and used in the studies (housed in the Animal Care Unit, University of Iowa, Iowa City, IA). Phenylephrine HCl U.S.P, (N-105-TO, Sterling-Winthrop Research Institute) was a gift and used as received. Sesame oil (lot no. 104F–0903, Sigma Chemical Co., St. Louis, MO.) and methylcellulose U.S.P. (400 cps, Hercules, Wilmington, DL) were purchased and also used as received. All other chemicals were reagent grade.

The composition and preparation of single dose formulations of 1 or 10 per cent prodrug (micronized) suspension of prodrug in sesame oil (PE) as well as an aqueous viscous formulation of 1 or 10 per cent (phospate buffer, pH 5·75; 30 centipoise, isotonic) phenylephrine HCl (PE) is described elsewhere.[1]

Single dose studies

Drug (1, 10 per cent PO or 10 per cent PE) was instilled into the right eye of rabbits in a volume of 10 μl 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 carefully returned to its normal position within 10 s after instillation. At time intervals of 5, 10, 20, 40, 60, 90, 150, 240,

and 360 min following drug administration, rabbits (10 eyes/time interval) were sacrificed. Immediately after death, the eye was secured by grasping the superior latis rectus muscle with forceps and flushed gently with 2–3 ml of 0·9 per cent saline. Cornea and aqueous humor samples were removed and frozed immediately.

Topical infusion studies

Only 1 per cent PE was used in these experiments. Neither 10 per cent nor sesame oil could be maintained on the cornea for a sufficient time period without observing dehydration or swelling to the cornea, respectively. A specially designed cyclinder was fashioned from contact lens material and used to maintain 1 per cent PE in contact with the cornea until steady state aqueous humor levels were attained. This occurred within about 2h of constant rate input to the cornea. Consequently, the time period for infusion was studied from 0 to 180 min, whereas the post-infusion period was studied from 180 to 540 min. Rabbits (8–20 eyes/time interval) were sacrificed at various intervals. Aqueous humor and cornea samples were removed, frozen, and stored for drug extraction. Details describing the topical infusion technique have been reported previously.[4,5]

Tissue removal

The aqueous humor was removed from the experimental eye by paracentesis with a 27-gauge needle attached to a 1·0 ml disposible syringe (BD Pastipak, Becton-Dickinson and Co., Rochelle Park, NJ) inserted through the corneal-scleral junction. Care was taken not to puncture the iris. About 150 ml of aqueous humor was removed from each rabbit eye and frozen. The cornea was then removed with a 9 mm trephine (Stortz Instr. Co., St. Louis, MO) and a curved corneal scissors (E 3220, Stortz). Once removed, the corneal tissue sample was blotted gently on glassine paper, weighed, and stored in a 2 ml screw capped plastic vial (# 655, Sarstadt, West Germany). Both aqueous humor and cornea samples were immediately frozen and stored at -78°C.

Tissue extraction of drug

Upon thawing, a volume of 0·1 ml of aqueous humor containing drug was quantitatively transferred to a test tube containing 0·1 ml of distilled water. To this mixture was added 0·8 ml of methanol which served as a protein precipitant. The mixture was shaken vigorously for 2 min, and then allowed to stand for 10 min before centrifuging at 3000 rev min^{-1} for 15 minutes at 0°. A volume of 0·8 ml of supernatant solution was transferred to a second tube and diluted to a total volume of 3·2 ml with distilled water and assayed by HPLC using flourescence detection.[6] Drug connections were expressed in terms of phenylephrine HCl

following instillation of either PO or PE. Standard concentrations and aqueous humor samples devoid of drug were also treated in this manner and used in constructing a standard curve.

The thawed cornea was placed into a 10 ml tissue grinding tube (Potter-Elvehjem, Kontes, Morton Grove, IL) along with 0·6 ml of pH 7·4 phosphate buffer for extracting drug. A teflon coated rod (K-885450, Kontes) was inserted into the grinding tube containing the cornea and buffered solution. The cornea was vigorously mixed with the buffer at 30 rev min^{-1} for one min. A volume of 0·4 ml of buffer containing the extracted drug was transferred to a clean test tube and mixed with 0·1 ml of distilled water. Similarly, 0·1 ml of solution containing known concentrations of drug were used in place of distilled water in order to construct a standard curve. A volume of 2·0 ml of methanol was added to precipitate proteins. The mixture was further treated as described for the aqueous humor samples.

Calculations

The data resulting from the topical infusion of phenylephrine HCl over time was treated by non-compartmental methods using equations specific for the eye and this method of administration.[4,5] The steady state tissue levels that result from this technique obviate the need for modeling and permit the calculation of the absorption rate constant, k_a, ocular clearance, Q_e, ocular volume of distribution at steady state, V_{ss}, and the mean residence time for drug in aqueous humor following topical administration MRT.

$$k_a = \frac{V_C\,(dC_C/dt)_I}{C_W V_W} \tag{1}$$

The term, k_a, represents the first order rate constant for penetration across the outer layer of the cornea, the epithelium. The permeability of various compounds across epithelium, stroma and endothelium have been determined for beta blocking agents,[7,8] cyclophosphamide[9], tobramycin, and phenylephrine.[10] Expressed as a percent contribution to barrier resistance, the epithelium represents 95·6 per cent for phenylephrine HCl. The remaining layers, the stroma and endothelium, contribute 4·4 per cent to barrier resistance which can be considered negligible. Consequently, these two layers could be kinetically lumped together with the aqueous humor.

$$Q_e = \frac{K_o T}{AUC} \tag{2}$$

$$V_{ss} = \frac{K_o T\,AUMC}{(AUC)^2} - \frac{K_o T^2}{2\,AUC} \tag{3}$$

$$MRT = \frac{AUMC}{AUC} - \frac{T}{2} \quad (4)$$

The constant input rate, K_o, can be calculated from the following equation:[5]

$$K_o = (dC_C/d_t)_I V_C \quad (5)$$

The input rate, K_o', can also be calculated from the excised corneal permeability experiments.

$$K_o' = CP_e\, AC_W\, 60 \quad (6)$$

Theoretically, equations (5) and (6) are equivalent, only their methods of calculation are different. The terms, A and 60, in equation (6) represent the area of the cornea exposed by the well (0·5025 cm^2) and 60 converts seconds into minutes. Additional terms used in equations (1)–(6) are defined as follows:

V_W: Volume of drug solution in well (0·7ml)

AUC and AUMC: Areas (to infinity) under the concentration-time curve and concentration X time-time curve, respectively.

C_W: Constant concentration of phenylephrine HCl applied to the cornea over time, T, to anesthetized rabbits (10 mg/ml^{-1}).

C_C, C_A: Concentration of phenylephrine HCl in aqueous humor (A) or cornea (C).

$(dC_C/dt)_I$: Initial slope of the curve representing C_C vs t.

V_C: Volume of excised cornea used in the permeability experiments, but excluding the epithelial barrier (39 ml).

C_{SS}: Apparent steady state concentration of phenylephrine HCl.

CP_e: Permeability coefficient across the corneal epithelium.

Statistical treatment of the results from the topical single dose studies were based upon area considerations and discussed in detail in another report.[11]

RESULTS AND DISCUSSION

Single dose studies

Following the topical instillation of 10 μl of 1 or 10 per cent PO and 10 per cent PE solutions to rabbit eyes, drug concentrations in cornea and aqueous humor were removed and assayed for phenylephrine HCl. The results appear in figures 1 and 2 for cornea and aqueous humor, respectively.

The 10 per cent PO preparation shows approximately 6–10 times higher drug levels than 10 per cent PE in both cornea and aqueous humor at most time

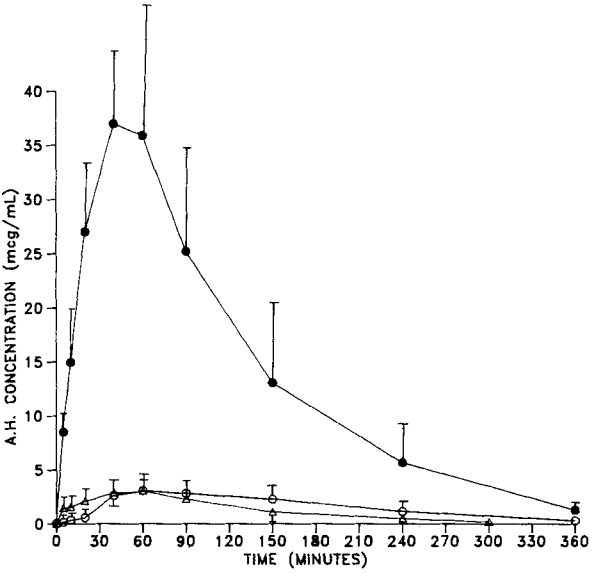

Figure 1. Mean phenylephrine concentration in the cornea at various time intervals after a single topical instillation of 10 μl of 1 per cent PO (- Δ -), 10 per cent PO (- ● -), and 10 per cent PE (- O -) to the rabbit eye

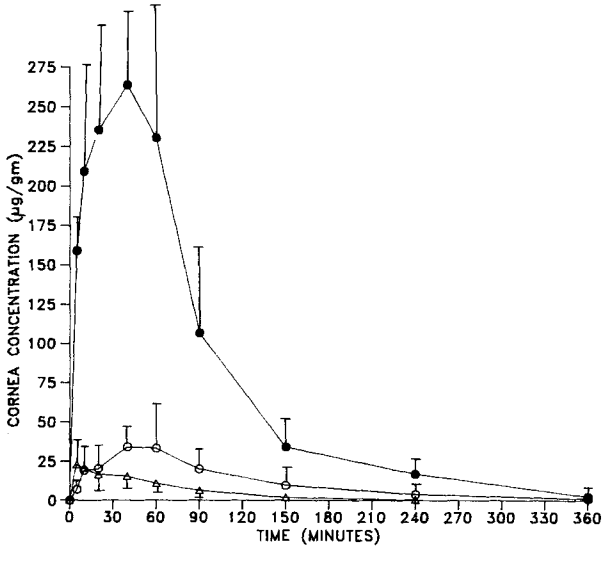

Figure 2. Mean phenylephrine concentration in aqueous humor at various time intervals after a single topical instillation of 10 μl of 1 per cent PO (-Δ-), 10 per cent PO (-●-) and 10 per cent PE (- O -) to the rabbit eye

intervals whereas a comparison of the AUCs indicate about 6–8 fold greater, respectively. Both calculations indicate that the prodrug is absorbed more extensively.

Table 1. Statistical analysis of phenylephrine in the cornea and aqueous humor following topical instillation of 1 per cent or 10 per cent phenylephrine oxazolidine suspension (PO) and 10 per cent viscous phenylephrine HCl (PE) solution

	Cornea ($M\mu g\ g^{-1}$)			Aqueous humor ($M\mu g\ ml^{-1}$)		
	1% PO	10% PO	10% PE	1% PO	10% PO	10% PE
Group AUC ($\mu g, g^{-1} \times$ min)	1564·5	26377·4	4190·2	427·0	5100·5	615·8
Group SE (AUC)*	178·0	1517·3	375·5	62·8	325·5	107·8
df†	39·1	36·2	42·1	21·8	38·7	24·6
95% CI‡	1215–1913	23403–29351	3450–4930	304–550	5738–4463	827–505
	1% PO vs	1% PO vs	10% PO vs	1% PO vs	1% PO vs	10% PO vs
t-statistic	10% PO	10% PE	10% PE	10% PO	10% PE	10% PE
AUC difference§	24812·9	1145·3	22187	4674	189	4485
S difference§	1528	415·5	1563	332	125	343
df differencesπ	37·2	59·8	40·6	41·6	43·0	46·5
t-Value§	16·2	6·3	14·2	14·1	1·51	13·1
Probability	$p<0{\cdot}001$	$p<0{\cdot}001$	$p<0{\cdot}001$	$p<0{\cdot}001$	$p<0{\cdot}1$	$p<0{\cdot}001$

*Standard error (SE) of estimate of the group AUC.
†Approximate degrees of freedom for SE of the estimate of AUC.
‡95% confidence interval for AUC differences.
§*t*-Value determined from: $t = \dfrac{\text{AUC difference}}{\text{S difference}} = \dfrac{AUC_1 - AUC_2}{[(SE_{AUC1})^2 + (SE_{AUC2})^2]^{1/2}}$
πApproximate degrees of freedom.[11,22]

Table 1 summarizes the bioavailability data of PE and PO after topical application to the rabbit eye. A comparison of the AUC for either cornea or aqueous humor shows that 10 per cent PO is statistically greater ($p<0{\cdot}001$) than 10 per cent PE. When the AUCs for 1 per cent PO and 10 per cent PE are compared, corneal AUCs are statistically different but aqueous humor AUCs show no statistical difference. The latter comparison agrees with the mydriatic results[1] which clearly showed that the responses for 1 per cent PO (10 μl) when compared to 10 per cent PE (10 μl) in the same group of rabbits were equal. As expected, comparisons between AUC for cornea and aqueous humor showed a statistically significant increase for data representing 10 per cent versus 1 per cent PO.

The MRT (see Table 2) for 10 per cent PE in either aqueous humor or cornea is longer than that of 1 or 10 per cent PO which is likely due to the slower rate of penetration for phenylephrine HCl across the epithelium barrier. The MRT for 10 and 1 per cent PO are nearly identical for aqueous humor but somewhat less for 1 per cent PO when compared to 10 per cent PO in the cornea. Theoretically, no difference in MRT is expected with a change in the administered dose. However, the cornea is acting as a reservoir as well as a barrier and the release kinetics under these conditions is slower than at lower concentrations.

From the cornea or aqueous humor concentration-time profiles for 10 per cent PE, the half-life representing elimination was calculated. The values, which were calculated from the last four time intervals were 63·0 and 83·5 minutes for cornea and aqueous humor, respectively. The difference in half-lives is considered small, within experimental error and therefore representative of parallel elimination.

Topical infusion studies

Since the cornea cannot be subjected to pH and tonicity values which deviate much from physiological range,[12,13] care must be taken in choosing a vehicle and drug concentration remaining in contact with the cornea for the 3-hour period of the experiment. In preliminary experiments, we observed that sesame oil which is acceptable for single dose instillation cannot be used with the topical infusion technic because of cornea swelling induced by the oil. Also, 10 per cent PE solution is hypertonic and had to be lowered in concentration to 1 per cent or a significant dehydration of the cornea would occur.

Figures 3 and 4 present the corneal and aqueous humor concentrations of drug over time following constant application of 1 per cent (aqueous, pH 7·6, isotonic) phenylephrine HCl to the cornea. Steady state drug levels were approached by 80 min for cornea and 150 minutes for aqueous humor samples. Nevertheless, constant drug concentration remained on the cornea through 180 min so that steady state could be estimated.

The steady state concentration attained for cornea and aqueous humor samples were 123·4 $\mu g\ g^{-1}$ and 16·55 $\mu g\ ml^{-1}$, respectively. Once drug was removed from the cornea (at 180 min), levels declined sharply for corneal samples but not initially for aqueous humor. The aqueous humor concentration was 16·3 $\mu g\ ml^{-1}$ at 180 min and 16·03 $\mu g\ ml^{-1}$ at 210 min. These results illustrate the ability of the cornea to partially act as a reservoir.

Very hydrophilic drugs such as phenylephrine HCl will accumulate in the stroma, whereas very lipophilic drugs such as the cannabinoids, will accumulate in the epithelium.[14] In the case of the very lipophilic drugs, the stroma acts as a barrier which because of its thickness and high resistance to penetration can significantly prolong the length of time drug continues to enter the aqueous humor. For example, delta-9-tetrahydrocannabinoid, when dosed topically to rabbit eyes in single doses shows no significant decline in aqueous humor levels

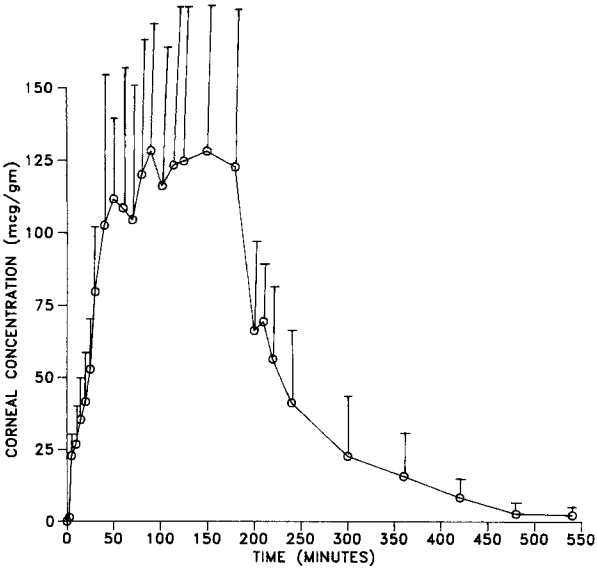

Figure 3 Mean phenylephrine concentration in the cornea at various time intervals following constant application of 1 per cent PE to the cornea of anesthetized rabbits for 180 min

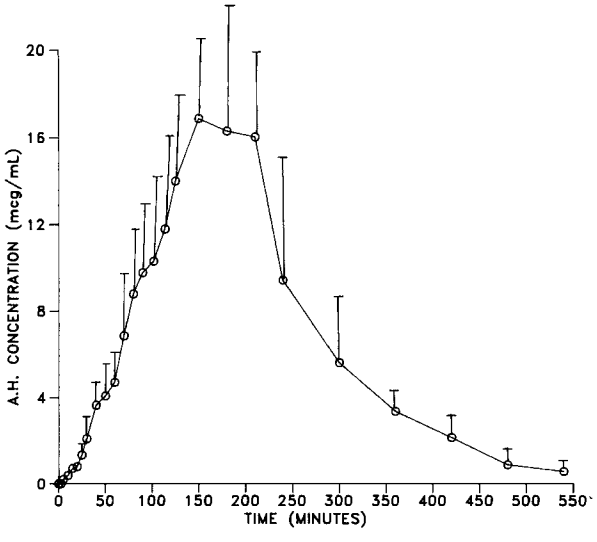

Figure 4 Mean phenylephrine concentration in aqueous humor at various time intervals following constant application of 1 per cent PE to the cornea of anesthetized rabbits for 180 min

even after six hours.[14] Very hydophilic drugs are less likely to show this prolonged effect because the endothelium is relatively thin and has aqueous channels.

From the latter linear time intervals in the post-infusion time period, elimination half-lives of 69 and 75 min were calculated for the decline of drug in cornea and aqueous humor, respectively. These values obtained for half-life are nearly identical to half-lives calculated from the single dose studies indicating that the ocular pharmacokinetics of phenylephrine are essentially linear and that the elimination half-life can be determined from either a single dose or from constant rate input to the cornea.

Table 2. Ocular pharmacokinetic values determined from topical infusion of phenylephrine HCl (1 per cent) to the rabbit eye

k_a (min^{-1})*	Q_e ($ml\ min^{-1}$)†	MRT (min)‡	V_{ss} (ml)§
$4{\cdot}15 \times 10^{-5}$	14·6	118·9	0·423

*First order absorption rate constant; calculated from equation (1).
†Ocular clearance; calculated from equation (2).
‡Mean residence time for drug in aqueous humor; calculated from equation (4).
§Ocular volume of distribution at steady state; calculated from equation (3).

The data shown in Figures 3 and 4 were used to calculate k_a, MRT, V_{ss}, and Q_e from equations (1)–(6). Table 2 summarizes the results. Equations (1) and (5), used to calculate k_a and K_o, required that the initial slope from Figure 3 be determined. This was accomplished by fitting the time intervals from 2·5 through 30 min to a third order polynomial equation:

$$C_C = -12{\cdot}61 + 7{\cdot}98\ t - 0{\cdot}472\ t^2 + 0{\cdot}0103\ t^3 \qquad (7)$$

At $C_C = 0$, a lag time of 1·76 min was calculated by solving for the root of equation (7). In order to calculate the initial slope of the early time points, equation (7) was differentiated and the lag time of 1·76 substituted into the resulting differential equation. An initial slope of 6·41 $\mu g\ min^{-1}\ g^{-1}$ was determined. From equation (5), a value of 0·250 $\mu g\ min^{-1}$ was calculated for K_o using 39 ml for the volume of cornea exposed to the well (but excluding the epithelial barrier) and assuming that 1 g of tissue equals 1 ml. By comparison K was calculated as 0·287 $\mu g\ min^{-1}$ from equation (6) in which CP_e was equal to $9{\cdot}53 \times 10^{-7}$ cms[10].

The initial slope from the C_C versus t plot was used in equation (1) to determine k_a yielding a value of $4{\cdot}15 \cdot 10^{-5}\ min^{-1}$. For lipophilic drugs higher values for k_a have been obtained namely, 0·0014 min^{-1} for clonidine,[5] 0·004 min^{-1} for pilocarpine,[15] and a range of 0·000015 to 0·0013 min^{-1} for various carbonic anhydrase inhibitors.[4,16] Without exception, all drugs studied using the

topical infusion technique show a much smaller absorption rate constant when compared to their respective elimination rate constants.

These results are typical of a 'flip-flop' model[17] and can lead to misinterpretation in assigning the correct value to parameters resulting from non-linear curve fitting of apparent biexponential profiles when only single drop instillation is studied. However, when a topical drop is applied, the flip-flop model is not apparent because of the rapid loss from the absorption site which is a consequence primarily of the relatively large drainage rate constant. The use of the topical infusion method permits the much smaller corneal absorption rate constant to be detected without interference from the much larger drainage rate constant.

A Q_e value of 14·6 μl min^{-1} was obtained from equation (2). This value is comparable to values determined for clonidine[5] (14·9 μl min^{-1}) and by Miller *et al.*[18] for pilocarpine (12–13 μl min^{-1}) and by Tang-Liu *et al.*[19] for flurbiprofen (14·4 μl min^{-1}). Bulk flow of aqueous humor in the rabbit eye is about 1·5 per cent of the volume of the anterior chamber each minute.[20] If a value of 0·311 ml is used for the volume of aqueous humor in the calculation, a Q_e of 4·67 μl min^{-1} is determined for aqueous humor clearance by bulk flow. Values of Q_e obtained for the above mentioned drugs are approximately 2·5 to 3 times greater than bulk flow. This would indicate that drug loss is occurring by additional pathways, possibly by uptake into the tissues of the anterior uvea which is highly vascular and possibly into the lens without significant reverse diffusion during the time of the experiment.

V_{SS} for phenylephrine, 0·423 ml, is slightly smaller than 0·575, 0·62 and 0·530 ml, previously reported for pilocarpine,[21] flurbiprofen,[19] and clonidine,[5] respectively. The smaller V_{SS} calculated for phenylephrine may be a consequence of reduced tissue binding capability or increased protein binding in aqueous humor. Compared to physiologically real volumes of 0·045 and 0·311 ml for the cornea and aqueous humor, V_{SS} is relatively small. Although extensive V_{SS} values are lacking for ophthalmic drugs, the present evidence indicates that drug binding and/or tissue distribution is not as significant in the eye compared to systemic relationships of volume of distribution to the physiological real volume (i.e., plasma volume).

Although not reported here, preliminary treatment of the results from single and topical infusion of phenylephrine HCl permitted a fit of the data to classical pharmacokinetic models. Reasonable fits were obtained with the data to two-compartment open models when the cornea and aqueous humor were separated into reversibly connected compartments. However, the calculated parameter values were not consistent between the single instillation model and the topical infusion model. Also, little relevance of the parameter values could be realized.

The use of the topical infusion method along with application of statistical moment theory and the initial slope calculation appears to reliably describe absorption, distribution and elimination of phenylephrine HCl independent of model considerations.

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