Pharmaceutical Research, Vol. 7, No. 5, 1990

Research Article

Ocular Pharmacokinetics and Pharmacodynamics of Phenylephrine and Phenylephrine Oxazolidine in Rabbit Eyes

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Received September 12, 1989; accepted November 30, 1989

The aqueous humor concentration of phenylephrine and its corresponding mydriatic response were measured over time in New Zealand albino rabbit eyes following a 10-μl topical instillation of a phenylephrine HCl viscous solution (10%) or a phenylephrine oxazolidine (prodrug) suspension in sesame oil (1 and 10%). The bioavailability of a 1% prodrug suspension in the rabbit eye (AUC of aqueous humor concentration vs time) was 30% lower than that of a 10% phenylephrine solution ($P < 0.1$) with the exception that the peak time occurred 34 min earlier with the prodrug. A 10% prodrug suspension increased the aqueous humor bioavailability approximately eightfold but improved the mydriatic activity (AUC of mydriasis vs time) only fourfold. The pharmacokinetic parameters, apparent absorption, and elimination rate constants, of phenylephrine and the prodrug were determined from aqueous humor concentration–time and mydriasis–time profiles. The study showed that the kinetic parameters of phenylephrine estimated from its mydriasis profile do not accurately reflect the kinetics of drug distribution in the iris. These parameters also varied with the instillation of phenylephrine solution or prodrug suspensions. A mydriatic tolerance of the pupil response was apparent after the topical instillation of phenylephrine solution. The mydriatic tolerance may be due to the decrease in receptor number in the iris dilator muscle.

KEY WORDS: phenylephrine; prodrug; ocular pharmacokinetics; ocular pharmacodynamics; mydriatic tolerance; rabbits.

INTRODUCTION

The rate constants associated with drug absorption, distribution, and elimination are important parameters in the design of an appropriate dosage regimen to achieve a desired therapeutic effect in patients. In the eye, these parameters may be estimated from the biological response after topical administration (1). For example, the apparent absorption and elimination rate constants that describe drug kinetics in the human iris for several commonly used ophthalmic drugs, such as tropicamide (2,3), pilocarpine (3,4), homatropine (5), and phenylephrine (6,7), have been determined from their pupil response-time profiles. These results are based on the assumption that the extent of the mydriasis is an instantaneous response of the quantity of the drug residing in the biophasic tissue (iris dilator muscle), so that the time course of the pupil response directly reflects the change of the drug in the iris (7). However, the kinetics of phenylephrine in the aqueous humor and its relation to the corresponding mydriasis have never been thoroughly investigated.

Phenylephrine is routinely used for mydriasis during cataract surgery and ophthalmoscopic examination. Its pharmacokinetics in the human eye was estimated from its mydriatic activity (6,7). However, a rebound miosis in some patients has been observed during the period of phenylephrine treatment (8–10). A subsequent instillation of phenylephrine in these patients resulted in a reduction of mydriasis (8), suggesting that mydriatic tolerance may develop in the iris muscle; therefore mydriasis measurements may not accurately reflect the pharmacokinetic behavior of the drug.

The objectives of this study are (i) to investigate the possible tolerance (or hysteretic) effect developed by phenylephrine in the rabbit eye and (ii) to correlate the kinetics of phenylephrine in rabbit aqueous humor to its corresponding mydriatic response after topical instillation.

Because the iris is a porous tissue with a large surface area in direct contact with the aqueous humor, distribution equilibrium of drug between these tissues can be assumed to occur rapidly. Therefore, aqueous humor is chosen as a sampling tissue to monitor the drug kinetics of phenylephrine in the eye, so that the time course of the drug concentration in the iris can be directly related to its concentration in the aqueous humor (1).

Phenylephrine oxazolidine was recently developed as a prodrug that rapidly penetrates the cornea and therefore allows for a 10- to 15-fold reduction in the phenylephrine dose. Consequently, it is possible to minimize the undesired systemic side effects after a topical administration into the eye (11). The oxazolidine prodrug was designed by masking the amine functionality with a bulky pivalyl moiety, so that the

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0724-8741/90/0500-0476$06.00/0

prodrug would be considerably devoid of α-adrenergic activity in ocular tissues (12). We describe here the relationship of the kinetics of the mydriatic response with drug absorption from topical administration of phenylephrine and its lipophilic prodrug.

MATERIALS AND METHODS

Materials

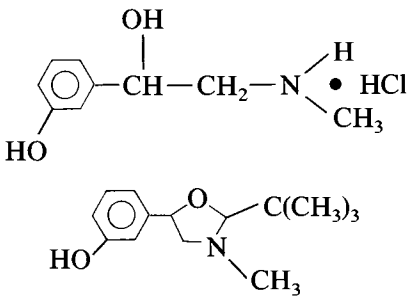
Phenylephrine HCl was a gift from Sterling–Winthrop Research Institute and used as received. The synthesis of phenylephrine oxazolidine, 2-t-butyl-3-methyl-5-(m-hydroxyphenyl)-1,3-oxazolidine, has been described elsewhere (11,13). Their chemical structures are represented in Scheme I. Ten percent of phenylephrine HCl was prepared in the methylcellulose viscous solution with a pH and viscosity of 5.75 and 30 cP, respectively (11). Phenylephrine oxazolidine (1 and 10%) was micronized and suspended in sesame oil (11). All other chemicals and reagents were of analytical grade and were used as received.

Determination of Phenylephrine Concentration

New Zealand albino rabbits, 3–4 months old, of either sex, were used in the study. Ten microliters of each preparation was carefully instilled into the right eye of each rabbit. The rabbits were sacrificed at intervals of 5, 10, 20, 40, 60, 90, 150, 240, and 360 min following drug instillation. Immediately after death, approximately 200 μl of aqueous humor was collected and mixed with methanol to precipitate protein. The mixture was then vortexed and centrifuged at 1000*g* for 10 min at 0°C. The supernatant was transferred to a clean tube and analyzed by HPLC for phenylephrine content (14). Since phenylephrine oxazolidine is rapidly hydrolyzed to phenylephrine (11), only phenylephrine concentration was monitored after the instillation of prodrug suspension. At least 10 rabbit eyes were used for each time interval.

Measurement of Mydriatic Response

Six rabbits were used to measure mydriasis after a topical dose of 10 μl of each preparation. The pupil diameter (including a millimeter rule in the same plane as the pupil) was photographed at a fixed distance using a flood of diffuse light and measured to the nearest 0.1 mm (11). The diameter determined prior to drug instillation served as a baseline. Mydriatic response was measured at 0, 5, 18, 35, 65, 95, 125, 155, 185, 245, 305, and 365 min following topical application.



Scheme I. Top: phenylephrine hydrochloride. Bottom: phenylephrine oxazolidine.

DATA ANALYSIS

Drug Kinetics in Aqueous Humor

Since phenylephrine and its oxazolidine prodrug were formulated in different vehicles (viscous aqueous solution vs sesame oil suspension), they may show a different absorption process (first order vs zero order) into the aqueous humor. To minimize the different kinetics that may occur between these two formulations, a small volume (10 μl) of the drug formulation was administered into the rabbit eyes. It was assumed that both formulations were rapidly diluted by tears, which was followed by first-order absorption kinetics of drug into the ocular anterior chamber.

Since the kinetics of the drug absorption into ocular anterior chamber could be quite complicated due to the precorneal loss factors, nonproductive drug absorption, and the intraocular drug distribution (15–18), determination of the intrinsic kinetic parameters through classical or physiological pharmacokinetic approach was not made in this study. Instead, the time course of phenylephrine concentration in aqueous humor, $C_a(t)$, can be generally described by a biexponential equation with absorption and elimination phases (1,19). The absorption phase is directly related to the drug permeability across the cornea and the elimination phase is the lumped first-order clearance of the drug from aqueous humor (20). The apparent absorption (A_1) and elimination (B_1) rate constants were determined from the nonlinear least-squares regression curve fitting (NONLIN) according to the following equation:

$$C_a(t) = M[e^{-B_1(t-t')} - e^{-A_1(t-t')}] \quad (1)$$

where M is the value of C_a at the interception of the A_1 and B_1 components of the curve and depends on the initial dose of the drug, the fraction absorbed, and the kinetic parameters, A_1 and B_1. t' is the time lag between the instillation and the first appearance of phenylephrine in the aqueous humor. This equation, although simplified, describes the basic drug kinetics in the aqueous humor and allows us to compare the drug absorption and elimination between the aqueous humor and the iris without deriving a complex pharmacokinetic model.

Assuming rapid equilibration of drug between the iris and the aqueous humor, the drug concentration in iris tissue (C_i) can be directly correlated to the concentration of phenylephrine in aqueous humor by the partition coefficient (PC) of phenylephrine between the iris and the aqueous humor.

$$C_i(t) = C_a(t) \times PC \quad (2)$$

Measurement of Mydriasis

The mydriatic response of phenylephrine at time t, $\Delta E(t)$, is determined from the increase in pupil diameter.

$$\Delta E(t) = E(t) - E_o \quad (3)$$

where $E(t)$ is the measurement of the pupillary diameter at time t, and E_o is the measurement of pupil diameter prior to the drug instillation. The maximal mydriatic response (ΔE_{max}) of phenylephrine over time occurs at t_{max}.

Drug Kinetics in Iris

If we assume that the mydriasis induced by phenylephrine is directly related to the drug concentration in the iris, the kinetics of phenylephrine can be directly expressed by the time course of mydriatic response after the instillation. The apparent absorption (A_2) and elimination (B_2) rate constants of phenylephrine in the iris are determined from the response–time curve by biexponential curve fitting.

$$\Delta E(t) = N[e^{-B_2(t-t'')} - e^{-A_2(t-t'')}] \quad (4)$$

where N is the value of the mydriatic response at the interception of A_2 and B_2 components of the curve. t'' is the lag time between instillation and the first observation of mydriatic response.

Aqueous Humor Drug Bioavailability and Mydriatic Activity

Since the apparent absorption (A) and elimination (B) rate constants can be simultaneously determined by curve fitting, the time at which phenylephrine concentration in aqueous humor or iris reaches its maximum (t_{max}) is calculated (independent of lag time) by

$$t_{max} = \frac{\ln (A/B)}{A - B} \quad (5)$$

The bioavailability of phenylephrine in aqueous humor refers to the extent of absorption and is determined from the area under the concentration–time curve (AUC_a). Mydriatic activity of phenylephrine is calculated from the area under the mydriasis–time curve (AUC_i). Since each drug concentration determined in aqueous humor at each time point is generated from one rabbit, the AUC_a of the drug concentration–time curve is calculated by Satterthwaite's procedure (21–23). In contrast, the time course of the mydriatic activity is generated by a continual measurement from the same rabbit, therefore AUC_i is calculated by the linear trapedoizal rule from time 0 to time infinity (24).

Aqueous Humor Drug Concentration and Corresponding Mydriatic Response

If mydriasis is induced only when phenylephrine is bound to the adrenoreceptor in the iris, the measurement of mydriatic response at time t can be related to the corresponding phenylephrine concentration in the iris by a Michaelis–Menton relationship (or classic pharmacodynamic E_{max} model).

$$\Delta E(t) = \frac{\Delta E_{max} \times C_i(t)}{K_m + C_i(t)} \quad (6)$$

where K_m is the drug concentration in the iris required to perform half of the maximal mydriatic response (½ ΔE_{max}). Similarly, the mydriatic response can be correlated to the drug concentration in aqueous humor according to Eq. (2):

$$\Delta E(t) = \frac{\Delta E_{max} \times C_a(t)}{K_m' + C_a(t)} \quad (7)$$

where K_m', the drug concentration in aqueous humor required to produce ½ ΔE_{max}, is equal to K_m/PC.

If mydriatic tolerance is developed by phenylephrine following topical instillation, the value of K_m' is no longer a constant throughout the experiment. The K_m' at each time interval can be calculated by rearranging Eq. (7):

$$K_m'(t) = \left(\frac{\Delta E_{max}}{\Delta E(t)} - 1\right) \times C_a(t) \quad (8)$$

RESULTS

Drug Kinetics

The time course of phenylephrine concentration in aqueous humor and the corresponding mydriasis profile following topical instillation of phenylephrine viscous solution or prodrug suspension are presented in Fig. 1. The pharmacokinetic parameters determined from the drug concentration–time curves and from the mydriatic response profiles are summarized in Table I.

The apparent absorption and elimination rate constants of phenylephrine in aqueous humor following the instillation of 10% phenylephrine viscous solution were 0.017 and 0.010 (min^{-1}), respectively. The elimination rate constants determined from the prodrug formulation (1 and 10%) were equal to that obtained from the phenylephrine solution. However, prodrug suspension improved the absorption into aqueous humor about 2.8-fold (see Table I). The lag time associated with phenylephrine concentration in aqueous humor following instillation of the viscous solution was approximately 7 min. No lag time was observed when prodrug was instilled. The peak time (t_{max}) of the prodrug appeared about 40 min after its instillation and was about 34 min earlier than that determined from phenylephrine solution.

These results indicated that the more lipophilic prodrug penetrated the cornea faster than phenylephrine. The log octanol/buffer (pH 7.4) distribution coefficient of the prodrug was 3 units greater than that of phenylephrine: 1.38 vs −1.67, respectively (11). The similar elimination rate constants obtained for phenylephrine solution (10%) and its oxazolidine prodrug (1 and 10%) indicated that the prodrug was converted to phenylephrine during the absorption phase.

The apparent absorption rate constants obtained from mydriatic response curves also showed that the prodrug produced a mydriasis about twofold faster than phenylephrine. However, the prodrug, at 10% but not at 1%, showed slower drug elimination from the iris than the phenylephrine solution. The 10% prodrug formulation increased the absorption rate by 55% over that obtained with the 1% prodrug but decreased the drug elimination rate about twofold. The t_{max} of the prodrug suspension occurred 8 min earlier than that of the phenylephrine solution. These results indicate that the

Fig. 1. Time course of phenylephrine concentration in rabbit aqueous humor (mean ± SE; n = 10) and the corresponding mydriasis profile (mean ± SE; n = 6) following 10-μl topical instillation of (A) 10% phenylephrine HCl viscous solution, (B) 1% prodrug suspension, and (C) 10% prodrug suspension in rabbit eyes. The dotted line represents the curve of best fit for aqueous humor concentration. The solid line represents the curve of best fit for mydriasis measurement.

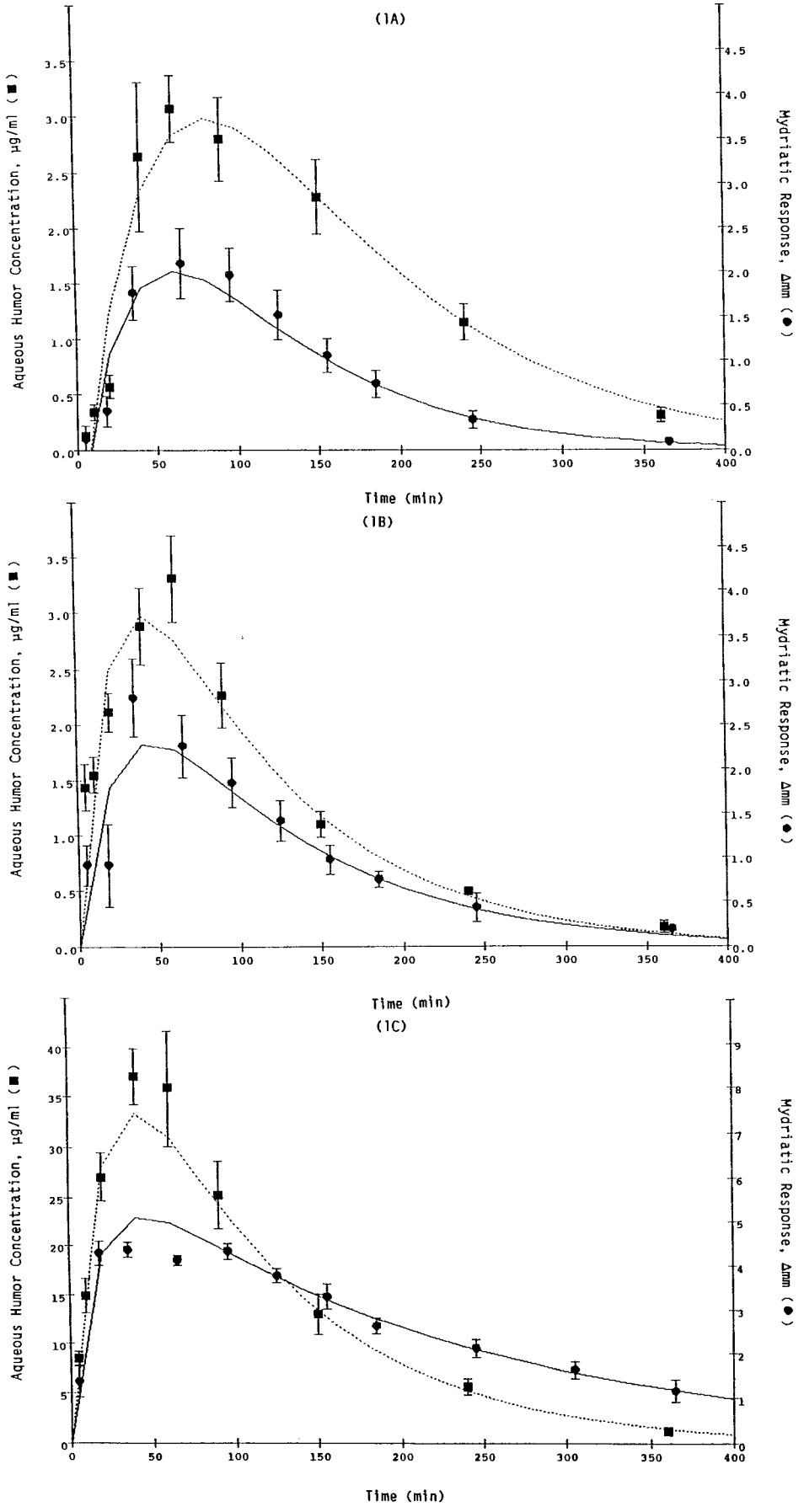

(1A)
Aqueous Humor Concentration, µg/ml (■)
Mydriatic Response, Δmm (●)
Time (min)
(1B)
Aqueous Humor Concentration, µg/ml (■)
Mydriatic Response, Δmm (●)
Time (min)
(1C)
Aqueous Humor Concentration, µg/ml (■)
Mydriatic Response, Δmm (●)
Time (min)

Table I. Pharmacokinetic Parameters[a] of Phenylephrine Following the Topical Instillation of Phenylephrine HCl (PE) Viscous Solution and Phenylephrine Oxazolidine (PO) Suspension[b]

	Determined from drug concentration in aqueous humor				Determined from mydriatic response			
	A_1 (min^{-1})	B_1 (min^{-1})	t' (min)	t_{max} (min)	A_2 (min^{-1})	B_2 (min^{-1})	t'' (min)	t_{max} (min)
10% PE	0.0173 (0.0017)	0.0103 (0.0010)	6.6 (2.1)	74.1	0.0268 (0.0016)	0.0125 (0.0004)	6.8 (1.9)	53.3
1% PO	0.0475 (0.0039)	0.0108 (0.0008)	0.0	40.4	0.0406 (0.0055)	0.0100 (0.0004)	0.0	45.8
10% PO	0.0489 (0.0034)	0.0105 (0.0004)	0.0	40.1	0.0631 (0.0032)	0.0048 (0.0002)	0.0	44.2

[a] A and B, apparent absorption and elimination rate constant; t' and t'', lag time; $t_{max} = \ln(A/B)/(A - B)$.
[b] Mean ± SE.

pharmacokinetic parameters determined from the mydriatic response curves were not always equivalent when the lipophilic prodrug was used.

Aqueous Humor Drug Bioavailability and Mydriatic Activity

Table II compares the aqueous humor bioavailability and mydriatic activity results for phenylephrine and its prodrug. Based upon the AUC measurements, the 1% prodrug suspension produced a lower bioavailability of phenylephrine in aqueous humor than the 10% phenylephrine solution but showed about 15% greater mydriatic activity; however, the difference was not statistically significant ($P < 0.1$). Ten percent prodrug improved the aqueous humor bioavailability (AUC_a) about eightfold compared to the results for the 10% phenylephrine solution; however, it increased the mydriatic activity (AUC_i) by only fourfold. No increase in the mydriatic response was observed for 10% prodrug at the plateau period of maximal response from 18 to 95 min (Fig. 1C), during which the pupil response has likely reached a maximal dilation from the high concentration of phenylephrine in the iris.

Mydriatic Tolerance

The pupil diameter (E_o) prior to drug instillation was 3.29 ± 0.51 mm ($n = 18$) and reached maximal mydriasis about 18 min after the instillation of 10% prodrug suspension. The mydriatic response remained maximal for another 77 min and then began to decline. The maximal mydriatic response (ΔE_{max}) for 10% phenylephrine suspension was 4.27 ± 0.51 mm ($n = 18$).

Table II. Aqueous Humor Bioavailability (AUC_a) and Mydriatic Activity (AUC_i) of Phenylephrine Following the Topical Instillation of Phenylephrine HCl Viscous Solution and Phenylephrine Oxazolidine Suspension[a]

	AUC_a (μg/ml · min)	AUC_i (mm · min)
10% phenylephrine solution	615.8 (107.8)	319.6 (16.2)
1% prodrug suspension	427.0* (62.8)	373.6* (22.0)
10% prodrug suspension	5100.5** (325.5)	1265.4** (46.2)

[a] Mean ± SE.
* $P < 0.1$, Student's t test (compared to 10% PE).
** $P < 0.001$, Student's t test (compared to 10% PE).

The drug concentration in aqueous humor at each time interval was plotted against its corresponding mydriatic response following the instillation of 10% phenylephrine viscous solution as shown in Fig. 2. If the drug concentration was not sampled at the time when mydriatic response was measured, the concentration was estimated by interpolating between the nearest two observations. A clockwise hysteresis loop was noted from the time course of the drug concentration–mydriasis curve. At the 240-min time interval, the phenylephrine concentration (1.15 μg/ml) was twofold higher than that measured at the 20-min time interval ($P < 0.05$). Nevertheless, at these two time intervals a similar mydriatic response (~0.4 mm) was observed. Since a higher drug concentration is needed to produce an equivalent mydriasis at later time points, the term "mydriatic tolerance" was used in this study to describe this phenomenon (25).

The induction of the mydriatic tolerance vs time was also reflected by the increase of the K_m' value during the time course of the experiment (Fig. 3). The K_m' values of the 10% phenylephrine solution were relatively constant at about 3.2 μg/ml through 90 min and then steadily increased to 12 μg/ml at 240 min postinstillation. One percent prodrug appeared to maintain a constant K_m' longer than the 10% phenylephrine solution. The K_m' values obtained from the 10% prodrug suspension prior to 90 min ranged from 17.9 μg/ml to zero after the instillation. This was because the observed mydriatic response reached ΔE_{max} during this period, therefore the K_m' value could not be accurately estimated from Eq. (8). Overall, the prodrug suspension showed a different profile of K_m'–time value compared to the phenylephrine solution.

DISCUSSION

The study results indicate that the parameters estimated from the mydriatic measurements do not accurately reflect the drug kinetics of phenylephrine in the iris. Although the drug concentration in the iris was not determined in this study, a previous report has demonstrated that the drug concentration–time curve of phenylephrine in the rabbit

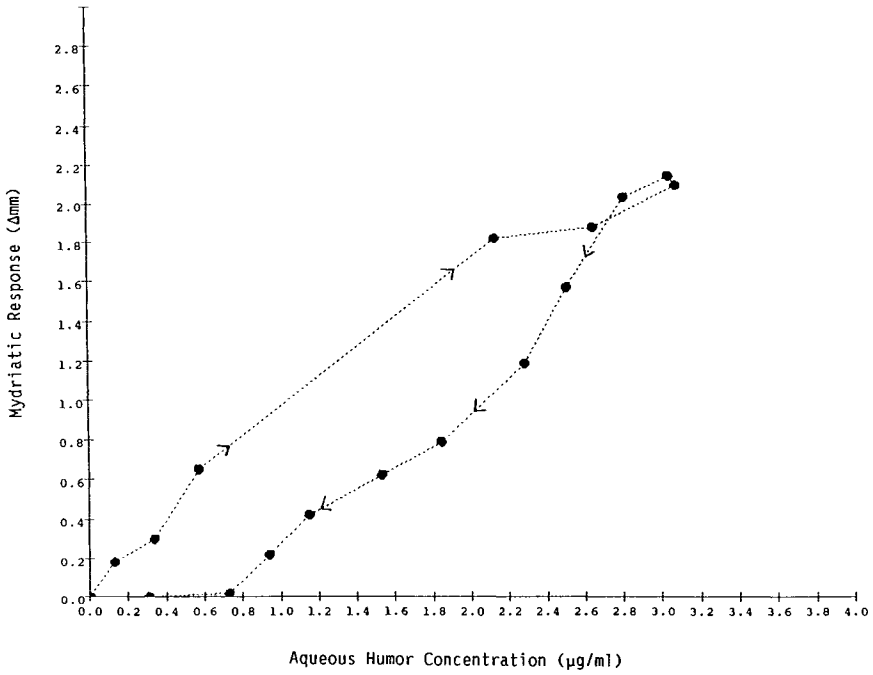


Fig. 2. Mean mydriasis measurement vs mean aqueous humor concentration of phenylephrine (clockwise hysteresis loop) following 10-μl topical instillation of 10% phenylephrine HCl viscous solution.

aqueous humor was apparently in parallel with that in the iris/ciliary body following topical instillation of 0.1% radiolabeled phenylephrine aqueous solution (26). The observation of parallel concentration profiles suggests that the distribution equilibrium of phenylephrine occurs rapidly between the rabbit aqueous humor and the iris/ciliary body. The estimated partition coefficient (*PC*) of phenylephrine between the iris and aqueous humor concentrations of drug at distribution equilibrium is approximately 15.

The absorption and elimination rate constants obtained

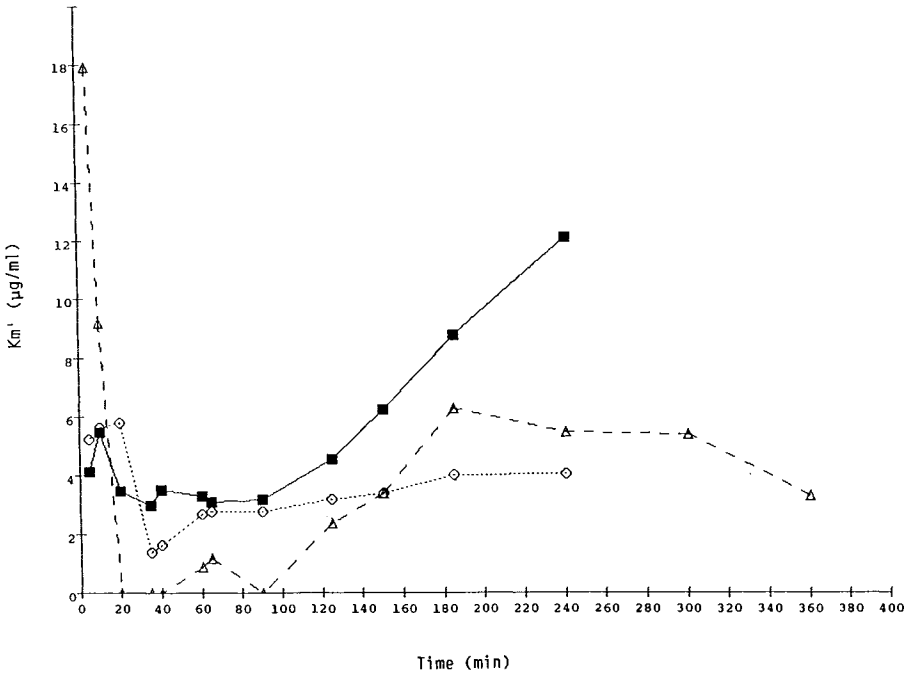


Fig. 3. Value of K_m' vs time plot following 10-μl topical instillation of (—■—) 10% phenylephrine HCl viscous solution, (·····○·····) 1% prodrug suspension, and (—△—) 10% prodrug suspension in rabbit eyes.

from mydriasis varied with the instillation of phenylephrine solution and prodrug suspension. As expected for drugs which have nonlinear dose–response curves, these parameters were also not in agreement with those determined from the drug concentration–time curves.

Since the presence of the prodrug in the aqueous humor may favor the equilibrium uptake of phenylephrine into the iris and consequently the response will be greater than expected from the aqueous humor drug concentration, the prodrug disposition was also studied in aqueous humor. The phenylephrine prodrug readily partitioned into the corneal epithelium because of its favorable lipophilicity. Conversion of the prodrug should likely have occurred largely prior to reaching the iris tissue, during the corneal penetration, because of its aqueous instability (11). The enzymatic activity in the cornea as well as hydrolysis in aqueous humor may further enhance conversion to phenylephrine. As a result, the distribution of the prodrug into iris tissue was assumed to be negligible.

According to the clockwise hysteresis effect of mydriasis versus concentration, the pharmacokinetic parameters of a mydriatic agent determined from its biological response could be affected by the administration of the drug itself via biological supersensitivity or tolerance, delayed distribution equilibrium between biophasic tissue and receptors, or the presence of active metabolites (25).

Biological tolerance after drug administration may result from (i) a decrease in the availability of receptors in the target tissue and/or (ii) a rapid decrease in receptor activity (25). The former process is dependent on drug concentration and duration of exposure and may take several hours to develop. The decrease in the receptor activity may involve the translocation of receptors within the membrane without a decrease in number. The combination of these processes may be part of a cellular regulation system capable of adaptation to stimulation (25).

A typical example of tolerance is the cocaine euphoria after intranasal administration in "euphoria-experienced" volunteers (27). When the degree of euphoria was plotted against the cocaine concentration in plasma, a clockwise hysteresis loop was noted following the time sequence. However, the causes of cocaine's euphoric tolerance remain unclear (27).

The clockwise hysteresis can also be partially attributed to the distribution characteristics of a drug, such as nicotine (28), which occurs when the effect tissue, e.g., brain or heart, of nicotine equilibrates with arterial drug concentration faster than does the sampling site, e.g., forearm venous blood. However, this does not likely occur for phenylephrine when inducing mydriatic tolerance, because drug distribution between the iris (effect site) and the aqueous humor (sampling site) was expected to reach equilibrium rapidly.

The clockwise hysteresis may also occur if an antagonistic metabolite accumulates. Studies with metoprolol suggest that its metabolite may reduce the effects of metoprolol on heart rate in man (29). Antoine *et al.* demonstrated the presence of phenylephrine metabolites in the rabbit corneal epithelium, probably also in the iris/ciliary body (26). The influence of the phenylephrine metabolites on the mydriatic tolerance needs further evaluation.

The mydriatic tolerance developed by phenylephrine may be due to the effect of the drug on the iris and, in particular, the dilator muscle, resulting in a decrease in the receptor number in the dilator muscle cells (8). This was evidenced from the fact that pigment floater has been observed in the aqueous humor after a topical instillation of 5% phenylephrine solution (10). Pigment floaters are present in the epithelial cells of the iris and are released to aqueous humor by a rupture of the cells. Recovery of this effect on iris usually requires more than 12 hr. Rebound miosis, a compensatory result of the damage occurring to the iris dilator muscle, may also reduce the mydriatic response when phenylephrine is used to induce mydriasis (8). Damage to the iris dilator muscle and subsequent rebound miosis may cause a significant mydriatic tolerance of phenylephrine. The mydriatic tolerance is of clinical importance for the patients who use phenylephrine for examination prior to retinal detachment or cataract surgery.

Several pharmacodynamic models have been applied to describe quantitatively the development of tolerance for nicotine (30), furosemide (31), and cocaine (32). These models were modified from the classic pharmacodynamic E_{max} concept (25) with assumptions of the presence of noncompetitive antagonists, e.g., a metabolite (30,31), a distribution lag (33), or the biochemical transformation of the active agonists (34). Since the mechanism of mydriatic tolerance developed by phenylephrine must be further investigated, no pharmacodynamic model is developed in this study to describe the tolerance phenomena of phenylephrine in the rabbit eye. Instead, the K_m' value of the E_{max} model was used to monitor the formation of mydriatic tolerance following the time sequence for the phenylephrine formulations.

ACKNOWLEDGMENT

The work was partially funded by Angelini Pharmaceuticals, Inc., River Edge, New Jersey 07661.

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