Novel Site-Specific Chemical Delivery System as a Potential Mydriatic Agent: Formation of Phenylephrine in the Iris-Ciliary Body from Phenylephrone Chemical Delivery Systems

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ABSTRACT: The objective of this study was to test the three novel ester derivatives of phenylephrone (isovaleryl, phenylacetyl, and pivalyl esters) as potential site-specific chemical delivery systems. The mydriatic effect and ocular distribution/metabolism of these compounds were studied by topical application to the eyes of normal rabbits. It was assumed that a reduction–hydrolysis sequence could produce the active phenylephrine in the iris-ciliary body tissues. All the derivatives showed a more pronounced mydriatic effect than that of phenylephrine, whereas phenylephrone was completely devoid of any mydriatic activity. Phenylacetyl ester was the most potent drug, with short duration of action, and showed maximum activity in the presence of 0.01% benzalkonium chloride without causing any visible irritation to the rabbit eye. Administration of the novel compounds to the eyes of the rabbits showed no traces of phenylephrine in the systemic circulation, contrary to topical administration of phenylephrine. Phenylephrone was detected in different compartments of the eye, whereas phenylephrine was present only in the iris-ciliary body tissues following administration of phenylacetyl ester. The conversion of phenylephrone esters to the active drug, phenylephrine, and thus their subsequent activity was dependent on the physicochemical characteristics of the drugs. The results suggest the potential use of phenylacetyl ester as a potent short-term mydriatic agent without systemic side effects. © 2001 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 90:12–22, 2001.

Keywords: phenylephrone derivatives; chemical delivery system; site-specific; mydriatic activity

INTRODUCTION

Phenylephrine HCl is a α_1-selective adrenergic agonist used in the eye for its mydriatic effect. Because of its hydrophilic nature, it poorly penetrates the epithelium of the cornea. Therefore, it is typically administered at very high concentrations (2.5 or 10%) to the eye to achieve a clinical effect. As a result of applying a large dose that is poorly absorbed, significant local and systemic side effects are common in some individuals. The systemic side effects after ocular administration are due to the drainage of the applied drug into

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the nasolacrimal duct and subsequent systemic distribution. Even a dosage as low as one drop of 0.125% phenylephrine HCl has been shown to elicit an adverse reaction.[1] Several systemic side effects, such as severe hypertension, syncope, subarachnoid hemorrhage, ventricular arrhythmia, and myocardial infarction, have been reported following topical ocular application of phenylephrine HCl.[2–5]

Bodor et al. proposed and successfully applied a novel chemical delivery system (CDS) concept to overcome toxicity and transport-related problems in the delivery of active compounds to the target sites in the body.[6–11] The CDS specific to iris-ciliary body in the eye are obtained by molecular modifications based on differential distribution of metabolic enzymes, esterases and reductases, inherent to the eye; esterases are present ubiquitously, whereas reductases are localized only in the iris-ciliary body tissues.[12] Because the iris-ciliary body is considered to be the primary site of drug action, targeting drugs selectively to this site is highly desirable. Previous reports showed that esters of adrenalone but not adrenalone itself could be converted via a reduction–hydrolysis sequence by reductases and esterases to the active adrenaline (epinephrine) only at the iris-ciliary body, the site of action. However, adrenalone was found to be an inactive species.[13–15] This result suggested that lipophilic ketones could be reduced in the iris-ciliary body. Accordingly, ketone ester precursors of phenylephrine, which is also a β-hydroxylamine like adrenaline, could then possibly be converted by the metabolic enzymes, reductases and esterases, to active phenylephrine by reductive–hydrolysis processes at the site of action, thus avoiding systemic side effects due to exposure of drug to nontarget sites. Thus, novel bioreversible agents of phenylephrine were designed in accordance with the adrenalone CDS.[16] The structures of these compounds are provided in Figure 1. We have previously reported the physicochemical properties, enzymatic stability, and the transport characteristics of these novel compounds.[17] Our studies have shown that these compounds are converted to intermediates with free phenol-ketone following ester hydrolysis, and the transport characteristics were influenced by the physicochemical properties of the drugs.[17,18]

The present paper reports the studies on the relative mydriatic activities of novel phenylephrone derivatives (isovaleryl, phenylacetyl, and pivalyl esters; Figure 1) and their *in vivo* disposition studies to assess the site-specific delivery of phenylephrine to the target tissues, based on the CDS concept.

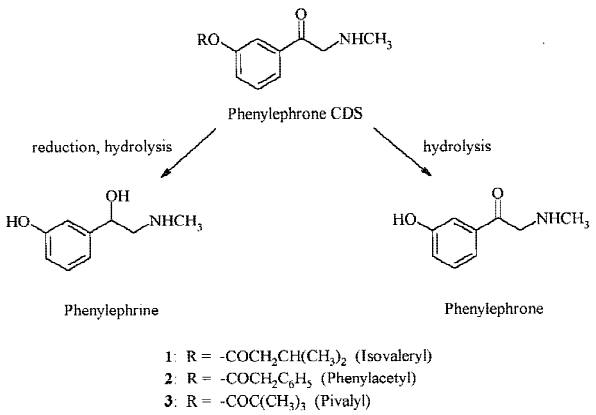

Figure 1. Structures of phenylephrone CDS.

EXPERIMENTAL SECTION

Materials

Synthesis and characterization of the phenylephrone CDS have been previously reported.[16] Phenylephrine hydrochloride was purchased from Spectrum Chemical Company (St. Louis, MO). Benzalkonium chloride was purchased from Aldrich Chemical Company (Milwaukee, WI) and triethylamine was obtained from Spectrum Chemical Company (Gardena, CA). All other chemicals used in the study were of analytical reagent or HPLC grade and were used as received without further purification. Male New Zealand albino rabbits purchased from Myrtles Rabbitry (Thompson Road, TN) weighing 2.0–2.5 kg were used for the *in vivo* distribution–metabolism and pharmacological activity studies.

Analytical Method

A high-pressure liquid chromatography system (HPLC) was used for the determination of the three phenylephrone esters and for the analysis of the regeneration profile of phenylephrone. The liquid chromatographic system consisted of ISCO model 2350 HPLC pump, ISCO autoinjector with a loop of 20 μL, and a PARC model 400 electrochemical (EC) detector. The EC detector with the glassy carbon cell was operated in the d.c. mode at +1150 mV versus an Ag/AgCl electrode. The column was kept under constant temperature conditions using a waters TMC temperature control

system. A Microsorb-MV C18 column (4.6 × 150 mm, 5-μm particle size and 100 Å pore size) with a similar 6-cm guard column and a mobile phase (pH 3.6) consisting of acetonitrile:acetate buffer: triethylamine (25:75:0.05) with a flow rate of 1 mL/min were used for the resolution of the phenylephrone CDS. The mobile phase used for the analysis of the time course of phenylephrone and phenylephrine production consisted of 12% acetonitrile, 0.1% triethylamine, and 0.2 M acetate buffer (pH 3.6), and the flow rate was maintained at 1 mL/min. To minimize the background noise, the solvent mixture was pre-filtered with 0.45-μm membrane and degassed using a magnetic stirrer in vacuum. The injection volume was 20 μL. The sensitivity of the assay of phenylephrone esters was ~0.2 ng/mL. The sensitivity of phenylephrine HCl and phenylephrone HCl was 0.15 ng/mL. Calibration curves were obtained by plotting the peak area as a function of drug concentration.

Mydriatic Effect

Adult male New Zealand albino rabbits weighing ~2.0–2.5 kg were used. Animals were kept in individual cages with free access to food and water. The experiment was designed to compare the mydriatic effects of phenylephrine HCl, phenylephrone, and esters of phenylephrone HCl. The measurement was done using locally fabricated and calibrated Haab's scale (4–16 mm) with increments of 0.2 mm. In a group of three rabbits, 50 μL of phenylephrine, phenylephrone, and esters of phenylephrone were administered in one eye. The esters were evaluated for mydriatic effect in isotonic phosphate buffer (pH 6.0) at concentrations 0.1, 0.5, 1.0, and 2.5% phenylephrone in a light- and temperature-controlled room. The formulations containing 0.1, 0.5, and 1% phenylacetyl ester with 0.01% benzalkonium chloride (BAC) in isotonic phosphate buffer (pH 6.0) were also tested for pharmacological activity. The mydriatic effects of phenylephrone and phenylephrone esters were compared with that 2.5% solution of phenylephrine HCl. The untreated eye served as a control and received vehicle only. Pupillary changes were measured with Haab's scale held at a constant distance at time intervals of 0, 5, and 15 min, and 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, and 6.0 h. During the measurement, rabbits were placed in tabletop restraint box to minimize head movement. All pupillary measurements were obtained from restrained rabbits and were carried out by the same operator. Values are given as mean ± standard error of the mean (SEM). Rabbits were also observed for any obvious manifestation of irritation caused by the compounds under investigation, such as swelling, redness, and lacrimation.

In Vivo Distribution–Metabolism Studies

Groups of at least three adult male New Zealand albino rabbits weighing ~2.0–2.5 kg were used for the studies. A 50-μL volume of 0.5% phenylacetyl ester in isotonic phosphate buffer (pH 6.0) solution was administered topically to both eyes of each rabbit. After appropriate time intervals of 15, 45, and 150 min, the animals were sacrificed by peripheral ear vein injection of 3 mL of sodium pentobarbital (65 mg/mL), and the treated eyes were removed and rinsed in a beaker of normal saline. Aqueous humor was collected by making a single puncture at the limbus with a 25-gauge × 1.6-cm needle attached to a 1-mL syringe. Then, the cornea and the iris-ciliary body were isolated. The tissues were pooled and homogenized with a tissuemizer in ice-cold perchloric acid (0.05 M), which contained 0.05% sodium metabisulfite as an antioxidant. Samples were re-homogenized in methanol to prepare 10% homogenates, transfered to microfilters (Millex units, Millipore Products Division), and centrifuged for 20 min at 10,000 rpm to precipitate proteins. Aqueous humor and plasma samples were analyzed as such without any furthur dilution. Aliquots of 20 μL of the 10% tissue homogenate samples were analyzed by HPLC using an electrochemical detector. Quantitation was done by using a calibration curve obtained by the addition of known amounts of the compounds to aqueous humor, iris-ciliary body, or cornea isolated from a control rabbit after topical administration of the solution without the drug.

The procedure to assess the plasma levels in the rabbit after topical administration of the compounds is as follows: The blood samples (1.0 mL) were withdrawn from the marginal ear vein at 5, 10, 15, 45, and 60 min following drug instillation. The samples were placed in citrated tubes and centrifuged, and the plasma immediately frozen. To 100 μL of sample (after equilibrating to room temperature), 1 mL of acetonitrile was added to precipitate the proteins. Following thorough mixing and filtering through 0.2-μm filter unit, the samples were centrifuged for 5 min at 6000 rpm. The clear supernatant was then analyzed by HPLC.

RESULTS AND DISCUSSION

Mydriatic Activity

The effect of phenylephrone and the three esters of phenylephrone on the pupillary dilation was evaluated and compared with their parent compound, phenylephrine HCl. The dose–response curves for mydriatic effect were obtained by administering drug at increasing concentrations. The doses of the novel compounds administered were at concentrations equivalent to 0.1, 0.5, 1.0, and 2.5% phenylephrone. The mydriatic effect of the compounds both in the treated and control eye were compared with those that received 2.5% phenylephrine. Phenylephrone at 2.5% concentration was also tested for possible mydriatic activity.

The maximum mydriatic activity (mydriatic$_{max}$), time to reach the maximum mydriatic activity (t_{max}), and the area under the curve (AUC) of the compounds tested at various concentrations are listed in Table 1. Figures 2 and 3 represent the change in pupil diameter over time in the treated eye for the compounds under study at 0.5 and 1.0% concentrations, respectively. The maximal dilation was achieved 45 min after instillation of phenylephrone esters and 1 h after instillation of phenylephrine HCl. The faster attainment of maximal activity may be explained on the basis of rapid corneal penetration of the phenylephrone esters compared with phenylephrine. The ester derivatives of phenylephrone showed significantly ($p < 0.01$) greater effect than phenylephrine HCl at all concentrations except at 0.1% concentration. Phenylephrone showed no activity in the treated or control eye. The higher activity of the phenylephrone CDS may be attributed to their greater lipophilicity. The log octanol/pH 7.4 buffer distribution coefficient for the esters were in the range 1.92–2.35[17] (in comparison with 0.67 for phenylephrine), which is optimal for rapid corneal absorption for many ophthalmic drugs.[19,20]

The extent of pupillary dilation increased for all the compounds under study with an increase in concentration (dose equivalent to 0.1 to 1.0%

Table 1. Maximum Mydiatic Activity (mydriatic$_{max}$), Time to Reach the Maximum Mydriatic Activity (t_{max}), and Area Under the Curve (AUC) of the Compounds Tested at Various Concentrations[a]

Compound (Conc.)	Mydriatic$_{max}$ (mm)	t_{max} (min)	AUC (h mm)
Phenylephrine HCl			
2.5%	7.8 (0.3)	60	4.27 (0.9)
Isovaleryl ester			
0.1%	5.5 (0.8)	45	4.23 (0.7)
0.5%	8.7 (1.2)	45	4.71 (0.6)
1.0%	9.6 (1.3)	45	6.67 (0.9)
2.5%	9.7 (1.0)	45	7.01 (1.1)
Phenylacetyl ester			
0.1%	6.8 (0.5)	45	4.55 (0.4)
0.5%	9.3 (0.7)	45	7.06 (0.5)
1.0%	11.2 (1.0)	45	12.75 (1.4)
2.5%	11.4 (0.9)	45	12.81 (1.9)
Pivalyl ester			
0.1%	5.1 (0.4)	45	4.22 (0.6)
0.5%	8.1 (0.9)	45	9.08 (1.5)
1.0%	8.5 (1.1)	45	8.37 (1.4)
2.5%	8.5 (0.8)	45	9.28 (1.8)
BAC (0.01%) + Phenylacetyl ester			
0.1%	8.0 (1.2)	45	3.97 (0.5)
0.5%	11.2 (1.5)	45	12.08 (2.1)
1.0%	11.3 (0.9)	45	12.88 (1.7)

[a] Concentrations are equivalent to percent phenylephrone. Figures in parenthesis are ± standard error of mean ($n = 3$).

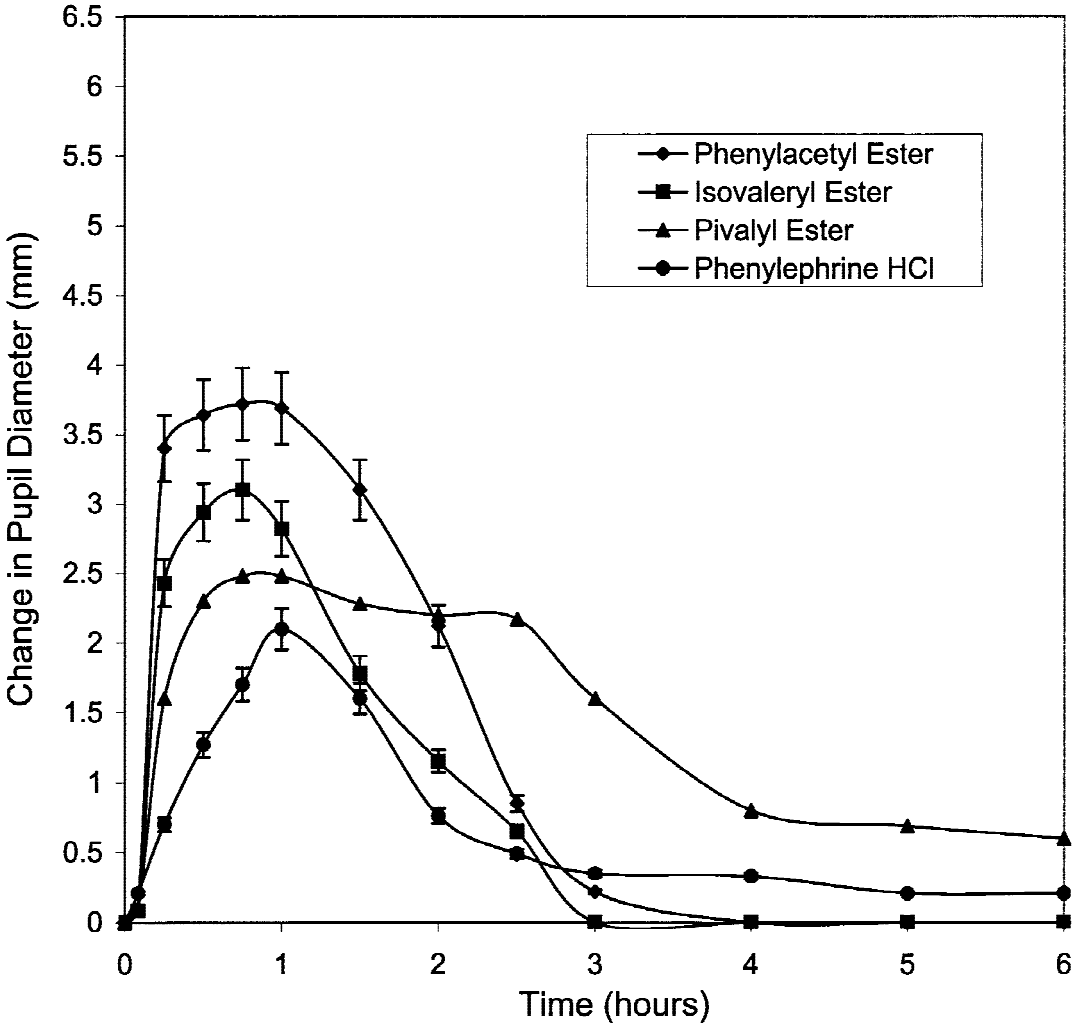

Figure 2. Mydriatic responses of phenylephrine HCl (2.5%) and esters of phenylephrone HCl (0.5%) in the test eye. Each value represents average (±SEM) of three experiments.

phenylephrone). However, increasing the concentrations beyond 1.0% did not give rise to a proportional increase in mydriatic activity. This result probably could be due to reaching a maximum in transport as well as enzymatic process rates and also due the nature of the eye with regard to maximum attainable dilation. Phenylacetyl and isovaleryl esters, chemically and enzymatically the most labile derivatives,[17] were more potent than pivalyl ester.

The eyes treated with phenylacetyl ester showed maximum change in pupillary diameter approaching 3.7 ± 0.3 and 5.5 ± 0.6 mm at 45 min for concentrations of 0.5 and 1.0%, respectively, compared with 2.1 ± 0.3 mm in eyes treated with 2.5% phenylephrine HCl. The rate of rise of the peak pupillary dilation was also rapid for phenylacetyl ester. At the 15-min time point, the test drug treated eyes with 0.5 and 1.0% concentrations achieved near maximal change in dilation of 3.4 ± 0.2 and 3.8 to 0.4 mm, respectively, in contrast to only 0.7 ± 0.1 mm dilation obtained by those eyes treated with 2.5% phenylephrine HCl.

It is required by the Food and Drug Administration (FDA) that all multidose ophthalmic preparations should contain preservatives such as BAC. BAC was reported to increase the corneal permeability of a number of compounds.[21–25] Because phenylacetyl ester showed the highest activity with relatively shorter duration of action, this compound was further tested for its interac-

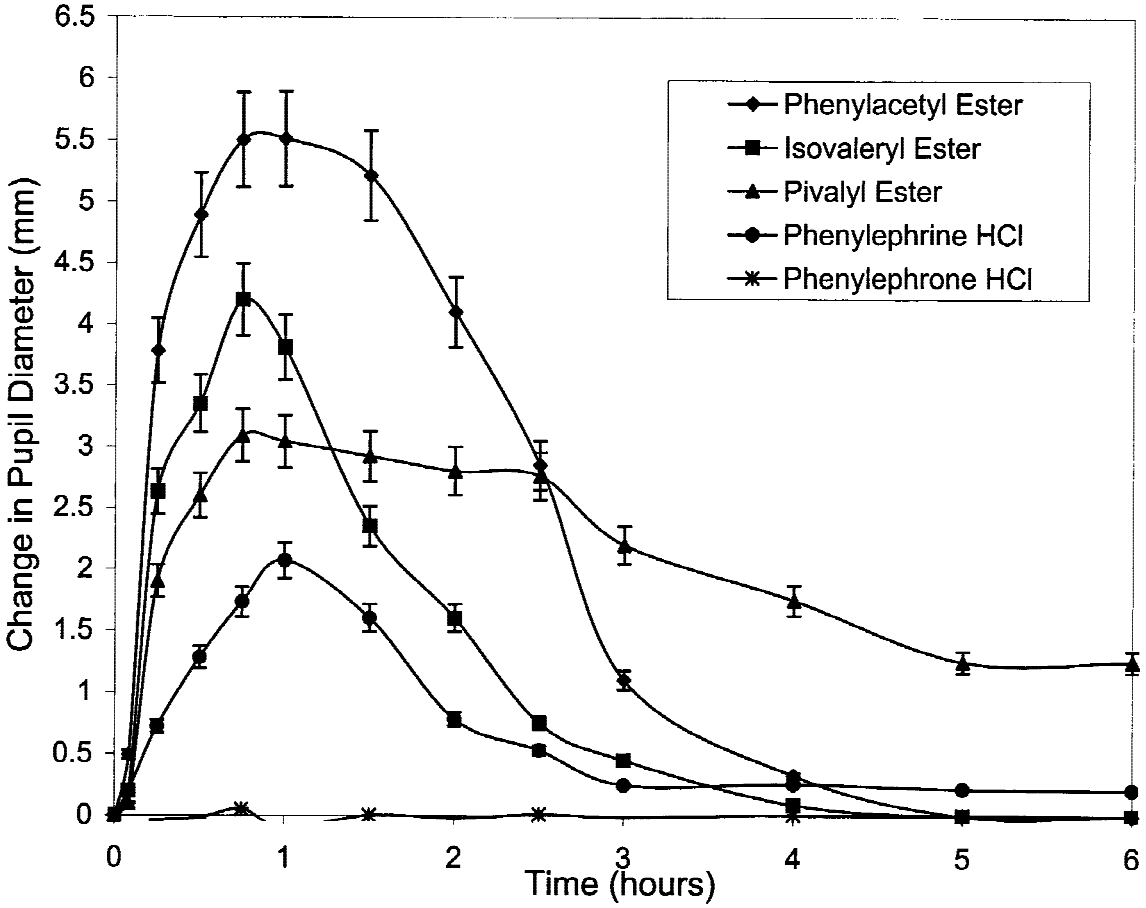

Figure 3. Mydriatic responses of phenylephrine HCl (2.5%), phenylephrone HCl (2.5%), and esters of phenylephrone HCl (1.0%) in the test eye. Each value represents average (±SEM) of three experiments.

tions with BAC, a commonly used preservative in ophthalmic formulations. The mydriatic activity of phenylacetyl ester at concentrations equivalent to 0.1, 0.5, and 1.0% phenylephrone was studied in the presence of 0.01% BAC (Figure 4, Table 1). The rabbit eyes treated with the drug solution containing BAC did not show any signs of visible irritation, such as swelling, redness, and lacrimation. Phenylacetyl ester at 0.1 ($p < 0.05$) and 0.5% ($p < 0.005$) concentrations showed a significant increase in activity in the presence of BAC compared with that treated without BAC. But the compound at 1.0% concentration showed comparable mydriatic activity in the presence and absence of BAC (Table 1). The results also showed no significant ($p < 0.001$) difference in the mydriatic activity between 0.5 and 1.0% concentrations of phenylacetyl ester in the presence of BAC. Both concentrations showed a maximum change in pupil diameter of 5.6 ± 0.6 mm at 45 min, whereas the eyes treated with 0.1% phenylacetyl derivative showed a maximum change in pupil diameter of 2.4 ± 0.3 mm.

The time course of mydriatic activity of the compounds under investigation in the control eye is depicted in Figure 5. The control eye into which only the vehicle was administered did not show any significant dilation except for phenylephrine HCl. At 45 min, phenylephrine HCl produced a significant ($p < 0.01$) dilation in the control eye compared with phenylephrone CDS. Unilateral instillation of 2.5% phenylephrone into the rabbit eyes did not show a significant change in pupil diameter both in the test and control eye (Figures 3 and 5). This result strongly supports the hypothesis that the rapid hydrolysis of esters of phenylephrone leads to the production of inactive compound, phenylephrone, which is devoid of any local or systemic effects. For further confirmation, the blood samples drawn at predetermined inter-

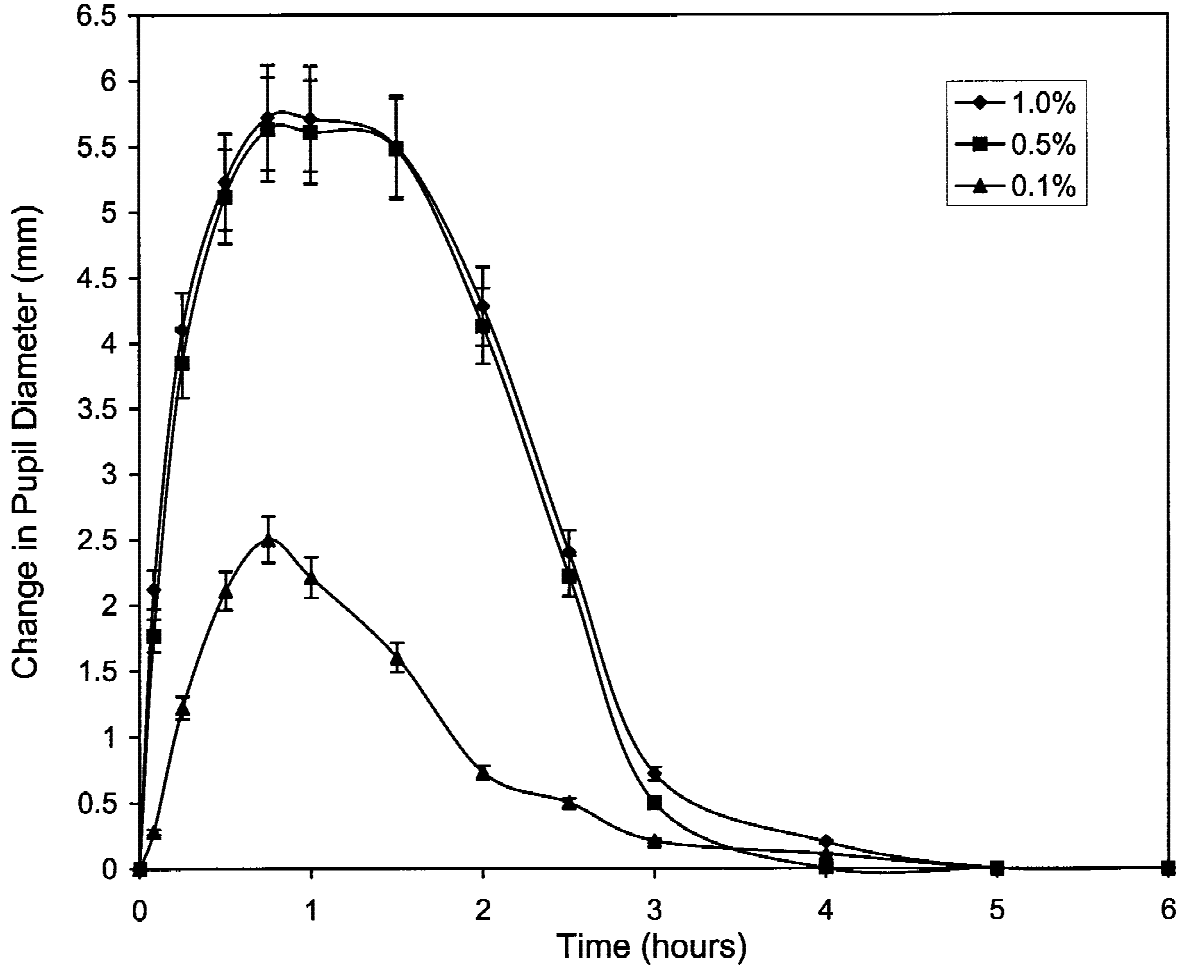

Figure 4. Mydriatic responses of phenylacetyl phenylephrone HCl in the presence of 0.01% BAC at pH 6.0 in the test eye. Each value represents average (±SEM) of three experiments.

vals were analyzed for the presence of phenylephrine after topical administration of the compounds. Results of this study revealed that phenylephrine was not detected as a biotransformation product of phenylephrone CDS in blood. However, significant amounts of phenylephrine were observed in the blood samples after its topical application (Figure 6).

The extent of mydriasis depends on the quantity of the drug residing in the target tissue (iris-ciliary body) and, hence, the time course of the pupil response directly reflects the change of drug in the iris-ciliary body.[26] More than 90% of topically administered drugs have been reported to be drained into systemic circulation through nasolacrimal duct without entering the inner eye. This drainage results in high incidence of systemic side effects after ocular administration of drugs. In the present investigations, the control eye that received only the vehicle without the drug served as an indicator of systemic absorption of the drug and its subsequent side effects. Pupillary dilation was observed in the control eye in phenylephrine HCl-treated animals but not with phenylephrone CDS-treated animals. This result could be due to the persistence of phenylephrine in systemic circulation in comparison with the rapid systemic inactivation of phenylephrone CDS, which was further confirmed by testing the blood samples for the presence of phenylephrine.

In most of the clinical conditions, mydriatics are used as diagnostics rather than for therapeutic purposes. Hence, persistence of the mydriatic action after a certain required period of time is both unnecessary and inconvenient. The ubiqui-

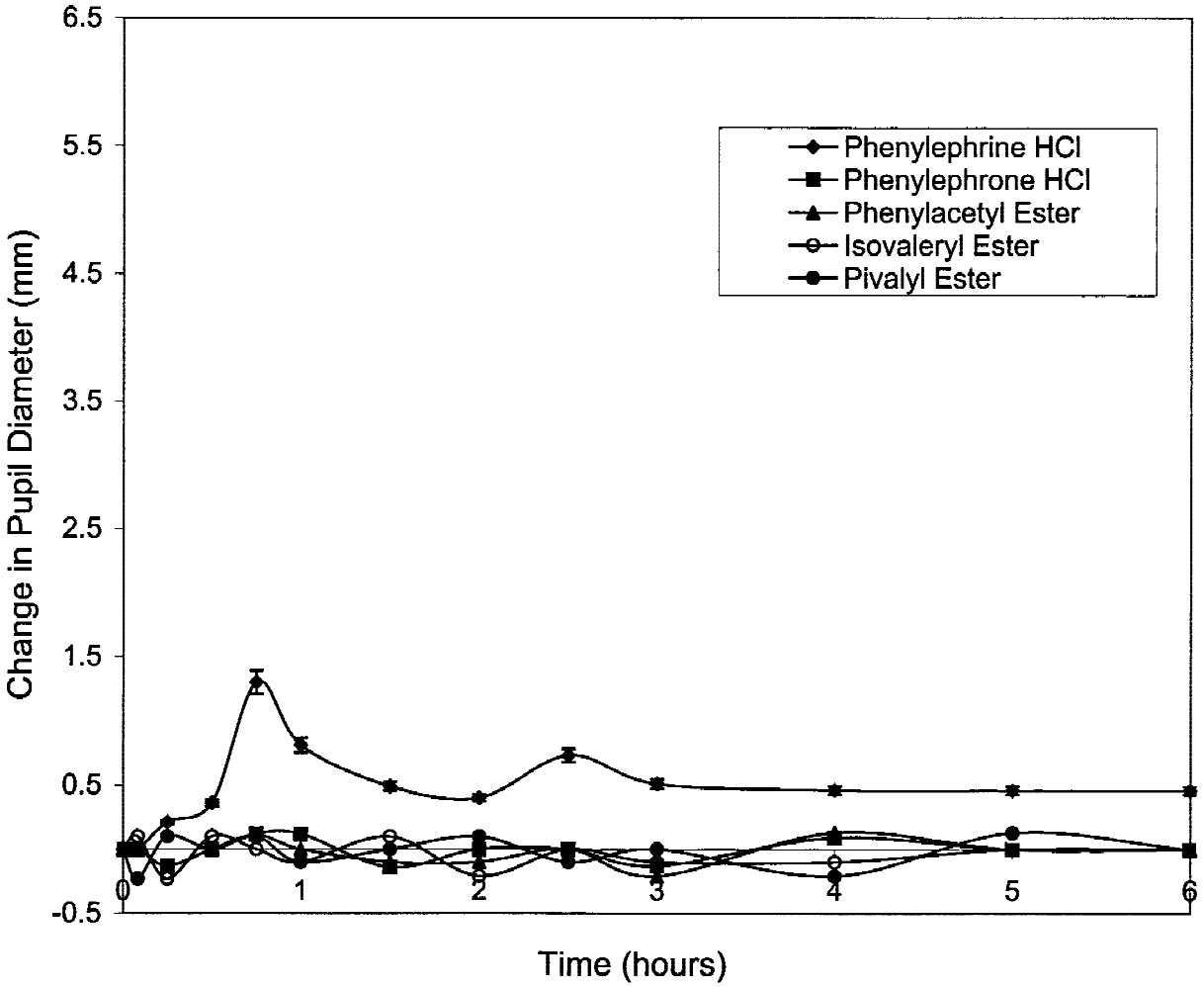

Figure 5. Mydriatic responses of phenylephrine HCl, phenylephrone HCl, and esters of phenylephrone HCl at 2.5% in the control eye. Each value represents average (±SEM) of three experiments.

tous presence of esterases will aid in the metabolism of the phenylephrone CDS to an inactive metabolite, phenylephrone. In the present study both the isovaleryl and phenylacetyl ester showed shorter duration of mydriatic activity. The presence of the bulky group in pivalyl phenylephrone HCl resulted in increased duration of action because of slower hydrolysis of pivalyl ester by esterases. All the compounds studied did not exhibit any visible irritant action, such as swelling, redness, or lacrimation, even at the highest concentrations tested. Phenylacetyl ester was extremely potent in eliciting mydriatic activity of all the compounds tested. Both isovaleryl and phenylacetyl esters showed a short duration of action (150 min). The reasons for these differences could be that isovaleryl and phenylacetyl esters, being more lipophilic, have greater permeation across the corneal membranes, which results in delivery of higher concentrations at the target site (i.e., iris-ciliary body). Furthermore, these derivatives, which are highly labile to enzymatic activity, undergo facile reduction–hydrolysis process to generate the active compound, phenylephrine, at the site of action (iris-ciliary body). Pivalyl ester, which is relatively more stable chemically and enzymatically, showed low mydriatic effect with a prolonged duration of action.

In Vivo Distribution/Metabolism Studies

The results from the pharmacological activity studies of the novel compounds indicate that phenylacetyl derivative is a potent mydriatic

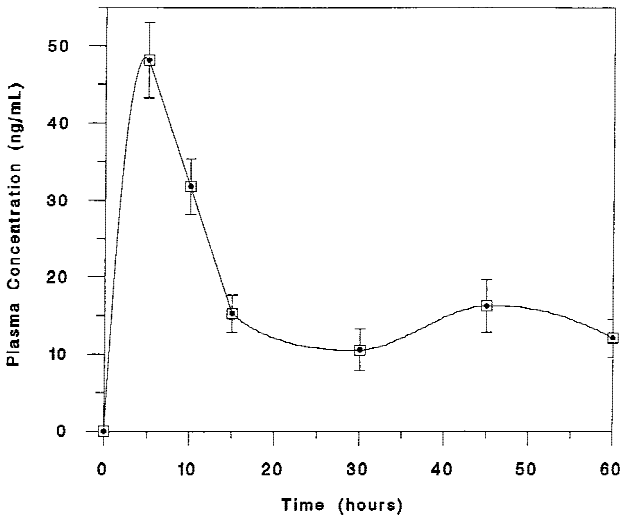

Figure 6. Plasma concentrations of phenylephrine following topical application of phenylephrine HCl (2.5%) to the rabbit eyes. Each value represents average (±SEM) of three experiments.

Table 3. Tissue Concentrations of Phenylephrine at Various Time Intervals Following Topical Administration of Phenylacetyl Phenylephrone HCl (0.5% Solution)[a]

	Concentration of Phenylephrine		
Location	15 min (μg/g)	45 min (μg/g)	150 min (μg/g)
Cornea	—	—	—
Conjunctiva	—	—	—
Aqueous humor	—	—	—
Iris-ciliary body	0.23 ± 0.11	2.89 ± 0.57	0.07 ± 0.04

[a] Figures represent the mean ± SEM ($n = 6$).

agent. To assess the site-specificity of this agent, the *in vivo* disposition in different ocular tissues of rabbits was investigated.

Tissue concentrations of phenylephrone and phenylephrine following topical administration of phenylacetyl ester are depicted in Tables 2 and 3, respectively. The concentrations of phenylephrone in the tissues increased and then decreased rapidly. Higher levels of phenylephrone were found in corneal and conjunctival tissues (Table 2). The reasons for these findings could be that the cornea, being the major barrier to penetration, has the capacity to act as drug reservoir. On the other hand, conjunctiva presents a larger surface area to the tears than the cornea and was reported to accumulate large number of drugs in unusually large quantities due to binding in the mucin granules of goblet cells.[27,28] Phenylephrone concentration in aqueous humor was significantly lower compared with other tissues. This result could be due to various reasons, including continuous formation and drainage of aqueous humor, low enzyme activity, and absorption into the tissues of the anterior uvea, the blood circulating through them, and the lens. *In vivo* studies also showed that the peak concentrations of phenylephrone in the iris-ciliary body tissues coincided with those of cornea, conjunctiva, and aqueous humor. As summarized in Table 2, phenylephrone levels were higher in iris-ciliary body than in aqueous humor. Phenylephrone is formed by the hydrolytic cleavage of phenylacetyl ester by the action of esterases. Thus, the higher levels of phenylephrone in iris-ciliary body are due the high esterase activity in the tissue. The most significant finding in the present study was the presence of the active compound, phenylephrine, in high concentrations in the iris-ciliary body (Table 3). Interestingly, phenylephrine could not be detected in any other ocular tissues tested. The iris-

Table 2. Tissue Concentrations of Phenylephrone at Various Time Intervals Following Topical Administration of Phenylacetyl Phenylephrone HCl (0.5% Solution)[a]

	Concentration of Phenylephrone		
Location	15 min (μg/g)	45 min (μg/g)	150 min (μg/g)
Cornea	9.13 ± 2.17	21.85 ± 4.09	3.26 ± 0.96
Conjunctiva	17.41 ± 4.86	38.91 ± 5.37	11.53 ± 2.80
Aqueous humor	0.65 ± 0.14	2.10 ± 0.75	0.98 ± 0.23
Iris-ciliary body	0.97 ± 0.31	9.64 ± 2.29	1.94 ± 0.55

[a] Figures represent the mean ± SEM ($n = 6$).

Table 4. Tissue Concentrations of Phenylephrone at Various Time Intervals Following Topical Administration of Phenylephrone HCl (2.5% Solution)[a]

Location	Concentration of Phenylephrone		
	15 min (μg/g)	45 min (μg/g)	150 min (μg/g)
Cornea	16.11 ± 3.08	34.69 ± 4.56	4.62 ± 1.14
Conjunctiva	26.12 ± 4.21	43.52 ± 4.89	15.16 ± 2.80
Aqueous humor	1.01 ± 0.23	2.45 ± 1.07	1.18 ± 0.42
Iris-ciliary body	0.45 ± 0.16	2.52 ± 0.42	0.51 ± 0.13

[a] Figures represent the mean ± SEM ($n = 6$).

ciliary body tissues appear to be one of the major sites of drug metabolism because of high enzymatic activity. The presence of phenylephrine in the iris-ciliary body tissues is indicative of the bioactivation via a reduction followed by hydrolytic cleavage, suggesting the site-specificity of novel CDS.

The tissue concentration of phenylephrone following topical instillation of 2.5% phenylephrone is depicted in Table 4. Phenylephrone concentration was detected in all the ocular tissues including iris-ciliary body. However, phenylephrine could not be detected in the ocular tissues/fluids. Furthermore, phenylephrone did not show any mydriatic activity on topical administration, suggesting an apparent lack of *in vivo* conversion of phenylephrone to phenylephrine.

CONCLUSION

All the esters tested showed significant dilation of the pupil in the treated eye on unilateral administration. A significant pupillary dilation was observed in the untreated eye after unilateral administration of phenylephrine. The absence of pupil dilation in the control eye of the animals treated with phenylephrone CDS indicates the facile metabolism of these compounds in systemic circulation. This result was further confirmed by testing the presence of the active compound, phenylephrine, in the rabbit blood after topical instillation of phenylephrone CDS. Phenylephrine was not detected in the blood after topical administration of all the CDS studied. Phenylacetyl ester of phenylephrone exhibited greater activity, yet it is metabolically more labile. High concentrations of phenylephrone were observed in cornea and conjunctiva followed by iris-ciliary body and aqueous humor on instillation of phenylacetyl ester, and the active compound, phenylephrine, was detected only in the iris-ciliary body tissues. These results suggest the potential use of phenylacetyl ester derivative as a short-acting mydriatic agent.

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REFERENCES

1. Weiss DI, Shaffer RN. 1962. Mydriatic effects of one eighth percent phenylephrine. Arch Ophthalmol 68:727–729.
2. Lansche RK. 1966. Systemic reactions: to topical epinephrine and phenylephrine. Am J Ophthalmol 61:95–98.
3. Solosko D. 1972. Hypertension following 10 percent phenylephrine ophthalmic. Anesthesiology 36: 187–189.
4. Wilensky JT, Woodward HJ. 1973. Acute systemic hypertension after conjunctival instillation of phenylephrine hydrochloride. Am J Ophthalmol 76:156–157.
5. Fraunfelder FT, Scafidi AF. 1978. Possible adverse effects from topical ocular 10% phenylephrine. Am J Ophthalmol 85:447–453.
6. Bodor N. 1996. The use of retrometabolic drug design concepts in ophthalmic drug discovery. In: Reddy IK, editor. Ocular therapeutics and drug delivery. Pennsylvania: Technomic, pp 335–361.
7. Reddy IK, Bodor NS. 1994. Novel approaches to

design and deliver safe and effective anti-glaucoma agents to the eye. Adv Drug Deliv Rev 14:251–267.
8. Bodor N. 1984. Novel approaches to the design of safer drugs: Soft drugs and site specific chemical delivery systems. In: Testa B, editor. Advances in drug research. London: Academic Press, pp 255–331.
9. Bodor N, Brewster E. 1983. Problems of delivery of drugs to the brain. Pharm Ther 19:337–386.
10. El-Koussi AA, Bodor N. 1989. Formulation of propranolol in the iris-ciliary body from its propranolol ketoxime precursor—a potential antiglaucoma drug. Int J Pharm 53:189–194.
11. Bodor N, El-Koussi A, Kano M, Nakamura T, Khalifa M. 1988. Design, synthesis and pharmacological activity of novel chemical delivery systems of β-blockers. J Med Chem 31:100–108.
12. Lee VHL, Chein D-S, Sasaki H. 1988. Ocular ketone reductase distribution and its role in the metabolism of ocularly applied levobunolol in the pigmented rabbit. J Pharmacol Exp Ther 246:871–878.
13. Bodor N, Kaminski JJ, Roller RG. 1978. Improved delivery through biological membranes VI. Potent sympathomimetic adrenalone derivatives. Int J Pharm 1:189–196.
14. Bodor N, Visor G. 1984. Formation of adrenaline in the iris-ciliary body from adrenalone diesters. Exp Eye Res 38:621–626.
15. Bodor N, Visor G. 1984. Improved delivery through biological membranes. XVII. A site-specific chemical delivery system as short-acting mydriatic agent. Pharm Res 1:168–172.
16. Reddy I. 1989. Design and evaluation of site-specific chemical delivery systems to the eye. Ph.D. Dissertation, University of Florida, Gainesville, FL.
17. Goskonda VR, Khan MA, Bodor NS, Reddy IK. 1999. Chemical delivery systems: Evaluation of physicochemical properties and enzymatic stability of phenylephrone derivatives. Pharm Dev Tech 4(2):189–198.
18. Goskonda VR, Khan MA, Hutak CM, Reddy IK. 1999. Permeability characterisics of novel mydriatic agents using an in vitro cell culture model that utilizes SIRC rabbit corneal cells. J Pharm Sci 88: 180–184.
19. Huang HS, Schoenwald RD, Lach JL. 1983. Corneal penetration behavior of β-blocking agents II. Assessment of barrier contributions. J Pharm Sci 72:1272–1279.
20. Chien DS, Bundgaard H, Lee VHL. 1988. Influence of corenal epithelial integrity on the penetration of timolol prodrugs. J Ocular Pharmacol 4:137–145.
21. Burstein NL, Klyce SD. 1977. Electrophysiologic and morphologic effects of ophthalmic preparations on rabbit cornea epithelium. Invest Ophthalmol 16:899–911.
22. Green K, Tonjum A. 1971. Influence of various agents on corneal permeability. Am J Ophthalmol 72:897–905.
23. Keller N, Moore D, Carper D, Longwell A. 1980. Increased corneal permeability induced by the dual effects of transient tear film acidification and exposure to benzalkonium chloride. Exp Eye Res 30: 203–210.
24. Green K, Downs SJ. 1974. Prednisolone phosphate penetration into and through the cornea. Invest Ophthalmol 13:316–319.
25. Tonjum AM. 1975. Permeability of rabbit corneal epithelium to horseradish peroxidase after the influence of benzalkonium chloride. Acta Ophthalmol 53:335–347.
26. Mishima S. 1981. Clinical pharmacokinetics of the eye. Proctor lecture. Arch Ophthalmol Vis Sci 21: 504–541.
27. Salazar M, Patil PN. 1975. An explanation for the long duration of mydriatic effect of atropine in eye. Invest Ophthalmol 15:671–675.
28. Sieg J, Robinson J. 1976. Mechanistic studies on transcorneal permeation of pilocarpine. J Pharm Sci 65:1816–1821.