Pharmacokinetics of Topical Ocular Phenylephrine HCl

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The rates of corneal penetration and efflux of phenylephrine (PE) and its metabolites were found to be limited by the epithelium. The rate constant for corneal penetration as measured in a lucite block perfusion system was $1.06 \times 10^{-3} \text{ hr}^{-1}$ when the epithelium was present and $1.25 \times 10^{-2} \text{ hr}^{-1}$ when the epithelium was denuded. Epithelial removal reduced the half-time ($t/2$) for corneal efflux of PE from 24 min to 6 min.

Ocular absorption of topically applied 0.1% PE (three 30-μl instillations at five-minute intervals) was increased when the corneal epithelium was removed prior to application. Corneal concentrations of PE increased threefold, aqueous humor concentrations increased ten to 13-fold and iris/ciliary body concentrations increased sixfold upon epithelial removal. HPLC analysis suggested that the corneal epithelium was responsible for the metabolic degradation of PE, which occurred following topical instillation of PE. Invest Ophthalmol Vis Sci. 25:48-54, 1984

Phenylephrine · HCl (PE) is a synthetic sympathomimetic used to obtain mydriasis for routine fundoscopic examinations, for refractions, and during ocular surgery. Systemic side effects from topical administration of 10% PE have been reported and include elevated blood pressure, ventricular arrhythmia, myocardial infarction and subarachnoid hemorrhage.1 More frequently, adverse ocular reactions occur, such as the corneal clouding, sometimes observed following prolonged use of PE during vitrectomy.2

Previous studies in our laboratory demonstrated that topical administration of either 2.5% or 10% PE causes epithelial cell vacuolization in rabbit corneas; removal of the epithelium prior to treatment results in a drug-induced corneal edema as well as reversible vacuolization of stromal keratocytes and endothelial cells.3 Our studies in cell culture confirmed the toxicity of PE to the corneal endothelium.4 In addition, we showed that PE reduces the rate of glucose oxidation by the endothelium of isolated rabbit corneas.5

Despite the wealth of studies that demonstrate the adverse effects of PE, little data is available to characterize its pharmacokinetics and metabolism. In a study on human volunteers, Matsumoto et al.6 determined the pharmacokinetic coefficients for the elimination rate, absorption, and the least effective concentration based on pupil responses; however, the study did not include data on the actual tissue levels of PE or its metabolites. The goals of the present study were to measure the corneal penetration rate of PE, to examine its absorption and distribution in ocular tissue, and to determine if PE is metabolized during corneal penetration.

Materials and Methods

Phenylephrine · HCl was obtained as [14C-7]PE from Pathfinder's Laboratories (St. Louis, MO) with a specific activity of 15.36 μCi/μmole. High-performance liquid chromatography (HPLC) conducted in our laboratory showed that >99% of the label was associated with PE. Unlabeled PE was obtained from Sigma Chemical Co. (St. Louis, MO) and dihydroxybenzylamine·HBr (DHBA) was purchased from Aldrich Chemical Co. (Milwaukee, WI). DHBA was used as an internal standard for HPLC analysis as well as a recovery marker for tissue fractionation procedures. Stock solutions of DHBA and PE were prepared in 0.05 N HClO4 containing 0.1% sodium bisulfite and 0.05% Na2 EDTA. DHBA solutions were prepared daily while PE solutions were stored under N2 at 4°C for up to 3 weeks.

In Vitro Corneal Penetration

New Zealand white rabbits weighing 2 to 3 kg were euthanized by injection of sodium pentobarbital into the marginal ear vein. The eyes were enucleated with the conjunctiva and lids intact, and the corneas were mounted in a lucite-block perfusion chamber7,8 with a holder modified according to Dikstein and Maurice.9

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The exposed area of the cornea was estimated at 1.1 cm² in all experiments. Paired corneas were used, one with the epithelium intact and the other with the epithelium removed by scraping with a Gill knife. Six milliliters of glutathione bicarbonate Ringer’s (GBR) were added to the reservoirs bathing either side of the cornea: NaCl, 6.52 gm/L; KCl, 0.359 gm/L; CaCl₂ · 2H₂O, 0.153 gm/L; MgCl₂ · 6H₂O, 0.159 gm/L; NaH₂PO₄, 0.103 gm/L; NaHCO₃, 2.454 gm/L; glucose, 0.903 gm/L; and reduced glutathione, 0.092 gm/L. Phenylephrine at various concentrations was added to the reservoir bathing the epithelial surface (2.6 μCi/µmole). Constant mixing and pH 7.6 were maintained by means of an airlift siphon operated with 95% air-5% CO₂. The temperature was maintained at 37°C by a circulating water bath.

The diffusion rate of ¹⁴C-labeled molecules across the cornea was measured by removing aliquots from the reservoir bathing the endothelial surface at various times after the addition of ¹⁴C-PE to the reservoir bathing the epithelial surface. Samples of the bathing media were placed in Aquasol (New England Nuclear, Boston, MA), counted to <5% error, and corrected for quench by the external standard method. The rate of penetration of ¹⁴C-labeled molecules was calculated by plotting total dpm/endothelial reservoir/cm² corneal area versus time. Regression analysis showed a linear diffusion rate over 4 hours. The rate of PE penetration was determined by multiplying the rate of measured ¹⁴C flux by the percent of ¹⁴C positively identified as PE by HPLC analysis.

In Vitro Corneal Eflux

To determine the corneal washout rate of PE, paired rabbit corneas were mounted in the perfusion block system previously described. For these studies, paired corneas had either intact or scraped epithelial surfaces. Following perfusion for three hours with PE (2.6 μCi/µmole), one cornea was removed from the perfusion system, rinsed, blotted, weighed, and solubilized in 1 ml of Protosol (New England Nuclear, Boston, MA) at 55°C. The paired cornea remained in the perfusion system; however, the bathing media was replaced with fresh GBR every 15 min. At intervals following the initial reservoir rinse, the paired cornea was removed and handled in a manner similar to its mate. Tissue digests were placed in Econofluor (New England Nuclear, Boston, MA) and the isotope quantitated by liquid scintillation counting with quench correction by the external standard method. The corneal half-life (t₁/₂) of ¹⁴C-labeled molecules was determined from plots of the dpm/mg tissue wet weight versus time. Half-lives were also calculated according to equation 1, where Cᵣ = corneal radioactivity at time = 0, and α = the rate constant of “efflux”.

\[ C_t = C_0 e^{-\alpha t} \]

In Vivo Penetration and Tissue Distribution

Phenylephrine-¹⁴C was topically applied to both eyes of 2-3-kg New Zealand white rabbits in 30-µl drops three times at 5-minute intervals as either a 0.1% (15.36 μCi/µmole) or a 2.5% (3.07 μCi/µmole) solution in phosphate-buffered saline (PBS). Both corneas of an individual rabbit were either scraped or the epithelium was left intact. Epithelial removal was achieved by scraping the cornea with a Gill knife following topical anesthesia with 0.5% proparacaine. Rabbits were euthanized by a sodium pentobarbital overdose at specific times following the final instillation of PE, and the ocular tissues were harvested and prepared for either determination of total isotope content or for HPLC analysis. At the time of harvest, eyes were proptosed, irrigated, and the excess fluid absorbed. Aqueous humor was obtained by limbal puncture with a 25-gauge needle and collected into a tuberculin syringe. The corneal and iris/ciliary body were next excised as one unit. The cornea was separated from the iris/ciliary body and each treated separately. Each tissue was rinsed for 3 seconds in GBR, blotted, and placed in preweighed vials. To obtain dry weights, the vials of tissue were frozen in methanol-dry ice and lyophilized. Samples were rehydrated and solubilized in Protosol as previously described in order to determine total isotope content.

Preparation of Samples for HPLC Analysis

Samples of GBR obtained from the perfusion block system were adjusted to a final concentration of 0.05N HClO₄, and a known amount of DHBA was added as an internal standard. Additional samples were prepared to account for solvolysis of PE in GBR by incubating known quantities of PE in GBR under the conditions of perfusion for 4 hours. No significant solvolysis was observed in GBR at pH 7.6 and 37°C during a 4-hour period.

Samples of aqueous humor from PE-treated eyes were diluted 1:1 with 0.05 N HClO₄ containing a known amount of DHBA, centrifuged for 10 minutes at 4°C, and 27,000 Xg. The supernatant was analyzed directly. Aqueous humor was also obtained from PBS-treated controls and analyzed by HPLC to detect the presence of any interfering substances. Finally, known quantities of DHBA and ¹⁴C-PE were added to aqueous humor samples from PBS-treated eyes and analyzed to insure that no change in retention time or molar response occurred when aqueous humor extracts were chromatographed.
Fig. 1. In vitro penetration rate of radioactive molecules to the endothelial side of the cornea. Initial phenylephrine concentrations in the epithelial reservoirs were A, 120 μM (N = 9) and B, 593 μM (N = 5). The equations of the lines are reported with the slope ± standard error of the slope in brackets; r² is the correlation coefficient. (●) Epithelium intact, (Δ) Epithelium scraped.

High-Performance Liquid Chromatography

Separation was achieved on a Microbondapak C-18 reversed phase column (Waters Associates, Milford, MA) with a mobile phase of 0.1 M sodium phosphate buffer:methanol (90:10) containing 5 mM 1-heptanesulfonic acid (Eastman Kodak, Rochester, NY), and 0.1 mM Na₂EDTA. The complete solvent was adjusted to pH 5.0 with concentrated phosphoric acid. Chromatography was performed on a Beckman HPLC system with model 421 controller and Beckman model 110A pumps. The conditions of chromatography were room temperature and a flow rate of 1.6 ml/min. Detection was achieved with a model LC-4A amperometric detector and model LC-17 flow cell equipped with a glassy carbon electrode run at 1.1 V (Bioanalytical Systems, Lafayette, IN). Detector responses were quantitated by peak height analysis and compared to known standards. Column eluent was collected in 0.5 ml fractions and the radioactivity quantitated by liquid scintillation counting in Aquasol as previously described.

Results

In Vitro Corneal Penetration

The penetration rate of ¹⁴C-labeled molecules through corneas with intact epithelia (84.1 ± 0.1 dpm/cm²/min) was increased about ninefold (784.0 ± 0.7 dpm/cm²/min) by removal of the corneal epithelium (Fig. 1A). An increase of the initial PE concentration in the epithelial reservoir from 120 to 593 M (fivefold) produced corresponding increases in the rates of corneal penetration (Fig. 1B). It is clear that penetration was linear during 4-hour perfusion experiments. The rate constant for the appearance of ¹⁴C-labeled molecules on the endothelial side of the cornea was 1.06 ± 0.21 × 10⁻³ hr⁻¹, with the epithelium intact, and 1.25 ± 0.08 × 10⁻² hr⁻¹ with the epithelium absent.

At the conclusion of in vitro penetration experiments, the solutions bathing both the corneal epithelium and endothelium were analyzed by HPLC. When the corneal epithelium was present, only 34% to 48% of ¹⁴C-labeled molecules present in the endothelial reservoir could be identified as PE. For perfusions in which the epithelium was removed by scraping, 89% to 95% of ¹⁴C-labeled molecules present in the endothelial side of the cornea were identified as PE. The latter was not substantially different from controls in which no corneal tissue was present during the incubation of PE in GBR. The chromatograms of PE solutions incubated in GBR indicated >90% of ¹⁴C was recovered in fractions coincident with the electrochemical peak of PE (Fig. 2A). The residual ¹⁴C (2.61 ± 2.19%) was eluted near the solvent front in
fractions 3–5. Chromatograms of the solutions bathing the epithelial surface were not significantly different from controls (Fig. 2B). Chromatograms of the solution bathing the endothelial surface clearly showed the emergence of two radioactive peaks. Fractions 3–5 at the front contained 22.8 ± 1.5% of total $^{14}$C, while fractions 6–9 contained 15.8 ± 4.9% of total counts (Fig. 2C).

The rates of accumulation of PE on the endothelial side of the cornea were calculated based on the amount of $^{14}$C label, which could be identified as PE by HPLC analysis. The corneal penetration rates when 120 $\mu$M PE was placed in the epithelial reservoirs were 5 pmole/cm$^2$/min with the epithelium intact and 120 pmole/cm$^2$/min with the epithelium removed. When 593 $\mu$M PE was added to the epithelial reservoirs, these rates increased to 24 and 609 pmole/cm$^2$/min respectively. The apparent large increase in the rate of accumulation upon removal of the corneal epithelium was apparently a result of two factors: (1) removal of the diffusion barrier and (2) removal of the tissue responsible for the metabolism of PE.

Corneal Efflux of PE

The role of the corneal epithelium as a barrier to the efflux of PE and its metabolites was emphasized in corneal washout experiments (Fig. 3). Corneas denuded of epithelium and perfused for 3 hours with 120 $\mu$M PE contained six times more PE and metabolites than corneas with intact epithelium. Replacement of the media bathing the corneal surfaces with fresh GBR containing no PE caused a rapid efflux of $^{14}$C-labeled molecules. The efflux of isotope from corneas, which had been denuded of epithelium prior to equilibration, had apparent $t_{1/2}$ of 6 min; for those with an intact epithelium, the $t_{1/2}$ was 24 min. In these studies, frequent replacement of the bathing media prevented the cornea from reaching PE equilibrium with its environment.

In Vivo Corneal Penetration and Distribution in Ocular Tissues

Following the topical instillation of 0.1% PE (three times at 5-minute intervals) into the eyes of rabbits, the amount of PE and metabolites present in the cornea 30 min after the last instillation was three times greater in corneas with the epithelium removed (Figs. 4A and B). The corneal washout rate in each case yielded a $t_{1/2}$ of approximately 1.5 hours. Under the conditions of this study, the estimated concentration of PE in the cornea 1 hour after the last instillation of 0.1% was 20.6 $\mu$M for those corneas with the epithelium intact.
Epithelium debrided
\[ T_{1/2} = 6 \text{ minutes} \]

Epithelium intact
\[ T_{1/2} = 24 \text{ minutes} \]

Fig. 3. Washout of radioactivity from corneas following an initial 3-hour in vitro corneal perfusion with 120 \( \mu \text{M} \) PE in the epithelial reservoir. (A) Epithelium intact. (B) Epithelium debrided. Brackets indicate the number of determinations for each point.

and 57.7 \( \mu \text{M} \) for those corneas with the epithelium removed by scraping.

Peak aqueous humor concentrations were achieved in less time and were 13-fold greater in those eyes with the epithelium removed, implying a greater rate of corneal penetration and corneal efflux than in corneas with intact epithelia (Figs. 4A and B). HPLC analysis of the aqueous humor indicated that metabolites of PE, as well as PE itself, were present in the anterior chamber (Figs. 5A and B). The distribution of radioactivity in the chromatograms of aqueous humor were similar to those from the in vitro system in that the retention time of the metabolites was the same. PE was metabolized to the greatest extent when the corneal epithelium was present. One hour after the instillation of the last drop of 0.1%, the concentration of PE in the anterior chamber was 0.8 \( \mu \text{M} \) when the epithelium was present and 8.2 \( \mu \text{M} \) when the epithelium was absent. When 2.5% PE was instilled into the eyes of rabbits, the concentration in the anterior chamber was 20.2 \( \mu \text{M} \) when the epithelium was intact, and 347.0 \( \mu \text{M} \) when the epithelium was denuded. The instillation of 2.5% resulted in a lower percentage of drug metabolism than when 0.1% solutions were instilled. The values reported here for aqueous humor drug levels were calculated based on the amount of PE actually accounted for in HPLC analyses following topical instillation.

The iris/ciliary body reached peak concentrations one hour after instillation of the last drop whether the epithelium was present or not. The amount of \(^{14}\text{C}\)-labeled drug present in irides from eyes of animals with intact epithelium was 4.9 \( \mu \text{mole/mg wet weight} \), while those from animals denuded of corneal epithelium was 31.19 \( \mu \text{mole/mg wet weight} \).

**Discussion**

These studies clearly indicate that the corneal epithelium is a barrier to the penetration and flux of PE, both in and out of the cornea. Removal of the epithelium resulted in a tenfold increase in the rate constant of the penetration of PE and its metabolites through the cornea during in vitro perfusions. This observation is consistent with the fact that PE has low lipid solubility and exists as a weak base at physiologic pH. The low lipid solubility accounts for the observation that the epithelium is a barrier, while the ionic character of PE at the pH's below its pKa's accounts for its rapid diffusion through the stroma. The corneal washout studies emphasize the fact that the epithelium serves as a barrier to the efflux of PE.

The corneal epithelium appears not only to be a barrier to the penetration and efflux of PE but it also contributes to the metabolic degradation of PE. In the in vitro perfusion system, which is a closed system, the removal of the epithelium appeared to eliminate the agent responsible for metabolism. In vivo, the removal of the corneal epithelium greatly reduced the metabolism of PE but did not eliminate it, suggesting that other ocular tissue may contribute to PE metabolism. When 0.1% PE was topically applied (\( \times 3 \)), 56% of total \(^{14}\text{C}\) in the aqueous humor was identified by HPLC as PE when the epithelium was intact as compared with 67% when the epithelium was scraped. Topical application of 2.5% (\( \times 3 \)) increased these percentages to 70% and 95%, respectively. The metabolites have not yet been positively identified, but they appear to be the same both in vivo and in vitro. PE is a substrate for monoamine oxidase (MAO), an enzyme known to be present in the corneal epithelium.
Fig. 4. Absorption and elimination of radioactivity from rabbit eyes after topical application of 0.1% 14C-PE. Cornea (●–●), Aqueous humor (●), Iris/ciliary body (●••). Each point is the mean ± standard error of the mean for four or five determinations.

Fig. 5. High-pressure liquid chromatograms of aqueous humor samples 1 hour after topical treatment with 0.1% 14C-PE in PBS. Chromatographic conditions are as stated in Materials and Methods. Radioactive counts in eluent fractions are superimposed (——) on chromatograms (—). A, epithelium intact. B, epithelium scraped. 1 = unidentified peaks. DHBA = dihydroxybenzylamine·HBr; PE = phenylephrine·HCl.
however, we have not yet established whether MAO is responsible for the metabolism seen in these studies.

Topical instillation of 0.1% (×3) PE into the eyes of rabbits from which the epithelium was removed resulted in a 3.5-fold greater corneal concentration of PE and its metabolites 1 hour following the final instillation. At peak concentrations, the aqueous and irides of eyes with denuded epithelia contained 13-fold and sixfold more PE and metabolites than those tissues from eyes with intact epithelia.

Earlier studies in our laboratory established that topical instillation of 2.5% PE (three 30-μl instillations at 5-minute intervals) into rabbit eyes resulted in damage to the corneal endothelium when the epithelium was denuded prior to topical application. The present study enabled us to estimate the aqueous humor PE concentrations achieved following such treatment. As noted earlier, these concentrations were 25 μM with the epithelium intact and 340 μM when the epithelium was scraped. These studies together allowed a comparison of endothelial ultrastructural changes in vivo with an actual PE concentration in the aqueous humor. This was previously achieved in a cell culture study in which the observation was made that bovine corneal endothelial damage was not significant at a PE concentration in the culture medium of 0.001% (49 nM), while 0.01% PE (490 nM) was, indeed, toxic to the endothelium.

The data reported here not only establish that the corneal epithelium is a barrier to the diffusion of PE and its metabolites but also indicate that the epithelium contributes to the metabolic degradation of PE. When low concentrations (<0.1%) are instilled into eyes, metabolism can significantly reduce intraocular PE concentrations.

Key words: phenylephrine, edema, HPLC, ocular drug absorption, corneal penetration, metabolism

References