Ocular Absorption of Pz-Peptide and Its Effect on the Ocular and Systemic Pharmacokinetics of Topically Applied Drugs in the Rabbit

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Purpose. To determine the corneal and conjunctival penetration of 4-phenylazobenzyloxycarbonyl-L-Pro-L-Leu-Gly-L-Pro-D-Arg (Pz-peptide) and to evaluate its effect on the corneal and conjunctival penetration of hydrophilic solutes as well as on the ocular and systemic absorption of topically applied atenolol and propranolol in the rabbit. The hydrophilic solutes were mannitol, fluorescein, FITC-dextran 4,000, and FITC-dextran 10,000.

Methods. Drug penetration across the rabbit cornea and conjunctiva was evaluated using the modified Ussing chamber. Ocular and systemic absorption of topically applied atenolol and propranolol was evaluated by analyzing the drug concentration in various anterior segment tissues at 45 min and in the blood over 240 min, respectively, following topical instillation of 25 μl of 20 mM atenolol or propranolol solution to the rabbit eye.

Results. The conjunctiva was 29 times more permeable than the cornea to 3 mM Pz-peptide. Conjunctival Pz-peptide transport was 1.7 times more extensive in the mucosal-to-serosal than in the opposite direction, whereas corneal Pz-peptide transport showed no directionality. The apparent permeability coefficient of Pz-peptide across the cornea and the conjunctiva increased over the 1–5 mM range, suggesting that Pz-peptide enhanced its own transport across both epithelial tissues. The cornea appeared to be more sensitive than the conjunctiva to the penetration enhancement effect of Pz-peptide. Thus, whereas Pz-peptide elevated the corneal transport of mannitol, fluorescein, and FD4 by 50%, 57%, and 106%, respectively, it did not affect the conjunctival transport of mannitol and fluorescein, while enhancing FD4 transport by only 46%. Moreover, while Pz-peptide enhanced the ocular absorption of topically applied hydrophilic atenolol, it did not affect the ocular absorption of lipophilic propranolol. Interestingly, Pz-peptide did not affect the systemic absorption of either beta adrenergic antagonist.

Conclusions. Pz-peptide appears to facilitate its own penetration across the cornea and the conjunctiva. Pz-peptide appears to increase the ocular absorption of topically applied hydrophilic but not lipophilic drugs, while not affecting the systemic absorption of either type of drugs.

KEY WORDS: Pz-peptide; paracellular transport; cornea; conjunctiva; hydrophilic solutes.

INTRODUCTION

The relative impermeability of the corneal epithelium to polar drugs is well known. Approaches based on formulation changes, prodrug derivatization, and penetration enhancers have been found to afford varying degrees of enhancement in corneal drug permeability (1–3). In recent years, conjunctival drug penetration has also gained attention (4–8), possibly because topically applied drugs will come into contact with not only the cornea but also the conjunctiva. Compared with the cornea, the conjunctiva is 2–30 times more permeable to drugs of varying physicochemical characteristics (4). As such, it is less sensitive to formulation changes (4) and prodrug derivatization (5).

4-Phenylazobenzoxycarbonyl-Pro-Leu-Gly-Pro-D-Arg (Pz-peptide) is a hydrophilic (log PC = −0.88), collagenase-labile pentapeptide with a molecular weight of 777 daltons that appears to opt for the paracellular pathway for transport across rabbit intestinal segments and Caco-2 cell monolayers (9,10). This peptide facilitates its own transport and that of paracellular markers up to 4,000–5,000 daltons by triggering opening of tight junctions in a transient, reversible manner (9). Pz-peptide was found to stimulate transepithelial Na^+ flux across the colonic segments at the level of the amiloride-sensitive Na^+ channel, thereby triggering intracellular biochemical changes that ultimately resulted in tight junctional opening and enhanced paracellular solute transport (11).

The present study was conducted to determine whether the corneal and conjunctival penetration of Pz-peptide behaved similarly as its intestinal penetration. Thus, the objectives of this study were: (a) to determine the relative permeability of the cornea and the conjunctiva to Pz-peptide, (b) to determine whether Pz-peptide enhanced its own transport as well as that of polar solutes across the cornea and the conjunctiva, and (c) to evaluate the effect of Pz-peptide on the ocular and systemic absorption of topically applied hydrophilic atenolol and lipophilic propranolol in the rabbit. The polar solutes were mannitol (M.W. 182, molecular radius (m.r.) 3.6 Å), atenolol (M.W. 266, m.r. 3.8 Å), fluorescein (M.W. 376, m.r. 5.5 Å), FITC-dextran 4,000 (FD4; M.W. 4,000, m.r. 14 Å) and FITC-dextran 10,000 (FD10; M.W. 10,000, m.r. 22 Å). For comparison, EDTA and cytochalasin B, two known paracellular penetration enhancers, were also evaluated. EDTA is known to increase tight junctional permeability by chelating extracellular Ca^{2+} (12), whereas cytochalasin B is known to do so by inhibiting polymerization of actin filaments (13).

MATERIALS AND METHODS

Materials

Male, Dutch-belted pigmented rabbits, 1.8–2.2 kg, were purchased from Irish Farm Rabbitry (Los Angeles, CA). The investigations utilizing rabbits described in this report conformed to the Principles of Laboratory Animal Care (NIH Publication #85-23, revised 1985).

4-Phenylazobenzyloxycarbonyl-L-Pro-L-Leu-Gly-L-Pro-D-Arg (Pz-peptide) and its hydrolytic dipeptide product, 4-phenylazobenzyloxycarbonyl-L-Pro-L-Leu (Pz-product) were purchased from Sigma (St. Louis, MO) and Bachem (Philadelphia, PA), respectively. ^{3}H-Mannitol (specific activity, 26 mCi/nmol) was purchased from New England Nuclear (Boston, MA). Mannitol, atenolol, propranolol, ethylenediaminetetraacetic acid (EDTA), cytochalasin B, fluorescein, FITC-dextran

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4,000 and FITC-dextran 10,000, amiloride, hexamethylene amiloride, and ouabain were obtained from Sigma (St. Louis, MO). All other reagents were of either analytical or HPLC grade.

HPLC Assay of Pz-peptide, Pz-product, Atenolol, and Propranolol

Pz-peptide and Pz-product were assayed by reverse phase HPLC on a Beckman ODS C_{18} column (Beckman Instruments, Fullerton, CA) that was interfaced with a Shimadzu HPLC system. This system consisted of a model LC-6A pump, an autoinjector model SIL-6A, an UV-VIS detector, and a Chromatopac model C-R3A data station (Shimadzu Instruments, Kyoto, Japan). The mobile phase was a mixture of acetonitrile and 0.1% phosphoric acid in doubly deionized water (pH 3.0). The flow rate was 1 ml/min. The column was first equilibrated with 40% acetonitrile for 4 min, followed by a linear increase of acetonitrile to 60% for the next 5 min and holding it at 60% for the final 10 min. Thereafter, the column was reequilibrated with 40% acetonitrile for 5 min before the next injection. Pz-peptide in the eluate was monitered spectrophotometrically at 318 nm. The retention times of propranolol (internal standard), Pz-peptide and Pz-product were 5.4 ± 0.6 min, 8.6 ± 0.7 min, and 13.4 ± 0.5 min, respectively.

For atenolol, the mobile phase was 10% acetonitrile in 90% of 0.2% w/v triethylamine (pH 3.0). It was monitored fluorometrically at an excitation wavelength (λ_{ex}) of 225 nm and an emission wavelength (λ_{em}) of 300 nm. The retention time was 4 ± 0.3 min for atenolol and 7 ± 0.4 min for bamethane (internal standard). Propranolol was also assayed on reversed phase HPLC using 50% acetonitrile in 50% of 10 mM phosphate buffer (pH 3.0) as the mobile phase and was monitored fluorometrically (λ_{ex}: 295 nm, λ_{em}: 360 nm). The retention time was 4 ± 0.3 min for labetalol (internal standard) and 7 ± 0.5 min for propranolol.

Corneal and Conjunctival Penetration of Pz-Peptide and Pz-Product

Rabbit cornea and conjunctiva were excised and mounted in modified Ussing chambers as described by Ashton *et al.* (4). Two and one-half ml of glutathione-bicarbonate Ringer's solution (GBR) (14), preadjusted to pH 7.4 and 300 ± 30 mOsm/kg, were added to either the mucosal or the serosal side, depending on the direction of transport to be tested. An equal volume of the same solution containing Pz-peptide or Pz-product (1–5 mM) was then added to the opposite (receiver) side. The contents of each chamber were mixed by bubbling a 95% O_2-5% CO_2 mixture at the rate of three to four bubbles per second, and the temperature within each chamber was maintained at 37°C by a circulating water bath. Periodically up to 240 min, a 100 μl aliquot was taken from the receiver side for analysis and was replaced immediately by an equal volume of GBR solution.

The apparent permeability coefficient (Papp, cm/sec) was calculated from the following equation: Papp = flux / (A × C_0 × 60), where flux (nmol/min) is the slope of the linear portion of a plot of amount of drug accumulated vs. time, A is the surface area of the cornea (1.089 cm^2) or conjunctiva (0.95 cm^2), C_0 is the initial drug concentration in the donor compartment (nmol/ml), and 60 is the factor for conversion from minutes to seconds.

Corneal and Conjunctival Penetration of Polar Solutes

Essentially the same procedure as for Pz-peptide and Pz-product was used. The dosing solution was 0.5 Ci/ml of ^{3}H-mannitol; or 0.5 mg/ml of fluorescein, FD4, FD10, atenolol, or propranolol, with or without 3 mM of enhancers (Pz-peptide, EDTA, and cytochalasin B). ^{3}H-Mannitol was assayed in a liquid scintillation counter (Beckman LS1801, Fullerton, CA) after mixing with 5 ml of liquid scintillation cocktail (Ecoscint, National Diagnostics, Manville, NJ). Fluorescein, FD4, and FD10 were measured in a fluorescence spectrophotometer (Perkin-Elmer, 10S Fluorescence Spectrophotometer, Norwalk, CT) at λ_{ex} of 490 nm and λ_{em} of 530 nm.

Ocular Absorption of Topically Applied Atenolol and Propranolol

Twenty-five microliters of a dosing solution (20 mM atenolol or propranolol, with or without 3 mM enhancers—Pz-peptide, EDTA and cytochalasin B) were instilled into both eyes of each unanesthetized rabbit. Dosing solutions were prepared in a 10 mM Tris HCl buffer and adjusted to pH 7.4 with an osmolality of 300 ± 20 mOsm/kg. At 45 min after dosing, the rabbit was euthanized with an overdose of a sodium pentobarbital solution (Eutha-6, Western Medical Supply, Arcadia, CA) administered via a marginal vein. Aqueous humor was collected and anterior segment tissues were excised as previously described (15). All excised tissues were rinsed with ice-cold KCl solution, blotted dry, transferred to preweighed microcontrifuge tubes. After being soaked in 200 μl of 0.6% $HClO_4$ at 4°C for at least 12 hr, the mixture was extracted as described below for plasma for assay of atenolol and propranolol.

Systemic Absorption of Topically Applied Atenolol and Propranolol

Fifteen minutes before solution instillation, each unanesthetized rabbit was cannulated in a central ear artery with a polyethylene tubing (PE-50, Intramedic) and was heparinized with 100 μl of 1,000 U/ml of Na heparin (Western Medical Supply, Arcadia, CA). Thereafter, 25 μl of a dosing solution were instilled into both eyes of each rabbit. At designated times, 2.5 ml of blood samples were collected into heparinized tubes, centrifuged at 1,500 × g for 10 min to yield 1 ml of plasma, and stored at −20°C until assayed within one week. An i.v. bolus experiment using the same amount of drug was conducted to yield the control data for calculation of systemic bioavailability following ocular administration.

For the assay of atenolol, 0.6 ml of plasma was mixed with 100 μl of bamethane solution (0.1 mg/ml) and 1.2 ml of NaOH for adjustment of pH to 12.3. The mixtures were then extracted with CH_2Cl_2 and butanol (95:5%) for 15 min, and centrifuged at 1,500 × g for 15 min. Seven ml of the supernatant was transferred to a conical tube. After evaporating off the organic layer with N_2 gas, the residue was dissolved in 100 μl of mobile phase, and 50 μl of the resulting solution was injected into the HPLC. In the case of propranolol, 0.2 ml plasma was mixed with 100 μl of labetalol solution (10 μg/ml) and 400 μl of carbonate buffer (pH 9.8), vortexed with 3 ml ether for 15 min, and centrifuged at 1,500 × g for 15 min. Two and half ml of the supernatant was concentrated and injected into the HPLC.

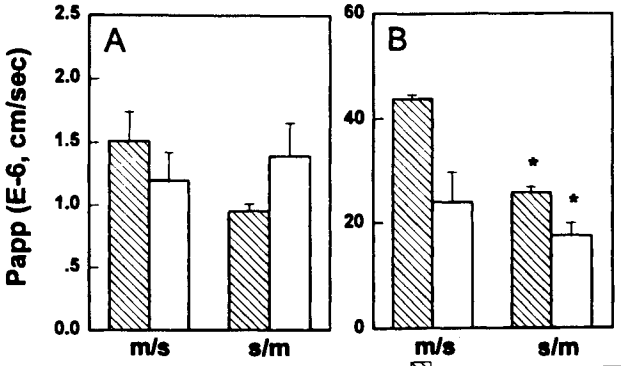

Fig. 1. Directionalilty of 3 mM Pz-peptide (▨) and Pz-product (□) transport across the cornea (A) and the conjunctiva (B) in the rabbit. Each value represents mean ± S.E. of four different experiment. Key: m/s, mucosal-to-serosal direction; s/m, serosal-to-mucosal direction. *Significantly different from the m/s direction ($P < 0.05$).

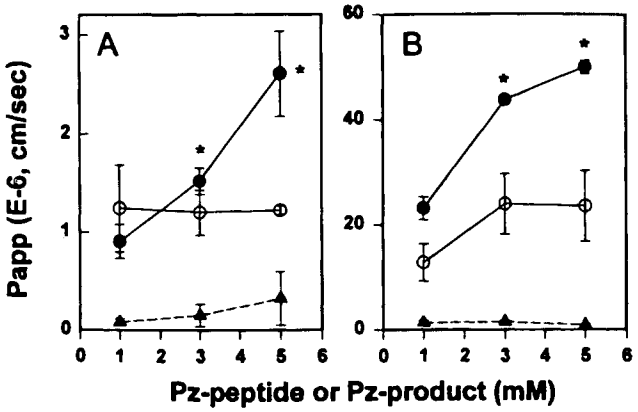

Fig. 2. Concentration dependency of Pz-peptide (●) and Pz-product (○) penetration across the cornea (A) and the conjunctiva (B) in the rabbit. Pz-Pro-Leu (Pz-product) contribution (▲) formed from Pz-peptide was less than 5% in the conjunctiva and less than 10% in the cornea. Each point represents mean ± S.E. of four different experiments. *Significantly different from 1 mM ($P < 0.05$).

The drug concentrations versus time data for topical solution instillation were fitted to a one-compartment pharmacokinetic model using SIPHAR (PC/MS-DOS, Version 4.0-May 1991, Simed, Creteil Cedex, France), and the following pharmacokinetic parameters were obtained: peak concentration (C_{max}), peak time (t_{max}), and apparent absorption rate constant (k_a). In addition, the area under the plasma concentration-time curve (AUC) from time zero to infinity for both topical solution instillation and i.v. administration was calculated by the trapezoidal rule-extrapolation method. Bioavailability (BA) was calculated from the ratio of AUC's between ocular instillation and intravenous bolus administration and expressed as a percentage.

Statistical Analysis

Comparison between two means was performed using the unpaired Student's t-test. One-way analysis of variance was used to test for significant difference between groups. Statistical significance was defined as $p < 0.05$.

RESULTS

Pz-Peptide and Pz-Product Penetration Across the Cornea and the Conjunctiva

The Papp for both Pz-peptide and Pz-product at 3 mM was about 29 times higher in the conjunctiva than the cornea ($p < 0.05$) (Fig. 1). In the conjunctiva, peptide penetration from the mucosal to the serosal side (m/s) was 1.7 times larger than that in the opposite direction ($p < 0.05$). In the cornea, there was no directionality in transport ($p > 0.05$). Pz-peptide was 1.5–1.9 times more permeable than Pz-product in the conjunctiva in the both directions ($p < 0.05$). By contrast, no significant difference in the transport of Pz-peptide and Pz-product was observed in the cornea ($p > 0.05$).

The Papp of Pz-peptide in the cornea and the conjunctiva increased with concentration over the 1–5 mM range (Fig. 2), there being a 3- and 2-fold difference, respectively. By contrast, the Papp of Pz-product was concentration-independent in both epithelia. The percentage of Pz-product contributing as the proteolytic metabolite to the total flux of Pz-peptide was less than 10% in the cornea and less than 5% in the conjunctiva.

As shown in Figure 3, Pz-peptide penetration was not affected by the mucosal addition of either 10 μM amiloride (a Na^+ channel blocker) or 10 μM hexamethylene amiloride (a Na^+/H^+ exchange blocker), by the serosal addition of 100 μM ouabain (a Na^+/K^+ ATPase inhibitor), or by the mucosal replacement of Na^+ with choline chloride in the mucosal buffer ($P > 0.05$ by one-way ANOVA).

Effect of Pz-Peptide on the Corneal and Conjunctival Penetration of Atenolol and Propranolol

Over the 1–5 mM concentration range, Pz-peptide increased the Papp values of atenolol and propranolol in both

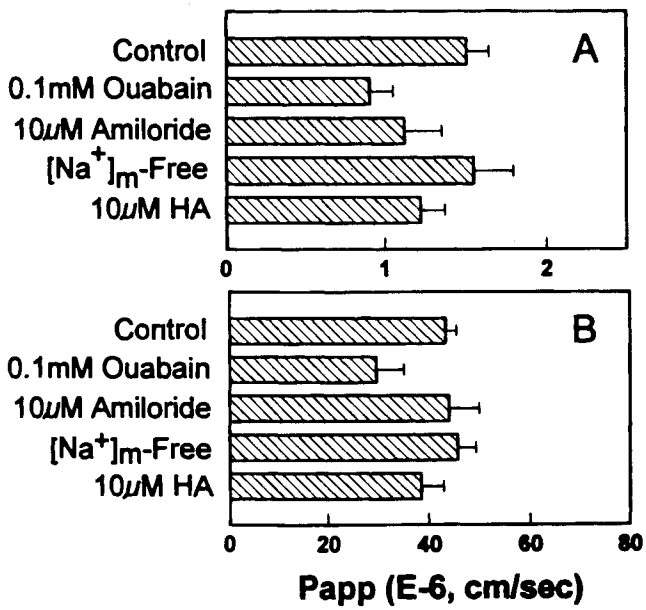

Fig. 3. Effect of perturbation of transepithelial Na^+ transport on the corneal (A) and conjunctival (B) penetration of 3 mM Pz-peptide. Key: (a) control; (b) 100 μM ouabain on the serosal side; (c) 10 μM amiloride on the mucosal side; (d) mucosal Na^+-free solution; (e) 10 μM hexamethylene amiloride on the mucosal side. Each value represents mean ± S.E. of four experiments.

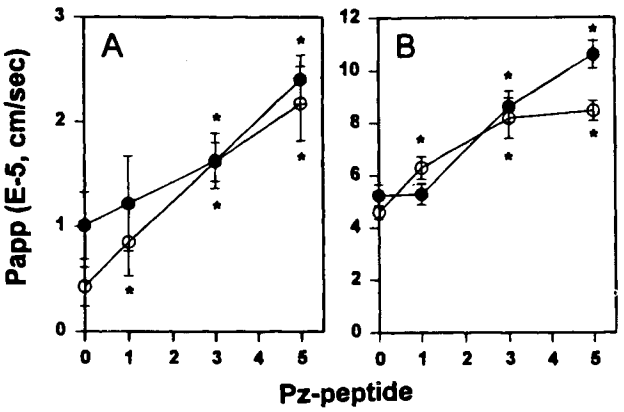

Fig. 4. Effect of 1–5 mM Pz-peptide on the corneal (A) and conjunctival (B) penetration of atenolol (○) and propranolol (●). Each point represents mean ± S.E. of four different experiments. *Significantly different from control (0 mM) ($P < 0.05$).

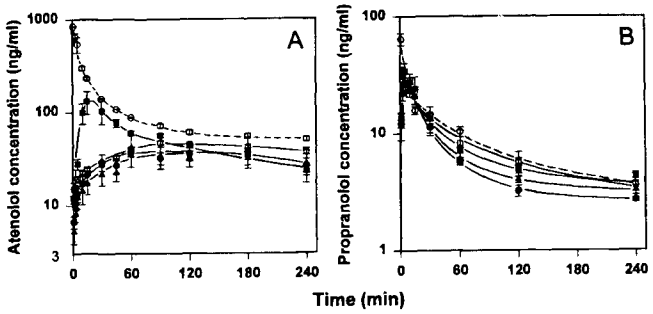

Fig. 6. Effect of 3 mM enhancers on the systemic absorption of atenolol (A) and propranolol (B) following topical instillation of 25 μl of 20 mM drug solution into both eyes of the rabbit. Key: (●), control, (▲), with 3 mM Pz-peptide; (■), with 3 mM EDTA; and (□), 3 mM cytochalasin B; and (○), i.v. bolus administration. Each point represents mean ± S.E. (n = 3).

the cornea and the conjunctiva, more so in the cornea (2.4–5.1 times) than the conjunctiva (1.8–2.0 times) (Fig. 4).

Effect of Pz-Peptide on the Corneal and Conjunctival Penetration of Polar Solutes

Pz-peptide at 3 mM elevated the corneal transport of mannitol by 50%, fluorescein by 57%, and FD4 by 106% (Fig. 5A). However, no enhancement was seen in FD10. In the conjunctiva (Fig. 5B), a 46% and 39% increase was seen, respectively, in the Papp of FD4 and FD10. However, no increase was observed for mannitol and fluorescein. By contrast, both EDTA and cytochalasin B generally increased polar solute transport to a greater degree than Pz-peptide, especially in the case of FD10 (Fig. 5).

Effect of Pz-Peptide on the Ocular and Systemic Absorption of Topically Applied Atenolol and Propranolol

Neither the ocular (Table II) nor the systemic absorption (Fig. 6B, Table I) of topically applied propranolol was affected by the addition of Pz-peptide, EDTA or cytochalasin B. The exception was the increase in propranolol concentration in the iris-ciliary body by 3 mM EDTA (Table II). Unlike 3 mM EDTA, neither Pz-peptide nor cytochalasin B altered the systemic bioavailability of topically applied atenolol (Fig. 6A, Table I). Specifically, EDTA increased the absorption rate constant (k_a), C_{max}, and AUC values of atenolol, corresponding to a 49% increase in systemic bioavailability. Interestingly, Pz-peptide was as effective as EDTA and cytochalasin B in enhancing the ocular absorption of topically applied atenolol (Table II).

DISCUSSION

A significant finding in this study is that Pz-peptide (log PC = −0.88), a paracellularly transported pentapeptide in the intestine (9,10), is able to penetrate the cornea and the conjunctiva at an efficiency of 15% and 83% that of propranolol (log PC = 3.21), respectively. As is the case in the intestine, its transport pathway is probably also paracellular. Such a possibility is supported by (a) the less than 10% of Pz-peptide being in the metabolite form following corneal and conjunctival transport (Fig. 2), and (b) enhancement in the transport of polar solutes up to approximately 4,000 daltons in the cornea and

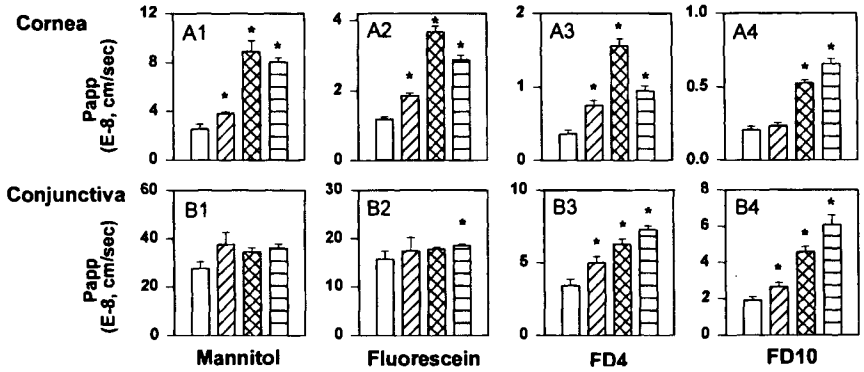

Fig. 5. Effect of 3 mM enhancers on the transport of mannitol, fluorescein, FITC-dextran 4,000 (FD4) and FITC-dextran 10,000 (FD10) across the cornea (A) and the conjunctiva (B). Key: (□) control; (▨) with Pz-peptide; (▩) with EDTA; (▤) with cytochalasin B. The initial concentration of all polar solutes was 0.5 mg/ml. Each value represents mean ± S.E. of four different experiments. *Significantly different from the control ($P < 0.05$).

Table I. Plasma Pharmacokinetic Parameters of Atenolol and Propranolol Following Topical Ocular Administration[a]

Drug	Parameters	Control	+Pz-Peptide	+EDTA	+Cytochalasin B
Atenolol	C_{max}(ng/ml)	38.6 (4.8)	45.8 (13.5)	136 (34.6)[c]	49.7 (0.73)
	t_{max}(min)	80.0 (16.3)	80.0 (16.3)	25.0 (8.16)[c]	120 (24.5)
	k_a (min^{-1})	0.023 (0.004)	0.024 (0.010)	0.104 (0.026)[c]	0.070 (0.024)
	AUC (μg min/ml)	12.2 (0.3)	14.0 (3.5)	18.2 (0.6)[c]	16.0 (1.8)
	BA (%)[†]	26.7 (0.6)	30.6 (7.7)	39.7 (1.2)[c]	34.9 (4.0)
Propranolol	C_{max}(ng/ml)	35.7 (1.7)	30.5 (1.9)	30.8 (1.7)	34.8 (1.5)
	t_{max}(min)	7.7 (3.0)	3.7 (0.5)	6.0 (1.7)	4.3 (0.5)
	k_a (min^{-1})	0.32 (0.11)	0.59 (0.13)	0.48 (0.17)	0.56 (0.11)
	AUC (μg min/ml)	2.28 (0.05)	2.69 (0.21)	2.68 (0.21)	2.85 (0.44)
	BA (%)[b]	75.5 (1.8)	89.1 (7.0)	88.6 (7.1)	94.4 (14.4)

[a] Atenolol and propranolol were administrated at a dose of 0.4 μmole/kg rabbit. The instilled concentration of Pz-peptide, EDTA and cytochalasin B was 3 mM.
[b] Bioavailability (BA, %) = $AUC_{ocular}/AUC_{iv} \times 100$. The AUC_{iv} of atenolol and propranolol was 45.8 (0.57) and 3.02 (0.35) μg min/ml, respectively.
[c] Significantly different from the control of ocular administration ($P < 0.05$).

10,000 daltons in the conjunctiva (Fig. 5). Indeed, Pz-peptide enhances its own transport across the cornea 2.9 times and the conjunctiva 2.2 times over the 1–5 mM concentration range. This contrasts with the 1.5-2.0-fold increase in the intestine (11). Compared with EDTA and cytochalasin B of equimolar concentration, 3 mM Pz-peptide is 1.5–5.0 times less effective in enhancing the corneal penetration of mannitol, fluorescein, and FD4 (Fig. 4) and is incapable of altering corneal FD10 penetration. Although 3 mM Pz-peptide is capable of enhancing the conjunctival penetration of FD4 and FD10, it is 1.8–5.6 times less effective when compared with EDTA and cytochalasin B (Fig. 4). This generally lower potency of Pz-peptide as an enhancer in the cornea and the conjunctiva relative to EDTA and cytochalasin B is similar to the situation in the intestine (11). The more pronounced effect of EDTA and cytochalasin B on polar solute transport, especially towards FD4 and FD10 (Fig. 5), is probably due to its additional effect on membrane integrity (16,17). Such a milder and more size-discriminant action of Pz-peptide on paracellular permeability may be advantagous from the safety point of view, should Pz-peptide be used as an ocular paracellular penetration enhancer with further testing.

The *in vitro* penetration enhancement effect of Pz-peptide on propranolol is unexpected (Fig. 4), given that this lipophilic beta adrenergic antagonist most probably undergoes transcellular transport and that, as such, its transport should not be enhanced by the increase in paracellular permeability elicited by Pz-peptide. Because propranolol is a possible substrate for the gp170 drug efflux pump in the rat liver canalicular membrane (18), it is conceivable that Pz-peptide may indirectly inhibit this drug efflux pump, thereby enhancing propranolol transport. Recently, the gp170 drug efflux pump has been reported to be present in the rabbit conjunctiva epithelial cells (19).

The penetration enhancement effect of Pz-peptide on corneal and conjunctival transport observed *in vivo* (Fig. 6 and Table II) is less pronounced than that which occurred *in vitro* (Fig. 4). This may be attributed to the dilution of the topically applied dose through mixing with the resident tears and through binding of Pz-peptide to mucin and other tear proteins. Thus,

Table II. Effect of 3 mM Enhancers on the Ocular Tissue Concentration of Propranolol and Atenolol at 45 min Following Topical Instillation of 25 μl of 20 mM Drug Solution into Both Eyes of the Rabbit[a]

Drug	Tissues	Control	+Pz-Peptide	+EDTA	+Cytochalasin B
Atenolol	Corneal epithelium	5.87 (0.69)	35.0 (5.36)[b]	44.7 (13.5)[b]	35.2 (7.33)[b]
	Corneal stroma	3.85 (1.92)	4.76 (0.66)	7.61 (0.28)[b]	8.02 (0.98)[b]
	Aqueous humor	0.16 (0.051)	0.51 (0.15)[b]	0.44 (0.029)[b]	0.48 (0.091)[b]
	Lens	0.24 (0.18)	0.36 (0.032)	0.48 (0.17)	0.36 (0.048)
	Iris-ciliary body	0.40 (0.17)	0.63 (0.091)	0.81 (0.21)	0.55 (0.10)
	Conjunctiva	7.53 (1.08)	15.7 (3.03)[b]	16.3 (2.24)[b]	17.7 (2.57)[b]
	Sclera	2.78 (0.82)	2.64 (0.48)	2.35 (0.25)	3.59 (0.73)
Propranolol	Corneal epithelium	162 (25.3)	261 (70.1)	209 (44.4)	191 (43.9)
	Corneal stroma	18.2 (5.79)	18.6 (3.86)	18.3 (2.85)	24.7 (1.23)
	Aqueous humor	0.97 (0.031)	1.02 (0.14)	0.77 (0.028)	1.17 (0.18)
	Lens	0.22 (0.020)	0.16 (0.011)	0.21 (0.001)	0.26 (0.042)
	Iris-ciliary body	3.37 (0.77)	4.26 (0.73)	5.75 (0.35)[b]	6.85 (0.87)[b]
	Conjunctiva	10.8 (1.63)	13.9 (2.70)	16.9 (2.61)	17.3 (6.22)
	Sclera	4.16 (1.52)	6.63 (3.31)	5.55 (0.58)	7.06 (1.35)

[a] Each value (μg/g tissue) represents mean ± S.E. ($n = 4$).
[b] Significantly different from the control of ocular administration ($P < 0.05$).

the effective concentration of Pz-peptide in tears following topical solution instillation may be closer to 1 mM than 3 mM. As can be seen in Fig. 4, at 1 mM, whereas neither the corneal nor the conjunctival penetration of propranolol is affected by Pz-peptide, atenolol penetration across both epithelia is enhanced 1.4–2.0 times. Herein lies a possible explanation for the lack of effect of Pz-peptide on the ocular and systemic absorption of topically applied propranolol. That systemic atenolol concentration is not affected by 3 mM Pz-peptide is consistent with the relatively minor contribution of the conjunctival pathway to systemic absorption of topically applied atenolol (20). This finding also suggests that the nasal mucosa, that contributes 83% to the systemic absorption of topically applied atenolol (21), may be even less sensitive than the conjunctiva to the penetration enhancement effect of Pz-peptide.

The mechanism by which Pz-peptide increases paracellular permeability in the cornea and the conjunctiva requires further study. Stimulation of transepithelial Na^+ transport through either Na^+/H^+ exchanger or the amiloride-sensitive Na^+ channel, an element in Pz-peptide's paracellular enhancement mechanism in the intestine (13), does not appear to be involved. This is because none of the conditions designed to perturb transepithelial Na^+ movement exerted an effect on Pz-peptide transport (Fig. 3). This finding should not be surprising, since the amiloride-sensitive Na^+ channel is not known to exist in either the cornea or the conjunctiva (22). It remains to be seen whether the lack of such a mechanism in the cornea and the conjunctiva is responsible for the 2-fold difference in transport between the m/s and the s/m direction in the conjunctiva and for the lack of directionality in transport in the cornea, as compared with the 25-fold difference in the intestine (11). Nevertheless, we cannot rule out the involvement of other ion transport processes on the tear side of the cornea and the conjunctiva that may be affected by Pz-peptide.

In summary, Pz-peptide appears to facilitate its own transport and that of polar solutes on the order of 4,000 daltons across the rabbit cornea and conjunctiva by a mechanism that is different from that in the intestine. The attractiveness of Pz-peptide as a possible penetration enhancer for topically applied hydrophilic drugs is its preferential effect on ocular absorption, as the results with topically applied atenolol seem to suggest.

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