Different Effects of Absorption Promoters on Corneal and Conjunctival Penetration of Ophthalmic Beta-Blockers

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Received August 8, 1994; accepted March 30, 1995

Purpose. The purpose of this study was to investigate the improvement in corneal penetration of ophthalmic beta-blockers of various lipophilicities afforded by absorption promoters and to compare the corneal against conjunctival penetration in response to absorption promoters. ***Methods.*** The penetration of the beta-blockers, atenolol, carteolol, tilisolol, timolol, and befunolol, in the presence of absorption promoters, across the isolated corneal and conjunctival membranes of albino rabbits was measured using a two-chamber glass diffusion cell. EDTA, taurocholic acid, capric acid, and saponin were used as the absorption promoters. ***Results.*** The absorption promoters significantly increased the corneal permeability of most beta-blockers, especially the hydrophilic agents. The absorption promoters also enhanced the conjunctival permeability of beta-blockers, although their effect in promoting conjunctival penetration was less than that on corneal penetration. There was a differing penetration of instilled beta-blockers in the cornea and conjunctiva in response to absorption promoters. Capric acid and saponin showed significant promoting action on corneal penetration, but not on conjunctival penetration. Taurocholic acid had a significant effect on conjunctival penetration but not on corneal penetration. Saponin caused slight irritation. ***Conclusions.*** Absorption promoters can improve the ocular delivery of beta-blockers and a selective use of absorption promoter can improve the extent and pathway of drug ocular absorption.

KEY WORDS: beta-blocker; drug delivery system; ocular penetration; absorption promoter; eye.

INTRODUCTION

In recent years, certain beta-blockers that decrease aqueous humor formation by the ciliary processes have been commonly used for the treatment of glaucoma and are very often indispensable in this treatment (1). However, the usefulness of the instilled beta-blockers is often limited, due to their systemic absorption and subsequent induction of cardiovascular and respiratory side effects (1). Polymer vehicles, liposomes, nanoparticles, and polymeric inserts have been used successfully to prevent drainage of the drugs from the precorneal area and to improve the ocular delivery (2,3). Another approach is to increase the corneal penetration of the drug by the use of transient derivatives (prodrugs) or by the use of absorption promoters that exhibit little toxicity (2,4).

The use of absorption promoters seems to be advantageous in the use of ophthalmic preparations of most drugs. Further, the different responses of corneal and conjunctival barriers to absorption promoters may be useful for controlling the extent and the pathway of the ocular and systemic absorption of drugs instilled into the eye. Determination of the *in vitro* corneal and conjunctival permeability has been reported to be useful for predicting the *in vivo* ocular and systemic absorption of beta-blockers (5).

The purpose of this study was to investigate the improvement in corneal penetration of ophthalmic beta-blockers of various lipophilicities afforded by absorption promoters and to compare the corneal against conjunctival penetration in response to absorption promoters.

MATERIALS AND METHODS

Chemicals

Tilisolol hydrochloride, befunolol, and carteolol were supplied by Nisshin Flour Milling Co., Ltd. (Tokyo, Japan), Kaken Pharmaceutical Co., Ltd. (Tokyo, Japan), and Otsuka Pharmaceuticals Co., Ltd. (Tokyo, Japan), respectively. Capric acid, pindolol hydrochloride, and methyl *p*-hydroxybenzoate were purchased from Katayama Chemical Co., Ltd. (Osaka, Japan), Sigma Chemical Company (St. Louis, MO), and Kishida Chemicals. Co., Ltd. (Tokyo, Japan), respectively. Salicyl methionine was prepared in our laboratory. Timolol, atenolol, saponin, taurocholic acid, EDTA, salicylic acid, *o*-ethoxybenzamide, and other chemicals were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Phosphate-buffered saline (pH 7.4) was prepared by mixing an isotonic phosphate buffer with an equal volume of saline.

In Vitro Penetration Experiment

Male Nippon albino rabbits, weighing 2.0–3.0 kg, were used throughout the study. They were fasted for 24 hr prior to use for experiments but had free access to water. All experiments in the present study adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

The glass apparatus used for *in vitro* diffusion and the procedures used for preparing the corneal and conjunctival membranes have been described in previous reports (4,6). The rabbits were sacrificed by administering an overdose of pentobarbital sodium solution. The corneal or conjunctival membranes of the rabbits were dissected and mounted in diffusion chambers.

Glutathione bicarbonated Ringer's solution, containing 5 mM beta-blockers (4 ml) with or without an absorption promoter (0.5% saponin, 0.5% EDTA, 1% taurocholic acid, or 0.5% capric acid) was added to the epithelial side (donor side) of the diffusion chamber. Ringer's solution without promoter or penetrant (4 ml) was added to the endothelial side (receiver side). The contents of each chamber were stirred gently and bubbled with a 95% O_2/5% CO_2 mixture. The diffusion apparatus was jacketed at 35 ± 0.5°C (cornea) (7)

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0724-8741/95/0800-1146$07.50/0

and 37 ± 0.5°C (conjunctiva). Samples (50 μl) were withdrawn from the receiver side at 30-min intervals over a 4-hr period and the drug concentration was determined by HPLC.

Local Toxicity

Local irritation was determined on the basis of blinking counts in albino rabbits, in which counts were made for 5 min in the instillation eye and non-instillation (control) eye after the instillation of 25 μl of absorption promoter. Buffer (pH 7.4) was instilled as a control.

The rabbits' eyes were gently rinsed 6 hr after the instillation of absorption promoter, and were examined and scored according to the scale of Draize *et al.* (8) 1, 2, 3, and 7 days after the instillation.

Drug Determination

Samples of beta-blockers for *in vitro* experiments (50 μl) were mixed with isotonic phosphate-buffered saline (pH 7.4) or with 0.1 M HCl (50 μl) and methanol (100 μl), including the internal standard (300 μg/ml *o*-ethoxybenzamide for tilisolol, 500 μg/ml methyl *p*-hydroxybenzoate for timolol, 20 μg/ml pindolol for carteolol, 30 μg/ml salicylic acid for atenolol, and 50 μg/ml salicyl methionine for befunolol). The mixture was centrifuged at 12000 × *g* for 10 min, and 50 μl of the supernatant was injected into HPLC system.

The HPLC system (LC-6A; Shimadzu Co., Ltd., Kyoto, Japan) was used in the reverse phase mode for assay. The stationary phase used was a Cosmosil 5C18-P packed column (4.6 mm i.d. × 150 mm length, Nacalai Tesque Inc.). A mixture of methanol and 50 mM NaH_2PO_4 (tilisolol 37:63; atenolol 20:80; timolol 40:60; befunolol 45:55; carteolol 25:75, v/v) was used as the mobile phase, the flow rate being 1.0 ml/min. Retention of the drug was monitored with a UV spectrophotometric detector (SPD-10A, Shimadzu Co., Ltd.; 220 nm for atenolol; 290 nm for timolol; 250 nm for carteolol) and a fluorescence HPLC monitor (RF-535, Shimadzu Co., Ltd.; excitation wavelength 315 nm and emission wavelength 420 nm for tilisolol; excitation wavelength 300 nm and emission wavelength 500 nm for befunolol).

RESULTS

Corneal Permeability of Beta-Blockers with Absorption Promoters

The apparent permeability coefficient and lag time of beta-blockers were estimated from the slope and X-intercept of the corneal penetration profiles. The results are summarized in Table I. The permeability of the cornea to atenolol was enhanced by capric acid (20.3-fold) and by saponin (31.9-fold). Capric acid and saponin also enhanced the permeability of the cornea to carteolol and tilisolol (5.1- to 13.2-fold), although they had little effect in increasing the penetration of timolol and befunolol. EDTA and taurocholic acid significantly increased the corneal permeability to some beta-blockers, but their promoting activity was lower than that of capric acid and saponin.

Table I. Corneal Permeability Coefficient (Papp) and Lag Time of Beta-Blockers with Absorption Promoters

Promoter	*N*	Lag time (hr)	Papp ($\times 10^5$, cm/sec)	Ratio[a]
Atenolol				
Control	6	−0.1 ± 0.2	0.072 ± 0.011	1.0
0.5% EDTA	3	0.2 ± 0.1	0.12 ± 0.03	1.7
1% Taurocholic acid	4	0.4 ± 0.2	0.17 ± 0.04	2.4
0.5% Capric acid	4	0.5 ± 0.1	1.46 ± 0.13*	20.3
0.5% Saponin	5	0.3 ± 0.1	2.30 ± 0.08*	31.9
Carteolol				
Control	6	0.3 ± 0.0	0.16 ± 0.02	1.0
0.5% EDTA	4	0.8 ± 0.1	0.46 ± 0.08*	2.9
1% Taurocholic acid	3	0.5 ± 0.1	0.24 ± 0.08	1.5
0.5% Capric acid	4	0.5 ± 0.0	1.42 ± 0.15*	8.9
0.5% Saponin	3	0.3 ± 0.0	2.11 ± 0.05*	13.2
Tilisolol				
Control	6	0.2 ± 0.0	0.30 ± 0.04	1.0
0.5% EDTA	6	0.5 ± 0.0	0.34 ± 0.07	1.1
1% Taurocholic acid	4	0.6 ± 0.1	0.41 ± 0.05	1.4
0.5% Capric acid	5	0.2 ± 0.1	1.52 ± 0.10*	5.1
0.5% Saponin	3	0.3 ± 0.1	2.29 ± 0.51*	7.6
Timolol				
Control	6	0.2 ± 0.1	0.76 ± 0.13	1.0
0.5% EDTA	3	0.4 ± 0.1	1.76 ± 0.13*	2.3
1% Taurocholic acid	3	0.2 ± 0.1	1.56 ± 0.24*	2.1
0.5% Capric acid	4	0.1 ± 0.0	2.31 ± 0.11*	3.0
0.5% Saponin	3	0.3 ± 0.0	2.54 ± 0.15*	3.3
Befunolol				
Control	4	0.2 ± 0.0	1.07 ± 0.08	1.0
0.5% EDTA	3	0.4 ± 0.0	1.66 ± 0.04*	1.6
1% Taurocholic acid	4	0.2 ± 0.1	0.99 ± 0.09	0.9
0.5% Capric acid	4	0.1 ± 0.0	1.22 ± 0.02	1.1
0.5% Saponin	4	0.2 ± 0.0	2.84 ± 0.12*	2.7

[a] Ratio of Papp on control.
Values represent means ± SE of at least three experiments.
* $P < 0.05$ significantly different from control; ANOVA, followed by Fisher's PLSD.

Conjunctival Permeability of Beta-Blockers with Absorption Promoters

The findings for conjunctival permeability are summarized in Table II. The conjunctival permeability to beta-blockers with or without absorption promoters was greater than the corneal permeability. Improvements in conjunctival drug permeability by absorption promoters were mostly smaller than improvements in corneal drug permeability. Taurocholic acid significantly increased conjunctival permeability to the beta-blockers (2.1- to 3.2-fold), except for befunolol. Saponin and EDTA significantly increased conjunctival permeability to atenolol. Capric acid did not significantly increase conjunctival permeability to any beta-blocker. The ratio of corneal to conjunctival penetration (CR/CJ ratio) was markedly increased by capric acid and saponin, but was decreased by taurocholic acid.

Effect of Drug Lipophilicity on Permeability of Beta-Blockers with Absorption Promoters

The beta-blockers selected were all very similar in molecular weight and pKa (7). However, their lipophilicity var-

Table II. Conjunctival Permeability Coefficient (Papp) and Lag Time of Beta-Blockers with Absorption Promoters

Promoter	N	Lag time (hr)	Papp ($\times 10^5$, cm/sec)	Ratio[a]	CR/CJ[b]
Atenolol					
Control	5	0.2 ± 0.1	2.78 ± 0.68	1.0	0.03
0.5% EDTA	5	0.1 ± 0.0	6.05 ± 0.31*	2.2	0.02
1% Taurocholic acid	4	−0.1 ± 0.0	8.76 ± 0.97*	3.2	0.02
0.5% Capric acid	4	−0.1 ± 0.0	3.98 ± 0.27	1.4	0.37
0.5% Saponin	3	−0.2 ± 0.1	7.04 ± 0.38*	2.5	0.33
Carteolol					
Control	5	0.2 ± 0.1	3.97 ± 0.82	1.0	0.04
0.5% EDTA	4	0.0 ± 0.1	5.92 ± 0.58	1.5	0.08
1% Taurocholic acid	3	0.4 ± 0.2	9.04 ± 1.84*	2.3	0.03
0.5% Capric acid	3	0.1 ± 0.0	3.56 ± 0.22	0.9	0.40
0.5% Saponin	3	0.1 ± 0.0	5.86 ± 0.14	1.5	0.36
Tilisolol					
Control	6	0.2 ± 0.1	2.95 ± 0.86	1.0	0.10
0.5% EDTA	4	0.4 ± 0.0	5.15 ± 1.02	1.7	0.07
1% Taurocholic acid	4	0.1 ± 0.0	7.90 ± 0.76*	2.7	0.05
0.5% Capric acid	5	−0.1 ± 0.1	3.45 ± 0.32	1.2	0.44
0.5% Saponin	4	0.1 ± 0.0	4.82 ± 1.03	1.6	0.48
Timolol					
Control	6	0.2 ± 0.0	3.55 ± 0.75	1.0	0.21
0.5% EDTA	3	0.0 ± 0.1	6.54 ± 0.85*	1.8	0.27
1% Taurocholic acid	4	0.1 ± 0.0	7.60 ± 0.64*	2.1	0.21
0.5% Capric acid	4	−0.2 ± 0.1	3.54 ± 0.39	1.0	0.65
0.5% Saponin	4	0.0 ± 0.0	5.73 ± 0.45*	1.6	0.44
Befunolol					
Control	4	0.1 ± 0.0	6.38 ± 0.18	1.0	0.17
0.5% EDTA	5	0.0 ± 0.0	7.18 ± 0.35	1.1	0.23
1% Taurocholic acid	3	0.0 ± 0.0	5.70 ± 0.67	0.9	0.17
0.5% Capric acid	4	0.0 ± 0.1	2.59 ± 0.14*	0.4	0.47
0.5% Saponin	5	−0.2 ± 0.1	8.54 ± 0.40*	1.3	0.33

[a] Ratio of Papp on control.
[b] Ratio of corneal to conjunctival Papp.
Values represent means ± SE of at least three experiments.
* $P < 0.05$ significantly different from control; ANOVA, followed by Fisher's PLSD.

ied (logarithmic value of apparent partition coefficients between 1-octanol and pH 7.4 buffer; −1.5 for atenolol; −0.81 for carteolol; −0.24 for tilisolol; −0.16 for timolol; −0.009 for befunolol) (6). Figure 1 shows the relationship between the partition coefficient and the permeability coefficient of beta-blockers in the cornea and conjunctiva. Corneal permeability to the beta-blockers depended on drug lipophilicity. This dependency was retained in the presence of taurocholic acid and EDTA, but was reduced by capric acid and saponin. The conjunctival permeability to a drug, with or without absorption promoters, did not show a dependency on drug lipophilicity.

Local Irritation

The local irritation caused by the absorption promoters, determined by the blinking count method, is shown in Table III. The blinking counts in the instillation eye were significantly increased by the instillation of saponin. The eye damage evoked by the absorption promoters was also evaluated, according to the Draize score; no damage was observed, except with saponin, in which a slight irritation was observed 12 hr after instillation, the irritation disappearing by 24 hr (data not shown).

DISCUSSION

On instillation, most of an ophthalmic drug is rapidly eliminated from the precorneal area *via* drainage by the nasolacrimal duct and dilution by tear turnover, and is easily absorbed into the systemic circulation (2). The ocular absorption of an instilled drug is predominantly dependent on corneal permeability. Newton *et al.* (9) reported that Azone, a transdermal absorption promoter, increased the delivery of instilled cyclosporine into the cornea, enhancing immunosuppression in severe case of graft rejection. Smolen *et al.* (10) demonstrated that the permeability-enhancing effect of preservatives or cationic polymers on carbachol was beneficial at the active site inside the eye ball. The absorption promoters used in the present experiment increased the corneal penetration of most beta-blockers (Table I).

Carteolol, timolol, and befunolol are used clinically as ophthalmic drops for glaucoma. Tilisolol was reported to

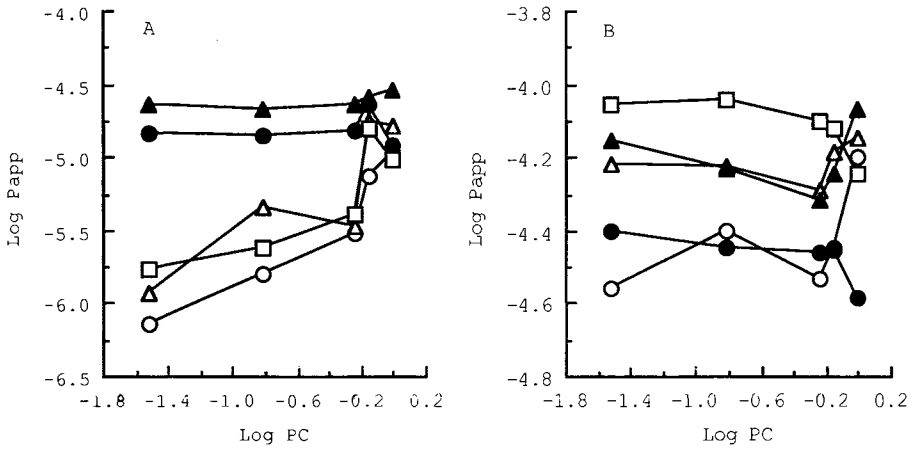

Fig. 1. Relationship between logarithmic value of apparent partition coefficient (PC) and permeability coefficient (Papp) of beta-blockers in cornea (A) and conjunctiva (B). Key: (○) control; (△) EDTA; (□) taurocholic acid; (●) capric acid; (▲) saponin.

decrease intraocular pressure after its instillation in rabbit (11). Atenolol did not evoke a sufficient decrease in intraocular pressure after instillation, an effect that may have been due to the poor permeability of the cornea to this agent (1). Capric acid and saponin increased the corneal permeability of atenolol, compared to the corneal permeability of timolol and befunolol without an absorption promoter. The ocular delivery of tilisolol and carteolol, both of which are hydrophilic beta-blockers, was also improved by capric acid and saponin. These results indicate the usefulness of capric acid and saponin for the ocular delivery of hydrophilic beta-blockers. The use of capric acid and saponin, however, resulted in only a slight improvement in the ocular delivery of lipophilic beta-blockers, such as timolol and befunolol. The cornea consists of three primary layers: the epithelium, stroma, and endothelium. The epithelium, being lipoidal in nature, is considered to contribute to the corneal barrier that prevents the absorption of hydrophilic drugs (2,7). The decrease in this barrier function against hydrophilic drugs produced by capric acid or saponin caused the dependency of corneal penetration on drug lipophilicity to disappear (Fig. 1).

The conjunctiva consists of a thin mucous membrane and vascularized tissue lining the inside of the eyelids and the anterior sclera. The permeability of the conjunctiva contributes to non-corneal ocular absorption and systemic absorption. The conjunctiva is leakier than the cornea because of the preponderance of the paracellular route in the former. Huang *et al.* (12) showed that the conjunctiva was much more permeable to hydrophilic macromolecules and ^{3}H-mannitol than the cornea. The paracellular permeability of beta-blockers could explain the low sensitivity of conjunctival permeability to absorption promoters (Table II).

Irrespective of the insensitivity of the conjunctival membrane to absorption promoters, taurocholic acid had the most potent effect on the conjunctival penetration of beta-blockers. Taurocholic acid, a weak biological surfactant, has been reported to increase the conjunctival permeability of even macromolecules such as insulin (13). EDTA, a calcium chelator, showed slight promoting activity on both corneal and conjunctival penetration. Calcium chelators are thought to loosen tight junctions by chelating Ca^{2+} (2). Saponin, a strong surfactant, markedly affected the corneal penetration of beta-blockers and had a slight effect on conjunctival penetration. Surfactants have been reported to affect membrane fluidity and to increase the permeability of cell membranes (14). Capric acid has been reported to affect both cell membranes and tight junctions, and to form ion-pair complexes with cationic drugs (15). Under the present conditions, capric acid markedly affected corneal penetration, but did not affect conjunctival penetration. It is worth noting the different responses of the cornea and conjunctiva to absorption promoters. The mechanisms of the absorption promoters and the barrier properties of membranes must define drug permeability. The ratio of corneal to conjunctival penetration (CR/CJ ratio) reflected the different responses.

Our findings show that absorption promoters improved the corneal penetration of beta-blockers. An absorption promoter may control the extent and the pathway of the ocular and the systemic absorption of instilled beta-blockers by altering corneal and conjunctival drug penetration. Slight local irritations in the eye were observed with saponin. Further investigations of the *in vivo* penetration behavior of beta-blockers and the safety of absorption promoters are necessary before these promoters can be employed for clinical use.

Table III. Blinking Count in Rabbit Eye in 5-min Period After Instillation (25 μl) of Absorption Promoter

Promoter	Control eye	Instillation eye
Control	0.5 ± 0.5	1.0 ± 0.4
0.5% EDTA	2.5 ± 0.9	13.3 ± 4.0
1% Taurocholic acid	3.0 ± 1.8	17.3 ± 4.1
0.5% Capric acid	1.5 ± 1.2	5.0 ± 2.4
0.5% Saponin	3.3 ± 2.6	55.3 ± 15.0*

Values represent means ± SE of four experiments.
* $P < 0.05$ significantly different from control; ANOVA, followed by Fisher's PLSD.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, by a Grant-in-Aid from the Mochida Memorial Foundation for Medical and Pharmaceutical Research, and by a Grant-in-Aid from the Uehara Memorial Foundation. The authors wish to thank Akemi Yamakita and Naoko Ishiyama for technical assistance in portions of this work.

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