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Ophthalmic Preservatives as Absorption Promoters for Ocular Drug Delivery

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Abstract

The effects of ophthalmic preservatives on the drug permeability through isolated ocular membranes of albino rabbits were investigated using a two-chamber glass diffusion cell. Tilisolol and fluorescein isothiocyanate (FITC)-dextrans (average molecular weights 4400 and 9400 Da; FD-4 and FD-10, respectively) were used as model penetrants of ophthalmic β-blockers and peptide drugs. Preservatives significantly enhanced the corneal penetration of not only tilisolol but also FITC-dextrans. Especially, benzalkonium chloride increased the corneal permeability of FD-4 and FD-10 by 28·8 and 37·1 times, respectively. These results indicate the usefulness of ophthalmic preservatives as absorption promoters for the ocular delivery of β-blockers and hydrophilic macromolecules. Preservatives also enhanced the conjunctival permeability of tilisolol, FD-4 and FD-10. The promoting effect of preservatives on the conjunctival drug penetration was smaller than that on the corneal one. Preservative increased the ratio of corneal to conjunctival permeability of tilisolol, FD-4 and FD-10.

The different responses of corneal and conjunctival drug penetrations to ophthalmic preservatives may be useful to control the extent and pathway for the ocular and systemic absorptions of instilled drugs.

Upon instillation of ophthalmic drugs, most of the instilled amount is rapidly eliminated from the pre-corneal area due to drainage by the naso-lacrimal duct and dilution by tear turnover, and easily absorbed into the systemic circulation (Shell 1982; Schoenwald 1990). Numerous attempts have been reported to improve the ocular delivery of topically applied drugs and diminishing their systemic adverse effects (Lee & Robinson 1986; Newton et al 1988; Lee 1990; Sasaki et al 1993a, b; Lee et al 1994). Among them, the use of absorption promoters seems to be advantageous to ophthalmic application for most drugs.

Marsh & Maurice (1971) reported that the concentration of fluorescein in aqueous humour of man was enhanced by an instillation of dye with non-ionic surfactant. Smolen et al (1973) demonstrated that the enhancing effect of preservative or cationic polymer on carbachol permeability is beneficial in the active site inside the eye globe. Absorption promoters also increased absorption of peptide drugs. Newton et al (1988) reported that Azone, a transdermal absorption promoter, increased the ocular delivery of instilled cyclosporin and enhanced the immunosuppression activity. Chiou & Chuang (1989) and Yamamoto et al (1989) demonstrated that absorption promoters increased the systemic absorption of instilled insulin and enhanced its hypoglycaemic activity.

However, the use of absorption promoter has been limited by its local toxicity to ocular tissues. Some reports have demonstrated the effects of ophthalmic preservatives on corneal irritability and enhancement of a drug penetration into the eye (Stern et al 1983; Burstein 1984; Lee & Robinson 1986; Camber & Edman 1987; Green 1993). These ophthalmic preservatives are considered to be safe as absorption promoters in ophthalmic formulations because they have been used for clinical ophthalmic disease for extended periods.

The purpose of this study was to investigate the improvement brought about by ophthalmic preservatives in the corneal penetration of tilisolol and fluorescein isothiocyanate (FITC)-dextrans as model penetrants of ophthalmic β-blockers and peptide drugs. We also compared the corneal penetration in response to ophthalmic preservatives with the conjunctival penetration in response to these agents. The different responses of corneal and conjunctival barriers to preservatives may be useful for controlling the drug absorption.

Materials and Methods

Materials

Tilisolol hydrochloride was supplied by Nisshin Flour Milling Co., Ltd (Tokyo, Japan). FITC-dextrans (average molecular weights 4400 and 9400 Da: FD-4 and FD-10), sorbic acid, 2-phenylethanol, methylparaben and propylparaben were purchased from Sigma Chemical Co. (St Louis, MO, USA). Paraben was used as a mixture of methylparaben and propylparaben (13:7, w/w). Benzyl alcohol, benzalkonium chloride and all other chemicals were of reagent grade, and were obtained 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, 2·0-3·0 kg, were individually housed in cages in an air-conditioned room and maintained on a standard laboratory diet (ORC4, Oriental Yeast Co., Ltd, Tokyo, Japan). The rabbits were starved for 24 h before

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use but had free access to water. All experiments in the present study conformed to the Guideline for Animal Experimentation in Nagasaki University. The glass apparatus for in-vitro diffusion and the procedure for preparing ocular membranes were according to previous reports (Lee et al 1988; Sasaki et al 1993b). Rabbits were killed with an overdose of a sodium pentobarbitone solution administered via a marginal ear vein. The ocular membranes were dissected and mounted in the diffusion chambers. Glutathione bicarbonated Ringer's solution was used throughout the diffusion studies. Penetrant solution (5 mM tilisolol or 0·05 mM FITC-dextran, 4 mL) containing ophthalmic preservative (0·01% benzalkonium chloride, 0·04% paraben, 0·5% 2-phenylethanol, 0·5% benzyl alcohol or 0·25% sorbic acid) was added to the epithelial side (donor side). Glutathione bicarbonated Ringer's solution (4 mL) without penetrant and preservative 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 to maintain the ocular membrane, donor solution and receiver solution at 35 ± 0.5°C (cornea) and 37 ± 0.5°C (conjunctiva). At 30-min intervals for 4 h, a sample (50 μL) was withdrawn from the receiver side and the concentrations of tilisolol or FITC-dextran were determined.

Drug determination

The samples of tilisolol for in-vitro experiments (50 μL) were mixed with pH 7·4 phosphate-buffered saline (50 μL) and methanol (100 μL) including internal standard (300 μg mL^{-1} *o*-ethoxybenzamide). The mixture was centrifuged at 12 000 *g* for 10 min and 50 μL of supernatant was injected into an HPLC system. An HPLC system (LC-6A, Shimadzu Co., Ltd, Kyoto, Japan) was used in the reverse phase mode for assay. The stationary phase used was Cosmosil 5C18-P packed column (4·6 mm i.d.×150 mm length, Nacalai Tesque Inc.). Mixtures of methanol and 50 mM NaH_2PO_4 (37:63, v/v) were used as the mobile phase with a flow rate of 1·0 mL min^{-1}. Retention of drug was monitored with a fluorescence HPLC monitor (RF-535, Shimadzu Co., Ltd; excitation wavelength 315 nm and emission wavelength 420 nm).

FITC-dextran was determined with a spectrofluorophotometer (RF-510, Shimadzu Co., Ltd; excitation wavelength 489 nm and emission wavelength 515 nm) in pH 7·4 phosphate-buffered saline.

Data analyses

The apparent permeability coefficient (P_{app}) and the lag time of tilisolol and FITC-dextran through ocular membranes were estimated from the slope and X-intercept of the linear portion in plotting accumulated drug amount in the receiver cell vs time.

All data were statistically evaluated by analysis of variance followed by the Fisher PLSD test. $P < 0.05$ was considered significant.

Results

Effect of preservative on corneal permeability

The penetration profiles of tilisolol, FD-4 and FD-10 with ophthalmic preservatives across the isolated ocular membranes are shown in Fig. 1. Corneal penetration without preservatives (control) was previously reported (Sasaki et al 1995a, b). The apparent permeability coefficient and the lag time are summarized in Table 1. Benzalkonium chloride, paraben, 2-phenylethanol, benzyl alcohol and sorbic acid were used as ophthalmic preservatives at the clinical concentration of commercial eye drops.

Benzalkonium chloride and 2-phenylethanol showed significant promoting effects on the corneal penetration of tilisolol by 3·5 and 2·7 times, respectively. Paraben, benzyl alcohol and sorbic acid did not show a significant increase on the corneal penetration of tilisolol. FD-4 and FD-10 showed similar corneal permeabilities through membranes in the presence of preservatives. Their corneal permeabilities were markedly lower than that of tilisolol. Some preservatives significantly enhanced the permeability of even hydrophilic macromolecules. Especially, benzalkonium chloride increased the permeability of FD-4 and FD-10 by 28·8 and 37.1 times, respectively. 2-Phenylethanol and benzyl alcohol enhanced the permeability of FD-4 and FD-10 by 2·6–8·1 times.

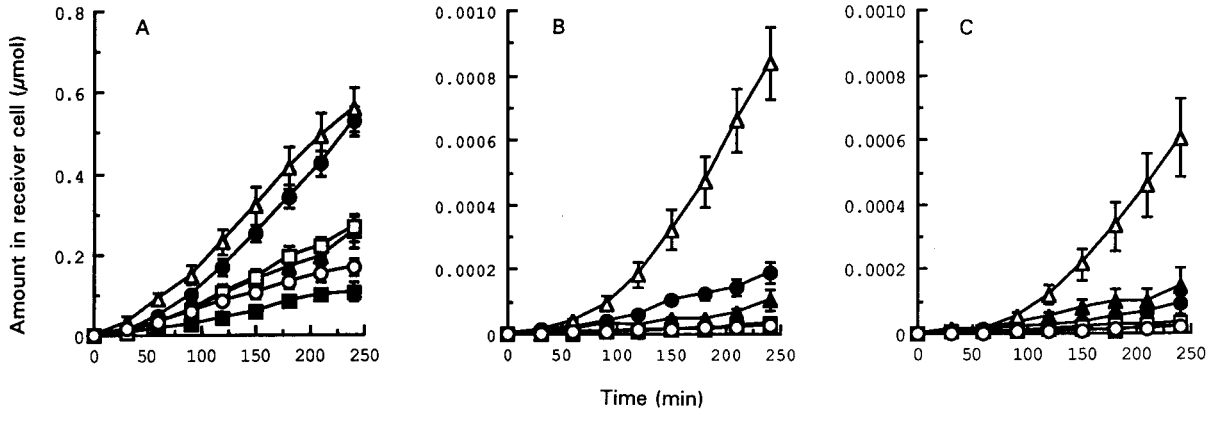

FIG. 1. Effect of preservatives on penetration of tilisolol (A), FD-4 (B) and FD-10 (C) through cornea. ○ Control, △ benzalkonium chloride, □ paraben, ● 2-phenylethanol, ▲ benzyl alcohol, ■ sorbic acid. Each point represents the average ± s.e.m.

Table 1. Apparent permeability coefficient (P_{app}) and lag time of tilisolol, FD-4 and FD-10 with ophthalmic preservatives through cornea.

Preservative	n	Lag time[a] (h)	P_{app}[a] ($cm\ s^{-1}$)	Ratio[b]
Tilisolol				
Control	6	0·2 ± 0·0	3·0 ± 0·4	1·0
0·01% Benzalkonium chloride	4	0·4 ± 0·2	10·4 ± 2·1*	3·5
0·04% Paraben	3	0·5 ± 0·2	3·7 ± 0·5	1·2
0·5% 2-Phenylethanol	4	0·7 ± 0·0	8·1 ± 0·5*	2·7
0·25% Sorbic acid	4	0·6 ± 0·0	2·3 ± 0·2	0·8
FD-4				
Control	5	0·8 ± 0·4	0·057 ± 0·016	1·0
0·01% Benzalkonium chloride	4	1·0 ± 0·1	1·64 ± 0·17*	28·8
0·04% Paraben	4	1·5 ± 0·3	0·067 ± 0·007	1·2
0·5% 2-Phenylethanol	3	0·4 ± 0·3	0·32 ± 0·06*	5·6
0·5% Benzyl alcohol	3	0·3 ± 0·3	0·15 ± 0·05*	2·6
0·25% Sorbic acid	4	1·0 ± 0·2	0·045 ± 0·006	0·8
FD-10				
Control	4	0·6 ± 0·1	0·031 ± 0·003	1·0
0·01% Benzalkonium chloride	5	1·0 ± 0·0	1·15 ± 0·26*	37·1
0·04% Paraben	4	0·2 ± 0·2	0·059 ± 0·010	1·9
0·5% 2-Phenylethanol	3	0·3 ± 0·2	0·15 ± 0·05	4·8
0·5% Benzyl alcohol	3	0·4 ± 0·2	0·25 ± 0·10	8·1
0·25% Sorbic acid	3	−0·4 ± 0·1	0·028 ± 0·006	0·9

[a] Average ± s.e.m. [b] Ratio of P_{app} to control. *$P < 0·05$ compared with control.

Effect of preservative on conjunctival permeability

Fig. 2 shows the penetration profiles of tilisolol, FD-4 and FD-10 with ophthalmic preservatives through conjunctiva. Conjunctival penetrations without preservatives (control) were previously reported (Sasaki et al 1995a, b). Table 2 summarizes the apparent permeability coefficient and the lag time. The conjunctival permeabilities of penetrants with or without preservatives were higher than the corneal permeabilities. Benzalkonium chloride significantly enhanced the conjunctival penetration of tilisolol. The conjunctival permeability of FD-4 or FD-10 was also increased by benzalkonium chloride, 2-phenylethanol or benzyl alcohol. The promoting effect of preservative on the conjunctival penetration was mostly smaller than that on the corneal penetration.

The ratio of corneal to conjunctival permeability of FD-4 and FD-10 was lower than that of tilisolol. Benzalkonium chloride markedly increased the ratio for both FD-4 and FD-10.

Discussion

In the ophthalmic field, many preservatives have been used for a long time to prevent the patient from introducing microbiologically contaminated drugs into his eyes and to maintain the potency of the ophthalmic drug (Mullen et al 1973; Green 1993). An injured eye has reduced resistance to infection. Benzyl alcohol and 2-phenylethanol are used rarely because of their slow activities and irritation. Paraben and sorbic acid are more effective against moulds and fungi than bacteria. Parabens are only effective at concentrations near the limit of their solubility. Benzalkonium chloride is most frequently used in commercial eye drops because of its rapid bactericidal efficacy and low toxicity under properly

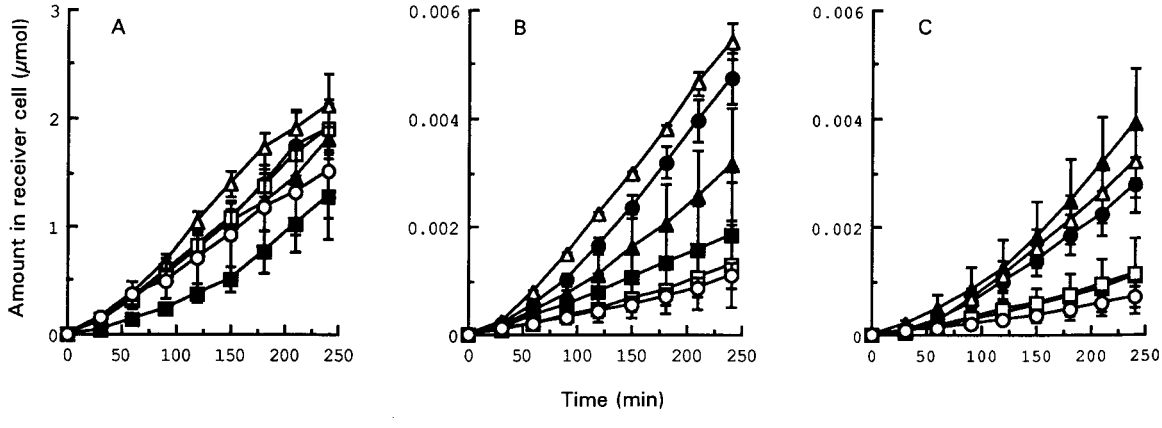

FIG. 2. Effect of preservatives on penetration of tilisolol (A), FD-4 (B) and FD-10 (C) through conjunctiva. ○ Control, △ benzalkonium chloride, □ paraben, ● 2-phenylethanol, ▲ benzyl alcohol, ■ sorbic acid. Each point represents the average ± s.e.m.

Table 2. Apparent permeability coefficient (P_{app}) and lag time of tilisolol, FD-4 and FD-10 with ophthalmic preservatives through conjunctiva.

Preservative	n	Lag time[a] (h)	P_{app}[a] ($cm\ s^{-1}$)	Ratio[b]	CR/CJ[c]
Tilisolol					
Control	6	0·2 ± 0·1	29·5 ± 8·6	1·0	0·102
0·01% Benzalkonium chloride	6	0·4 ± 0·1	46·5 ± 3·1*	1·6	0·224
0·04% Paraben	3	0·4 ± 0·1	28·1 ± 3·1	1·0	0·132
0·5% 2-Phenylethanol	3	0·3 ± 0·1	29·8 ± 4·6*	1·0	0·272
0·5% Benzyl alcohol	3	0·3 ± 0·0	27·2 ± 1·6*	0·9	0·132
0·25% Sorbic acid	4	0·6 ± 0·1	22·7 ± 4·7	0·8	0·101
FD-4					
Control	5	0·9 ± 0·4	2·8 ± 0·7	1·0	0·020
0·01% Benzalkonium chloride	4	0·5 ± 0·0	10·7 ± 0·6*	3·8	0·153
0·04% Paraben	4	0·1 ± 0·2	2·3 ± 1·3	0·8	0·029
0·5% 2-Phenylethanol	4	0·6 ± 0·0	9·4 ± 0·9*	3·4	0·034
0·5% Benzyl alcohol	4	0·6 ± 0·1	6·0 ± 2·0	2·1	0·025
0·25% Sorbic acid	4	−1·0 ± 1·0	3·5 ± 1·9	1·3	0·013
FD-10					
Control	6	0·5 ± 0·2	1·56 ± 0·50	1·0	0·020
0·01% Benzalkonium chloride	5	0·7 ± 0·0	6·57 ± 1·32*	4·2	0·175
0·04% Paraben	3	0·4 ± 0·1	2·14 ± 0·10	1·4	0·028
0·5% 2-Phenylethanol	3	0·6 ± 0·1	5·52 ± 1·04*	3·5	0·027
0·5% Benzyl alcohol	4	0·8 ± 0·1	7·95 ± 2·15*	5·1	0·031
0·25% Sorbic acid	3	0·2 ± 0·2	1·87 ± 1·23	1·2	0·015

[a] Average ± s.e.m. [b] Rabio of P_{app} to control. [c] Ratio of corneal to conjunctival permeability. *$P < 0·05$ compared with control.

controlled conditions. The toxicity of some preservatives may be tolerated by the use of low concentrations in clinical use (Mullen et al 1973; Green 1993).

The corneal composite structure is indicated by three primary layers: the epithelium, stroma and endothelium. The epithelium, being lipoidal in nature, is considered to contribute to the corneal penetration barrier of particularly hydrophilic drugs (Huang et al 1983). The corneal permeability predominantly contributes to ocular absorption of instilled drug. Many researchers have demonstrated that some preservatives significantly increased the corneal permeability of ophthalmic drugs (Burstein 1984; Lee & Robinson 1986; Camber & Edman 1987; Green 1993). Ophthalmic preservatives at the clinical concentration increase the corneal penetration of tilisolol, an ophthalmic β-blocker. Ophthalmic preservatives also increase the corneal penetration of even hydrophilic macromolecules such as FITC-dextran. Recently, some peptide drugs have been introduced to be used clinically in ophthalmic fields although biological membranes are impermeable to most of them because of their large molecular weight and hydrophilicity (Harris et al 1992).

Benzalkonium chloride shows the highest promoting effect on corneal drug penetration of currently used preservatives. It is a powerful cationic detergent which destroys bacteria after ionic attraction. The detergent effect on plasma membranes accounts for its epithelial toxicity. Benzalkonium chloride 0.01% causes cells of the corneal epithelium to peel at their borders (Pfister & Burstein 1976). Benzalkonium chloride also enlarges intercellular spaces in superficial cells of the cornea (Green & Tønjum 1971; Tønjum 1975). Klyce & Crosson (1985) demonstrated that the superficial surface of the corneal epithelium contributes to the drug penetration barrier. The degree of the changes of epithelium was dependent upon the concentration and the exposure time of benzalkonium chloride (Tønjum 1975; Stern et al 1983).

The cornea in-vivo is more resistent to the destruction caused by benzalkonium chloride than in-vitro because the dilution and elimination of benzalkonium chloride and the protective effect of the tear film take place in-vivo. The physiological change–that is, the inhibition of the electrical potential difference across the corneal epithelium–was observed with benzalkonium chloride concentrations of 0·0004 and 0·005% in-vitro, and 0·01% in-vivo (Green & Tønjum 1975; Burstein & Klyce 1977). The instillation of 0·01% benzalkonium chloride (100 μL) showed no irritation according to the Draize scoring (Griffith et al 1980), and a 0·004 to 0·01% had no influence on the epithelial aerobic metabolism (Burton & Hill 1981).

Benzalkonium chloride instilled is preferentially absorbed by corneal epithelium and conjunctiva (Green 1993). The corneal exposure to multiple drops of benzalkonium chloride leads to epithelial accumulation but no penetration into the anterior chamber (Green 1993). Benzalkonium chloride could be a safe absorption promoter for the ocular drug delivery. Further investigations are also necessary on in-vivo potential use and long-term safety of benzalkonium chloride instilled.

Conjunctiva is a thin mucous membrane and a vascularized tissue lining the inside of the eye lids and the anterior sclera. The conjunctival permeability contributes to the non-corneal ocular absorption and the systemic absorption (Doane et al 1978; Ahmed & Patton 1987). The conjunctiva is leakier than the cornea through a significant paracellular route. Huang et al (1989) showed that the conjunctiva was much more permeable to hydrophilic macromolecules and [^{3}H]mannitol than the cornea. The paracellular permeabilities of tilisolol, FD-4 and FD-10 can explain the low sensitivity of the conjunctival permeability to the preservatives. Furthermore, the different responses of corneal and conjunctival barriers to ophthalmic preservatives may be useful in controlling the extent and pathway of the ocular and systemic absorption of instilled drugs.

Thus, ophthalmic preservatives improve the corneal penetration of not only β-blockers but also of hydrophilic macromolecules. An adequate selection of preservative may be able to control the ocular and systemic absorptions of instilled drugs. These results also indicate that preservatives must be carefully used in ophthalmic eye drops.

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