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Ocular Membrane Permeability of Hydrophilic Drugs for Ocular Peptide Delivery

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Abstract

The purpose of this study is to investigate the ocular membrane permeability and the permeation mechanism of hydrophilic drugs such as thyrotropin-releasing hormone (TRH), *p*-nitrophenyl β-cellopentaoside (PNP) and luteinizing hormone-releasing hormone (LHRH).

The penetration of hydrophilic drugs was measured across the isolated corneal and conjunctival membranes of albino rabbits using a two-chamber diffusion glass cell. The corneal permeabilities of hydrophilic drugs were much lower than those of beta blockers reported previously. The corneal penetration of TRH was the highest among the hydrophilic drugs studied. Scraping the corneal epithelium increased the penetration of hydrophilic drugs. Conjunctival membranes showed higher permeability to hydrophilic drugs compared with corneal membranes. The permeability of drugs was also analysed by Fick's equation. The partition parameter and diffusion parameter of TRH, PNP and LHRH in the cornea were lower than those in scraped cornea and conjunctiva. In addition to the data of fluorescein isothiocyanate-dextran reported previously, the permeability coefficient of hydrophilic drugs through the cornea, scraped cornea and conjunctiva correlated with molecular weight of the drugs. The diffusion parameters of hydrophilic drugs decreased with an increase of molecular weight for all ocular membranes. The extent of dependency of partition parameters on the molecular weights of drugs varied according to the ocular membrane. These results indicate that ocular membranes are sufficiently different in permeation character and mechanism to control the extent and pathway for ocular absorption of hydrophilic drugs.

In ophthalmology, various peptide drugs have begun to be used clinically. Peptide drugs such as cyclosporin, various growth factors, interferons and interleukins are also expected to become potential therapeutic agents for uveitis, wound healing, herpes simplex infections and modification of immune response (Harris et al 1992).

The ocular route is also a possible route for systemic delivery of peptides, since the mucous membrane in the conjunctiva and nasal cavity are permeable to even hydrophilic macromolecules. Some peptide drugs such as thyrotropin-releasing hormone (TRH), luteinizing hormone-releasing hormone (LHRH) and insulin have been found to be well absorbed systemically after instillation with or without absorption promoters (Chiou & Chuang 1989; Yamamoto et al 1989).

The instilled drug is absorbed in the periocular tissues such as the cornea and the conjunctiva, and is distributed to effective sites. Most peptide drugs penetrate biological membranes with difficulty because of high molecular weight and hydrophilicity. The permeability of instilled drugs through ocular membranes should be considered as an important factor to control the extent and pathway for ocular and systemic delivery of drugs. However, little information on barrier properties is available for hydrophilic drugs.

Maurice (1960) showed the usefulness of diffusion equation to characterize the movement of fluorescein in the cornea in detail. The purpose of this study is to investigate ocular membrane permeability and permeation mechanism of hydrophilic drugs in-vitro using diffusion equation.

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Materials and Methods

Materials

TRH (M.W. 362.4) and LHRH (M.W. 1182.3) were kindly supplied by Tanabe Pharmaceutical Co. Ltd. (Osaka). *p*-Nitrophenyl β-cellopentaoside (PNP; M.W. 949.8) was purchased from Seikagaku Kogyo Co. (Tokyo). Protocatechuic acid, *O*-ethoxybenzamide and all other chemicals were of reagent grade obtained from Nacalai Tesque Inc. (Kyoto). Phosphate-buffered saline (pH 7·4) was prepared by mixing isotonic phosphate buffer with an equal volume of 0·9% NaCl (saline).

Animals

Male Nippon albino rabbits (2·0–3·0 kg) were housed in cages in an air-conditioned room and maintained on a standard laboratory diet (ORC4, Oriental Yeast Co. Ltd., Tokyo). The rabbits were fasted for 24 h before use but had free access to water. All experiments in the present study conformed to Guideline for Animal Experimentation in Nagasaki University.

In-vitro penetration experiment

The glass apparatus for in-vitro diffusion and the procedure for preparing ocular membranes were reported previously (Lee et al 1988; Sasaki et al 1993), where the viability of the cornea and conjunctiva was confirmed over the time course of the experiment by the measurement of hydration and by scanning electron microscopy. Rabbits were killed with an overdose of sodium pentobarbitone administered via a marginal ear vein. The ocular membranes were dissected and mounted in the diffusion chambers. In some experiments, the epithelium of cornea was scraped with a surgical knife to prepare a stromal

and endothelial membrane (scraped cornea). Glutathione bicarbonated Ringer's solution was used throughout the diffusion studies (Schoenwald & Huang 1983).

Penetrant solution (0·5 mM TRH, 0·2 mM PNP or 0·5 mM LHRH, 4 mL) and penetrant-free solution (4 mL) were added to the epithelial side (donor side) and the endothelial side (receiver side), respectively. 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 $35 \pm 0{\cdot}5$°C (cornea) and $37 \pm 0{\cdot}5$°C (conjunctiva). At appropriate time intervals, a sample (200 μL) was withdrawn from the receiver side and the concentration of drugs was assayed by HPLC.

To examine metabolism of penetrating species in the penetration experiment, drug solutions (0·5 mM) were added to both donor and receiver sides in diffusion chamber with ocular membrane. A sample (200 μL) was withdrawn from both sides 240 min after incubation and the concentration of drugs was assayed by HPLC.

Drug determination

The samples of TRH, PNP and LHRH for in-vitro experiments (200 μL) were mixed with methanol (50 μL) including internal standard (10 μg mL^{-1} protocatechuic acid for TRH, 50 μg mL^{-1} *O*-ethoxybenzamide for both PNP and LHRH). The mixture was centrifuged at 12 000 g for 10 min and 80 μL of supernatant was injected into an HPLC system (LC-6A, Shimadzu Co., Ltd., Kyoto) used in the reverse-phase mode. The stationary phase used was Cosmosil 5C18-P packed column (150 mm length × 4·6 mm i.d., Nacalai Tesque Inc.). Mixtures of acetonitrile and 50 mM phosphate buffer (pH 3·0) (TRH 0·5:99·5; PNP 8:92; LHRH 18:82 v/v) were used as a mobile phase with a flow rate of 1·0 mL min^{-1}. Retentions of drugs were monitored with a UV spectrophotometric detector (SPD-10A, Shimadzu Co. Ltd.; 220 nm for TRH; 290 nm for PNP, 280 nm for LHRH). These samples for determination were stored below 5°C until assay.

Data analyses

The obtained penetration profiles were analysed based on a diffusion model for the infinite dose system which considers the ocular membrane to be a one-plane barrier membrane (Sasaki et al 1995a). This membrane model assumes a constant drug concentration in the donor solution and a sink condition in the receiver phase, since the cumulative amount of drug transferred to the receiver phase was much smaller than the donor amount. According to the model, the Laplace transform for the total amount of drug appearing in the receiver phase (Q) is expressed as follows:

$$Q = K'.D'^{0.5}.C_0/s^{1.5}/\sinh(s^{0.5}/D'^{0.5}) \quad (1)$$

where: $K' = K.V$; $D' = D/L/L$; $Kp = K'.D'/A$; $LT = 1/6/D'$ and D is the diffusion coefficient in membrane, K is the partition coefficient of drug between membrane and donor solution, L is the effective length of diffusion through membrane, V is the effective membrane volume, s is the Laplace variable with respect to time, A is the effective diffusion area, C_0 is the drug concentration in the donor phase, Kp is the permeability coefficient and LT is the lag time. Since it is difficult to determine correctly the real diffusion length (L) for each penetrant, the diffusion parameter (D′) and the partition parameter (K′) were defined.

The penetration parameters were estimated by fitting the penetration profiles to equations using MULTI(FILT), a non-linear least-squares computer program based on a fast inverse Laplace transform algorithm (Yano et al 1989). This program was written in MS-FORTRAN and run on a personal computer (PC-9801 BX, NEC, Tokyo).

Results

Permeability of hydrophilic drugs through ocular membranes

The hydrophilicity of drugs was confirmed by determination of apparent partition coefficient between pH 7·4 buffer and 1-octanol (0·5 mM). Less than 0·5% partitioned to organic phase and oil/water interface. The profiles of cumulative amount of hydrophilic drugs in the receiver phase through ocular membranes showed a lag phase followed by a linear rise. Penetration parameters according to Fick's equation were calculated from the average profiles. The results are summarized in Table 1. The parameters thus obtained varied with different penetrants and membranes. The permeability coefficients of hydrophilic drugs were extremely low compared with those of beta blockers reported previously (Sasaki et al 1995a). Scraping the corneal epithelium enhanced the permeability coefficient of the intact cornea by 34–100 times. The scraping increased partition parameters, but not diffusion parameters, of corneal permeations of TRH, PNP and LHRH. The conjunctival permeability coefficients of hydrophilic drugs were 17–47 times higher than those in the cornea. The partition parameters of these drugs in the conjunctiva were also higher than those in the cornea.

In the metabolism experiment, a significant decrease of penetrating species was not detected in donor and receiver sides of cornea and conjunctiva for 240 min incubation except

Table 1. Penetration parameters of TRH, PNP and LHRH through cornea (CR), scraped cornea (SCCR) and conjunctiva (CJ).

Parameter	TRH	PNP	LHRH
CR			
n	5	6	7
LT (h)	0·16	0·15	0·39
D′ (h^{-1})	1·03	1·11	0·43
K′ (10^2 cm^3)	0·13	0·04	0·03
Kp (10^2 cm h^{-1})	0·15	0·06	0·02
SCCR			
n	3	4	4
LT (h)	0·09	0·23	0·44
D′ (h^{-1})	1·92	0·73	0·38
K′ (10^2 cm^3)	2·30	4·23	4·49
Kp (10^2 cm h^{-1})	5·10	3·54	1·99
SCCR/CR[a]	34	59	100
CJ			
n	4	6	6
LT (h)	0·14	0·14	0·19
D′ (h^{-1})	1·22	1·20	0·88
K′ (10^2 cm^3)	2·40	0·66	0·84
Kp (10^2 cm h^{-1})	3·73	1·02	0·94
CJ/CR[b]	25	17	47

n: number of experiment, LT: lag time, D′ (D/L/L): diffusion parameter, K′ (K.V): partition parameter, Kp: permeability coefficient. [a]Ratio of scraped corneal Kp to corneal Kp. [b]Ratio of conjunctival Kp to corneal Kp.

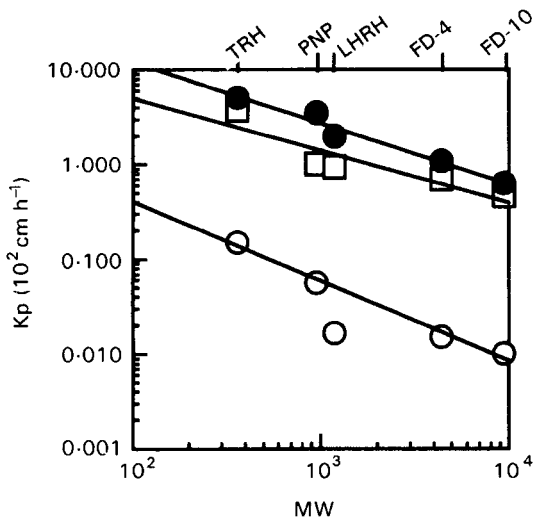

FIG. 1. Relationship between molecular weight (MW) and permeability coefficient (Kp) in cornea (○), scraped cornea (●) and conjunctiva (□). The solid lines through the data were obtained from a linear regression fit. Cornea (except for LHRH): $Y = -0.839\ X - 0.710$ ($\gamma = 0.995$, $P < 0.05$); scraped cornea: $Y = -0.647\ X + 0.379$ ($\gamma = 0.983$, $P < 0.05$); conjunctiva: $Y = -0.553\ X + 0.195$ ($\gamma = 0.911$, $P < 0.05$).

for LHRH. A little LHRH ($11 \pm 2\%$) left the donor side of the cornea under the present conditions.

Relationship between molecular weight and permeability coefficient in hydrophilic drugs

Fig. 1 shows a relationship between log values of molecular weight and permeability coefficient in hydrophilic drugs. Permeability coefficients of fluorescein isothiocyanate-dextrans (M.W.s 4400 and 9400; FD-4 and FD-10) reported previously (Sasaki et al 1995b) were added in the figure. The permeability coefficients of hydrophilic drugs showed a significant correlation with molecular weight of drugs in all ocular membranes, except for LHRH in cornea.

The relationships between log values of molecular weight and diffusion parameter or partition parameter are shown in Figs 2A and 2B, respectively. A significant correlation was obtained between molecular weight and diffusion parameter in conjunctiva. On the other hand, the cornea showed a significant correlation between molecular weight and partition parameter.

Discussion

The corneal membrane consists mainly of three membranes; an epithelial layer, a stromal layer and an endothelial layer. The epithelium is considered to be the primary barrier that prevents absorption of hydrophilic drugs (Huang et al 1983; Chien et al 1988). The lipophilicity of drugs has been reported to be important for transcellular penetration of lipoidal corneal epithelium (Schoenwald & Huang 1983). The poor permeability of hydrophilic drugs such as TRH, PNP and LHRH was observed in the present study.

In general, hydrophilic drugs that have little interaction with membranes cannot distribute to the lipid membrane of cell surfaces. (Grass & Robinson 1984, 1988a,b; Grass et al 1988.) demonstrated that transport of hydrophilic compounds through the cornea was by an aqueous diffusional pathway. Ultrastructural analysis of the cornea by scanning and transmission electron microscopy suggests that the intercellular space of the corneal epithelium may in part be the anatomical location of this pathway. They estimated the limiting molecular dimension of epithelium to be < 5 nm for intercellular pathways. The high value of diffusion parameters of hydrophilic compounds for scraped cornea and intact cornea suggest the rapid diffusion through an aqueous route not the transcellular membrane.

In the corneal epithelial layer, adjacent cells combine very tightly and the number of aqueous pore pathways are very few.

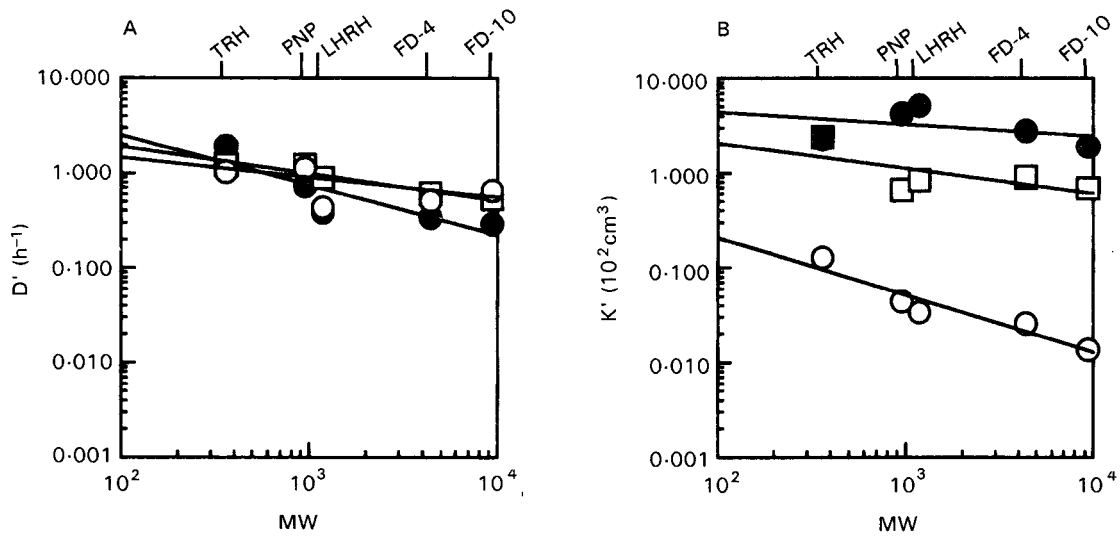

FIG. 2. Relationship between molecular weight (MW) and diffusion parameter (D')(A) or partition parameter (K')(B) in cornea (○), scraped cornea (●) and conjunctiva (□). The solid lines through the data were obtained from a linear regression fit. (A) Cornea (except for LHRH): $Y = -0.210\ X + 0.584$ ($\gamma = 0.825$, not significant); scraped cornea: $Y = -0.528\ X + 1.459$ ($\gamma = 0.877$, not significant); conjunctiva: $Y = -0.287\ X + 0.854$ ($\gamma = 0.958$, $P < 0.05$) (B) Cornea: $Y = -0.604\ X - 1.473$ ($\gamma = 0.950$, $P < 0.05$); scraped cornea: $Y = -0.119\ X - 1.142$ ($\gamma = 0.407$, not significant); conjunctiva: $Y = -0.226\ X - 1.926$ ($\gamma = 0.643$, not significant).

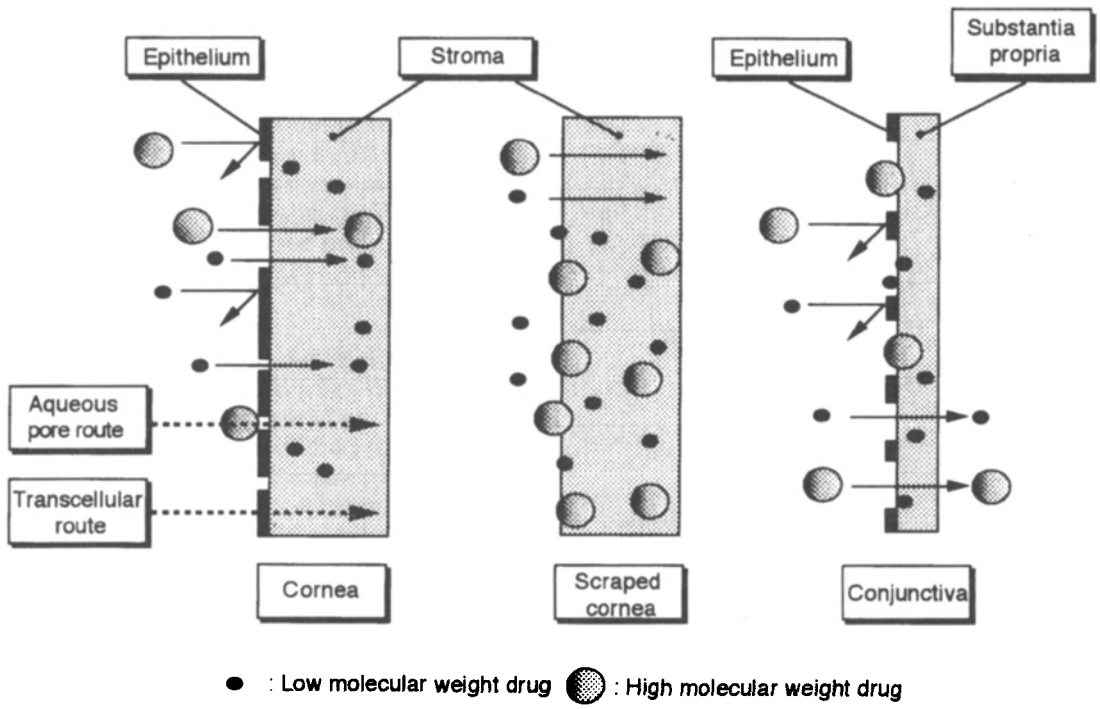

FIG. 3. Schematic representation of corneal, scraped corneal and conjunctival penetration of hydrophilic drugs.

These characteristics agreed with the low partition parameters for corneal penetrations of TRH, PNP and LHRH (Table 1). The partition coefficient of hydrophilic compounds from donor solution to aqueous route in the interface should be almost unity. The partition parameters for hydrophilic drugs are considered to mainly reflect an available area of aqueous pathway.

The lower diffusion parameters of LHRH than those of PNP were observed regardless of their similar physicochemical properties such as molecular weight and hydrophilicity. This may be in part explained by metabolism of LHRH during membrane penetration and by LHRH-membrane interactions such as hydrogen bonding, charge and conformational issue (Lee et al 1986; Rojanasakul et al 1990).

When the corneal epithelial layer was scraped, the permeability coefficient and partition parameter of TRH, PNP and LHRH were markedly increased. The hydrophilic drugs directly partition to stroma which has a hydrophilic nature. Diffusion parameters were slightly affected by scraping the epithelium (Table 1). These results indicate that the absorption behaviours of hydrophilic drugs may be easily and extremely affected by ocular disease or application of absorption enhancer.

The conjunctiva is a thin mucous vascularized membrane lining the inside of the eye lids and anterior sclera. The conjunctival absorption of drugs contributes to systemic absorption and also to the ocular absorption known as non-corneal absorption. Huang et al (1989) reported that the conjunctiva was much more permeable to hydrophilic macromolecules and [^{3}H]mannitol than the cornea. Inulin penetrated through the non-corneal route in a quantity amounting to as much as 40% of the total absorbed in the eye (Ahmed & Patton 1987). TRH, PNP and LHRH also showed much higher conjunctival permeability coefficient compared with corneal permeability coefficient owing to an increase of partition parameter (Figs 1 and 2B, Table 1). These results indicate a richness of paracellular route in conjunctival membrane.

Hydrophilic drugs that have little interaction with membranes may diffuse through the aqueous pathways of cornea. The diffusion process is influenced by molecular weight. In fact, the permeability coefficients of hydrophilic drugs in all ocular membranes significantly correlated with molecular weights of drugs except for LHRH in cornea (Fig. 1). The diffusion parameters of hydrophilic drugs decreased with an increase of molecular weight of drugs (Fig. 2A). On the other hand, it is worth noting that the partition parameters of hydrophilic drugs also showed a dependency on molecular weight in conjunctiva and cornea (Fig. 2B). The dependency of permeability coefficient on molecular weight of drugs in cornea, in particular, is predominantly caused by a dependency of partition parameter.

The penetration behaviours of hydrophilic drugs through aqueous pathway of ocular membranes are considered in Fig. 3. The cornea has a lot of small aqueous pathways accessible to low molecular weight drugs and a few larger aqueous pathways for high molecular weight drugs. The partition parameters which reflect the available area of aqueous pathways are affected by molecular weights of drugs. Conjunctiva possesses a lot of large aqueous pathways that high molecular weight drugs enable to permeate. Scraped cornea has enough aqueous surface for distribution of hydrophilic drugs regardless of their molecular weights. The hydrophilic drugs that partition to the membrane diffuse the aqueous pathway according to their molecular weights.

Thus, ocular membranes are sufficiently different in permeation character and mechanism to control the extent and pathway for ocular absorption of hydrophilic drugs.

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