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Corneal Critical Barrier against the Penetration of Dexamethasone and Lomefloxacin Hydrochloride: Evaluation by the Activation Energy for Drug Partition and Diffusion in Cornea

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The cornea is a solid barrier against drug permeation. We searched the critical barrier of corneal drug permeation using a hydrophobic drug, dexamethasone (DM), and a hydrophilic drug, lomefloxacin hydrochloride (LFLX). The activation energies for permeability of DM and LFLX across the intact cornea were 88.0 and 42.1 kJ/mol, respectively. Their activation energies for permeability across the cornea without epithelium decreased to 33.1 and 16.6 kJ/mol, respectively. The results show that epithelium is the critical barrier on the cornea against the permeation of a hydrophobic drug of DM as well as a hydrophilic drug of LFLX. The activation energy of partition for DM (66.8 kJ/mol) was approximately 3-fold larger than that of diffusion (21.2 kJ/mol). The results indicate that the partition for the hydrophobic drug of DM to the corneal epithelium is the primary barrier. Thermodynamic evaluation of activation energy for the drug permeation parameters is a good approch to investigate the mechanism of drug permeability.

Keywords corneal permeability; *in vitro*; dexamethasone; lomefloxacin hydrochloride; partition; diffusion; activation energy

INTRODUCTION

Topically applied ophthalmic drugs are often used in ocular therapy. However, the poor bioavailability of drugs is a problem in the ophthalmic area (Lee, 1990). Although the major route by which most ophthalmic drugs enter the eye is via the cornea, poor bioavailability may be due to precorneal processes such as the rapid removal of the drug from the absorption site and the existence of a cornea that prevents the passage of the drug molecules (Lee et al., 1986). The cornea is a three-layer structure consisting of a hydrophilic stromal layer sandwiched between a very lipophilic epithelial layer on the outside and a much less lipophilic endothelial layer on the inside. The epithelium contributes to the corneal permeability barrier of highly hydrophilic drugs (Huang et al., 1983).

Many experimental works have characterized corneal permeability (Prausnitz et al., 1998), and some models have been developed to describe transcorneal drug transport (Cooper et al., 1987; Edward et al., 2001; Grass et al., 1988a; Huang et al., 1983; Worth et al., 2000; Yoshida et al., 1996). Drug movement in the eye may be well described by a simple passive diffusion model based on Fick's second law of diffusion, since the events taking place in the various eye tissues depend usually upon the local concentration of drugs (Tojo, 2004). The diffusion coefficient of a drug across ocular tissues depends on the chemical structure and the physicochemical properties as well as the molecular weight of the drug (Tojo, 2004). Permeability consists of diffusion and partition (Martin et al., 1993). The partition coefficient demonstrates the relative solubility in the membrane and surrounding medium depending on the physicochemical properties of the drug and the membrane. Activation energy for permeability was determined to reveal mechanisms of transmembrane of molecules (Grass et al., 1988b; Kawazu et al., 1999). We expected to obtain information about the critical barrier of corneal drug permeation by determining individual activation energy for diffusion and partition.

The effects of ophthalmic preservatives on corneal irritability and enhancement of a drug permeability into the eye were demonstrated (Burstein, 1984; Camber et al., 1987; Green, 1992; Lee et al., 1986). Benzalkonium chloride (BAK) is the most frequently used preservative in commercial eye drops because of its rapid bactericidal efficacy and relatively low toxicity. It is a powerful cationic detergent, which kills bacteria after ionic attraction. It has high boosting effect on corneal drug permeability (Sasaki et al., 1995).

In this report, we examined the critical barrier to corneal permeability of drugs by evaluation of activation enagy for diffusion

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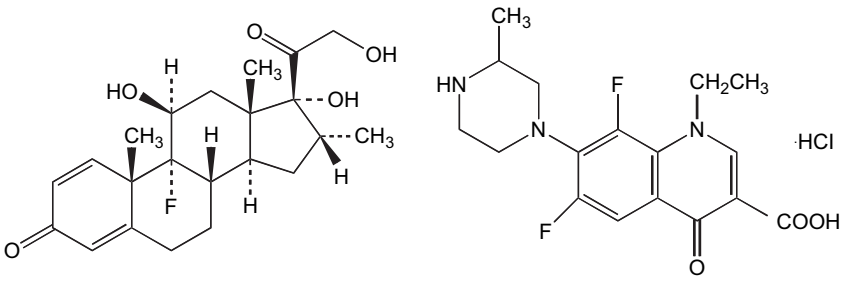

DM LFLX

FIGURE 1. Structure of DM and LFLX. Dexamethasone (DM): 9-fluoro-11β, 17, 21-trihydroxy-16α-methylpregna-1, 4-diene-3, 20-dione, lomefloxacin hydrochloride (LFLX): (±)-1-ethyl-6, 8-difluoro-1, 4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid hydrochloride.

and partition in intact and de-epithelialized corneas. The time-dependent permeation of the drugs was measured across the isolated rabbit cornea using a diffusion chamber at various temperatures. Dexamethasone (DM, 9-fluoro-11β, 17, 21-trihydroxy-16α-methylpregna-1, 4-diene-3, 20-dione, MW: 392) is an anti-inflammatory steroidal drug, and lomefloxacin hydrochloride (LFLX, (±)-1-ethyl-6, 8-difluoro-1, 4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid hydrochloride, MW: 388) is a fluoroquinolone anti-microbial drug (Figure 1). They have the potential to be used in the topical treatment of ocular disease. DM and LFLX are model pharmaceuticals of hydrophobic and hydrophilic drugs to investigate the corneal permiation, since their octanol-water partition coefficients (Log P) are 1.49 (Schoenwald et al., 1978) and −1.03 (pH7.4) (Abbott Japan Co., Ltd., 2005), respectively.

We also examined the effect of BAK on activation energy for diffusion and partition of DM and LFLX in intact and de-epithelialized corneas.

EXPERIMENTAL SECTION

Materials

Lomefloxacin hydrochloride (LFLX) was obtained from Abbott Japan (Minato-ku Tokyo, Japan). Dexamethasone (DM), methanol (HPLC grade), and acetonitrile (HPLC grade) were purchased from Nacalai Tesque (Nakagyo-ku, Kyoto, Japan). Benzalkonium chloride (BAK) solution (10%, JP grade) was purchased from Nihon Pharmaceutical (Chiyoda-ku, Tokyo, Japan). All reagents were of the highest grade commercially available or HPLC grade. Water was purified with a Milli-Q purification system (Millipore, Tokyo, Japan). Male Nippon albino rabbits (about 2 kg) were supplied from Japan Laboratory Animals (Nerima-ku, Tokyo, Japan). Standard solutions of DM and LFLX were prepared by dissolving each of them (20 mg) in 50% acetonitrile (20 mL) or 50% methanol (20 mL), respectively. A portion of each solution was diluted with water for various concentrations of working standard solutions.

In Vitro Corneal Permeation Experiments

Rabbits were sacrificed by intravenous injection of excess sodium pentobarbital. Their corneas with surrounding stroma were dissected and mounted in a side-by-side diffusion chamber (Ohtori et al., 1994). For some experiments, the corneas were de-epithelialized by carefully scraping away the corneal epithelium with a surgical knife until the stroma was exposed. All experimental procedures were approved by the institutional Committee for the Care and Use of Laboratory Animals.

Test solution (0.3% LFLX in 2.6% glycerine (pH5.0) or 0.008% DM in receptor buffer (pH7.2), 4.5 mL) with or without 0.01% BAK was added to the epithelial side (donor side) of diffusion chamber. Receptor buffer (4.5 mL) was added to the endothelial side (receiver side). Receptor buffer was composed of 0.013% calcium chloride, 0.04% potassium chloride, 0.02% magnesium sulfate, 0.019% sodium dihydrogenphosphate dihydrate, 0.787% sodium chloride, and 0.1% glucose, and adjusted to pH 7.2. The temperature of the diffusion chamber was maintained at 20C, 34C, and 40°C. Samples (100 μL) were withdrawn from endothelial solution of each diffusion chamber at specified time points for assay of the drugs by HPLC. After the sampling, 100 μL of fresh receptor buffer was added to maintain a constant endothelial volume.

Data Analysis

Permeation parameters were calculated according to Fick's second law of diffusion. The steady-state rates of drug permeation (dQ/dt) and the lag time (td) were estimated from the slope and x-intercept of linear regression line obtained from permeation profile of cumulative amount in receiver chamber. The permeability coefficient P (cm/sec), diffusion coefficient D (cm^2/sec) and partition coefficient K (no unit) are given by Eqs. (1–3):

$$P = \frac{dQ}{dt} \cdot \frac{1}{C} \tag{1}$$

$$D = \frac{h^2}{6td} \tag{2}$$

$$K = \frac{dQ}{dt} \cdot \frac{h}{D \cdot C} \tag{3}$$

where C is the drug concentration in the donor solution (DM, 80 μg/mL; LFLX, 3000 μg/mL), and h is the thickness of the cornea (0.04 cm).

The energy of activation (Ea) was estimated using the Arrhenius equation (4):

$$k = Ae^{-Ea/RT} \tag{4}$$

where k is the specific reaction rate, A is a constant commonly referred to as the frequency factor, R is the gas constant, and T is temperature. Plots of log P, log D, and log K versus $1/T$ were constructed, and their slopes determined. The slope of the plot can be related to Ea in the following manner (5):

$$m = -Ea / 2.303R \tag{5}$$

where m is the slope of the plot for the drugs.

High-Performance Liquid Chromatography (HPLC)

An HPLC system (TOSOH 8020, Tosoh, Minato-Ku, Tokyo, Japan) was composed of an autosampler (AS-8020), a pump (DP-8020), a column oven (CO-8020), a UV detector (UV-8020), and a data processing software (LC-8020). An octadecylsilica column (TSK-gel ODS-80Ts, 150 × 4.6 mm i.d., Tosoh) was used. Analysis of DM was carried out using 50% acetonitrile as mobile phase at a flow-rate of 0.7 mL/min at 40°C. Analysis of LFLX was also carried out using a mixture of 30 mM ammonium phosphate buffer (pH 2.8) containing 5 mM sodium 1-pentanesulfonate and methanol (62.5:37.5, v/v) as mobile phase at a flow-rate of 0.8 mL/min at 40°C. Detection of DM and LFLX were performed at 240 and 280 nm, respectively. The injection volume was 50 μL.

RESULTS

Corneal Permeability of DM and LFLX

The time dependent permeation of DM and LFLX across the isolated rabbit cornea was examined using a diffusion chamber. The corneal permeability of DM across the intact cornea was increased by increasing the temperature (Figure 2a).

The corneal permeability of DM across the de-epithelialized cornea was also increased by increasing the temperature (Figure 2b). The cumulative amounts of DM across the de-epithelialized cornea were larger than that across the intact cornea at each temperature. Similar to the corneal permeability of DM, the corneal permeabilities of LFLX across both the intact and de-epithelialized corneas were increased by increasing the temperature, and the cumulative amounts of LFLX across the de-epithelialized cornea were larger than that across the intact cornea at each temperature (Figure 2c, d).

Permeation parameters (permeability coefficient P, diffusion coefficient D and partition coefficient K) of DM and LFLX are calculated from the slope and x-intercept of linear regression line obtained from each permeation profile. These parameters are summarized in Table 1.

The temperature-dependent permeability coefficient of DM across the intact cornea was 10.38-fold increased from 5.05×10^{-7} cm/sec at 20°C to 5.24×10^{-6} cm/sec at 40°C.

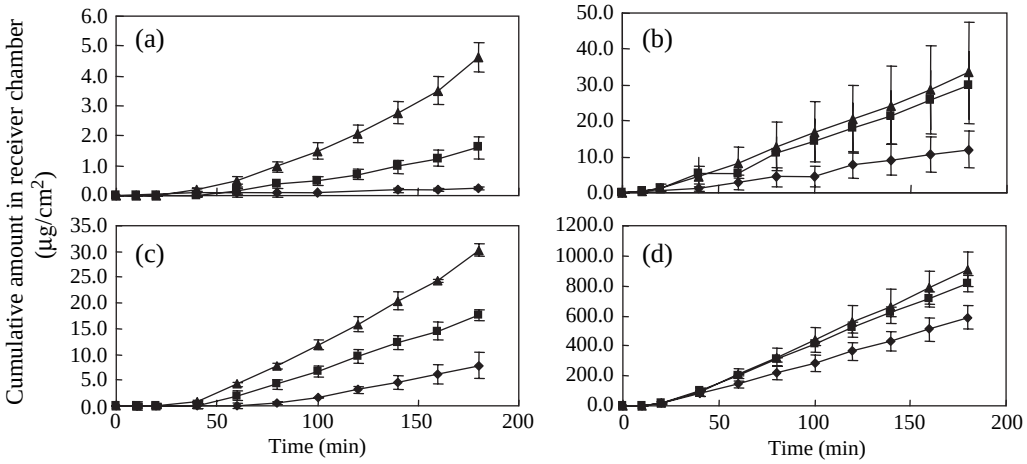

FIGURE 2. Permeation profiles for DM across (a) intact and (b) de-epithelialized cornea, and for LFLX across (c) intact, and (d) de-epithelialized. Each point represents the mean ± SD (n = 3). ◆, 20°C; ■, 34°C; ▲, 40°C.

TABLE 1
Permeation Parameters of DM and LFLX for the Intact and De-epithelialized Cornea

	Lag Time (h)	Permeability Coefficient (P, cm/sec)	Diffusion Coefficient (D, cm^2/sec)	Partition Coefficient (K, no unit)
DM				
Intact				
20°C	1.4 ± 0.1	$5.05 \times 10^{-7} \pm 3.98 \times 10^{-8}$ (1.00)	$5.22 \times 10^{-8} \pm 4.92 \times 10^{-9}$ (1.00)	$1.40 \times 10^{3} \pm 2.42 \times 10^{2}$ (1.00)
34°C	1.0 ± 0.1	$2.38 \times 10^{-6} \pm 1.38 \times 10^{-7}$ (4.71)	$7.71 \times 10^{-8} \pm 9.64 \times 10^{-9}$ (1.48)	$4.48 \times 10^{3} \pm 3.83 \times 10^{2}$ (3.20)
40°C	0.8 ± 0.1	$5.24 \times 10^{-6} \pm 5.98 \times 10^{-7}$ (10.38)	$9.11 \times 10^{-8} \pm 6.89 \times 10^{-9}$ (1.75)	$8.30 \times 10^{3} \pm 8.61 \times 10^{2}$ (5.93)
De-epithelialized				
20°C	0.5 ± 0.3	$1.76 \times 10^{-5} \pm 6.31 \times 10^{-6}$ (1.00)	$2.02 \times 10^{-7} \pm 1.30 \times 10^{-9}$ (1.00)	$1.34 \times 10^{4} \pm 3.19 \times 10^{3}$ (1.00)
34°C	0.3 ± 0.1	$2.26 \times 10^{-5} \pm 3.52 \times 10^{-6}$ (1.28)	$2.43 \times 10^{-7} \pm 4.43 \times 10^{-8}$ (1.20)	$1.56 \times 10^{4} \pm 7.59 \times 10^{2}$ (1.16)
40°C	0.3 ± 0.0	$4.52 \times 10^{-5} \pm 8.44 \times 10^{-6}$ (2.57)	$2.69 \times 10^{-7} \pm 6.93 \times 10^{-9}$ (1.33)	$2.42 \times 10^{4} \pm 5.13 \times 10^{3}$ (1.81)
LFLX				
Intact				
20°C	1.2 ± 0.1	$3.82 \times 10^{-7} \pm 1.51 \times 10^{-7}$ (1.00)	$6.15 \times 10^{-8} \pm 6.27 \times 10^{-9}$ (1.00)	$9.27 \times 10^{2} \pm 4.64 \times 10^{2}$ (1.00)
34°C	0.8 ± 0.1	$7.60 \times 10^{-7} \pm 6.06 \times 10^{-8}$ (1.99)	$9.08 \times 10^{-8} \pm 1.48 \times 10^{-8}$ (1.48)	$1.23 \times 10^{3} \pm 2.18 \times 10^{2}$ (1.33)
40°C	0.7 ± 0.0	$1.19 \times 10^{-6} \pm 2.45 \times 10^{-8}$ (3.12)	$1.03 \times 10^{-7} \pm 5.77 \times 10^{-9}$ (1.67)	$1.67 \times 10^{3} \pm 7.16 \times 10^{1}$ (1.80)
De-epithelialized				
20°C	0.3 ± 0.0	$1.91 \times 10^{-5} \pm 2.95 \times 10^{-6}$ (1.00)	$2.75 \times 10^{-7} \pm 3.74 \times 10^{-8}$ (1.00)	$1.00 \times 10^{4} \pm 8.13 \times 10^{2}$ (1.00)
34°C	0.2 ± 0.0	$2.63 \times 10^{-5} \pm 9.39 \times 10^{-7}$ (1.38)	$3.09 \times 10^{-7} \pm 0.00 \times 10^{0}$ (1.12)	$1.23 \times 10^{4} \pm 4.38 \times 10^{2}$ (1.23)
40°C	0.2 ± 0.1	$2.94 \times 10^{-5} \pm 4.21 \times 10^{-6}$ (1.54)	$3.22 \times 10^{-7} \pm 6.72 \times 10^{-8}$ (1.17)	$1.34 \times 10^{4} \pm 2.67 \times 10^{3}$ (1.34)

Values represent means ± *SD* (n = 3). Parenthesis indicate ratio to value at 20°C.

This was attributed primarily to the 5.93-fold increase of the partition coefficient from 1.40×10^3 at 20°C to 8.30×10^3 at 40°C. The DM permeability coefficient of the de-epithelialized cornea was 2.57-fold increased from 1.76×10^{-5} cm/sec at 20°C to 4.52×10^{-5} cm/sec at 40°C. Small changes in incremental ranges of diffusion and partition coefficients were observed on the de-epithelialized cornea by increasing the temperature compared to the intact cornea. When LFLX was used, the permeability coefficient across the intact and de-epithelialized cornea was 3.12-fold and 1.54-fold enhanced from 20 to 40°C, respectively (Table 1). In contrast to DM, little changes in incremental ranges of diffusion and partition coefficients of LFLX were observed on both the intact and de-epithelialized corneas by increasing the temperature. The results showed that corneal permeation for a hydrophobic drug of DM was more sensitive for temperature than that for a hydrophilic drug of LFLX, and that partition of DM to the corneal epithelium was specifically influenced by temperature.

Effect of BAK on Corneal Permeability of DM and LFLX

The time dependent permeation of DM and LFLX was examined across the isolated rabbit cornea at 34°C in the presence of 0.01% BAK. The permeation of DM and LFLX across the intact cornea was dramatically increased by the addition of BAK to the donor solution (Figure 3a, c).

However, no changes in permiation profiles were observed across the de-epithelialized cornea (Figure 3b, d). BAK had an effect on the epithelium to increase the permeation of the drugs.

We also examined the time dependent permeation of DM and LFLX with BAK in the donor solution across the intact and de-epithelialized cornea at 20 and 40°C to calculate the permeation parameters from each permeation profile (permeation profiles not shown). The permeation parameters are summarized in Table 2.

The DM permeability coefficient across the intact cornea was 3.40-fold increased from 9.37×10^{-7} cm/sec at 20°C to 3.19×10^{-6} cm/sec at 40°C. This increase was less than the 10.38-fold-increase in the absence of BAK (see Table 1). The smaller incremental range of the permeability coefficient was due to a decrease for partition coefficient of the 1.81-fold-increase with BAK in comparison with the 5.93-fold-increase without BAK (see Table 1). The DM permeability coefficient in the presence of BAK across the de-epithelialized cornea was 2.45-fold increased from 1.36×10^{-5} at 20°C to 3.33×10^{-5} cm/sec at 40°C. This incremental range of permeability coefficient was almost equivalent to that without BAK (2.57-fold, see Table 1). The effect of the temperature on the DM partition coefficient to the corneal epithelium was decreased by adding BAK to the donor solution. When LFLX was used, the permeability coefficient in the presence of BAK across the intact and de-epithelialized cornea was 4.24-fold and 2.47-fold increased from 20 to 40°C, respectively (Table 2). Marked changes in

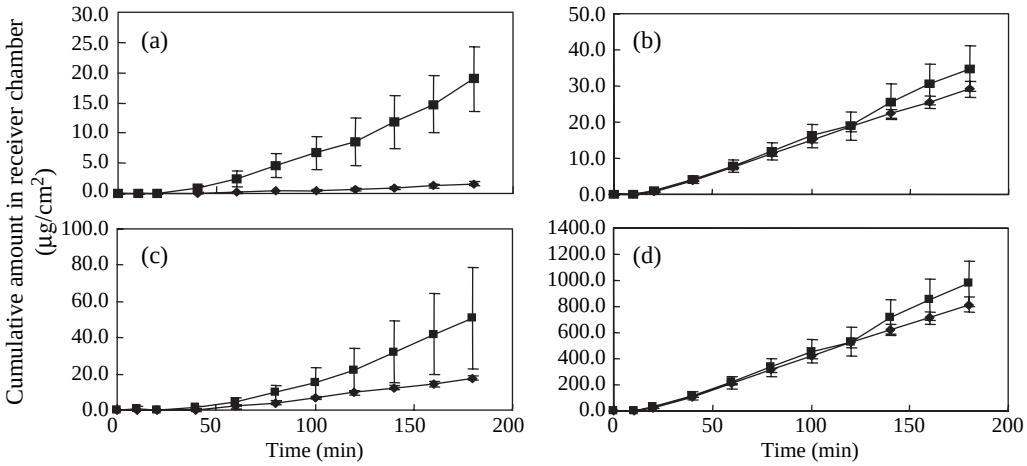

FIGURE 3. Effect of BAK on permeation profiles for DM across (a) intact and (b) de-epithelialized cornea, and for LFLX across (c) intact and (d) de-epithelialized. Each point represents the mean ± *SD* (*n* = 3). ◆, without; ■, 0.01% BAK.

TABLE 2
Effect of 0.01% BAK on Permeation Parameters of DM and LFLX for the Intact and De-epithelialized Cornea

	Lag Time (*h*)	Permeability Coefficient (P, cm/sec)	Diffusion Coefficient (D, cm^2/sec)	Partition Coefficient (K, no unit)
DM				
Intact				
20°C	1.1 ± 0.0	$9.37 \times 10^{-6} \pm 2.34 \times 10^{-6}$ (1.00)	$6.78 \times 10^{-8} \pm 1.30 \times 10^{-9}$ (1.00)	$2.00 \times 10^{4} \pm 5.29 \times 10^{3}$ (1.00)
34°C	0.7 ± 0.1	$2.53 \times 10^{-5} \pm 7.63 \times 10^{-6}$ (2.70)	$1.07 \times 10^{-7} \pm 1.29 \times 10^{-8}$ (1.58)	$3.35 \times 10^{4} \pm 6.11 \times 10^{3}$ (1.68)
40°C	0.6 ± 0.0	$3.19 \times 10^{-5} \pm 1.15 \times 10^{-5}$ (3.40)	$1.27 \times 10^{-7} \pm 3.30 \times 10^{-9}$ (1.87)	$3.62 \times 10^{4} \pm 1.32 \times 10^{4}$ (1.81)
De-epithelialized				
20°C	0.5 ± 0.1	$1.36 \times 10^{-5} \pm 7.44 \times 10^{-7}$ (1.00)	$1.52 \times 10^{-7} \pm 2.55 \times 10^{-8}$ (1.00)	$1.32 \times 10^{4} \pm 2.65 \times 10^{3}$ (1.00)
34°C	0.4 ± 0.0	$3.05 \times 10^{-5} \pm 8.50 \times 10^{-6}$ (2.24)	$1.93 \times 10^{-7} \pm 2.89 \times 10^{-9}$ (1.27)	$2.28 \times 10^{4} \pm 6.72 \times 10^{3}$ (1.73)
40°C	0.4 ± 0.0	$3.33 \times 10^{-5} \pm 9.79 \times 10^{-6}$ (2.45)	$1.84 \times 10^{-7} \pm 1.43 \times 10^{-8}$ (1.21)	$2.58 \times 10^{4} \pm 5.89 \times 10^{3}$ (1.95)
LFLX				
Intact				
20°C	1.1 ± 0.0	$9.50 \times 10^{-7} \pm 7.07 \times 10^{-7}$ (1.00)	$6.68 \times 10^{-8} \pm 2.80 \times 10^{-9}$ (1.00)	$2.01 \times 10^{3} \pm 1.41 \times 10^{3}$ (1.00)
34°C	0.9 ± 0.2	$1.96 \times 10^{-6} \pm 1.17 \times 10^{-6}$ (2.06)	$8.70 \times 10^{-8} \pm 2.23 \times 10^{-8}$ (1.30)	$3.61 \times 10^{3} \pm 2.41 \times 10^{3}$ (1.80)
40°C	0.7 ± 0.0	$4.03 \times 10^{-6} \pm 6.88 \times 10^{-7}$ (4.24)	$9.94 \times 10^{-8} \pm 5.32 \times 10^{-9}$ (1.49)	$5.82 \times 10^{3} \pm 6.98 \times 10^{2}$ (2.90)
De-epithelialized				
20°C	0.4 ± 0.0	$1.30 \times 10^{-5} \pm 1.32 \times 10^{-6}$ (1.00)	$2.12 \times 10^{-7} \pm 2.75 \times 10^{-8}$ (1.00)	$8.91 \times 10^{3} \pm 9.70 \times 10^{2}$ (1.00)
34°C	0.2 ± 0.0	$2.82 \times 10^{-5} \pm 5.35 \times 10^{-6}$ (2.17)	$3.19 \times 10^{-7} \pm 2.99 \times 10^{-8}$ (1.50)	$1.27 \times 10^{4} \pm 1.54 \times 10^{3}$ (1.43)
40°C	0.3 ± 0.0	$3.21 \times 10^{-5} \pm 4.14 \times 10^{-6}$ (2.47)	$2.93 \times 10^{-7} \pm 5.40 \times 10^{-8}$ (1.38)	$1.60 \times 10^{4} \pm 1.60 \times 10^{4}$ (1.80)

Values represent means ± *SD* (*n* = 3). Parenthesis indicate ratio to value at 20°C.

incremental ranges of the LFLX permeability coefficients were not observed compared to that of without BAK.

Activation Energies for the Movement of DM and LFLX Across Cornea

Activation energies for the movement of DM and LFLX across the intact and de-epithelialized corneas were estimated using the Arrhenius equation from the permeation parameters. The results are summarized in Table 3.

The activation energy for permeability of DM across the intact cornea was 88.0 kJ/mol, which consisted of 21.1 kJ/mol for diffusion and 66.8 kJ/mol for partition. By removing the epithelium, the activation energy for permeability decreased to 33.1 kJ/mol. The activation energies for diffusion and partition also decreased to 10.7and 19.8 kJ/mol, respectively. When LFLX was used, the

TABLE 3
Activation energy of DM and LFLX Permeation of the Intact and De-epithelialized Cornea

	Activation Energy (kJ/mol)			
	Without		0.01% BAK	
	Intact	De-epithelialized	Intact	De-epithelialized
DM				
Permeability	88.0 (1.00)	33.1 (0.38)	47.9 (0.54)	35.9 (0.41)
Diffusion	21.2 (0.24)	10.7 (0.12)	24.0 (0.27)	8.3 (0.09)
Partition	66.8 (0.76)	19.8 (0.23)	23.6 (0.27)	26.3 (0.30)
LFLX				
Permeability	42.1 (1.00)	16.6 (0.40)	52.0 (1.24)	35.8 (0.85)
Diffusion	19.9 (0.47)	6.1 (0.15)	15.0 (0.36)	14.2 (0.34)
Partition	21.0 (0.50)	11.1 (0.26)	38.8 (0.92)	21.7 (0.52)

Parenthesis indicate ratio to value of intact cornea without BAK.

activation energy for permeability across the intact cornea was 42.1 kJ/mol, which consisted of 19.9 kJ/mol for diffusion and 21.0 kJ/mol for partition. The activation energy for permeability across the de-epithelialized cornea decreased to 16.6 kJ/mol. It consisted of the activation energies for diffusion and partition that were 6.1and 11.1 kJ/mol, respectively. The activation energies of permeability for DM and LFLX became below the half by removing the epithelium. This suggested that the epithelium was a critical barrier in the cornea for permeability of a hydrophobic drug of DM as well as a hydrophilic drug of LFLX. The endothelium could not be a barrier against the permeability for drugs due to its single-layer film (Patrick et al., 1993). These findings support the conclusion that epithelium contributes to the corneal permeability barrier (Huang et al., 1983).

The activation energies for movement of DM and LFLX across the intact and de-epithelialized corneas in the presence of 0.01% BAK in the donor solution were estimated. The activation energy of permeability for DM across the intact cornea was 47.9 kJ/mol, which consisted of 24.0 kJ/mol for diffusion and 23.6 kJ/mol for partition. The activation energy of permeability for DM was decreased to about half from 88.0 to 47.9 kJ/mol by addition of BAK to the donor solution. The activation energy of diffusion was not changed, while the activation energy of partition was decreased to about one third from 66.8to 23.6 kJ/mol. The activation energy of permeability for DM against the de-epithelialized cornea was 35.9 kJ/mol. It consisted of the activation energies for diffusion and partition that were 8.3and 26.3 kJ/mol, respectively. No changes of the activation energies for each parameter were observed for the de-epithelialized cornea by the addition of BAK. The results indicated that BAK increased the partition of DM to the corneal epithelium. When LFLX was used in the presence of BAK in the donor solution, the activation energy for permeability across the intact cornea was 52.0 kJ/mol, which consisted of 15.0 kJ/mol for diffusion and 38.8 kJ/mol for partition. The activation energy of permeability for LFLX across the de-epithelialized cornea was 35.8 kJ/mol. It consisted of the activation energies for diffusion and partition that were 14.2and 21.7 kJ/mol, respectively. The activation energy of permeability for LFLX was increased in the presence of BAK. The activation energy of each parameter for the de-epithelialized cornea increased more remarkably. This suggested that stroma was a major barrier in the cornea for permeability of LFLX in the presence of BAK.

DISCUSSION

The poor bioavailability of drugs is a problem in the ophthalmic area (Lee, 1990). This is because the cornea prevents the passage of the drug molecules (Lee et al., 1986). There have been many reports on the characterization of corneal permeability (Prausnitz et al., 1998) and models of transcorneal drug transport (Cooper et al., 1987; Edward et al., 2001; Grass et al., 1988a; Huang et al., 1983; Worth et al., 2000; Yoshida et al., 1996). Although a methodology has been needed for an evaluation of mechanism of the drug permeability in the cornea, no standard methods have been developed.

A thermodynamic analysis is one of the approches to reveal mechanisms of trans-membrane of drug molecules. In this study, we applied a thermodynamic approach by determining individual activation energy for diffusion and partition to obtain information about the critical barrier of corneal drug permeation. The activation energies for permeability across the intact cornea were 88.0 and 42.1 kJ/mol for DM and LFLX, respectively. The values were significant larger than that of water, butanol and glycerol; approx. 17 to 27 kJ/mol (Grass et al., 1988b). It is probably that DM and LFLX need energy for diffusion steps as well as partitioning steps. In contrast, water, butanol, and glycerol are moving through an

aqueous medium only, since they are very small molecular weight and high hydrophilic compounds. Therefore, few or no partitioning steps for the compounds are required to pass through the cornea (Grass et al., 1988b).

We next evaluated individual activation energies of diffusion and partition for DM and LFLX. For DM, the activation energy of partition across the intact cornea was 3-fold larger than that of diffusion. For LFLX, the activation energies of diffusion and partition across the intact cornea were almost the same. However, decreasing of the activation energy of diffusion by removing the epithelium was larger than that of partition. It was suggested that the partition step in the epithelium of the cornea was the primary barrier for a hydrohobic drug of DM. On the other hand, the diffusion step in the epithelium was the primary barrier for a hydrophilic drug of LFLX.

Benzalkonium chloride showed the highest effect of promoting corneal permeation of DM. The partition of DM to the corneal epithelium was increased by the addition of 0.01% BAK in the donor solution. This is supported by a previous report that the superficial surface of the corneal epithelium contributes to the drug permeation barrier (Klyce et al., 1985). BAK causes cells of the corneal epithelium to peel at their borders (Pfister et al., 1976) and shows the promoting effect on corneal drug permeation (Sasaki et al., 1995). However, BAK does not always decrease the activation energy of permeability. Interestingly, the activation energy of permeability for LFLX was increased in the presence of BAK. Furthermore, the decreasing of the activation energy of the partition by removing the epithelium was larger than that of diffusion. This behavior of LFLX in the presence of BAK is like a hydrophobic drug. The diffusion and partition coefficient may be changed due to formation of an ion pair between carbonic acid of LFLX and quaternary ammonium salt of BAK (Higashiyama et al., 2004), although additional investigations will be needed to reveal the detailed mechanisms.

CONCLUSIONS

The effect of temperature on the corneal permeability of drugs using model pharmaceuticals DM and LFLX was examined. The permeability of both hydrophobic and hydrophilic drugs against the cornea was increased by the increase in temperature. The analysis of the mechanism of the corneal permeability became possible by the evaluation of activation energy from the data of *in vitro* corneal permeability experiments. It become clear that the partition step and the diffusion step in the epithelium of the cornea were the primary barrier for a hydrohobic drug of DM and a hydrophilic drug of LFLX, respectively. Furthermore, BAK increased the partition of DM to the corneal epithelium. A thermodynamic approach of activation energy for the permeation parameters of drugs is a good approach to investigate the mechanism of drug permeability.

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