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Estimation and enhancement of in vitro corneal transport of S-1033, a novel antiglaucoma medication

Kazutaka Higaki*, Masaharu Takeuchi, Masayuki Nakano

Developmental Research Laboratories, Shionogi and Co., Ltd., 3-1-1 Futaba-cho, Toyonaka, Osaka, 561, Japan

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Abstract

To improve the in vitro corneal transport of S-1033, a novel prostaglandin-derivative antiglaucoma medication, the effects of several factors such as benzalkonium chloride (BAC), concentration of S-1033, and lipophilicity on the corneal permeability of S-1033 were investigated. The apparent permeability coefficient (P_{app}) of S-1033 was 5.81×10^{-7} cm/s on application of 0.1 mM solution. The addition of BAC (0.005%) enhanced by 2.5-fold the transport percent and P_{app} of S-1033. Corneal transport tended to be improved by methyl esterification of S-1033 but not by the coexistence of BAC with S-1033 methyl ester. TLC analysis showed that S-1033 methyl ester was hydrolyzed to S-1033 during passage through the cornea. The therapeutic concentration of S-1033 (5.4 mM, the highest concentration in the experiments), greatly promoted its transport and increased P_{app}. However, there was little difference in the uptake percent, which showed the transcorneal percent and corneal accumulation percent at steady state, for these dosing conditions. The highest concentration gave the lowest corneal accumulation percent and the highest corneal clearance, indicating that the transport rate of S-1033 in the cornea to the receptor side was the largest, which may have been responsible for the marked improvement of the transcorneal transport of S-1033. Increases in corneal accumulation of S-1033 due to methyl esterification and BAC addition seem to be due to the increase of lipophilicity and ion pair formation, respectively. To investigate the relationship between corneal permeability and the partition coefficient (PC) with or without BAC, the permeabilities of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) were estimated. The larger the value of logPC in the range of -0.67–3.89, the larger was the value of P_{app} in the absence of BAC. However, in the presence of BAC, there was a parabolic relationship, which suggests that BAC can greatly affect the interaction between cornea and drugs.

Keywords: Corneal transport; Antiglaucoma medication; Prostaglandin derivative; Benzalkonium chloride; Lipophilicity

1. Introduction

Glaucoma is one of the most serious diseases of the eye, leading to irreversible blindness due to intraocular high pressure, if untreated. To reduce the intraocular pressure occurring in glaucoma,

* Corresponding author. Tel.: +81 06 331 8081, ext. 344; fax: +81 06 332 6385.

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three groups of drugs (miotics, adrenergic agonists, and carbonic anhydrase inhibitors) have been used (Akers et al., 1977). However, they have the disadvantages of a short therapeutic effect (Camber et al., 1986) and a systemic side effect (Fraunfelder and Meyer, 1987; Buclin et al., 1991). Therefore, a new type of antiglaucoma medication is needed. One candidate has been prostaglandin because prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and E_2 (PGE_2) have long-lasting and highly significant activity for decreasing intraocular pressure (Camras et al., 1977; Camras and Bito, 1981; Stern and Bito, 1982). However, they also have several adverse effects which must be overcome before they can be clinically used; they can cause initial hypertension (Eakins, 1977; Camras et al., 1977; Camras and Bito, 1981; Stern and Bito, 1982), inflammatory response, breakdown of the blood-aqueous barrier (Eakins, 1977), and systemic side effects.

S-1033, (5Z,9α,11α,13E)-9,11-dihydroxyprosta-5,13-dienoic acid sodium salt, is a prostaglandin derivative which is promising as an antiglaucoma treatment because it can reduce the intraocular pressure without causing initial hypertension (Goh et al., 1994) or an inflammatory response (Goh and Kishino, 1994). Also, S-1033 has extremely low autacoid activity and therefore would not cause adverse systemic effects (Goh and Kishino, 1994).

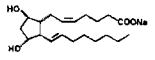
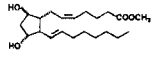
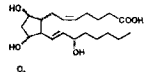
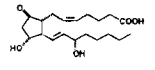
When [^{14}C]S-1033 was instilled into the eyes of white rabbits, the total radioactivity was distributed to the ocular tissues at high concentrations while the total radioactivity and S-1033 very rapidly appeared in the systemic plasma (Higaki et al., 1995). The highest level of total radioactivity was found in the cornea, and the uvea (iris and ciliary body), the probable target of S-1033, showed the second highest level among the ocular tissues (Higaki et al., 1995). The transport pathway of S-1033 to the uvea was suggested to be via the 'cornea — aqueous humor — uvea' route. As S-1033 in aqueous humor became concentrated in the uvea (Higaki et al., 1995), the corneal permeability of S-1033 would be responsible for its delivery to the uvea.

In the present study, we estimated the corneal permeability of S-1033 and tried to enhance its corneal transport in in vitro corneal transport experiments. We also studied the relationship between corneal permeability and lipophilicity, and the effect of benzalkonium chloride (BAC) on this relationship for prostaglandin derivatives including S-1033.

2. Materials and methods

2.1. Materials

S-1033 (Kishi et al., 1992), ^{14}C-labeled S-1033 ([^{14}C]S-1033, 6.96 MBq/mg as a free acid), and ^{14}C-labeled (5Z,9α,11α,13E)-9,11-dihydroxyprosta-5,13-dienoic acid methyl ester ([^{14}C]S-1033 methyl ester, 6.13 MBq/mg) (Scheme 1) were synthesized at the Developmental Research Laboratories, Shionogi and Co., Ltd. Their radiochemical purity was greater than 98.5% on TLC (silica gel HPTLC plate 60 F_{254}, Merck, Darmstadt, Germany; chloroform/methanol/acetic acid, 20:2:1 for S-1033; 40:2:1 for S-1033 methyl ester; v/v), and this high level of purity was maintained for several months. [^{3}H]-Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 20.6 GBq/mg) and [^{3}H]prostaglandin E_2 (PGE_2, 18.8 GBq/mg) were purchased from Amersham International plc (Amersham, UK). The chemical structures are shown in Scheme 1. All other reagents were of analytical grade and were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

	Chemical Structures	Molecular Weight	Log PC	
			without BAC	with BAC
S-1033		360	1.29	1.50
S-1033 methyl ester		352	3.89	3.87
PG F2α		354	-0.42	-0.30
PG E2		352	-0.67	-0.38

Scheme 1. Chemical structures, molecular weight, and logPC values with or without benzalkonium chloride. LogPC values are expressed as the means of three experiments.

2.2. Preparation of dosing solutions

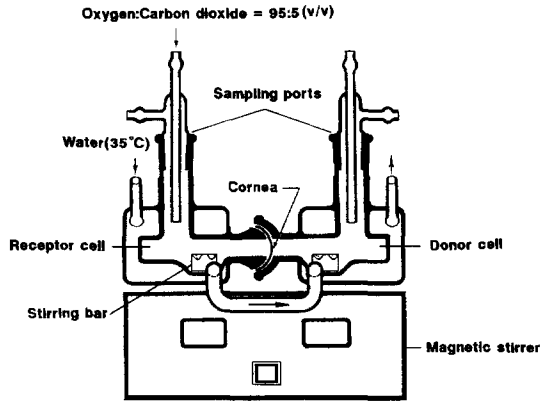
The test compound was dissolved in glutathione bicarbonate Ringer (GBR) solution (O'Brien and Edelhauser, 1977; Schoenwald and Huang, 1983) at 0.1 mM or 5.4 mM with or without 0.005% BAC, which was aerated with 95% oxygen and 5% carbon dioxide. The concentration of S-1033 methyl ester solution without BAC was 0.018 mM due to its low solubility. No degradation of [^{14}C]S-1033 in the dosing solution was recognized from estimation of the radiochemical purity of [^{14}C]S-1033 in the donor cell before and after the corneal transport study.

2.3. Animals

Male Japanese white rabbits (Kbl:JW; 11 weeks old, Kitayama Labes Co., Ltd., Japan) were used in all experiments.

2.4. In vitro corneal transport study

A rabbit was anesthetized by injecting 2 ml of 6.5% pentobarbital into the marginal or central ear vein. The abdomen was dissected at the midline and the rabbit was sacrificed by bleeding from the inferior vena cava and aorta. The eye was immediately proptosed, a small transverse hole was made by needle (25G) posteriorly from the limbus, and the cornea with the scleral ring was carefully cut out with a small scissors. The isolated cornea was carefully placed on the side-by-side diffusion cell system (Scheme 2), which maintained the cornea curvature. The diffusion cell system was kept at 35°C. First, 3.5 ml of GBR buffer at 35°C was added to the endothelial side (receptor), and the corneal transport experiment was started by adding 3.5 ml of test solution at 35°C to the epithelial side (donor). The solutions in both sides of the diffusion cell system were stirred with a magnetic stirrer and bubbled with 95% oxygen and 5% carbon dioxide throughout the experiments. Samples of 150 μl were drawn from the receptor side at set times over 4 h. An equal volume of GBR buffer was immediately added to the receptor side after each sampling. In some cases, 50-μl samples were drawn from the donor side every 1 h up to 4 h without addition of GBR buffer. At the end of the experiment, solutions from both sides and the cornea were tested for the remaining drug.



Scheme 2. Side-by-side diffusion cell system for in vitro corneal transport studies.

2.5. Analytical procedure

2.5.1. Radioactivity in GBR solution, 1-octanol, and cornea

The cornea after digestion with 2 ml of Soluene-350 (Packard), a portion of 1-octanol for the partition coefficient study, or a portion of GBR solution for the corneal transport study was dissolved in 10 ml of Picofluor 40 (Packard). Radioactivity was measured with a liquid scintillation counter (model Tri-Carb 2000CA, Packard Instrument Co., Downers Grove, IL).

2.5.2. Determination of radioactive unchanged drug and S-1033 in GBR solution and 1-octanol

An aliquot of radioactive GBR solution or radioactive 1-octanol was diluted with methanol and spotted on an HPTLC plate with unlabeled authentic drug. The plate was developed with chloroform/methanol/acetic acid (20:2:1, v/v for S-1033; 40:2:1, v/v for S-1033 methyl ester) or toluene/ethyl acetate/methanol/acetic acid (20:80:3:2, v/v for $PGF_{2\alpha}$, PGE_2). The plates were visualized under I_2 vapor and radioactive zones were located radioluminographically with a Bio-Imaging Analyzer (BAS2000; Fuji Photo Film

Co., Ltd., Tokyo, Japan). The silica gel of interest was scraped off the plate and transferred to the counting vial, to which 1 ml of distilled water had been added. Picofluor 40 (10 ml) was then added, and the radioactivity was measured on a Tri-Carb 2000CA liquid scintillation counter.

2.6. Calculation of apparent permeability coefficients, lag times and corneal clearances

The apparent corneal permeability coefficient (P_{app}) was determined according to Eq. (1) (Schoenwald and Huang, 1983; Camber, 1985):

$$P_{app} = \frac{\Delta Q}{\Delta t \cdot 60 \cdot A \cdot C_0} (cm/s) \quad (1)$$

where Q reveals the total amount of unchanged drug or S-1033 permeated at time t and $\Delta Q/\Delta t$, 60, A and C_0 represent the slope of the linear portion of the Q–time graph, the conversion of minutes to seconds, the corneal surface area (0.6 cm^2), and the initial concentration of drug in the donor cell, respectively. Lag time is the X intercept when the linear portion of the Q–time graph is extrapolated. Corneal clearance (CL_{cornea}) describing the transport from cornea to the receptor cell at steady state was calculated from Eq. (2).

$$CL_{cornea} = \frac{\Delta Q}{\Delta t \cdot corneal\ amount} (cornea/\text{min}) \quad (2)$$

2.7. Partition coefficient studies

Distilled water and 1-octanol were vigorously shaken and allowed to stand for 24 h at 25°C. The octanol phase was removed to another test tube and shaken with GBR solution of test compound for 1 h at 25°C. Initial concentrations of test compounds were equal to those in the in vitro corneal transport studies except for 5.4 mM S-1033. The two phases were separated by centrifugation at 3000 rev./min for 20 min. The partition coefficient (PC) for the unchanged drug was calculated according to Eq. (3) after determination of the unchanged drug concentrations in both phases.

$$PC = \frac{concentration\ of\ unchanged\ drug\ in\ octanol\ phase}{concentration\ of\ unchanged\ drug\ in\ aqueous\ phase} \quad (3)$$

2.8. Statistical analysis

Statistical significance was evaluated using Student's t-test. Results are expressed as mean ± S.D.

3. Results

3.1. Estimation of the corneal transport of S-1033

The corneal transport of S-1033 was studied with a solution of 0.1 mM (Figs. 1–3). The fraction of S-1033 penetrating to the receptor side within 4 h was 0.09 ± 0.03%, and P_{app} and lag time were 0.58 ± 0.18 × 10^{-6} cm/s and 92.4 ± 12.2 min, respectively (Fig. 2). The amount in the cornea at steady state and CL_{cornea}, describing the transport clearance of S-1033 in the cornea to the

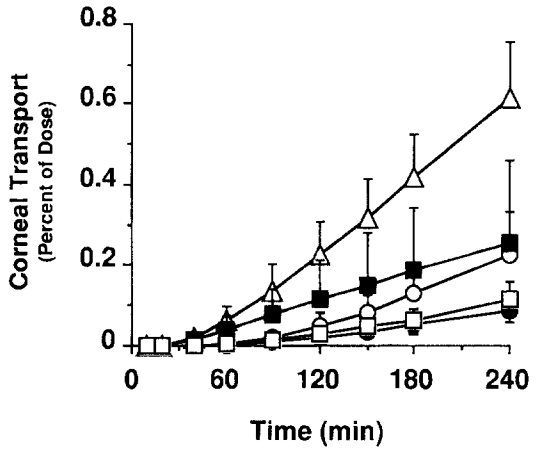


Fig. 1. Transcorneal transport of S-1033 and its lipophilic prodrug, S-1033 methyl ester, with or without benzalkonium chloride. Results are expressed as the means with vertical bars showing the S.D. of four experiments. Key: ●, 0.1 mM S-1033; ○, 0.1 mM S-1033 with BAC; △, 5.4 mM S-1033; ■, 0.018 mM S-1033 methyl ester; □, 0.1 mM S-1033 methyl ester with BAC.

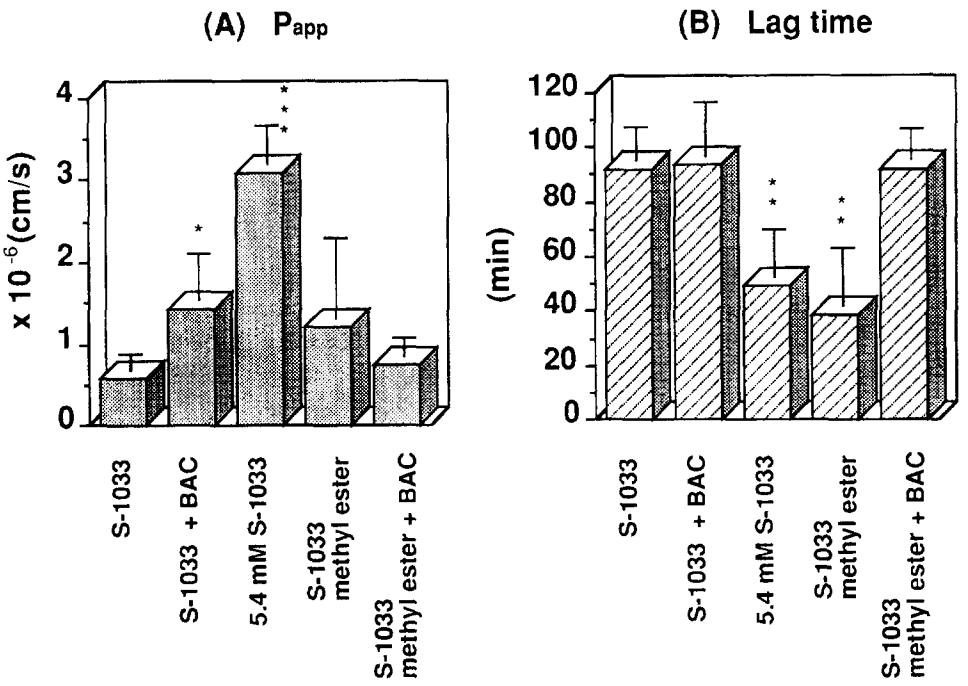


Fig. 2. Apparent corneal permeability coefficients and lag times for S-1033 and its lipophilic prodrug, S-1033 methyl ester, with or without benzalkonium chloride. Results are expressed as the means with vertical bars showing the S.D. of four experiments. Statistically significant differences compared with the value of 0.1 mM S-1033 are indicated as follows: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

receptor side, were 0.97 ± 0.14% and 0.65 ± 0.30 × 10^{-3} cornea/min, respectively (Fig. 3).

3.2. Enhancement of the corneal transport of S-1033

To improve the corneal permeability of S-1033, we examined the effects of BAC, the concentration of S-1033 itself, and the methyl esterification of S-1033 on its corneal transport (Figs. 1–3). Addition of BAC to the S-1033 solution tended to enhance the corneal transport 2.5 times and significant enhancement was observed for the value of P_{app} (Fig. 2A). The methyl esterification of S-1033 also tended to enhance its transport to the receptor side (Fig. 1) and produced an increasing tendency for P_{app} (Fig. 2A), a significant decrease in lag time (Fig. 2B) and significant enhancement in the corneal amount percent at steady state (Fig. 3B) and in the uptake percent (Fig. 3A). The addition of BAC to S-1033 methyl ester solution could not improve the transcorneal transport of S-1033 (Figs. 1 and 2A), although the corneal uptake tended to increase in this system (Fig. 3A,B). Application of a high concentration (5.4 mM) of S-1033 led to the highest transcorneal transport percent (0.62 ± 0.14) (Fig. 1) and the highest value of P_{app} (3.09 ± 0.47 × 10^{-6} cm/s) (Fig. 2A) among the various conditions, although the corneal amount percent was equal to that for the 0.1 mM S-1033 (Fig. 3B).

Although a similar extent of uptake enhancement of S-1033 was observed under the conditions of BAC addition, a high concentration of S-1033 and methyl esterification, the corneal amount percent significantly decreased only for the high concentration condition (Fig. 3B), which was responsible for the difference in the enhancement of transcorneal transport of S-1033. Fig. 3C indicates the marked enhancement of CL_{cornea} by the high concentration condition, which means that a high concentration promotes the diffusion and/or

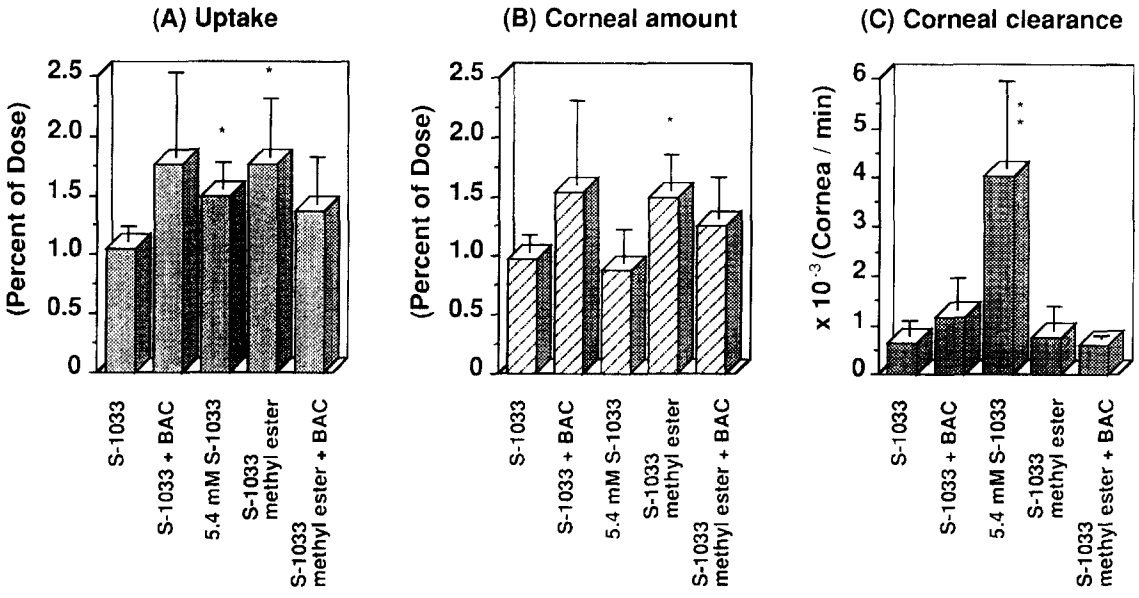


Fig. 3. Corneal uptake, accumulation, and clearance of S-1033 and its lipophilic prodrug, S-1033 methyl ester, with or without benzalkonium chloride. Results are expressed as the means with vertical bars showing the S.D. of four experiments. Statistically significant differences compared with the value of 0.1 mM S-1033 are indicated as follows: $^{**}P < 0.01$; $^{*}P < 0.05$.

efflux of S-1033 in the cornea. The BAC addition tended to enhance CL_{cornea} two fold but the methyl esterification did not improve the clearance (Fig. 3C). The effects of these conditions on CL_{cornea} coincided with those on P_{app}, revealing corneal-to-receptor transport to be the rate-limiting step for the transcorneal transport of S-1033.

3.3. Relationship between corneal permeability and the partition coefficient

To investigate the relationship between transcorneal transport and the partition coefficient for prostaglandin derivatives, we performed in vitro corneal transport experiments with $PGF_{2\alpha}$ and PGE_2. In the absence of BAC, P_{app} values for the two PGs were the smallest among the test compounds while the coexistence of BAC led to significant enhancement of PG transcorneal transport (Table 1). Fig. 4 shows the relationships between P_{app} and logPC of PG derivatives in the presence and absence of BAC. The values of logPC obtained between 1-octanol and GRB solution ranged from −0.67 to 3.89 (Scheme 1). The addition of BAC increased logPC for S-1033, $PGF_{2\alpha}$ and PGE_2, while decreasing that for S-1033 methyl ester. In the absence of BAC, the corneal permeability increased with increasing logPC in the range of −0.67–3.89. On the other hand, in the presence of BAC, P_{app} of S-1033, $PGF_{2\alpha}$ and PGE_2 increased while P_{app} of S-1033 methyl ester decreased, leading to parabolic relationship between P_{app} and logPC and an optimal logPC for the transcorneal transport of PG derivatives.

Table 1
Apparent corneal permeability coefficients of prostaglandin E_2 and $F_{2\alpha}$ with or without benzalkonium chloride

PG	P_{app} ($\times 10^{-6}$ cm/s)	
	Without BAC	With BAC
PGE_2	0.123 ± 0.024	0.538 ± 0.316*
$PGF_{2\alpha}$	0.127 ± 0.056	0.801 ± 0.244**

Data are expressed as the means ± S.D. of four experiments; BAC, benzalkonium chloride.
Statistical differences between studies with and without benzalkonium chloride are indicated as follows: $^{*}P < 0.05$; $^{**}P < 0.001$.

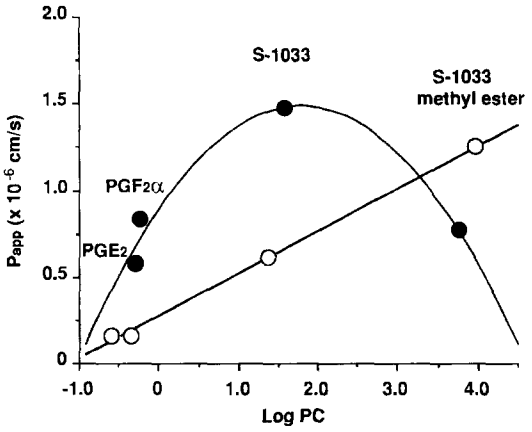


Fig. 4. Relationship between corneal permeability coefficient and logPC of prostaglandin derivatives with or without benzalkonium chloride. Regression curves are represented as follows: ○, without BAC, $P_{app} = 2.6033 \times 10^{-7} + 2.4512 \times 10^{-7} \times \text{logPC}$, $r = 0.9999$; ●, with BAC, $P_{app} = 9.067 \times 10^{-7} + 6.3943 \times 10^{-7} \times \text{logPC} - 1.869 \times (\text{logPC})^2$, $r = 0.9762$.

4. Discussion

To enhance the corneal transport of S-1033, the effects of BAC, the most widely used preservative (Green et al., 1987; Camber and Edman, 1987b) and/or methyl esterification and a high concentration of S-1033 (5.4 mM) on the corneal transport of S-1033 were investigated. The strongest promoting effect was observed after application of a high concentration of S-1033 (Figs. 1 and 2A). With the corneal transport of S-1033 taken as the corneal uptake, all of the above conditions increased the corneal uptake to the same extent, indicating that they had a similar promoting effect on the corneal uptake of S-1033. However, the addition of BAC and methyl esterification also tended to enhance the accumulation of chemicals at steady state, while no change of the corneal amount percent was observed on application of a high concentration (Fig. 3B). These findings suggest that the transport process from the cornea to the receptor side following S-1033 uptake is the rate-limiting step for the promoting effect on the transcorneal transport of S-1033. Addition of BAC would enhance the corneal uptake of S-1033 by modifying the integrity of the corneal epithelium (Lee, 1990) and/or widening the intercellular spaces of the superficial epithelial cell layers (Pfister and Burstein, 1976). $PGF_{2\alpha}$, which is structurally very similar to S-1033, was reported to possess surface activity (Roseman and Yalkowsky, 1973) and it was recognized that S-1033 had a critical micelle concentration of 3.22 mM. Therefore, S-1033 may play a similar but milder role than BAC, because no morphological change in the cornea was observed by scanning electron microscopy after 10.8 mM S-1033 instillation for a month (data not shown), while 0.01% BAC was reported to cause corneal damage such as loss of epithelial cells (Tonjum, 1975: Pfister and Burstein, 1976). The difference in the promoting effect between BAC addition and a high concentration of S-1033 may be explained by a difference in the interaction with the cornea. The increase in the corneal amount of S-1033 by BAC addition may be responsible for the formation of an ion pair between anionic S-1033 and cationic BAC (Lee, 1990), supported by an increase in logPC of S-1033 with the coexistence of BAC (Scheme 1) and the long retention of BAC in the cornea, particularly the epithelium (Green et al., 1987). The higher lipophilicity of S-1033 methyl ester compared with S-1033 would cause the enhancement of both corneal uptake and retention. The addition of BAC to S-1033 methyl ester did not enhance the transcorneal transport of S-1033 (Figs. 1 and 2A). Although BAC can improve the corneal penetration of a variety of hydrophilic compounds (Tonjum, 1975; Keller et al., 1980; Camber and Edman, 1987a,b), the transcorneal transport of the lipophilic prodrug of $PGF_{2\alpha}$ has been reported to decrease in the presence of BAC and its transport across the de-epithelized cornea has been also reported to decrease extensively (Camber and Edman, 1987a). Therefore, the decreasing effect caused by BAC would be due to a reduction in the lipophilic characteristics of the corneal epithelium (Lee, 1990) and/or to a decrease in the apparent lipophilicity of S-1033 methyl ester in the presence of BAC (Scheme 1). Another possible factor is the reduction of the hydrolysis rate ($0.070 \pm 0.023\ \text{min}^{-1}$, in the absence of BAC; $0.0063 \pm 0.0042\ \text{min}^{-1}$, in the presence of BAC; $P < 0.002$), because the

corneal permeability has been reported to be correlated with the rate of enzymatic hydrolysis (Chien et al., 1991; Suhonen et al., 1991a,b). BAC has been reported to inhibit the hydrolysis of $PGF_{2\alpha}$ esters in the cornea homogenate (Camber and Edman, 1987a). Chien et al. (1991) have reported that enzymatically labile prodrugs can penetrate the cornea more readily than stable ones, because the concentration gradient between the corneal epithelial cells and beyond is steeper for labile prodrugs. We propose another possible reason — a hydrophilic parent compound regenerated by hydrolysis may more readily be transferred across the stromal layer, the rate-limiting membrane for lipid-soluble compounds (Grass and Robinson, 1984), than the hydrophobic prodrug itself. The same explanation could be given for the parabolic relationship between P_{app} and logPC (Fig. 4).

The relationship between corneal permeability and lipophilicity has been investigated for various lipophilic prodrugs such as pilocarpine (Suhonen et al., 1991a,b), timolol (Chang et al., 1987; Chien et al., 1991), $PGF_{2\alpha}$ (Camber et al., 1986), and β-blockers (Schoenwald and Huang, 1983; Huang et al., 1983; Sasaki et al., 1993). Almost all of these studies indicated that the permeabilities vary parabolically along with the lipophilicity. The optimal value of the partition coefficient ranged from 1 to 5 for the lipophilic prodrugs because of the dependency on the chemical structure and/or the lipophilic moiety. In the absence of BAC, the relationship between P_{app} and logPC was linear in the range of -0.67 to 3.89 (Fig. 4), which coincides with the lipophilic prodrugs of $PGF_{2\alpha}$ (Camber et al., 1986). These different patterns of the P_{app}-logPC relationship may be attributed to the effect of other factors determining permeability, such as the fraction of the ionized form, molecular size, the balance between aqueous and lipid solubility (Lee, 1990), and the rate of hydrolysis (Chien et al., 1991; Suhonen et al., 1991a,b). These factors are also related to the lipophilicity for epithelial uptake and the hydrophilicity for trans-stromal transport, whose balance should be important for improving transcorneal transport by lipophilic prodrug derivatization.

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