Effects of Absorption Enhancers and Lipid Composition on Drug Permeability through the Model Membrane Using Stratum Corneum Lipids[1)]

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We have developed a model membrane system for estimating drug permeability of skin by fixing liposomes composed of stratum corneum (SC) lipids (ceramides, palmitic acid, cholesterol, and cholesterol-3-sulfate) onto a supporting filter, Biodyne B (*Chem. Pharm. Bull.*, 41, 575 (1993)). In both the model membrane and guinea pig skin experiments, the addition of absorption enhancers (Azone, decylmethylsulfoxide, oleic acid, and capric acid) caused an increase in cyclobarbital (a relatively hydrophilic drug) permeation, but had little effect on ibuprofen (a hydrophobic drug) permeation. Thus, the model membrane and guinea pig skin behave similarly in terms of permeability, suggesting that the main target of the enhancers is the SC intercellular lipid lamellae. The influence of SC lipid composition on permeability was also investigated. A lipid composition similar to that of skin SC showed the lowest permeability for the hydrophilic drug. Our model membrane system was found to be widely applicable for drug permeation studies.

Keywords stratum corneum lipid; absorption enhancer; permeability; model membrane; skin

For dozens of years, the skin permeability of drugs has been extensively studied as a drug delivery system. It is well recognized that the stratum corneun (SC) lipids play a crucial role in percutaneous absorption.[2)] A model membrane composed of SC lipids would be of help for both basic studies and practical application.

In our previous paper,[1)] we developed a model membrane system for skin by sandwiching liposomes composed of the SC lipids between two supporting filters, Biodyne B. The model membrane has both lipidic and aqueous pathways and closely mimics the skin in drug permeability for both hydrophilic and hydrophobic drugs. Several absorption enhancers act mainly on the SC lipid bilayers to fluidize them, facilitating drug permeation.[2d,g)] It is important in evaluating the usefulness of our model system to examine its barrier properties in the presence of enhancers.

In the present study, drug permeability through the model membrane containing an enhancer was compared with that through guinea pig skin pretreated with the enhancer. Furthermore, the influence of modification of SC lipid composition on drug permeability was investigated. We found our system valuable for the elucidation of the relationships between SC lipid composition (including the incorporation of absorption enhancers) and drug permeability.

Experimental

Materials Ceramides from bovine brain (type IV, approx. 99%), palmitic acid (sodium salt, approx. 99%), cholesterol (>99%), cholesterol-3-sulfate (sodium salt), lauric acid (99%—100%), capric acid (99%—100%), and oleic acid (approx. 99%) were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Azone was obtained from Nelson Research (Irvine, CA, U.S.A.). Decylmethylsulfoxide (DCMS) was a kind gift from Prof. Kimura (Okayama University). Cyclobarbital (CB) and Ibuprofen (IB) were manufacured by Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively. Membrane filters used were Biodyne B (pore size 0.45 μm, Pall Ultrafine Filtration Co., New York, U.S.A.). All other chemicals were of special grade. Buffers were prepared with water twice distilled from a glass still.

Preparation of Liposomes Individual lipids were dissolved in chloroform–methanol (2:1 or 1:1 by volume) solutions and aliquots were mixed to prepare an SC lipid mixure (ceramides : palmitic acid : cholesterol : cholesterol-3-sulfate = 3.25 : 4.5 : 3.25 : 1, mole ratio).[3)] Each absorption ehnancer (Azone, DCMS, oleic acid, lauric acid, and capric acid) in an ethanol solution was added to the SC lipid mixture (SC lipids : enhancer = 10 : 3, mole ratio). After evaporation of the solvent, the residual film was vacuum-dried overnight. The thin film was hydrated with a 5 mM Tris–HCl/1 mM EDTA buffer (pH 7.5) and then vortexed at 80—85 °C. To investigate the change in drug permeability by modifying SC lipid composition, each SC lipid component, except for cholesterol-3-sulfate, was added to the SC lipid mixture in excess (30 mol%), instead of incorporating enhancers.

Fixation of Liposomes An SC lipid suspension containing an enhancer or an additive lipid was freeze-thawed 7—15 times. The milky suspension was uniformly spread on a membrane filter (25 mm diameter) using an Amicon ultrafiltration cell (type 8010, Beverly, U.S.A.) under nitrogen gas pressure of 1—3 kg/cm^2 as described previously.[1)] The amount of the lipids loaded was 3.0 mg/cm^2.

Preparation of Skin Male guinea pigs (Hartley strain, weighing 250—300 g) from Shimizu Laboratory Supplies (Kyoto, Japan) were shaved with electrical clippers the preceding day. The dorsal skin of the anesthetized animal was excised. After removal of the subcutaneous fat, the skin was punched out into disks 24 mm in diameter. Six pieces of skin were obtained from each animal. To examine the effects of enhancers, each enhancer in an ethanol solution (0.2 ml) was applied on the skin (48 μmol enhancer/cm^2) and then incubated at 37 °C. Six hours later, ethanol was evaporated with a hair dryer and the skin was punched out into 18 mm diameter disks.

Membrane Permeation The procedures for membrane permeation have previously been described in detail.[1)] Briefly, a model membrane or a piece of skin was mounted on a Valia-Chien type skin permeation cell (0.636 cm^2 permeation area). The donor compartment was filled with a drug suspension in a 10 mM Tris–HCl/150 mM NaCl/1 mM EDTA buffer (pH 7.5) and the acceptor compartment was filled with the buffer (6.5 ml per compartment). The temperature was maintained at 37 ± 1 °C. At intervals, aliquots (0.2 ml) of the acceptor solution were sampled and the amount of the permeated drug was determined using HPLC.[2f)]

The permeability coefficient of the drug was calculated using a curve fitting procedure, as reported elsewhere.[4)] Statistical analysis was performed by use of the two sample t test, and a p value of <0.1 was considered significant.

Results and Discussion

First, we examined the effects of several enhancers on the drug permeability through the model membrane and guinea pig skin. CB and IB were used as relatively hydrophilic and hydrophobic model drugs, respectively. The molecular weights of CB and IB are very similar: 236.26 and 206.27, respectively. The enhancers tested (Azone, DCMS, oleic acid, lauric acid, and capric acid) are considered to act on the SC lipid membranes.[2d,g,5]

The permeability coefficients of the model drugs through the model membrane and guinea pig skin are listed in Table I and Table II, respectively. The values in parentheses are enhancing ratios against the control value. In model membrane experiments, a model membrane without enhancers was used as a control. In guinea pig skin experiments, a skin pretreated with ethanol was used as a control, because each enhancer in an ethanol solution was applied on the skin.

Azone, DCMS, and oleic acid increased the permeation of CB through the model membrane roughly fourfold, whereas lauric acid and capric acid showed smaller enhancing effects. No enhancers exhibited permeation enhancing effects for IB through the model membrane.

Pretreatment with the enhancers increased the CB permeation through guinea pig skin by 10—16 times, whereas it induced practically no enhancing effects for IB permeation.

In both the model membrane and guinea pig skin experiments, application of the enhancers increased the permeation of a relatively hydrophilic drug (CB), but had little effect on a hydrophobic drug (IB). Hori *et al.*[6] noted that this is a typical observation in penetration enhancement studies: larger effects are much more likely to be observed with penetrants which transport poorly without enhancers. Under this condition, the permeation coefficients of CB through both the model membrane and skin were one order magnitude smaller than those of IB.

In this context, the permeability properties of both the model membrane and skin agree qualitatively, suggesting that enhancers examined here mainly act on the intercellular lipid lamellae of SC. Plausible reasons for the quantitative inagreement are that (1) the quantity of enhancer applied on the skin was different from that incorporated into SC lipid liposomes, (2) each enhancer was applied with ethanol in the skin experiments, and (3) the actual epidermis consists of various layers (stratum granulosum, stratum spinosum, and stratum basale) other than SC, with different lipid compositions.

Table III shows the effects of lipid composition on the permeability coefficients of the model drugs through the model membrane. Modification of the lipid composition again resulted in the permeation enhancement of CB; especially, the addition of ceramides significantly increased the CB permeation. The IB permeation appeared to be little influenced by the augmentation of each SC lipid component.

Ceramides are considered to play a critical role in the barrier property of SC.[7] The content of hydroxyl groups and the presence of an amide group enables ceramide to act both as a hydrogen bond donor and acceptor.[8] The orientation of hydrogen bond donors and acceptors of the hydroxyl groups and amide groups allow lateral interaction with other lipid molecules, promoting condensation in the head group region.[8] Ceramides, whose hydrocarbon chains are straight and almost entirely saturated, form bilayers with closely packed interiors, and such highly ordered bilayers are suitable for the barrier properties of SC.[7] However, the addition of ceramides caused a large CB permeation.

Under our permeation study condition, the SC lipid mixtures were fully hydrated. If hydrogen bonds were directed to water molecules instead of lipid molecules, the lipid–water contact was increased, resulting in an incremental increase of the molecular area at the membrane interface. These effects would actually decrease membrane stability by reducing or preventing efficient packing of the hydrocarbon chains. Barry reported that water molecules associated with lipid polar head groups *via* hydrogen bonding, both loosening lipid packing and reducing intermolecular forces.[2d] Under a fully hydrated condition, the addition of ceramides may increase the

TABLE I. Permeability Coefficients of Model Drugs through the Model Membrane[a]

	CB ($\times 10^{-6}$ cm/s)	IB ($\times 10^{-6}$ cm/s)
No enhancer (control)	2.10±0.36 (1.0)	23.4±2.9 (1.0)
Azone	9.00±2.31 (4.3)[b]	25.2±8.3 (1.1)
DCMS	8.29±3.40 (3.9)[b]	20.2±1.7 (0.86)
Oleic acid	8.44±0.82 (4.0)[b]	19.4±1.8 (0.83)
Lauric acid	3.18±1.04 (1.5)	30.3±7.6 (1.3)
Capric acid	4.56±0.65 (2.2)[b]	35.3±2.2 (1.5)[b]

a) Mean of three experiments±S.D. Values in parentheses indicate enhancing ratios against the control value. *b*) $p<0.05$.

TABLE II. Permeability Coefficients of Model Drugs through Guinea Pig Skin[a]

	CB ($\times 10^{-6}$ cm/s)	IB ($\times 10^{-6}$ cm/s)
No enhancer	0.337±0.014	5.71±0.78
Ethanol (control)	1.22±0.14 (1.0)	12.8±2.5 (1.0)
Azone	11.8±6.4 (9.7)[b]	22.5±1.9 (1.8)[b]
DCMS	19.4±7.4 (15.9)[b]	22.3±3.8 (1.7)[b]
Oleic acid	12.5±5.0 (10.2)[b]	20.1±1.3 (1.6)[b]
Lauric acid	13.2±3.4 (10.8)[b]	27.8±10.9 (2.2)[c]
Capric acid	15.4±3.7 (12.6)[b]	23.9±3.3 (1.9)[b]

a) Mean of three experiments±S.D. Values in parentheses indicate enhancing ratios against the control value. *b*) $p<0.05$, *c*) $p<0.1$.

TABLE III. Effects of Lipid Composition[a] on Permeability Coefficients[b] of Model Drugs through the Model Membrane

Lipid composition	Permeability coefficient ($\times 10^{-6}$ cm/s)	
	CB	IB
Control	2.10±0.36 (1.0)	23.4±2.9 (1.0)
+Ceramides	28.5±3.1 (13.6)[c]	23.7±6.1 (1.0)
+Cholesterol	4.70±1.84 (2.2)[d]	27.8±5.2 (1.2)
+Palmitic acid	5.40±0.70 (2.6)[c]	18.4±5.5 (0.79)

a) Each lipid was added 30% in excess of the control lipid composition (ceramides : palmitic acid : cholesterol : cholesterol-3-sulfate = 3.25 : 4.5 : 3.25 : 1, mole ratio). *b*) Mean of three experiments±S.D. Values in parentheses indicate enhancing ratios against the control value. *c*) $p<0.05$, *d*) $p<0.1$.

hydration of the membrane, causing membrane fluidization rather than stabilizing the membrane *via* intermolecular hydrogen bonding between head groups. This may account for the increase of CB permeation by the addition of ceramides.

Abraham *et al.*[9] investigated SC lipid mixtures using solid state ^{2}H-NMR spectroscopy, varying the mole ratio of cholesterol to ceramides from 1 to 0. The bilayers which contained the largest amounts of cholesterol were most fluid. The addition of cholesterol fluidized the lipids of the model membrane, resulting in enhanced CB permeation. Our experiments indicated that the SC lipid composition close to that of skin SC exhibits the lowest permeability for the hydrophilic drug.

In conclusion, the drug permeation behavior of our model membrane system is similar to that of guinea pig skin, even in the presence of enhancers. Furthermore, our system was found to be useful for investigating the relationships between lipid composition and drug permeability. Structural aspects of the model membrane in the presence of enhancers are under investigation using various spectroscopic techniques.

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