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Permeation of several drugs through keratinized epithelial-free membrane of hamster cheek pouch

Keiko Tsutsumi [a,*], Yasuko Obata [a], Kozo Takayama [a], Koichi Isowa [b], Tsuneji Nagai [a]

[a] *Department of Pharmaceutics, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142-8501, Japan*
[b] *The Center of Japan Biological Chemistry, Fukue 52, Kaizu-cho, Kaizu-gun, Gifu 503-0628, Japan*

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Abstract

The hamster cheek pouch mucosa was selected as a substitute for the human buccal mucosa in an in vitro permeation study. Considering that a keratinized layer is not present in the human buccal mucosa, keratinized epithelial-free hamster cheek pouch (KEF-membrane) was prepared by chemical splitting. To confirm the usefulness of the KEF-membrane, a permeation study was conducted using several drugs with different lipophilicities. The permeability coefficient of hydrophilic drugs through the KEF-membrane (P_{kef}) was significantly greater than that through a viable KEF-membrane ($P_{kef\text{-}viable}$), which was estimated by using the permeability coefficient of the viable full-thickness membrane and that of the keratinized layer. On the other hand, the P_{kef} values of lipophilic drugs were comparable with the $P_{kef\text{-}viable}$ values. Furthermore, the ratio of these P values ($P_{kef}/P_{kef\text{-}viable}$) decreased with increasing lipophilicity of drugs. These findings indicate that the KEF-membrane may be useful for buccal permeation studies of lipophilic drugs.

Keywords: Buccal permeation; Keratinized epithelial-free membrane; Pathological study

1. Introduction

Buccal absorption of drugs is an attractive route for systemic delivery when the bioavailability of drugs administered orally is insufficient, due to such events as first-pass hepatogastrointestinal metabolism. The oral mucosa is roughly classified into three types: masticatory, lining and specialized mucosa. The major difference among the types is the presence or absence of a keratinized outermost layer as part of the epithelium. The non-keratinized epithelium of the buccal mucosa is more permeable than the keratinized one.

* Corresponding author. Tel.: +81-3-5498-5783; fax: +81-3-5498-5783.

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Therefore, the buccal region is a more suitable site for drug delivery (Hoogstraate et al., 1996; Taylan et al., 1996; Voorspoels et al., 1996). In vitro permeation experiments employing an animal buccal membrane, which is mounted in the diffusion cells, are useful for revealing the absorption characteristics of drugs. For this purpose, the animal buccal membrane selected should have a buccal tissue structure comparable to that of the human buccal mucosa. Hamster cheek pouch has often been used as a model membrane for buccal drug absorption because a wide surface area is available for in vitro experiments (Tanaka et al., 1980; Ishida et al., 1983; Kurosaki et al., 1991). However, one of the drawbacks of using hamster cheek pouch is that the membrane is fully covered with a thick keratinized layer. Thus, it is desirable to carry out permeation experiments using a keratinized epithelial-free membrane (KEF-membrane) to obtain reliable data, comparable to data obtained using human mucosa. In this study, we prepared the KEF-membrane using a chemical splitting technique, and evaluated its possible uses and limitations as a model membrane for buccal drug absorption.

2. Materials and methods

2.1. Materials

Ibuprofen was purchased from Tokyo Chemical Industries, Japan. Lidocaine, aminopyrine and caffeine were purchased from Wako Pure Chemical Industries, Japan. Verapamil hydrochloride and indomethacin were purchased from Sigma Chemical, USA. The other chemicals used were of reagent grade.

2.2. Determination of partition coefficients

An excess amount of drug was suspended in phosphate buffer solution (pH 7.4) or isopropyl myristate (IPM) at 37°C for 1 h under sonication. pH values of the drug-suspended buffer solution were measured after saturation, since solubilities were high enough to induce the change in pH in the buffer solution. An aliquot was withdrawn from the suspension and filtered through a 0.45 μm disposable filter unit (Ekikuro-Disk 13, Gelman Science Japan, Japan). The sample solution was then diluted with methanol. The concentration of drugs in phosphate buffer solution (C_w) or IPM (C_{oil}) was determined using high pressure liquid chromatography (HPLC). The apparent partition coefficient (K) of the drugs was calculated as the solubility ratio; C_{oil}/C_w.

2.3. Permeation study

An appropriately sized area of the cheek pouch of a male golden hamster (body weight approximately l00 g; Saitama Laboratory Animals, Japan) was excised, and the keratinized epithelium (KE-membrane) and the KEF-membrane were separated from the full-thickness mucosa using a chemica1 splitting technique (immersion in a 2 M sodium bromide solution for 18 h at 7°C) (Scott et al., 1986). An in vitro permeation study was performed using a two-chamber diffusion cell equipped with a water jacket (37°C; available diffusion area, 0.785 cm^2; volume of each half-cell, 3.0 ml), in which the full-thickness, KE- or KEF-membranes had been mounted (Tojo et al., 1987). The drug suspension (pH 6.4–7.7 listed in Table 2) in phosphate buffer solution was transferred to the donor cell. The receiver cell was filled with phosphate buffer solution and both cells were stirred using a magnetic stirrer. Every 30 min, 0.3 ml samples were taken from the receiver cell and replaced by the same volume of fresh phosphate buffer solution (pH 7.4) to maintain a constant volume. The concentration of drugs was determined using HPLC. In the case of the permeation experiment using the full-thickness membrane, the receiver cell was bubbled with oxygen gas (95% O_2, 5% CO_2) to maintain tissue viability. Separately, the electric resistance of the full-thickness membrane was measured to confirm tissue viability using an Ussing chamber apparatus, in which a short circuit had been incorporated (Kubo et al., 1993; Maitani et al.,

Table 1
HPLC conditions for the analysis of drugs used in this study

Drug	Mobilephase	Detection (nm)	Internal standard	Column
Lidocaine	Methanol:0.057% phosphoric acid (65:35)	254	*p*-Hydroxybenzoic acid hexyl ester	C_8 4.6×200 mm
Ibuprofen	Methanol:0.1% phosphoric acid (80:20)	262	*p*-Hydroxybenzoic acid butyl ester	C_{18} 4.6×250 mm
Indomethacin	Methanol:0.057% phosphoric acid (65:35)	254	*p*-Hydroxybenzoic acid hexyl ester	C_8 4.6×200 mm
Aminopyrine	Acetonitrile:0.1% phosphoric acid (40:60) +5 mM sodium dodecylsulfate	254	*p*-Hydroxybenzoic acid methyl ester	C_{18} 4.6×250 mm
Caffeine	Methanol:0.057% phosphoric acid (65:35)	274	*p*-Hydroxybenzoic acid hexyl ester	C_8 4.6 × 200 mm
Verapamil	Methanol:0.057% phosphoric acid(65:35)	278	*p*-Hydroxybenzoic acid hexyl ester	C_8 4.6 × 200 mm

1997). For this measurement, a phosphate buffer saline (PBS) solution (pH 7.4) was used as the solvent in the receiver cell.

2.4. Determination of drug concentration

Determination of drug concentration in the sample solution was performed using HPLC. The sample solution was injected onto the column using an autoinjector equipped with a system controller (SIL10A, SCL10A; Shimadzu, Japan), a pump (LCl0AS; Shimadzu) and a UV detector (SPD6A; Shimadzu). In all cases, the flow rate of the mobile phase was l.0 ml/min. Other analytical conditions are summarized in Table 1.

2.5. Pathological study

The excised full-thickness, KE- and KEF-membranes were fixed in a 10% formalin solution for at least 24 h before routine processing and then cut vertically against the mucous surface at the central region in 4 mm widths. Each section was dehydrated using a graded series of ethanol solutions and embedded in paraffin wax. Tissues were divided into small pieces (about 3 μm in thickness) and stained with hematoxylin and eosin. All sections were examined by optical photolight microscopy.

3. Results and discussion

3.1. Microscopic observation of the full-thickness, KE- and KEF- membranes

In order to obtain the KEF-membrane, we selected a chemical splitting method to separate the keratinized layer. It was considered to be an easier technique, compared with mechanical methods such as stripping and slicing, for routine use. In the report by Reid et al. (1986), a large standard error in ion transport across the tape-stripping hamster cheek pouches on the Ussing chamber system was observed. It has also been reported that membrane resistance of dermatomed membrane depended on the thickness of membrane using porcine buccal mucosa in vitro. Furthermore, a clear relationship was observed between membrane resistance and permeability of acebutolol and bupranolol (de Vries et al., 1991). Scott et al. (1986) reported several chemical splitting methods which can be used with rat skin; i.e. enzymatic digestion with trypsin or hyaluronidase, exposure to dithiothreitol and immersion in aqueous solutions of calcium chloride or sodium bromide. However, the majority of these splitting techniques are unsuccessful because of enzymatic digestion of tissues or imperfect separation. Among these splitting techniques, the best separation was accomplished by immersion of rat full-thickness skin in a 2 M sodium bromide

solution (using 4–5-week-old rats) and 0.1 M dithiothreitol solution (using 1-day-old rats). The latter solution was considered to be unsuitable for routine use because it could only be applied to very young rat skin. Furthermore, chemical splitting using a 2 M sodium bromide solution separated the epidermis from the dermis in the case of human full-thickness skin. This effect appears to be due to loosening the plane of the junction between the KE-membrane and the KEF-membrane (Baumberger et al., 1942; Scott et al., 1986). Similarly, we prepared hamster cheek pouch KEF-membrane using the same splitting technique so that the membrane would be comparable to human buccal mucosa, which has no keratinized layer, because hamster cheek pouch is fully covered with a keratinized epithelium.

Fig. 1 shows the microphotographs of cross-sections of each tissue. The full-thickness membrane is composed of the keratinized epithelium, basal lamina, lamina propria and submucosa (Fig. 1A). The cross-section of the KE-membrane indicated the presence of a basal lamina (Fig. 1B). The KEF-membrane, which consisted of a lamina propria and submucosa, did not show any pathological damage caused by the chemical splitting treatment (Fig. 1C).

3.2. Permeation of drugs through the full-thickness and KE-membranes

The degree of permeation through the full-thickness, KE- and KEF-membranes was evaluated using several drugs with different lipophilicities. Basic properties such as the molecular weight, the solubility in phosphate butter solution or IPM, and the partition coefficient (K) between phosphate buffer solution and IPM are summarized in Table 2. The differences in the molecular weights were slight, whereas a wide range of K values of these drugs was observed. Fig. 2 shows typical results of the cumulative amount of drugs which permeated into the receiver cell as a function of time. Steady-state permeation was immediately attained following the start of the permeation study. The steady-state flux (J) was determined from the slope of the linear line shown in Fig. 2. The permeability

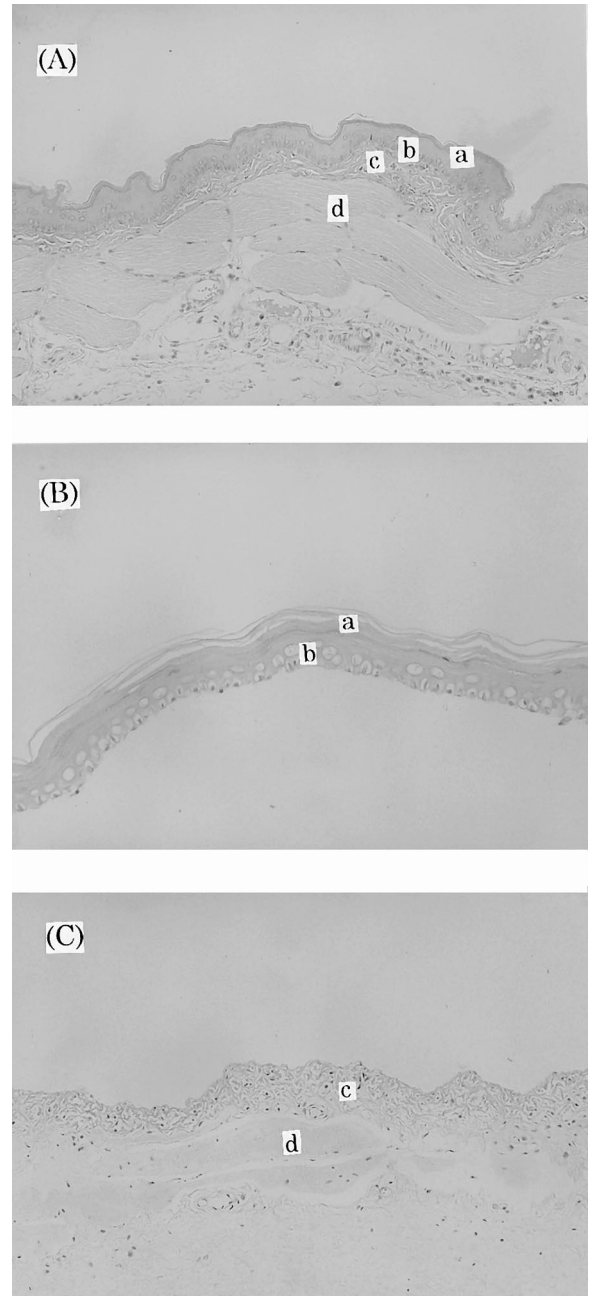

Fig. 1. Microphotographs of vertical sections of hamster cheek pouch mucosa. (A) Full-thickness membrane (H & E stain. × 100); (B) KE-membrane (H & E stain. × 200); (C) KEF-membrane (H & E stain. × 100). a, Keratinized epithelium; b, basal lamina; c, lamina propria; d, submucosa.

Table 2
Physicochemical parameters of drugs used in this study

Drug	MW	Log K[a]	pK_a	Solubility(mg/ml)		pH[b]	F_u[c]
				IPM	Phosphate buffer		
Lidocaine	234	1.6	7.9	200.40	5.10	7.7	0.39
Ibuprofen	206	1.4	5.2	193.26	7.79	6.7	0.03
Indomethacin	358	0.41	4.5	4.27	1.65	7.2	0.00
Aminopyrine	231	−0.38	5.0	18.47	44.53	7.5	1.00
Caffeine	194	−1.6	0.8	0.79	33.65	7.4	1.00
Verapamil	455	−2.9	8.9	0 05	36.34	6.4	0.00

[a] Logarithm of IPM/phosphate buffer solution partition coefficient at 37°C.
[b] pH of drug-suspended buffer solution.
[c] Nonionized fraction, $F_u = 1/[1 + \text{antilog}\ (\text{pH}-\text{p}K_a)]$ in acidic drugs, $F_u = 1/[1 + \text{antilog}\ (\text{p}K_a-\text{pH})]$ in alkaline drugs.

coefficient (P) was estimated using the J value and the solubility of each drug. Identical permeation experiments were conducted employing the KE- and KEF-membranes, which had been separated from the full-thickness membrane, and the results are given in Table 3. Permeation of drugs in the full-thickness membrane increased with an increase in lipophilicity of drugs, and a linear relationship was observed between log P and log K values ($r = 0.923$). The same tendency was seen in the KE-membrane ($r = 0.927$). The outermost layer of the hamster cheek pouch is relatively lipophilic, therefore it is more permeable to lipophilic drugs than to hydrophilic drugs. Furthermore, the degree of permeation of each drug through the KE-membrane was greater than that through the full-thickness membrane. This suggests that the KEF-membrane, in addition to the KE-membrane, also acts as a diffusion barrier. The P values of KEF-membrane were also influenced by the K values of drugs ($r = 0.755$). Furthermore, an influence of dissociation degree of drugs in the donor suspension on the KEF-membrane permeation was investigated. The nonionized fraction (F_u) of drugs was estimated from pK_a and pH values of the drug suspension, using a Henderson–Hasselbalch equation (Table 2). As a result, lidocaine and ibuprofen molecules were not fully ionized ($F_u = 0.39$ in lidocaine and 0.03 in ibuprofen). Indomethacin and verapamil molecules were fully ionized in the donor suspension. On the other hand, aminopyrine and caffeine molecules were completely nonionized in the donor suspension. No clear relation between P values and ionized degrees of each drug was observed; therefore, it might be difficult to evaluate the effect of ionization degree of permeants from the results observed with different drugs.

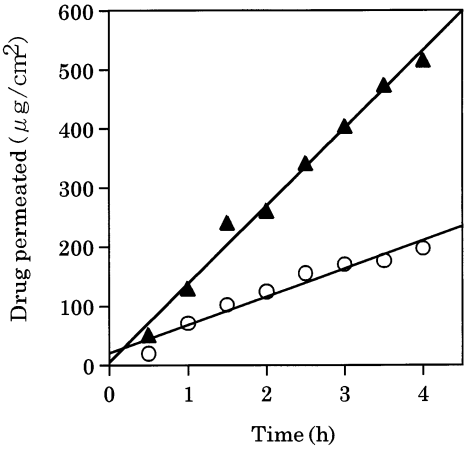

Fig. 2. Permeation profiles of ibuprofen (▲) and caffeine (○) through the full-thickness membrane. Each point represents the mean of three determinations.

3.3. Effect of the viability of the KEF-membrane on drug permeation

In contrast to the horny layer of skin, the human buccal membrane is viable. Therefore, it is important to clarify the effect of the viability of the KEF-membrane on drug permeation. The viability of the excised full-thickness membrane of the hamster cheek pouch was investigated using

Table 3
Permeability coefficient P (cm/h) of drugs through full-thickness membrane, KE- membrane and KEF- membrane[a]

Drug	Full-thickness membrane	KE-membrane	KEF-membrane
Lidocaine	39.1 ± 20.5	96.3 ± 34.2	26.9 ± 6.81
Ibuprofen	31.0 ± 8.99	98.9 ± 22.9	32.4 ± 2.83
Indomethacin	17.4 ± 5.98	25.3 ± 12.2	52.3 ± 13.5
Aminopyrine	2.55 ± 1.35	20.4 ± 10.9	23.1 ± 1.14
Caffeine	1.26 ± 0.122	3.80 ± 0.727	22.4 ± 2.67
Verapamil	1.54 ± 0.908	6.3 ± 3.59	10.8 ± 0.338

[a] Each value represents the mean ± S.D. of three determinations.

an Ussing chamber apparatus. From the values of spontaneous transmucosal potential difference and short-circuit current, the membrane electrical resistance was calculated according to Ohm's law. As a result, the electrical resistance of the full-thickness membrane was maintained at an almost constant level of approximately 1300 Ω cm^2 up to 4 h after the start of experiments by bubbling with oxygen gas. This suggests that the viability of the full-thickness membrane can be maintained during the permeation study. On the other hand, the outermost layer of hamster cheek pouch is covered with 'dead' keratinized epithelium, therefore drug permeation through the KE-membrane may not be affected by the viability of mucous cells.

The resistance to diffusion in each layer is equal to the reciprocal of the P value of that particular layer. The total value of the resistance to diffusion is given as the sum of the reciprocals of P values for a series of layers (Flynn et al., 1972). Thus, the P value for the viable KEF-membrane ($P_{\text{kef-viable}}$) can be estimated using the following equation:

$$1/P_{\text{kef}-\text{viable}} = 1/P_{\text{f}} - 1/P_{\text{ke}} \quad (1)$$

where P_{f} is the permeability coefficient of the full-thickness membrane, of which the viability was maintained by oxygen gas bubbling, and P_{ke} is the permeability coefficient of the KE-membrane which was prepared using the chemical splitting technique.

Fig. 3 shows the relationship between the ratio of the P values ($P_{\text{kef}}/P_{\text{kef-viable}}$) and the K values of each drug. P_{kef} values were determined using the KEF-membrane prepared by chemical splitting, therefore, P_{kef} represents drug permeation through 'dead' KEF-membrane. $P_{\text{kef-viable}}$ values were significantly smaller than P_{kef} values for all drugs tested. The $P_{\text{kef}}/P_{\text{kef-viable}}$ values clearly decreased with increasing K values of the drugs. It was observed that the $P_{\text{kef}}/P_{\text{kef-viable}}$ values of hydrophilic drugs with $\log K < 0$ were approximately 10-fold larger than those of lipophilic drugs with $\log K > 0$. The viable KEF-membrane acted as a strong barrier to the permeation of hydrophilic drugs such as aminopyrine, caffeine and verapamil hydrochloride.

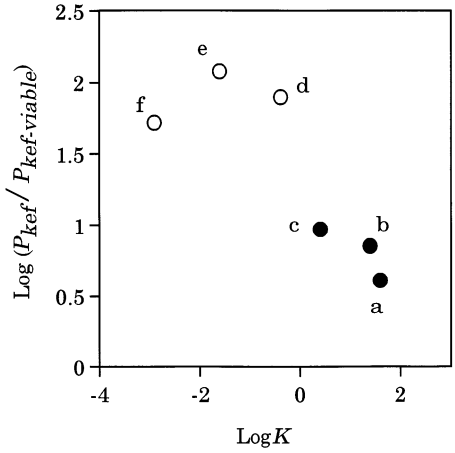

Fig. 3. Relationship between the partition coefficient (K) of drugs and the permeability coefficient (P) ratio. a, Lidocaine; b, ibuprofen; c, indomethacin; d, aminopyrine; e, caffeine; f, verapamil.

On the other hand, the difference in P values between the dead and viable KEF-membranes was not marked in the case of lipophilic drugs such as lidocaine, ibuprofen and indomethacin.

Maitani et al. (1997) investigated the effect of oxygen bubbling in excised rabbit nasal mucosa on the permeation of lucifer yellow as a hydrophilic drug and diazepam as a lipophilic drug. In the absence of oxygen bubbling, the permeation of lucifer yellow was significantly greater than that of diazepam compared with the permeation observed in the presence of oxygen bubbling. This result indicated that hydrophilic drugs such as lucifer yellow are more affected by the viability of membranes than are lipophilic drugs such as diazepam. This finding suggested that the pathways of absorption of hydrophilic and lipophilic drugs may be different. Similar to the results observed with nasal mucosa, it was shown that the viability of the KEF-membrane plays an important role in the permeation of hydrophilic drugs. This suggests that the viable KEF-membrane was a significantly stronger diffusion barrier than was the KEF-membrane for hydrophilic drugs, since hydrophilic drugs might permeate the viable KEF-membrane in a particular route. On the other hand, lipophilic drugs might permeate both the viable and the dead KEF-membranes to similar degrees based on a passive diffusion mechanism (Higuchi 1960). Accordingly, the permeation of lipophilic drugs is predominantly controlled by the degree of distribution of drugs at the surface of the KEF-membrane. We suggest that the permeation of lipophilic drugs through the buccal membrane might be independent of the viability of the membrane. Our results indicate that the KEF-membrane is useful for the in vitro buccal permeation study of lipophilic drugs.

Acknowledgements

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