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Buccal absorption of ergotamine tartrate using the bioadhesive tablet system in guinea-pigs

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Abstract

The buccal administration of ergotamine tartrate (ET) combined with polyvinyl alcohol (PVA) gel brought about higher plasma concentration of ET compared with that of oral administration of capsules in guinea-pigs. T_{max} of ET in plasma of buccal administration was significantly smaller than that of oral administration. For the buccal dosage form of ET, the bioadhesive tablet system (BTS) was newly developed. It consisted of a reservoir of drug and an adhesive region. BTS showed better absorption of ET compared with PVA gel in guinea pigs. Among several pharmaceutical bases in the reservoir of BTS, Witepsol W-35 was most effective. It is likely that the high lipophilic property of Witepsol W-35 in which ET was dissolved facilitated the drug release by its relatively low melting point (around 35 °C), consequently a rapid absorption. In addition, the enhancing activity of the cod-liver oil extract (CLOE) in hydrophilic ointment on the in vivo buccal ET absorption was clarified to be comparable to that in the in vitro study utilizing the keratinized epithelial-free membrane (KEF-membrane) of the hamster cheek pouch.

Keywords: Buccal absorption; Ergotamine tartrate; Bioadhesive tablet system (BTS); Keratinized epithelial-free membrane (KEF-membrane); In vitro–in vivo correlation

1. Introduction

Ergotamine tartrate (ET) has been used for curing migraine. The oral bioavailability of ET was fairly poor because of its high first-pass extract by the liver. Up to 99% of the orally administered dose may be cleared by this process (Bobik et al., 1981; Little et al., 1982; Wyss et al., 1991). Hence, the oral administration of ET shows marginal and slow onset of efficacy. A route of alternative administration that avoids injection but leads to a rapid efficacy would be advantageous for the patients. This study was initiated with the intention of developing a new dosage form, a buccal adhesive tablet system (BTS), to improve ET absorption. Buccal mucosa is an attractive route for systemic delivery of drugs that is rela-

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tively permeable with a rich blood supply (Hoogstraate et al., 1996; Voorspoels et al., 1996). Moreover, it has high robustness and accessibility. A drug can be easily applied and localized to the application site, and also can be removed from there if necessary. The human buccal mucosa consists of, in series: an outermost layer of stratified squamous epithelium; a basement membrane; a lamina propria and submucosa. In general, the epithelium (about 40–50 cell layers thick) has been thought to be a principal permeability barrier for hydrophilic and polar permeants. The hamster cheek pouch has widely been used as a model membrane for the buccal drug absorption study because it has a large area for drug administration (Tanaka et al., 1980; Kurosaki et al., 1991). Considering that the human buccal mucosa does not have a keratinized epithelial layer, we prepared a keratinized epithelial-free membrane of hamster cheek pouch (KEF-membrane) by chemical splitting technique. We evaluated the KEF-membrane as a model of the human buccal membrane, and found that the KEF-membrane was useful to test the permeation behavior of lipophilic drugs (Tsutsumi et al., 1999). For a formulation strategy, we have also reported the physical property of polyvinyl alcohol (PVA) gel, a non-ionic polymer, as a principal excipient for the buccal dosage form to contain a cationic drug such as ET (Tsutsumi et al., 1994).

The aim of the present study was to develop the efficient buccal ET formulation using several pharmaceutical bases. Furthermore, the effect of absorption enhancers on the buccal ET absorption in vivo was investigated. Guinea-pigs were selected as a model animal because the biological composition of buccal mucosa (i.e. the degree of keratinization) is relatively similar to that of humans among other rodent animals (Ibrahim and Meyer, 1985).

2. Materials and methods

2.1. Materials

ET, Sodium caprate (CA) and glycocholic acid sodium salt (GC) were purchased from Tokyo Chemical Industries, Japan. PVA (n = between 1500 and 1800), polyoxyethylene octylphenyl ether (Triton X 100) and carboxyvinyl polymer (HIVISWAKO 105®) were purchased from Wako Pure Chemical Industries, Japan. Polyoxyethylene hydrogenated castor oil (Nikkol HCO 60) was supplied by Nikko Chemical, Japan. Cod-liver oil extract (CLOE) was a gift from Dr. Loftsson in Iceland University, Iceland. The fatty acid components of CLOE were described previously by Loftsson et al. (1995). The pharmaceutical bases used in the present study were hydrophilic ointment (JP XIII; oil in water (o/w) type ointment containing water: white petrolatum 25%, stearyl alcohol 20%, propylene glycol 12%, HCO 60 4%, glycerine monostearate 1%, p-methyl hydroxybenzoate 0.1%, p-propyl hydroxybenzoate 0.1%, purified water 37.8%; Yamada Pharmaceutical Industries, Japan); macrogol 1500 (JP XIII; water-soluble base: polyethylene glycol 1500 100%; Sanko Pharmaceutical Industries, Japan); Witepsol W-35 (oleaginous base: lauric acid (C_{12}) 40–50% and the other composed of caprylic acid (C_8)–stearic acid (C_{18}); Mitsuba Trading Company, Japan). The other chemical products used were of reagent grade.

2.2. Preparation of miniature capsules and oral administration

ET was directly filled into a capsule (MINI CAPSULES®, Japan Elanco, Japan) using the filling device. The capsules were administered orally to guinea-pigs using the sound.

2.3. Preparation of PVA gels

PVA powder was dissolved in purified water at around 80–100 °C, and added 0.3% Triton X 100 and ET, and then poured into a plastic container (diameter 23 mm, height 15 mm). This PVA solution in the container was then frozen at −20 °C for 90 min and subsequently thawed at 25 °C for 90 min. This process was repeated three times, and the gel produced was stored at 4 °C prior to use.

2.4. Preparation of bioadhesive tablet system (BTS)

Fig. 1 shows the schematic design of BTS that was used in this study. The BTS was prepared as follows: carboxyvinyl polymer and hydroxypropylcellulose (1:1, w/w) were mixed in a polyethylene bag. The mixture was directly compressed using a pressing machine (100 kg/cm^2; Shimadzu, Japan). The tablet was flat faced (diameter 10 mm, thickness 2 mm; weight 150 mg) and had bioadhesiveness. In the center of the tablet, a hollow was made (0.28 cm^2; diameter 6 mm, thickness 1 mm) by employing a punch equipped with a projection in its center. A mixture of 3 mg of ET and propylene glycol (PG) corresponded to 10% (w/w) of the total core amount were added to each base while stirring. Thirty milligrams of the mixture of pharmaceutical base containing 3 mg of ET were filled into the hollow of tablet. In the case of Witepsol W-35, it was prepared by heating at 60 °C for a few minutes and then mixed with ET while stirring continuously.

2.5. In vivo absorption from the buccal mucosa in guinea-pigs

Male guinea-pigs weighing approximately 350 g (Saitama Laboratory Animals, Japan) were used. After anesthetization with urethane saline solution (25%; 4 ml/kg i.p.), the guinea-pigs were secured on their back. The administration region of the buccal mucosa of each guinea-pig was wiped with an absorbent cotton immersed in saline, and then BTS was applied. Blood samples (0.3 ml) were taken via the jugular vein at 0.17, 0.5, 0.75, 1, 2 and 3 h after administration. Each collected blood was centrifuged, and the plasma (0.1 ml) was thoroughly mixed with methanol (0.3 ml) containing an appropriate amount of *p*-butyl hydroxybenzoate as an internal standard. The mixture was again centrifuged for 1 min and the supernatant solution was filtered using a disposable filter unit (Ekikuro-Disk 3CR, Gelman Science Japan Ltd., Japan). The concentration of ET in each of the sample solutions was then determined using the HPLC method described below.

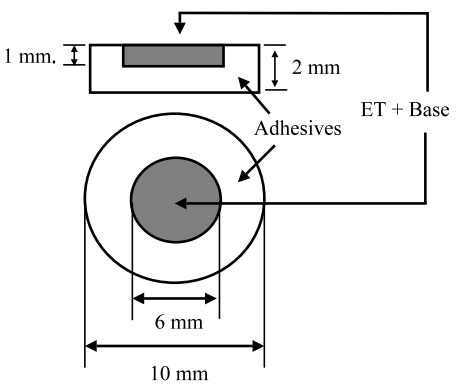

Fig. 1. Diagram of the BTS.

2.6. Pharmacokinetics parameters of ET in guinea-pigs

Phosphate buffer solution (pH 2.5) of ET was administered via the jugular vein at a dose of 0.1 mg/body. Sample solutions to determine the plasma concentration of ET was prepared as described above. The concentration of ET in the sample solution was determined by the HPLC method. Pharmacokinetic parameters of ET in guinea-pigs were obtained from the plasma concentration–time curve.

2.7. In vitro permeation study using KEF-membrane of the hamster cheek pouch

The cheek pouch of a male golden hamster (body weight approximately 100 g, Saitama Laboratory Animals, Japan) was excised and immersed in a 2 M sodium bromide solution for 18 h at 7 °C to obtain the KEF-membrane (Scott et al., 1986). The prepared KEF-membrane was then placed in a Franz-type permeation cell equipped with a water jacket (37 °C; available diffusion area 1.77 cm^2; volume of receiver cell 16.0 ml) (Merritt and Cooper, 1984). The receiver cell was filled with a mixture of pH 7.4 phosphate buffer solution and PG (1:1, v/v), stirring with a magnetic stirrer to maintain the

sink condition. After application of BTS to the donor side, the permeation experiment was started immediately. At an appropriate interval, a sample solution (0.3 ml) was taken from the receiver cell and replaced by the same volume of fresh PG–phosphate buffer mixture to maintain a constant volume. The concentration of ET in the sample solution was determined using the HPLC method.

2.8. HPLC determinations

Determination of the ET concentration in sample solution was performed by the HPLC method with a fluorescence detector (Cieri, 1987). The sample solution was injected onto a column (YMC Packed A-303 S-5 120A ODS, 4.6 × 250 mm, Yamamura Chemical Laboratories, Japan) using an autoinjector equipped with a system controller (SIL10A, SCL10A, Shimadzu, Japan), a pump (LC10AS, Shimadzu, Japan) and a fluorescence detector (RF-10AXL, Shimadzu, Japan) operating excitation wavelength at 250 nm and emission wavelength at 430 nm. Elution was carried out at room temperature with a mobile phase consisting of acetonitrile–water (1:1, v/v) containing 0.005 M sodium 1-pentanesulfonate at a flow rate of 1.0 ml/min. In the case of in vitro experiments, UV detector (SPD6A, Shimadzu, Japan) operating at 313 nm was used.

2.9. Data analysis

Area under the curves of the plasma ET concentration (AUC) was determined with the trapezoidal rule up to 3 h after administration. The observed oral or buccal bioavailability of ET were estimated using the following equation, in which F' is the observed bioavailability; D is the administered dose; and t is the administered time:

$$F' = (\mathrm{AUC}_{0-t,\mathrm{buccal}} D_{\mathrm{iv}} / \mathrm{AUC}_{\mathrm{iv}} D_{\mathrm{buccal}}) \times 100\% \quad (1)$$

The means of all data were presented with their standard error (mean ± S.E.). The significant difference between two groups was determined by a non-paired Student's t-test.

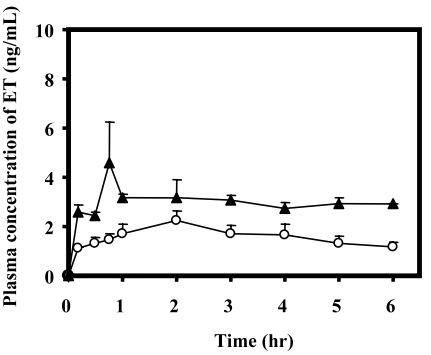

Fig. 2. Absorption profiles of ET after oral and buccal administration in guinea-pigs at a dose of 3 mg/body. ○, Oral; ▲, Buccal. Each point represents the mean ± S.E. ($n = 3$–6).

3. Results and discussion

3.1. Comparison between oral and buccal administration with PVA gel formulation

The pharmacokinetic parameters after an intravenous administration of ET at a dose of 0.1 mg/body were: an average elimination half-life of 0.245 ± 0.041 h, a volume of distribution of 7.29 ± 0.23 l/kg, and the first-order elimination rate of 2.92 ± 0.56 h^{-1}. The $t_{1/2}$ of ET was considered to be fairly short. These findings were comparable with those of dihydroergotamine by intramuscular administration in humans (Humbert et al., 1996).

As shown in Fig. 2 and Table 1, the AUC_{0-6h} value of ET from PVA gel by buccal administration was two fold greater ($P < 0.001$) than that of the capsule administered orally at the same dose of 3 mg/body. This result indicated that the buccal absorption of ET was significantly greater compared with that of gastrointestinal absorption.

The release profile of ET from PVA gel was regarded as an apparent first-order mechanism because a certain amount of insoluble ET was homogeneously incorporated in the hydrophilic gel. Although the amount of ET release from the PVA gel was about 40% until 6 h (Tsutsumi et al., 1994), the buccal absorption was considerably efficient as compared to that of the gastrointestinal tract. It is suggested that the release rate from a device might be one of the rate-determining

Table 1
Pharmacokinetic parameters of ET after oral and buccal administration at a dose of 3 mg/body

Formulation	C_{max} (ng/ml/cm^2)	t_{max} (min)	AUC_{0-6h} (h · ng/ml/cm^2)	F' (%)[a]
Capsule	2.95 ± 0.53	88 ± 33	5.11 ± 1.38	2.61 ± 0.70
PVA gel	5.08 ± 2.56	58 ± 46	11.6 ± 1.6[b]	5.90 ± 1.45

Each value represents the mean ± S.E. of 3–6 determinations.
[a] Percent ratio of AUC_{0-6h} values versus intravenous injection with the equivalent dose of ET.
[b] Significant difference from the capsule ($P < 0.001$).

factors for ET. The mucosal dosage form that has an adhesive region and a drug reservoir was previously designed and improved the human gingival absorption of lidocaine (Ishida et al., 1982). In the present study, a mixture of carboxyvinyl polymer and hydroxypropyl cellulose (1:1, w/w) was selected as an adhesive component for BTS, of which the compatibility and safety has been evaluated (Ishida et al., 1981, 1982, 1983; Nagai, 1985). Witepsol W-35, hydrophilic ointment or macrogol 1500 was chosen as a core material to incorporate ET in a suspended condition. Fig. 3 and Table 2 show the plasma concentration–time profile and the AUC_{0-3h} values of ET for four buccal formulations. The AUC_{0-3h} values of three types of BTS were significantly greater than that of PVA gel ($P < 0.05$ each), and various profiles were observed depending on the bases in the formulations. Among these bases, the highest ET absorption was obtained when Witepsol W-35 was applied ($P < 0.05$ each to hydrophilic ointment and macrogol 1500). The observed bioavailability, F', of ET was largely increased (20.5%) by Witepsol W-35, which was 3.5 fold greater than that of PVA gel (5.9%). Tamura et al. (1997) have reported that an oily base, petrolatum, might inhibit the partitioning of cyclosporine toward the skin due to its high affinity to the drug. One of the plausible explanations for our finding is that the low melting point of Witepsol W-35 played an important role on the fast ET absorption. The lipophilic nature of Witepsol W-35 (hydroxyl value 40–50) could have highly dissolved ET, thereby, ET was immediately released from the base to distribute toward the mucosal surface at its melting point (34–35 °C) where the matrix structure of Witepsol W-35 was readily destroyed. The melting point of macrogol 1500 (hydroxyl value 264–300) is also around body temperature (37–41 °C), however, the dissolution of ET might be slow at the mucosal surface under the hydrophilic circumstances. The emulsified ointments that have the intermediate lipophilicity, absorption ointment (w/o type) or hydrophilic ointment (o/w type), have been observed to accelerate the absorption rate of salicylic acid in hamster cheek pouch membrane (Tanaka et al., 1980). They suggested that some active agent in the emulsified ointments could be involved in the drug absorption at the mucosal surface. According to the slow ET absorption from hydrophilic ointment displayed in Fig. 3, the release rate of ET from the matrix of the hydrophilic ointment was supposed to be a rate-limiting (the melting point of main components, white petrolatum and stearyl alcohol, are at 35–60 °C and 56–62 °C, respectively) regardless of its active agent. It is complex to interpret how the base influences ET solubility, distribution and/or diffusion, including

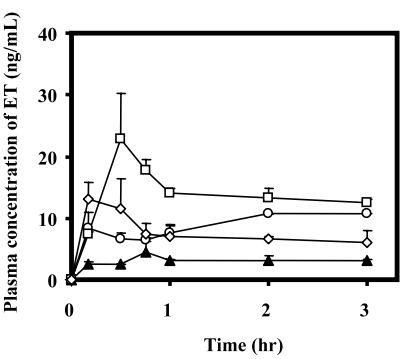

Fig. 3. Absorption profiles of ET after buccal administration of each formulation in guinea-pigs at a dose of 3 mg/body. ▲, PVA gel; □, Witepsol W-35; ◇, Macrogol 1500; ○, Hydrophilic ointment. Each point represents the mean ± S.E. of three determinations.

Table 2
Pharmacokinetic parameters of ET after buccal administration of BTS at a dose of 3 mg/body

Formulation	Enhancer	C_{max} (ng/ml/cm^2)	t_{max} (min)	AUC_{0-3h} (h · ng /ml/cm^2)	F' (%)[a]
Witepsol W-35	–	24.3 ± 6.8	40 ± 4	40.4 ± 5.0[b]	20.5 ± 2.6
Macrogol 1500	–	14.9 ± 3.6	20 ± 5	23.0 ± 1.5	11.7 ± 0.7
Hydrophilic ointment	–	14.0 ± 1.6	93 ± 51	25.5 ± 1.6	13.0 ± 0.8
	CLOE	77.9 ± 24.4	38 ± 12	79.6 ± 4.9[c,e]	40.6 ± 2.5
	GC	33.1 ± 4.3	50 ± 8	43.3 ± 2.4[d]	22.1 ± 1.1
	CA	29.0 ± 3.2	80 ± 41	48.3 ± 2.2[d]	24.5 ± 1.1
	HCO 60	22.2 ± 3.4	140 ± 16	46.5 ± 2.3[d]	23.7 ± 1.2

Each value represents the mean ± S.E. of three determinations.
[a] Percent ratio of AUC_{0-3h} values of each formulation versus intravenous injection with the equivalent dose of ET.
[b] Significant difference from other excipients ($P<0.05$ each).
[c] Significant difference from the hydrophilic ointment without enhancer ($P<0.001$).
[d] Significant difference from the hydrophilic ointment without enhancer ($P<0.01$).
[e] Significant difference from other enhancers ($P<0.01$ each).

changing thermodynamic activity of ET in the base. However, these findings demonstrated that the melting point and lipophilicity of the base were essential factors to consider for a rapid buccal absorption of ET.

3.2. Promoting activity of enhancers in hydrophilic ointment

The promoting activity of absorption enhancers in the buccal ET absorption was tested with incorporating each enhancer in the base. In this study, hydrophilic ointment was selected as a core base that has an affinity to both hydrophilic and lipophilic enhancers in order to minimize a thermodynamic interference for enhancing action toward the buccal mucosa. The enhancing ability of CLOE was already expressed in a comparison with those of well-known absorption enhancers, HCO 60, GC and CA through the KEF-membrane in vitro (Tsutsumi et al., 1998a).

The permeation of ionizable molecules is generally believed to follow the pH-partition theory across the buccal membrane (Shurmann and Turner, 1978; Chen et al., 1999). ET is expected to exist as nonionized and ionized forms by approximately 50% each in the oral cavity (pH 6.2–7.6) due to its pK_a value of 6.7. Although the mechanism of most absorption enhancers is not clarified yet, generally they are considered to act specifically at the transcellular or the paracellular pathways in the epithelium. CLOE extracted from the cod-liver oil (consisted mainly of triglycerides) was a mixture of 16 kinds of unsaturated (ca. 83%) and saturated acids (ca. 17%). The composition of fatty acids in CLOE is almost the same as that of the cod-liver oil such as: oleic acid (16%; 18:1, ω-9), docosahexaenoic acid (DHA; 12%; 22:6, ω-3), eicosapentaenoic acid (EPA; 9%; 20:5, ω-3), gondoic acid (9%; 20:1, ω-9), cetoleic acid (8%; 22:1, ω-11), palmitoleic acid (6%; 16:1, ω-7) and *cis*-vaccenic acid (4%; 18:1, ω-7). Loftsson et al. (1995) have reported that the permeation enhancing effect of CLOE appeared to be associated with the unsaturated acids: palmitoleic acid, *cis*-vaccenic acid, EPA, DHA and oleic acid enhanced the permeation of hydrocortisone through hairless mouse skin. Additionally, linoleic acid (2%; 18:2, ω-6) has also been shown the enhancement for salicylic acid permeation in human abdominal skin (Cooper, 1984). In terms of promoting buccal absorption, the considerable effect of oleic acid has been observed in porcine buccal membrane (Manganaro and Wertz, 1996). As a result of the previous in vitro transport examination of ET, the overall action mode of CLOE was mainly for a permeation enhancement of the nonionized form of ET (Tsutsumi et al., 1998b). Since no comparable effect of ET permeation enhancement was observed by individual major components of CLOE, oleic acid, EPA and DHA, at the donor concentration determined by

the composition ratios corresponding to 5% CLOE (Tsutsumi et al., 1998a), the promoting action of CLOE was suspected to present a synergistic mode occurred by its various components.

Results are shown in Fig. 4 and Table 2. When 5% (w/w) of each enhancer was added to the hydrophilic ointment, ET absorption was significantly increased compared with that of the control. CLOE exhibited the greatest enhancing activity among those enhancers in which the AUC_{0-3h} value was 3.1 fold greater than that of the control ($P < 0.001$). The F' values of CLOE and the control were 40.6 and 13.0%, respectively. Moreover, the T_{max} and C_{max} values of CLOE were markedly faster and higher than those of the control, respectively. Takeuchi et al. (1998) have investigated that the comparison between that *cis*-unsaturated fatty acids, oleic acid (ω-9) and petroselinic acid (ω-12), which differ from each other in the position of a double bond while maintaining the same number of aliphatic carbon atoms, by Fourier transform/attenuated total reflection analysis, with respect to their action as skin penetration enhancers. Their results clarified that differences in the physicochemical properties of fatty acids that originate from differences in the double bond position most likely determine the efficacy as penetration enhancers: petroselinic acid in which a double bond is at an even numbered position more rapidly affects the perturbation of the structures of both epidermis and dermis than oleic acid in which the double bond is at an odd numbered position.

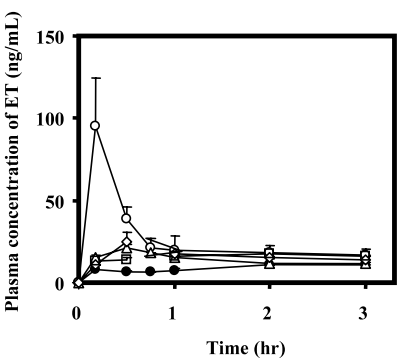

Fig. 4. Absorption profiles of ET after buccal administration in hydrophilic ointment containing several enhancers in guinea-pigs at a dose of 3 mg/body. ●, Control; ○, CLOE; ◇, GC; □, CA; △, HCO 60. Each point represents the mean ± S.E. of three determinations.

In contrast, the enhancement of buccal ET absorption by GC, CA and HCO 60 were statistically meaningful but relatively modest (1.7, 1.9 and 1.8 folds, respectively). CA (C_{10}) has been utilized as an absorption enhancer in an approved suppository of ampicillin in Sweden and Japan (Doktacillin®, Astra, Sweden). A possible explanation for this result is partly provided by the fact that the enhancing action of CA has been reported to act primarily with altering the barrier of polar pathway in various mucosal epithelium (Tomita et al., 1988; Hayashi et al., 1999). Likewise, the treatment of the human epidermis with surfactant, decylmethyl sulfoxide, slightly increased the transport of salicylic acid at pH 2.65 (for nonionized form of salicylic acid), while greatly increasing that at pH 9.9 (for ionized form of salicylic acid) (Cooper, 1982). Assumably, the permeation of the ionized ET could not be promoted effectively by those absorption enhancers through the restricted diameter of aqueous paracellular pathway (Tomita et al., 1988) due to its relatively large molecular size (MW 582 for ionized ergotamine). In the case of bile salts, its enhancing capability is thought to become pronounced above the critical micelle concentration (CMC 2–5 mM for GC) because the formation of micelles involves in an interaction with membrane components, thereby increasing membrane fluidity and permeability. Senel et al. (1998) have suggested that glycocholic acid begins to accumulate in the tissue after exposure to mucosa and then open both the paracellular and transcellular pathways in the epithelium for the passage of drugs at 100 mM. On the other hand, 5% (equivalent to 100 mM) of sodium deoxycholate combined with a carbopol–poloxamer gel has shown only 1.7 fold increase of triamcinolone acetate permeation in the excised porcine buccal membrane (Shin and Kim, 2000). Similarly, although the applied GC concentration was approximately 100 mM, our result did not show great enhancement of ET absorption by GC.

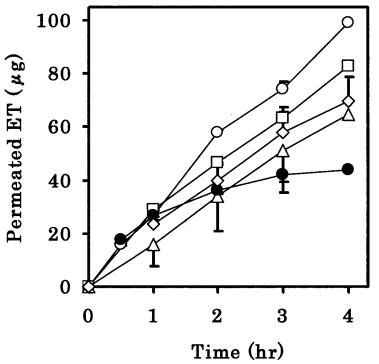

Fig. 5. Permeation profiles of ET in hydrophilic ointment containing several enhancers through the KEF-membrane at a dose of 3 mg. ●, Control; ○, CLOE; ◇, GC; □, CA; △, HCO 60. Each point represents the mean ± S.E. of three determinations.

3.3. In vitro–in vivo correlation of ET absorption

We have previously found that the apparent permeability coefficients (P) of drugs increased with lipophilicity through the viable full-thickness membrane of hamster cheek pouch which attained a plateau value for highly lipophilic drugs with log K (logarithm of partition coefficient in isopropyl myristate/pH 7.4 phosphate buffer solution, 37 °C) ≥ 1 in the in vitro transport study (Tsutsumi et al., 1999). The plateau P-values were quantitatively identical to those of the KEF-membrane (keratinized epithelium-removed: the lamina propria and the submucosa composed of collagen and elastic fibers and cellular components in a hydrated ground substances), while hydrophilic drugs (log $K \leq 1$) passed through the KEF-membrane seven times or more faster than those through the viable full-thickness membrane. Flynn et al. (1981) has formerly reported that the dermis was a major resistance to diffusion for the series of lipophilic alkanols in hairless mouse skin. Accordingly, it is hypothesized that the passive diffusion of ET (log $K = 2.3$) via transcellular pathway was sufficiently rapid, thus the submucosal layer became a dominant permeation barrier.

Fig. 5 shows the in vitro permeation profile of ET from BTS containing hydrophilic ointment across the KEF-membrane. The relative efficacy of absorption enhancers in vitro is in full agreement with that in vivo. ET permeation markedly improved by the addition of absorption enhancers, specifically CLOE. The in vivo F' values (taken from Table 2) and the in vitro flux, J, values of ET arising from BTS containing each enhancer in hydrophilic ointment were compared. A good correlation of the relative efficacy among those enhancers was observed between the F' and J values of ET, as shown in Fig. 6 ($r = 0.958$). This result supports the hypothesis. It has been known that the submucosal tissue of buccal membrane acts as a storage depot in the drug absorption (Tanaka et al., 1980). Consequently, CLOE was appeared to enhance ET permeation in the submucosal layer in vivo as well. From these findings, the action mode of CLOE in vivo was identified with that of the in vitro absorption studies. In another word, the effects of enhancers in vivo could be predicted by utilizing the KEF-membrane as a model membrane in the in vitro experiments for highly lipophilic drugs.

In conclusion, the in vivo buccal ET absorption was significantly greater than that of oral administration in guinea-pigs. For the buccal dosage form, BTS that had separate adhesive and drug release parts was better than homogeneous PVA gel on the buccal ET absorption. Furthermore, Witepsol W-35 most improved the buccal ET absorption as a pharmaceutical base. In addition, the enhancing mechanism of CLOE through the buccal membrane in vivo was clarified to decrease a diffusional resistance of the submucosal layer. Although many kinds of purified fatty acid have

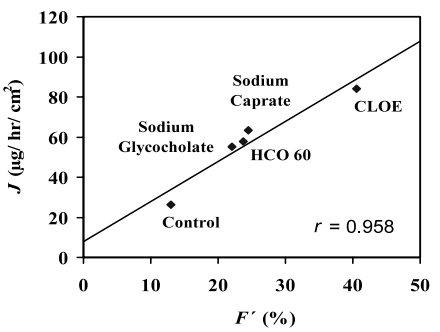

Fig. 6. Relation between F' (%) and J values (μg/h/cm^2) of ET in hydrophilic ointment.

strong potency of enhancing drug permeation in various membranes, their harmfulness remains too problematic (e.g. irreversible membrane damage) to be clinically useful in the majority of extensive absorption enhancer search (Van Hoogdalem et al., 1990; Swenson et al., 1994; Lindmark et al., 1997). CLOE derived from a natural marine product with absorption enhancing capability is feasible to fulfill this requirement.

Acknowledgements

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