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Effect of the cod-liver oil extract on the buccal permeation of ionized and nonionized forms of ergotamine using the keratinized epithelial-free membrane of hamster cheek pouch mucosa

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Abstract

The effect of cod-liver oil extract (CLOE) on the buccal permeation of ionized and nonionized forms of ergotamine (ERG) was investigated in vitro and compared with that of oleic acid which was a major component (ca. 16%) of CLOE. The apparent partition coefficient (K) values of ERG were slightly increased upon the addition of enhancers, however, a significant difference was not observed between CLOE and oleic acid. To investigate the mode of action of CLOE, the permeability coefficients of the nonionized (P^n) and ionized forms (P^i) of ERG were determined in the presence and absence of enhancers. The P^n values of ERG were markedly increased, especially CLOE; however, the P^i values of ERG were almost constant upon the addition of each enhancer. It was clarified that CLOE mainly enhanced the permeation of the nonionized form of ERG.

Keywords: Buccal permeation; Ergotamine; Cod-liver oil extract; Oleic acid

1. Introduction

Ergotamine (ERG) is used clinically as tartrate in the treatment of migraines. However, the bioavailability of ERG is rather poor following oral administration. We consider that buccal administration of ERG is one of the most attractive routes for systemic delivery when the bioavailability following oral administration is insufficient due to such effects as first-pass hepatogastrointestinal metabolism (Hoogstraate et al., 1996; Taylan

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et al., 1996; Voorspoels et al., 1996). However, the buccal permeation rate was usually in the range of one or more orders of magnitude lower than those found for other mucosal sites, in particular the nasal mucosa.

We have previously reported the enhancing effect of several permeation enhancers which had low irritancy of the mucosa and cod-liver oil extract (CLOE) on the permeation of ERG tartrate in a mixture of propylene glycol (PG) and phosphate buffer solution (1:1, v/v) through the keratinized epithelial-free membrane of hamster cheek pouch mucosa in vitro (Tsutsumi et al., 1998). Considering that the human buccal mucosa does not have a keratinized layer, we prepared keratinized epithelial-free membrane of hamster cheek pouch mucosa by chemical splitting. It was considered that the enhancing activity of CLOE which exhibited the greatest effect among these enhancers was a result of a direct action on the mucosal membrane together with the solubilizing effect of CLOE in the donor solution. The purpose of this study was to investigate the mode of action of CLOE in the transbuccal delivery of the ionized and nonionized forms of ERG.

2. Materials and methods

2.1. Materials

ERG tartrate and oleic acid were purchased from Tokyo Chemical Industries, Japan. CLOE was a gift from T. Loftsson in the University of Iceland. The fatty acid components of CLOE were described in a previous report (Loftsson et al., 1995). The other chemical products used were of reagent grade.

2.2. Determination of the apparent partition coefficient of ERG

An excess amount of drug was added to phosphate buffer solution, pH 7.4, or isopropyl myristate (IPM) at 37°C for 1 h under sonication. 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 ERG in the phosphate buffer solution (C_w) or IPM (C_{oil}) was determined using high performance liquid chromatography (HPLC). The apparent partition coefficient (K) of ERG was calculated as the solubility ratio: C_{oil}/C_w.

2.3. Permeation study

An in vitro permeation study was carried out using the cheek pouch of a male golden hamster (body weight ca. 100 g: Saitama Laboratory Animals, Japan) as a model membrane for the human buccal mucosa (Tanaka et al., 1980; Ishida et al., 1983; Kurosaki et al., 1991). An appropriately sized area of the cheek pouch was excised and then immersed in a 2 M sodium bromide solution for approximately 18 h to obtain the keratinized epithelial-free membrane (Scott et al., 1986). This membrane was placed in 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) (Tojo et al., 1987). Both cells were stirred using a magnetic stirrer. The donor cell was filled with a suspension of ERG tartrate in a mixture of phosphate buffer solution and PG (1:1, v/v), and then the pH of the ERG suspension was measured after being saturated. The receiver cell was filled with the PG-phosphate buffer solution mixture.

2.4. Determination of ERG concentration

Determination of ERG concentration in the sample solution was performed using HPLC. The sample solution was injected onto the column (YMC Packed A-303 S-5 120A ODS, 4.6×250 mm, Yamamura Chemical Laboratories, Japan) by an autoinjector equipped with a system controller (SIL10A, SCL10A, Shimadzu, Japan), a pump (LC10AS, Shimadzu) and a UV detector (SPD6A, Shimadzu) operating at 313 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.

2.5. Data analysis

The pK_a values of ERG in the PG-phosphate buffer solution mixture were obtained using the solubility data at 37°C.

$$pK_a = pH + \log \{(S - S_0)/S_0\} \quad (1)$$

where S is the solubility of ERG at the current pH and S_0 denotes the solubility of the nonionized form of ERG (which was determined at pH 9).

The permeability coefficient (P) of the nonionized and ionized forms of ERG was determined separately in order to clarify the effect of enhancers on the buccal permeation of ERG (Swarbrick et al., 1984; Obata et al., 1993a,b). The following equation can be derived under the assumption that the total flux (J) was composed of the individual J of the nonionized and ionized forms using the experimental values obtained using the two kinds of pH lower or higher to pK_a value of the PG-phosphate buffer solution mixture.

$$J^t = P^n C^n + P^i C^i \quad (2)$$

where J^t is the total J of ERG calculated using the experimental data, and P^n and P^i are the P values of the nonionized and ionized forms, respectively. C^n and C^i are the concentrations of the nonionized and ionized forms in the donor solution, respectively.

$$C^n = C^t - C^i \quad (3)$$

$$C^i = \frac{C^t}{1 + 10^{(pH - pK_a)}} \quad (4)$$

where C^t is the total concentration of ERG in the donor solution. Since ERG was suspended in the donor solution, the total solubility of ERG was used as the C^t value for estimating C^n and C^i values.

3. Results and discussion

The effect of CLOE on ERG permeation through the keratinized epithelial-free membrane separated from hamster cheek pouch was investigated and compared with that of oleic acid. Oleic acid was selected as an active control because it is a major component (ca. 16%) of CLOE. The influence of enhancers on the apparent partition coefficient (K) values of ERG was clarified using phosphate buffer solution and IPM containing various concentrations of each enhancer. The results are given in Fig. 1. The K values were slightly increased upon the addition of enhancers; however, a significant difference was not observed between CLOE and oleic acid.

Fig. 2 shows the flux and apparent permeability coefficient of ERG from the PG-pH 7.4 phosphate buffer solution mixture (1:1) through the keratinized epithelial-free membrane of hamster cheek pouch. The permeation rate was markedly increased by the addition of 3% CLOE or oleic acid to the donor solution when compared with the control solution not containing enhancers. The greatest enhancing activity of CLOE was seen at 5%; however, a further increase in the CLOE concentration from 7 to 10% resulted in a decrease in enhancing activity. On the other hand, the enhancing activity of oleic acid was maximized at 3% and then both J and P values gradually decreased with an increase in oleic acid concentration from 5 to 10%. Niazy (1991) has reported a similar phenomenon of oleic acid in

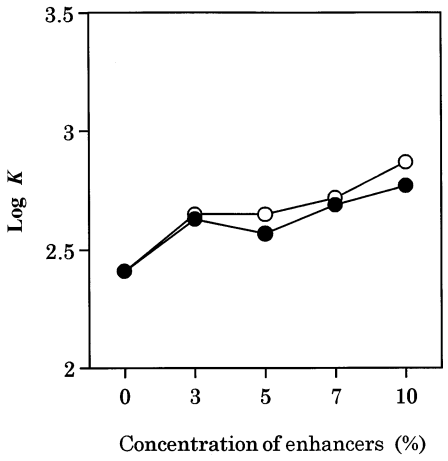

Fig. 1. Effect of permeation enhancers at various concentrations on the partition coefficients (K) of ERG using phosphate buffer solution (pH 7.4) and IPM containing enhancers at 37°C. ●, CLOE; ○, oleic acid.

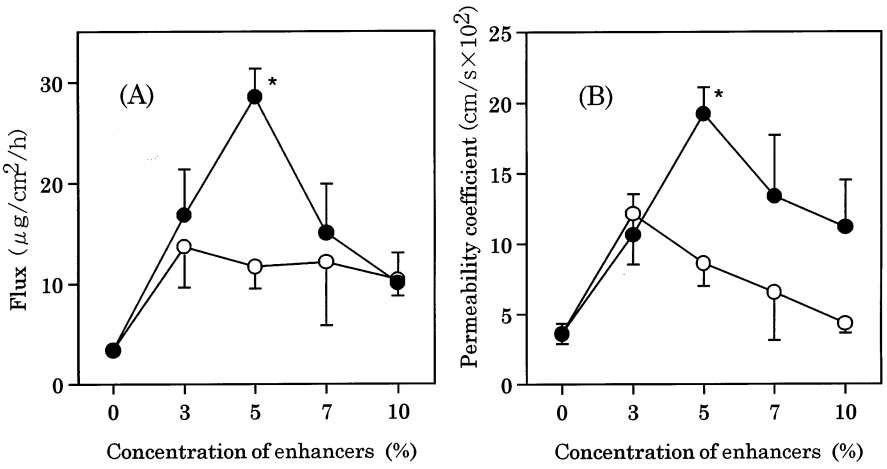

Fig. 2. Effect of permeation enhancers on the flux (*A*) and permeability coefficient (*B*) of ERG through epithelial-free hamster cheek pouch membrane. ●, CLOE; ○, Oleic acid. * $p < 0.01$. Each point represents the mean ± S.D. of three determinations.

PG for the transport of dihydroergotamine through rabbit skin. Further, Stoughton and McClure (1983) have reported that Azone has an optimal concentration for the enhancing effect due to the formation of a physiologically inactive complex between Azone at high concentrations and several drugs such as fusidic acid, triamcinolone and 5-flurouracil. Although the reason that the enhancing activity of CLOE decreased at higher concentrations is not clear, there is some possibility of physiologically inactive complex formation between CLOE and ERG, because the solubility of ERG was somewhat lowered at the higher concentrations of CLOE as summarized in Table 1. The enhancing activity of CLOE was greater than that of oleic acid, suggesting that the other components of CLOE may act as effective absorption enhancers. For this point, Loftsson et al. (1995) have previously reported that palmitoleic acid, *cis*-vaccenic acid, EPA and DHA which were some components of CLOE as well as oleic acid enhanced the permeation of hydrocortisone through hairless mouse skin in vitro. Therefore, it was considered that these components of CLOE also affected the enhancing activity of CLOE.

ERG was partially dissociated in the donor solution because the pK_a value of ERG was estimated to be 6.7. To investigate the mode of action of CLOE more precisely, we estimated P^n and P^i values of ERG using Eqs. (2)–(4) and the solubility data of ERG in the PG-phosphate buffer solution containing various concentrations of enhancers (Table 1). As shown in Fig. 3, the P^n values of ERG were markedly increased, especially with CLOE; however, the P^i values of ERG were almost constant and the data points in Fig. 3 were overlapped on each other upon the addition of each enhancer. It was suggested that CLOE mainly enhanced the permeation of the nonionized form of ERG. Consequently, the increase in the J and P values of ERG upon the addition of CLOE, as shown in Fig. 2, is attributable to the enhancement of the permeation of nonionized ERG. It is likely that nonionized ERG permeates the membrane via a lipophilic route based on a passive-diffusion mechanism (Higuchi et al., 1960). CLOE may distribute in the lipid-rich region of the buccal membrane and may change the barrier structure of the lipoidal pathway in the keratinized epithelial-free membrane separated from hamster cheek pouch.

In our permeation study of ERG, PG-phosphate buffer solution mixture was employed as both donor and receiver solutions. Further, ERG was used as a salt form with tartaric acid. Gao and Singh (1998) has reported that PG enhanced the transepidermal water loss which was a rele-

vant parameter of the functional state of the cutaneous barrier through porcine epidermis in vitro. Synergy of PG with several enhancers such as Azone and terpenes on the drug permeation through the skin has also been reported (Okamoto et al., 1987; Michniak et al., 1998; Ho et al., 1998). On the other hand, Okada et al. (1982) reported that carboxylic acid such as tartaric acid enhanced the vaginal absorption of leuprolide in rats in vivo. Similar enhancement action of tartaric acid on the permeation of FITC-dextran 4000 in hamster colon in vitro has also been reported by Hayashi et al. (1998). In addition to the results observed with CLOE, the promoting activities of PG and tartaric acid on the permeation of ERG should be investigated in future in order to understand the mechanism of mode of action of CLOE in detail. However, our present results demonstrated that CLOE could be promising compound as a permeation enhancer for the buccal absorption of ERG.

Table 1
Influence of various concentrations of enhancers on the pH and solubility of ERG

Enhancer (w/w%)		pH of ERG suspensions	C^{t} [a] (mg/ml)
Control	—	7.83	$9.36\times10^{-2}\pm0.33\times10^{-2}$
		4.82	$2.28\times10\pm0.09\times10$
CLOE	3	6.67	$1.58\times10^{-1}\pm0.13\times10^{-1}$
		4.18	3.13 ± 0.11
	5	6.14	$1.49\times10^{-1}\pm0.10\times10^{-1}$
		4.13	5.37 ± 0.21
	7	6.25	$1.13\times10^{-1}\pm0.09\times10^{-1}$
		4.15	4.15 ± 0.20
	10	5.75	$8.99\times10^{-2}\pm0.16\times10^{-2}$
		4.05	3.39 ± 0.54
Oleic acid	3	6.50	$1.13\times10^{-1}\pm0.23\times10^{-1}$
		4.14	3.24 ± 0.27
	5	5.82	$1.36\times10^{-1}\pm0.10\times10^{-1}$
		4.13	5.57 ± 0.05
	7	5.91	$1.85\times10^{-1}\pm0.27\times10^{-1}$
		4.14	3.62 ± 0.46
	10	5.53	$2.40\times10^{-1}\pm0.20\times10^{-1}$
		4.10	3.21 ± 0.29

[a] Total concentration of ERG in the donor solution. Each value represents the mean ± S.D. for three determinations.

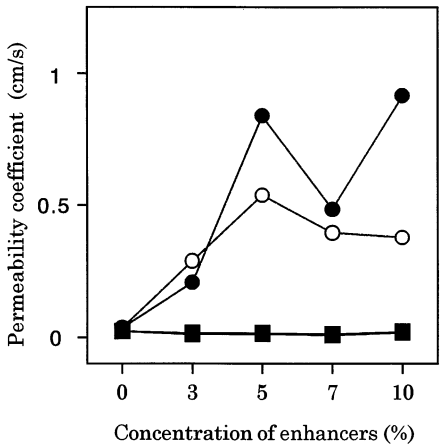

Fig. 3. Effect of permeation enhancers on the permeability coefficient (P) of nonionized and ionized ERG through epithelial-free hamster cheek pouch membrane. ●, P^{n} in CLOE; ○, P^{n} in oleic acid; ■, P^{i} in CLOE; □, P^{i} in oleic acid.

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