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RESEARCH PAPER

Effect of Cod-Liver Oil Extract on the Buccal Permeation of Ergotamine Tartrate

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ABSTRACT

Ergotamine tartrate (ET) is used clinically in the treatment of migraines. However, the bioavailability of ET is rather poor following oral administration. Therefore, we tried to improve ET delivery using buccal administration. The purpose of this study was to investigate the characteristics of the permeation of ET through the hamster cheek pouch in vitro using a two-chamber diffusion cell, and to evaluate the effect of permeation enhancers on the transbuccal delivery of ET.

Cod-liver oil extract (CLOE), polyoxyethylene hydrogenated castor oil (HCO 60), sodium glycocholate (GC), and sodium caprate (CA) were selected as permeation enhancers considering their low irritancy of the mucosa. When the enhancers were added to the donor cell at a 5% concentration each, the ET permeation rate markedly increased compared with that in a control not containing enhancer. Among these enhancers, CLOE exhibited the greatest effect.

Because CLOE is composed of 16 kinds of fatty acids, the enhancement action of each of the major components was separately determined. As major fatty acids, palmitic acid, oleic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) were selected and their enhancing effects were studied. The enhancing effect of each fatty acid was significantly lower than that of CLOE.

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INTRODUCTION

In recent years, there has been a renewed interest in using buccal, nasal and eye mucos as sites for non-invasive drug delivery. The administration of drugs via mucosa makes it possible to bypass the first-pass hepato-gastrointestinal metabolism of drugs following oral administration (1–4).

We selected the buccal mucosa as an administration site for a drug. The buccal mucosa has the advantage over other mucosa that a drug can be easily applied and localized there, and then can also be removed from the application site (5–8).

The buccal mucosa consists of an outermost layer of stratified squamous epithelium, below which lies a basement membrane, and below this, in turn, a lamina propria and submucosa. The basement membrane acts as a permeation barrier to large molecules and lipophilic drugs.

Ergotamine tartrate (ET) is used clinically in the treatment of migraines. However, ET has a large molecular weight (MW 1313) and the bioavailability of ET is rather poor following oral administration. Therefore, we tried to improve ET delivery using buccal administration. 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.

Several absorption enhancers have been developed to enhance the absorption of drugs by the gastrointestinal, rectal, and other mucosal membranes. For example, sodium salts of medium-chain fatty acids, such as caprate, are some of these enhancers. Other enhancers, various fatty acids such as oleic acid and palmitoleic acid, have been shown to increase the skin permeation rates of many drugs (9).

The purpose of this study was to investigate the characteristics of the permeation of ET through the hamster cheek pouch in vitro (10,11), and to evaluate the effect of permeation enhancers on the transbuccal delivery of ET.

MATERIALS AND METHODS

Materials

ET, sodium caprate (CA), glycocholic acid sodium salt (GC), oleic acid, and palmitic acid were purchased from Tokyo Chemical Industries, Ltd., Japan. Polyoxyethylene hydrogenated castor oil (Nikkol HCO 60) was supplied by Nikko Chemical Co. Ltd., Japan. cis-5,8,11, 14,17-Eicosapentaenoic acid (EPA) and cis-4,7,10,13, 16,19-docosahexaenoic acid (DHA) were purchased from Sigma Chemical Co. (St. Louis, MO). Cod-liver oil extract (CLOE) was gift from T. Loftsson, Iceland University, Reykjavik, Iceland. Fatty acid com-

Table 1

The Fatty Acid Component of Cod-Liver Oil and Its Fatty Acid Extract (CLOE)

Fatty Acid		Composition (%)	
Name	Number	Cod-Liver Oil	CLOE
Myristic acid	14:0	3.6	3.6
Palmitic acid	16:0	10.5	10.4
Palmitoleic acid	16:1 n-7	6.5	6.4
Stearic acid	18:0	2.6	2.6
cis-Vaccenic acid	18:1 n-7	4.4	4.4
Oleic acid	18:1 n-9	16.3	16.2
Linoleic acid	18:2 n-6	1.6	1.5
Moroctique acid	18:4 n-3	2.4	2.4
cis-11-Eicosenoic acid	20:1 n-7	0.4	0.5
Gondoic acid	20:1 n-9	9.6	9.4
Gadoleic acid	20:1 n-11	1.5	1.6
Eicosapentaenoic acid (EPA)	20:5 n-3	9.6	9.3
Erucic acid	22:1 n-9	0.6	0.6
Cetoleic acid	22:1 n-11	7.7	7.8
Clupandonic acid	22:5 n-3	1.4	1.4
Docosahexaenoic acid (DHA)	22:6 n-3	12.5	11.9

ponents in CLOE are given in Table 1 (12). Oleic acid, palmitic acid, EPA, and DHA were of extra pure grade. The other chemical products were of reagent grade.

Determination of ET Solubility

An excess amount of ET (about 30-fold the solubility) was added to phosphate buffer (PB), pH 7.4 or a mixture of PB and propylene glycol (PG) or glycerine. The resulting ET suspension was placed in a water bath (37°C) for 24 hr and stirred with a magnetic stirrer. An aliquot was withdrawn from the suspension and filtered through a 0.45 μm disposable filter unit (Ekikuro-Disk 13, Gelman Science Japan Ltd.). The sample solution was then diluted with methanol containing an appropriate amount of *p*-hydroxybenzoate *n*-butyl ester as an internal standard. The concentration of ET in the sample solution was determined using HPLC.

Determination of ET Concentration

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

Permeation Study Using the Buccal Membrane of the Hamster Cheek Pouch

An in vitro permeation study was carried out using the cheek pouch of a male golden hamster (body weight approximately 100 g, Saitama Laboratory Animals, Saitama, Japan) as a model membrane for the human buccal mucosa. An appropriately sized area of the cheek pouch was excised and immersed in a 2 M sodium bromide solution for 18 hr to obtain the epithelial-free membrane (13–15). This membrane was then 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) (16). Both cells were stirred using a magnetic stirrer. The donor cell was filled with a suspension of ET in the PG/PB mixture. The receiver cell was filled with the PG/PB mixture.

Pretreatment of the Buccal Membrane with Enhancer

The donor cell was filled with a pretreatment solution containing 5% of each of the permeation enhancers. The receiver cell was filled with the PG/PB mixture not containing enhancer. Both cells were stirred by a magnetic stirrer. After the pretreatment (1 or 3 hr), the solutions in both cells were removed. The donor and receiver cells were rinsed several times using a fresh PG/PB mixture. After the cells were rinsed, the permeation experiment was started immediately.

RESULTS AND DISCUSSION

Effect of PG or Glycerine on the Solubility of ET

In a preliminary study, the apparent partition coefficient (K) of ET was determined employing an isopropyl myristate/PB, pH 7.4 system at 37°C for 24 hr (17). The K value of ET was about 200, indicating the highly lipophilic nature of ET. Therefore, we selected PG or glycerine as a solubilizer. The effect of PG or glycerine on the solubility of ET at 37°C is shown in Fig. 1. The solubility of ET in PB (pH 7.4) containing 50% PG was approximately 60-fold higher than that in PB not containing PG. Moreover, the effect of PG was greater than that of glycerine. The results indicated that the solubility of ET was significantly improved by the addition of PG. Therefore, we used a PG/PB (1:1 v/v) mixture as donor and receiver solutions in the permeation study.

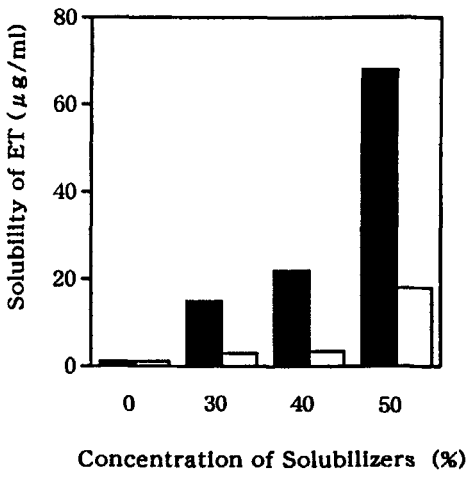

Figure 1. Effect of the concentration of solubilizers on the solubility of ET in PB, pH 7.4. ■, propylene glycol; □, glycerine.

Effect of Enhancers on Buccal Permeation Rate of ET

As shown in Fig. 2, the flux of ET through the hamster buccal mucosa was low, suggesting the poor permeation of ET through the membrane.

CLOE, HCO 60, GC, and CA were selected as permeation enhancers. When 5% each of the enhancers was added to the donor cell, the ET permeation rate was markedly increased compared with that in a control not containing enhancers. Among these permeation enhancers, CLOE exhibited the greatest effect. The solubility of ET in the donor solution containing enhancers is shown in Fig. 3. The solubility of ET was markedly increased by the addition of HCO 60, which may lead to a decrease in the partitioning of ET to the mucosa, resulting in a relatively low flux of ET. Similar phenomena were observed for GC and CA. On the other hand, the flux and the solubility of ET increased about eightfold and twofold, respectively, following the addition of CLOE. The maximum p value of ET was obtained following the addition of CLOE. These results suggest that the enhancing action of CLOE results from a direct action on the mucosa together with an ET solubilizing effect in the donor solution.

Figure 4 shows the effect of additive concentrations of CLOE in the donor cell on the ET permeation rate. Regardless of the additive concentration of CLOE, the p value of ET was almost constant. Therefore, the amount

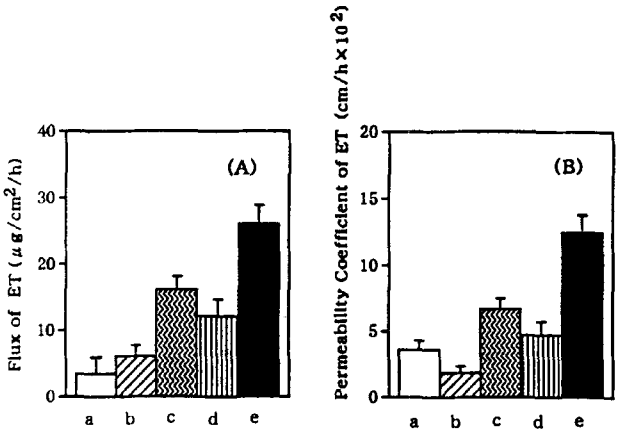

Figure 2. Effect of permeation enhancers on the flux (a) and permeability coefficient (b) of ET through epithelial-free hamster cheek pouch membrane. (a) Control; (b) HCO 60; (c) GC; (d) CA; (e) CLOE. Each point represents the mean ± SD of three determinations.

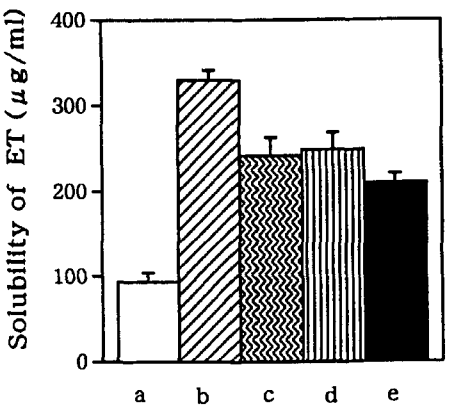

Figure 3. Effect of permeation enhancers on the solubility of ET in the donor solution. (a) Control; (b) HCO 60; (c) GC; (d) CA; (e) CLOE. Each point represents the mean ± SD of three determinations.

of CLOE required to exhibit the enhancing action was considered to be approximately 3%.

Mode of Enhancing Action of CLOE

To investigate the mode of action of CLOE, the buccal membrane was pretreated with CLOE for several

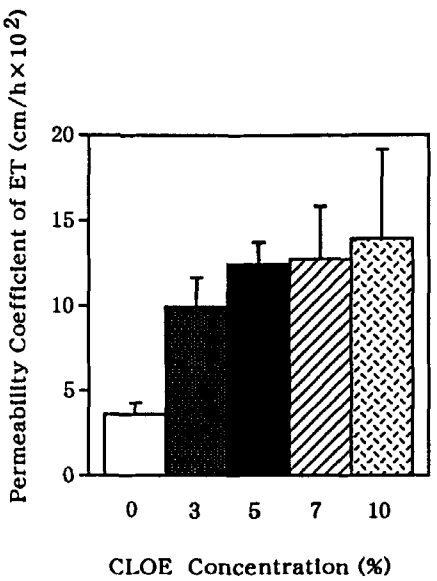

Figure 4. Effect of CLOE at various concentrations on the permeability coefficient of ET through epithelial-free hamster cheek pouch membrane. Each point represents the mean ± SD of three determinations.

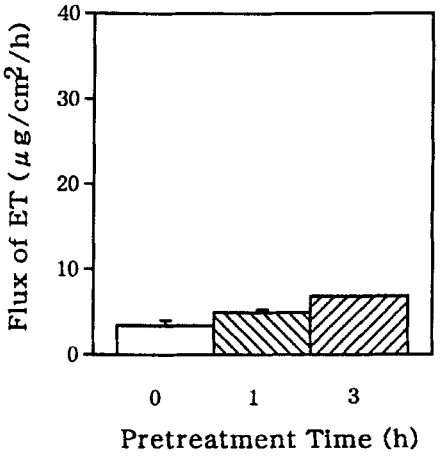

Figure 5. Effect of pretreatment with 5% CLOE on the flux of ET through epithelial-free hamster cheek pouch membrane. Each point represents the mean ± SD of three determinations.

hours. As shown in Fig. 5, the flux of ET was almost constant with or without pretreatment. Furthermore, the extension of the pretreatment period had no effect on the flux of ET. The results suggest that the enhancing effect of CLOE was transient.

CLOE is composed of 16 kinds of fatty acids which we summarized in Table 1 (12). Among these, palmitic acid, oleic acid, EPA, and DHA are major components. Thus, we selected these fatty acids to evaluate the enhancing effect of CLOE. The results are shown in Fig.

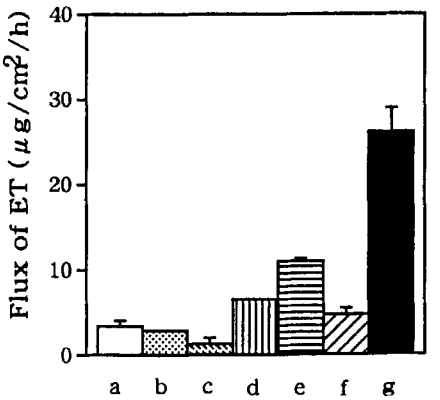

Figure 6. Effect of each fatty acid in CLOE on the flux of ET through epithelial-free hamster cheek pouch membrane. (a) Control; (b) palmitic acid; (c) EPA; (d) DHA; (e) oleic acid; (f) the mixture; (g) CLOE. Each point represents the mean ± SD of three determinations.

6. The concentration of each fatty acid in the donor solution was determined based on the composition ratios in Table 1, corresponding to 5% CLOE. The effects of each fatty acid and the mixture were significantly lower than that of 5% CLOE. It is likely that the enhancing action of CLOE results from the synergistic action of these major fatty acids or the effects of other lower percentage components in CLOE.

In conclusion, CLOE is promising as a naturally occurring enhancer of the buccal absorption of ET, although the precise enhancement mechanism has not yet been clarified.

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