ELSEVIER

International Journal of Pharmaceutics 174 (1998) 19–28

international journal of pharmaceutics

Buccal absorption through golden hamster cheek pouch in vitro and in vivo of 17β-estradiol from hydrogels containing three types of absorption enhancers

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Received 13 April 1998; received in revised form 25 May 1998; accepted 24 June 1998

Abstract

The combined effects of hydrogels and absorption enhancers on the permeability of 17β-estradiol (E_2) through buccal membrane were investigated by measuring the rate of permeation of E_2 through hamster cheek pouch buccal mucosa in vitro and in vivo. Glycerylmonolaurate (LAU), *l*-menthol (MEN) and sodium caprate (CAP) were selected as absorption enhancers in hydrogels containing ethanol and propylene glycol (PG). Based on the rate of penetration through the buccal mucosa of E_2 in the PG and ethanol gels containing the enhancers, the permeability of E_2 was observed to be greater in the hydrogel containing ethanol than in that containing PG. CAP increased the permeability of E_2 compared with that of the control in both the PG and ethanol gels. MEN increased the permeability of E_2 in a 40% (w/w) ethanol gel, and LAU was more effective in the ethanol gel than in the PG gel without decreasing the gel strength. Regarding the mechanisms of these absorption enhancers in the ethanol gel, CAP and MEN mainly contributed to the diffusion of E_2 in the mucosa, but LAU increased the solubility of E_2 in the hydrogel. The buccal administration of E_2 in the 40% (w/w) ethanol hydrogels containing 2% (w/w) LAU allowed the maintenance of the plasma level at above 300 ng/ml cm^2 for 7 h after application in hamster. No primary morphological change of buccal membrane was detected using scanning electron microscopy 7 h after application. These findings suggest that the combination of LAU and ethanol is useful and safe in hydrogels containing E_2 for application to the buccal mucosa.

Keywords: Buccal absorption; 17β-estradiol; Glycerylmonolaurate; *l*-Menthol; Sodium caprate; Hydrogel

1. Introduction

Osteoporosis of the old is a major problem in aged societies and is particularly serious for post-menopausal women. Treatment to prevent os-

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0378-5173/98/$ - see front matter
PII S0378-5173(98)00234-8

teoporosis involves delaying menopause artificially and supplying female hormones.

In the latter case, oral, percutaneous (Williams and Barry, 1991; Liu et al., 1992; Megrab et al., 1995) and nasal absorption (Schipper et al., 1990; Maitani et al., 1997) of estradiol (E_2) have been studied extensively by many investigators. The oral administration of E_2 results in very low bioavailability because of its high first-pass effect and thus buccal administration may present an alternative. Products for the transdermal delivery of E_2 are currently on the market (Santus and Baker, 1993). Recently, Estradarm TTS® has become commercially available in Japan. High permeability may not be expected for the transdermal delivery of E_2 without damaging the skin because the skin has the stratum corneum which is a barrier to drug absorption.

We focused on the buccal mucosa as a drug transport route, since the buccal mucosa is comparatively thin, contains many blood vessels, and drugs absorbed from the buccal mucosa bypass the liver and systemic vascular system. However, drugs in the term of buccal or sublingual tablets administered orally are absorbed to a very low degree. Many enhancers (Williams and Barry, 1991), cosolvents (Liu et al., 1992; Obata et al., 1993; Kim et al., 1996), and gel type formulations exhibiting adhesiveness to the mucosa (Voorspoels et al., 1996) have been studied to enhance the absorption. The buccal mucosa in hamster cheek pouch may be similar in part to the skin and the human oral mucosa having the stratum corneum.

Therefore, we investigated combined effects of hydrogels and absorption enhancers on the buccal permeation of E_2 in vitro and in vivo.

2. Materials and methods

2.1. Materials

E_2, *l*-menthol (MEN, M_W 156.27) and sodium caprate (CAP, M_W 194.25) were purchased from Tokyo Kasei (Tokyo, Japan). Lauricidin®, glyceryl monolaurate (LAU, M_W 274.38) was kindly supplied by Lauricidin (Galena, IL). Soybean-derived sterol mixture and its glucoside were kindly supplied by Ryukakusan (Tokyo, Japan). Sodium glycocholate was purchased from Nacalai Tesque (Kyoto, Japan). Water used throughout the experiments was membrane-filtered (Milli-Q, Nihon, Tokyo, Japan). Sodium taurocholate, propylene glycol 400 (PG), the carboxyvinylpolymer, marked as 'Hiviswako 103' and ethanol were of guaranteed reagent grade (Wako, Osaka, Japan). All other chemicals were of analytical reagent grade. Estradarm TTS® was purchased from Novartis (Tokyo, Japan).

2.2. Preparation of hydrogel ointments

The hydrogel ointments were prepared as follows: E_2 (0.5% (w/w)) and the enhancers (1–3% (w/w)) were dissolved in PG or ethanol. Separately, carboxyvinylpolymer (1.5% (w/w)) and triethanolamine (2% (w/w)) were dissolved in distilled water. Both solutions were then well mixed to produce hydrogels containing PG (20, 40% (w/w); PG gel) or ethanol (20, 40% (w/w); ethanol gel) (Table 1). The resulting gel ointment was stored at room temperature for 24 h under air-tight conditions prior to use.

2.3. In vitro permeation study

In the case of hydrogel ointments, the permeation through buccal mucosa was measured in a

Table 1
Formulae of the hydrogels containing estradiol

Hydrogels	(g)
PG gel	
Carboxyvinylpolymer	1.5
Triethanolamine	2.0
Propylene Glycol	20.0 or 40.0
Estradiol	0.5
Enhancer	1.0, 2.0 or 3.0
Water	ad. 100.0
Ethanol gel	
Carboxyvinylpolymer	1.5
Triethanolamine	2.0
Ethanol	20.0 or 40.0
Estradiol	0.5
Enhancer	1.0, 2.0 or 3.0
Water	ad. 100.0

Franz-type diffusion cell apparatus. The effective area available for diffusion was 1.44 cm^2. The receptor compartment was filled with 16 ml of 30% (w/w) PG solution, kept at 37°C, and agitated with a magnetic stirrer to maintain the receptor side sink condition. A piece of suitably sized cheek pouch buccal mucosa from a male golden hamster (90–100 g, Tokyo Laboratory Animals, Japan) was excised immediately before the permeation experiments. Approximately 2.0 g of hydrogel was spread on the epithelial cell side of the excised buccal mucosa, which was then mounted on the diffusion cell. Every hour up to 7 h, 200 μl samples were withdrawn from the receptor compartment. The same volume of 30% (w/w) PG solution from the same stock solution was added to the receptor compartment to keep the volume constant.

In the case of E_2 suspension in water, the following conditions were different; the cell apparatus used was a water-jacket type-2 chamber diffusion cell (0.785 cm^2), both donor (E_2 suspension) and receptor solution volume was 3 ml, and 50 μl samples were withdrawn from the receptor compartment.

The sample for permeation studies was filtered using a disposable filter unit (Ekikuro-Disc 3, Gelman, Tokyo, Japan). The concentration of E_2 in the filtrate was analyzed using an HPLC system (Shimadzu model LC-10AS, Shimadzu, Kyoto, Japan) equipped with a variable wavelength UV monitor (SSC UV 3000B, Sensyu, Tokyo, Japan). The flow rate was 1 ml/min and elution was carried out at 40°C. Other analytical conditions were as follows: column, YMC-Pack A-302 ODS, 150 × 4.6 mm i.d.; UV detection, 280 nm; mobile phase, methanol/water (65/35 v/v) (Maitani et al., 1997).

The cumulative flux (J) through the membrane was evaluated as a pseudo-steady-state slope of the plot of the cumulative amount of drug absorbed per unit surface area vs time at eight points for a period of 7 h. The apparent permeability coefficient (P) was calculated from $P = J/C$, where C is the apparent drug solubility in the hydrogel. The apparent diffusion coefficient (D) was calculated from $P = KD$, where K is the partition coefficient.

2.4. In vitro determination of gel strength

The physical strength of hydrogel ointments was evaluated by measuring the detachment force of an adapter with a diameter of 15 mm which entered the gel surface at a rate of 30 cm/min using a rheometer (NRM-2002D-D, Fudo, Tokyo, Japan). Sample gel (around 23 g) for strength measurement was prepared in a container.

2.5. Determination of solubility of E_2 and enhancers

The solubility of E_2 was determined in PG/water (20/80, 40/60 w/w) or ethanol/water (20/80, 40/60 w/w) solutions, and was defined as the C value. An excess amount of E_2 was added to the solution and the mixture was thoroughly agitated at 37°C for 24 h until it reached equilibrium. The solution layer was taken and filtered using a disposable filter unit. The concentration of E_2 in the filtrate was determined by HPLC. Quantitative conditions were the same as previously described for the determination of the E_2 concentration in in vitro permeation studies. The solubility of MEN was determined by gas chromatography according to a previously described method (Maitani et al., 1996). The solubility of LAU was determined by HPLC (Maruyama and Yonese, 1986). The analytical conditions were as follows: column, YMC-Pack A-303 ODS, 250x4.6 mm i.d.; UV detection, 215 nm; mobile phase, water/acetonitrile (20/80 v/v); 20 μl injection; flow rate, 1 ml/min. The solubility of CAP was so high that it could not be determined accurately in PG/water and ethanol/water solutions.

2.6. Determination of the partition coefficient of E_2

The K value of E_2 is represented as the ratio of the concentration in isopropyl myristate as the organic phase to that in the water phase, namely, the PG/water or ethanol/water solution containing each enhancer (1% (w/w)). E_2 was dissolved in the water phase, the same volume of the two equilibrated phases was added to suitable vials

and the mixture was agitated with a magnetic stirrer at 37°C for 48 h until it reached equilibrium. The mixture was then allowed to separate into both phases by standing for 24 h. The equilibrium concentration of E_2 in the water phase was determined by HPLC. The E_2 concentration in the organic phase was considered to be the amount by which the concentration had decreased in the water phase. Quantitative conditions were the same as previously described for the determination of E_2 concentration in in vitro permeation studies.

2.7. In vivo absorption study

Male golden hamsters weighing 90–100 g were used. After anesthetization with carbamic acid ethyl ester solution (25% (w/w); 4 ml/kg; i.p.), the hamsters were secured on their backs and the hair on the abdominal skin was gently removed with an electric animal clipper for percutaneous absorption. The Estradarm TTS® (2 mg E_2, 5 cm^2) was attached to the shaved skin. The hydrogel ointments (total of 1 g of 0.5% (w/w) E_2, total available application area 1.5 cm^2) were applied to both cheek pouches for buccal absorption. The absorption experiments were performed over a period of 7 h. Blood samples (300 μl) were taken periodically via the jugular vein. The concentration of E_2 in the plasma was determined by the automatic immunoassay system IM × ™ (Dainabot, Tokyo, Japan).

2.8. Morphological study of the buccal mucosa

After the in vivo absorption study, the buccal mucosa was excised from the male golden hamsters. The mucosal surface was washed with phosphate-buffered saline (pH 7.0) and prefixed with 2% (w/v) glutaraldehyde for 2 h. After being soaked in 7.5% (w/v) saccharose buffer solution for 24 h, the mucosa was again fixed with a 1% (w/w) osmic acid solution for 2 h. The mucosa was then dehydrated using a graded series of acetone solutions (60–100% (v/v)). After drying at the critical point using a critical point dryer (Model HCP-2, Hitachi, Tokyo, Japan), the mucosal surface was coated with gold and examined using a scanning electron photomicroscope (Model JSM-T200 Jeol, Tokyo, Japan).

2.9. Statistical analysis

Statistical analysis was performed using ANOVA; system (1) consisted of seven enhancers in water, and system (2) consisted of four hydrogels and three enhancers for E_2.

3. Results

3.1. In vitro permeability and gel strength

The effects of enhancer(1% (w/w)) on the solubility, steady-state fluxes and P values of a 0.5% (w/w) E_2 suspension in water absorbed through hamster cheek pouch at 37°C in vitro were investigated (Table 2). LAU(1% (w/w)) and 1% (w/w) MEN were in the suspension state but 1% (w/w) CAP was soluble in water. The E_2 permeated through hamster cheek pouch linearly vs time almost without lag time and the metabolite was not detected. LAU, MEN and CAP were significantly effective enhancers of E_2 permeation compared with the control ($p < 0.05$).

We used hydrogels containing PG and ethanol as ointments, and LAU, MEN and CAP as enhancers. Fig. 1 shows the effect of 1–3% (w/w) of enhancer in the hydrogels containing 0.5% (w/w) E_2 on the steady-state flux through the hamster buccal mucosa. The solubility of LAU in 20% PG/water and 40% (w/w) PG/water, and 20% ethanol /water and 40% (w/w) ethanol/water was 16.02, 17.69, 32.96 mg/ml and very soluble, respectively, and the solubility of MEN was 0.80, 2.75, 2.30 and 19.0 mg/ml, respectively. CAP is very soluble in these solutions. Fluxes of E_2 from most of the hydrogels were increased due to the addition of each enhancer to the hydrogel with increasing concentration of enhancer. However, it was observed that the flux of E_2 with LAU did not depend on the LAU concentration.

Table 2
Effect of 1% enhancer on the solubility, steady-state fluxes and permeability coefficients of estradiol (0.5%) in water absorbed through hamster cheek pouch at 37°C in vitro

Enhancer	Solubility of E_2 (μg/ml)	Cumulative flux (μg/cm^2 h) $\pm$ S.D.	P (cm/s)[a] $\times 10^{-4}$ S.D.
Control	1.04	0.42 ± 0.13	1.12 ± 0.35
SS[b]	1.22	0.59 ± 0.23	1.35 ± 0.52
SG[c]	1.08	0.36 ± 0.07	0.92 ± 0.17
Sodium glycocholate	7.59	0.44 ± 0.22	0.16 ± 0.08*
Sodium taurocholate	6.97	0.53 ± 0.16	0.21 ± 0.06*
Laurate	3.82	0.96 ± 0.29*	0.70 ± 0.21
Caprate	2.44	1.83 ± 0.67*	2.08 ± 0.76
Menthol	2.20	3.46 ± 0.43*	4.37 ± 0.55*

[a] Permeability coefficient.
[b] Soybean-derived sterol mixture.
[c] Soybean-derived sterylglucoside mixture.
* $p < 0.05$ vs control.

We determined the gel strength of hydrogels containing enhancers. Fig. 2 shows the effects of 1–3% (w/w) of enhancer on the gel strength of hydrogel ointments containing 0.5% (w/w) E_2. The gel strength was almost the same as that of the control or increased slightly due to the addition of LAU and MEN to the hydrogel. However, it was greatly decreased when CAP was added to the hydrogels.

3.2. In vivo absorption

Fig. 3 shows a comparison of the blood concentration of E_2 absorbed from a 40% (w/w) ethanol gel containing 2% (w/w) LAU and 0.5% (w/w) E_2 through hamster buccal mucosa and from Estradarm TTS® through the skin. The hydrogel E_2 was present in blood at a significantly higher concentration than was Estradarm TTS® E_2. The E_2 doses in both formulations were different, but E_2 was in the suspension state.

The primary morphological changes of the mucosa were examined microscopically at 7 h, i.e. after the in vivo absorption study was complete (Fig. 4). No visible change was observed after 7 h compared with before application of the ethanol gel.

4. Discussion

4.1. In vitro permeability of E_2 from E_2 suspension and gel strength of hydrogel containing enhancer

One of the strategies for designing buccal absorption formulations involves combining the effects of enhancers and cosolvents such as ethanol/water or PG/water to increase the rate of permeation of a drug. MEN (Williams and Barry, 1991), LAU (Maitani et al., 1996), sodium glycocholate and taurocholate for percutaneous absorption (Senel et al., 1994) and for buccal absorption (Zhang et al., 1994), CAP for intestinal absorption (Tomita et al., 1995) and soybean-derived sterol mixture and soybean-derived sterylglucoside mixture for nasal absorption (Maitani et al., 1995) have been reported to be effective enhancers. Among these enhancers, LAU, MEN and CAP were effective in enhancing E_2 permeation through hamster cheek pouch (Table 2).

If the gel strength of a hydrogel containing enhancer is high, it will adhere to the mucosa to achieve sustained release of a drug. Although the fluxes of E_2 were markedly increased upon the addition of CAP to hydrogels containing PG or

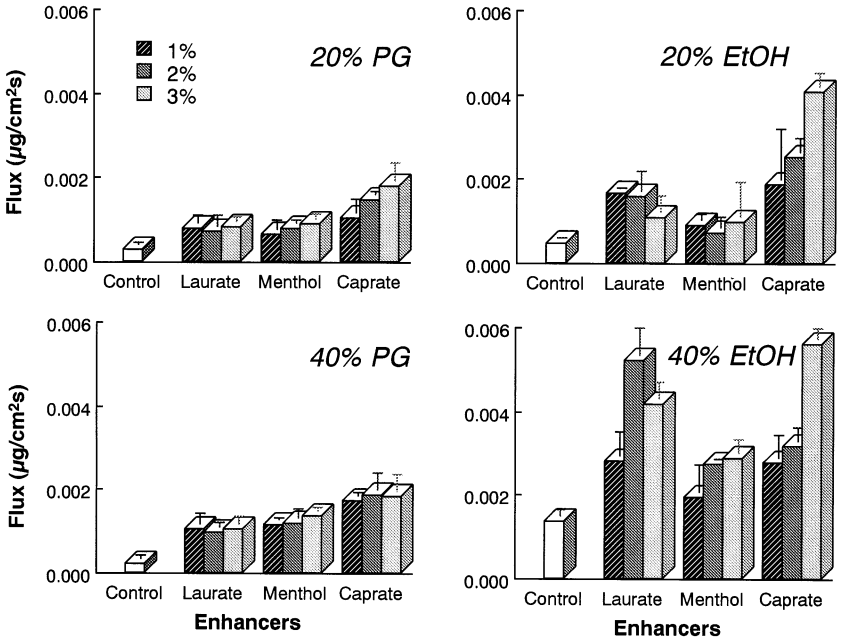

Fig. 1. Effect of 1–3% (w/w) enhancer in the hydrogels containing 0.5% (w/w) E_2 on the steady-state flux of E_2 through excised hamster buccal mucosa. Each column represents the mean $\pm$S.D. for three determinations.

ethanol (Fig. 1), CAP decreased the gel strength with increasing concentration (Fig. 2). It is possible that CAP, as a surfactant, changed the gel structure by interacting with the carboxyvinyl-polymer in the hydrogel. LAU enhanced the permeability of E_2 in proportion to the ethanol concentration in the ethanol gel without changing the gel strength (Figs. 1 and 2). This may be due to the fact that the gel was constructed using liquid crystal action of monoglyceride so that it disperses in water as a lamellar type liquid crystal (Larsson, 1967). It is expected that a hydrogel containing LAU would adhere well to mucosa.

4.2. Effect of enhancers on E_2 penetration in hydrogels

Table 3 summarizes the influence of 1% (w/w) of enhancer on the solubility, flux, P, K and D values of 0.5% (w/w) E_2 in hydrogels absorbed through excised hamster cheek pouch. E_2 is in the suspension state in the donor. The solubility of E_2 in PG/water and ethanol/water solutions containing 1% (w/w) of enhancer increased slightly in the order MEN < LAU < CAP.

CAP (1% (w/w)) increased the flux values of E_2 compared with LAU and MEN ($p < 0.05$, in the 40% (w/w) PG gel and 20% (w/w) ethanol gel) except in 40% (w/w) ethanol gel where the flux values increased in the order MEN < CAP $\leq$ LAU. The P values increased in the order LAU < CAP < MEN, except in the 40% (w/w) ethanol gel in which the solubility of E_2 was extremely high. K values were not increased by enhancers except for the 20% (w/w) PG/water solution for which the K values increased corresponding to the decrease in the solubility of E_2.

Damage to the buccal mucosa caused by enhancers may be estimated from a comparison of the D values. It was observed that the D values increased in the order of LAU < MEN < CAP, except 40% (w/w) ethanol gel where LAU increased slightly compared with MEN. From these results, it was shown that LAU added to ethanol hydrogels results in the high absorption of E_2 with a low degree of damage to the buccal mucosa. The differences of mechanisms in absorption enhancement in hydrogels is discussed in Section 4.3.

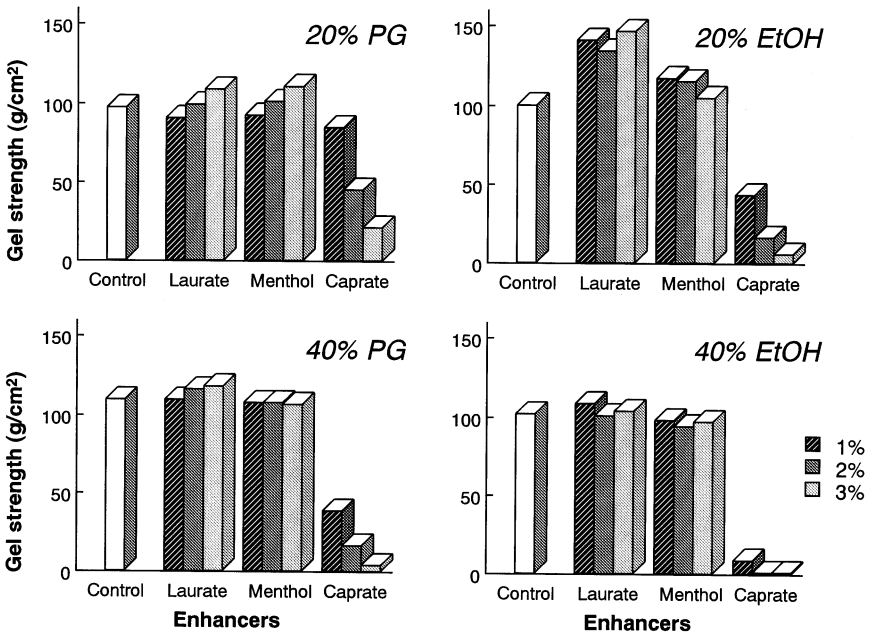

Fig. 2. Effects of 1–3% (w/w) enhancer on the gel strength in hydrogels containing 0.5% (w/w) E_2. Each column represents the mean for duplicate determinations.

4.3. Effect of hydrogels on E_2 penetration

Regarding the penetration of E_2 in hydrogels, the absorption of a drug through the buccal mucosa can be determined by the following three steps; (1) the extent of dissolution of the drug in a hydrogel, (2) the rate of penetration of the drug, and (3) the rate of penetration of PG or ethanol through the mucosa. Since PG and ethanol can penetrate mucosa, drugs dissolved in PG or ethanol transport with PG (Bendas et al., 1995) or ethanol (Liu et al., 1992). The type of cosolvent used affects (1) the solubility of a drug in a hydrogel, (2) the D and/or K values and (3) the degree of cotransport.

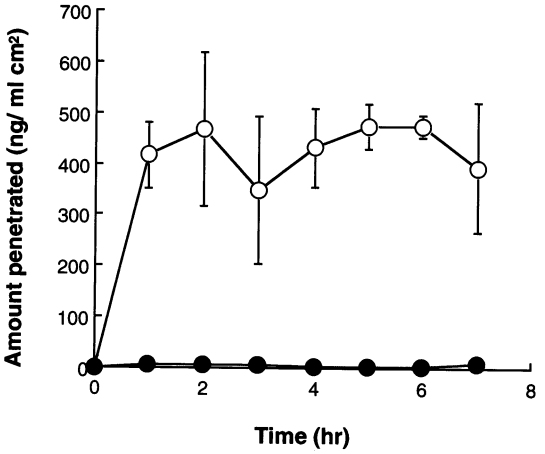

Fig. 3. Comparison of fluxes of 0.5% (w/w) E_2 in a 40% (w/w) ethanol gel containing 2% (w/w) laurate through hamster buccal mucosa and in Estradarm TTS® across the skin of hamsters. ($p < 0.01$) (○, 40% (w/w) ethanol gel containing 2% (w/w) laurate; ●, Estradarm TTS®.)

Based on the results of the penetration through buccal mucosa of E_2 in PG and ethanol gels containing enhancers, the permeability of E_2 was found to be greater in hydrogels containing ethanol than in those containing PG.

In the PG gel, the solubility of E_2 in the absence of enhancer (shown as the control in Table 3) was increased by increasing the PG concentration from 20 to 40% (w/w); on the contrary, the P values were significantly decreased ($p < 0.05$) and thus the cumulative fluxes were not increased. However, the solubility of E_2 was kept constant or decreased slightly upon the addition of enhancers, except CAP in the PG gel. Enhancers in the 20% (w/w) PG

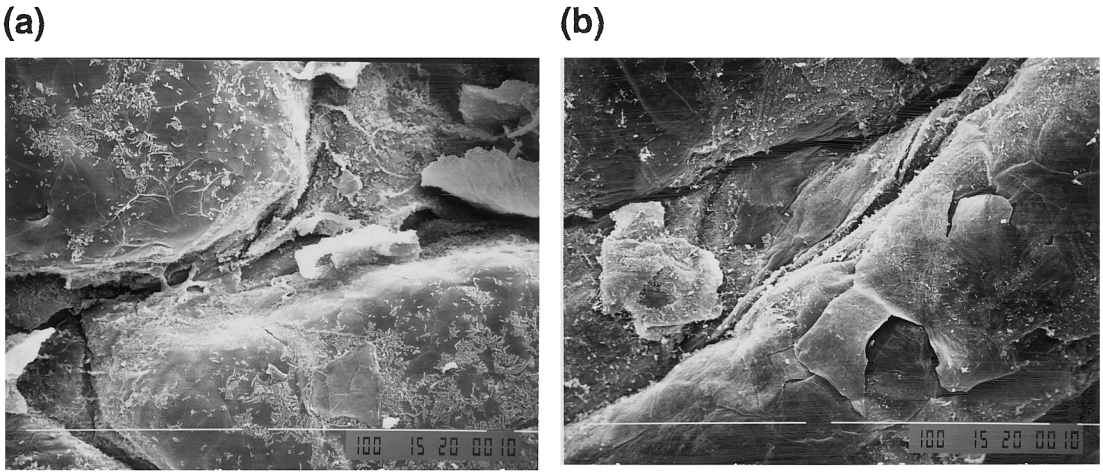

Fig. 4. Scanning electron micrographs of hamster buccal mucosa before (a) and (b) 7 h after application of a 40% (w/w) ethanol hydrogel containing 2% (w/w) laurate. Magnification: × 500.

gel increased the cumulative flux and P values by increasing K values of E_2 with respect to the mucosa. MEN and CAP in the 40% (w/w) PG gel increased the cumulative flux and P value significantly by increasing D values of E_2 with respect to the mucosa ($p < 0.01$), but LAU in the 40% (w/w) PG gel increased the cumulative flux and P value by increasing K values of E_2.

In the 20% (w/w) ethanol gel, the solubility of E_2 increased markedly upon the addition of enhancers except MEN. The cumulative flux of E_2 in the 20% (w/w) ethanol gel was significantly increased by all enhancers compared with control ($p < 0.05$), and that of E_2 in the 40% (w/w) ethanol gel was also significantly increased upon the addition of LAU and CAP ($p < 0.05$). MEN and CAP in the ethanol gel increased the cumulative flux of E_2 by contributing to the diffusion of the drug since their D values increased. LAU in the ethanol gel increased the cumulative flux of E_2 by increasing its solubility. It was suggested that the fraction of E_2 penetrating the mucosa as a result of cotransport may increase as the solubility in ethanol gel increases (Liu et al., 1992). Furthermore, a higher ethanol content in the hydrogel might lead to increased importance of the cotransport pathway (Megrab et al., 1995).

The mechanism by which the enhancers increase the flux of E_2, for LAU and MEN in the 20% (w/w) PG gel is thought to be that they contribute to the partitioning of E_2, for MEN and CAP in the 40% (w/w) PG gel that they contribute to the diffusion of E_2, and for LAU that it increases the partitioning. In addition MEN and CAP in the ethanol gel may mainly contribute to the diffusion of drug and LAU may increase the solubility of E_2.

4.4. In vivo study

From the results of the flux and gel strength, the best formulation was determined to be 2% (w/w) LAU in a 40% (w/w) ethanol gel and was applied to an in vivo study. The application of a buccal hydrogel containing 5 mg E_2/1.5 cm^2 in hamsters showed that E_2 was absorbed in less than 1 h and that plasma levels (above 300 ng/ml cm^2) were maintained and were significantly higher than those obtained after the percutaneous administration in 2 mg E_2/5 cm^2 in Estraderm TTS® (Fig. 3).

One of the reasons for this difference may be due to the difference between skin and mucosa. Megrab et al. (1995) reported that human skin showed a maximum flux of E_2 (1.45 ± 0.38 $\mu g/cm^2$ h) at ethanol vehicle contents between 40 and 60% (w/w). Transdermal delivery of E_2 has been investigated and products for this are currently on the market, for example Estraderm TTS® containing 8 mg E_2 and 0.6 ml ethanol per 20 cm^2. Alza's estraderm patch also contains ethanol and it was

Table 3
Effect of 1% enhancer on the permeation of estradiol (0.5%) in various hydrogels through hamster cheek pouch at 37°C in vitro

Hydrogel (%)	Enhancer	Solubility[a] (μg/ml)	Flux (μg/cm^2 s) $\times 10^{-4}$ $\pm$ S.D.	P (cm/s)[b] $\times 10^{-5}$ $\pm$ S.D.	K[c]	D (cm^2/s)[d] $\times 10^{-6}$
PG						
20	Control	1.48	2.89 $\pm$ 0.28	1.73 $\pm$ 0.17	10.29	4.36
	Laurate	0.47	7.84 $\pm$ 1.98	2.10 $\pm$ 0.53	78.85	0.27
	Menthol	0.37	6.48 $\pm$ 2.03	4.44 $\pm$ 1.39	38.14	1.17
	Caprate	2.07	10.52 $\pm$ 3.07	4.17 $\pm$ 1.22	11.19	3.72
40	Control	9.69	2.26 $\pm$ 0.56	0.23 $\pm$ 0.06	9.38	0.24
	Laurate	10.92	10.38 $\pm$ 2.58*	0.70 $\pm$ 0.17**	12.60	0.56
	Menthol	8.62	11.44 $\pm$ 0.45*	1.15 $\pm$ 0.05*	10.51	1.10
	Caprate	22.82	17.41 $\pm$ 0.69*	1.02 $\pm$ 0.04*	6.46	1.58
EtOH						
20	Control	2.44	4.70 $\pm$ 0.06	0.86 $\pm$ 0.01	21.46	0.40
	Laurate	8.33	16.38 $\pm$ 0.05*	0.91 $\pm$ 0.00*	20.65	0.44
	Menthol	2.44	8.93 $\pm$ 1.41**	2.09 $\pm$ 0.33**	16.50	1.27
	Caprate	14.00	18.80 $\pm$ 1.19*	1.28 $\pm$ 0.81	9.47	1.35
40	Control	229.64	13.78 $\pm$ 1.38	0.11 $\pm$ 0.01	4.54	0.24
	Laurate	334.64	27.95 $\pm$ 5.93**	0.15 $\pm$ 0.03	4.30	0.34
	Menthol	336.66	19.24 $\pm$ 6.69	0.11 $\pm$ 0.04	4.29	0.25
	Caprate	455.86	27.64 $\pm$ 5.47**	0.20 $\pm$ 0.04**	2.06	0.96

[a] Solubility of estradiol in PG/water or ethanol/water vehicles at 37°C.
[b] Permeability coefficient.
[c] Partition coefficient from isopropyl myristate/PG or ethanol solution at 37°C.
[d] Diffusion coefficient.
* $p < 0.01$,
** $p < 0.05$ compared with control.

reported that the E_2 flux increased to 0.1–0.2 μg/cm^2 h in the presence of the flux of ethanol (Santus and Baker, 1993). Our data are comparable to those obtained using commercial products. In addition, LAU in the ethanol gel caused no primary morphological change to the mucosa 7 h after application (Fig. 4). These high flux and sustained release profiles in in vivo buccal absorption reflected adhesiveness and the combined effects of LAU and the ethanol gel in the in vitro permeation study. The irritation caused by the chronic administration of this formulation containing ethanol should be studied further for the application for humans.

5. Conclusions

In the in vitro study, CAP markedly increased the flux of E_2 in the PG or ethanol gel, but decreased the gel strength with increasing concentration. LAU greatly enhanced the permeability of E_2 in proportion to the ethanol concentration in the ethanol gel without decreasing the gel strength. In the in vivo study, the flux of E_2 was higher than 300 ng/cm^2 in hamster up to 7 h after the application of 2% (w/w) LAU in a 40% (w/w) ethanol gel, and no visible change or primary morphological damage to the mucosa was observed. These findings suggest that use of a combination of 2% (w/w) glycerylmonolaurate and a 40% (w/w) ethanol gel was the most effective method of increasing the buccal permeability of 17β-estradiol.

Acknowledgements

This work was supported by the Ministry of Education, Science, Sports and Culture of Japan.

The authors are grateful to Miss N. Sasaki and Miss K. Miyagi for their assistance with the experiments. Special thanks are due to Dainabot Co. Ltd. for providing us with the automatic immunoassay system IM × ™.

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