International Journal of Pharmaceutics, 84 (1992) 117–128

IJP 02736

Combined effects of pH, cosolvent and penetration enhancers on the in vitro buccal absorption of propranolol through excised hamster cheek pouch

Anne Coutel-Egros [a,b], Yoshie Maitani [a], Michel Veillard [b], Yoshiharu Machida [a] and Tsuneji Nagai [a]

[a] *Department of Pharmaceutics, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142 (Japan)*
[b] *Rhône-Poulenc Rorer, Institut de Biopharmacie, Department of Pharmaceutics, 20 av. Raymond Aron, 92165 Antony Cedex (France)*

(Received 11 July 1991)
(Modified version received 4 October 1991)
(Accepted 12 December 1991)

Key words: Buccal absorption; Permeability; Partitioning; Penetration enhancement; Propranolol

Summary

The combined effect of pH and vehicle composition on the buccal absorption of propranolol has been evaluated in vitro using hamster cheek pouch. Ethanol was used as cosolvent in various binary-water mixtures. Lauric acid, 1-menthol, sodium salicylate and phosphatidylcholine were evaluated as penetration enhancers. Isopropyl myristate was used as a model of the mucosal lipid phase in partitioning experiments. Permeation of propranolol increased in extent with increasing pH of the vehicle, as compared with the unionized lipophilic fraction of the drug. Permeation of propranolol was decreased at pH 9.4 and increased at pH 6.8 on the addition of ethanol. Ethanol increases the solubility of propranolol in the aqueous vehicle and reduces the oil/vehicle partitioning of the drug through a thermodynamic effect. In addition, at low concentration, ethanol is assumed to increase the permeability of the buccal mucosa to the polar ionized form of propranolol, while higher concentration is required to enhance the diffusivity of the nonpolar unionized form. Menthol and lauric acid were effective at promoting the buccal absorption of propranolol only when the drug was ionized. It was assumed that the mechanism of permeation enhancement was an interaction with the membrane components for 1-menthol, increasing the diffusibility of propranolol, whereas lauric acid may form a more partitionable complex with the drug without any action on membrane permeability.

Introduction

Buccal administration is one of the possible drug delivery routes to avoid oral first-pass metabolism (Hussain et al., 1986) and, despite the lower absorption capacity compared to other mucosal routes (Aungst et al., 1988), the oral cavity is easily accessible and much less sensitive than other mucosa. The buccal absorption of numerous compounds has been studied and most of the investigators have explained their results on the basis of simple passive diffusion through a lipid

Correspondence: A. Coutel-Egros, Rhône-Poulenc Rorer, Institut de Biopharmacie, Department of Pharmaceutics, 20 Av. Raymond Aron, 92165 Antony Cedex, France.

membrane (Moffat, 1972; Siegel, 1984; Le Brun et al., 1989). The fundamental physicochemical factors influencing buccal absorption are pK_a and pH for ionic drugs, drug solubility in the vehicle, drug transport from the vehicle to the membrane and diffusion through the membrane.

Various adjuvants have been used to improve the buccal absorption of drugs, including surfactants (Siegel and Gordon, 1985), bile salts (Nakada et al., 1989; Wolany et al., 1990) or Azone (Kurosaki et al., 1988, 1989). However, the mechanism of penetration enhancement for the buccal route has been much less extensively investigated compared to that for the skin. The rate of drug transfer across biological membranes may be enhanced in two major ways. The first is by modification of the physicochemical properties of the drug to optimize membrane/vehicle partitioning. For example, lauric acid (Green et al., 1989) and sodium salicylate (Young et al., 1988) are considered to enhance the skin partitioning of cationic drugs by a mechanism of ion-pairing. The second involves interaction with the components of the biological membrane to increase its permeability. Sodium salicylate, by an interaction with membrane proteins, increases cell membrane permeability (Kajii et al., 1986). Interaction with the lipid components of the membrane has also been proposed for fatty acids (Golden et al., 1987).

The objective of the present study was to evaluate the combined effects of pH and cosolvent on the efficacy of a number of penetration enhancers in promoting buccal absorption of propranolol. To study such complex interactions in a comprehensive fashion, we evaluated each factor in vitro on drug permeation across excised hamster cheek pouch and on the isopropyl myristate/vehicle partitioning of the drug. Propranolol was used as a model compound, since it is a weak base which allows the study of particular species by pH control. Various aqueous mixtures of ethanol were used as binary-cosolvent systems. Lauric acid, 1-menthol, sodium salicylate and phosphatidylcholine were chosen for their ability to enhance drug absorption through the skin and various mucosa.

Materials and Methods

Reagents and chemicals were used as received. Propranolol was used as the hydrochloride salt (Sigma Chemical Co., St Louis, U.S.A.), sodium salicylate was obtained from Wako Pure Chem. Ind. Ltd (Japan). Lauric acid and 1-menthol were obtained from Tokyo Kasei Kogyo Co. Ltd (Japan) and egg yolk phosphatidylcholine from Sigma (Type XV-E). Other reagents were analytical grade.

Preparation of excised hamster cheek pouch

Hamster cheek pouch was chosen as a model of keratinized buccal mucosa. Male golden hamsters (Saitama Experimental Animal Supply Co., 90–100 g) were killed by inhalation of an overdose of ether vapor. The cheek pouch was cleaned with saline, and then the tissue was excised and rinsed in saline. The mean thickness ($\pm$SD) determined with a micrometer ($n = 69$) was 0.017 ± 0.003 cm. After excision, the tissue was immediately mounted between two glass disks, giving a diffusion area of 0.785 cm^2, in a vertical glass diffusion cell.

Preparation of vehicles

Various isotonic buffers were used to adjust the pH of the vehicle; phosphate buffer for pH between 6.8 and 7.8, alkaline borate buffer for pH between 8.3 and 9.0 and bicarbonate buffer for pH 9.4. Cosolvent mixtures were prepared by mixing ethanol and isotonic buffers in various ratios from 0 to 30% (w/v) ethanol. Isotonic phosphate buffer initially adjusted to pH 6.8 and isotonic bicarbonate buffer adjusted to pH 9.4 were used to study the combined effect of pH and cosolvent. The adjuvants studied as penetration enhancers were added at a concentration of 0.5% (w/v) in 30% (w/v) ethanol-buffer mixtures. pH 6.8 phosphate buffer and pH 9.4 bicarbonate buffer were used to examine the combined effect of adjuvant and pH.

In vitro hamster cheek pouch permeation studies

A Franz-type permeation cell was used (Merrit and Cooper, 1984). The receiver side of the diffu-

sion cell was filled with 16.5 ml of a pH 7.4 isotonic phosphate buffer and stirred continuously with a magnetic stirrer. The diffusion cell was equilibrated in a water bath at 37°C prior to the experiment. Propranolol hydrochloride was dissolved in the vehicle and the initial concentration was chosen to maintain sink conditions during experiments. The donor side was filled with 5 ml of the studied solution. Samples were withdrawn from the receiver chamber over a 6 h period and an equal volume of buffer was immediately added to maintain a constant volume. The permeation was determined by measuring the amount of drug in the receiver side. Each experiment was conducted at least in triplicate.

Isopropyl myristate / vehicle partition experiments

Isopropyl myristate (IPM) was used, since it has been proposed as a model of the lipophilic phase to simulate the skin/vehicle partitioning of numerous compounds (Surber et al., 1990) and since the keratinized buccal mucosa is a stratified squamous epithelium similar to the skin. The vehicle studied and IPM were mutually saturated prior to the partitioning experiment. IPM (3 ml) was added to 3 ml of the vehicle containing 1 mM propranolol hydrochloride. Two or three test tubes were prepared for each determination. The tubes were placed in an incubator and shaken at 37°C overnight to allow them to equilibrate. The tubes were then centrifuged and the concentration of the drug in the aqueous phase was determined by HPLC. The pH of the aqueous phase was measured after partitioning.

Determination of the pK_a of propranolol

The pK_a of propranolol in the various ethanol-water mixtures was determined by titration at 37°C. The studied solutions containing 1 mM propranolol hydrochloride were titrated with 0.01 N NaOH. The pK_a values were obtained from the half-neutralization point of the titration curves.

Determination of drug solubility

The apparent solubility of propranolol (S_{app}) was determined in triplicate in a given cosolvent mixture, using pH 9.4 isotonic bicarbonate buffer as aqueous phase. An excess of propranolol hydrochloride was dissolved in the mixture and shaken at 37°C for 24 h. The pH was measured after equilibrium and the intrinsic solubility of the unionized form (S) was calculated by using the pK_a value of the drug and the medium pH according to the Henderson-Hasselbalch equation: $S = S_{app}/[1 + \text{antilog}(pK_a - pH)]$.

Drug assay

The concentration of propranolol was determined by high-performance liquid chromatography (HPLC) based on the method of Koshakji and Wood (1986). A μ-Bondapack phenyl column (4 mm × 30 cm, 10 μm average particle size) protected by a guard column μ-Bondapack phenyl guard-pack (Waters Associates, Milford) was used. The mobile phase, pH 3.5, consisted of acetonitrile/ water/ acetic acid/ triethylamine (450 : 600 : 10 : 0.15 by vol.) and was run through the HPLC system at a rate of 2 ml/min at room temperature. A Shimadzu RF530 fluorescence detector was used, with excitation wavelength set at 236 nm and emission wavelength at 340 nm. Chromatograms were recorded using a CR6A integrator (Shimadzu, Kyoto, Japan).

Calculation of the unionized fraction

The unionized fraction of propranolol (F_u) was calculated from the drug pK_a and medium pH using the Henderson-Hasselbalch equation: $F_u = 1/[1 + \text{antilog}(pK_a - pH)]$.

Calculation of the permeability coefficient

The flux through hamster cheek pouch (J) was evaluated as the steady-state slope of the plot of cumulative amount of propranolol per unit surface area (Q) vs time (t); $J = dQ/dt$. The apparent permeability coefficient ($K_{p,app}$) was calculated from $K_{p,app} = J/C$ where C is the drug concentration in the donor medium.

Calculation of the partition coefficient

The apparent partition coefficient (P_{app}), was calculated based on the expression $P_{app} = (C_b - C_a)/C_a$, where C_b and C_a are the respective overall aqueous concentrations of the drug, before and after partition into IPM.

Results and Discussion

Effect of pH

The effect of the pH of the donor medium on the permeation of propranolol through hamster cheek pouch was studied using isotonic buffers. $K_{p,app}$ increased as the pH of the donor solution increased (Fig. 1). These results were in accordance with the pH dependence of human buccal absorption of propranolol (Schürmann and Turner, 1978), suggesting that the diffusion of propranolol depends on the ionization state of the molecule.

A pK_a value of 9.02, determined from three titration curves at 37°C, was used to calculate F_u. Since pK_a values for bases decrease with temperature (Perrin et al., 1981), our value was in good agreement with that of 9.45 determined at 24°C by Schürmann and Turner. $K_{p,app}$ of propranolol increased while the percentage of unionized propranolol increased (Table 1). As the partition coefficient (P_{app}) determined with isopropyl myristate also increased with F_u (Table 2), these results were attributed to the greater liposolubility of the unionized molecule. A linear relationship ($r = 0.999$) between P_{app} and F_u was observed, whereas $K_{p,app}$ values showed a small deviation from linearity (Fig. 2). $K_{p,app}$ depends on the permeability coefficients for the unionized form (K_{pu}) and ionized form (K_{pi}), and depends

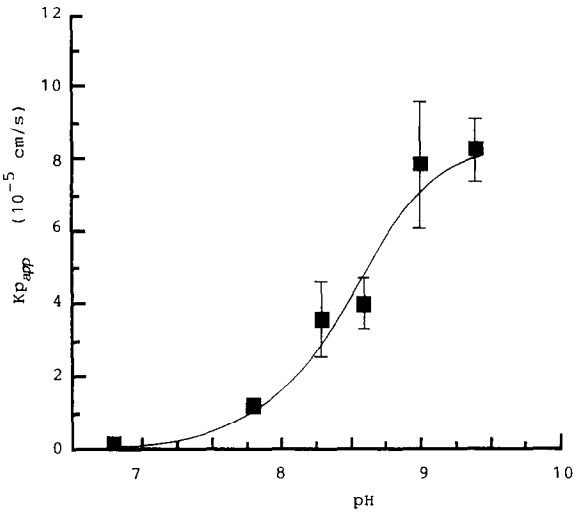

Fig. 1. Relationship between apparent permeability coefficient ($K_{p,app}$) and pH of the donor medium for the permeation of propranolol across hamster cheek pouch. Each point represents the mean ± SD of 3–6 experiments.

on the fraction of drug in each state according to Eqn 1:

$$K_{p,app} = F_u K_{pu} + (1-F_u) K_{pi} \quad (1)$$

At pH 6.8, the drug is totally ionized and the term ($F_u K_{pu}$) in Eqn 1 can be neglected. Then K_{pi}, calculated from the ratio $K_{p,app}/(1-F_u)$, was found to be equal to 0.14×10^{-5} cm/s. At other pH values, K_{pu} was calculated by replacing the

TABLE 1

Influence of pH and drug unionized fraction (F_u) on the apparent permeability coefficient ($K_{p,app}$) and the permeability coefficient calculated for the unionized form (K_{pu}) in excised hamster cheek pouch for propranolol

pH	F_u [a]	$K_{p,app}$ (± SD) ($\times 10^{-5}$) (cm/s)	$K_{p,app}/F_u$ (± SD) ($\times 10^{-5}$) (cm/s)	K_{pu} [b] ($\times 10^{-5}$) (cm/s)	n
6.8	0.006	0.14 ± 0.03	23.33 ± 5.00	ND [c]	4
7.8	0.057	1.20 ± 0.16	21.05 ± 2.81	18.74	3
8.3	0.160	3.54 ± 1.07	22.12 ± 6.69	21.39	6
8.6	0.275	3.98 ± 0.78	14.15 ± 2.84	14.10	5
9.0	0.489	7.83 ± 1.80	16.03 ± 3.67	15.87	4
9.4	0.696	8.24 ± 0.90	11.84 ± 1.29	11.78	3

[a] F_u was calculated using a pK_a value of 9.02 (determined in triplicate at 37°C).
[b] K_{pu} was determined using a K_{pi} value of 0.14×10^{-5} from $K_{pu} = [K_{papp}/F_u] - [K_{pi}(1-F_u)/F_u]$ (see text, Eqn 1).
[c] Not determined as F_u is almost equal to 0. Data are given as the mean of n experiments ± SD.

TABLE 2

Influence of the pH of the vehicle on the isopropyl myristate / vehicle partitioning of propranolol

pH	F_u	P_{app}	P_u [a]
6.62	0.004	0.57	142.50
6.67	0.005	0.59	132.67
7.65	0.041	5.10	124.66
8.13	0.114	12.86	112.68
8.43	0.205	27.44	134.19
8.79	0.371	52.48	141.46
9.32	0.666	98.12	147.30

[a] P_u denotes the partition coefficient of the unionized form, calculated from the ratio P_{app}/F_u. Data are given as the mean of two to three experiments.

value of K_{pi} in Eqn 1. The K_{pu} values listed in Table 1 show that the permeation of the unionized form is at least 85-times greater than that for the ionized form and K_{pu} is almost equal to the ratio $K_{p,app}/F_u$. However, K_{pu} decreased while the pH of the medium increased whereas P_u calculated from the ratio P_{app}/F_u was found to be almost constant (Table 2). A similar non-linear relationship between the permeability coefficient of propranolol and the unionized fraction has been observed in cultured hamster pouch buccal epithelium (Tavakoli-Saberi and Audus, 1989), with no further increase in permeation rate at higher pH. These results are similar to those obtained by Shürmann and Turner who assumed the presence of a buffering layer at the membrane surface, hence modifying the pH locally.

Effect of cosolvent

Various ethanol-buffer mixtures containing from 0 to 30% ethanol were studied at pH 6.8 and 9.4. $K_{p,app}$ at pH 9.4 decreased as ethanol concentration in the vehicle increased whereas the value at pH 6.8 was increased by ethanol (Fig. 3).

With organic solvent, the ionic equilibriums of weak bases and acids shift toward the least charged species. Then, pK_a increases for acids and decreases for bases (Rubino, 1987). Therefore, when buffer systems are used in cosolvent-water mixtures, the fraction of the unionized form will be influenced by both the change in pH of the medium and by the shift of the pK_a of the drug. The pK_a values of propranolol decreased from 9.02 to 8.58 as ethanol content increased from 0 to 30%. At the same time, the pH of the donor media prepared with bicarbonate and phosphate buffers increased from 9.4 to 10.4 and from 6.8 to 7.4, respectively (Tables 3 and 4). Then, the proportion of unionized form increased as much as about 10-fold when 30% ethanol was added to phosphate buffer. To analyse the effect of ethanol independently of the change in F_u, the permeability coefficients, K_{pu} and K_{pi}, were calculated using Eqn 1. At pH 9.4, ethanol increased F_u, the term $K_{pi}(1-F_u)$ could be neglected and K_{pu} was then obtained from the ratio $K_{p,app}/F_u$. At pH 6.8, F_u also increased and the term $(K_{pu}F_u)$ was greater. K_{pi} was then determined by substituting the K_{pu} value obtained at pH 9.4 into Eqn 1. The K_{pu} and K_{pi} values are listed in Tables 3 and 4, respectively. At pH 9.4, the values for K_{pu} were lowered by ethanol whereas at pH 6.8, K_{pi} values were increased with a maximum for 10% ethanol and then decreased. The above results suggest that ethanol may exert different effects on the permeation behavior of the ionized and unionized species.

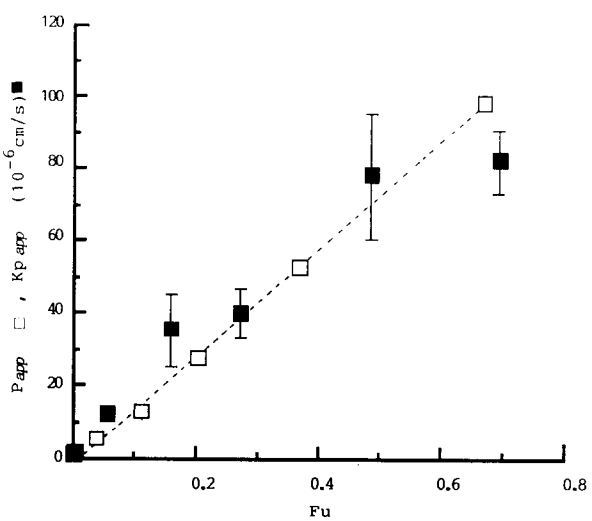

Fig. 2. Apparent isopropyl myristate/vehicle partition coefficient (P_{app} □) and apparent permeability coefficient for propranolol across hamster cheek pouch ($K_{p,app}$ ■) as a function of unionized fraction of propranolol (F_u). Each point represents the mean ± SD of 3–6 experiments.

TABLE 3

Effect of ethanol concentration on the permeation of the unionized form of propranolol across hamster cheek pouch from pH 9.4 bicarbonate buffer

% ethanol (w/v)	$K_{p,app}$ (± SD) ($\times 10^{-5}$) (cm/s)	pH [a]	pK_a [b]	F_u [c]	K_{pu} [d] (± SD) ($\times 10^{-5}$) (cm/s)
0	8.24 ± 0.90	9.38	9.02	0.696	11.84 ± 1.29
10	4.71 ± 0.92	9.70	8.93	0.855	5.51 ± 1.08
20	4.47 ± 0.37	10.08	8.77	0.953	4.26 ± 0.39
30	3.43 ± 0.52	10.43	8.58	0.986	3.48 ± 0.52

[a] pH of the vehicle measured after permeation experiment.
[b] pK_a of propranolol measured in triplicate at 37°C.
[c] Fraction of drug unionized, calculated with the corresponding pK_a values.
[d] K_{pu} denotes the permeability coefficient of the unionized form and was calculated from $K_{pu} = K_{p,app}/F_u$. Data are given as the mean of three experiments ± SD.

The permeability coefficient (K_p) is described according to Fick's first law as a composite variable which includes membrane/vehicle partitioning (P_m) and the drug diffusivity into the membrane (D):

$$K_p = P_m D \quad (2)$$

Therefore, according to Eqn 2, ethanol could modify K_p via two mechanisms: modification either of membrane-vehicle partitioning or of D by interaction with membrane components. The membrane-vehicle partitioning of propranolol was estimated on the basis of the apparent partition coefficient with isopropyl myristate (P_{app}). At both pH 9.4 and 6.8, P_{app} was decreased on addition of ethanol (Fig. 4).

Yalkowsky and Roseman (1981) have shown that the log solubility in a water-cosolvent mixture (log S_m) may be related to the volume fraction of solvent, F_c, by:

$$\log S_m = \log S_w + aF_c \quad (3)$$

where a represents the slope of the semi-logarithmic plot of the molar solubility vs F_c and is defined as the solubilizing power of the cosolvent for the solute. Log S_w denotes the log solubility in water. The partition coefficient between two immiscible phases can be described as the ratio of

TABLE 4

Effect of ethanol concentration on the permeation of the ionized form of propranolol across hamster cheek pouch from pH 6.8 phosphate buffer

% ethanol (w/v)	$K_{p,app}$ (± SD) ($\times 10^{-5}$) (cm/s)	pH [a]	pK_a [b]	F_u [c]	K_{pi} [d] (± SD) ($\times 10^{-5}$) (cm/s)
0	0.14 ± 0.03	6.80	9.02	0.006	0.07 ± 0.03
10	0.79 ± 0.32	6.85	8.93	0.008	0.75 ± 0.32
20	0.63 ± 0.21	7.08	8.77	0.020	0.55 ± 0.21
30	0.66 ± 0.29	7.40	8.58	0.062	0.47 ± 0.31

[a] pH of the vehicle measured after permeation experiment.
[b] pK_a of propranolol measured in triplicate at 37°C.
[c] Fraction of drug unionized, calculated with the corresponding pK_a values.
[d] K_{pi} denotes the permeability coefficient of the ionized form and was calculated from $K_{pi} = [K_{p,app}/(1-F_u)]-[K_{pu}F_u/(1-F_u)]$ with the corresponding K_{pu} values listed in Table 3 (see text, Eqn 1). Data are given as the mean of three experiments ± SD.

the drug solubility in the oil phase divided by the solubility of the solute in the vehicle. If the solvent mixture does not affect the drug solubility in the oil phase, then the partition coefficient decreases while the solubility in the vehicle increases (Sloan et al., 1986; Pardo et al., 1990). Therefore, by analogy with Eqn 3, the log partition coefficient of a water-cosolvent mixture (log P_m) may be related to F_c and the log partition coefficient when the vehicle is water (log P_w) (Turi et al., 1979) according to:

$$\log P_m = \log P_w - aF_c \quad (4)$$

The relationship between P_m and S_m for propranolol was studied at pH 9.4. The values were normalized with respect to the unionized fraction. Fig. 5 demonstrates a linear increase in log solubility ($r = 0.967$) and a linear decrease in log partition coefficient ($r = 0.993$), respectively.

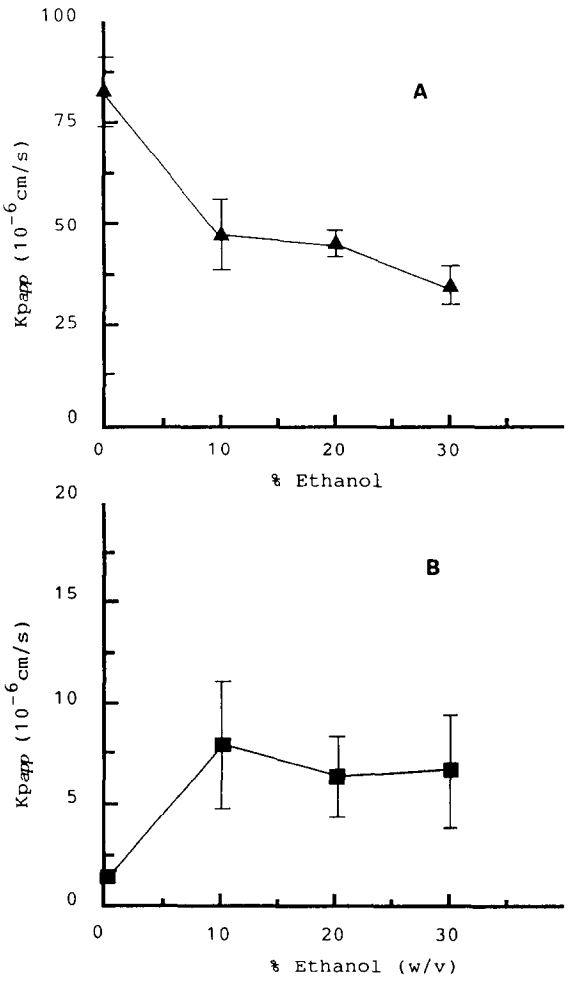

Fig. 3. Relationship between apparent permeability coefficient ($K_{p,app}$) and ethanol concentration in ethanol-buffer mixtures with (A) pH 9.4 bicarbonate buffer and (B) pH 6.8 phosphate buffer. Each point represents the mean ± SD of three experiments.

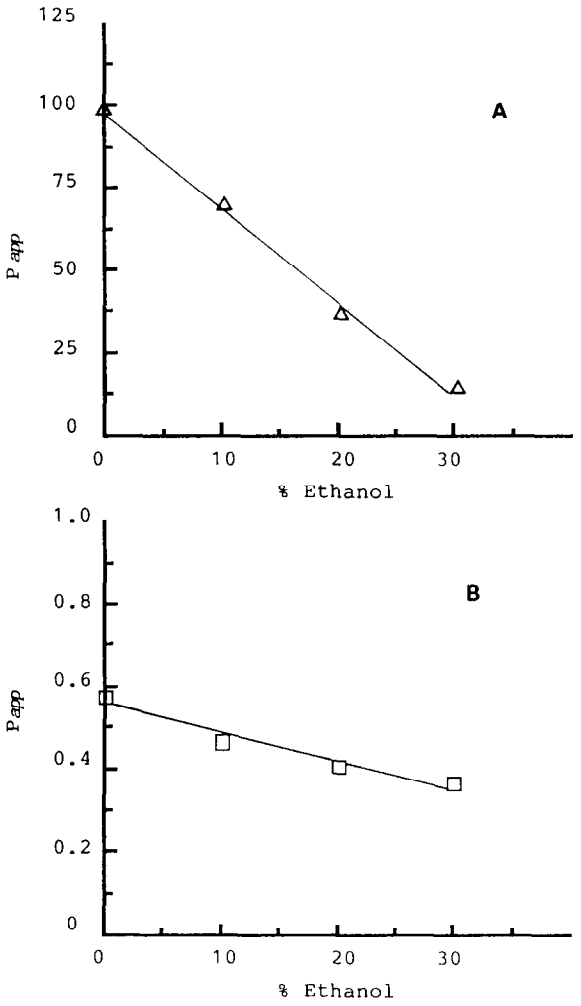

Fig. 4. Relationship between apparent partition coefficient (P_{app}) and ethanol concentration in ethanol-buffer mixtures with (A) pH 9.4 bicarbonate buffer and (B) pH 6.8 phosphate buffer. Each point represents the mean of three experiments.

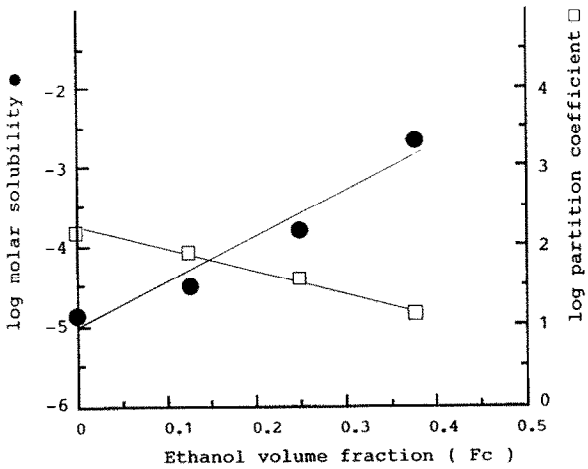

Fig. 5. Log isopropyl myristate/vehicle partition coefficient (□) and log molar solubility (●) determined for the unionized propranolol as a function of ethanol volume fraction in ethanol-pH 9.4 bicarbonate buffer mixtures.

These results were in accordance with the satisfactory linear fit obtained for several non-polar drugs in aqueous cosolvent mixtures (Yalkowsky and Roseman, 1981; Gould et al., 1984; Irwin et al., 1990). However, the *a* values (values of the slopes in Fig. 5) were equal to 5.63 and 2.70 for log solubility and log partition coefficient, respectively. This difference means that the above assumption that the solvent mixture does not affect drug solubility in the oil phase was not appropriate and that the smaller slope value obtained for the log partition coefficient indicates greater solubility of the drug in the oil phase. Turi et al. (1979) have shown that propylene glycol leads to a linear increase in the solubility of diflorasone diacetate in water with a lower rate of decrease in the skin/vehicle partition coefficient, resulting from an enhancement in solubility in the skin. As the permeation profiles through hamster cheek pouch (Fig. 3) were not totally reflected by the IPM-vehicle partition profiles (Fig. 4), it was assumed that according to Eqn 2, K_p was affected by a modification of D. To evaluate quantitatively the action of ethanol on the membrane, independently of the modification of partitioning, the relative enhancement ratio (RER) was calculated according to Eqn 5 (Pardo et al., 1990):

$$\mathrm{RER} = (K_{pm}/K_{pw})/(P_m/P_w) \qquad (5)$$

where K_{pm} and P_m denote the permeability and partition coefficients when the vehicle is a cosolvent mixture and K_{pw} and P_w correspond to those in water. The data were preliminarily nor-

TABLE 5

Relative enhancement ratio (RER) of the permeation of propranolol through hamster cheek pouch from various cosolvent mixtures of ethanol in isotonic buffers

% ethanol (w/v)	P_{app}	pH [a]	F_u [b]	P_m/P_w [c]	K_{pm}/K_{pw} [c]	RER $[(K_{pm}/K_{pw})/(P_m/P_w)]$
pH 9.4 bicarbonate buffer						
0	98.12	9.3	0.671	1	1	1
10	63.35	9.6	0.821	0.57	0.47	0.82
20	35.79	9.9	0.925	0.26	0.36	1.37
30	13.77	10.2	0.977	0.10	0.29	3.05
pH 6.8 phosphate buffer						
0	0.57	6.6	0.004	1	1	1
10	0.46	6.8	0.008	0.40	4.23	10.53
20	0.40	7.1	0.019	0.15	1.35	8.92
30	0.36	7.3	0.048	0.05	0.46	8.57

[a] pH of the vehicle measured after partitioning.

[b] F_u was calculated with the corresponding pK_a values, listed in Table 3.

[c] The subscript w refers to the pure buffers as donor media without ethanol, the subscript m corresponding to any other mixtures. The values were normalized with respect to F_u in $K_{p,app}/F_u$ and P_{app}/F_u. Each value is the mean of three experiments.

malized with respect to F_u, the values of RER being listed in Table 5. At pH 9.4, RER was significantly increased, with a value of 3, only when a minimum of 30% ethanol was added. At pH 6.8, a more marked effect was observed with only 10% ethanol giving an RER value of 10. These results suggest that the action of ethanol is specific to the diffusion pathway of the ionized hydrophilic species at low concentrations, whereas higher concentrations are required to affect the diffusion pathway of the unionized lipophilic form. Keratinized hamster cheek pouch is a stratified squamous epithelium like the skin and the mechanisms by which ethanol interacts with the membrane may be similar. It has been shown that water-ethanol mixtures enhance skin permeation of salicylate ions through a mechanism involving alteration of the lipidic and proteinaceous regions of the stratum corneum (Kurihara-Bergstrom et al., 1990). These authors have shown that lipid extraction occurred at higher ethanol concentration than alteration of the proteins. It has also been reported that ethanol can induce a significant increase in the skin diffusion of hydrophobic compounds, such as β-estradiol, at concentrations above those required for polar compounds (Ghanem et al., 1987).

Effect of penetration enhancers

The effect of various adjuvants on the permeation of propranolol through hamster cheek pouch was studied at pH 9.4 and 6.8 at a concentration of 0.5% in a 30% ethanol-aqueous mixture. The $K_{p,app}$ values are listed in Table 6. At pH 9.4, when the drug is almost totally unionized, all the adjuvants tested were ineffective or led to only a slight decrease in $K_{p,app}$. At pH 6.8, when the drug is almost completely ionized, $K_{p,app}$ was significantly increased only by 1-menthol. However, since the pH of the medium was affected by some adjuvants, especially lauric acid, the values of $K_{p,app}$ were normalized with respect to F_u. The enhancement ratio (ER) for each adjuvant was calculated from the ratio of K_p obtained with the adjuvant divided by that determined for the control vehicle without adjuvant. The results

TABLE 6

Influence of various adjuvants on the permeation of propranolol through hamster cheek pouch and relative enhancement ratio (RER) from vehicles containing 0.5% adjuvant in 30% ethanol-buffer mixtures

Adjuvants	$K_{p,app}$ ($\pm$ SD) ($\times 10^{-5}$) (cm/s)	pH vehicle	F_u [a]	ER [b,d] (K_{px}/K_{pc})	(P_x/P_c) [c,d]	RER (K_{px}/K_{pc})/(P_x/P_c)
pH 9.4 bicarbonate buffer						
Control	3.03 $\pm$ 0.20	10.3	0.98	1	1	1
Lauric acid	1.62 $\pm$ 0.84	9.1	0.75	0.7	0.5	1.4
Sodium salicylate	2.33 $\pm$ 0.30	10.3	0.98	0.8	0.6	1.2
Menthol	2.58 $\pm$ 0.15	10.2	0.97	0.9	0.7	1.2
Phosphatidylcholine	2.76 $\pm$ 0.15	10.2	0.98	0.9	0.7	1.3
pH 6.8 phosphate buffer						
Control	0.66 $\pm$ 0.29	7.4	0.06	1	1	1
Lauric acid	0.68 $\pm$ 0.20	6.7	0.01	4.9	3.4	1.4
Sodium salicylate	0.42 $\pm$ 0.08	7.3	0.05	0.8	0.8	1.0
Menthol	2.40 $\pm$ 0.32	7.4	0.06	4.0	1.1	3.8
Phosphatidylcholine	0.35 $\pm$ 0.04	7.4	0.06	0.6	2.7	0.2

[a] F_u was calculated using a pK_a value of 8.58.
[b] Enhancement ratio, ER, for the permeation coefficients, with normalized values ($K_{p,app}/F_u$).
[c] Enhancement ratio of the partition coefficients with normalized values (P_{app}/F_u).
[d] The subscript c refers to control vehicle without adjuvant, the subscript x corresponding to any other vehicles. Data are the mean of three experiments.

are listed in Table 6. At pH 9.4, ER was not significantly affected by the adjuvants tested. At pH 6.8, ER was increased by approx. 5- and 4-fold by lauric acid and 1-menthol, respectively. To evaluate the mechanisms involved, modification of partitioning behavior or interaction with membrane components, the IPM/vehicle partition coefficients were determined, the data being tabulated in Table 6. The relative enhancement ratio, RER, was estimated analogously with Eqn 5 from $(K_{px}/K_{pc})/(P_x/P_c)$ where the subscript c refers to the control vehicle without adjuvant and the subscript x corresponds to any other vehicles with the various adjuvants. As shown by the results in Table 6, a positive enhancement effect on RER was only achieved with 1-menthol at pH 6.8 when propranolol is ionized. Menthol has been shown to exert a similar enhancement effect on the in vivo percutaneous absorption of diclofenac sodium, a water-soluble drug without effect on the octanol/vehicle partition coefficient (Obata et al., 1990). Williams and Barry (1991) also reported that other terpenes, including the alcohol class, enhanced the in vitro permeation of a polar drug without any modification of the stratum corneum/water partitioning of the drug. No enhancement effect was observed at pH 9.4, when propranolol was unionized. The percutaneous absorption of ketoprofen, a lipophilic drug, was also unaffected by menthol (Okabe et al., 1990). Polar and non-polar solutes presumably diffuse through the stratum corneum via different pathways involving proteinaceous regions and the polar head groups of lipids for water-soluble drugs and the hydrophobic lipid phase for lipophilic compounds (Elias, 1981; Barry, 1987). From our results, it may be assumed that, at the concentration used, 1-menthol altered the polar route for the ionized hydrophilic form of propranolol. However, further studies are required in order to specify the exact mechanism involved. Since lauric acid had almost no effect on RER, it was concluded that the enhancement effect resulted essentially from a partitioning mechanism without any influence on the membrane. Several others have also demonstrated that fatty acids could increase the permeation of cationic drugs via a mechanism involving the formation of a more partitionable complex by ion-pairing (Green et al., 1989; Ogiso and Shintani, 1990). Among the other adjuvants studied, none was found to be effective at enhancing the buccal absorption of propranolol. The decrease in $K_{p,app}$ observed was related to modification of partitioning with an increase in the affinity for the ethanol-aqueous phase. Phosphatidylcholine, which has been proposed as penetration enhancer in transdermal drug delivery (Kurosaki et al., 1991), did not increase the transport of propranolol through hamster cheek pouch (ER = 0.6), although the extent of partitioning into IPM was enhanced 2.7-fold (Table 6). This could indicate the formation of a more partitionable complex which has greater affinity for IPM but not for hamster buccal mucosa.

Conclusion

The present results suggest that the efficacy of adjuvants in enhancing buccal absorption across keratinized hamster cheek pouch is dependent on the physico-chemical properties of the permeant. Without any adjuvant, propranolol can only permeate significantly through hamster cheek pouch when it is unionized and lipophilic. Ethanol increases the intrinsic solubility of propranolol in ethanol-aqueous mixtures with reduction of the partitioning behavior by a thermodynamic effect. In addition, low concentrations of ethanol may increase the permeability of keratinized buccal mucosa for the polar ionized propranolol, while a higher concentration is required in order to enhance the diffusivity of the nonpolar unionized form. Menthol and lauric acid are adjuvants that effectively promote the absorption of propranolol only when the drug is ionized. Using isopropyl myristate as a model of the mucosal lipid phase in partitioning experiments, we assumed that the mechanism of permeation enhancement was an interaction with the components of the membrane for 1-menthol, increasing the diffusivity of propranolol in the buccal mucosa, whereas lauric acid could form a more partitionable complex without any action on the membrane permeability. Further studies are required to determine the mechanisms by which ethanol and 1-menthol in-

teract with the membrane components. However, we assumed that 1-menthol and low concentrations of ethanol may affect the polar diffusion pathway involving the polar domain of the lipid region and proteins, whereas at a concentration of 30%, ethanol may affect the non-polar pathway with interaction with non-polar lipids.

References

Aungst, B.J., Rogers, N.J. and Shefter, E., Comparison of nasal, rectal, buccal, sublingual and intramuscular insulin efficacy and the effects of a bile salt absorption promoter. *J. Pharmacol. Exp. Ther.*, 24 (1988) 23–27.

Barry, B.W., Mode of action of penetration enhancers in human skin. *J. Controlled Release*, 6 (1987) 85–97.

Elias, P.M., Epidermal lipids, membranes, and keratinization. *Int. J. Dermatol.*, 20 (1981) 1–19.

Ghanem, A.-H., Mahmoud, H., Higuchi, W.I., Rohr, U.D., Borsadia, S., Liu, P. and Fox, J., The effects of ethanol on the transport of β-estradiol and other permeants in hairless mouse skin. II. A new quantitative approach. *J. Controlled Release*, 6 (1987) 75–83.

Golden, G.M., McKie J.E. and Potts, R.O., Role of stratum corneum lipid fluidity in transdermal drug flux. *J. Pharm. Sci.*, 76 (1987) 25–28.

Gould, P.L., Goodman, M. and Hanson, P.A., Investigation of the solubility relationships of polar, semi-polar and non-polar drugs in mixed co-solvent systems. *Int. J. Pharm.*, 19 (1984) 149–159.

Green, P.G., Hadgraft, J. and Ridout G., Enhanced in vitro skin permeation of cationic drugs. *Pharm. Res.*, 6 (1989) 628–632.

Hussain, M.A., Aungst, B.J. and Shefter, E., Buccal and oral bioavailability of nalbuphine in rats. *J. Pharm. Sci.*, 75 (1986) 218–219.

Irwin, W.J., Sanderson F.D. and Li Wan Po A., Percutaneous absorption of ibuprofen: Vehicle effects on transport through rat skin. *Int. J. Pharm.*, 66 (1990) 193–200.

Kajii, H., Horie, T., Hayashi, M. and Awazu, S., Effects of salicylic acid on the permeability of the plasma membrane of the small intestine of the rat: a fluorescence spectroscopic approach to elucidate the mechanism of promoted drug absorption. *J. Pharm. Sci.*, 75 (1986) 475–478.

Koshakji, R.P. and Wood, A.J.J., A modified, sensitive liquid chromatographic method for measurement of propranolol with fluorescence detection. *J. Pharm. Sci.*, 75 (1986) 87–89.

Kurihara-Bergstrom, T., Knuston, K., DeNoble, L.J. and Goates C.Y., Percutaneous absorption enhancement of an ionic molecule by ethanol-water systems in human skin. *Pharm. Res.*, 7 (1990) 762–766.

Kurosaki, Y., Takatori, T., Kitayama, M., Nakayama, T. and Kimura, T., Application of propranolol to the keratinized oral mucosa: Avoidance of first-pass elimination and the use of 1-Dodecylazacycloheptan-2-one (Azone) as an absorption enhancer of bioadhesive film-dosage form. *J. Pharmacobio-Dyn.*, 11 (1988) 824–832.

Kurosaki, Y., Hisaichi, S-I., Hong, L-Z., Nakayama, T., and Kimura, T., Enhanced permeability of keratinized oral mucosa to salicylic acid with 1-dodecylazacycloheptan-2-one(Azone), in vitro studies in hamster cheek pouch. *Int. J. Pharm.*, 49 (1989) 47–55.

Kurosaki, Y., Nagahara, N., Tanizawa, T., Nishimura, H., Nakayama, T., and Kimura, T., Use of lipid disperse systems in transdermal drug delivery: comparative study of flufenamic acid permeation among rat abdominal skin, silicone rubber membrane and stratum corneum sheet isolated hamster cheek pouch. *Int. J. Pharm.*, 67 (1991) 1–9.

Le Brun, P.P.H., Fox, P.L.A., De Vries, M.E. and Bodde, H.E., In vitro penetration of some β-adrenoreceptor blocking drugs through porcine buccal mucosa. *Int. J. Pharm.*, 49 (1989) 141–145.

Merrit, E.W. and Cooper, E.R., Diffusion apparatus for skin penetration. *J. Controlled Release*, 1 (1984) 161.

Moffat, A.C., Absorption of drugs through the oral mucosa. *Top. Med. Chem.*, 4 (1972) 1–26.

Nakada, Y., Awata, N., Ikuta, Y. and Goto, S., The effect of bile salts on the oral mucosal absorption of human calcitonin in rats. *J. Pharmacobio-Dyn.*, 12 (1989) 736–743.

Obata, Y., Takayama, K., Okabe, H. and Nagai, T., Effect of cyclic monoterpenes on percutaneous absorption in the case of a water-soluble drug (diclofenac sodium). *Drug Design. Del.*, 6 (1990) 319–328.

Ogiso, T. and Shintani, M., Mechanism for the enhancement effect of fatty acids on the percutaneous absorption of propranolol. *J. Pharm. Sci.*, 79 (1990) 1065–1071.

Okabe, H., Obata, Y., Takayama, K. and Nagai, T., Percutaneous absorption enhancing effect and skin irritation of monocyclic monoterpenes. *Drug Design. Del.*, 6 (1990) 229–238.

Pardo, A., Shiri, Y., and Cohen, S., Percutaneous absorption of physostigmine, optimization of delivery from binary solvent by thermodynamic control. *J. Pharm. Sci.*, 79 (1990) 573–578.

Perrin, D.D., Dempsey, B. and Serjant, E.P., *In pKa Prediction for Organic Acids and Bases*, Chapman and Hall, London, 1981, pp. 8–9.

Rubino, J.T., The effects of cosolvents on the action of pharmaceutical buffers. *J. Parenter Sci. Technol.*, 41 (1987) 45–49.

Schürmann, W. and Turner, P., A membrane model of the human oral mucosa as derived from buccal absorption performance and physicochemical properties of the β-blocking drugs, atenolol and propranolol. *J. Pharm. Pharmacol.*, 30 (1978) 137–147.

Siegel, I.A., Permeability of the rat oral mucosa to organic solutes measured in vivo. *Arch. Oral. Biol.*, 29 (1984) 13–16.

Siegel, I.A. and Gordon, H.P., Surfactant-induced increases

of permeability of rat oral mucosa to non-electrolytes in vivo. *Arch. Oral. Biol.*, 30 (1985) 43–47.

Sloan, K.B., Siver, K.G., and Koch, S.A., The effect of vehicle on the diffusion of salicylic acid through hairless mouse skin. *J. Pharm. Sci.*, 75 (1986) 744–749.

Surber, C., Wilhelm, K.-P., Hori, M., Maibach, H.I. and Guy, R.H., Optimization of topical therapy: Partitioning of drugs into stratum corneum. *Pharm. Res,* 7 (1990) 1320–1324.

Tavakoli-Saberi, M.R. and Audus, K.L., Physicochemical factors affecting β-adrenergic antagonist permeation across cultured hamster pouch buccal epithelium. *Int. J. Pharm.*, 56 (1989) 135–142.

Turi, J.S., Danielson, D. and Wolterson, J.W., Effect of polyoxypropylene 15 stearyl ether and propylene glycol on percutaneous penetration rate of diflorasone diacetate. *J. Pharm. Sci.*, 68 (1979) 275–280.

Williams, A.C. and Barry, B.W., Terpenes and lipid-protein-partitioning theory of skin penetration enhancement. *Pharm. Res.*, 8 (1991) 17–24.

Wolany, G.J.M., Munzer, J., Rummelt, A. and Merkle, H.P., Buccal absorption of sandostatin(octreotide) in conscious beagle dogs. *Proc. Int. Symp. Controlled Release Bioactive Mater.*, 17 (1990) 224–225.

Yalkowsky, S.H. and Roseman T.J., Solubilization of drugs by cosolvents. In Yalkowsky, S.H. (Ed.) *Techniques of Solubilization of Drugs*, Dekker, New York, 1981, pp. 91–134.

Young, C.-H., Shim, C.-K., Lee, M.H. and Kim, S.K., Effect of sodium salicylate on in vitro percutaneous penetration of isopropamide iodide through mouse skin. *Int. J. Pharm.*, 45 (1988) 59–64.