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Enhanced *Ex Vivo* Buccal Transport of Propranolol: Evaluation of Phospholipids as Permeation Enhancers

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The aim of the present study was to evaluate the effects of two phospholipid permeation enhancers, lysophosphatidylcholine (LPC) and didecanoylphosphatidylcholine (DDPC), along with a fusidic acid derivative, sodium taurodihydrofusidate (STDHF) and ethanol (EtOH) on the buccal transport of propranolol hydrochloride (PPL) using an *ex vivo* buccal diffusion model. The permeation rate of [^{3}H]PPL as measured by steady-state fluxes increased with increasing EtOH concentration. A significant flux enhancement ($P<0.05$) was achieved by EtOH at 20 and 30 %v/v concentrations. At a 0.5 %w/v permeation enhancer concentration, the buccal permeation of [^{3}H]PPL was significantly enhanced by all the enhancers studied (i.e., LPC, DDPC and STDHF) compared to the control (phosphate-buffered saline pH 7.4, PBS). LPC and DDPC displayed a greater degree of permeation enhancement compared with STDHF and EtOH-PBS mixtures with an enhancement ratio of 3.2 and 2.9 for LPC and DDPC, respectively compared with 2.0 and 1.5 for STDHF and EtOH:PBS 30:70 %v/v mixture, respectively. There was no significant difference between LPC and DDPC for the flux values and apparent permeability coefficients of [^{3}H]PPL. These results suggest that phospholipids are suitable as permeation enhancers for the buccal delivery of drugs.

Key words: Buccal delivery, Propranolol, Permeation enhancer, Lysophosphatidylcholine, Didecanoylphosphatidylcholine, Sodium taurodihydrofusidate

INTRODUCTION

The buccal mucosa is considered one of potential sites for the delivery of drugs to the systemic circulation. Advantages of the buccal mucosa as a drug delivery route have been detailed by several investigators (Chidambaram and Srivatsava, 1995; De Vries *et al.*, 1991; Harris and Robinson, 1992). Some of the benefits include: (i) the bypass of the hepatic first-pass metabolism as the drug is directly absorbed into the systemic circulation, (ii) a rich blood supply, (iii) the robustness of the mucosa, (iv) the ease of access (i.e., precise and reproducible localization of dosage forms can be achieved), (v) the provision of relatively large surface area available for absorption, (vi) the possibility of localized modification of tissue permeability, and (vii) non-sex specific.

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Propranolol hydrochloride (PPL) is a non-selective beta blocker and used for the management of hypertension, angina pectoris, myocardial infarction, and cardiac arrhythmias (Parfitt, 1999). Although PPL is almost completely absorbed from the gastro-intestinal tract, it undergoes an extensive and highly variable hepatic first-pass metabolism after oral administration, resulting in a low systemic bioavailability of between 15 and 23% and considerable variation in initial plasma levels of PPL (Cid *et al.*, 1986; Iwamoto and Watanabe, 1985; Shand *et al.*, 1970; Walle *et al.*, 1978). Thus, the buccal delivery of PPL is highly advantageous.

The buccal mucosa with regard to permeability may not be as efficient as the other potential mucosal routes such as nasal, pulmonary, rectal, and vaginal (Anders and Merkle, 1989; Aungst *et al.*, 1988). The permeation of drugs through the buccal mucosa has been prevented by various permeability barriers. Wertz and Squier have summarized the potential barriers as the saliva and its components, mucin, keratin, cell junctions, intercellular lipids, and membrane thickness (Wertz and Squier, 1991). This relatively low buccal permeability can be overcome by the use of

permeation enhancers. There has been extensive research to identify compounds that can improve the absorption of drugs exhibiting poor permeation characteristics. There are numerous compounds known to have absorption enhancing effects and classification and mechanisms of oral mucosal permeation enhancers are well documented (Ganem-Quintanar *et al.*, 1997; Şenel and Hincal, 2001). From the literature, it can be seen that the most widely studied types of permeation enhancer have been bile salts and surfactants. However, bile salts and non-ionic surfactants can permanently damage the integrity of the mucosal tissue and have an effect on reducing the ciliary beat frequency (Hirai *et al.*, 1981; Hermens *et al.*, 1990). Thus, there is a need for the investigation of new enhancers which are less damaging to the mucosal tissue. One area receiving increased attention is the use of phospholipids as permeation enhancers (Hovgaard *et al.*, 1995; Vermehren and Hansen, 1998). The aim of the present study was to evaluate the effect of two phospholipid permeation enhancers (lysophosphatidylcholine and didecanoylphosphatidylcholine), together with a fusidic acid derivative, sodium taurodihydrofusidate and ethanol on the buccal transport of PPL using an *ex vivo* diffusion model (Lee and Kellaway, 2000).

MATERIALS AND METHODS

Materials

Tritium labeled PPL (DL-[4-^{3}H]PPL, [^{3}H]PPL) was purchased from PerkinElmer Life Sciences (Middlesex, UK). Sodium taurodihydrofusidate (STDHF) was obtained from Leo Pharmaceuticals (Ballerup, Denmark). The phospholipids, lysophosphatidylcholine (LPC) and didecanoylphosphatidylcholine (DDPC), unlabeled PPL, phosphate-buffered saline pH 7.4 (PBS) tablets and ethanol (EtOH) were obtained from Sigma-Aldrich Company (Poole, UK). HiSafe 3 liquid scintillation cocktail was obtained from Fisher Chemicals (Loughborough, UK). Fresh distilled water was used throughout.

Preparation of porcine buccal tissue

Fresh pig heads were supplied by a local abattoir on the day of slaughtering. The buccal tissue was obtained by utilizing pig heads within 24 h of slaughter. The buccal tissue was cut away with a Stanley knife. The mucosal membrane was separated by removing the underlying connective tissue with tweezers and surgical scissors. The porcine buccal mucosa was then washed with cold PBS and blot-dried with tissue paper to remove surface-associated water prior to being mounted in a Franz diffusion cell.

Ex vivo transport study

The porcine buccal mucosa was equilibrated in a Franz diffusion cell by placing 0.5 mL of PBS in the donor compartment and 2.2-2.4 mL of PBS in the receiver compartment for 30 min. The transport experiment was initiated by replacing the PBS in the donor compartment with 0.5 mL of test solutions. The test solutions were prepared using an EtOH-PBS mixture containing 30 %v/v EtOH to contain labeled PPL of known radioactivity (5 μCi/mL) and unlabeled PPL (2 mg/mL) together with 0.5 %w/v of the enhancers (LPC, DDPC and STDHF). The Franz diffusion cell was placed on a magnetic stirring block immersed in a water bath at 37 °C and the donor compartment was tightly sealed with a silicone-greased cover glass to prevent moisture loss. Samples (0.2 mL) were withdrawn from the receiver compartment at predetermined time intervals up to 8 h and immediately replaced with an equal volume of pre-warmed fresh PBS. HiSafe 3 liquid scintillation cocktail (3 mL) was added to each sample and liquid scintillation counting (Wallac 1409 DSA liquid scintillation counter, EG&G Wallac, Turku, Finland) was used to determine levels of [^{3}H]PPL transported. Results are expressed as apparent permeability coefficient (P_{app}) ± standard deviation (SD), which was calculated with the following equation (Dowty *et al.*, 1992):

$$P_{app}\ (cm/s) = (R \times V)/(A \times 100\% \times 60)$$

where, R is the rate of transfer (%/min), V is the volume of the donor compartment (cm^3), A (cm^2) is the exposed surface area of the tissue to transport, 100% is the initial amount in the donor compartment, and 60 (s/min) is the conversion factor for minutes to seconds.

Statistical analysis

The *ex vivo* buccal transport results were statistically analyzed using one-way ANOVA and *P* values of 0.05 or less were considered statistically significant.

RESULTS AND DISCUSSION

The *ex vivo* buccal transport study was performed using a fresh porcine buccal tissue obtained within 24 h of slaughter since the barrier properties of the tissue is maintained only for 24 h at 4 °C (Lee *et al.*, 2002). PPL has been suggested to have poor buccal permeability in the beagle dog model (DeGrande *et al.*, 1996) and a permeability coefficient value of 6.42×10^{-6} cm/s has been reported using porcine buccal mucosa (Le Brun *et al.*, 1989). The permeability coefficient of [^{3}H]PPL in PBS vehicle recorded in this study was 2.97×10^{-6} cm/s which was significantly different from the permeability coefficient value of 6.42×10^{-6} cm/s at the confidence limits tested (95%). The difference between our current study and the other study for the permeability coefficients may be attributable to the experimental models employed.

The permeation characteristics of [^{3}H]PPL in EtOH-PBS mixtures containing 0 to 30% EtOH across porcine buccal mucosa were initially studied and are shown in Fig. 1. This study indicates that the buccal transport rate of [^{3}H]PPL can be promoted by EtOH. The permeation rate of [^{3}H]PPL as measured by steady-state fluxes obtained from the linear portion (2-5 h) of the permeation per unit surface area-time curves increased with increasing EtOH concentration.

A significant flux enhancement ($P<0.05$) was achieved by EtOH at 20 and 30 %v/v concentrations (Fig. 3). There has been little information about the direct impact of EtOH on the oral mucosa or its permeability. Some studies, however, have demonstrated that alcohols may exert a topical effect increasing absorption into the underlying tissue by mainly lipid extraction and/or changing the physico-chemical properties of the permeant such as solubility and partition coefficient in the vehicle and in the membrane (Ganem-Quintanar *et al.*, 1997). PPL with a pKa value of 9.45 at 24 °C (Schürmann and Turner, 1978) has shown a pH-dependence of human buccal absorption indicating that the permeation of PPL depends on the ionization state of the molecule (Coutel-Egros *et al.*, 1992). As more ethanol was added to phosphate buffer (PBS), the fraction of unionized PPL increased, subsequently leading to an increased permeability coefficient (Coutel-Egros *et al.*, 1992). Indeed, PPL in its unionized form is highly lipophilic so that the buccal transport of unionized

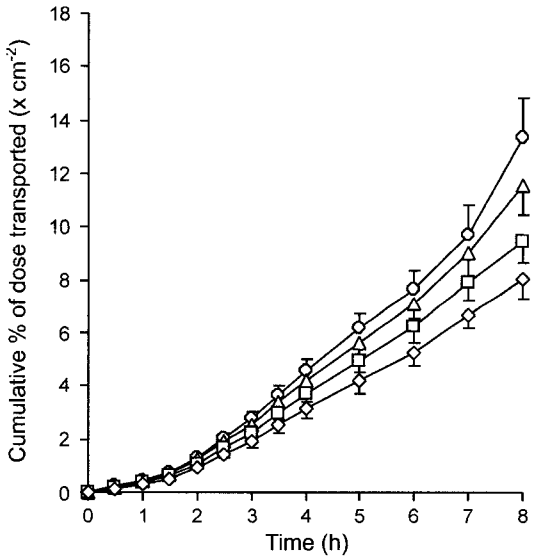

Fig. 1. The effect of ethanol on the permeation of [^{3}H]propranolol across porcine buccal mucosa *ex vivo*: ○, 30 %v/v EtOH solution; △, 20 %v/v EtOH solution; □, 10 %v/v EtOH solution; ◇, 0 %v/v EtOH (PBS alone). Mean±SD, n=7.

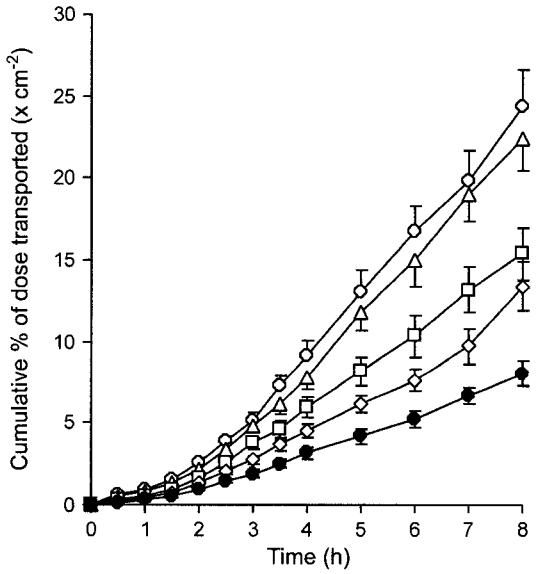

Fig. 2. The effect of different permeation enhancers on the permeation of [^{3}H]propranolol across porcine buccal mucosa *ex vivo*: ○, 0.5 %w/v LPC in EtOH:PBS (30:70 %v/v) solution; △, 0.5 %w/v DDPC in EtOH:PBS (30:70 %v/v) solution; □, 0.5 %w/v STDHF in EtOH:PBS (30:70 %v/v) solution; ◇, 30 %v/v EtOH solution; ●, 0 %v/v EtOH (i.e., PBS alone). Mean±SD, n=7.

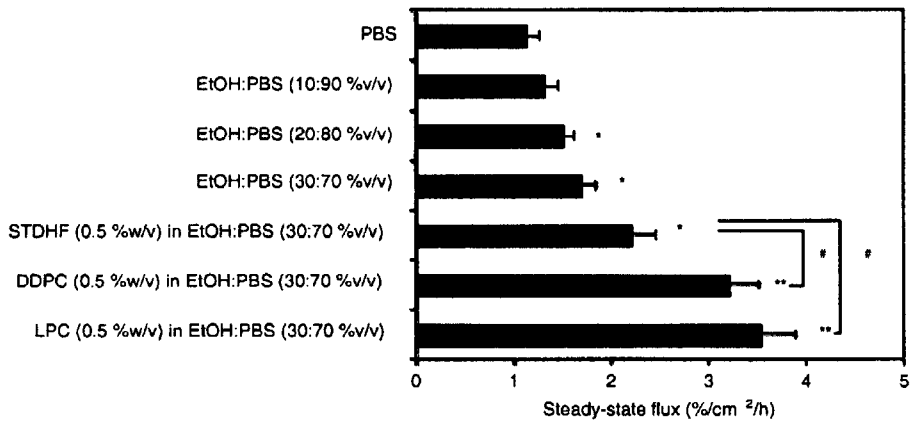

Fig. 3. Steady-state fluxes of [^{3}H]propranolol. Mean±SD, n=7. *Significantly different from PBS group at $P<0.05$. **Significantly different from PBS group at $P<0.01$. #Significantly different from STDHF group at $P<0.05$.

Table I. Permeabilities of [^{3}H]propranolol in various vehicles containing different permeation enhancers. Mean±SD, n=7

	Apparent permeability coefficient ($P_{app} \times 10^{-6}$ cm/s)	ER#
PBS	2.97 ± 0.39	–
EtOH:PBS (10:90 %v/v)	3.50 ± 0.38	1.2
EtOH:PBS (20:80 %v/v)	4.01 ± 0.29*	1.4
EtOH:PBS (30:70 %v/v)	4.52 ± 0.42*	1.5
STDHF (0.5 %w/v) in EtOH:PBS (30:70 %v/v)	5.88 ± 0.66*	2.0
DDPC (0.5 %w/v) in EtOH:PBS (30:70 %v/v)	8.53 ± 0.87**	2.9
LPC (0.5 %w/v) in EtOH:PBS (30:70 %v/v)	9.45 ± 0.98**	3.2

*Significantly different from PBS group at P<0.05.
**Significantly different from PBS group at P<0.01.
#Enhancement ratio = P_{app} obtained with permeation enhancers/P_{app} obtained with PBS.

PPL is generally influenced by its transcellular permeability.

At a 0.5 %w/v permeation enhancer concentration, the buccal permeability of [^{3}H]PPL was significantly enhanced by all the enhancers studied (i.e., LPC, DDPC and STDHF) as compared to the control (i.e., without enhancer, PBS alone) (Fig. 2-3, Table I).

From the *ex vivo* diffusion study, it was observed that LPC and DDPC showed a greater degree of permeation enhancement compared with STDHF and EtOH-PBS mixtures with an enhancement ratio of 3.2 and 2.9 for LPC and DDPC, respectively compared with 2.0 and 1.5 for STDHF and EtOH:PBS 30:70 %v/v mixture, respectively (Table I). For the steady-state permeation period (2-5 h), [^{3}H]PPL with LPC or DDPC also demonstrated significantly better permeation than with STDHF ($P<0.05$) (Fig. 3). There was no significant difference between LPC and DDPC for the flux values and apparent permeability coefficients of [^{3}H]PPL.

As shown in Fig. 2 and 3, the permeation enhancing effect of STDHF was not as effective as that of phospholipid enhancers (LPC and DDPC). A possible explanation for this is that, although PPL diffuses mainly through the transcellular route, STDHF generally causes an improvement of passive paracellular drug transport by the interference with the connections between cells (Aungst and Rogers, 1989; Kissel *et al.*, 1992). Hence, the transcellular permeability of PPL was not greatly affected by the addition of STDHF.

The effectiveness of LPC as a permeation enhancer demonstrated using in vitro models is substantiated in the literature. LPC is a surface-active, amphiphilic molecule present in biological membranes. It has been previously evaluated as a penetration enhancer for buccal, nasal, vaginal, and intestinal transport of drugs (Fisher *et al.*, 1991; Hovgaard *et al.*, 1995; Richardson *et al.*, 1989; Zhang *et al.*, 1994). It has been believed that LPC may offer an effective permeation enhancement without considerable tissue damages that can be judged by microscopic observation of the tissue integrity and appearance of the mucosal surface. In the mucosal barrier study using the back permeation of glucose into the oral cavity, the permeation enhancing effect exhibited by LPC was transient and reversible resulting in the prompt recovery of the mucosal permeation barrier function (Zhang *et al.*, 1994). The appropriate level of LPC as a permeation enhancer was reported as <1% since LPC at concentrations greater than 1% may lead to morphological changes in the epithelial cells (Bolin *et al.*, 1986). DDPC was also suggested to have a high degree of membrane reactivity that can make DDPC a potential permeation enhancer (Vermehren and Hansen, 1998). Incorporation of exogenous phospholipids like LPC and DDPC into the biological membranes can cause membrane packing defects leading to leaky regions (Vermehren and Hansen, 1998). This may therefore be employed to explain how phospholipids have an impact on increasing the buccal membrane permeability as observed in the present study. For PPL therapy, it is thought that the buccal PPL administration with permeation enhancers, especially bile salts and non-ionic surfactants that can cause significant tissue damage or permanent alteration of the membrane structure may not be desirable since buccal PPL administration in humans is anticipated to be a long-term, multiple daily therapy.

CONCLUSIONS

The phospholipid-type permeation enhancers investigated appear to be effective in facilitating the buccal transport of PPL. LPC was the most effective permeation enhancer for PPL. A concentration of 0.5 %w/v of LPC and DDPC gave rise to significant permeation enhancement compared with 0.5 %w/v STDHF and EtOH-PBS mixtures containing 0 to 30 %v/v EtOH. Therefore it can be concluded that phospholipids are suitable as permeation enhancers for the buccal delivery of drugs.

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