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Ex Vivo Permeability Characteristics of Porcine Buccal Mucosa to Drugs with Various Polarity

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ABSTRACT – The aim of this study was to analyze characteristics of the barrier function of excised porcine buccal mucosa to the test compounds, estradiol, propranolol HCl, melatonin, and mannitol with a wide range of partition coefficient values. The permeability of melatonin was measured through frozen, stored, and fresh porcine buccal mucosa to examine the impact of storage conditions on the permeability of porcine buccal mucosa. The results demonstrated that the ex vivo permeability of the porcine buccal mucosa was greater for more lipophilic solutes, which was consistent with a series of molecules transported by passive transepithelial diffusion. The melatonin permeation profiles through frozen, stored, and fresh mucosa illustrated that damage was incurred by the freezing process of the mucosal tissue, leading to loss of the barrier function and thereby an increased permeation coefficient. It can be observed that the influence of compound lipophilicity on the association of the compounds with buccal mucosa was clear. The relationship between permeation coefficient and Log P values for the four compounds investigated demonstrated a proportional relationship, further confirming the importance of the lipophilicity of a compound to permeate the buccal mucosa. These results showed that the ex vivo porcine buccal mucosa model is a suitable tool to screen oral mucosal permeability.

Key words – Buccal drug delivery, Porcine buccal mucosa, Drug polarity, Permeability

The possibility of utilizing the oral mucosa as a potential delivery site for systemic drugs has lead to much interest in this field. In this context, the buccal mucosa offers a number of advantages over other mucosal routes such as nasal, pulmonary, ocular, and vaginal; absorption is directly into the systemic circulation, therefore avoiding hepatic first-pass metabolism, it has a rich blood supply, the area is easily accessible, and it is not sex specific.[1-3]

Several studies have been carried out to assess the permeability properties of buccal mucosa.[4] Compared to in vivo assessment, in vitro or ex vivo permeability measurement gives a number of advantages and has been a useful means to study the mechanisms of buccal mucosal drug absorption. Generally, freshly excised buccal tissue from a test animal is used as a membrane to study solute diffusion. Selection of a suitable animal tissue for the in vitro or ex vivo permeability study is made on the basis of the following criteria[5,6]: (a) the morphological similarities to human tissue in terms of its degree of keratinization, lipid composition, and relative thickness; (b) adequate surface area available for absorption; (c) availability and ease of acquisition of the buccal mucosa; (d) economy. One should consider that in order to obtain good correlation to human buccal absorption, permeability experiments must be performed with non-keratinized epithelia. Of several animal buccal tissues, rabbit, canine, and porcine buccal tissues are stated to be non-keratinized.[5] Many investigators have used the hamster cheek pouch mucosa or the rat buccal mucosa.[7,8] This is due mainly to the advantage of the availability of such tissues. If used, correlations should be carried out carefully for these tissues since they are keratinized.

The objective of the present study was to develop and characterize an ex vivo model to allow the investigation of solute permeability across buccal mucosal tissue, which could be related to the human situation.[9] Porcine buccal mucosa was selected as the chosen model, as it has non-keratinized epithelium and has been shown to have structural similarities with human buccal mucosa. The feasibility of the model can be evaluated by measuring the permeability characteristics of drugs having different polarity.

Experimental

Materials

[^{14}C]-Mannitol, [^{14}C]-estradiol, [^{3}H]-propranolol HCl, and [^{3}H]-melatonin (specific activity, 50 mCi/mmol) were obtained from DuPont Company (Hertfordshire, UK). Phosphate-buffered saline, pH 7.4 (PBS) tablets were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). NCS-II Tissue Sol-

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ubilizer and HiSafe 3 liquid scintillation cocktail were obtained from Amersham Corporation (Arlington Heights, IL, USA) and Fisher Chemicals (Loughborough, UK), respectively. Fresh distilled water was used throughout. Fresh pig heads were supplied by a local abattoir on the day of slaughtering.

Determination of Partition Coefficient of Drugs

The *n*-octanol/PBS partition coefficient was measured prior to investigating drug transport across porcine buccal mucosa. The shake-flask method was used. PBS and *n*-octanol were shaken to co-saturate in a 50 ml screw capped test tube for 24 hr at 37°C. The two phases were then separated by centrifugation at 2000 rpm for 10 min. The aqueous phase was used to prepare drug solutions (0.5 mCi/ml PBS). This aqueous solution (3 ml) was added to the organic phase (3 ml) and shaken in a shaker-bath at 37°C. After equilibration (24 h), the two phases were separated following centrifugation at 2000 rpm for 10 min. Samples were taken from the aqueous phase and analyzed by liquid scintillation counting.

Preparation of Porcine Buccal Mucosa

Fresh pocine buccal tissue was obtained by utilizing pig heads within 24 hours of slaughter. The buccal tissue was cut away with a 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 standard Franz cell. Separately, stored and frozen buccal tissues were used to evaluate tissue viability, which were obtained after the storage of the tissue intact in the pig heads at 4 °C (stored tissue) and −20°C (frozen tissue) for 24 hr.[10]

Ex Vivo Buccal Permeation Study

The porcine buccal mucosa was equilibrated in a Franz 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 0.5 hr. The experiment was initiated by replacing the PBS in the donor compartment with 0.5 ml of the test solution of drugs (1 mCi/ml PBS). The assembled Franz cell was placed on a magnetic stirring block in a water bath at 37°C and the donor compartment was sealed with a silicone-greased cover slip to prevent moisture loss. Samples (0.2 ml) were withdrawn from the receiver compartment at pre-determined time intervals and replaced with the same volume of pre-warmed fresh PBS. Liquid scintillation counting (Wallac 1409 DSA liquid scintillation counter, EG&G Wallac, Turku, Finland) was used to determine levels of drugs permeated. Results are expressed as permeability coefficient (P_c) ± standard deviation, which are calculated from the % flux of the permeants into the receiver compartment over a given time period:

$$P_c(cm/s) = \frac{R \times V}{A \times 100 \times 60}$$

where, R is the rate of transfer (%/min), V is the volume of donor compartment (cm^3) and A is the area of tissue exposed (cm^2). At the end of the 8 hr experiment, the buccal tissue was thoroughly washed with distilled water. The tissue was then digested with 1 ml of NCS-II Tissue Solubilizer at 37°C for 3 days. Acetic acid (30 μl) was added to neutralize the buccal tissue solution. Assay for the determination of radioactivity found in the buccal tissue was carried out by liquid scintillation counting after the addition of 3 ml of liquid scintillation cocktail.

Statistical Analysis

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

Results and Discussion

The porcine buccal tissue was found to be permeable to the four compounds investigated with permeability coefficients listed in Table I. It can be seen from the relatively low variation observed for the different tissue samples that the present experiment used was a good ex vivo model for measuring the diffusion of the drug across buccal tissue. From the tissue morphology observed with light microscope, it was found that tissue breakdown was seen at 6 hr incubation and was more extensive after 8 hr incubation. However, measurements of tissue ATP level and electrical resistance exhibited that there was no significant evidence of tissue breakdown (data not shown). Thus, the viability of the buccal tissue was considered to maintain eight hours for which the transport study was carried out.

Table I and Figure 1 show that the ex vivo permeability of the porcine buccal mucosa is greater for more lipophilic sol-

Table I–*Permeability of Drugs Having Various Log P Values through Fresh Porcine Buccal Mucosa. Mean ± SD, n=7*

Solute	Log P	Permeability coefficient ($\times 10^{-6}$ cm s^{-1})
Mannitol	-3.07	0.24 ± 0.03
Melatonin	0.98	2.41 ± 0.43
Propranolol HCl	2.75	3.74 ± 0.52
Estradiol	3.78	6.11 ± 0.95

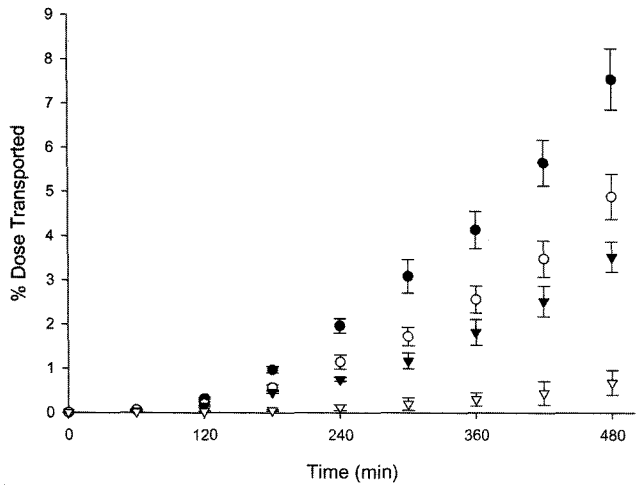

Figure 1–Permeation profiles of estradiol (●), propranolol (○), melatonin (▼), and mannitol (▽) through fresh porcine buccal mucosa. Mean ± SD, n=7.

utes, which is consistent with a series of molecules transported by passive transepithelial diffusion.[11] The paracellular marker molecule, mannitol, which is often used to assess tight junction integrity in epithelial cell monolayers produced a P_c value of $0.24 \pm 0.03 \times 10^{-6}$ cm s^{-1} through fresh porcine buccal mucosa. Mannitol, hydrophilic molecule having very low Log P value had very low permeation, with less than 1% flux over the eight hour study period.

Melatonin is a pineal neurohormone which has been reported to have high buccal bioavailability in beagle dogs using a patch delivery system.[12] As shown in Figure 2, fresh and stored buccal tissue showed similar permeabilities for melatonin, producing P_c values of $2.41 \pm 0.43 \times 10^{-6}$ cm s^{-1} and $2.64 \pm 0.46 \times 10^{-6}$ cm s^{-1}, respectively, which were not significantly different at the confidence limits tested (95%). However, frozen buccal tissue resulted in a considerably increased P_c value of $5.49 \pm 1.10 \times 10^{-6}$ cm s^{-1}, which was significantly different at the confidence limits tested (95%). This illustrates that damage was incurred by the freezing process. Upon freezing the tissue in an uncontrolled manner, ice crystals are formed within the tissue. It can permanently damage the intracellular matrix and/or the structure of cell layers so that the permeability of a variety of compounds being transported through paracellular and transcellular routes is increased.[13,14]

The beta-blocker propranolol has been suggested to have poor permeability in the beagle dog model; however, a P_c value of 6.42×10^{-6} cm s^{-1} has previously been reported using porcine buccal mucosa.[15] In the current study, a P_c value of $3.74 \pm 0.52 \times 10^{-6}$ cm s^{-1} was recorded for propranolol through fresh porcine buccal mucosa. Our value observed is pretty less than in the study carried out by Le Brun *et al.*[15] The reason for this difference might be explained by the different permeation experiment setting and Franz diffusion cell used.

Among the four compounds with a wide range of Log P values, estradiol being the most lipophilic compound produced the greatest P_c value of $6.11 \pm 0.95 \times 10^{-6}$ cm s^{-1}. It has been suggested that the oral mucosa has a depot function and association of a number of drugs to the mucosa following mucosal administration has been reported.[15] It can be seen in Figure 3 that the influence of compound lipophilicity on the association of the compounds with buccal mucosa is clear. The associated amount for estradiol, propranolol, melatonin, and mannitol was $1.33 \pm 0.34\%$, $0.72 \pm 0.28\%$, $0.42 \pm 0.12\%$, and $0.13 \pm 0.05\%$ to a gram of mucosa tissue, respectively. Remaining amount of

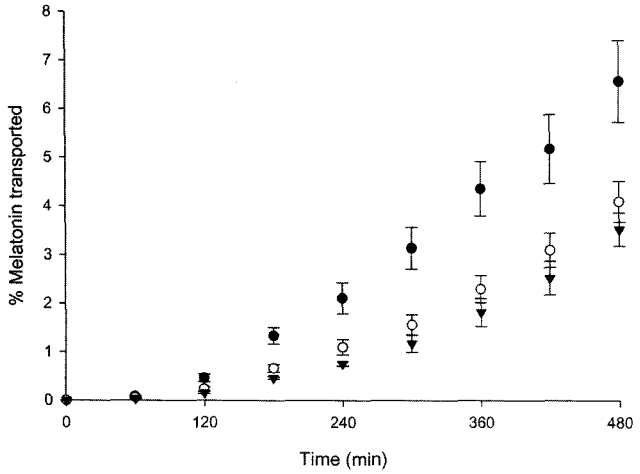

Figure 2–Permeation profiles of melatonin through fresh (●), stored (○) and frozen (▼) porcine buccal mucosa. Mean ± SD, n=7.

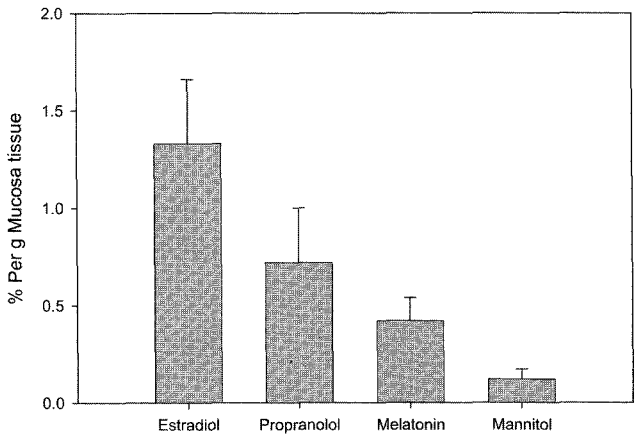

Figure 3–Amount of the four drugs investigated (estradiol, propranolol, melatonin, and mannitol) remaining in the porcine buccal mucosa after 8 h. Mean ± SD, n=7.

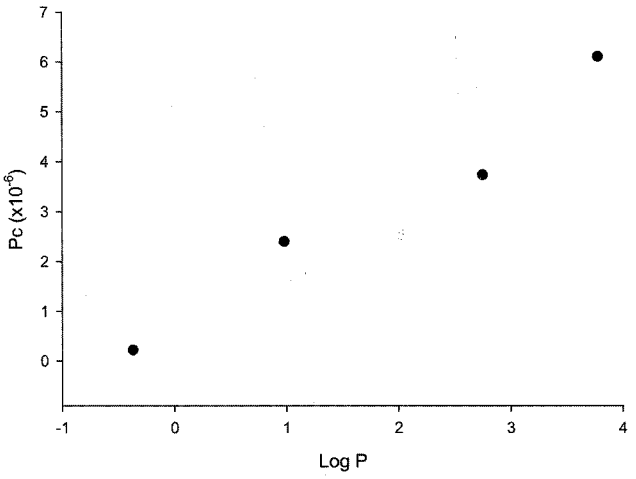

Figure 4–Plot of Log P versus permeability coefficient for estradiol, propranolol, melatonin, and mannitol in the ex vivo porcine buccal mucosa model.

the four compounds in the buccal mucosa at the end of the experimental period which is considered to be binding (or association) of the compounds to the buccal mucosa increased with increasing lipophilicity (Log P). It is, therefore, evident that in order for a drug to be more permeable through the mucosa it needs to firstly be associated with the mucosa, which tends to occur by more lipophilic drug.[16] This finding can also supported by the fact that the main barrier to the permeation has been found to be localization of the permeant in the upper epithelial layers.[6] In the case of mannitol, it is known to traverse via the paracellular route. However, prior to being transported mannitol has to be associated with the mucosa but due to its hydrophilic nature, the association with the mucosa is low, necessarily causing low permeability. In Figure 4, plotting permeation coefficient versus Log P values for the four compounds investigated demonstrated a proportional relationship with $R^2 = 0.963$. This further confirms the importance of the lipophilicity of a compound being investigated to permeate the buccal mucosa.

Conclusions

The ex vivo porcine buccal mucosa model was successfully shown to be good at discriminating between the transmucosal transport of hydrophilic and lipophilic drugs. These results also indicate that the ex vivo porcine buccal mucosa model is a suitable tool to screen oral mucosal permeability. Although the permeability of more lipophilic compound increases with increasing lipophilicity in the current ex vivo model, this is not reflected by the in vivo bioavailability as the drug is probably subject to other factors such as metabolism and tissue binding.

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