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The Effect of Storage Conditions on the Permeability of Porcine Buccal Mucosa

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The impact of storage conditions on the permeability of porcine buccal mucosa to [^{3}H]water and [^{14}C]mannitol was assessed. The fresh pocine buccal tissue (fresh tissue) was obtained by utilizing pig heads within 24 hours of slaughter. The stored and frozen porcine buccal tissues (stored tissue and frozen tissue) were obtained after the storage of the tissue intact in the pig heads at 4°C or -20°C, respectively, for 24 h. The results demonstrated that the barrier properties of the porcine buccal mucosa were maintained with regard to [^{3}H]water permeability when stored at 4°C for 24 h. However, freezing the tissue resulted in tissue damage illustrated by a significant increase in [^{3}H]water permeability. [^{14}C]Mannitol does not appear to be a suitable model solute to assess the ex vivo permeability of porcine buccal mucosa due to its extremely low permeability.

Key words: Buccal permeability, Porcine buccal mucosa, Storage, Freezing, Water, Mannitol

INTRODUCTION

The oral cavity has been selected extensively as a potential site for local and systemic delivery of therapeutic agents. Local therapy has treated conditions such as gingivitis, oral candidiasis, oral lesions, dental caries and xerostomia (Nantwi et al., 1997). Since the successful delivery of glyceryl trinitrate across the sublingual mucosa for the treatment of angina pectoris, the oral cavity (mainly buccal route) has been investigated for systemic absorption of drugs (De Vries et al., 1991).

As a tool to study a drug transport across oral mucosal membrane, in vitro or ex vivo assessment of the diffusivity of the drug offers considerable advantages over in vivo methodology. For example, the experimental set up is simple and controlling experimental factors such as pH, osmolarity and temperature is also readily achieved (Zhang and Robinson, 2000). These methods can be used to explore the mechanism of drug transport across the oral mucosa, to screen rapidly drug candidates for oral mucosal delivery, and to examine permeation enhancement. Oral mucosal tissues of animals are routinely used as an alternative to the human oral mucosa. The main concern of choosing the animal tissue model is resemblance of the ultrastructures and metabolic properties of the animal tissue to man. Pigs, dogs, rabbits, rhesus monkeys, guinea pigs, rats and hamsters have been commonly studied. Of these, the oral mucosae of pigs, dogs, rabbits and rhesus monkeys are known to be similar to that of humans with regard to the non-keratinization of the oral epithelium (Squier, 1973). The surface area of the buccal and sublingual mucosae of pigs and dogs is relatively large, which leads to the minimized individual variation (Zhang and Robinson, 2000).

The viability and integrity of the excised tissue directly affect the permeability of a drug. So far, the ATP level within the oral mucosal cell has been an important parameter to evaluate tissue viability and integrity (Dowty et al., 1992). Testing the barrier function of mucosal membrane using tritiated water has also been a useful way to measure the tissue viability since, in the passive transport process, the tissue is regarded as viable if the barrier function of the tissue remains unchanged during certain experimental conditions (Yazdanian, 1994). One of the difficulties associated with developing an ex vivo buccal transport model is the storage of excised tissue specimens. Several methods can be used including freezing and storage in transport medium (Lesch et al., 1989). The objective of this research was to examine the

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impact of storing and freezing the porcine buccal tissue upon barrier properties to develop an ex vivo transport mode for the investigation of drug permeability across porcine buccal mucosa.

MATERIALS AND METHODS

Materials

Tritiated water ([^{3}H]water, specific activity, 5 Ci/ml) and [1-^{14}C]-D-mannitol ([^{14}C]mannitol, 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 (Poole, UK). NCS-II Tissue Solubilizer and 'HiSafe' 3 liquid scintillation cocktail were obtained from Amersham Corporation (Arlington Heights, IL) 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.

Preparation of porcine buccal tissue

The fresh pocine buccal tissue (fresh tissue) was obtained by utilizing pig heads within 24 hours of slaughter. The stored and frozen porcine buccal tissues (stored tissue and frozen tissue) were obtained after the storage of the tissue intact in the pig heads at 4°C or -20°C, respectively, for 24 h. 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.

Transport 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 h. The experiment was initiated by replacing the PBS in the donor compartment with 0.5 ml of the test solution containing [^{3}H]water (1 mCi/ml PBS) or [^{14}C]mannitol (0.5 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

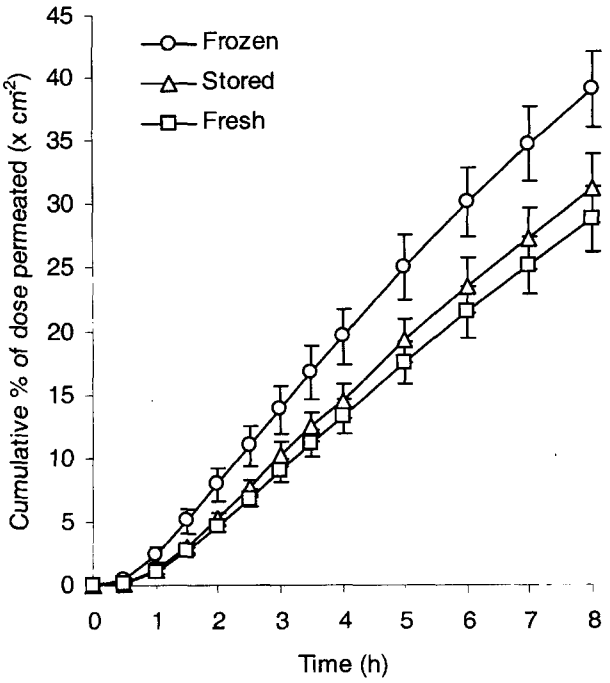

Fig. 1. Permeation of [^{3}H]water through fresh, stored and frozen porcine buccal mucosa. Mean ± SD, n=7.

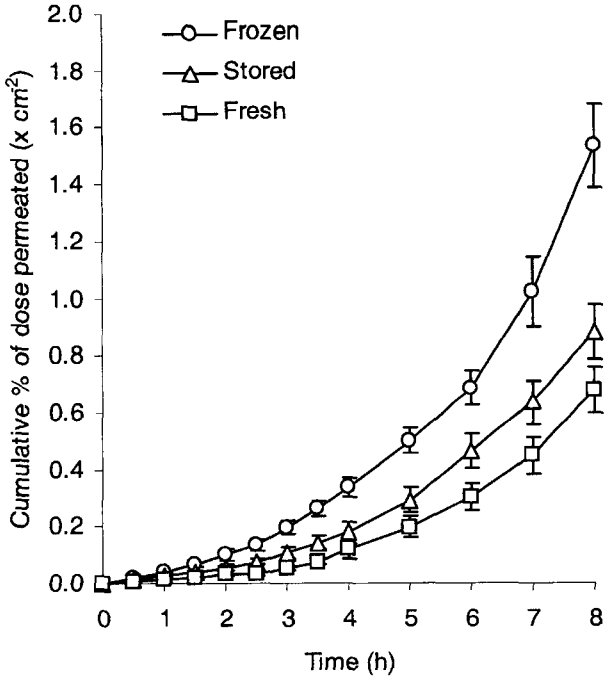

Fig. 2 Permeation of [^{14}C]mannitol through fresh, stored and frozen porcine buccal mucosa. Mean ± SD, n=7.

levels of [^{3}H]water and [^{14}C]mannitol permeated. Results are expressed as permeability coefficient (Pc) ± standard deviations, which are calculated from the % flux of the permeant 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 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

Fig. 1 and 2 report the effect of buccal tissue storage upon the permeability of porcine buccal mucosa to water and mannitol. The steady-state flux and permeability coefficient (P_c) values of [^{3}H]water and [^{14}C]mannitol obtained for fresh, stored and frozen porcine buccal mucosae are summarized in Table I. Permeability coefficients were calculated from the linear portion of the permeation profiles collected between 1 and 4 h. The permeability of [^{3}H]water across porcine buccal tissue was significantly higher ($P < 0.05$) than that of [^{14}C]mannitol at all storage conditions including fresh tissue.

The permeation results show that storing the porcine buccal tissue at 4°C for 24 h intact in the pig heads produced a slight increase in [^{3}H]water permeability. However, the value of the permeation coefficient was not significantly different from the control (fresh tissue) at the confidence limits tested (95%). This suggests that the barrier properties are maintained during the storage period. Freezing the tissue at -20°C for 24 h resulted in an increase in the [^{3}H]water permeability, which was significantly different from those for fresh and stored tissues ($P < 0.05$), illustrating that damage is 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 (Swarbrick et al., 1982; Hadzija et al., 1992).

Table I. Permeabilities of [^{3}H]water and [^{14}C]mannitol through fresh, stored and frozen porcine buccal mucosa. Mean± SD, n=7.

Tissue	[^{3}H]Water		[^{14}C]Mannitol	
	$Flux_{1\text{-}4\,h}$ (%/cm^2/h)	Permeability coefficient (P_c) ($\times 10^{-6}$ cm/s)	$Flux_{1\text{-}4\,h}$ (%/cm^2/h)	Permeability coefficient (P_c) ($\times 10^{-7}$ cm/s)
Fresh	4.3 ± 0.4	11.4 ± 1.0	0.04 ± 0.01	1.0 ± 0.3
Stored	4.7 ± 0.4	12.6 ± 1.1	0.06 ± 0.01	1.5 ± 0.3
Frozen	5.8 ± 0.5*	15.6 ± 1.3*	0.11 ± 0.01*	2.9 ± 0.2*

* $P < 0.05$: Significantly different from control group (fresh tissue).

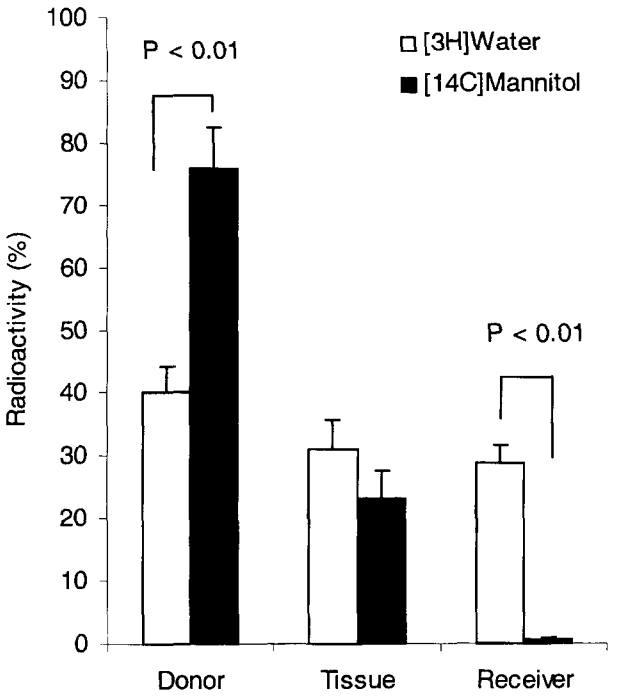

Fig. 3. Amounts of [^{3}H]water and [^{14}C]mannitol found in donor and receiver compartments, and digested tissue obtained from the experiment employing fresh buccal tissue after 8 h. The amounts in the sample taken for the measurement of radioactivity were combined with the amounts found in the receiver compartment. Mean ± SD, n=7.

Mannitol is a hydrophilic marker of the paracellular transport pathway and is often used to assess tight junction integrity in single cell monolayer (Jacobsen et al., 1995). It had very low permeability, with far less than 1.0 % flux over the 8 h study period. The permeation profiles of [^{14}C]mannitol were different from those of [^{3}H]water. Also, the lag times of [^{14}C]mannitol were significantly longer than those of [^{3}H]water (data not shown). This implies that it is difficult for [^{14}C]mannitol to permeate across porcine buccal mucosa. However, [^{14}C]mannitol also showed a storage condition dependency with regard to its permeability with an order of frozen tissue > stored tissue > fresh tissue. Using this marker in the buccal model is considered unsuitable since the permeation of [^{14}C]mannitol is very low and incomplete over the 8 h experiment period. Therefore, the permeability of [^{14}C]mannitol may not be reflective of tissue barrier integrity.

The quantity of [^{3}H]water and [^{14}C]mannitol found in the donor compartment, fresh buccal tissue, and receiver compartment after 8 h of the experiment is presented in Fig. 3 as the percentage of total amount applied to the tissue. The radioactivity distribution profile suggested that the porcine buccal tissue acts as a depot for [^{3}H]water and [^{14}C]mannitol. However, the dose of [^{14}C]mannitol transported to the buccal tissue was trapped between cell layers within the tissue, probably leading to a substantial decrease in the permeation rate.

It is reasonable to assume that the freshly excised buccal tissue from animals immediately after killing retains most of the barrier properties of the buccal tissue, required for the development of an ex vivo model to study buccal drug permeability. However, the availability of fresh tissue is often limited by the method and schedule of experimentation and the amounts of tissues supplied. In those cases, it is suggested that porcine buccal tissue can be preserved at 4°C for 1 day with expectation to show similar barrier properties to those exhibited by fresh tissue.

In conclusion, the results presented in this paper demonstrated that the barrier properties of the porcine buccal mucosa can be maintained with regard to [^{3}H]water permeability when stored at 4°C for 24 h. However, freezing the tissue in an uncontrolled manner resulted in tissue damage illustrated by a significant increase in [^{3}H]water permeability. [^{14}C]Mannitol does not appear to be a suitable model solute to validate an ex vivo permeability of porcine buccal mucosa due to its extremely low permeability.

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