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An in vivo dog model for studying recovery kinetics of the buccal mucosa permeation barrier after exposure to permeation enhancers: apparent evidence of effective enhancement without tissue damage

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Summary

This work proposes a method to study mechanisms of action of permeation enhancers and demonstrates that it is possible to effectively, transiently and reversibly increase mucosal permeability without causing any visible mucosal irritation and damaging the permeation barrier. Permeation enhancers have been used to increase mucosal permeability in buccal, sublingual and nasal drug delivery. However, it is unclear whether they act by transiently altering the mucosa or by damaging it. When an enhancer increases mucosal permeability, the permeability to glucose, which is extremely low without an enhancer, is also increased. Glucose in the submucosal interstitial fluid can thus permeate across the mucosa (back permeation) and be collected. For transient mucosal alteration, the permeation barrier will begin to recover after the enhancer is removed, resulting in a gradual decrease in glucose back permeation flux. If the mucosa is damaged, it is assumed that the flux will not decrease for some time. The effectiveness of sodium cholate (C), sodium taurocholate (TC) and lysophosphatidylcholine (LPC) as enhancers was demonstrated in the buccal administration of insulin in anesthetized dogs. Recovery kinetics were studied in the same animals by measuring glucose back permeation fluxes following exposure to these enhancers. Glucose back permeation flux showed no decrease following exposure to C, but began to decrease immediately following exposure to TC and LPC, and decreased by 80% in 5–8 h. No visible mucosal irritation was observed in any case. These results suggest that under the given conditions, TC and LPC transiently altered the mucosal barrier function while C altered the mucosal barrier function for prolonged times which may be the result of either extended enhancer stay in the mucosa or mucosa damage.

Introduction

In oral transmucosal drug delivery, drugs are directly exposed to the oral (buccal and sublingual) mucosa and permeate across the mucosal tissues to reach the systemic circulation. For many drugs, this route of delivery offers many advantages over traditional routes of delivery, including absence of trauma, increased speed of delivery (compared to oral and intramuscular routes), improved bioavailability due to avoidance of degradation in the GI tract and hepatic first-pass

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metabolism. A number of small molecular weight drugs including the nitrates, morphine, fentanyl and buprenorphine, have been found to penetrate through oral mucosa tissues at sufficient rates to achieve effective plasma concentrations within clinically reasonable times (Murrell, 1879; Altman et al., 1960; Bell et al., 1985; McAllister, 1986; Shah et al., 1986). Oral transmucosal delivery may also provide the attractive advantage of allowing the administration of some higher molecular weight drugs, including some peptides, which must currently be delivered via injections. Unfortunately, oral mucosal permeabilities of these high molecular weight compounds are usually too low to allow plasma concentrations to reach therapeutic levels. To facilitate the transmucosal delivery of large molecules, a number of permeation enhancing agents have been used in attempts to significantly increase buccal, sublingual and nasal mucosal permeability (Wieriks, 1964; Tolo and Jonsen, 1975; Ishida et al., 1981; Gordon et al., 1985; Merkle et al., 1985; Nagai, 1985; Aungst et al., 1988; Ritschel et al., 1989; O'Hagan et al., 1990).

However, the mechanisms by which these agents enhance permeation are unclear. It is believed some enhancers increase mucosal permeability by damaging the mucosa tissues (Squier, 1984; Aungst and Rogers, 1989), while others may offer effective enhancement without significant tissue damage (Squier, 1984; Fix et al., 1986). Harris and Robinson (1992) have suggested that the line between effective enhancement and tissue damage is necessarily fine, since enhancement of permeability implies, by definition, some alteration of the protective permeation barrier of the mucosa.

The speed of recovery of the mucosal permeation barrier after exposure to an enhancer can reveal important information regarding the mechanism of action of the enhancer. If the mucosa is significantly damaged by an enhancer, recovery of barrier function would only result from tissue turnover, or other self-repairing mechanisms, and would be slow. If the enhancer only transiently alters tissue structure, the recovery process may be much faster. In addition, recovery kinetics of the mucosal permeation barrier should enable evaluation of the safety of an enhancer and its clinical utility.

The objectives of this work are to test an in vivo method to assess the speed of recovery of the buccal mucosa permeation barrier after exposure to permeation enhancers, and to study the possibility of effective permeability enhancement without tissue damage.

The buccal mucosa in its natural state is practically impermeable to glucose (Stanley et al., 1992). When a permeation enhancer increases mucosa permeability (either transiently or destructively), the permeability to glucose is dramatically increased. As a result, glucose in the submucosal interstitial fluid, which is in rapid equilibrium with blood glucose, can permeate across the mucosa into the oral cavity, which in turn can be collected into a receiving medium (such as water) held in a diffusion cell (Stanley et al., 1992). This permeation (referred to hereafter as back permeation) is driven by the glucose concentration gradient between the submucosal interstitial fluid and the aqueous solution (receiving medium) in contact with the mucosa, and the 'sink condition' maintained in the receiving medium. If the alteration of the mucosal structure is transient, the permeation barrier of the mucosa will begin to recover after the enhancer solution is removed from the mucosa, resulting in a gradual decrease in glucose back permeation flux. On the other hand, if the alteration is destructive and 'permanent', recovery will be slow and may even be absent for a long time, resulting in slow or no decrease in the glucose back permeation flux for the entire experiment. If an experimental animal's blood glucose concentration is maintained stable and constant, the recovery process of the mucosal permeation barrier should be quantitatively reflected in a decrease in glucose back permeation flux.

In this work, the mongrel dog was used as the model animal due to the histological similarities with human buccal mucosa (Ebert, et al., 1987). Sodium cholate (C), sodium taurocholate (TC) and lysophosphatidyl choline (LPC) were selected as model permeation enhancers for the study. All three compounds have been previously reported to be effective permeation enhancers in transnasal

or transbuccal delivery of either insulin or human growth hormone (Ishida et al., 1981; Gordon et al., 1985; Ritschel et al., 1989; O'Hagan et al., 1990). To prove they are also effective in our animal model, transbuccal insulin absorption was evaluated with each enhancer. Blood glucose changes were measured as an indicator of insulin absorption and systemic bioavailability.

Experimental

Dog handling

Two healthy mongrel dogs of either sex (25–32 kg) were used. The dog was anesthetized with sodium thiopental (20 mg/kg, i.v.) and then maintained on 1–2% halothane in oxygen. Respirations of the dog were maintained with a mechanical ventilator. An intravenous infusion of lactated Ringer's solution was initiated in a cephalic vein to maintain proper hydration and gain access to the vein. The femoral artery was catheterized for continuous measurements of arterial blood pressure and collection of blood samples. Heart rate was monitored with a standard ECG.

The anesthetized dog's mouth was kept open with a retractor for either insulin administration or mucosa barrier recovery experiments. After the experiment, the dog was allowed to recover from anesthesia and was returned to the Animal Resources Center for observation and future use.

Devices

The diffusion cell used for insulin administration was made of Teflon (Fig. 1). When attached to the buccal mucosa, donor solutions can be introduced into the cell for direct contact with the mucosa through an area of 18 cm^2.

The stainless-steel diffusion cell used in the mucosal barrier recovery experiments (Fig. 2) has four completely segregated compartments, each acting as an independent cell and allowing the solution in it to directly contact the mucosa through an area of 2.0 cm^2.

Chemicals

Sodium cholate, sodium taurocholate, lysophosphatidylcholine and bovine insulin were obtained from Sigma Chemical Co., St. Louis, MO, and used as received.

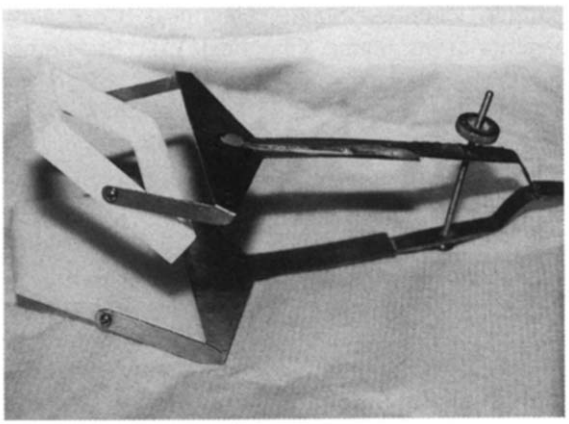

Fig. 1. Teflon diffusion cell for buccal administration of insulin. When clamped onto the dog's buccal mucosa, the solution can be held in it and have direct contact with the mucosa through an area of 18 cm^2.

Glucose concentration measurements

Blood glucose was measured with commercially available glucose strips and a matching reflectance meter (Glucostix and Glucometer, Ames Division, Miles Lab. Inc., Elkhart, IN). Glucose concentrations in the receiving medium (water) were measured with Trinder's method (1991

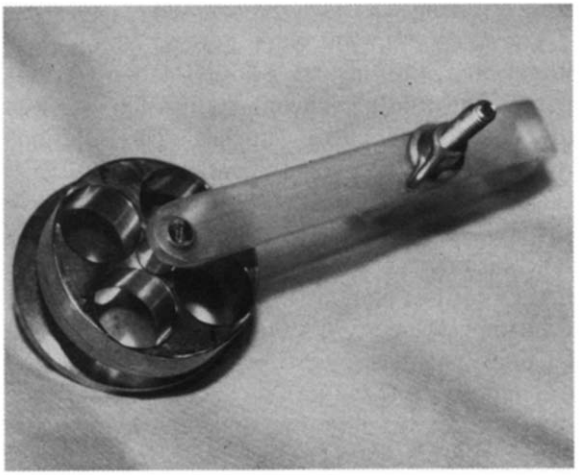

Fig. 2. Stainless-steel cell used for glucose back permeation measurements. The cell has four segregated compartments each having an area of 2.0 cm^2.

Product Catalog, Sigma Chemical Co.) with reagents from Sigma Chemical Co., St. Louis, MO.

Effectiveness of the permeation enhancers

The effectiveness of the three permeation enhancers were tested by measuring their ability to facilitate the transmucosal permeation of insulin. 2% C, 3% TC or 2% LPC was added to 2% (w/w) insulin in saline, the pH was adjusted to 9.0. For control measurements, a 2% insulin in pH 9.0 saline solution containing no enhancer was also made. Insulin in these solution is stable for at least 6 h (unpublished results) and all insulin solutions were freshly made immediately before they were used. The Teflon diffusion cell was attached to the dog's buccal mucosa. Three baseline blood samples were taken for glucose quantitations in a 40 min period prior to insulin administration. 4 ml of a given insulin or insulin/enhancer solution was introduced into and left in the diffusion cell for 10 min (C and TC) or 20 min (LPC) before it was withdrawn. The buccal mucosa area was then rinsed with water five times to remove remaining insulin and enhancer. Blood samples were taken and glucose concentrations in them determined every 5–15 min for 90 min from the moment the solution was introduced into the cell.

Recovery of buccal mucosa permeation barrier

These experiments were performed on days other than the insulin administration experiments. The four-compartment diffusion cell was attached to the buccal mucosa. The solutions used in these experiments contained 2% C, 3% TC or 2% LPC, respectively, dissolved in 0.9% $NaCl/H_2O$ with pH adjusted to 9.0, analogous to the solutions in insulin absorption studies. 0.8 ml of each of the solutions were introduced into the diffusion cell, one solution per compartment. The TC and C solutions were kept in the cell for 10 min and the LPC solution for 20 min, before they were withdrawn. The mucosa and the compartments were rinsed five times with water thereafter to remove remaining enhancers from the mucosa surface. To start the measurement of mucosal barrier recovery, 0.8 ml of water was introduced into and kept within each compartment for 30 min before being withdrawn for glucose concentration determinations. The compartments were again rinsed five times with water before the next 30 min measurement cycle was begun. The same procedure was repeated 10–12 times to measure the glucose back permeation flux for 7–8 h. Arterial blood samples (0.5 ml each) for glucose concentration measurements were collected every 2 h throughout the experiment to ensure that blood glucose levels remained unchanged (fluctuation within $\pm 10\%$), as is expected from healthy fasted dogs.

Average glucose back permeation flux during each 30 min period was calculated according to:

$$J = (C \cdot V)/(A \cdot t)$$

where C is the glucose concentration in water in the compartment at the end of the 30 min period, V denotes the volume of water in the diffusion cell (0.8 ml), A is the area of the diffusion cell compartment (2.0 cm^2) and t is 0.5 h (30 min).

Results

Fig. 3 shows that in the insulin administration experiments, blood glucose concentrations started to decrease in 15 min from the commencement of all three insulin/enhancer solutions and decreased to below 50 mg/dl in 30–80 min. However, when the insulin solution containing no enhancer was applied, no decrease in blood glucose was observed. No visible irritation was observed after exposure to any of the solutions, as judged by visual observation of integrity and appearance (color, smoothness) of the mucosal surfaces, in comparison with surrounding mucosal surfaces that did not have any contact with enhancers.

Table 1 provides the average glucose back permeation flux in each 30 min measurement period for each enhancer, along with blood glucose concentrations and apparent permeability coefficient at selected time points throughout the experiment. Blood glucose concentrations remained stable throughout the experiment while

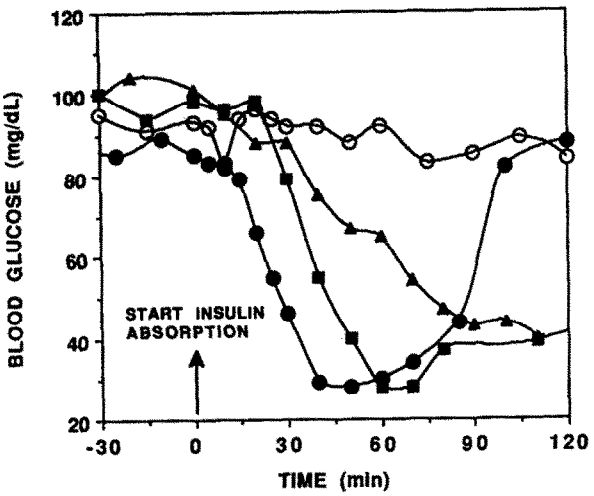

Fig. 3. Blood glucose changes resulted from buccally administrated insulin using permeation enhancers; average values from two dogs. Insulin/C and insulin/TC solutions were in contact with buccal mucosa from $t = 0$ to $t = 10$ min. Insulin/LPC solution was in contact with buccal mucosa from $t = 0$ to $t = 20$ min. (○) No enhancer, (●) sodium taurocholate, (■) cholate, (▲) lysophosphatidylcholine.

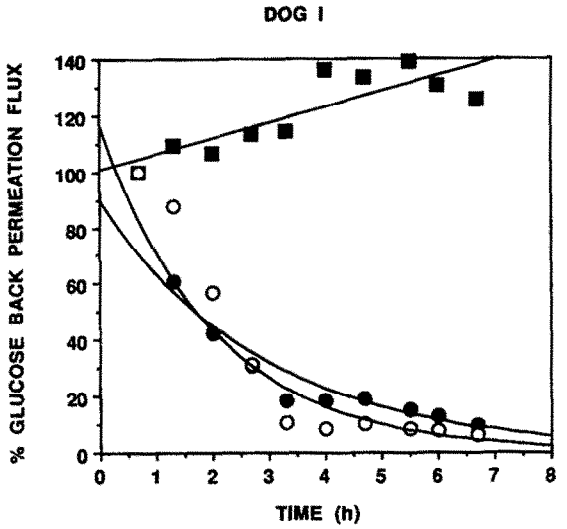

Fig. 4. Glucose back permeation flux vs time after exposure to permeation enhancers; dog I. The curves represent best fit single exponentials (see Results). (●) Sodium taurocholate, (■) cholate, (○) lysophosphatidylcholine.

glucose back permeation flux started to decrease immediately after the removal of the enhancers, except in the case of C in which no decrease was observed. Figs 4 and 5 show the percentage decreases (the flux in the first 30 min period was set as baseline or 100%) in glucose back permeation flux vs time from last exposure to the enhancer solutions. Following removal of TC and LPC solutions, fluxes began to decrease immediately and decreases were approximately first order processes. Rates of decrease in glucose back permeation after exposure to TC and LPC solutions

TABLE 1

Blood glucose, back permeation flux, and apparent permeability coefficient vs time after exposure to permeation enhancers

Time (h) [a]	Dog I							Dog II						
	Blood glucose (mg/dl)	Flux (μg/h per cm^2)			$P(\times 10^{-6})$ (cm/s)			Blood glucose (mg/dl)	Flux (μg/h per cm^2)			$P(\times 10^{-6})$ (cm/s)		
		LPC	TC	C	LPC	TC	C		LPC	TC	C	LPC	TC	C
0.7	102	29.1	36.7	31.4	7.9	10.0	8.6	89	40.0	42.0	34.2	12.5	13.1	10.7
1.3		17.7	32.2	34.4					30.3	38.9	37.8			
2.0	98	12.3	20.9	33.5	3.4	5.9	9.5	94	23.8	30.4	40.3	7.0	9.0	11.9
2.7		9.0	11.5	35.6					23.4	25.3	42.1			
3.3		5.4	3.8	35.9					19.2	22.7	41.0			
4.0	97	5.4	3.0	42.8	1.5	0.8	12.3	91	15.9	18.5	41.7	4.9	5.6	12.7
4.7		5.5	3.6	41.8					12.7	15.3	41.0			
5.5		4.4	3.1	43.6					12.4	13.7	41.7			
6.0	103	3.8	2.9	40.9	1.1	0.8	11.7	88	11.1	12.0	42.6	3.4	3.7	13.0
6.7		2.8	2.3	39.4					10.1	10.3	42.1			
7.3									10.1	10.6	41.6			
8.0								92	8.4	9.9	42.0	2.5	3.0	12.7

[a] Time between last exposure to enhancer and the midpoint of each 30 min period.

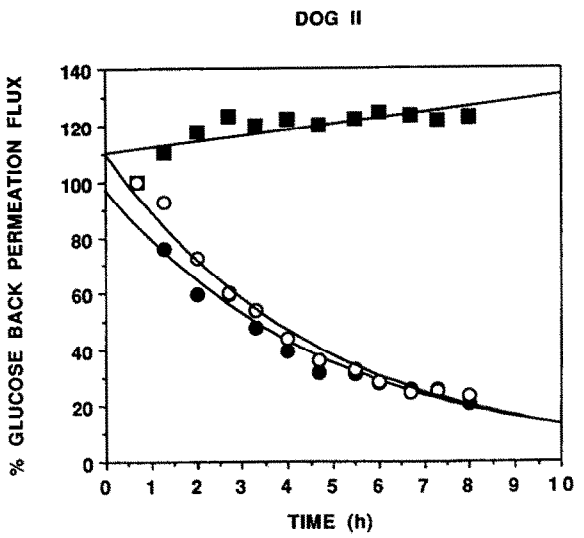

Fig. 5. Glucose back permeation flux vs time after exposure to permeation enhancers; dog II. The curves represent best fit single exponentials (see Results). (●) Sodium taurocholate, (■) cholate, (○) lysophosphatidylcholine.

were similar. 50 and 80% decreases occurred in 2–3 and 5–8 h, respectively. The solid lines in the Figs 4 and 5 are the best fit single exponential curves, with rate constants ranging from 0.087 to 0.216 h^{-1}. In the case of C, glucose back permeation flux remained unchanged in both dogs over the 7–8 h experiments.

Discussion

As indicated by the results in Fig. 3, no significant transbuccal mucosal insulin absorption occurred with the insulin solution containing no enhancer, while sufficient quantities of insulin were systemically absorbed to cause hypoglycemia with any of the three insulin/enhancer solutions. These results indicate that the buccal mucosa in its unaltered state is practically impermeable to insulin, but all three enhancers under the conditions specified were able to effectively increase buccal mucosal permeability to insulin.

In the insulin absorption studies, blood glucose concentrations did not start to decrease significantly until the insulin/enhancer solution was removed from the mucosa and did not reach minima for another 20–60 min. The authors believe that this phenomenon may be attributable to the diffusional lag time, a possible insulin depot in the mucosa, and the fact that the animal's pancreas normally reduces endogenous insulin production to counteract the exogenous insulin. Blood glucose concentrations do not decrease from normal levels (80–120 mg/dl) until exogenous insulin concentrations alone are sufficiently high, which takes time to occur.

In the mucosal barrier recovery experiments, the enhancers were used at the same concentrations and in the same solutions as those in the insulin administration experiments except that they contained no insulin. This was carried out in order to match the experimental conditions employed during the insulin absorption studies. Since blood glucose concentrations were stable throughout the experiment, decreases or lack of decreases in glucose back permeation flux can only be attributed to recovery or lack of recovery of the mucosa permeation barrier. The recovery of the mucosal permeation barrier can only be the result of two possible processes – tissue turnover (or other repair mechanisms) or return from the transiently altered state to its unperturbed state. Buccal mucosa turnover time in the dog is unknown to the authors, but it is 14 days in man (Squier et al., 1976). If tissue turnover or other mechanisms of repairing the mucosa in the dog take place over a similar duration of time to that in man, significant recovery of the permeation barrier within a few hours suggests that only transient and reversible alteration of mucosal structure has occurred. On the other hand, lack of significant recovery in 7–8 h indicates that either the enhancer has a slow clearance from the mucosa or more severe or permanent damage of the mucosa has occurred.

Our results suggest that TC and LPC in the specified solutions enhance buccal permeability by transiently altering the mucosal barrier function, rather than by damaging it. In contrast, C either stayed in the mucosa for extended times, or significantly damaged or 'permanently' altered the mucosa structure. The speedy recovery of the mucosa permeation barrier after exposure to TC

and LPC also suggests that it is possible to transiently and reversibly increase the mucosal permeability sufficiently that macromolecules such as insulin can permeate with enough speed to achieve therapeutic plasma concentrations.

It should be pointed out that the conditions under which a given permeation enhancer is used, such as concentration, solution pH, ionic strength, and exposure time, may also determine the speed of recovery of the permeation barrier. Thus, it is possible that changes in these parameters can turn a speedy recovery into a slow one, and vice versa, after exposure to the same enhancer. Therefore, in evaluating a given enhancer, the conditions under which it is used must also be considered. Harris and Robinson (1992) pointed out that mucosa damage and effective enhancement without mucosa damage may simply be different degrees of the same phenomenon. In this respect, the rate of mucosal barrier recovery may serve as a criterion in selecting these parameters.

It should also be pointed out that in this method, only glucose was used to characterize the recovery of the mucosa permeation barrier. While glucose may be a good representative of small hydrophilic molecules, it may not reflect the recovery of the permeation barrier to lipophilic molecules or macromolecules. However, hydrophilic compounds in general are known to have lower mucosal permeability than lipophilic compounds and are therefore more likely to require permeation enhancement. In addition, it is reasonable to expect that the permeation barrier to larger hydrophilic molecules recovers faster than that to the much smaller glucose. In this regard, glucose is useful as an indicator to estimate the lower limit of recovery rate.

In summary, this work proposes an in vivo method to evaluate the recovery kinetics of the buccal mucosal permeation barrier after exposure to permeation enhancers. This method may provide an easy way to evaluate the safety of permeation enhancers as well as a technique to select exposure time, enhancer concentration, vehicle pH and other parameters in transmucosal drug formulations containing permeation enhancers. In addition, this study suggests that effective enhancement of the buccal mucosa permeability without significant mucosal damage may be possible.

With necessary modifications and improvements, this method may be extended to in vivo human tests.

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