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Research Paper

Transport of Fentanyl Through Pig Buccal and Esophageal Epithelia *in Vitro*. Influence of Concentration and Vehicle pH

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Purpose. To validate pig esophageal epithelium as a model for the permeability barrier of the buccal mucosa, the transport of fentanyl across the two tissues was compared *in vivo*.
Methods. The epithelia were separated by immersing the excised mucosae into an isotonic saline solution at 60–65°C. Fentanyl was delivered as the citrate salt at a concentration of 1 or 2 mg/mL buffered at one of four pH values (between 6.0 and 7.4).
Results. Across both barriers, drug transport increased proportionally with concentration as expected. However, drug flux was not linearly related to the unionized fraction of the drug; indeed, fentanyl delivery was significant even when 98.5% of the drug was in the ionized form.
Conclusions. Buccal and esophageal fluxes were very similar under all conditions suggesting that the esophageal epithelium is a representative tool for buccal transport studies *in vitro*.

KEY WORDS: buccal epithelium; diffusion; esophageal epithelium; fentanyl; permeability; transport.

INTRODUCTION

Systemic drug delivery across the oral mucosa can be achieved either sublingually, via the floor of the mouth and the ventral side of the tongue, or buccally via the mucosal lining of the cheeks. Although sublingual administration is well suited for rapid absorption and a fast onset of action, the less permeable cheek mucosa is a potential platform for sustained drug delivery. In addition, drug administration through the buccal or sublingual mucosae avoids presystemic metabolism in the gastrointestinal tract and liver, as well as the acid environment of the stomach. Another advantage of these routes is the facile application, localization, and removal of a putative drug delivery system (1).

In vitro buccal absorption studies are typically conducted using pig cheek mucosa, which is generally recognized as a representative model of the human tissue (2). However, this model has some important limitations in that the available, usable surface is small and is also frequently damaged by mastication. Moreover, its excision is fastidious and time-consuming as the buccal mucosa is tightly bound to the underlying muscular tissue.

To overcome these drawbacks, the pig buccal mucosa can be substituted with the adjacent esophageal tissue, the structure and biochemical features of which are remarkably similar. Both mucosae manifest a squamous, stratified, nonkeratinized epithelium supported by connective tissue (3); the only significant difference is that the thickness of the esophageal epithelium is smaller and less variable (409 ± 104 *vs.* 767 ± 279 μm) (4). The buccal and esophageal permeability barrier is located in the epithelium and related to the intercellular lipid material, the composition of which is very similar qualitatively and quantitatively in both epithelia (5).

From a practical point of view, the use of the pig esophageal model has several advantages: (1) it has a larger ($\geq$ 150 cm^2) and essentially undamaged epithelium, (2) its excision is facile being much less firmly bound to the underlying muscular tissue, and (3) its thickness is relatively constant both within and between tissue samples.

Fentanyl, a synthetic opioid, was chosen as a model drug for this study. It is extremely potent and is generally administered intravenously for anesthesia and moderate-to-severe pain relief (6). Fentanyl undergoes extensive hepatic first-pass metabolism and is therefore ineffective after oral administration. Presystemic inactivation can be circumvented if the drug is delivered via the buccal mucosa across which it is rapidly absorbed (7–9).

Previously, we have shown that the *in vitro* permeability of fentanyl across buccal and esophageal mucosae is similar (4). Here, the influence of drug concentration and vehicle pH on the transport of fentanyl across buccal and esophageal epithelia *in vitro* was evaluated.

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MATERIALS AND METHODS

Materials

Fentanyl citrate was purchased from Macfarlan Smith Ltd. (Edinburgh, UK). $Na_2HPO_4 \cdot 2H_2O$, KH_2PO_4, NaCl, and ammonium acetate were obtained from Fluka (Buchs, Switzerland). Acetonitrile (HPLC grade) was acquired from Riedel-de Haën (Seelze, Germany).

Tissue Preparation

Pig cheeks and esophagus were obtained from animals sacrificed at the local slaughterhouse (SODEXA, Annecy, France) and were transported to the laboratory in isotonic phosphate buffer at pH 7.4. The buccal mucosa was carefully removed from the underlying muscle and connective tissue with scissors. Adipous residues and part of the connective tissue were discarded. The esophagus was opened longitudinally with scissors and rinsed with saline. The mucosa was isolated from the outer muscle layer by cutting the loosed connective fibers with a scalpel. Circular pieces of buccal and esophageal tissues 24 mm of diameter were punched out. Epithelium was then isolated by immersing the full-thickness mucosae into an isotonic saline solution at 60–65°C for 60 s. The epithelium was then peeled inward from the edges of the mucosa. Samples were rinsed quickly in deionized water to remove superficial exogenous salts, drained on a cellulose filter, and frozen at −20°C until use. This procedure has been shown to have no significant effect on either the morphology or the permeability characteristics of the tissues (4). All experiments were conducted using tissue from at least two animals with three to five replicates.

Permeation Studies

Fentanyl transport was studied using vertical diffusion cells (diffusion area = 1.77 cm^2) at 37°C. Epithelial samples were previously thawed in isotonic phosphate buffer for 15 min at room temperature. Each tissue was then spread over a filter membrane with pore diameter of 0.45μm (Millipore HAWP 2500, Saint Quentin en Yvelines, France) and clamped between the chambers of the diffusion cells with the epithelial side facing the donor compartment. The donor and receptor chambers were filled with 1 and 6 mL of isotonic phosphate buffer (pH 7.4), respectively. The cells were equilibrated at 37°C for 30 min following which the donor solution was discarded and replaced with 1 mL of fentanyl citrate solution. The drug concentration was either 1 or 2 mg/mL at pH 6.0, 6.6, 7.0, or 7.4. The temperature of the donor solution was 33°C. Diffusion experiments ($n \geq 6$) were conducted for 8 h. Samples (200 μL) were periodically taken from the receptor phase and replaced with the same volume of fresh buffer.

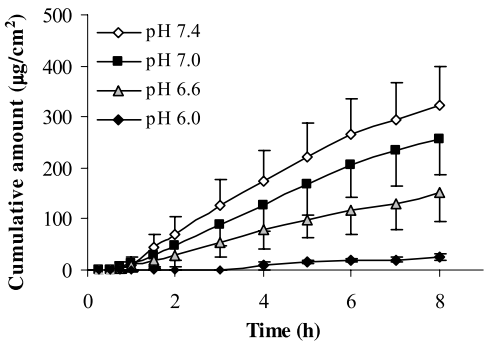

Fig. 1. Cumulative permeation of fentanyl from a solution of the citrate salt at 2 mg/mL across buccal epithelium as a function of donor solution pH.

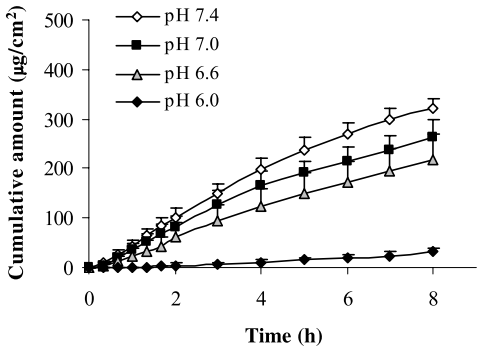

Fig. 2. Cumulative permeation of fentanyl from a solution of the citrate salt at 2 mg/mL across esophageal epithelium as a function of donor solution pH.

Fentanyl Assay

Samples were analyzed by HPLC using a Waters chromatographic system (Waters France, Saint Quentin Yvelines, France) fitted with a reverse-phase column (Nucleosil 100-5 C18 AB, Macherey-Nagel, Hoerdt, France) heated at 40°C. The mobile phase was acetonitrile/acetic ammonium buffer (50:50) pumped at 1 mL/min. Fentanyl was detected at 256 nm.

Data Analysis

Mucosal drug transport was analyzed according to a passive diffusion mechanism mathematically described by Fick's first law (10). According to this model, the steady-state rate of appearance (J) of fentanyl in the receptor compartment is given by Eq. (1):

$$J = \frac{AKD}{h}(C_d - C_r) \cong \frac{AKD}{h} \bullet C_d \quad (1)$$

where C_d and C_r are the drug concentrations in the donor and receiver compartments, respectively; under the conditions of the experiments performed in this study, $C_d \gg C_r$, allowing simplification of the equation as shown. A is the epithelial surface area available for transport, and h is the thickness of the barrier. D and K are, respectively, the drug's diffusivity in the membrane, and its partition coefficient between the membrane and the aqueous buffer in the donor compartment. The permeability coefficient (K_p) of fentanyl across the epithelium is defined as $K_p = KD/h$. The cumulative amount of drug per unit area of mucosa (Q/A) reaching the receptor phase as a function of time (t) is given by:

$$\frac{Q}{A} = \int_0^t \frac{J}{A} dt = K_p \bullet C_d \bullet t \quad (2)$$

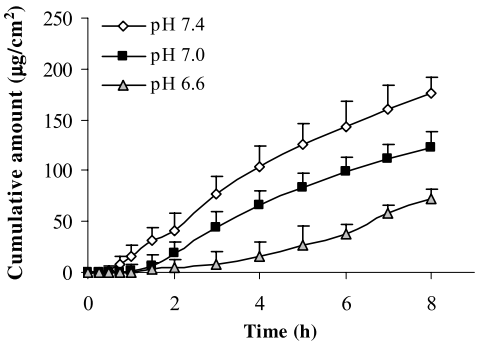

Fig. 3. Cumulative permeation of fentanyl from a solution of the citrate salt at 1 mg/mL across buccal epithelium as a function of donor solution pH.

It follows that, at steady state, a graph of Q/A *vs.* time should be linear with a slope equal to K_pC_d. The lag times were obtained from back extrapolation of this straight line to the time axis. The degree of ionization of fentanyl at the different pH values of the donor compartment was calculated from the Henderson–Hasselbach equation using a pK_a of 8.43 (11). All results were expressed in terms of fentanyl base using a fentanyl/fentanyl citrate weight ratio of 0.64. ANOVA and Tukey's multiple comparison test were used to compare the results obtained ($p < 0.05$, significant differences).

RESULTS

The transport of fentanyl across both epithelial tissues increased with the pH of the donor solution (Figs. 1, 2, 3, 4). Drug fluxes and permeability coefficients across the two barriers were not significantly different at any pH and concentration (Table I). As a result, graphs of esophageal *vs.* buccal fluxes as a function of pH and concentration were linear with slopes close to unity, especially at 2 mg/mL (Fig. 5).

At pH 6.0, fentanyl transport was very low across both epithelia (Figs. 1 and 2), compared to that at pH 6.6 and above. Remarkably, the change in the degree of ionization of the drug from pH 6.0 to 6.6 (99.6–98.5%) resulted in a more than fivefold increase in flux (Table I). Further increase in pH and the concomitant change in the degree of fentanyl ionization resulted in considerably less dramatic

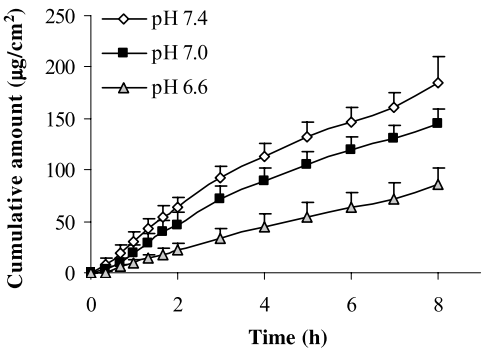

Fig. 4. Cumulative permeation of fentanyl from a solution of the citrate salt at 1 mg/mL across esophageal epithelium as a function of donor solution pH.

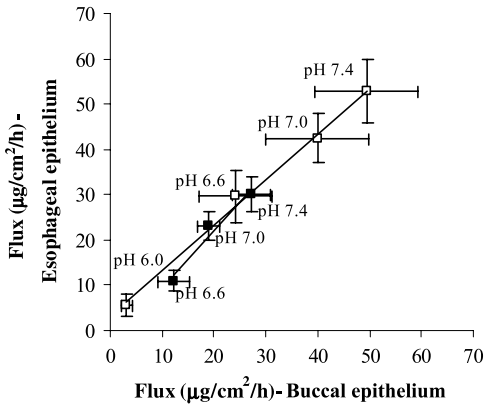

Fig. 5. Correlation between fentanyl fluxes across buccal and esophageal epithelia (as a function of pH) when applied as the citrate salt at either 1 mg/mL (■) or 2 mg/mL (□); the corresponding lines of regression are: $y = 1.268x + 3.223$ ($r^2 = 0.953$) and $y = 1.006x + 3.327$ ($r^2 = 0.995$).

changes in flux and permeability coefficient (Table I and Fig. 6).

As predicted by the Fick's first law of diffusion (Eq. 1), drug flux increased proportionally and significantly when the concentration of fentanyl citrate in the donor solution was increased from 1 to 2 mg/mL (Table I, $p < 0.05$). As a result, the deduced permeability coefficients (i.e., the ratio of the measured flux to the applied concentration) were constant for each experimental condition studied, as illustrated in Fig. 6.

Lag times for fentanyl transport across the mucosal barriers were significantly shorter at pH 6.6 and above, as compared to those at pH 6.0 ($p < 0.001$, Table II), when the drug donor concentration was 2 mg/mL. With the lower drug level in the vehicle, lag times across the esophageal membrane were significantly shorter ($p < 0.01$, Table II) than those across the buccal barrier at all pHs tested.

DISCUSSION

First of all, as shown in Table I, fentanyl transport was proportional to the drug concentration in the donor com-

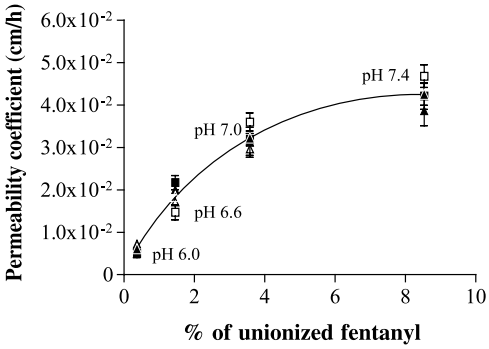

Fig. 6. Relationship between fentanyl permeability coefficient across pig buccal and esophageal epithelia and the percent unionized drug in the donor phase, at donor concentrations of 1 or 2 mg/mL. —■—: esophageal epithelium, 2 mg/mL; —▲— buccal epithelium, 2 mg/mL; —□—: esophageal epithelium, 1 mg/mL; —△—: buccal epithelium, 1 mg/mL.

Table I. Steady-state Fluxes of Fentanyl (μg/cm^2/h) Across Buccal and Esophageal Epithelia as a Function of pH and Concentration (Mean±SD; n = 6–10)

pH	Ionized fraction	1 mg/mL*		2 mg/mL	
		Buccal	Esophageal	Buccal	Esophageal
7.4	91.5	27.2 ± 3.7	30.0 ± 3.4	49.9 ± 9.9	52.9 ± 7.1
7.0	96.4	18.9 ± 2.2	23.6 ± 3.0	39.9 ± 10.0	42.5 ± 5.2
6.6	98.5	12.3 ± 3.0	10.9 ± 2.6	24.2 ± 7.1	29.6 ± 5.7
6.0	99.6	ND	ND	3.0 ± 1.1*	5.5 ± 2.5*

At fixed concentration, no differences between fluxes across buccal and esophageal tissues were observed.
* Transport was influenced significantly by pH (values at pH 6.0 at 2 mg/mL being less than those at pH 6.6 and above) and drug donor concentration ($p < 0.05$).

partment, in accord with Fick's first law of diffusion. Clinical data from the literature show that after buccal transmucosal administration of ≈1 mg of fentanyl citrate, plasma concentrations in the therapeutic range (≈2 ng/mL) were achieved (9). Fluxes of fentanyl achieved following application of a solution either 1 or 2 mg/mL fell in the range of 10–50 μg/cm^2/h. Practically speaking, for a buccal delivery system with a surface area of 1.5 cm^2, and containing an equivalent drug concentration, this corresponds to a fentanyl input rate of 15–75 μg/h. Given that the clearance of fentanyl is about 40 L/h (9), such a system would lead to a steady-state plasma concentration of 1–2 ng/ml, which is within the therapeutic range.

The transport of fentanyl across both buccal and esophageal epithelia increased with pH (and hence with the unionized fraction of the drug). Qualitatively, this is consistent with the pH-partition hypothesis, which predicts that the unionized form of the drug, being more lipophilic than the ionized form, more readily transfers through the lipoidal mucous membranes. The correlation between buccal and esophageal fluxes at all pH values considered testifies that the permeability barrier of these tissues to fentanyl is very similar (Fig. 5). These findings agree with a previous study showing that the epithelia possess common biochemical features (5).

Fentanyl transport was significant across both tissues even when 98.5% of the drug was ionized. At higher degrees of ionization, the flux dropped sharply, reaching either negligible or not measurable levels. Absorption of highly ionized drugs, such as sodium diclofenac (12) and codeine phosphate (13) across human buccal mucosa, has been reported. Likewise, fentanyl at a very high degree of ionization, was considerably absorbed across dog buccal mucosa *in vivo* (14).

Table II. Lag Times (min) for Fentanyl Transport Across Buccal and Esophageal Epithelia as a Function of pH and Concentration (Mean ± SD; n = 6–10)

pH	1 mg/mL		2 mg/mL	
	Buccal	Esophageal*	Buccal	Esophageal
7.4	32 ± 16	11 ± 4	42 ± 23	15 ± 5
7.0	51 ± 18	18 ± 10	53 ± 22	23 ± 11
6.6	96 ± 67	31 ± 8	60 ± 42	18 ± 10
6.0	ND	ND	207 ± 44**	143 ± 55**

*Significantly different from buccal epithelium ($p < 0.01$).
**Significantly different from values at all other pHs tested ($p<0.001$).

This relatively high permeability of nonkeratinized mucosae to ionized and/or hydrophilic drugs merits further discussion. Microscopic visualization of the route taken by the hydrophilic, ultrastructural tracer, horseradish peroxidase across rabbit, monkey, or pig buccal mucosa revealed an intercellular pathway (15,16). Laser scanning confocal microscopy has also indicated an intercellular pathway for the buccal transport of fluorescein isothiocyanate-labeled dextrans (17). The permeability barrier located in the intercellular space of nonkeratinized epithelia substantially differs from that in the skin in that it contains about 50% of polar lipids, namely, phospholipids and glycosylceramides, which are absent in the skin barrier (18). The polar head groups of these lipids entrain numerous molecules of water such that within the intercellular space of a nonkeratinized epithelium, there probably exists two environments: a hydrophobic region within the lipid domains and a hydrophilic zone associated with the hydrated head groups of polar lipids (19). The latter environment has been suggested to offer a permeation route for ionized drugs (12,13).

An alternative possibility is that the fentanyl cation crosses the membrane associated with a counterion in the form of an ion pair, a potentially efficient mechanism that has been proposed for transport of certain drugs across the skin (20,21). Moffat (22) suggested that buccal absorption can occur even if the unionized fraction of drug is very small, because the ionized–unionized conversion reaction is very fast.

Whatever the mechanism involved, the absorption of fentanyl from a solution, in which it is predominantly ionized, is clear. *In vivo*, a tenfold increase in the unionized concentration of fentanyl in the donor solution resulted in only up to a fivefold increase in K_p (14). Similarly, in *in vitro* experiments reported in this work, a sixfold increase in the unionized concentration of fentanyl in the donor solution (the result of changing the pH from 6.6 to 7.4) almost tripled the value of K_p (Fig. 6). Parenthetically, it is also noted that the K_p of fentanyl across human skin (values ranging from 0.5×10^{-2} to 1.3×10^{-2} cm/h over the pH range 6.0–7.4) is significantly less than those across buccal and esophageal epithelia (23). This difference is consistent with the highly lipophilic nature of the permeability barrier in keratinized tissues such as the skin.

Finally, lag times were quite variable and, although the differences observed sometimes achieved statistical signifi-

cance, the practical consequences are unlikely to be important. Coefficients of variation were smaller for esophageal tissue (30–50%) than buccal (30–70%), probably because the former epithelium is of smaller and more reproducible thickness than the latter (270–500 *vs.* 300 to almost 1000 μm) (4).

CONCLUSIONS

The value of the esophageal epithelium as an *in vitro* model of the buccal mucosal permeability barrier was demonstrated. Over the range of pH and concentrations tested, the permeability of fentanyl across buccal and esophageal barriers was essentially identical. The transport of fentanyl from solutions, in which the drug was mostly in the ionized form, supported the hypothesis that a polar environment existed in the intercellular milieu of both mucosal epithelia. It follows that the esophageal epithelium is a useful membrane to evaluate prototypes of buccal drug delivery systems.

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