Transbuccal Delivery of Acyclovir: I. In Vitro Determination of Routes of Buccal Transport

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Purpose. To determine the major routes of buccal transport of acyclovir and to examine the effects of pH and permeation enhancer on drug permeation.

Methods. Permeation of acyclovir across porcine buccal mucosa was studied by using side-by-side flow through diffusion cells at 37°C. The permeability of acyclovir was determined at pH range of 3.3 to 8.8. Permeability of different ionic species was calculated by fitting the permeation data to a mathematical model. Acyclovir was quantified using HPLC.

Results. Higher steady state fluxes were observed at pH 3.3 and 8.8. The partition coefficient (1-octanol-buffer) and the solubility of acyclovir showed the same pH dependent profile as that of drug permeation. In the presence of sodium glycocholate (NaGC) (2–100 mM), the permeability of acyclovir across buccal mucosa was increased 2 to 9 times. This enhancement was independent of pH and reached a plateau above the critical micelle concentration of NaGC. The permeabilities of anionic, cationic, and zwitterionic forms of acyclovir were 3.83 × 10^-4, 4.33 × 10^-4, and 6.24 × 10^-6 cm/sec, respectively.

Conclusions. The in vitro permeability of acyclovir across porcine buccal mucosa and the octanol-water partitioning of the drug were pH dependent. A model of the paracellular permeation of the anionic, cationic, and zwitterionic forms of acyclovir is consistent with these data. The paracellular route was the primary route of buccal transport of acyclovir, and the enhancement of transbuccal transport of acyclovir by sodium glycocholate (NaGC) appeared to operate via this paracellular route.

KEY WORDS: acyclovir; membrane transport; buccal mucosal delivery; in vitro permeation; zwitterion.

INTRODUCTION

Two pathways for passive drug transport through oral mucosa have been investigated—the paracellular route consisting of hydrophilic intercellular spaces and the transcellular route requiring transport across lipophilic cell membranes (1). The lipophilicity of the permeant determines the dominant route.

For hydrophilic compounds such as acyclovir, the area fraction of intercellular space and the resulting tortuosity are the main limitations for this route. That is, the steady-state flux of a hydrophilic drug (Jp) through the paracellular route may be modelled as (2)

\[ J_p = \frac{D_p \varepsilon}{h_p} \cdot C_D \]

where \( \varepsilon \) is the area fraction of the paracellular route, \( h_p \) is the length of the paracellular route, \( D_p \) is the diffusion coefficient in the intercellular spaces, and \( C_D \) is the concentration of drug in the donor chamber. This model neglects the effects of surface charge on partitioning and diffusion and assumes the permeant does not alter its own transport.

For lipophilic drugs, the transcellular pathway is preferred, and the steady-state flux of a lipophilic drug (Jt) across the transcellular route is given by (2)

\[ J_t = \frac{(1 - \varepsilon)D_t \kappa_p}{h_t} \cdot C_D \]

where \( \kappa_p \) is the partition coefficient between the lipophilic phase (cell membrane) and the aqueous hydrophilic donor phase, \( h_t \) is the length of the transcellular route, and \( D_t \) is the diffusion coefficient in the lipophilic phase.

The routes of drug transport have been visualized directly by scanning electron microscopy (3,4), autoradiography (5,6), and confocal laser scanning microscopy (7). An indirect way to examine the routes of drug transport across buccal mucosa is to study permeation in vitro. Selection of an appropriate animal species is essential for the applicability of the results. In light of the many similarities to human, porcine buccal mucosa was selected for the present in vitro permeation studies (8–14).

Previous drug absorption studies have demonstrated that oral mucosal absorption of amines and acids at constant concentration is proportional to their partition coefficients (15). Similar dependencies on partition coefficients were obtained for \( \beta \)-adrenoceptor blocking agents (16), substituted acetanilides (17), carboxylic acids (18), and other drugs (19). On the other hand, there are drugs that primarily cross the oral mucosa through the paracellular route. Dowty and Robinson (6) studied the transport of thyrotropin releasing hormone (TRH) in rabbit buccal mucosa in vitro, and found that the main pathway of transport for TRH is the paracellular route.

Acyclovir is an antiviral, which is similar in structure to the purine nucleoside, guanosine. It is a zwitterion with both weak acid and basic moieties. Because of the poor oral and transdermal absorption of acyclovir, buccal mucosa is a logical choice for systemic delivery of acyclovir. The present study attempts to understand the in vitro transport of acyclovir across porcine buccal mucosa based on varying pH and employing the permeation enhancer, sodium glycocholate (NaGC).

MATERIALS AND METHODS

Buccal Tissue Collection and Preparation

Porcine buccal tissue was kindly provided by Long Ranch, Inc. (Manteca, CA) and was used within 2 hours after slaughter. The tissue was stored in Krebs buffer at 4°C upon removal. The mucosal membrane was separated by removing the underlying connective tissue with surgical scissors. Buccal mucosa of an approximate area of 0.75 cm² were then mounted between the donor and the receiver chambers.
**In Vitro Permeation Studies**

Side-by-side flow-through diffusion cells (Crown Glass Co., NJ) with a diffusional area of 0.69 cm² were used. Both chambers were stirred with Teflon coated magnetic stirring bars. After the buccal membranes were equilibrated with Krebs buffer (310 mM) at 37°C, the receiver and donor chambers were filled with fresh buffer solution and acyclovir solution (0.8 to 2.4 mg/ml, respectively). The flow rate of buffer was controlled at 1.23 ml/hr with a peristaltic pump (Minipuls 3, Gilson, Middleton, WI). The samples were collected every 90 minutes by using a fraction collector (Gilson-203, Middleton, WI) and analyzed using HPLC method for acyclovir. The permeability coefficients (P) were calculated as follows:

\[
P = \frac{\left(\frac{dQ}{dt}\right)}{(\Delta C \cdot A)}
\]  

where \(\frac{dQ}{dt}\) is cumulative amount permeated per unit time, \(\Delta C\) is the concentration difference across the buccal mucosa, and \(A\) is the diffusional area. The effects of pH of the buffer on permeation were identified by using isotonic McIlvaine buffer (IMB) solutions in the pH range of 3.3 to 8.8 (containing 2.4 mg/ml acyclovir). Analysis of variance (ANOVA) was used for statistical evaluations. Data are presented as mean ± standard deviation (n = 3).

The permeability of acyclovir was also evaluated in the presence of the permeation enhancer, sodium glycocholate (NaGC) at 2, 10, 20, 50, 75, and 100 mM of NaGC. The enhancer was added with the drug solution (2.4 mg/ml of acyclovir) into the donor chamber, while the receiver chamber contained only Krebs buffer. Enhancement ratios (ER) were calculated according to the following expression:

\[
ER = \frac{P_{\text{enh}}}{P_{\text{no enh}}}
\]

where \(P_{\text{enh}}\) is enhanced permeability coefficient and \(P_{\text{no enh}}\) is permeability coefficient of acyclovir without co-administration of the bile salt. Note that this definition is valid for a porous paracellular pathway, but that the thermodynamic activities are obscure for a partitioning mechanism.

**Determination of Partition Coefficients**

Mutually saturated 1-octanol and isotonic McIlvaine buffer solutions (pH 3.3–8.8) at 37°C were employed. An aliquot (10-μl) of 1-octanol saturated IMB containing 200 μg/ml of acyclovir was mixed with an equal volume of IMB saturated 1-octanol. Two phases were then allowed to equilibrate at 37°C for 24 hours in a shaker. The concentration of acyclovir in the aqueous phase was determined by using HPLC. The apparent partition coefficients (\(K_p\)) were calculated as the ratio of the concentration of acyclovir in each phase by using the following equation:

\[
K_p = \frac{C_{aq} - C_{eq}}{C_{eq}}
\]

where \(C_{aq}\) is the initial concentration of acyclovir in the aqueous phase and \(C_{eq}\) is the concentration of acyclovir at equilibrium in the aqueous phase. Dissolved water in the octanol phase is not accounted for separately in this measurement.

**Determination of Critical Micelle Concentration (CMC) of Sodium Glycocholate**

The CMC of NaGC was determined from the measured surface tension at 25°C by the capillary height method (20). Samples were prepared by dissolving 2.4 to 144 mg of NaGC in 2.4 mg/mL acyclovir in Krebs buffer. The solutions were then placed in a Petri dish in which a capillary tube (r = 0.05 cm) was immersed vertically. The height of the liquid in the capillary tube was measured and surface tension (γ) was calculated in accordance with the following formula:

\[
\gamma = \frac{0.5(h \cdot g \cdot r)}{\cos \delta}
\]

where \(h\) is the height (cm) of the liquid in the capillary tube, \(g\) is the density (g/ml) of the solvent, \(r\) is the gravitational constant (cm/s²), \(r\) is the radius (cm) of the capillary tube, and \(\delta\) is the contact angle between the solution and the glass of the capillary tube. Since the only variable in the formula is \(h\), the CMC was then extrapolated from the plot of capillary height (h) vs. concentration of NaGC.

**Determination of pH-Solubility Profile**

Excess acyclovir was added to 3 ml of deionized water in screw-capped vials. Variable volumes of either hydrochloric acid or sodium hydroxide were added to adjust the pH. The vials were shaken horizontally in a water bath shaker (Aquatherm, New Brunswick Scientific Co., Inc., Edison, NJ) for 24 hours at 37 ± 0.5°C. The suspension was passed through a 0.2 μm membrane filter (Acrodisc®, Gelman, Ann Arbor, MI) with the initial portion of the filtrate discarded to ensure saturation of the filter. An aliquot of the filtrate was diluted and analyzed by HPLC, while the rest of the filtrate was used for pH determination (Fisher Accumet pH meter Model 825MP, Pittsburgh, PA).

**RESULTS**

The mean steady-state flux (\(J_{ss}\)) increased with the concentration of acyclovir. Excellent linearity (\(r^2 = 0.993\)) was observed between \(J_{ss}\) and concentration (Fig. 1). There is a significant non-zero intercept, but this may reflect the weighting of the highest concentration by the linear regression. Such linearity is consistent with permeation of acyclovir by a passive diffusion process, over the concentration range (0.8–2.4 mg/ml) studied.

To probe into the mechanism of transport of acyclovir through porcine buccal mucosa, the effect of pH on permeation was investigated. Over the pH range from 3.3 to 8.8 (Fig. 2), the permeation of acyclovir is markedly affected by pH. There was a significant (P < 0.05) increase in flux and permeability coefficient at the extremes of the pH range investigated (as compared to values at pH 4.1, 5.8, and 7).

The apparent partition coefficients of acyclovir between 1-octanol and various buffer pH were determined. Figure 3a shows the effect of McIlvaine buffer pH on the 1-octanol/buffer partition coefficients of acyclovir. The same trend was seen