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

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Received February 24, 1998; accepted May 16, 1998

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 species were 3.83×10^{-5}, 4.33×10^{-5}, 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 (J_P) through the paracellular route may be modelled as (2)

$$J_P = \frac{D_P \varepsilon}{h_P} \cdot C_D \tag{1}$$

where ε 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 (J_T) across the transcellular route is given by (2)

$$J_T = \frac{(1 - \varepsilon) D_T K_P}{h_T} \cdot C_D \tag{2}$$

where K_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 β-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 cm^2 were then mounted between the donor and the receiver chambers.

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0724-8741/98/0800-1182$15.00/0

In Vitro Permeation Studies

Side-by-side flow-through diffusion cells (Crown Glass Co., NJ) with a diffusional area of 0.69 cm^2 were used. Both chambers were stirred with Teflon coated magnetic stirring bars. After the buccal membranes were equilibrated with Krebs buffer (310 mOsm) 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

$$P = \frac{\left(\frac{dQ}{dt}\right)}{(\Delta C \cdot A)} \tag{3}$$

where dQ/dt is cumulative amount permeated per unit time, ΔC is the concentration difference across the buccal mucosa, and A is the diffusional area. The effects of pH of the buffer on permeation were investigated 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

$$ER = \frac{P_{(enh)}}{P_{(ctrl)}} \tag{4}$$

where $P_{(enh)}$ is enhanced permeability coefficient and $P_{(ctrl)}$ 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-ml) 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

$$K_P = \frac{C_{aq} - C_{eq}}{C_{eq}} \tag{5}$$

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:

$$\gamma = \frac{0.5(h\rho gr)}{\cos\theta} \tag{6}$$

where h is the height (cm) of the liquid in the capillary tube, ρ is the density (g/ml) of the solvent, g is the gravitational constant (cm/s^2), r is the radius (cm) of the capillary tube, and θ 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

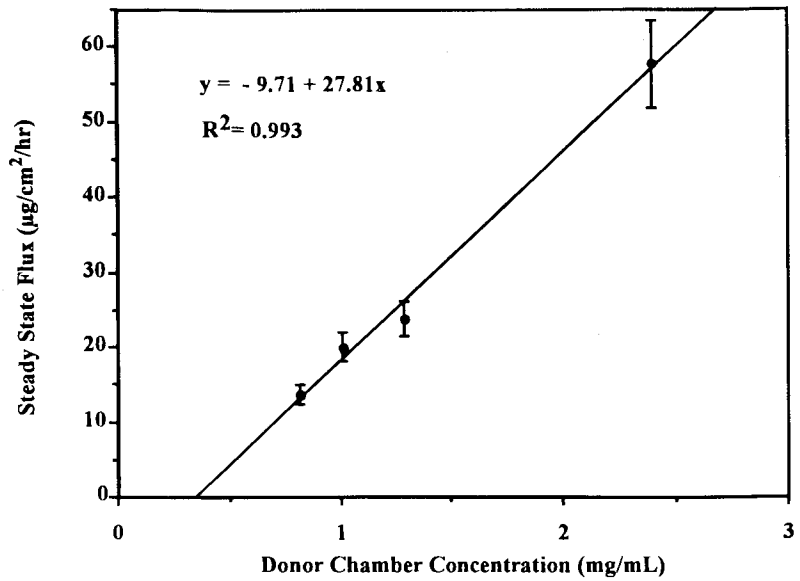

Fig. 1. Effect of donor chamber concentration of acyclovir on steady state flux.

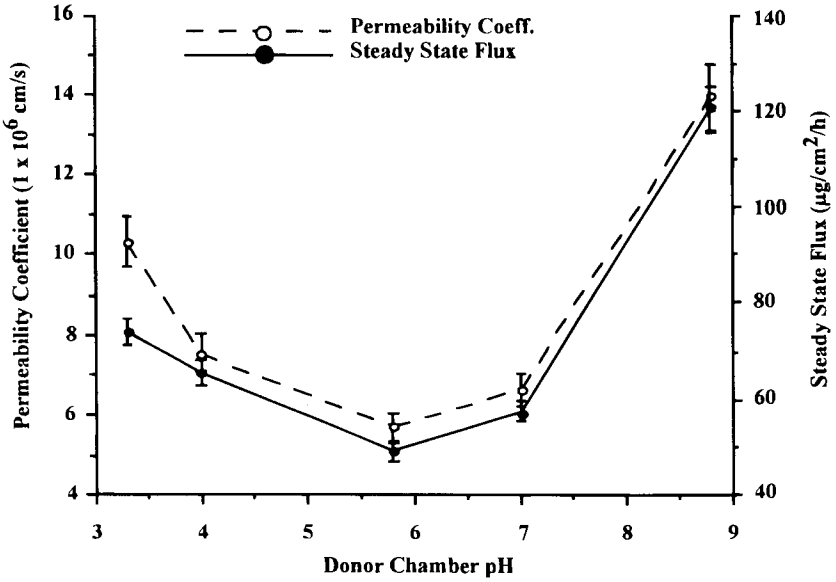

Fig. 2. Effect of donor chamber pH on *in vitro* permeability coefficient and steady state flux of acyclovir across buccal mucosa.

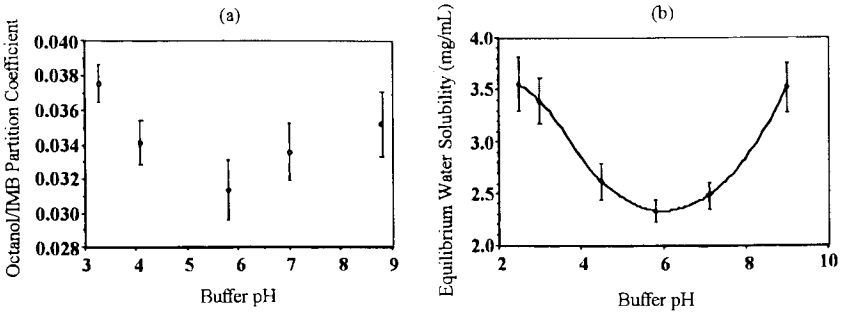

Fig. 3. (a) 1-Octanol/IMB equilibrium partitioning of acyclovir as a function of buffer pH. (b) Experimental pH-solubility profile of acyclovir in de-ionized water at 37°C.

with partition coefficients (K_P) as was observed with the steady-state flux and the permeability coefficient. For transport of acyclovir across buccal mucosa, the changes in flux and permeability did not reflect the changes in partition coefficient at different pH. The experimental results of permeation showed 1.76 and 2.33 times increase in the permeability of acyclovir across buccal mucosa, as the pH was changed from 5.8 to 3.3 and 8.8, respectively. However, for the same variation in pH, the partition coefficients increased by only 1.18 and 1.12 times.

The pH-solubility profile of acyclovir is shown in Fig. 3b. The equilibrium water solubility of acyclovir was significantly ($P < 0.05$) influenced by the pH with the highest solubility being at the two respective ends of the investigated range of pH.

Permeation enhancement studies conducted using the trihydroxy bile salt, NaGC, showed that co-administration of NaGC enhanced the steady state flux of acyclovir across porcine buccal mucosa to up to 9 times. This enhancement was independent of pH (Fig. 4), as no significant difference ($P > 0.05$) was found between the steady state fluxes at various values of pH. The enhancement ratio (ER) increased steadily with increasing NaGC concentration up to 20 mM, beyond this point ER leveled off to a plateau (Fig. 5). This concentration was just above the measured critical micelle concentration of NaGC. The CMC of NaGC was 18 mM in Krebs buffer in the presence of acyclovir at 25°C (Fig. 5).

DISCUSSION

The cellular structure of the oral mucosa suggests two possible transport routes, the paracellular route and the transcellular route. According to the pH-partition hypothesis, only the nonionized form of the drug is able to cross lipoidal membranes in significant amounts. The effect of pH on drug absorption for ionizable compounds has been extensively studied (21–26). If a single species of drug is transported via the paracellular route (Eq. 1), the permeability of the drug should be independent of partition coefficient. As shown in Figs. 2 and 3a, this is not the case for acyclovir. Conversely, if the drug is transported via the transcellular route (Eq. 2), the permeability of the drug should vary with the partition coefficient. Thus, the pH dependence of the permeation of ionizable drugs actually reflects the drug penetration route, because the partition coefficient of ionizable drugs is pH dependent. Therefore, if a drug is transported via the transcellular route, the drug absorption rate is also pH dependent. While the *in vitro* permeation of acyclovir varies with pH, the variation of the permeability coefficients of acyclovir with pH far exceeds that of the partition coefficients. Therefore, a model of permeation of a single species would not be consistent with the data (Figs. 2 and 3a).

Paracellular transport of all three species of acyclovir, anionic, cationic, and zwitterionic, appears to be the most likely mechanism for permeation. As a zwitterion, acyclovir is always ionized in aqueous media; however, the fractions of each species, cation, anion, and zwitterion vary with pH. To study the transbuccal permeation of different species of acyclovir, the fractions of each species of acyclovir at each pH were calculated based on the dissociation constants (27): $K_{a_1} = 3.98 \times 10^{-3}$ and $K_{a_2} = 5.01 \times 10^{-10}$. As shown in Fig. 6, the apparent *in vitro* permeability coefficient decreases as the fraction of zwitterion increases and the fraction of cation or anion decreases. The minimal permeability coefficient is observed near the isoelectric point (pH 5.8). It is hypothesized that the permeability coefficient of each ionic species may be different. Then, the total permeability coefficient of acyclovir, P_{tot}, may be modelled as three parallel independent fluxes (28):

$$P_{tot} = \frac{C_{cat}}{C_{tot}} P_{cat} + \frac{C_{zwi}}{C_{tot}} P_{zwi} + \frac{C_{ani}}{C_{tot}} P_{ani} \qquad (7)$$

where P and C are the permeability coefficient and concentration, and subscripts tot, cat, zwi, and ani indicate total, cation, zwitterion, and anion, respectively. Introducing the concentration of hydrogen ion, C_H^+, and dissociation constants, K_1 and K_2, into Equation 7, P_{tot} can be expressed as

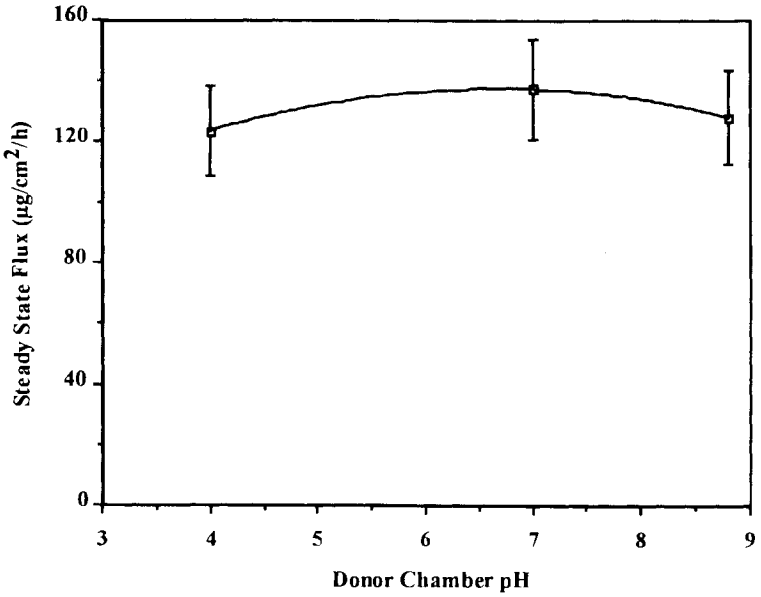

Fig. 4. Effect of donor chamber pH on NaGC permeation enhancement of acyclovir (in presence of 2 mM NaGC).

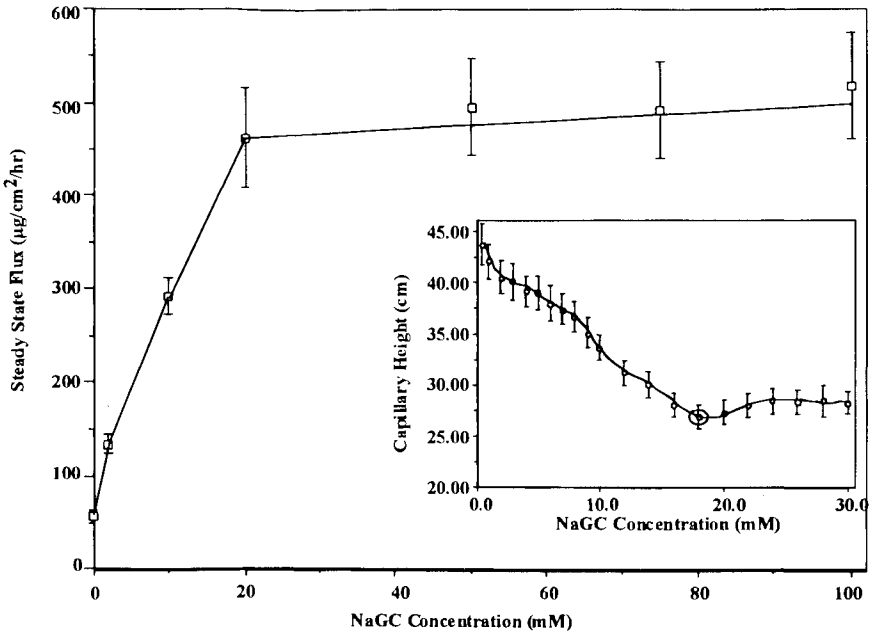

Fig. 5. Critical micelle concentration of NaGC at 25°C in Krebs buffer and the effect of NaGC concentration on steady state flux.

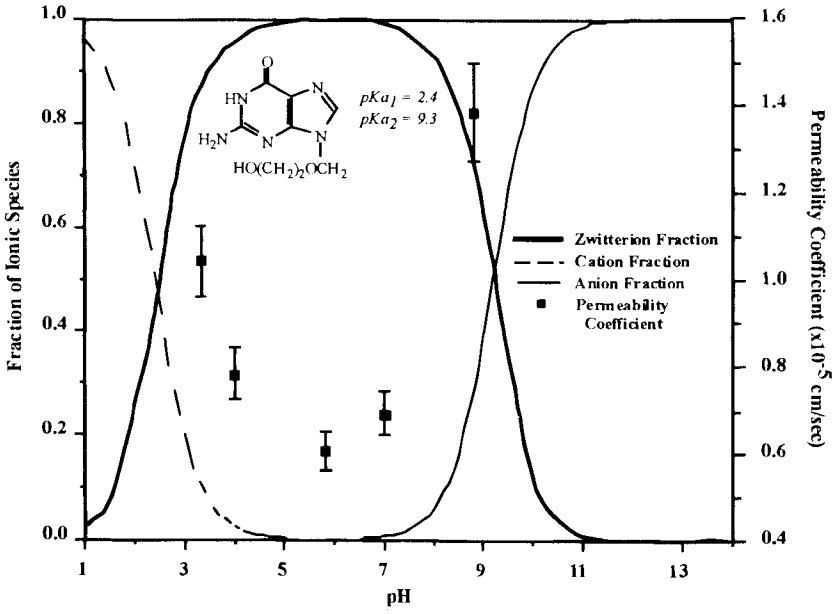

Fig. 6. Relationship between the *in vitro* permeability coefficient and the fraction of ionic species of acyclovir.

$$P_{tot} = \frac{C_{H^+}^2 P_{cat} + K_1 C_{H^+} P_{zwi} + K_1 K_2 P_{ani}}{C_{H^+}^2 + K_1 C_{H^+} + K_1 K_2} \quad (8)$$

The permeability coefficients of the ionic species were obtained by fitting the permeation data at various values of pH to Eq. 8 (Table I). The calculated curve is plotted with the *in vitro* experimental data in Fig. 7, and the agreement is excellent. The permeability coefficients of cation and anion were almost the same and about 6–7 times higher than that for the zwitterionic species. The comparability of the permeability coefficients of the anionic and cationic species is suggestive of "wide open" non-selective porous transport through buccal mucosa for ionized species in this molecular size range. Moreover, the perme-

Table I. Permeability Coefficient of Each Ionic Species of Acyclovir

Ionic species	Permeability coefficient (cm/sec)
Cation	4.33×10^{-5}
Zwitterion	6.24×10^{-6}
Anion	3.83×10^{-5}

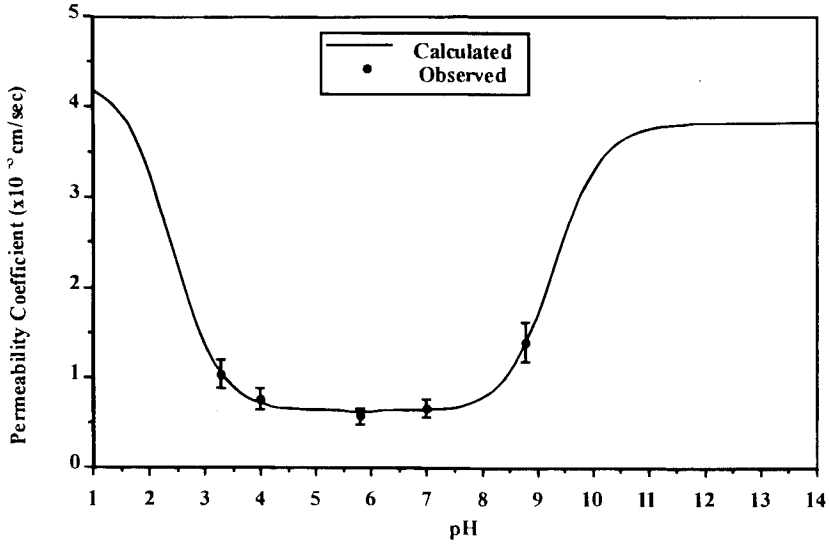

Fig. 7. Permeability coefficient of acyclovir at various pH values.

ability coefficients for the anionic and cationic species are nearly that of tritiated water through buccal mucosa (29) and are also consistent with a non-selective "wide open" porous model.

The reason for the considerably slower transport of the zwitterion through a non-selective paracellular route is not immediately obvious, but may be related to a lower diffusivity in aqueous media. Solvation of both ionized groups in the dipolar ion may contribute to this lower diffusivity, but it is unlikely to explain the 6 to 7-fold decrease. Dimerization or self-association may be another possible explanation. Nevertheless, it is apparent that the paracellular route of buccal permeation predominates for acyclovir.

The enhancement of permeation by NaGC provides further insight into the routes of buccal transport. The independence of ER on pH (Fig. 4) for all three species is indicative of a single mechanism of enhancement through the same route of transport, i.e., the paracellular route. Moreover, the steady-state fluxes reflect the same behavior as the surface tension (Fig. 5). Such behavior for a surfactant is consistent with its action in aqueous media, the paracellular route.

CONCLUSIONS

In vitro permeation of acyclovir across buccal mucosa is via the paracellular route through "wide open" non-selective pores, and the permeation is a function of pH. The permeability of acyclovir for the anionic and the cationic species were different compared to the zwitterionic species. Sodium glycocholate (NaGC) enhanced the permeation of acyclovir across porcine buccal mucosa by decreasing the resistance of this paracellular pathway.

REFERENCES

1. R. B. Gandhi and J. R. Robinson. Oral cavity as a site for bioadhesive drug delivery. *Adv. Drug Del. Rev.* **13**:43–74 (1994).
2. H. Zhang and J. R. Robinson. Routes of drug transport across oral mucosa in M. J. Rathbone (ed.), *Oral mucosal drug delivery*, Marcel Dekker, Inc., New York, 1996, pp. 51–64.
3. C. A. Squier and R. M. Hopps. A study of the permeability barrier in epidermis and oral epithelium using horseradish peroxidase as a tracer *in vitro*. *Br. J. Dermat.* **95**:123–129 (1976).
4. C. A. Squier and B. K. Hall. The permeability of mammalian non-keratinized oral epithelia to horseraddish peroxidase applied *in vivo* and *in vitro*. *Arch. Oral Biol.* **29**:45–50 (1984).
5. M. C. Alfano, A. J. Chasens, and C. W. Masi. Autoradiographic study of the penetration of radiolabelled dextrans and inulin through non-keratinized oral mucosa *in vitro*. *J. Periodont. Res.* **12**:368–377 (1977).
6. M. E. Dowty, K. E. Knuth, B. K. Irons, and J. R. Robinson. Transport of thyrotropin releasing hormone in rabbit buccal mucosa *in vitro*. *Pharm. Res.* **9**:1113–1122 (1992).
7. S. Senel, A. J. Hoogstraate, F. Spies, J. C. Verhoef, H. E. Junginger, and H. E. Bodde. Visualization of enhancing effects of bile salts on buccal penetration. *Eur. J. Morph.* **31**:35–41 (1993).
8. C. A. Lesch, C. A. Squier, A. Cruchley, D. M. Williams, and P. Speight. The permeability of human oral mucosa and skin to water. *J. Dent. Res.* **68**:1345–1349 (1989).
9. E. Quadros, J. Cassidy, K. Gniecko, and S. LeRoy. Buccal and colonic absorption of CGS 16617, a novel ACE inhibitor. *J. Control. Rel.* **19**:77–86 (1991).
10. C. A. Squier and B. K. Hall. The permeability of skin and oral mucosa to water and horseradish peroxidase as related to the thickness of the permeability barrier. *J. Invest. Dermat.* **84**:(1985).
11. A. J. Hoogstraate, C. Cullander, J. F. Nagelkerke, J. Verhoel, H. E. Junginger, and H. E. Bodde. Diffusion rates and transport pathways of FITC-labelled model compounds through buccal epithelium. *Proceed. Int. Symp. Control. Rel. Bioact. Mater.* **20**:234–235 (1993).
12. A. J. Hoogstraate, S. Senel, C. Cullander, J. Verhoef, H. E. Junginger, and H. E. Bodde. Effects of bile salts on transport rates and routes of FTIC-labelled compounds across porcine buccal epithelium *in vitro*. *J. Control. Rel.* **40**:211–221 (1996).
13. A. J. Hoogstraate, H. E. Bodde, C. Cullander, and H. E. Junginger. Diffusion rates and transport pathways of FITC-labelled model compounds through buccal epithelium. *Pharm. Res.* **9**:S-188 (1992).
14. A. J. Hoogstraate, J. C. Verhoef, B. Tuk, A. Pijpers, L. A. M. G. van Leengoed, J. H. M. Vheijden, H. E. Junjinger, and H. E. Bodde. Buccal delivery of fluorescein isothiocyanate-dextran 4400 and the peptide drug buserelin with glycodeoxycholate as an absorption enhancer in pigs. *J. Control. Rel.* **4**:77–84 (1996).
15. A. H. Beckett and A. C. Moffat. Correlation of partition coefficients in n-heptane-aqueous systems with buccal absorption data for a series of amines and acids. *J. Pharm. Pharmacol.* **21**:144S–150S (1969).
16. P. P. H. Le Brun, P. L. A. Fox, M. E. de Vries, and H. E. Bodde.

In vitro penetration of some β-adrenoreceptor blocking drugs through porcine buccal mucosa. *Int. J. Pharm.* **49**:141–145 (1989).
17. K. W. Garren and A. J. Repta. Buccal drug absorption. II. *In Vitro* diffusion across the hamster cheek pouch. *J. Pharm. Sci.* **78**:160–164 (1989).
18. A. H. Beckett and A. C. Moffat. Kinetics of buccal absorption of some carboxylic acids and the correlation of the rate constants and n-heptane:aqueous phase partition coefficients. *J. Pharm. Pharmacol.* **22**:15–19 (1970).
19. I. A. Siegel, K. T. Izutsu, and E. Watson. Mechanisms of non-electrolyte penetration across dog and rabbit oral mucosa in vitro. *Arch. Oral Biol.* **26**:357–361 (1981).
20. A. Martin, P. Bustamante, and A. H. C. Chun. *Physical Pharmacy*, Lea & Febiger, Malvern, PA, 1993, pp. 362–393.
21. J. Zhang, J. Streisand, S. Niu, D. Coleman, B. Hague, L. Maland, and T. Stanley. Buccal mucosal absorption of esmolol in an *in vivo* dog model. *Pharm. Res.* **9**:S-177 (1992).
22. O. Al-Sayed-Omar, A. Johnson, and P. Turner. Influence of pH on the buccal absorption of morphine sulphate and its major metabolite, morphine-3-glucuronide. *J. Pharm. Pharmacol.* **39**:934–935 (1987).
23. A. H. Beckett and E. J. Triggs. Buccal absorption of basic drugs and its application as an *in vivo* model of passive drug transfer through lipid membranes. *J. Pharm. Pharmacol.* **19**:31S–41S (1967).
24. A. H. Beckett and A. C. Moffat. The influence of substitution in phenyacetic acids on their performance in the buccal absorption test. *J. Pharm. Pharmacol.* **21**:139S–143S (1969).
25. C. L. Barsuhn, L. S. Olanoff, D. D. Gleason, E. L. Adkins, and N. F. H. Ho. Human buccal absorption of flubriprofen. *Clin. Pharmacol. Ther.* **44**:225–231 (1988).
26. D. Hicks. The buccal absorption of some β-adrenoceptor blocking drugs. *Br. J. Pharmacol.* **47**:680P–681P (1973).
27. A. Kristl, A. Mrhar, and F. Kozjek. The ionisation properties of acyclovir and deoxyacyclovir. *Int. J. Pharm.* **99**:79–82 (1993).
28. A. Tsuji, E. Miyamoto, N. Hashimoto, and T. Yamana. GI absorption of beta-lactam antibiotics II: Deviation from pH-partition hypothesis in penicillin absorption through *in situ* and *in vitro* lipoidal barriers. *J. Pharm. Sci.* **67**:1705–1711 (1978).
29. C. A. Squier, P. Cox, and P. W. Wertz. Lipid content and water permeability of skin and oral mucosa. *J. Invest. Dermat.* **96**:123–126 (1991).