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Transbuccal delivery of 2′,3′-dideoxycytidine: in vitro permeation study and histological investigation

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Abstract

Permeation of 2′,3′-dideoxycytidine (ddC), an ionic compound, through buccal mucosa was investigated in this in vitro study to identify the major permeation barrier within the epithelium of buccal mucosa and explore the feasibility of transbuccal delivery of ddC. In vitro permeation of ddC across porcine buccal mucosa was conducted in isotonic McIlvaine buffer solution (IMB) using in-line flow through diffusion cells at 37 °C. Sodium glycodeoxycholate (GDC) was used as the permeation enhancer in the permeation enhancement studies. Light microscopy was used in the histological studies of buccal tissue. The steady-state flux of ddC permeating through buccal mucosa increased linearly ($R^2 = 0.96$, $P < 0.05$) as the donor concentration of ddC was increased from 1 to 20 mg/ml. The permeabilities for the full thickness buccal mucosa, the epithelium, and the connective tissue were determined to be $1.75 \pm 0.74 \times 10^{-7}$, $2.90 \pm 1.86 \times 10^{-7}$, and $3.49 \pm 1.19 \times 10^{-6}$ cm/s, respectively. The permeability of ddC was significantly ($P < 0.05$) enhanced by GDC at a concentration of 4 mM. The histological study revealed that the thickness of epithelium was greatly reduced after buccal tissues were immersed in IMB for 12 and 24 h but the basal lamina remained intact even after 24 h. A bilayer diffusion model was established to quantitatively describe the contributions of the epithelium and the connective tissue to the permeation barrier. In conclusion, ddC permeated through buccal mucosa by passive diffusion over the range of concentrations examined. The basal lamina layer within the epithelium of buccal mucosa acted as an important barrier to the permeation of ddC. GDC effectively enhanced the buccal permeability of ddC. The transbuccal delivery is a potential route for the administration of ddC.

Keywords: Oral mucosa; Transbuccal drug delivery; 2′,3′-Dideoxycytidine; Bile salt; Histological study; Diffusion modeling

1. Introduction

The transbuccal drug delivery route has been examined as an alternative route of administration for various biological active agents in recent years due to its attractive advantages. Compared to oral administration, drugs delivered through

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buccal mucosa can bypass enzymatic degradation in the gastrointestinal (GI) tract and the hepatic first pass effect. The permeability of the buccal mucosa is higher than that of skin (Squier and Hall, 1985b). Hence, a lower loading dose in a transbuccal device could provide the same therapeutic effect as a transdermal patch. The buccal mucosa has a larger area for drug application and has good accessibility compared to other mucosae such as the nasal, rectal and vaginal mucosa (Rathbone et al., 1994). The buccal mucosa is more resistant to tissue damage or irritation because of its rapid cell turn over (Squier and Wertz, 1996; de Vries et al., 1991a) and daily exposure to xenobiotics, such as food. In addition, transbuccal delivery devices can be easily applied and removed. Therefore, administering drugs through the buccal mucosa is an attractive route for systemic drug delivery.

The buccal mucosa consists of a superficial layer of epithelium and a layer of connective tissue (de Vries et al., 1991a) as shown in Fig. 1. The epithelium is covered with mucus and connected to lamina propria by basal lamina. Lamina propria and submucosa that is located underneath the lamina propria constitute the connective tissue. The connective tissue contains a network of blood capillaries. Drugs permeated through the epithelium layer can diffuse into this network and enter the systemic circulation.

Most reported transbuccal permeation studies were conducted in vitro, because in vitro permeation assessment provides a number of advantages over in vivo studies (Zhang and Robinson, 1996). In vitro studies can be easily set up using various types of diffusion apparatus and experimental conditions can be controlled. In addition, the costs for in vitro studies are much lower than that for in vivo testing, since only small pieces of animal oral mucosa are involved in the in vitro studies. Among various animal oral mucosae that have been studied for transbuccal permeation, porcine buccal mucosa was found to be a good model for in vitro transbuccal permeation experiments. The anatomy and metabolism of porcine buccal mucosa are similar to that of human buccal mucosa (Mount and Ingram, 1991). The epithelium of buccal mucosa was believed to be the rate-limiting layer for the permeation (Squier and Rooney, 1976; Squier and Hopps, 1976; Squier and Hall, 1985b,a; Squier, 1977). However, the location of a major permeation barrier to hydrophilic compounds within the epithelium layer was not completely clear (de Vries et al., 1991a). In addition, the roles of epithelium, connective tissue, and full-thickness buccal mucosa in drug transport have not been fully studied yet (de Vries et al., 1991b).

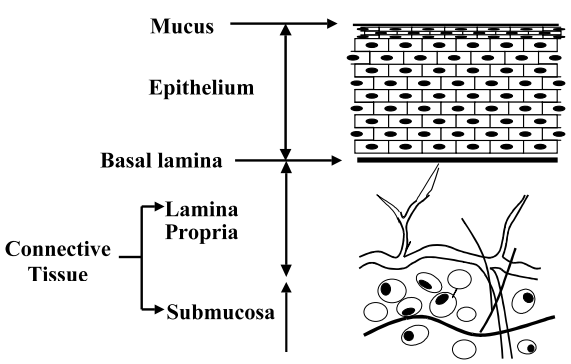

Fig. 1. Cross-sectional structure of buccal mucosa (de Vries et al., 1991a).

2′,3′-Dideoxycytidine (Zalcitabine, ddC), a nucleoside reverse transcriptase inhibitor, was approved by FDA to treat human immunodeficiency virus (HIV) infection in 1992. Clinically, ddC has been shown to increase patients' survival rate (Sifton, 1997). However, the dose-dependent adverse effect, such as peripheral neuropathy, is associated with current oral delivery. It was proposed by Chien and co-workers (Chien and Wearley, 1989; Kim and Chien, 1996) that a noninvasive zero-order delivery may reduce the side effects which result from the high drug concentration in plasma after conventional intravenous or oral administration. Transbuccal delivery of ddC has the potential to provide a constant therapeutic plasma level below the side effect concentration, which may prevent side effects such as peripheral neuropathy.

In this study, the major permeation barrier within the epithelium to the ionic compound, ddC, was determined by in vitro transbuccal permeation and histological studies. A diffusion model was established to describe the contribu-

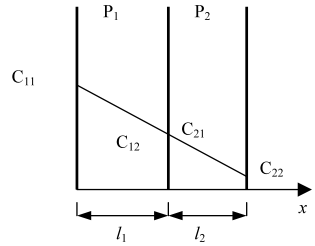

Fig. 2. Diffusion through a bilayer membrane with thickness of l_1, l_2, permeability of P_1, P_2, and surface concentration of C_{1i}, C_{2i} ($i = 1, 2$) for each layer.

tions of the epithelium and the connective tissue to the total permeability of the buccal mucosa. The feasibility of transbuccal delivery of ddC was also investigated.

2. Theory

For one-dimensional diffusion, the permeability of a diffusant through a solid membrane can be calculated as:

$$P = \frac{J_{SS}}{\Delta C} \tag{1}$$

where P is the permeability of the diffusant, ΔC is the concentration difference between the two surfaces of the membrane, and J_{SS} is the flux at steady state. The steady state flux is given in the following equation:

$$J_{SS} = \frac{\Delta M}{A \cdot \Delta t} \tag{2}$$

where ΔM is the amount of diffusant transported through the membrane during the time Δt at steady state, and A is the diffusional area.

For a bilayer membrane with thickness (l_1, l_2) and permeability (P_1, P_2) of each layer (Fig. 2), the following equation can be obtained at steady state for each layer of the membrane:

$$\Delta C_i = \frac{J_{SSi}}{P_i} \tag{3}$$

where ΔC_i is the concentration difference between the two surfaces of the ith layer C_{i1} and C_{i2}, J_{SSi} is the steady state flux through the ith layer, P_i is the permeability of the ith layer, and $i = 1, 2$. The distribution of diffusant between the two connecting surfaces can be described by (Ash et al., 1965):

$$C_{12} = K \cdot C_{21} \tag{4}$$

where K is the partition coefficient of the diffusant between the two layers. When $K = 1$, $C_{12} = C_{21}$. Thus we can write:

$$\Delta C_1 + \Delta C_2 = C_{12} - C_{11} + C_{22} - C_{21} = C_{22} - C_{11} = \Delta C_T \tag{5}$$

where ΔC_T is the total concentration difference between the two surfaces of the bilayer membrane. Combining Eqs. (3) and (5), the following equation can be obtained:

$$\frac{J_{SS1}}{P_1} + \frac{J_{SS2}}{P_2} = \frac{J_T}{P_T} \tag{6}$$

where J_T and P_T are the total flux and permeability through the bilayer membrane, respectively. At steady state, the flux through each layer should be the same and equal to the total flux through the bilayer member (Crank, 1995). Therefore, from Eq. (6), we have:

$$\frac{1}{P_1} + \frac{1}{P_2} = \frac{1}{P_T} \tag{7}$$

Eq. (7) indicates that the total permeability of a bilayer membrane with continuous concentration change can be calculated with the permeability of each layer of the membrane.

3. Methods

3.1. Tissue preparation

Porcine buccal tissues were obtained immediately after pigs were slaughtered (Long Ranch, Manteca, CA) and stored in normal saline at 4 °C. Buccal mucosae (including epithelium, lamina propria, and submucosa) were separated from the underlying tissues by surgical scissors. The diffusion studies and histological studies were carried out within 2 h after slaughtering. Thirty-nine tissue samples from six pigs were used for histological studies and 45 tissue samples from nine pigs were used for permeation studies. The buccal

mucosae were equilibrated with buffer solution prior to permeation study. The full-thickness buccal mucosae were used in histological studies and all permeation studies except the studies on determination of the rate limiting barrier. For the determination of the rate-limiting barrier of buccal mucosa, buccal epithelia (including mucus layer and basal lamina) were carefully separated from the connective tissues (including lamina propria and submucosa) by surgical scissors. Permeation studies were conducted using epithelia and connective tissues, respectively.

3.2. Histological studies

The buccal tissues were cut into 1×1 cm cross-sections and incubated in vials containing isotonic McIlvaine buffer solution (IMB, simulated gingival fluid without enzyme, pH 7.4) (McIlvaine, 1921; Deasy et al., 1989) or 10 mg/ml of ddC in IMB solution. The vials were kept in a water bath at 37 ± 1 °C. After 0.5, 1, 2, 4, 12, and 24 h, tissue samples (three buccal tissue samples for each time point) were fixed in 10% buffered formalin for 7 days at room temperature. The buccal mucosa in normal saline was used as the control and transferred directly to the buffered formalin without incubation. The following procedures for histological studies were carried out using the paraffin technique (Humason, 1962a,b). Tissue samples were dehydrated with a series of ethanol from 50% to absolute alcohol and embedded in paraffin. Paraffin preparations were cut into slides using a microtome (Model 820, American Optical Co., Buffalo, NY) and then stained by Harris Hematoxylin and Eosin Y Stain. The prepared slides were examined under a Nikon Eclipse E800 light microscope with planfluor objective lenses (Nikon, Inc., Melville, NY). The magnification of the microscope is $10 \times$. At least three microscope slides per tissue sample were prepared and examined. An Optronics DEI-750 three-chip CCD camera (Optronics Engineering, Goleta, CA) was used to capture images. Images were processed by Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The thickness of the epithelium of the tissue samples at each time point was estimated semi-quantitatively and compared with that of the control (set as 100%) using the ruler on the microscope.

3.3. Permeation studies

In vitro permeation studies were conducted at 37 ± 1 °C using in-line flow-through diffusion cells (PermeGear, Inc., Riegelsville, PA) (Squier et al., 1997) with a diffusional area of 0.126 cm^2. Porcine buccal membranes were mounted between donor and receiver chambers. The donor chambers were charged with ddC (Sigma®, St Louis, MO) IMB solution (pH 7.4) containing 0.01% (w/v) gentamicin. The receiver chambers were filled with fresh IMB solution containing 0.01% gentamicin and stirred with a Teflon-coated magnetic bar. The flow rates of fresh media entering receiver chambers were controlled by a Gilson Miniplus 3 peristaltic pump (Gilson, Inc., Middleton, WI) at 0.8 ml/h. Samples were collected every 90 min for 22.5 h using a Gilson FC205 fraction collector (Gilson, Inc., Middleton, WI). A dihydroxy bile salt, sodium glycodeoxycholate (GDC, Sigma®, St Louis, MO), was used as the permeation enhancer in the permeation enhancement studies. The concentration of GDC was varied from 0.6 to 50 mM. The steady state flux (J_{SS}) of ddC and the permeability coefficient (P) were calculated according to Eqs. (2) and (1), respectively. Permeation experiments were conducted in not less than triplicates. The results are presented in means $\pm$ S.D.

3.4. Analytical method

ddC concentrations of the collected samples were quantified using a high-performance liquid chromatographic system. The HPLC system consists of a Waters 590 HPLC pump (Waters, Milford, MA), a Hitachi L-7200 auto-sampler (Hitachi, Ltd., Tokyo, Japan), a Keystone ODS/A column (5 μm, 250×4.6 mm, Keystone Scientific, Inc., Bellefonte, PA), a Gilson 115 UV detector (Gilson, Inc., Middleton, WI), and EZChrom Chromatography data acquisition software (Scientific Software, Inc., San Ramon, CA). HPLC mobile phase was composed of 0.2 M phosphate buffer (pH 6.0) and acetonitrile with a ratio of

95:5. ddC was detected at a wavelength 270 nm with a retention time of 11.6 min.

4. Results and discussion

The average steady-state flux of ddC permeating through the buccal mucosa was significantly (ANOVA, $P < 0.05$) increased from 0.83 ± 0.25 to 13.42 ± 6.35 μg/cm^2 per h as the donor concentration of ddC was increased from 1 to 20 mg/ml (Fig. 3). A linear relationship was found between the steady-state flux and ddC concentration ($R^2 = 0.96$, $P < 0.05$). This indicates that passive diffusion occurred when ddC was transported through the buccal mucosa over the range of concentrations investigated.

The measured permeability coefficients for the full thickness buccal mucosa, the epithelium, and the connective tissue (as listed in Table 1), were $1.75 \pm 0.74 \times 10^{-7}$, $2.90 \pm 1.86 \times 10^{-7}$, and $3.49 \pm 1.19 \times 10^{-6}$ cm/s, respectively. The permeability of the full thickness buccal mucosa was not significantly (ANOVA, $P > 0.05$) different from that of the epithelium. However, the permeability of the connective tissue was significantly (ANOVA, $P < 0.05$) higher compared to the permeability of the other two membranes. To study the relations among the permeability of the epithelium, the connective tissue, and the full-thickness buccal mucosa, buccal mucosa was modeled as a bilayer as depicted in Fig. 2. The epithelium and the connective tissue were treated as two layers with intimate contact. The partition coefficient of ddC between the epithelium and the connective tissue is assumed to be unity, since the surfaces of both tissue cells are mainly composed of lipid bilayers. The change of diffusant concentration between the interfaces of epithelium and connective tissue was assumed to be continuous. When the permeabilities of the epithelium and the connective tissue were measured, the total permeability of the full-thickness buccal mucosa can be calculated according to Eq. (7). Based on the measured permeability of the epithelium and the connective tissue listed in Table 1, the calculated permeability of buccal mucosa was 2.67×10^{-7} cm/s. Although the calculated permeability was higher than the experimental result, this value was within the 99% confidence interval (i.e. 0.65×10^{-7} to 2.85×10^{-7} cm/s) of the experimental permeability coefficient. The steady state flux at various ddC concentrations can be simulated based on the measured permeability of the epithelium and the connective tissue using Eqs. (1) and (6) as shown in Fig. 3. Deviations of the simulated result from the experimental result were observed. Nonetheless, the simulated steady state fluxes were within the range of standard deviation except at a ddC concentration of 10 mg/ml.

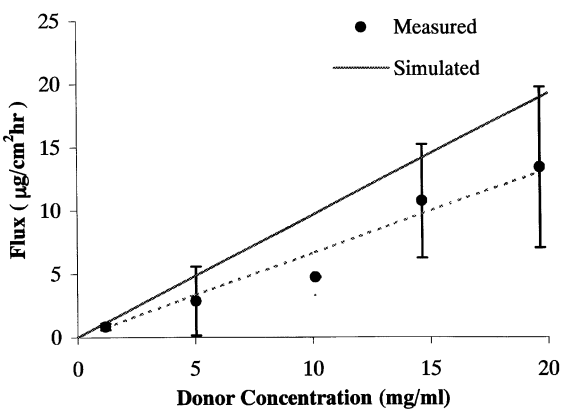

Fig. 3. Effect of donor concentration of ddC on the steady state flux of in vitro permeation through porcine buccal mucosa in isotonic McIlvaine buffer (pH 7.4). ●, measured data (means ± S.D., $n = 3$); solid line, simulated flux based on the measured permeability of the epithelium and the connective tissue using Eqs. (1) and (6); dashed line; linear regression based on measured data (regression $R^2 = 0.96$, $P = 0.003$).

Table 1
The permeability of ddC for the full thickness of buccal mucosa, epithelium, and connective tissue

Tissue layer	Permeability ($\times 10^7$ cm/s)	
	Measured[a]	Calculated
Buccal mucosa	1.75 ± 0.74	2.67[b]
Epithelium	2.90 ± 1.86	2.22[c]
Connective tissue	34.89 ± 11.91	35.37[c]

[a] The data are means ± S.D. ($n \geq 3$).
[b] Calculated based on the measured permeability of epithelium and connective tissue.
[c] Calculated by curve fitting according to Eqs. (1) and (6).

On the other hand, when the flux of ddC permeated through buccal mucosa was measured, the permeability of epithelium and connective tissue can be obtained by curve fitting based on the bilayer diffusion model as shown in Table 1. The results from curving fitting are in good agreement with the experimental results. Therefore, a bilayer diffusion model is applicable to the permeation studies of buccal mucosa. The measured permeability of the epithelium was about 12 times less than that of the connective tissue but about 1.7 times higher than that of the full thickness buccal mucosa. This indicates that the epithelium is the rate-limiting barrier for the permeation of ddC.

The results of the histological studies are shown in Fig. 4. The basal lamina remained intact and no nucleated cell leakage was found within 24 h. No visible change in the thickness of the superficial layer of the epithelium was observed after buccal tissues were immersed in IMB for up to 4 h. The thickness of the epithelium was greatly reduced after buccal tissues were immersed in IMB for 12 and 24 h. No difference was observed between the tissue samples incubated in IMB and ddC IMB solution.

Squier and his colleagues (Squier and Rooney, 1976; Squier and Hopps, 1976) studied the permeation barrier of the non-keratinized rabbit oral mucosa using lanthanum salts and horseradish peroxidase (HRPO) as tracers. It was found that the permeation barrier was located at the superficial layer of the epithelium in oral mucosa with a depth of about one-quarter to one-third of the epithelium. The same result was obtained using porcine buccal mucosa and HRPO as tracer (Squier and Hall, 1985b). They attributed the permeation barrier of non-keratinized oral mucosa to the membrane-coating granules in the epithelium (Squier and Rooney, 1976; Squier and Hopps, 1976; Squier, 1977; Squier and Hall, 1985b). However, the permeation barrier may not be truly identified in their studies, since the tissue was incubated in HRPO solution for only 1 h, which was about the lag time of HRPO based on their permeation studies. In this study, the histological investigation of porcine buccal mucosa showed no significant loss of superficial layers of the epithelium up to 4 h. About 30–40% of the superficial layer of the epithelium was sloughed off after buccal tissues were immersed in IMB for

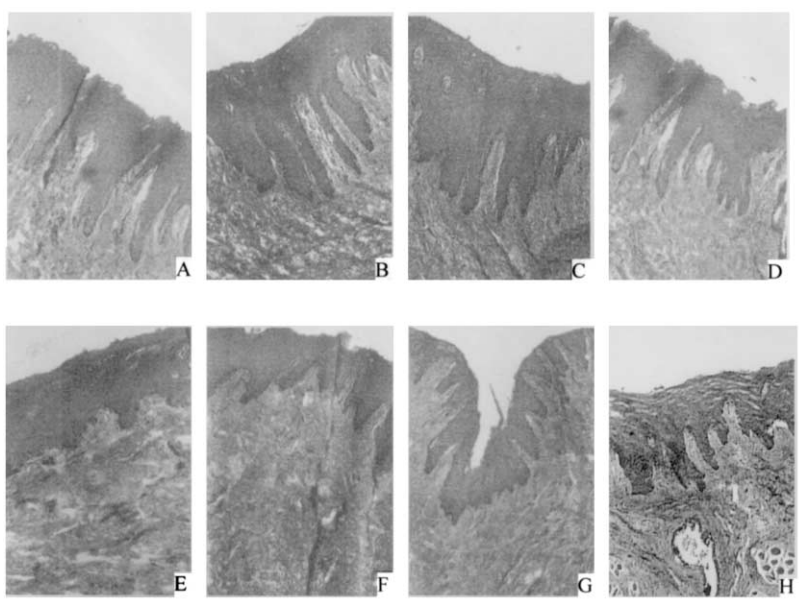

Fig. 4. Light microscopic view of buccal mucosa after immersion in isotonic McIlvaine buffer solution (IMB) for (A) control, (B) 0.5 h, (C) 1.0 h, (D) 2 h, (E) 4 h, (F) 12 h, (G) 24 h, and (H) in ddC IMB solution for 24 h (magnification, 10 ×).

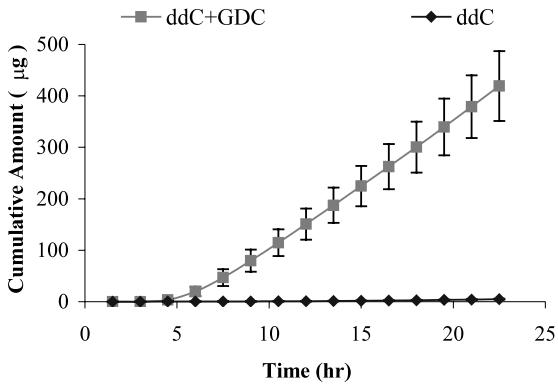

Fig. 5. Cumulative amount of ddC permeating through the porcine buccal mucosa without GDC (◆) and with co-administration of GDC (■). [GDC] = 4 mM; [ddC] = 10 mg/ml. Data are presented as means ± S.D. ($n = 3$).

12 h. If the permeation barrier for the transportation of ddC is located in the upper one-third of the epithelium, a large increase (e.g. 10 to 100 times increase) in flux due to the loss of the permeation barrier should be observed within this period. In the permeation studies, there is no such increase in flux as shown in Fig. 5. This demonstrates that the upper one-third of the epithelium is not the major barrier to the permeation of ddC, or that another barrier exists. Alfano and colleagues reported that the basal lamina of the oral mucosa may serve as a permeation barrier to endotoxin, inulin, dextran 70 (Alfano et al., 1975, 1977), and immune complexes (Brandtzaeg and Tolo, 1977). Squier and Hall found that the permeability for HRPO through the stripped sublingual epithelium was significantly higher than that of intact sublingual tissue, but the permeability of the stripped buccal epithelium was the same as that of the whole buccal tissue (Squier and Hall, 1985a). This indicates that the barrier was stripped off from the thin sublingual tissue, but the barrier from the thicker buccal mucosa remained (de Vries et al., 1991a). de Vries et al. (1991b) also reported that the main barrier for hydrophilic acebutolol was located in the deeper layers of the buccal epithelium, most likely the basal lamina. Our histological study showed that the basal lamina remained intact after being immersed in IMB for 24 h. Hence, the basal lamina within the epithelium acted as an important barrier to the permeation of ddC through the buccal mucosa.

The permeation enhancement study was conducted using GDC as the penetration enhancer. As shown in Fig. 6, the enhancement effect of GDC at low concentrations (0.6–1 mM) on the permeation of ddC through porcine buccal mucosa was not significant (ANOVA, $P > 0.05$) compared to without enhancer. However, the permeability of ddC significantly (ANOVA, $P < 0.05$) increased to $5.11 \pm 1.46 \times 10^{-6}$ cm/s at a GDC concentration of 4 mM. When GDC concentration was raised to 50 mM, the permeability of ddC increased to $5.61 \pm 1.06 \times 10^{-6}$ cm/s. The permeability obtained at a GDC concentration of 4 mM was not significantly (ANOVA, $P > 0.05$) different from that at GDC concentrations of 10 and 50 mM. GDC effectively enhanced the permeability of gdc up to 32 times compared to that without enhancer. In addition, the time for ddC to reach the steady state (T_{lag}) after using GDC was reduced from approximately 11 to 5 h (Fig. 5).

Squier et al. found that the permeability of buccal mucosa was greatly increased after lipid extraction (Squier et al., 1991). Therefore, lipid content is an important factor that determines the permeability of buccal mucosa. Bile salts were commonly selected as permeation enhancers due

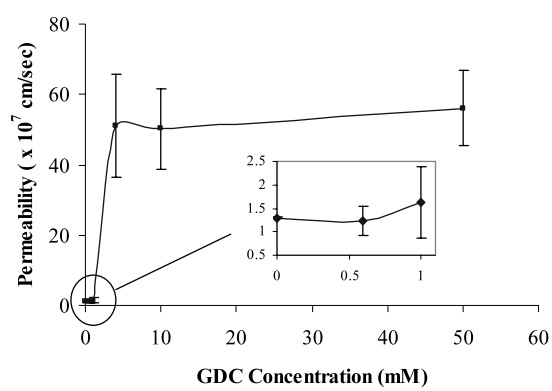

Fig. 6. The permeation enhancement effect of GDC on in vitro permeation of ddC through porcine buccal mucosa in McIlvaine buffer at pH 7.4 and ddC concentration of 10 mg/ml. Each point in the plot represents mean ± S.D. ($n = 3$).

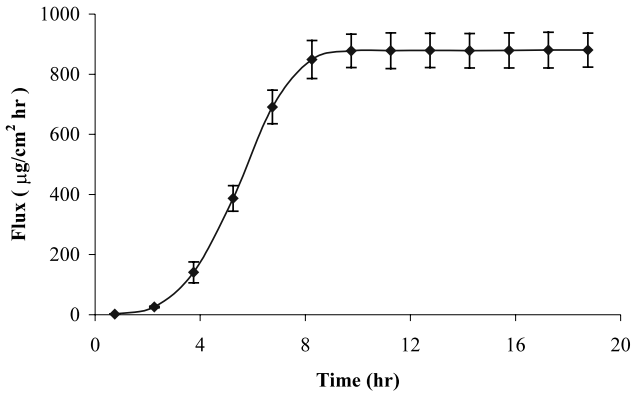

Fig. 7. Flux ($\Delta M/A\Delta t$) of ddC through the porcine buccal mucosa with a ddC concentration 50 mg/ml and GDC concentration 4 mM. Each point in the plot represents mean $\pm$ S.D. ($n = 3$).

to their capabilities to solubilize membrane lipids (Coleman et al., 1976; Vyvoda et al., 1977; Billington and Coleman, 1978). In this study, GDC significantly increased the permeability of ddC at a concentration of 4 mM, which was in the vicinity of the critical micelle concentration (CMC) of GDC (Martin et al., 1992; Gibaldi and Feldman, 1970). A limited enhancement effect was observed at lower GDC concentrations (< 4 mM). There was no further increase (ANOVA, $P > 0.05$) in permeation enhancement with GDC concentrations higher than 4 mM. This result can be explained by interfacial saturation theory (Hoogstraate et al., 1996). GDC solubilized the membrane lipids by incorporating lipids into GDC micelles. At concentrations below CMC, the lipid solubilization effect of GDC was very limited and a low enhancement effect was expected. Beyond the CMC, the interface between GDC micelles and lipid was saturated and hence further increase in enhancement was restricted. In Fig. 5, the flux of ddC was greatly increased after 4 mM GDC was co-administrated for about 6 h. This indicates the intercellular lipids were significantly solubilized by GDC after 6 h. Shojaei et al. (1999) studied transbuccal permeation of ddC using menthol as an enhancer. The permeability coefficient of ddC through the buccal mucosa increased 2.02 times at a menthol concentration of 0.3 mg/ml. This may be due to the limited effect of menthol on the intercellular lipid extraction over the range of concentrations studied.

It has been reported that the total body clearance of ddC is 0.336 l/h per kg and the minimum effective concentration of ddC is 0.1 μg/ml (Mitsuya and Broder, 1986). Based on a patient with body weight of 70 kg, the target rate of administration according to an i.v. infusion model is 2.35 mg/h. A similar target rate for transdermal delivery was achieved by Kim and Chien (1996). A total of 56.4 mg ddC will be delivered into the systemic circulation every 24 h according to this rate. However, based on the bioavailability of ddC (88%) (Klecker et al., 1988) and the current FDA-approved dose regimen of 0.75 mg oral every 8 h, only 1.98 mg would be delivered into the systemic circulation per day. It has been reported (Roche, 1996) that all patients receiving zalcitabine at about six times the current recommended dose (i.e. 13.5 mg per day) experienced peripheral neuropathy by week 10. Eighty percent of patients who received about two times the current recommended dose (i.e. 4.5 mg per day) experienced peripheral neuropathy by week 12. Hence, a lower drug plasma level would reduce the adverse effect of peripheral neuropathy while maintaining some therapeutic effect. A steady-state flux of 0.88 ± 0.06 mg/cm^2 per h was obtained, when the in vitro permeation was conducted with a combination of 50 mg/ml ddC and 4 mM GDC as shown in Fig. 7. Therefore, it can be estimated that a zero-order transbuccal delivery of ddC could be achieved by co-administration of 50 mg/ml ddC and 4 mM GDC with a

diffusional area of 2.67 cm^2 based on the minimum effective concentration or 0.1 cm^2 based on the current oral dose regimen.

5. Conclusion

ddC permeated through the buccal mucosa by passive diffusion over the range of concentrations examined. A bilayer diffusion model was established to quantitatively describe the contributions of the epithelium and the connective tissue to the total permeability of the drug across the buccal mucosa. The epithelium of the buccal mucosa acted as the major barrier to the permeation of ddC. GDC effectively enhanced the buccal permeability of ddC up to 32 times. Transbuccal delivery has been shown to be a potential route for the administration of ddC.

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