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Transbuccal permeation of a nucleoside analog, dideoxycytidine: effects of menthol as a permeation enhancer

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Abstract

The use of a safe and effective permeation enhancer is paramount to the success of a buccal drug delivery system intended for systemic drug absorption. The enhancing effects of menthol (dissolved in an aqueous buffer in the absence of co-enhancers) on buccal permeation of a model hydrophilic nucleoside analog, dideoxycytidine (ddC), were investigated. In vitro transbuccal permeation of ddC was examined using freshly obtained porcine buccal mucosa. The experiments were carried out in side-bi-side flow through diffusion cells. Permeation enhancement studies were performed with varying concentrations of l-menthol dissolved in Krebs buffer solutions containing ddC. Partition coefficient experiments were carried out to probe into the mechanism of permeation enhancing properties of l-menthol and DSC studies were conducted to determine if there is a eutectic formation between ddC and l-menthol at various concentrations. Permeation of ddC increased significantly ($P < 0.05$) in the presence of l-menthol independent of the concentration of the terpene. The apparent 1-octanol/buffer partition coefficient (log K_p) of ddC was significantly ($P < 0.05$) increased in presence of l-menthol and was also independent of the enhancer concentration. However, the tissue/buffer partition coefficient (log K'_p) data showed a concentration dependent increase of log K'_p in presence of l-menthol. Since log K'_p is a measure of drug binding to the tissue in addition to drug partitioning, binding of ddC to the buccal tissue may provide an explanation for the concentration dependent increase in these values.

Keywords: Transbuccal permeation; Dideoxycytidine; Menthol; Permeation enhancer

1. Introduction

The buccal mucosa, as a route for systemic drug delivery, offers distinct advantages over per-

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oral administration (Rathbone and Hadgraft, 1991). These advantages include escaping first pass effect, avoiding presystemic elimination within the GI tract, and a better enzymatic flora for certain drugs. Though these benefits make the buccal route attractive, the low flux associated with it most often makes the attainment of therapeutic plasma levels difficult. One approach in overcoming this problem has been the use of permeation/absorption enhancers. The tri- and dihydroxy bile salts are amongst the more commonly used permeation enhancers. However, using bile salts as enhancers may lead to significant and sometimes irreversible tissue damage at high concentrations. Ideally, an effective penetration enhancer should cause minimal tissue damage and toxicity, especially since the enhancer may also permeate across the tissue and yield detectable concentrations in the circulation (Senel et al., 1998).

Menthol is a monocyclic terpene with a pleasant taste and odor. It is widely consumed as a flavoring agent in oral dosage forms and as a fragrance in topical formulations. The effect of menthol on transdermal absorption of several drugs has been reported (Okabe et al., 1989; Williams and Barry, 1991; Kobayashi et al., 1994; Kaplun-Frischoff and Touitou, 1997; Gao and Singh, 1998; Kommuru et al., 1998). The effect of ethanol on the permeability of propranolol in the presence of menthol has also been reported (Coutel-Egros et al., 1992). A major benefit of using menthol as a permeation enhancer is its safety profile. The reported acute toxicity of menthol is of a very low order (Yamaguchi et al., 1994) with no carcinogenic effects. Menthol has also reported negative in a number of genotoxicity tests, including the Ames test and chromosomal aberration test in Chinese hamster fibroblasts (Ishidate, 1984). Furthermore, because of the pleasant taste associated with menthol, its use in a buccal drug delivery system may increase patient acceptability. In this study, we report the effects of l-menthol on the in vitro buccal permeation of a dideoxynucleoside type anti-HIV model drug.

2. Materials and methods

2.1. Materials

2′,3′-Dideoxycytidine (98 + %), l-menthol, and 1-octanol were purchased from Aldrich Chemical Co. (Ont., Canada). Gentamicin sulfate was purchased from ICN Pharmaceuticals, Inc. (Quebec, Canada). All other chemical reagents were analytical grade or better and were used as received. Krebs buffer (pH 7.1) had the following composition: sodium chloride 6.75 g/l, potassium chloride 0.31 g/l, calcium chloride dihydrate 0.37 g/l, sodium dihydrogen phosphate dihydrate 0.25 g/l, magnesium sulfate heptahydrate 0.29 g/l, HEPES 0.95g/l, sodium bicarbonate 1.83 g/l, and glucose 2.2 g/l. The buffers were isotonic for all studies.

2.2. Buccal tissue collection and preparation

Porcine buccal tissue was obtained from a local slaughterhouse and used within 2 h of slaughter. The tissue was stored in Krebs buffer at 4°C upon collection. The epithelium (with a thickness of 600–800 μm) was separated from the underlying connective tissues with surgical scissors and clamped in between the donor and the receiver chambers of the diffusion cells for permeation studies.

2.3. Transbuccal permeation studies

Buccal permeation experiments were conducted as described (Shojaei et al., 1998a). Briefly, side-bi-side flow through diffusion cells (Perme Gear Co., PA) with a diffusional area of 0.78 cm^2 and a capacity of 3.3 ml (for each chamber) were used. Temperature was maintained at 37°C by water jackets surrounding the two chambers that were stirred with Teflon coated magnetic stirring bars. After the buccal membranes were equilibrated with Krebs buffer (310 mOsm) in both chambers, the receiver chamber was filled with fresh buffer and the donor chamber was charged with ddC solution containing 0.01% (w/v) gentamicin to prevent possible bacterial degradation during permeation studies (Kim and Chien, 1995). The flow rate of the buffer was controlled at 1.2 ml/h with

a peristaltic pump (Minipuls 8, Gilson Inc., Middleton, WI). The samples ($n = 3$) were collected continuously using an automatic fraction collector (Gilson FC-203B) and analyzed by HPLC for ddC content. Tissue viability was verified, in terms of permeation, by using the same buccal tissues to conduct two consecutive permeation experiments (Shojaei et al., 1998b). The experiments were run under identical settings as indicated above, except that in between the first and second experiments, a 4-h wash-out period was included and both experiments were conducted using the same tissues. At the end of the first experiment, the solutions in the donor and the receiver chambers were replaced with fresh Krebs buffer. The mucosal membranes were re-equilibrated with Krebs for 4 h; the donor chamber was then charged with ddC solution and samples were collected continuously from the receiver chamber. The permeability coefficients (P) were calculated as follows

$$P = \frac{(\mathrm{d}Q/\mathrm{d}t)}{(\Delta C \cdot A)} \quad (1)$$

where $\mathrm{d}Q/\mathrm{d}t$ is cumulative amount permeated per unit time, ΔC is the concentration difference between the donor and receiver chambers, and A is the surface area of diffusion (0.78 cm^2).

The permeability of ddC was also evaluated in the presence of various concentrations of the permeation enhancer, l-menthol. Precisely weighed amounts of l-menthol were completely dissolved in the aqueous drug solution placed in the donor chamber, while the receiver chamber only contained Krebs buffer. Enhancement ratios (ER) were calculated according to the following expression (Gandhi and Robinson, 1992)

$$ER = P_{(\mathrm{enh})}/P_{(\mathrm{ctrl})} \quad (2)$$

where $P_{(\mathrm{enh})}$ is enhanced permeability coefficient and $P_{(\mathrm{ctrl})}$ is permeability coefficient of drug without the presence of the enhancer.

2.4. Analytical method

The concentration of 2′,3′-dideoxycytidine (ddC) following in vitro permeation studies was determined using reversed phase HPLC with a C_{18} column (Nova Pak, 4μ, 3.9×150 mm). The HPLC system consisted of a ChemMate pump (Rose Scientific, Edmonton, AB) coupled to a Marathon auto-injector (Spark Holland, Edmonton, AB), a Gilson UV-117 variable wavelength UV detector, and DataAlly software for data acquisition and integration. A mobile phase composed of 0.02 M Na_2HPO_4:acetonitrile (97:03) pH 7.3 was used for drug elution at a flow rate of 1.0 ml/min. The concentration of ddC was analyzed at 273 nm (Frijus-Plessen et al., 1990). The column was calibrated with ddC solutions of known concentrations.

2.5. Thermal analysis

To detect for possible eutectic formation between the drug and menthol, mixtures (various molar ratios) of menthol and ddC were prepared by dissolving the two components in methanol (Kaplun-Frischoff and Touitou, 1997). This was followed by evaporation of the solvent in a hood for about 36 h, with occasional stirring of the mixtures, until a dry solid powder was obtained. The solid mixtures were then thermally analyzed using a differential scanning calorimeter, DSC (Seiko SSC-5200).

2.6. Octanol/buffer partition coefficient determination

The partition coefficients of ddC between octanol and buffer solutions were determined by a shake-flask method. Krebs buffer solutions (pH 7.1) were previously saturated with 1-octanol. A precisely weighed quantity of ddC was dissolved in the buffer solutions (both with and without various concentrations of enhancer) to give final concentration of 200 μg/ml. Exactly 4 ml of 1-octanol was mixed with 4 ml of the ddC-buffer solution and placed in screw-capped glass scintillation vials. The two phases were allowed to equilibrate under constant shaking at 37°C for 24 h. The total volume of the samples was verified after the 24-h period to assure no loss had occurred as a result of evaporation. The aqueous phase was then separated using separatory funnels. After appropriate dilution of the aqueous

phase, the ddC content was determined using HPLC. The partition coefficient (K_p) was calculated as follows

$$K_p = \frac{C_{aq} - C_{eq}}{C_{eq}} \quad (3)$$

where C_{aq} and C_{eq} denote the initial and equilibrium concentrations of ddC in 1-octanol saturated buffer solution, respectively.

2.7. Tissue/buffer partition coefficient determination

Buccal tissue partition coefficients were determined by modifying the method of Chikhale et al. (1996). Dilute solutions of ddC, both with and without varying concentrations of menthol, in Krebs buffer were allowed to remain in contact with the mucosal side of the buccal tissue in the diffusion cell assembly at 37°C. The other side of the tissue was blocked with aluminum foil and the receiver chamber was kept empty to prevent diffusion through the buccal tissue. At various time intervals (up to 24 h) a 100 μl aliquot of the donor solution was sampled and quantified for ddC content by HPLC analysis. The partitioning of ddC into the buccal tissue was estimated from the decrease in solute concentration in the donor solution at equilibrium. The concentration of ddC in the membrane was divided by that remaining in the donor chamber at equilibrium to yield the buccal membrane partition coefficient K'_p. The following equation was used to calculate K'_p:

$$K'_p = \frac{(D_0 - D_t)V_d}{D_t \cdot V_B} \quad (4)$$

where, D_0 denotes the initial amount (mg) of drug at time zero, and D_t is the amount of drug at time t, V_d is the volume of the donor solution in cm^3, and V_B is volume of the buccal tissue membrane in cm^3. The volume of the buccal tissue membrane, V_B, was determined through buffer displacement experiments. The amount of buffer required to fill the donor chamber in presence of the tissue at equilibrium (V_{BE}) was subtracted from the total volume of the donor half-cell (without buccal tissue), V_{TOT}.

$$V_B = V_{TOT} - V_{BE} \quad (5)$$

2.8. Measurement of solubility

Excess amounts of ddC were added to 3 ml of Krebs buffer (pH 7.1) solutions containing 0, 0.1, 0.2, and 0.3 mg/ml of l-menthol and placed in screw-capped scintillation vials and mixed by vortexing. The vials were immersed in a shaking (at 100 rpm) water bath at 37°C for 48 h. The dispersions were then filtered using 0.2 μm Acrodisc® membrane filters (Gelman, Ann Arbor, MI) with the initial portions discarded to ensure filter saturation. An aliquot of the filtrate was appropriately diluted and analyzed by HPLC for drug content.

2.9. Data analysis

The steady state flux values were calculated from the linear portion of the plot of cumulative amount of ddC permeated across buccal tissue as a function of time. Lag times (t_{lag}) were calculated from the x-axis intercept of the linear portions. All experiments were run in triplicate and reported as mean ± SD. Statistical analyses were made using single factor ANOVA and differences were considered significant at a level of $P < 0.05$.

3. Results and discussion

To date, there are no standard measures to verify the viability and the integrity of the dissected tissues used in in vitro buccal permeation studies. In terms of tissue viability, drug permeation experiments themselves are perhaps the most useful measure (Zhang and Robinson, 1996a). If the permeability of the solute under investigation does not change significantly during the time course of the experiment, then the tissue is considered viable. In the present study, the permeability coefficients and t_{lag} values from tissue viability experiments were determined and no significant ($P > 0.05$) differences were found.

The permeability coefficient of ddC was determined to be 4.1×10^{-6} cm/s with a steady state flux of 26.4 μg/cm².h and a t_{lag} of 11 h. Perme-

ation of ddC increased significantly ($P < 0.05$) in the presence of l-menthol with an enhancement factor of 2.02 and a t_{lag} of 6 h (Fig. 1). This enhancement was not concentration dependent, as no significant ($P > 0.05$) difference was observed between the permeation enhancement of ddC in presence of 0.1, 0.2, and 0.3 mg/ml of l-menthol (Table 1).

To probe into the mechanism of menthol's permeation enhancement of ddC, thermal studies and partition coefficient determination experiments were conducted. Since menthol is known to form eutectic mixtures with certain compounds, thermal studies of various mixtures of ddC:menthol would assess for possible eutectic formation (Kommuru et al., 1998). If a eutectic is formed, the solubility of ddC is expected to increase based on the relationship between solute solubility and melting point, T_m, derived from the ideal solubility equation (Martin, 1993). Since solute solubility in a given medium depends on the strength of intermolecular forces, a decrease in the intermolecular forces would yield a lower heat of fusion and thus a lower melting point.

The melting points of ddC and l-menthol in various mixtures of ddC:menthol were determined and no eutectic formation was detected, within the investigated range of ddC:menthol concentrations. To determine whether the solubility of drug was affected by l-menthol even when no eutectic formation was found, the solubility of ddC in presence of various concentrations of l-menthol was measured. As shown in Table 2, the solubility of ddC did not significantly ($P > 0.05$) change in the presence of 0.1, 0.2, and 0.3 mg/ml of l-menthol in Krebs buffer at 37°C.

The apparent 1-octanol/Krebs partition coefficients (log K_p) of ddC increased significantly ($P < 0.05$) in presence of menthol, independent of the enhancer's concentration (Table 1). The log K_p results agree well with the permeation enhancement findings, where no significant difference in ER was seen between the three concentrations of l-menthol. This would indicate that the fraction of drug passing through the transcellular pathway was increased in the presence of l-menthol (since the solubility of the drug remained constant). However, when buccal tissue/Krebs buffer was used for the partition coefficient experiments, the findings were not in agreement with the K_p results. Compared to the octanol/buffer results, the tissue/buffer data (K'_p) were an order of magnitude

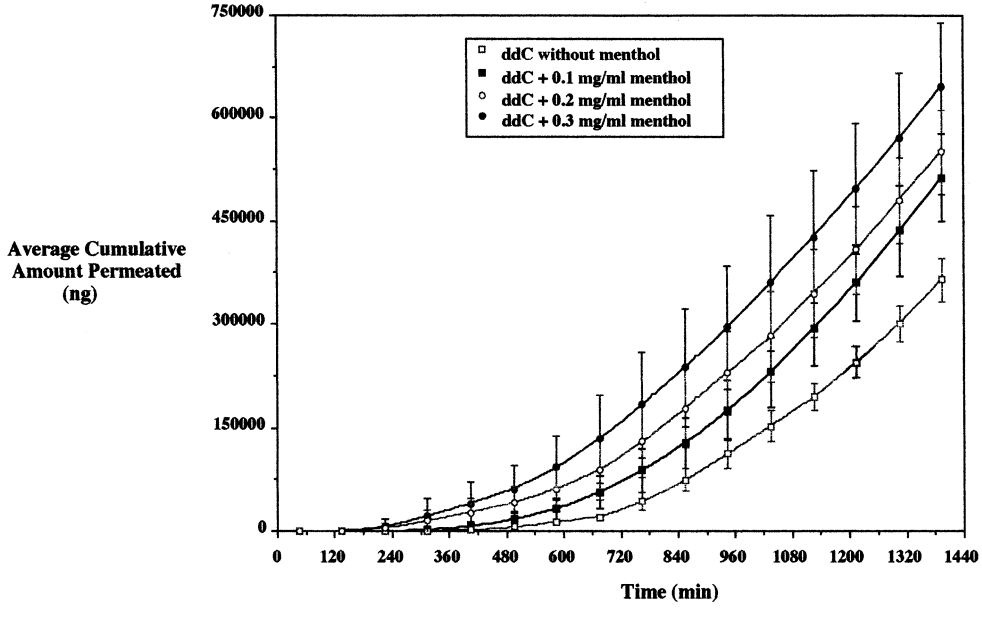

Fig. 1. In vitro transbuccal permeation of ddC with and without l-menthol.

Table 1
Effects of l-menthol on the in vitro buccal permeability coefficient and the apparent partition coefficient of dideoxycytidine

Permeability coefficient[a] ($\pm$SD) ($\times 10^{-6}$ cm/s)	Time lag ($\pm$SD) (h)	Concentration of l-menthol (mg/ml)	Permeability enhancement ratio (ER)	Octanol/buffer partition coefficient (Log K_p ($\pm$SD))	Tissue/buffer partition coefficient[b] (Log K'_p ($\pm$SD))
4.09 (0.37)	10.72 (0.81)	0.0	1.00	−1.302 (0.052)	−2.338 (0.080)
8.13 (0.77)	6.41 (0.64)	0.1	1.98	−1.151 (0.065)	−2.159 (0.072)
8.04 (0.71)	7.22 (0.59)	0.2	1.96	−1.125 (0.045)	−1.921 (0.054)
8.27 (0.84)	6.35 (0.71)	0.3	2.02	−1.151 (0.066)	−1.801 (0.098)

[a] Concentration of the donor cell was kept constant at 1 mg/ml ddC.
[b] Equilibrium tissue/buffer partition coefficients reported at 4 h.

smaller than the K_p data, which indicated that the octanol/buffer system under-estimated the polarity of ddC. Moreover, a linear ($r^2 = 0.986$) concentration dependence was observed for the apparent tissue/Krebs partition coefficients of ddC (Fig. 2).

The significant improvement in the partition coefficient of drug in the presence of l-menthol is in agreement with previously published literature on the percutaneous permeation enhancement mechanism of menthol (Gao and Singh, 1998). However, based on the K'_p data, the steady state flux should have also increased in a concentration dependent manner. This discrepancy between the K_p and K'_p values can be explained by considering that the K'_p values are not only a measure of the drug partitioning but also the binding of drug to the buccal tissue. Therefore, the concentration dependent increase in the tissue/buffer ratio (K'_p) is the result of an increase in partitioning as well as tissue binding of ddC. The octanol/buffer (K_p) data, on the other hand, are purely a measure of the drug's partitioning behavior.

To explain why the overall permeation enhancement was independent of the concentration of l-menthol, within the investigated range, the cellular framework of the buccal mucosa needs to be considered. The structure of the buccal mucosal barrier is such that drugs have two routes of transport for passive diffusion, paracellular and transcellular. Depending on the lipophilicity of the drug, one pathway is the dominant route. Polar permeants predominantly cross the barrier through the paracellular pathway (Eq. (6)) (Zhang and Robinson, 1996a) and non-polar, lipophilic solutes, pass through the transcellular pathway (Eq. (7)) (Zhang and Robinson, 1996b) as their dominant route.

$$J_p = \frac{D_p \varepsilon}{h_p} \cdot C_D \quad (6)$$

$$J_T = \frac{(1-\varepsilon) D_T K_p}{h_T} \cdot C_D \quad (7)$$

The flux (J_p) of a permeant passing through the paracellular route (Eq. (6)) is directly proportional to the diffusivity of the compound in the intercellular spaces (D_p), the donor chamber concentration of the drug (C_D), and the fractional area of the paracellular space (ε); and it is inversely proportional to the length of the paracellular route (h_p). In case of the transcellular route (Eq. (7)), aside from the diffusivity (D_T) and the donor concentration, the flux (J_T) is also dependent on the partitioning behavior (K_p) of the drug between the lipophilic phase and the aqueous phase. Given that ddC is a hydrophilic compound and highly polar at pH 7.1, the dominant route of its buccal transport is the paracellular pathway.

Table 2
Solubility of ddC in Krebs buffer at 37°C, with and without l-menthol

Menthol concentration (mg/ml)	ddC Solubility (mg/ml $\pm$ SD)
0.0	89.53 $\pm$ 0.62
0.1	87.74 $\pm$ 3.69
0.2	88.92 $\pm$ 3.44
0.3	88.44 $\pm$ 5.35

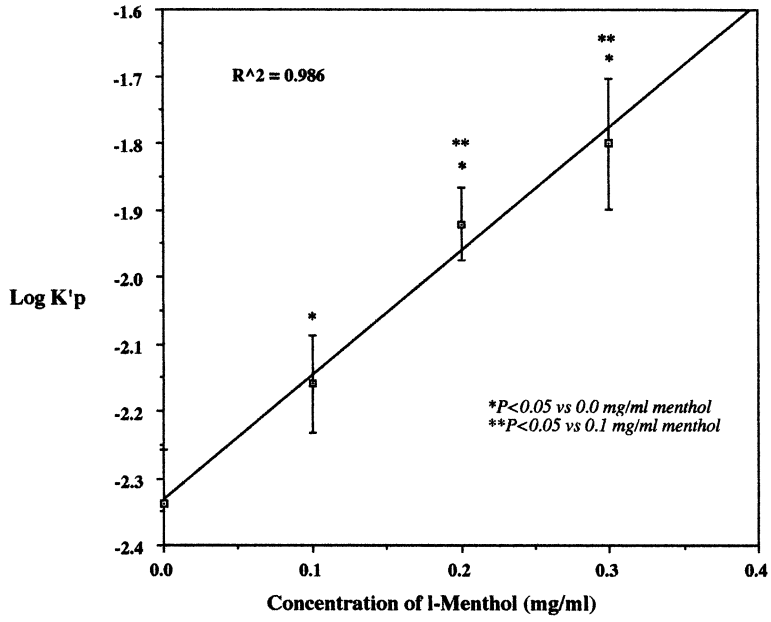

Fig. 2. Effect of l-menthol on the apparent partition coefficient of ddC.

However, l-menthol facilitated the fraction of the drug crossing through the transcellular pathway, since it significantly enhanced the partition coefficient of ddC. The dominant pathway of the drug is independent of K_p (as indicated by Eq. (6)) and enhancement of the partition coefficient of the drug would only improve the overall flux by a small extent.

4. Conclusions

The in vitro transbuccal permeation of dideoxycytidine was enhanced in the presence of very low concentrations of l-menthol. The mechanism of this enhancement was due, at least in part, to the partition coefficient enhancing effects of the terpene.

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