Transbuccal Delivery of 5-Aza-2′-Deoxycytidine: Effects of Drug Concentration, Buffer Solution, and Bile Salts on Permeation

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ABSTRACT

Delivery of 5-aza-2′-deoxycytidine (decitabine) across porcine buccal mucosa was evaluated as an alternative to the complex intravenous infusion regimen currently used to administer the drug. A reproducible high-performance liquid chromatography method was developed and optimized for the quantitative determination of this drug. Decitabine showed a concentration-dependent passive diffusion process across porcine buccal mucosa. An increase in the ionic strength of the phosphate buffer from 100 to 400 mM decreased the flux from 3.57 ± 0.65 to 1.89 ± 0.61 μg/h/cm^2. Trihydroxy bile salts significantly enhanced the flux of decitabine at a 100 mM concentration ($P > .05$). The steady-state flux of decitabine in the presence of 100 mM of sodium taurocholate and sodium glycocholate was 52.65 ± 9.48 and 85.22 ± 7.61 μg/cm^2/h, respectively. Two dihydroxy bile salts, sodium deoxytaurocholate and sodium deoxyglycocholate, showed better enhancement effect than did trihydroxy bile salts. A 38-fold enhancement in flux was achieved with 10 mM of sodium deoxyglycocholate.

KEYWORDS: Decitabine, transmucosal, buccal, ionic strength, bile salts.

INTRODUCTION

Decitabine, or 5-aza-2′-deoxycytidine, a deoxycytidine analog, is a potent inhibitor of DNA methylation that reactivates tumor suppressor genes that have been silenced by aberrant methylation.[1-3] Decitabine is being used in the clinical management of acute leukemia,[4-6] chronic myeloid leukemia,[7] myelodyplasia syndrome,[8,9] and sickle cell anemia.[10] It is more effective than other deoxycytidine analogs such as cytarabine and hence is one choice for treatment of acute myeloid leukemia.[11-14]

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Decitabine is now administered by intravenous infusion as a solution that must be freshly reconstituted every 4 to 6 hours.[15] A 4-hour infusion every 8 hours for 3 consecutive days with a starting dose of 15 to 50 mg/m^2/day is being administered in patients with myelodyplasia syndrome.[16,17] One of the reasons for this complex dosage regimen is the poor chemical stability of decitabine.[18,19] The most stable pH for decitabine is 7.0, and the rate of hydrolysis increases in alkaline or acidic media, and at higher temperatures (Ravivarapu H, Redkar S, 2003 unpublished data). Oral administration is also not ideal, as the acidic conditions in the stomach and the presence of hepatic deaminases degrade the drug.

A patient-friendly administration route for this therapeutically potent molecule would be a significant improvement over the current complex infusion therapy. Transmucosal administration may overcome some of the limitations of parenteral and oral delivery. Drugs administered through buccal or sublingual mucosa reach the systemic circulation in a relatively shorter time and can act more quickly. This route may also improve patient compliance, as it offers more comfort to the patient and minimizes the duration of hospitalization. Considering the stability issues, the buccal route may be preferable to the sublingual route, as it could avoid dilution and hydrolysis of the drug and possible swallowing.

The uppermost layer of buccal tissue is made up of 40 to 50 layers of epithelial cells. This layer is followed by the connective tissue layer or the lamina propria. Drugs that come into contact with the buccal epithelium will take either the transcellular route or the paracellular route to enter the lamina propria and gain access to the blood vessels.[20] The barrier nature of epithelium and the route of transport control the delivery of drugs across the buccal mucosa. The hydrophilic nature of cytoplasm and the intercellular spaces is a permeability barrier to lipophilic drugs, while the lipophilic cell membrane is a barrier to hydrophilic compounds. Although any single molecule can traverse both routes, the predominant pathway is based on the physicochemical properties of the molecule.[21]

Decitabine solubility in water is ~25 mg/mL at 25°C, and its octanol-water coefficient is 0.726 (log P: –0.32). Being a hydrophilic molecule, decitabine is likely to take the paracellular route during its passive diffusion through buccal

mucosa. One of the limitations encountered by hydrophilic molecules during their transbuccal transport is the limited surface area of the intercellular space and the tortuosity of the paracellular pathway. Under these circumstances, the use of a permeation enhancer becomes essential for delivering therapeutic quantities of decitabine into the systemic circulation. Bile salts have been widely used as permeation enhancers because of their biocompatibility, even though their long-term effect on buccal mucosa is not fully understood. The objective of the present study is, therefore, to evaluate the feasibility of transbuccal delivery of decitabine and the effect of bile salts on its permeability.

Materials and Methods

Materials

Decitabine, shown in Figure 1, was provided by SuperGen Inc (Dublin, CA). Sodium taurocholate (STC), sodium glycocholate (SGC), sodium deoxytaurocholate (SDTC), and sodium deoxyglycocholate (SDGC) were purchased from Sigma (Milwaukee, WI). All other materials and reagents were of analytical or high-performance liquid chromatography (HPLC) grades.

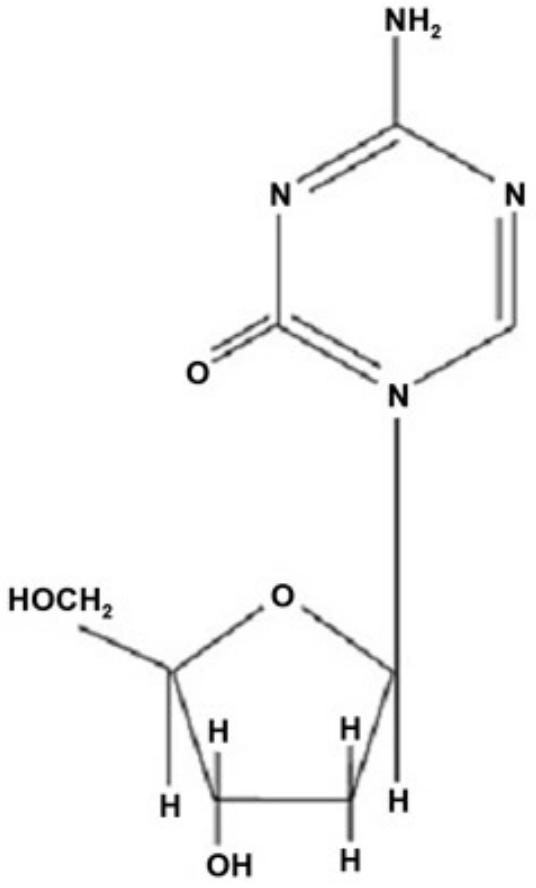

Figure 1. Structure of decitabine.

HPLC Method and Stability in Permeation Vehicles

Decitabine was quantified using a Beckman Coulter HPLC system with a photo diode array detector and a C_{18} reversed-phase column (Supelcosil LC18, 4.6 × 250 mm, 5 μm) (Milwaukee, WI). Phosphate buffer solution (200 mM, pH 6.8) was used as the mobile phase. The mobile phase was filtered using a 0.22 μm membrane filter and degassed prior to use. Samples equivalent to 50 μL were injected into the column using an auto-injector and eluted at a flow rate of 1.7 mL/min under the isocratic mode for 15 minutes. Decitabine was monitored at 220 nm using a UV detector. All chromatographic operations were carried out at room temperature (25 ± 2°C). The stability of decitabine in the mobile phase, water, and all permeation vehicles was evaluated for 24 hours.

Permeation Studies

The permeation characteristics of decitabine were studied using porcine buccal mucosa. Buccal tissues were obtained from freshly slaughtered pigs and used within 3 hours of animal sacrifice. Tissues were preserved in McIlvaine buffer during transportation. The underlying connective tissue was removed using surgical scissors, and a thickness of 500 ± 30 μm was used in permeation experiments. The effects of different donor drug concentrations (5, 13, and 18 mg/mL), buffer strengths (100, 200, and 400 mM), and hydrophilic enhancers (10 mM STC, 10 mM SGC, 100 mM STC, 100 mM SGC, 10 mM SDTC, and 10 mM SDGC) were studied. Franz diffusion cells with a 0.665 cm^2 diffusional area and a 7.5 mL receiver compartment volume were used for permeation experiments.

The processed buccal tissues were mounted between the donor and receptor compartments of permeation cells using a clamp. The mucosal side of the tissue was placed toward the donor compartment and the serosal side toward the receptor compartment. One milliliter of freshly prepared decitabine solution in phosphate buffer of pH 7.0 was transferred into the donor chamber. Except in the donor concentration effect studies, the concentration of decitabine in donor solution was maintained at 20 mg/mL. The receiver chamber was filled with phosphate buffer of pH 7.0 and stirred using a magnetic stirrer to avoid the formation of a stagnant diffusion layer. Samples were withdrawn at hourly intervals for 6 hours. At each time point, the receiver compartment solution was completely removed and replaced with fresh phosphate buffer solution. The quantity of drug permeated across the buccal mucosa was determined by HPLC. The steady-state flux and apparent permeability coefficient of decitabine under these experimental conditions were calculated and compared. All permeation experiments were performed at room temperature.

Results and Discussion

HPLC Method for Decitabine

A reproducible HPLC method for the quantification of decitabine was developed. The method was evaluated for its reproducibility, accuracy, limit of detection, and limit of quantification. A typical chromatogram of decitabine is shown in Figure 2. Under the specified conditions, the retention time of decitabine was found to be 9.14 ± 0.4 minutes, and its degradation peaks eluted after 10.60 ± 0.5 minutes. The current method exhibited linearity in the concentration range of 0.1 to 30 μg/mL. Upon least squares linear regression analysis of the peak area as a function of concentration, an r^2 value of 0.9997 was obtained between 3 interday calibrations.

Rapid degradation of decitabine was observed in all aqueous permeation vehicles. About 43% of the drug degraded in phosphate buffer of pH 7.0 within 24 hours at room temperature. The degradation rate was higher and multiple peaks were observed when samples were maintained at 37°C. Permeation studies were therefore conducted at room temperature. The extent of degradation of decitabine in the presence of the permeation test vehicles was in the range of 15% to 27% during the 6 hours of the permeation study, which was same as the range in the buffer during that time, suggesting that bile salts did not contribute to the degradation of decitabine. Complete removal of the fluid at each sample point, simultaneous replacement with fresh medium, and immediate HPLC analysis of the receiver samples were performed to minimize the errors due to degradation of permeated drug.

Effect of Donor Drug Concentration on Permeation

The permeation profile of decitabine with different donor concentrations is shown in Figure 3. Detectable quantities of decitabine were permeated into the receiver side within 1 hour, and steady state was achieved within 2 hours. The flux was found to increase with an increase in the concentration of drug in the donor compartment. The flux of decitabine at 5, 13, and 18 mg/mL donor concentrations was 0.25 ± 0.07, 1.37 ± 0.48, and 1.97 ± 0.43 μg/h/cm^2, respectively. A plot of steady-state flux as a function of donor concentration was linear (r^2 value of 0.9988), indicating that decitabine permeated by passive diffusion across the buccal mucosa. The mean permeability coefficient of decitabine was found to be 2.42 ± 0.91 × 10^{-8} cm/s.

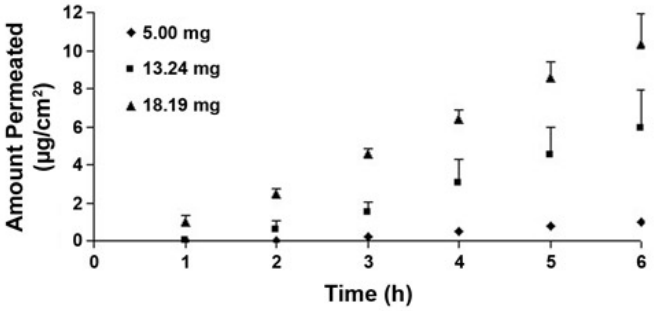

Figure 3. Permeation profile of decitabine showing the influence of donor drug levels on the cumulative amount of drug permeated across porcine buccal mucosa to the receiver side over 6 hours. The error bars indicate the SD of 3 observations.

Effect of Ionic Strength of Buffer

This study was conducted to evaluate the effect of ionic strength on the permeation of this cationic molecule through the buccal epithelium. Decitabine showed higher permeation at a lower ionic strength of buffer, as shown in Figure 4. The flux of decitabine dropped from 3.57 ± 0.65 to 1.89 ± 0.61 μg/h/cm^2 when the ionic strength of the buffer was increased from 100 to 400 mM. The lower permeability of decitabine in higher–ionic strength buffer can be attributed

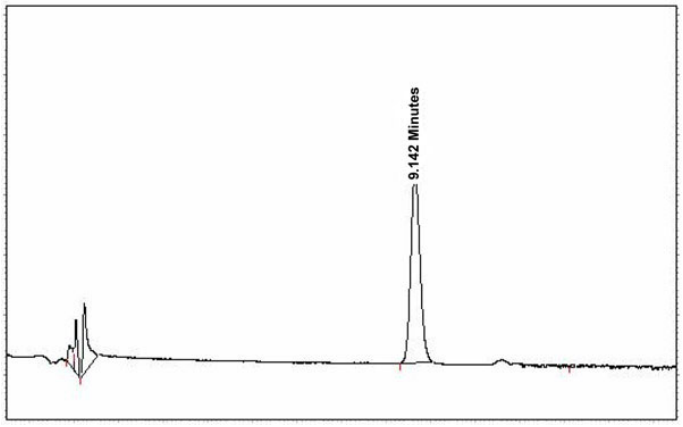

Figure 2. A typical chromatogram showing the decitabine peak at 9.14 ± 0.4 minutes with a tailing factor of 1. Linearity in the peak area was observed in the 0.1 to 30 μg/mL concentration range.

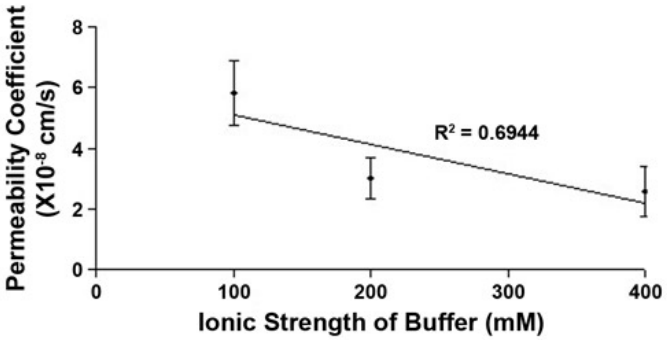

Figure 4. Influence of the ionic strength of the donor vehicle on the permeability of decitabine. The negative slope with an r^2 value of 0.6944 indicates a decrease in decitabine permeability in the presence of higher–ionic strength buffers. The error bars indicate the SD of 3 observations.

to ionic interactions and/or decitabine's stability in the buffer solution. A net decrease in the electronegative potential was observed when decitabine was dissolved in high–ionic strength buffers. The zeta potential of decitabine in buffers of 100, 200, and 400 mM buffers was –28.03 ± 5.12, –13.67 ± 5.12, and –6.75 ± 2.89 mV, respectively. Although the effect of the ionic strength of the vehicle on transcellular permeation of permeants is not clear, it may have considerable influence on the paracellular permeation of ionic drugs. Because the random molecular motion associated with diffusion is likely to be higher with charged systems, decitabine in lower–ionic strength buffer should have had a better permeation rate than it did in the presence of 400 mM buffer strength. Such ionic interactions were also reported by Sugawara et al.[22] The permeability of a series of cationic drugs across Caco2 cell lines decreased significantly when the molarity of potassium in the donor medium was increased. An increase in the ionic strength of the buffer from 2.5 to 125 mM, however, did not influence the permeability of neutral compounds such as acetanilide, antipyrine, and caffeine, or of a zwitterionic drug, enoxacin.

The ionic strength–dependent degradation of decitabine could also be one of the reasons for the decrease in flux on an increase in ionic strength. The extent of degradation of decitabine doubled when the ionic strength of the buffer was increased from 100 mM to 400 mM. The quantity of decitabine degraded in 100, 200, and 400 mM phosphate buffers of pH 7.0 over 6 hours was 15.07%, 20.65%, and 27.01%, respectively. As faster degradation is likely to decrease the thermodynamic activity of the system, the driving force for decitabine permeation could have decreased in the presence of higher–ionic strength buffers.

Effect of Bile Salts on Permeation

The permeation profiles of decitabine in the presence of 4 different bile salts are shown in Figure 5. All these bile salts enhanced the flux of decitabine across porcine buccal mucosa, except STC and SGC at the 10 mM concentration. The steady-state flux in the presence of these 2 trihydroxy bile salts at the 100 mM concentration was 52.65 ± 9.48 and 85.22 ± 7.61 μg/cm^2/h, respectively. The flux of decitabine in the presence of SDTC and SDGC at the 10 mM concentration was 41.87 ± 16.52 and 75.32 ± 3.73 μg/cm^2/h, respectively. These enhancements in the flux of decitabine in the presence of bile salts are believed to happen by a complex process. Some of the proposed modulation mechanisms of bile salts include solubilization and micellar entrapment of intercellular lipids, denaturation and extraction of proteins, enzyme inactivation, and tissue swelling.[23-25]

A comparison of the enhancing effects of bile salts on the flux of decitabine across buccal mucosa is shown in Table 1. The permeation enhancement of dihydroxy bile

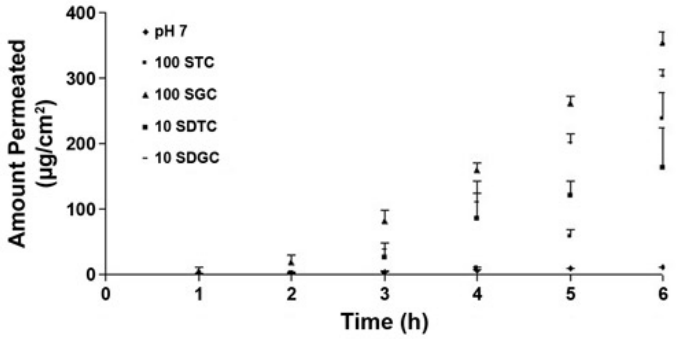

Figure 5. Permeation profile showing the enhancing effect of bile salts on the permeation of decitabine. The error bars indicate the SD of 3 observations.

salts (SDTC and SDGC) is better than that of trihydroxy bile salts (STC and SGC) for decitabine across porcine buccal mucosa. STC and SGC enhanced the flux ~28- to 43-fold only when their concentrations were 10-fold higher than their critical micellar concentrations (CMCs), whereas SDTC and SDGC showed comparable enhancements when their concentrations were ~3-fold higher than their CMC value. The reported CMCs of trihydroxy and dihydroxy bile salts were 10 mM and 4 mM, respectively.[26]

The flux enhancements in the presence of these 2 dihydroxy bile salts were in the range of 21- to 38-fold at the 10 mM concentration. Such differences in the enhancement effect of dihydroxy and trihydroxy bile salts were also found in the literature. SDGC provided greater permeation enhancement for mannitol than did STC and SGC in a TR 146 cell culture model. SDGC showed enhancement when its concentration was equal to or higher than its CMC, whereas STC and SGC improved the flux only when their concentration was ~2- to 3-fold greater than their CMC.[27] Better enhancing potential of a dihydroxy bile salt was related to its hydrophobicity, effective permeation into the cell membrane, and micellar solubilization of the membrane components. Similarly, Xiang et al have shown 32-fold enhancement in the permeability of 2′,3′-dideoxycytidine in the presence of SDGC across porcine buccal mucosa when its concentration was equal to its CMC.[28] In vivo bioavailability studies conducted in pigs have also shown the enhancement potential of SDGC. Five- to seven-fold enhancement in absolute bioavailability of buserelin and fluorescein isothiocyanate dextran was observed when 10 mM of SDGC was used.[29-31] Although dihydroxy bile salts showed a higher permeation-enhancing effect for decitabine than trihydroxy bile salts did, the dihydroxy bile salts' safety for buccal mucosa at these concentrations may be a factor in their selection. An earlier report published by Senel et al showed that the morphological changes caused by 10 mM of SDGC were comparable to those caused by 100 mM SGC.[32]

Table 1. Enhancing Effects of Bile Salts on the Steady-State Flux and Permeability of Decitabine Across Porcine Buccal Mucosa*

Enhancer	Permeability Coefficient† ($\times 10^{-8}$ cm/sec)	Steady-State Flux† ($\mu g/cm^2/h$)	Flux Enhancement Ratio
Control	3.00 ± 0.66	1.97 ± 0.43	1.00
10 mM STC	2.04 ± 0.34	1.42 ± 0.24	0.72
10 mM SGC	2.73 ± 0.45	1.90 ± 0.32	0.97
100 mM STC	67.54 ± 12.16	52.65 ± 9.48	26.77
100 mM SGC	124.70 ± 11.14	85.22 ± 7.61	43.32
10 mM SDTC	66.09 ± 26.07	41.87 ± 16.52	21.28
10 mM SDGC	118.90 ± 5.90	75.32 ± 3.73	38.29

*STC indicates sodium taurocholate; SGC, sodium glycocholate; SDTC, sodium deoxytaurocholate; SDGC, sodium deoxyglycocholate.
†Mean ± SD values from 3 permeation experiments.

The flux enhancement effect of bile salts also provided information on the permeation pathway of decitabine through buccal tissue. Based on the earlier reports, bile salts are believed to improve the permeation of ionic drugs, which predominantly traverse through the paracellular route. For instance, SGC enhanced the permeability of flecainide acetate and did not influence the permeation of flecainide base, which is more lipophilic than flecainide acetate.[33] It was also shown in our laboratory that the permeability of an ionic compound, ISIS 3082, that predominantly traverses through the paracellular route was enhanced by SGC.[34] Permeation enhancement of this hydrophilic drug in the presence of bile salts may therefore be related to its paracellular diffusion pathway across porcine buccal mucosa.

CONCLUSION

The feasibility of delivering decitabine through the buccal route was explored to overcome the limitations of the drug's parenteral and oral delivery. The current in vitro studies using isolated porcine buccal mucosa have demonstrated the feasibility of transbuccal delivery of decitabine. The concentration-dependent passive diffusion of decitabine was found to be influenced by the ionic strength of the buffer. Permeation of this hydrophilic drug was enhanced to ~40-fold by the use of SDGC and SGC.

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