Percutaneous Absorption of 2′,3′-Dideoxyinosine in Rats

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This study explored the topical route for administering of 2′,3′-dideoxyinosine (ddI), a nucleoside analog used for treating patients with acquired immunodeficiency syndrome. A dose of ddI (~180 mg/kg) dispersed in ~1 g ointment base was applied, with or without occlusion, to the back of high follicular density (HFD) and low follicular density (LFD) rats. The systemic ddI clearance was determined using a concomitant administration of an intravenous tracer dose of [^{3}H]ddI. At 24 hr, the experiment was terminated and skin sections at the application site were removed. After topical application, average plateau plasma levels of about 0.6 μg/ml were achieved within 1 to 2 hr and maintained for 24 hr. Occlusion gave a more uniform plasma profile but did not increase the bioavailability. The systemic bioavailability in HFD and LFD rats was about the same at 33%. In addition, a depot of about 16% of the dose was recovered by rinsing the application area and extracting the drug from the excised application site. These data indicate that about 50% of the dermal dose penetrated the skin barrier in 24 hr. The similar bioavailability in the HFD and LFD rats further suggests an unimportant role for the transfollicular absorption route for ddI. The effect of a mixture of penetration enhancers, Azone and propylene glycol (5:95), was studied in HFD rats. Coadministration of ddI with the enhancers did not increase the ddI bioavailability. However pretreatment and coadministration with the enhancers significantly increased the bioavailability to 62%, which is a conservative estimate because the plasma drug level was still at a plateau when the experiment was terminated at 24 hr. In summary, the transdermal bioavailability of ddI exceeded the 15% oral bioavailability found in previous studies by more than 3 folds and was further increased by the pretreatment with absorption enhancers. These data indicate the topical route as an attractive administration route.

KEY WORDS: 2′,3′-dideoxyinosine; transdermal; bioavailability; follicular density; penetration enhancer.

INTRODUCTION

2′,3′-Dideoxyinosine (ddI) is used to treat patients with acquired immunodeficiency syndrome. ddI inhibits the reverse transcriptase of the human immunodeficiency virus isolated from patients (1). Constant drug exposure is preferred for its antiviral activity (2). At present ddI is given to patients orally. However, ddI is acid labile and oral administration results in variable bioavailabilities even after coadministration with antacids (3–5). Our laboratory is interested in evaluating alternative noninvasive delivery routes. A previous publication describes the kinetics and absorption of the rectal administration route (6).

The transdermal delivery route has been used to provide a constant plasma drug level, improve patient compliance and avoid side effects. The properties of an ideal candidate for this route include a small molecular weight (≤300), and an octanol:water partition coefficient of 1 (7). ddI is a relatively small molecule with a molecular weight of 238 and an intrinsic solubility of 27 mg/ml in water at 25°C (8), and has a limited lipophilicity as suggested by its penetration across the blood-brain barrier (9). The present study evaluated the percutaneous absorption of ddI in rats and the effect of absorption enhancers, Azone (1-Dodecylazacycloheptan-2-one) and propylene glycol (PG).

MATERIALS AND METHOD

Chemicals. ddI (MW 238.2, Lot # 234-b-1) was provided by the National Institutes of Health (Bethesda, MD). [Ribose-2′,3′-^{3}H]ddI (specific activity 104 mCi/mg) was provided by the Research Triangle Institute (Research Triangle Park, NC) under contract with the National Institutes of Health. Ftorafur (N^1-(2-tetrahydrofuranyl)-5-fluorouracil) was a gift from Mead Johnson Research Laboratory (Evansville, IN) and Azone from Whitby Research Inc. (Richmond, VA). Unibase was purchased from Warner Chilcott Laboratories (Morris Plains, NJ), Lanolin from Paddock Laboratories (Minneapolis, MN), 1-octanol from Fluka Chemicals (Ronkonkoma, NY), and all other chemicals and solvents from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific Co. (Cincinnati, OH). The purities of radiolabeled and unlabeled ddI were evaluated by high pressure liquid chromatography (HPLC) and were 99.8% and 97.9%. All chemicals were used as received.

Dosage Formulation. Several formulations were evaluated to achieve optimal delivery of ddI. The first approach was to apply a ddI solution in saline (~27 mg/ml, pH ~7.0) to the treatment site using a syringe. However, the solution was not retained at the application site due to the large volume of the dosing solution required (~1.5 ml), and there was considerable loss of the solution during application. The second approach was to use an ointment base vehicle. Both o/w ointment base (Unibase) and w/o (Lanolin) ointment bases were evaluated. ddI (40–50 mg) was dissolved or suspended in 400 μl methanol and mixed with 1 g ointment base on a pill tile. This volume of methanol was close to the maximum volume that can be incorporated by the base. Dissolution of ddI in methanol was accomplished by the addition of 15 μl of 5N NaOH. Without the addition of NaOH, a suspension was obtained. The formulation was done immediately before the animal experiment. Five random samples of the ointments containing ddI in suspension or in solution were obtained and analyzed for drug content. The drug was evenly dispersed in the continuous phase as indicated by the 10% coefficient of variation between samples. We attempted to use an occlusive dermal delivery system (Hill Top Chamber, Hill Top Research Inc., Cincinnati, OH) by incorporating a solution of ddI in methanol in the chamber.

The partition coefficient of ddI was determined by thoroughly mixing a solution of ddI (10 μg/ml) in phosphate buffer (pH 7.0 or 8.5) with an equal volume of 1-octanol and equilibrating for 2 hr at 37°C. The fraction of ddI in the aqueous and octanol phases was determined by the UV ab-

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sorbance using a Beckman DU640 spectrophotometer (Beckman Instruments Inc., Irvine, CA).

Animal Protocol. Rats with different follicular densities, i.e. high density (female Fischer rats, Charles River Breeding Laboratories, Kingston, NJ) and low density (female fuzzy rats, Harlan Sprague-Dawley Indianapolis, IN) were used. The rats were 5–6 months old. The body weights were 209 ± 22 g (mean ± SD, n = 10) for the Fischer rats and 222 ± 30 g (n = 5) for the fuzzy rats. To determine follicular density, 1 cm^2 pieces of skin were removed from the dorsal surface, fixed in neutral buffered formalin and stained with methyl green. The number of hair follicles per unit area was counted under a microscope (Axioskop, Zeiss Instruments, Germany). The rats were housed in metabolism cages 2 days before an experiment. Permanent catheters were implanted in the right jugular vein under ether anesthesia 1 day before the study. For the high follicular density (HFD) rats, an area (8 cm^2) on the dorsal interscapular surface was clipped free of fur after cannulation. This was the dosing area. Care was exercised to avoid skin abrasion by the clipper. Hair removal was not necessary in the low follicular density (LFD) rats. Food and water were withheld from the onset of treatment for 6 hr. Treatments were given between 7 and 8 a.m. The ointment containing ddI was spread evenly over the dosing area with a spatula. After application, the residual ointment in the pill tile and spatula was dissolved in methanol and assayed for ddI. This represented the fraction of dose lost during formulation. The target dose was 200 mg ddI/kg animal body weight. About 10% of the dose was lost during formulation. The actual applied dose was 180 mg/kg. In most experiments the site was occluded with a plastic wrap. In some animals an intravenous dose of [^{3}H]ddI in tracer quantity, (20 μCi, equivalent to 192 ng), was administered over 0.5 min through the jugular catheter 5 min after applying the dermal dose. Serial blood samples (200 μl) were obtained over 24 hr and urine samples were collected at 12 and 24 hr. Samples were kept on ice to avoid ddI degradation by serum phosphorylases. After the last blood sample, the animal was anesthetized, and the application site was inspected visually for signs of damage or irritation. The residual ointment that remained on the occlusive wrap was recovered and the ointment on the skin at the application site was gently scraped off. This amount was estimated as the fraction remaining on the wrap and outside the skin. After rinsing with 500 μl methanol, the skin was excised and stored frozen at −70°C. The amount of ddI in the methanolic skin rinse and the skin was estimated as the amount remaining in the skin.

The effect of absorption enhancers, Azone and PG, was studied in HFD rats. Previous studies have shown that Azone at concentrations of 1 to 5% provided enhancement of percutaneous penetration of various drugs (10,11). The enhancement appears to be maximal when Azone is used in combination with a hydrophilic cosolvent such as PG (12,13). Pretreatment by Azone and other azacycloalkanone derivatives further potentiates the enhancement effects (14). In the present study, ddI was suspended in a mixture of Azone:PG (5:95) in polyethylene tubes. The administration of this mixture represented a coadministration of ddI with the enhancers. The control group received ddI in Azone:PG. The pretreatment group received an additional treatment with 500 μl of the Azone:PG (5:95) mixture (containing no ddI) at 24 hr prior to dose application. The pretreatment as well as the drug containing mixture was applied to an 8 cm^2 area on the dorsal surface of HFD rats with a spatula. Dose loss was estimated by rinsing the tube and spatula with methanol and assaying the rinses for ddI. The target dose was 200 mg/kg. About 35% of the dose was lost during formulation and application. The actual applied dose was ~130 mg ddI/kg. No occlusion was used in this study.

Sample Analysis. Biologic samples including plasma, urine, skin and wrap rinses, and skin extracts were analyzed for ddI. The plasma and urine samples and the methanolic rinses were extracted by solid phase extraction as described previously (15,16). The frozen skin sample was weighed, immersed in liquid nitrogen and pulverized to a fine powder. Two to three random samples, weighing approximately 100 mg, were homogenized and extracted with 10 ml of acetonitrile. The extraction efficiency, using homogenized skin samples spiked with known amounts of ddI, was greater than 90%. The extracts were analyzed by HPLC as previously described (15,16). The unlabeled ddI was quantitated by HPLC and UV absorbance and the [^{3}H]ddI by HPLC and liquid scintillation counting. The total concentration of the [^{3}H]ddI was less than 0.1% of the unlabeled ddI. Therefore, the UV absorbance represented the unlabeled drug and it was not necessary to correct for the contribution of [^{3}H]ddI. Standard curves for unlabeled ddI were linear in the range of 0.1 to 10 μg/ml (r^2 = 0.999) for plasma and urine samples, and from 1 to 30 μg/ml (r^2 = 0.999) for skin rinses and skin extract. The standard curves for [^{3}H]ddI were linear from 0.0002 to 1.6 μCi/ml (r^2 = 0.999).

Data Analysis. Some animals received simultaneously an intravenous tracer dose of [^{3}H]ddI and a dermal dose of unlabeled ddI. The plasma concentration-time profile of the intravenous dose was used to calculate the blood clearance ($CL_{i.v.}$). Data were analyzed using a three compartmental open model and by noncompartmental analysis as described previously (15). The area under the concentration-time curve (AUC) from time zero to time infinity, $CL_{i.v.}$, volume of distribution at steady state (VD_{ss}), and fraction of dose excreted in urine over 24 hr (Fe) were calculated according to standard procedures (17).

The bioavailable fraction (F) of the dermal dose was estimated by using plasma data (equation 1) and urine data (equation 2). For animals that received simultaneously intravenous tracer doses of [^{3}H]ddI and dermal dose of unlabeled ddI, F was determined using $CL_{i.v.}$ of individual animals. For animals that were given only a dermal dose of ddI, F was determined using the mean value of $CL_{i.v.}$ obtained from other animals. $Fe_{i.v.}$ and Fe_{dermal} are fractions of intravenous and dermal doses excreted unchanged in urine in 24 hr.

$$F = \frac{AUC_{dermal} \times CL_{i.v.}}{Dose_{dermal}} \qquad \text{(Equation 1)}$$

$$F = \frac{Fe_{dermal}}{Fe_{i.v.}} \qquad \text{(Equation 2)}$$

Flux of ddI across skin was calculated using the following equation:

$$Flux = \frac{Cp_{avg} \times CL_{i.v.}}{A} \qquad \text{(Equation 3)}$$

where Cp_{avg} is the average plasma concentration over 24 hr, and A is the area for absorption (18).

The mass balance was determined based on the dose fraction in the systemic circulation, the fraction remaining in the skin at the application site, the fraction lost during formulation (on pill tile and spatula), and the fraction recovered from the occlusion wrap and outside the skin. The unpaired Student's t-test was used to evaluate the statistical significance.

RESULTS

Effect of formulation and occlusion. The octanol:water partition coefficient of ddI at 37°C was 0.0635 ± 0.007 (range 0.0544 to 0.0686, n = 3) at pH 7.0 and 0.0559 ± 0.006 (range 0.0498 to 0.0617, n = 3) at pH 8.5. This is similar to the previously reported value of 0.0679 at pH 7.3 (19). The effect of formulation and occlusion on absorption was studied in HFD rats. In a pilot experiment using a saline solution of ddI, plasma levels were about 1 μg/ml at 30 min and about 0.35 μg/ml at 24 hr. Better control of the dose application to a defined area was achieved by formulating ddI in an ointment. Direct mixing of crystalline ddI with the ointment base yielded poor bioavailability and no drug was detected in the plasma. With an o/w base, incorporation of ddI as a solution (pH ~ 8.5) or as a suspension (pH ~ 6.9) gave similar bioavailabilities of 28.9% and 28.3%. Incorporation of a ddI suspension in an w/o base gave a significantly lower bioavailability of 11.6%. Application of a transdermal delivery device (Hilltop Chamber) gave inadequate release of ddI (data not shown). Subsequent studies were conducted using ddI suspension or ddI solution incorporated in an o/w base.

Figure 1 shows the plasma concentration-time profiles after the application of an o/w base containing ddI as a solution or as a suspension without occlusion. The plasma profiles display several peaks and troughs in the initial phases of absorption. With the use of occlusion, more uniform plasma profile was obtained (Figure 2). However, the bioavailabilities were not significantly affected by occlusion (see below).

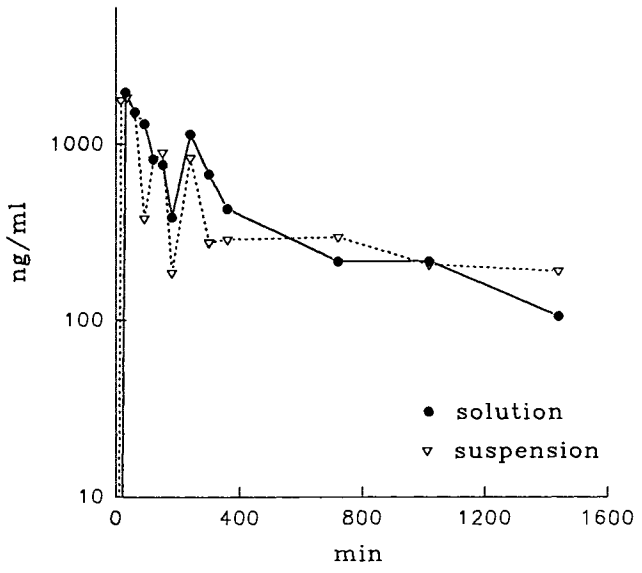

Figure 1. *Effect of formulation on the percutaneous absorption of ddI.* Forty to 50 mg of ddI either suspended or solubilized in methanol was incorporated in 1 g Unibase and applied to an 8 cm^2 area on the dorsal surface of HFD rats without occlusion. Data represent the average values of two rats in each group.

Pharmacokinetics of intravenous and dermal dose. The follicular density was 600 ± 75/cm^2 (n = 4) in HFD rats and 50 ± 35/cm^2 (n = 4) in LFD rats. Figure 2 (top panels) shows the mean plasma concentration-time profile of the intravenously administered [^{3}H]ddI in HFD and LFD rats. Similar to the data reported previously (15), computer analysis using a three compartmental model gave a correlation of 0.999 between the observed and the model predicted values, and the pharmacokinetic parameters obtained using compartmental and noncompartmental analysis were nearly identical (data not shown). The disposition of [^{3}H]ddI in HFD and LFD rats were similar (Table I).

The dermal application of ddI did not cause visible irritation in HFD and LFD rats. Figure 2 (bottom panels) shows the plasma concentration-time profiles of ddI dermally applied with occlusion. The ddI concentrations rose rapidly to peak plasma concentrations of about 1 μg/ml in 1 to 2 hr, declined to a plateau level of about 0.6 μg/ml from 4 to 16 hr, and further declined to about 0.4 μg/ml at 24 hr. The respective AUCs during the first 2 hr and from 4 to 16 hr were about 10% and 60% of the AUC over 24 hr.

Table II shows the bioavailability values and mass balance for the dermal studies using occlusion. The experi-

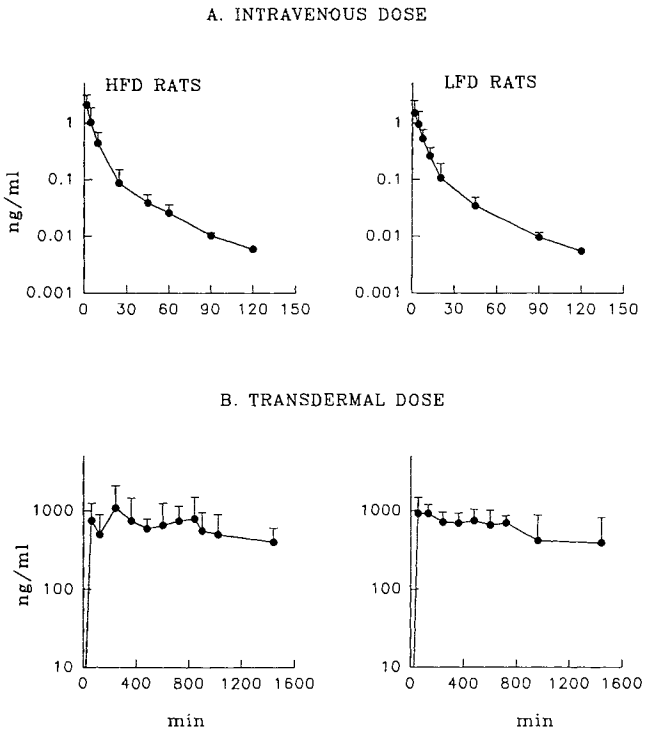

Figure 2. *Plasma concentration-time profile in HFD and LFD rats.* HFD (n = 6) and LFD (n = 5) rats received a dermal dose of ddI (180 mg/kg body weight) in 1 g ointment base (Unibase) applied to an 8 cm^2 area. Occlusion was used. Five min later, an intravenous tracer dose of [^{3}H]ddI was administered. Data represent mean + one SD. Note the differences in time scales for the intravenous and dermal data.

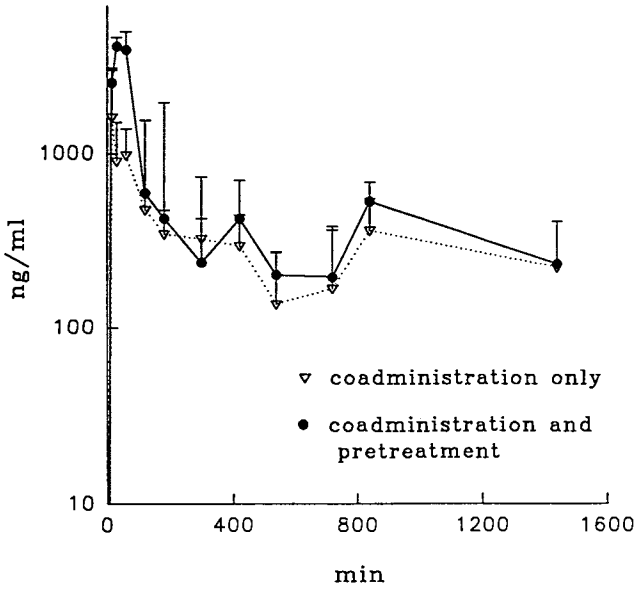

Figure 3. *Effect of penetration enhancers on the percutaneous absorption of ddI.* HFD rats received a 140 mg/kg dose of ddI suspended in a mixture of Azone:PG (5:95). The pretreated group received 500 μl of Azone:PG mixture 24 hr before application of ddI in Azone:PG (n = 4, circles). The control group received only ddI in Azone:PG (n = 5, triangles). The treatment area was 8 cm^2 and no occlusion was used. Data represent mean + one SD.

ments were terminated at 24 hr at which time the drug concentrations were still at 70% of the average level. In comparison, the intravenously administered [^{3}H]ddI declined exponentially with a terminal $t_{1/2}$ of 45 min. The relatively constant concentration derived from the dermal dose indicates continued absorption. The 24 hr bioavailability of the applied dose calculated using plasma data agreed with that calculated with the urine data, and was about 33% in both HFD and LFD rats. Mass balance accounted for about 80% of the dose in both groups of rats. The dose fraction found in the skin at 24 hr in the HFD rats was significantly higher than that in the LFD rats.

Effect of penetration enhancer. Figure 3 shows the effect of absorption enhancers. Upon coadministration with Azone:PG, the ddI concentration rose rapidly and peaked at 1 to 2 hr. The AUC during the first 2 hr was 15% of the AUC over 24 hr. In the rats that had additional Azone:PG pretreatment (pretreatment group), the peak concentrations occurring at 2 hr was 3 fold higher and the AUC during this time period was 47% of the AUC over 24 hr. The average plateau concentration was between 0.3 to 0.4 μg/ml in both groups. Compared to rats given ddI in o/w ointment base without enhancers, there was no difference in the plateau concentration attained with enhancer coadministration and/or pretreatment, when adjusted for the different applied doses. Coadministration of ddI with enhancers did not increase the bioavailability whereas the additional Azone:PG pretreatment significantly increased the bioavailability (Tables II and III).

Excretion of ddI in urine. The 24 hr urinary excretion of ddI after different treatments were compared. The Fe of the intravenous [^{3}H]ddI dose was similar to that found in our previous studies (15). The Fe after a ddI dose in o/w ointment with occlusion, or a ddI dose with enhancer coadministration and pretreatment but without occlusion, ranged from 4% to 7% of the applied dose. The ratio of Fe to the F calculated using plasma data represents the fraction of the bioavailable dermal dose excreted in the urine. This ratio remained constant at about 14% for all treatment groups, and is slightly lower than the Fe of 17% of the intravenous [^{3}H]ddI dose.

DISCUSSION

The goal of this study was to evaluate the dermal delivery route as an alternative to the oral route, to improve the systemic bioavailability and to maintain a constant therapeutic plasma level of ddI. The present study also evaluated the effects of formulation, occlusion, varying follicular densities, and penetration enhancers.

The oral bioavailability of an unbuffered solution of ddI (40 mg/kg) in rats was about 15% as shown in our laboratory and others (20,21). The present study shows that in rats, the dermal bioavailability of ddI over 24 hr was 33%, which was increased to 62% by coadministration and pretreatment with penetration enhancers. These bioavailability estimates are conservative values because the experiments were terminated at 24 hr, at which time the drug concentrations were at about 70% of the average level. In addition, a significant fraction of the dose (16%) was recovered in the skin at the end of the 24 hr experiment. Presumably, this amount represents an additional depot that eventually could be absorbed into the systemic circulation. This was confirmed in a separate study where 14% of the applied dose was recovered in the dermis layer (manuscript in preparation). Overall, the data suggests a final bioavailability of greater than 50% of

Table I. ***Pharmacokinetic parameters of intravenous dose of [^{3}H]ddI.*** The rats were given an intravenous injection of [^{3}H]ddI, at a dose of 20 μCi/rat, concomitantly with a dermal dose of 180 mg/kg. The parameters were calculated using noncompartmental analysis. Data are presented as mean ± SD. Values in parentheses indicate range.

Rat	$t_{1/2}$, α min	$t_{1/2}$, β min	$t_{1/2}$, γ min	Vd_{ss} ml/kg	CL ml/min/kg	Fe %
HFD (n = 6)	2.5 ± 1.1 (1.3–4.1)	5.7 ± 2.1 (4.4–9.8)	45 ± 19 (31.5–77.0)	890 ± 336 (685.6–1306)	67 ± 20 (53.7–103)	17 ± 7.8 (11.0–30.0)
LFD (n = 5)	2.0 ± 0.7 (1.2–2.3)	8.2 ± 1.2 (7.2–9.5)	42 ± 12 (30.5–55.7)	1087 ± 221 (867–1304)	80 ± 20 (53.3–108.2)	19 ± 3.7 (13.7–22.0)

Table II. ***Bioavailability and mass balance of dermally applied ddI.*** A dose of 180 mg/kg ddI incorporated in o/w ointment base was applied to the dorsal interscapular surface. The area was occluded with a plastic warp. The fraction of the dose recovered outside the skin was the sum of the amount remaining on the wrap and the amount removed by gently scraping the residual ointment from the surface of the application site. The fraction in the skin was the amount recovered from the methanolic skin rinses and the excised skin. Data are shown as mean ± SD. Values in parentheses indicate range. P is the level of significance obtained from the Student's t-test. NS, not significant.

Rat	Flux mg/kg/cm²/day	F, % applied dose	Fe, % applied dose	% loss in formulation	% recovery outside skin	% in skin	% accounted for
HFD (n = 6)	6.1 ± 2.2 (4.3–8.4)	32 ± 8.4 (24.6–44.3)	4.5 ± 2.1 (2.3–6.7)	12 ± 4.8 (8.6–18.1)	15 ± 6.1 (8.8–21.1)	20 ± 8.2 (12.7–30.0)	77 ± 10 (61.4–88.7)
LFD (n = 5)	7.1 ± 1.9 (4.4–9.1)	33 ± 12 (19.7–52.0)	5.1 ± 2.6 (3.0–8.7)	8.4 ± 3.5 (5.3–13.3)	24 ± 12 (10.2–39.9)	12 ± 4.2 (8.5–17.8)	79 ± 13 (59.1–88.9)
P	NS	NS	NS	NS	NS	0.05	NS

the topical dose, which was more than 3 times the oral bioavailability.

ddI incorporated in the o/w base as a methanolic suspension or as a solution gave similar bioavailabilities in the HFD rats, whereas a mixture of crystalline ddI in the o/w base showed negligible bioavailability. This suggests that the dissolution of ddI is necessary for dermal penetration, that the dissolution in the methanol and o/w ointment base was relatively rapid and was not rate limiting for absorption. The greater than 2 fold lower bioavailability of ddI in an w/o base may be due to poor partitioning of ddI from the w/o base into the skin. The reason for the significantly greater recovery of the dose in the skin at the application site in HFD rats as compared to the LFD rats is not known. One possibility is a greater amount of the drug adhering to or trapped in the hair follicles in HFD rats.

Literature reports have shown that occlusion occasionally increases percutaneous absorption due to increased skin temperature and hydration (22). An occlusive wrap may also prevent exfoliative losses of drug from the skin surface and pseudo-oral dosing via animal grooming. In the present study, the use of an occlusion did not significantly change the bioavailability or the fraction of the bioavailable dose excreted in the urine. The similar values of the Fe:F ratios after a dermal dose and the Fe of the intravenous [^{3}H]ddI dose rules out contamination of the urine by the shedding of drug-containing hair or pseudo-oral dosing.

The major pathways by which a drug may penetrate the skin are the trans-epidermal route through the stratum corneum, and the transfollicular route through hair follicles (23). Studies have shown that the skin of rabbits is most permeable followed closely by that of rats due to the high follicular density. For most compounds, percutaneous absorption in rabbits and in rats overpredicts the absorption in man (24). The current study compared the *in vivo* bioavailability in HFD and LFD rats. The major differences between these rats is the follicular density. Our observation of the similar bioavailability in HFD and LFD rats suggests that the transfollicular route is not the major transport route for ddI. However, it is conceivable that there may be other not-yet-reported structural differences between HFD and LFD rat skins that may affect percutaneous absorption. The pelage of the LFD rats bears a close resemblance to the distribution of human body hair and have been used extensively to study the transdermal migration of several drugs and carcinogens (25,26). The high bioavailability in LFD rats suggests that ddI may also be absorbed from human skin.

The percutaneous absorption of ddI is different from that of its structural analog, azidothymidine (AZT). *In vitro* studies using skin sections showed poor percutaneous absorption of AZT (18,27,28). Under *in vivo* conditions, AZT was not detected in blood following dermal administration to rats (18). The higher percutaneous absorption of ddI compared to AZT is unexpected as AZT is more lipophilic than

Table III. ***Effect of penetration enhancer.*** ddI was suspended in a vehicle of 5:95 Azone:PG and applied to HFD rats. The pretreated group received 500 μl of the vehicle 24 hr before application of ddI in Azone:PG, whereas the control group received only ddI in Azone:PG. No occlusion was used. Data presented are mean ± SD, with range shown in parentheses. P is the level of significance obtained from the Student's t-test. NS, not significant.

Treatment	Dose mg/kg	Cp_{max} (μg/ml)	AUC μg · min/ml	F % applied dose	Flux mg/kg/cm²/day	Fe % applied dose
Azone/PG Coadministration (n=5)	128 ± 12 (100–138)	1.8 ± 1.2 (0.7–3.5)	449 ± 118 (335–599)	27 ± 7 (20.0–36.2)	6.0 ± 1.7 (3.8–7.8)	3.5 ± 1.4 (2.9–5.5)
Azone/PG Pretreatment and coadministration (n=4)	124 ± 28 (117–138)	4.9 ± 0.8 (3.9–5.8)	948 ± 186 (669–1031)	62 ± 16 (45.0–89.1)	11.0 ± 3.8 (7.8–15.8)	7.0 ± 2.4 (4.6–9.0)
P	NS	0.005	0.0005	0.002	0.002	0.003

ddI indicated by the greater than 10 fold higher octanol:water partition coefficient of AZT (0.8) (29). The difference in the dermal bioavailabilities of AZT and ddI suggest different mechanisms of percutaneous absorption for these drugs. The percutaneous absorption of ddI is consistent with previous reports that the penetration of small polar compounds is through the "aqueous pores" in the stratum corneum, and may be dependent on the molecular volumes of the compounds and independent of the partition coefficient (30). AZT and ddI have been shown to have different transport mechanisms; AZT enters cells primarily by passive diffusion (31) whereas ddI permeates some cells partially by a nucleobase carrier and partially by passive diffusion (32). Active transport of purine nucleosides across epithelia has also been reported (33,34).

In the present study, the dermal dose was applied to an 8 cm^2 area. This application area is relatively large compared to the total surface area of a rat (~300 cm^2). For practicality and therapeutic application, it is necessary to increase the flux and to reduce the size of the application site. One approach is to use absorption enhancers. It has been shown that coadministration of drug with absorption enhancers may or may not significantly increase percutaneous absorption but pretreatment with enhancers can significantly increase penetration (18,35). The absorption enhancer, Azone, enhances the permeability of some lipophilic and hydrophilic drugs in human skin (36). Azone is water insoluble and its penetration across the stratum corneum is facilitated by the cosolvent PG (13). Our results showed that coadministration of ddI with 5% Azone in PG did not improve the bioavailability whereas additional pretreatment by the Azone:PG 24 hr before dosing significantly improved the flux and bioavailability. The enhancement by the pretreatment is consistent with literature data of a 10 to 12 hr lag time in the enhancement effect of Azone. It has been proposed that this lag time is due to a step-wise mechanism, i.e. the stratum corneum is first saturated with the cosolvent and subsequently with Azone (11), which causes structural disorder of the lipid layer (13).

The desired therapeutic concentration of ddI is 1 μg/ml (4). Results of the present study showed that topical administration of ddI gave peak concentrations at or above this level during the early time points indicating that the topical route can deliver the desired therapeutic concentration. The subsequent decline of concentration to about 0.6 μg/ml at later time points was in part due to reduced flux because of the extensive absorption and depletion of the dose. For example, about 50% of the bioavailable dose or 30% of the applied dose was absorbed after 2 hr in rats given enhancer pretreatment and coadministration. A sustained release formulation that maintains a constant flux may permit long term maintenance of the therapeutic concentration.

In conclusion, the topical route represents an attractive route to provide a high systemic bioavailability and for maintaining plateau viral inhibitory concentrations of ddI. Enhancement of the percutaneous absorption may be obtained by coadministration of suitable penetration enhancers.

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