Intestinal Absorption and First-Pass Elimination of 2′,3′-Dideoxynucleosides Following Oral Administration in Rats

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Intestinal absorption and first-pass elimination of 2′,3′-dideoxynucleosides (ddNs), including 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine (DDI) and 2′,3′-didehydro-3′-deoxythymidine (D4T), following oral administration was investigated in rats. Enzymatic degradation of ddNs in rat intestinal washing and in the intestinal homogenate showed them to be stable in the washing with half lives of more than 140 h, whereas degradation of DDI in the intestinal homogenate was more than ten times as rapid as those of AZT and D4T. Intestinal absorption was studied in three segments of the rat intestine (duodenum, jejunum and colon) using an *in situ* closed-loop method. The area under plasma ddN concentration curve (*AUC*) and the residual percent of dose 1 h after dosing indicated a greater absorption of AZT and D4T in the upper intestinal tract than in the colon, very poor absorption of DDI in all segments, and considerable absorption of AZT in the colon. The *AUC* and the mean residence time (*MRT*) of ddNs following four different routes (intravenous: i.v., intra portal vein: i.p.v., intra duodenal: i.d. and intra gastric: i.g.) were measured using the *in vivo* multiple sites of input method in rats. AZT and D4T were rapidly absorbed from the gastrointestinal tract and their bioavailability was more than 90%. DDI was less absorbed (33.02%) following i.d. administration compared with AZT and D4T. This poor absorption of DDI was partly attributable to its metabolism in the intestine.

Key words 2′,3′-dideoxynucleoside; intestinal absorption; AIDS

2′,3′-Dideoxynucleosides (ddNs), including approved anti-AIDS drugs, 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine (DDI) and 2′,3′-didehydro-3′-deoxythymidine (D4T), have exhibited potent impact against human immunodeficiency virus (HIV), although they are accompanied by several undesired side effects. AZT has produced dose-limiting bone marrow toxicity[1,2]; the major toxicities observed with DDI are peripheral neuropathy and pancreatitis.[3,4] The major dose-limiting toxicity of D4T is sensory peripheral neuropathy.[5,6] Thus, not only a single drug regimen but the coadministration of ddNs may be needed, since combining or alternating drugs is useful in reducing toxicity, exploring therapeutic synergy and lowering the risk of HIV resistance.[7–9]

Since anti-HIV therapy will have to continue for the life of the patient, the process of administration should be simple. Oral administration is a conventional route for systemic drug delivery; however, these ddNs have several drawbacks which prohibit successful drug delivery, such as rapid elimination[10,11] and low bioavailability.[12] It is desirable to design controlled release oral formulations which enable controlled and certain delivery of ddNs. This makes it necessary to understand the destiny of these ddNs before they reach the systemic circulation after their oral administration.

We have reported the absorption and elimination of ddNs for several years.[13,14] The present study was undertaken to investigate the intestinal absorption and elimination of ddNs in rats, including AZT, DDI and D4T, *in vitro*, *in situ* and *in vivo*. ddN metabolism was evaluated in rat intestinal homogenates and intestinal washing *in vitro*. In order to avoid the effect of intestinal content on metabolism and drug diffusion, an *in situ* closed-loop method was employed for a site dependency study. A method using multiple sites of input was used to determine *in vivo* absorption, and the *AUC* and *MRT* following four different routes were evaluated.

MATERIALS AND METHODS

Chemicals AZT was purchased from Yamasa Shoyu Co. (Chiba, Japan). DDI was purchased from Sigma Co. (St. Louis, MO, U.S.A.). D4T was synthesized from thymidine according to the procedure of Horwitz *et al.*[15] D4T was purified by silica-gel column chromatography and identified on the basis of its melting point, NMR and MS. 5-Fluoro-2′-deoxyuridine (FudR) was a gift from Yamasa Shoyu Co. (Chiba, Japan). All other chemicals were of reagent grade and were used as received.

Analytical Method The HPLC system used to determine the presence of the drugs in samples was composed of a Shimadzu LC-10AT pump, a Shimadzu SPD-10A UV detector, a Rheodyne 7125 injector and a reversed phase column (LiChrospher RP-18e, 250 × 4 mm). The mobile phase was a mixture of water, acetonitrile, and acetic acid at 85 : 15 : 0.2 for AZT, water containing 1 mM tetra-*n*-butyl ammonium phosphate as a counter ion, and acetonitrile at 92 : 8 for DDI and water, methanol, and acetic acid at 83 : 17 : 0.2 for D4T. The wavelength of the detection was 265 nm for AZT and D4T, and 254 nm for DDI. The eluant was pumped at a rate of 1.0 ml/min and ambient temperature was used.

Animal Protocols Male Wistar rats (body weight, 180—250 g) were fasted for 16 h, but were allowed free access to drinking water. The rats were anesthetized intraperitoneally with 25% (w/v) of ethyl carbamate (1 ml/200 g), and the left carotid was cannulated with polyethylene tubing (PE50, 0.58 mm, i.d., Becton Dick-

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inson Co.). The surgical procedure for the absorption study was carried out as follows.

***In Situ* Absorption Study** A closed-loop method[16] was used for this study. A midline abdominal incision allowed gentle exposure of the intestine. A 10 cm portion of the duodenum, jejunum or colon was rinsed well with pH 7.4 isotonic phosphate buffer (20 ml) and the two ends of the segments were tied to make a closed-loop. The biliary tract was tied to prevent any effect of enterohepatic circulation. Drugs (AZT, DDI and D4T) were dissolved at 44.6 μmol/ml in pH 7.4 isotonic phosphate buffer. One of the three drug solutions (1 ml/kg) was administered into the loop, with care taken not to cause any mucosal damage. The midline abdominal incision was covered with gauze pads which were moistened frequently with isotonic saline. Systemic artery blood (200 μl) was collected periodically in a heparinized tube and centrifuged at $9000 \times g$ for 3 min, then 50 μl of plasma was collected. Sixty min after dosing, the rats were decapitated, and the loop was excised and placed in hot saline (80 °C) to stop the enzymatic degradation of the drug. The loop was cut in the saline, the residual contents recovered, and these diluted to 50 ml with methanol. The loop was then homogenized in ice-cold saline and the homogenate-saline mixture was extracted with two 10 ml portions of methanol to collect the sorptional drugs in/on the loop. All samples were stored at −20 °C until being analyzed.

***In Vivo* Absorption Study** The multiple sites of input method[17] was used in this study. A midline abdominal incision was made and a 23-gauge needle threaded with polyethylene tubing (PE50) was connected to the portal vein for intra portal vein administration (i.p.v.), to the upper distal of the duodenum for intra duodenal administration (i.d.) or to the stomach for intra gastric administration (i.g.). The midline abdominal incision was sewn closed. Rats were administered one of the three drug solutions (50 μmol/ml) at a dose of 50 μmol/kg *via* one of the four different routes, the fourth being intravenously (i.v.). A drug solution was injected into the tail vein for i.v. administration. After dosing, systemic artery blood (200 μl) was collected periodically in a heparinized tube and centrifuged at $9000 \times g$ for 3 min, followed by the collection of 50 μl of plasma. Plasma samples were stored at −20 °C until analysis.

Sample Preparation The preparation of a plasma sample for AZT,[18] DDI[19] and D4T[20] was previously reported. Samples of the residual and sorptional amount of drug for an *in situ* absorption study were injected directly onto the HPLC column.

Metabolism in Intestinal Washing and Intestinal Homogenate Male Wistar rats were used for the *in vitro* metabolism studies. Animals were fasted for 16 h but allowed water *ad libitum*. Rats were sacrificed by decapitation, then the small intestine (from duodenum to ileum) was immediately removed and the mucosal side of the intestine was flushed/rinsed with pH 7.4 isotonic phosphate buffer (20 ml) and used as the intestinal washing. The intestine was slit open and the mucosal layer was peeled off with a razor. This layer was homogenized in a four-fold volume of ice-cold pH 7.4 isotonic phosphate buffer and was centrifuged at $100 \times g$ for 15 min at 0 °C. The supernatant was used as intestinal homogenate. Enzymatic metabolism was observed by the addition of 10 μl of stock solution (1 mg/ml: AZT, DDI, D4T or FudR) in pH 7.4 isotonic phosphate buffer to 1 ml of preincubated intestinal washing or intestinal homogenate at 37 °C. A 50 μl portion of the reaction mixture was collected periodically and deprotenized with the same volume of methanol. The resulting sample was injected onto the HPLC column, and the concentration of drug in each sample was determined. Susceptibility to enzymatic metabolism was evaluated on the basis of the pseudo-first-order rate constants obtained from the slopes of semilogarithmic plots of drug concentrations *versus* time.

RESULTS AND DISCUSSION

Susceptibility to Enzymatic Degradation *in Vitro* The enzymatic degradation rates of the ddNs and FudR, which is known as a substrate of nucleoside phosphorylase,[21] were measured in the presence of the rat intestinal washing (from duodenum to ileum) and the intestinal homogenate. Rate constants and half lives for the degradation are shown in Table 1. All of these drugs were quite stable in the washing, with half lives of more than 140 h. The effect of chemical degradation of the drugs was neglected because of their chemical stability under a neutral pH condition.[18,22-24] However, their enzymatic degradation in the intestinal lumen *in vivo* should be more rapid, because the washing was prepared with a large volume (20 ml) of pH 7.4 isotonic phosphate buffer, and the dilution would have been high.

The enzymatic degradation of these drugs in 20% (w/v) of the intestinal homogenate was more rapid than those in the intestinal washing. The site dependency of enzymatic degradation of ddNs in intestinal tissue showed no significant difference, except between the duodenum and colon in DDI.[25]

The susceptibility of FudR and DDI was compared with that of AZT and D4T; the half lives of the former two were 1.01 h and 3.58 h, respectively, and those values are more than ten times shorter than those of AZT and D4T. The activity of FudR is limited *in vivo*[26,27] because of its rapid degradation by pyrimidine nucleoside phosphorylase.[28] Although the type of enzyme which catalyzes the

Table 1. Enzymatic Reactivity of ddN

Compound	Intestinal washing		Intestinal homogenate	
	Rate constant $(h^{-1})^{a)}$	Half life $(h)^{b)}$	Rate constant $(h^{-1})^{a)}$	Half life $(h)^{b)}$
AZT	1.97×10^{-3} (5.6×10^{-4})	351.85	1.59×10^{-2} (3.90×10^{-3})	43.59
DDI	4.90×10^{-3} (1.18×10^{-3})	141.06	1.98×10^{-1} (2.20×10^{-3})	3.50
D4T	4.66×10^{-3} (1.65×10^{-3})	148.79	2.80×10^{-2} (5.00×10^{-4})	24.76
FudR	3.93×10^{-3} (8.90×10^{-4})	176.37	6.89×10^{-1} (3.00×10^{-4})	1.01

a) at 37 °C, *b*) at 37 °C. Values in parentheses indicate standard error of the mean value, $n=3$.

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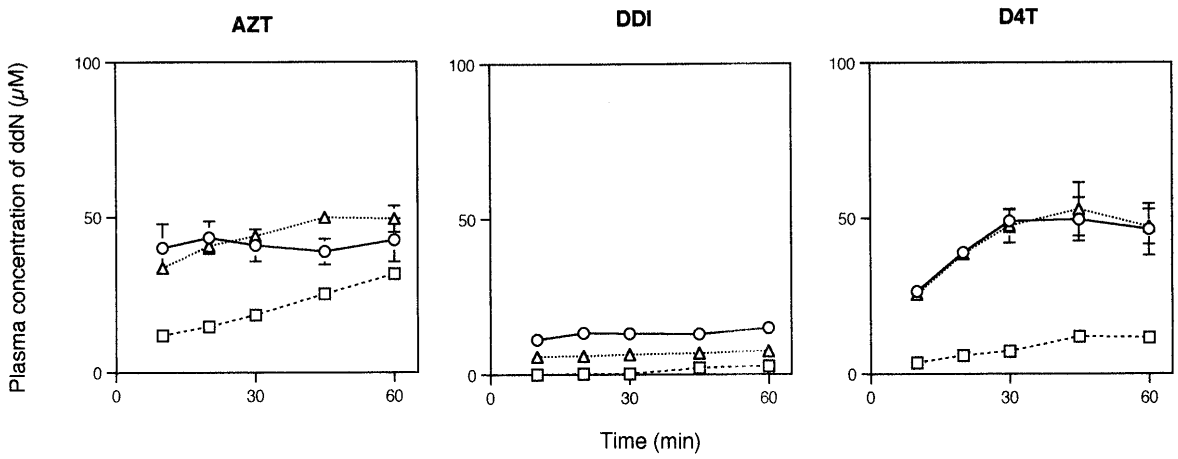

Fig. 1. Plasma Concentration–Time Profiles of ddNs in Rat *in Situ*

Following administration into the duodenum (○), jejunum (△) and colon (□), $n=3$. Vertical bars indicate the standard errors.

Table 2. ddN Absorption from the Loop

Compound	Site of the loop	Remaining at 60 min[a]	$AUC_{0\to60}$[b]
AZT	Duodenum	6.10 (3.44)	35.00 (5.50)
	Jejunum	10.78 (1.23)	40.00 (1.00)
	Colon	20.13 (3.90)	18.33 (2.00)
DDI	Duodenum	30.77 (4.82)	12.33 (1.17)
	Jejunum	63.72 (3.50)	5.83 (1.17)
	Colon	79.45 (6.34)	1.00 (0.50)
D4T	Duodenum	12.16 (3.44)	43.83 (0.83)
	Jejunum	14.39 (1.73)	39.83 (4.50)
	Colon	90.08 (9.08)	7.50 (1.00)

a) Sum of residual and sorptional percent of dose in/on the loop (%). *b*) Area under the curve of plasma ddN concentration–time from time zero to 60 min (μM·h). Values in parentheses indicate standard error of the mean value, $n=3$.

degradation of ddNs is unknown, since the metabolites of ddNs were not identified, DDI showed high susceptibility next to FudR in the intestinal homogenate. From these results, it is believed that the intestinal first pass elimination of DDI is responsible for its low oral bioavailability.

***In Situ* Absorption Study** Site dependency of rat intestinal mucosal transport of ddNs was investigated. Figure 1 shows plasma concentration–time profiles of ddNs, and Table 2 shows the $AUC_{0\to60}$ calculated from these profiles by trapezoidal rule and the sum of the residual and sorptional percent of the dose in/on the loop. The $AUC_{0\to60}$ following administration into the loop indicated that the transported amount in systemic circulation of AZT and D4T was greater in the upper intestinal tract (duodenum and jejunum) than in the colon. The $AUC_{0\to60}$ of DDI was rather small in all segments studied. The amount of residual and sorptional drug also showed similar tendencies. It was considered that the difference between AZT and D4T, and DDI was caused mainly by differences in their lipophilicity. The amount which disappeared from the loop is regarded as the amount transported into systemic circulation and the degradation amount in/on the loop and liver. The disappearance percent of DDI in the jejunum and colon was 36.28% and 20.55%, respectively, whereas the $AUC_{0\to60}$ of DDI following jejunum administration was approximately 6 times as high as that following colon administration. The cause of this inconsistency cannot be clarified, though the difference in diffusion time and metabolism in the intestinal membranes may partly attribute to the results.

AZT absorbed from colon was shown to be more than 2-fold and 18-fold in the *AUC* than those of D4T and DDI, respectively. The use of the closed-loop mesenteric-sampling *in situ* technique in rabbit resulted in minimal AZT absorption from the colon, because of its reduced surface area and drug diffusion to the absorbing membrane resulting from the increased viscosity of its luminal contents.[25] The difference in animals used and the presence of intestinal contents may have caused the different results. Our results indicate the intrinsic membrane transport of the drugs, since the metabolism and inhibition of drug diffusion by intestinal contents can be neglected in this study.

The evaluation of permeation through the silicon membrane (Silastic 500, 0.127 mm in thickness, Dow Corning Co.), in which transport occurs only by passive diffusion, showed that the permeation of AZT is 8 and 60 times higher than that of D4T and DDI, respectively (data not shown). Therefore, the higher lipophilicity of AZT[18] compared with D4T[20] and DDI[19] may partly reflect the higher colon absorption of the former drug.

***In Vivo* Absorption Study** Moment analysis was used for *in vivo* data treatment. At doses of 10—50 μmol/kg, the linear kinetics of these ddNs in systemic elimination were confirmed by the total body clearance following i.v. administration. It was assumed that the absorption of ddNs from the administration sites to systemic circulation was completed by the end of each experiment, because the slopes of the terminal phase of the plasma concentration–time profile following i.p.v., i.d. and i.g. administration were almost the same as those of i.v. administration (Fig. 2). Table 3 shows the *AUC*, *MRT* and absolute bioavailability following each administration.

All the ddNs administered by i.p.v. showed high bioavailability (>75%), and the *MRT* of these ddNs was almost the same as that following i.v. administration. After i.d. administration, the bioavailability of DDI was notably smaller (33%) than that of AZT or D4T (>90%). An increase in $MRT_{i.d.}$ from $MRT_{i.p.v.}$ was more evident in DDI compared with that in AZT and D4T. DDI is acid

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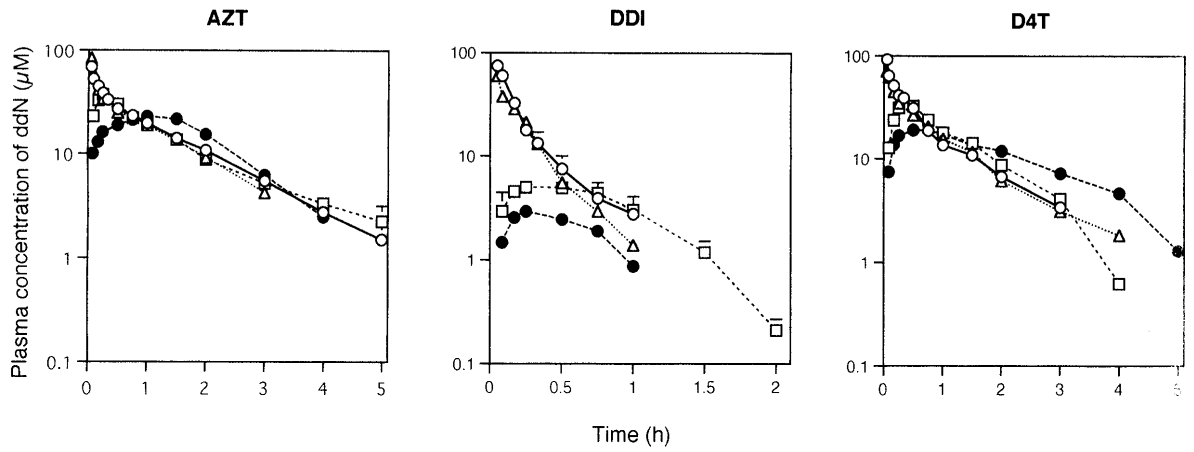

Fig. 2. Plasma Concentration–Time Profiles of ddNs in Rat *in Vivo*

Following i.v. (○), i.p.v. (△), i.d. (□), and i.g. (●), $n=3$. Vertical bars indicate the standard errors.

Table 3. Pharmacokinetic Parameters of ddN Following Administration

Compd.	Route of administration	$AUC^{a)}$	$MRT^{b)}$	$BA^{c)}$
AZT	i.v.	61.72 (2.53)	1.42 (0.14)	—
	i.p.v.	56.61 (4.56)	1.23 (0.06)[1]	91.72
	i.d.	56.62 (1.15)	1.54 (0.20)	91.73
	i.g.	56.36 (3.53)	1.71 (0.09)[1]	91.32
DDI	i.v.	17.20 (3.38)[2,4,5]	0.29 (0.06)[7,9]	—
	i.p.v.	13.46 (0.78)[3,6]	0.30 (0.03)[8,10]	78.26
	i.d.	5.68 (0.71)[4,5,6]	0.70 (0.05)[9,10]	33.02
	i.g.	2.35 (0.08)[2,3,4]	0.59 (0.02)[7,8]	13.66
D4T	i.v.	54.62 (6.58)	1.07 (0.08)[11,12]	—
	i.p.v.	50.93 (4.64)	1.21 (0.04)[13]	93.24
	i.d.	49.76 (2.75)	1.34 (0.04)[12,14]	91.10
	i.g.	50.58 (2.89)	1.89 (0.05)[11,13,14]	92.60

a) Area under the curve of plasma ddN concentration–time from time zero to infinity (μM·h). *b*) Mean residence time (h). *c*) Absolute bioavailability (%); Values in parentheses indicate standard error of the mean value, $n=3$. Matching superscripts (1, 2, 3—) indicate statistically significant differences ($p<0.05$) between data.

labile[24]; therefore the pH value of the site of i.d. administration was measured before and after the administration at 3 and 7 min, because the site was located in the upper end of the duodenum. The pH value changed from 6.18 to 6.81 and 6.93 at designated intervals. This indicated that the low bioavailability of DDI following i.d. administration was not attributable to its chemical degradation by the leakage of gastric juices.

The results of the *in situ* absorption study showed a large percentage of dosing DDI was unabsorbed and remained in the loop 1 h after dosing (Table 2). In contrast, the plasma concentration–time profile of DDI following i.d. administration *in vivo* suggested that the absorption was completed after 1.5 h (Fig.2). The difference between the *in situ* and *in vivo* results may have been due to the presence of intestinal contents, because in the *in situ* study, the inside of the loop was washed well before dosing. Bramer *et al.* reported the enzymatic degradation of DDI in intestinal contents, *e.g.*, microflora.[29] The half-life calculated from their data in standardized 100% fecal material was 1.033 h. They found that the metabolism of DDI in intestinal contents appears to be one of the major routes of pre-systemic first-pass elimination. The results of the *in situ* study also showed that DDI was best absorbed from the duodenum based on its *AUC* and residual percent; additionally, Sinko *et al.* reported site-specific intestinal absorption of DDI *in situ.*[30] These observations suggest that following i.d. administration, DDI moved to the lower intestine with decreasing absorption and increasing enzymatic degradation in the intestinal contents.

Following i.g. administration, the bioavailability of DDI was smaller (13.66%) than that of AZT and D4T (>90%). Differences between $MRT_{i.g.}$ and $MRT_{i.d.}$ were evident in D4T, although there was no significant difference in DDI. Larger $MRT_{i.g}$ than $MRT_{i.d.}$ was observed in AZT, though the difference was not statistically significant. Since the difference between $MRT_{i.g.}$ and $MRT_{i.d.}$ reflects the effect of gastric emptying time, GET, the small GET of DDI may be attributable to its acid lability, because GET can be expressed as: $1/(K_{degradation}+K_{GET_{int}})$, where $K_{degradation}$ indicates the rate constant of chemical degradation and $K_{GET_{int}}$ the rate constant of intrinsic gastric emptying time.

In conclusion, our results suggested that: 1) AZT was absorbed in a considerable amount from the colon. Therefore, the lower intestinal tract can become the target of an absorption site of AZT following oral administration. 2) D4T showed rapid absorption and high bioavailability after i.g. administration, although rapid elimination from plasma was similar to that after i.v. administration. This may suggest its higher absorbability in the upper intestinal tract. The sustained release dosage formulation of D4T which remained in the upper intestinal tract would provide better clinical treatment. 3) The present data also confirmed that DDI shows low bioavailability because of chemical degradation in an acidic environment and its metabolization in intestinal enzymes and/or contents. To overcome this, a combination of enteric coating and enzyme inhibitor, together with an adhesive dosage formulation on the upper intestinal tract, could be beneficial.

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