Prodrugs of 2',3'-Dideoxyinosine (DDI): Improved Oral Bioavailability *via* Hydrophobic Esters

Takeo Kawaguchi,[*,a] Tetsuya Hasegawa,[a] Toshinobu Seki,[a] Kazuhiko Juni,[a] Yasunori Morimoto,[a] Akira Miyakawa[b] and Mineo Saneyoshi[c]

Faculty of Pharmaceutical Sciences, Josai University,[a] 1–1 Keyakidai, Sakado, Saitama 350–02, Japan, Medical Services Center, Kyowa Hakko Kogyo Co.,[b] 1–6–1 Ohtemachi, Tokyo 100, Japan and Department of Biological Science, The Nishi-Tokyo University,[c] Uenohara, Kitatsuru, Yamanashi 409–01, Japan.　Received November 8, 1991

Five ester prodrugs of 2'3'-dideoxyinosine (DDI) were synthesized for the purpose of improving oral bioavailability. The prodrugs, acetate (C2-DDI), octanoate (C8-DDI), stearate (C18-DDI), benzoate (Bz-DDI), and hemisuccinate (Suc-DDI) were proved to quantitatively regenerate their parent drug by enzymatic hydrolysis. Though the chemical stability of the prodrugs under acidic conditions was not improved, their solubility in water was significantly decreased by esterification, except for Suc-DDI. Bioavailability was evaluated by oral administration to rats. Two hydrophobic prodrugs (C8-DDI and Bz-DDI) showed higher absolute bioavailability (23.5% and 31.0%, respectively) than did DDI (15.2%), though that of C2-DDI (11.5%) and Suc-DDI (4.5%) was poor.

Keywords　DDI; prodrug; dideoxynucleoside; AIDS; oral; administration; rat; *AUC*

Introduction

2',3'-Dideoxyinosine (DDI) is a purine dideoxynucleoside with potent activity against human immunodeficiency virus (HIV) in both T cells[1,2] and monocytes.[3] Like other dideoxynucleoside analogues (*e.g.*, zidovudine), DDI is converted through a series of reactions in the body to its active metabolite, 2',3'-dideoxyadenosine-5'-triphosphate, and in this form it is thought to inhibit HIV reverse transcriptase activity perferentially,[4,5] and thereby to suppress HIV infection by blocking the synthesis of a deoxyribonucleic acie (DNA) copy from the viral genome ribonucleic acid (RNA). Its mechanism of action is thought to be chain termination, competitive inhibition of reverse transcriptase, or both.[6] DDI appeared to have a higher *in vitro* therapeutic index than zidovudine,[1,2] and induced relatively little bone marrow toxicity in human hematopoietic progenitor cells.[7] In contrast to the results with zidovudine, the anti-HIV activity of DDI was not inhibited by naturally occurring deoxynucleosides.[8] These observations suggested that DDI was worth testing in patients with HIV infection, and several clinical studies have been done.[9–11] Though those clinical experiments have shown pharmacologically and/or toxicologically promising results, the problem of stability remains. DDI is quite labile to hydrolysis under acidic conditions on the C–N bond,[12] so that the drug has been administered with an antacid agent when given orally. In this report, we synthesized several ester prodrugs of DDI for the purpose of improving its bioavailability *via* oral administration.

Experimental

Analytical Instrumentation　Melting points were determined on a micro melting point apparatus (MP-S3, Yanagimoto, Kyoto, Japan) and are recorded uncorrected. Mass spectra (MS) were taken on a mass spectrometer (JMS DX-300, JEOL, Tokyo, Japan). Proton nuclear magnetic resonance (¹H-NMR) spectra (2 mg in 0.5 ml solvent) were obtained on an NMR spectrometer (JNM GX-270FT, JEOL) at 270 MHz, and chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard. A high-performance liquid chromatography (HPLC) system consisting of a pump (LC-9A, Shimadzu, Kyoto, Japan), a variable-wavelength detector (SPD-6A, Shimadzu), and a 20-μl fixed loop injector (model 7125, Rheodyne, Cotati, CA, U.S.A.) was used. Ultraviolet (UV) absorption spectra were taken on a Shimadzu 265 UV-VIS spectrophotometer.

Chemicals　DDI was purchased from Sigma Co. (St. Louis, MO,

U.S.A.). 5'-Esters of DDI (Fig. 1) were synthesized according to the method for the 5'-esters of zidovudine as reported previously.[13] Briefly, DDI suspension in anhydrous pyridine was treated at 4 °C with a corresponding acid anhydride and 4-dimethylaminopyridine. The mixture was held at room temperature overnight, then the pyridine was evaporated off and the residue was chromatographed on a silica gel column with a dichloromethane–ethanol mixture. The derivatives were more than 98% pure, as shown by one major peak by HPLC, and identified by nuclear magnetic resonance and mass spectrum.

5'-Acetyl-DDI (C2-DDI)　mp 181 °C. ¹H-NMR (CDCl₃) δ: 2.03 (s, 3H, CH₃), 2.13—2.18 (m, 2H, H-3'), 2.51—2.55 (m, 2H, H-2'), 4.22—4.26 (m, 2H, H-5'), 4.27—4.32 (m, 1H, H-4'), 6.26 (apparent t, *J*=4.40, 5.49 Hz, 1H, H-1'), 7.97 (s, 1H, H-8), 8.10 (s, 1H, H-2), 12.25 (br s, 1H, NH). UV λ$_{max}^{ethanol}$ nm: 245.5, 250.0. MS, *m/z*: 278 (M⁺).

5'-Octanoyl-DDI (C8-DDI)　mp 99 °C. ¹H-NMR (CDCl₃) δ: 0.86 (t, *J*=6.96, 6.59 Hz, 3H, CH₃), 1.27 (br s, 10H, (CH₂)C), 1.59—1.62 (m, 2H, (CH₂)CO), 2.08—2.19 (m, 2H, H-3'), 2.52—2.57 (m, 2H, H-2'), 4.25—4.44 (m, 3H, H-4',5'), 6.27—6.30 (m, 1H, H-1'), 8.02 (s, 1H, H-8), 8.09 (s, 1H, H-2), 11.87 (br s, 1H, NH). UV λ$_{max}^{ethanol}$ nm: 245.5, 250.0. MS *m/z*: 362 (M⁺).

5'-Stearoyl-DDI (C18-DDI)　mp 114 °C. ¹H-NMR (CDCl₃) δ: 0.88 (t, *J*=6.23, 6.96 Hz, 3H, CH₃), 1.25 (br s, 30H, (CH₂)C), 1.59 (br s, 2H, CH₂CO), 2.03—2.19 (m, 2H, H-3'), 2.52—2.61 (m, 2H, H-2'), 4.25—4.43 (m, 3H, H-4',5'), 6.27—6.30 (m, 1H, H-1'), 8.04 (s, 1H, H-8), 8.10 (s, 1H, H-2), 12.12 (br s, 1H, NH). UV λ$_{max}^{ethanol}$ nm: 245.5, 250.0. MS *m/z*: 502 (M⁺).

5'-Benzoyl-DDI (Bz-DDI)　mp 101 °C. ¹H-NMR (CDCl₃) δ: 2.08—2.19 (m, 2H, H-3'), 2.52—2.57 (m, 2H, H-2'), 4.27—4.32 (m, 1H, H-4'), 4.55—4.62 (m, 2H, H-5'), 6.27—6.30 (m, 1H, H-1'), 7.50—7.66 (m, 5H, C₆H₅), 8.02 (s, 1H, H-8), 8.09 (s, 1H, H-2), 11.98 (br s, 1H, NH). UV λ$_{max}^{ethanol}$ nm: 245.5, 250.0. MS *m/z*: 340 (M⁺).

5'-Succinoyl-DDI (Suc-DDI)　mp 99 °C. ¹H-NMR (CDCl₃–CD₃OD, 9:1) δ: 2.09—2.21 (m, 2H, H-3'), 2.52—2.63 (m, 6H, H-2', CH₂CO), 4.27—4.35 (m, 2H, H-5'), 4.37—4.48 (m, 1H, H-4'), 6.24—6.28 (m, 1H, H-1'), 7.93 (s, 1H, H-8), 8.14 (s, 1H, H-2). UV λ$_{max}^{ethanol}$ nm: 245.5, 250.0. MS *m/z*: 336 (M⁺).

Measurement of Solubility　An excess of each compound was added to a glass tube filled with distilled water (10 ml), and the water was stirred by a magnetic stirrer at 40 °C. An aliquot of the supernatant was withdrawn periodically, filtered through a membrane filter (Gelman Science, 0.45 μm), and the concentration of the compound measured by HPLC
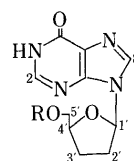
DDI	: R=H
C 2 -DDI	: R=COCH₃
C 8 -DDI	: R=CO(CH₂)₆CH₃
C18-DDI	: R=CO(CH₂)₁₆CH₃
Bz-DDI	: R=COC₆H₅
Suc-DDI	: R=CO(CH₂)₂COOH

Fig. 1.　Chemical Structures of DDI and Its Ester Prodrugs

(column: Nucleosil RP-18, 4.7 × 300 mm) at 250 nm with the mobile phase of water–CH$_3$CN (3 : 1) for C2-DDI, (3 : 7) for C8-DDI, (3 : 17) for C18, (13 : 7) for Bz-DDI, and a phosphate buffer (0.01 M, pH 7.0)–CH$_3$CN (96 : 4) for Suc-DDI. The solubility was determined as that point at which the change in concentration reached equilibrium. Hypoxanthine, supposedly a degradation product, was not detected by separate HPLC measurements in the experimental period (30—40 h).

Measurement of Partition Coefficient The apparent partition coefficient of the drugs was determined in a chloroform/water system at 24 °C.

Measurement of Hydrolysis Rates Chemical stability was evaluated in 0.05 N HCl at 25 °C. A reaction was initiated by the addition of a 10 μl stock solution (0.4 mM in ethanol) to a preheated HCl solution (2 ml) in a screw-capped glass tube. A 20 μl portion of the reaction mixture was periodically (at about 10 min intervals) injected into an HPLC column. The chemical stability was evaluated on the basis of the half lives obtained from the slopes of semilogarithmic plots of concentration vs. time.

Susceptibility to enzymatic hydrolysis was measured in the presence of three rat enzyme systems (liver, duodenum, and plasma) at 37 °C. Male Wistar rats (180—230 g) were obtained from Tokyo Laboratory Animal Co. (Tokyo, Japan) and were sacrificed to obtain the tissues. Their blood was centrifuged at 1000 × g for 15 min, and the resulting plasma was stored at −80 °C until use. Liver and duodenum were rinsed and homogenized with ice-cold saline to give a concentration of 5% (w/v), and the homogenates were stored at −80 °C until use. Homogenates were thawed 10 min before the experiments and diluted with an isotonic phosphate buffer (pH 7.0). Enzymatic hydrolysis was initiated by the addition of an ethanol solution of the drug (0.4 mM, 10 μl) to the preincubated homogenate (1 ml) at 37 °C. It was confirmed that 1% (v/v) ethanol had no measurable inhibitory effect on the enzyme activity. A 100 μl portion of the reaction mixture was withdrawn periodically, mixed with the same volume of CH$_3$CN for deproteinization, centrifuged at 3000 × g, and the supernatant was assayed for both the prodrug and DDI by HPLC. Susceptibility to enzymatic hydrolysis was evaluated on the basis of the pseudo-first-order rate constants obtained from the slopes of semilogarithmic plots of prodrug concentration vs. time.

In Vivo Studies DDI was administered to male Wister rats (180—220 g) intravenously (i.v.), orally (p.o.), and to the duodenum as a saline solution (42.4 mM) at a dose of 1 ml/kg. For the i.v. and p.o. experiments, a silicone catheter (PE50, 0.58 mm, i.d., Becton Dickinson) was inserted into the carotid of rats under anesthesia with sodium pentobarbital (50 mg/kg). The rats were fasted, but allowed free access to drinking water, in a Bollman cage for 16 h after the surgical operation to protect the inserted catheter from being gnawed. Blood samples (200 μl) were collected periodically from the catheter, centrifuged, and the resulting plasma was mixed with CH$_3$CN containing 1-(2-deoxy-3,5-epoxy-(β-D-threo-pentofuranosyl)thymine (1 μg/ml) as an internal standard. The DDI concentration was measured by HPLC with the mobile phase of water–CH$_3$CN (19 : 1). A separate experiment with anesthetized rats (sodium pentobarbital) was done for i.v. administration to clarify the effect of anesthesia on the kinetics of DDI. Intraduodenal administration was done by direct injection by surgically opening the abdominal region under sodium pentobarbital anesthesia. Blood samples were collected from the subclavian vein.

The prodrugs were administered orally (42.4 μmol/kg) to rats operated on as described above. Since the administration volume was the same (1 ml/kg), C8-DDI, C18-DDI and Bz-DDI were well ground (passed through a 280-mesh screen) and administered as suspensions in water because of their low water solubility, and the others (C2-DDI and Suc-DDI) were administered as solutions. Blood was collected and treated as described above.

Results and Discussion

Table I shows the physicochemical properties of DDI and its ester prodrugs. The very high melting point of DDI (>270 °C) was decreased by modification, though the effect was mininal in acetylation. The partition coefficients increased and the solubility in water decreased with the modification, except for in Suc-DDI, which showed very high solubility while the partition coefficient was still higher than that of DDI. The column on the far right in Table I shows the chemical stability of the derivatives at 25 °C. Since the solubility of C18-DDI is

TABLE I. Physicochemical Properties of DDI and Its 5'-Ester Prodrugs

Compound	Molecular weight	mp (°C)	Solubility[a] (μg/ml)	Partition coefficient[b]	Chemical stability[c]
DDI	236	>270	27300	−1.88	0.228[d] 2962[e]
C2-DDI	278	181	10400	−0.54	0.225[f] >2000[g]
C8-DDI	362	99	273	1.17	0.212[f] >2000[g]
C18-DDI	502	114	0.21	5.19	0.237[f] >2000[g]
Bz-DDI	340	101	442	1.08	0.215[f] >2000[g]
Suc-DDI	336	99	100000	−1.50	0.225[f] >2000[g]

a) In water at 40 °C. b) Chloroform/water at 24 °C. c) Half-life (h) of pseudo-first-order reaction. d) pH 2.34 chloroformate buffer at 25 °C, reported by Anderson et al.[12] e) pH 6.63 phosphate buffer at 25 °C, lit.[12] f) 0.05 N HCl at 25 °C, pH 1.49. g) pH 7.0 phosphate buffer at 25 °C.

TABLE II. Susceptibility of the DDI Prodrugs to Enzymatic Hydrolysis[a]

Compound	Liver		Duodenum		Plasma	
C2-DDI	0.59	(0.04)	1.63	(0.18)	1.95	(0.08)
C8-DDI	0.0021	(0.0003)	0.0051	(0.0002)	0.015	(0.003)
C18-DDI	0.17	(0.03)	0.65	(0.01)	0.11	(0.02)
Bz-DDI	0.042	(0.012)	0.027	(0.001)	51.3	(0.8)
Suc-DDI	1.94	(0.15)	12.6	(0.9)	315	(44)

a) Half-life (min) at 37 °C; values in parentheses indicate standard error of the mean value, $n = 4$.
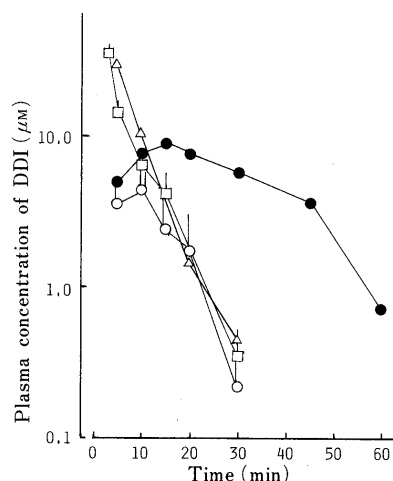

Fig. 2. Plasma Concentration–Time Profiles of DDI in Rat

Following i.v. (□), i.v. with anesthesia (△), p.o. (○) and intraduodenal (●), $n = 3$. Vertical bars indicate the standard errors.

very low (0.21 μg/ml), degradation should not involve a pseudo-first-order-reaction for the compound. The 5'-position seems to play a minor role in the chemical stability of the glycosyl bond of DDI, so no improvement was observed in its stability under acidic conditions by esterification on the 5'-position.

Table II shows the susceptibility of the ester prodrugs to enzymatic hydrolysis. Because of variation in susceptibility among the esters, each experiment was done using arbitrary enzyme concentrations with appropriate half-lives (1—1.5 h), and standardized 100% to homogenate or plasma. The hydrolysis of the prodrugs resulted in quantitative regeneration of their parent drug, DDI. C8-DDI showed the highest susceptibility and the dicarboxylic hemiester (Suc-DDI) the lowest of all the enzyme systems studied. This order of susceptibility to enzymatic hydrolysis is similar to that in other dideoxynucleoside esters such as
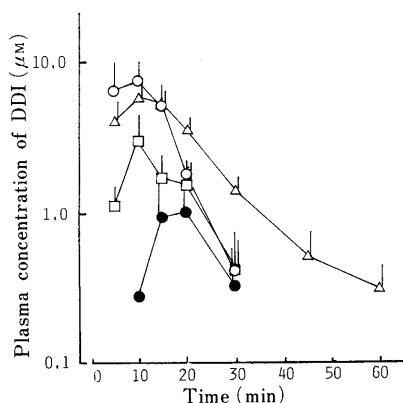
Fig. 3. Plasma Concentration–Time Profiles of DDI in Rat

Following *p.o.* administration of C2-DDI, $n=4$ (□), C8-DDI, $n=5$ (△), Bz-DDI, $n=5$ (○), and Suc-DDI, $n=3$ (●). Vertical bars indicate the standard errors.

$2',3'$-didehydro-$3'$-deoxythymidine esters[14,15] and zidovudine.[13]

Figure 2 shows plasma concentration–time profiles of DDI after administration by three different routes (*p.o.*, i.v., and intraduodenal) with the same dose of DDI (42.4 μmol/kg). The area under the blood concentration–time curve (*AUC*) was calculated by the trapezoidal rule-extrapolation method. An *AUC* of i.v. administration was $448.2 \pm 85.3 \mu$M min calculated from a two-compartment model (elimination rate constant, k_{el}: α phase, 1.076 ± 0.082 min^{-1}; β phase, 0.383 ± 0.032 min^{-1}). No effect of anesthesia was observed on the pharmacokinetic parameters following i.v. administration (Fig. 2). Absolute bioavailability of *p.o.* administration was only 15.2%, while that of intraduodenal was 70.0%. This observation suggests that the low bioavailability following oral administration is attributable, as expected, to the considerable degradation in the stomach. Relatively prolonged plasma concentration following intraduodenal administration may indicate that absorption is saturated or that a site-specific, protein-mediated transport is responsible for the nucleoside analogue,[16–18] though the absorption rate of DDI has to be directly measured for further discussion.

Figure 3 shows DDI concentration–time profiles in plasma following oral administration of the DDI prodrugs. The prodrugs themselves were not detected in the plasma in these experiments. Relative bioavailability of two of the hydrophobic esters, C8-DDI (32.0%, $AUC = 143.2 \pm 18.0$ μM min) and Bz-DDI (31.0%, $AUC = 139.0 + 42.6$ μM min) was higher than that of DDI (15.2%, $AUC = 68.2 \pm 7.0$ μM min), and could be attributable to their low water solubility or to some form of protection from chemical degradation, since the most of the dose is supposed to remain as solid in the stomach solution. Significant differences (Student's *t*-test) were observed between C8-DDI and DDI ($p < 0.01$), and between Bz-DDI and DDI ($p < 0.05$). On the other hand, the water soluble or hydrophilic esters C2-DDI and Suc-DDI showed poor bioavailability (11.5%, $AUC = 51.7 \pm 6.7 \mu$M min and 4.5%, $AUC = 20.1 \pm 10.8 \mu$M min, respectively). DDI was not detected by the administration of the most hydrophobic ester, C18-DDI. Its very low

solubility did not allow the ester to regenerate any great amount of its parent-drug by enzymatic hydrolysis, though the ester demonstrated susceptibility to hydrolysis at a very low concentration (Table II); C18-DDI itself may not be absorbed well from the gastric and intestinal tract. Though the chemical stability of Suc-DDI in an acidic condition is comparable to that of DDI, its bioavailability was even lower than DDI. Since Suc-DDI has the least susceptibility to enzymatic hydrolysis, Suc-DDI may not regenerate DDI even if some amount can go through the stomach; and Suc-DDI itself may not be absorbed from the intestinal tract, posssibly because of its negative change and/or far different chemical structure compared to a natural nucleoside.

Though the behavior of C18-DDI and Suc-DDI remains to be explained, the hydrophobicity and susceptibility to enzymatic regeneration of these ester prodrugs seem to be important in their improved bioavailability. The prodrugs may go through the stomach as an intact prodrug because of high hydrophobicity or low water solubility, enzymatically regenerate DDI at the intestinal tract according to its susceptibility, then be absorbed as DDI. The absorption mechanism of DDI is now being investigated and will be reported in the near future.

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