IN VIVO PHARMACOKINETICS AND METABOLISM OF ANTI-HUMAN IMMUNODEFICIENCY VIRUS AGENT D4T-5'-[p-BROMOPHENYL METHOXYALANINYL PHOSPHATE] (SAMPIDINE) IN MICE

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ABSTRACT:

Sampidine, d4T-5'-[p-Sampidine, bromophenyl methoxyalaninyl phosphate] (HI-113), a novel aryl phosphate derivative of stavudine (d4T), is substantially more potent than d4T in inhibiting HIV replication in human peripheral blood mononuclear cells or thymidine kinase-deficient T cells (Venkatachalam et al., 1998; Vig et al., 1998; Uckun and Vig, 2000). HI-113 has been shown to inhibit the replication of HIV-2 and zidovudine (ZVD/AZT)-resistant HIV-1 strains in human peripheral blood mononuclear cells at nanomolar concentrations (Venkatachalam et al., 1998; Vig et al., 1998; Uckun and Vig, 2000). Previous results have also demonstrated that the presence of a single para-bromine group in the phenyl moiety of HI-113 markedly enhances its ability to undergo hydrolysis and thereby produce substantially more of the key metabolite alaninyl-d4T-monophosphate (ala-d4T-MP) and d4T. The T_{max} values for ala-d4T-MP and d4T derived from intravenously administered HI-113 were 5.1 and 17.4 min, respectively. The elimination half-life for synthetic ala-d4T-MP was 38.9 min after intravenous administration. Ala-d4T-MP was metabolized to form d4T (T_{max} = 5.0 min). The elimination half-life of d4T derived from intravenously administered ala-d4T-MP (32.4 min) was similar to the elimination half-life of intravenously administered d4T (26.6 min). In contrast, the elimination half-life of ala-d4T-MP derived from HI-113 (138.8 min) was markedly longer than the elimination half-life of ala-d4T-MP given intravenously (38.9 min). Following oral administration of HI-113, the elimination half-lives of ala-d4T-MP (56.1 min) and d4T (102.6 min) were also prolonged.

Materials and Methods

Chemicals. We used HPLC-grade reagents and deionized distilled water. Acetonitrile was purchased from Burdick & Jackson Company (Muskegon, MI). Hydrochloric acid was purchased from Fisher Scientific (Fair Lawn, NJ). Ammonium phosphate and phosphoric acid were purchased from Sigma (St. Louis, MO). The synthetic procedures for the preparation of HI-113, ala-d4T-MP, and d4T (structures shown in Fig. 1) have been previously described in detail (Venkatachalam et al., 1998; Vig et al., 1998; Uckun and Vig, 2000).

Quantitative HPLC for Detection of HI-113 and Its Metabolites. Each plasma sample (200 μl) was mixed 1:4 with acetone (800 μl) and vortexed for at least 30 s. Following centrifugation, the supernatant was transferred into a clean tube and dried under nitrogen. A 50-μl solution of 50% methanol in 200 mM HCl was used to reconstitute the extract residue, and 40 μl of the reconstituted sample was subjected to analytical HPLC. The HPLC system used for these studies was a Hewlett Packard (Palo Alto, CA) series 1100 instrument equipped with a quaternary pump, an autosampler, an automatic electronic degasser, an automatic thermostatic column compartment, a diode array detector, and a computer with Chemstation software for data analysis (Chen et al., 1999a,c,e). The analytical column was a Zorbax SB-Phenyl (250 × 4.6 mm; 5 μm; Hewlett Packard) column attached to a guard column (Hewlett Packard). The column was equilibrated prior to data collection. The linear gradient mobile phase (flow rate = 1.0 ml/min) was 100% A/0% B at 0 min, 88% A/12% B at 20 min, and 8% A/92% B at 30 min (A: 10 mM...
ammonium phosphate buffer, pH 3.7; B: acetonitrile). The detection wavelength was 268 nm. The peak width, response time, and slit were set at >0.03 min, 0.5 s, and 4 nm, respectively.

**Stability of HI-113 and ala-d4T-MP in Plasma.** Plasma samples were spiked with HI-113 and ala-d4T-MP to yield final concentrations of 250 μM for HI-113 and 100 μM for ala-d4T-MP. Spiked plasma samples were stored at −20°C. At a predetermined time, an aliquot (100 μl) of the spiked plasma sample was extracted by adding 400 μl of acetonitrile to induce the precipitation of proteins.

**Stability of HI-113 in Plasma in Presence of Selective Esterase Inhibitors.** Plasma samples were preincubated with the esterase inhibitors paraoxon (final concentration = 0.1 mM), physo stagmine (final concentration = 0.1 mM), and EDTA (final concentration = 1 M) at 37°C for 30 min. Subsequently, HI-113 was added to yield a final concentration of 250 μM. At a predetermined time, an aliquot (100 μl) of the spiked plasma sample was extracted by adding 400 μl of acetonitrile to induce the precipitation of proteins.

**Stability of HI-113 in Murine Liver Homogenates.** Freshly obtained livers of female BALB/c mice were homogenized in 1× phosphate-buffered saline (1:1, w/v) by using a Polytron (PT-MR2000) homogenizer (Kinematical AG, Littau, Switzerland). HI-113 was added to the liver homogenate to yield a final concentration of 100 μM and was incubated at 37°C. At a predetermined time, an aliquot (100 μl) of the spiked liver homogenate sample was extracted by adding 400 μl of acetonitrile to induce the precipitation of proteins.

**Stability of HI-113 and ala-d4T-MP in Gastric and Intestinal Fluids.** The simulated gastric and intestinal fluids that were prepared following the U.S. Pharmacopeia XXII methods were spiked with HI-113 and ala-d4T-MP to yield final concentrations of 100 μM for each compound. The spiked fluids were then placed in a 37°C water bath. At a predetermined time, 100-μl aliquots of the spiked gastric or intestinal fluid were extracted by adding 400 μl of acetonitrile.

**Pharmacokinetic Studies in Mice.** Female BALB/c mice (6–8 weeks old) (Tacconic, Germantown, NY) were housed in a United States Department of Agriculture-accredited animal care facility under standard environmental conditions (12-h light/12-h dark photoperiod, 22 ± 1°C, 60 ± 10% relative humidity). All rodents were housed in microisolator cages (Lab Products, Inc., Maywood, NJ) containing autoclaved bedding. Mice were allowed free access to autoclaved pellet food and tap water throughout the study. All animal studies are approved by the Parker Hughes Institute Animal Care and Use Committee, and all animal care procedures conformed to the principles outlined in the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, Washington DC, 1996).

A 50-μl solution of HI-113 (100 mg/kg) dissolved in dimethyl sulfoxide was administered i.v. via the tail vein. This volume of dimethyl sulfoxide is well tolerated by mice when administered by rapid i.v. or intraperitoneal injection (Rosenkrautz et al., 1963; Wilson et al., 1965). Four to five mice per time point were used for pharmacokinetic studies. Blood samples (~500 μl) were obtained from the ocular venous plexus by retro-orbital venipuncture at 0, 2, 5, 10, 15, 30, 45, 60, 120, 240, and 360 min after i.v. injection. To study the pharmacokinetics of ala-d4T-MP and d4T following systemic administration of these compounds, mice were injected with 75 mg/kg ala-d4T-MP and 40 mg/kg d4T, respectively (these doses are equimolar to the 100-mg/kg dose of HI-113).

To determine the pharmacokinetics of HI-113 following its oral administration, 12-h fasted mice were given a bolus dose of 100 mg/kg HI-113 via gavage using a no. 21 stainless steel ball-tipped feeding needle. Blood sampling time points were 0, 2, 5, 10, 15, 30, 45, 60, 120, 240, and 360 min after the gavage.

All collected blood samples were heparinized and centrifuged at 7000g for 2 min to separate the plasma fraction from the whole blood. The plasma samples were then processed immediately using the extraction procedure described above.

**Pharmacokinetic Analysis.** Pharmacokinetic modeling and parameter calculations were carried out using the WinNonlin Professional version 3.0 (Pharsight, Inc., Mountain, CA) pharmacokinetics software (Chen et al., 1999b,d,f; Uckun et al., 1999a,b). An appropriate model was chosen on the basis of the lowest sum of weighted squared residuals, the lowest Schwartz Criterion, the lowest Akaikes Information Criterion value, the lowest standard errors of the fitted parameters, and the dispersion of the residuals. F test was also used to discriminate between these hierarchical models (Gabrielsson and Weiner, 1997). The elimination half-life was estimated by linear regression analysis of the terminal phase of the plasma concentration-time profile. The area under the concentration-time curve (AUC) was calculated according to the linear trapezoidal rule between the first sampling time (0 h) and the last sampling time plus Ck, where C is the concentration of the last sampling and k is the elimination rate constant. The systemic clearance (CL) was determined by dividing the dose by the AUC. The clearance of each metabolite was estimated by simultaneous fitting of the concentration versus time curves of the parent drug and metabolites to pharmacokinetic models specified as a system of differential equations (Gabrielsson and Weiner, 1997).

**Results**

**Chromatographic Separation of HI-113 and Potential Metabolites of HI-113.** We established standard HPLC conditions for simultaneous separation of HI-113 and its metabolites ala-d4T-MP and d4T in plasma. Using the chromatographic separation conditions described under *Materials and Methods*, the retention times (R<sub>t</sub>) measured for HI-113 and its metabolites in spiked samples were 28.7 ± 0.02 min (HI-113A; n = 13; Fig. 2B), 28.9 ± 0.02 min (HI-113B; n = 13; Fig. 2C), 15.3 ± 0.2 min (ala-d4T-MP; n = 30), and 18.5 ± 0.1 min (d4T; n = 30), respectively. At these retention times, no significant interference peaks were observed in the blank plasma samples (Fig. 2, A and B). There was another peak eluting at approximately 27 min (Fig. 2B). The chemical identity of this peak, which may represent another metabolite of HI-113, is currently unknown.

The hydrochloric acid component of the reconstituted solutions plays a key role in the chromatography of HI-113 and its metabolites, as the acid protonates ala-d4T-MP and no peak appears in the chromatogram for this metabolite if there is no hydrochloric acid in the reconstituted solution. The acidic solution renders ala-d4T-MP less stable, however. Therefore, all of the extracted samples were analyzed immediately after reconstitution.

The HPLC methods for quantitation of the plasma levels of the parent compound HI-113 and its metabolites were fully validated using previously published protocols (Chen et al., 1999a,c,e). The lowest limit of detection was 0.25 μM at signal-to-noise ratio of ~4. Good linearity (r > 0.995) was observed between concentrations ranging from 0.5 to 12.5 μM and from 12.5 to 100 μM in 200 μl of plasma (standard curves and linear equations are not shown). Intra- and interassay variabilities were less than 8%.

**Stability of HI-113 and ala-d4T-MP.** The results shown in Fig. 3A indicate that HI-113 is unstable in plasma. Following incubation with plasma, >95% of HI-113 decomposes within 5 min (Table 1).
The decomposition of HI-113 in the plasma samples was complete within 30 min. Hence, immediate extraction of the samples is required after collection to accurately measure the HI-113 levels. In contrast, ala-d4T-MP was stable in the plasma for at least 24 h.

Decomposition of HI-113 in plasma was significantly inhibited by paraoxon, partially inhibited by physostigmine, but not affected by EDTA (Table 1). HI-113 decomposed after incubation with the liver homogenate within 30 min as it did in plasma (Fig. 3B). However, unlike in plasma, significant amounts of d4T were detected after incubation of HI-113 with the liver homogenate.

The results of the stability studies also revealed that HI-113 is relatively stable in gastric fluid for 8 h, but it is not stable in intestinal fluid (Fig. 3, C and D). HI-113 quickly decomposed to yield ala-d4T-MP in intestinal fluid (approximately 94% of the HI-113 decomposed within 2 h). Ala-d4T-MP was stable in intestinal fluid yielding only trace amounts of d4T as a metabolite.

Metabolism and Pharmacokinetic Profile of HI-113 Following Intravenous Administration. Following intravenous administration, HI-113 (100 mg/kg) was metabolized to yield HI-113-M1 ($R_T = 15.3$ min) and HI-113-M2 ($R_T = 18.5$ min) (Fig. 2C). HI-113-M1 had the same retention time as ala-d4T-MP, whereas HI-113-M2 had the same retention time as d4T (Fig. 2, B and C). The UV spectra of these two metabolites were identical to those of ala-d4T-MP (spectrum match factor representing the degree of similarity between the spectra was 996) and d4T (spectrum match factor = 998), respectively.

The plasma concentration versus time curves of nonmetabolized HI-113, ala-d4T-MP, and d4T after i.v. injection of HI-113 were mono-, bi-, and monoexponential, respectively. The initial model (model 1) was tentatively assigned as illustrated in Fig. 4A. After fitting model 1 to the pooled plasma concentration versus time data, we found that the coefficient of variation for some of the parameters such as $Cl_{m1}$ was over 100%. Model 2 with no $Cl_{m1}$ resulted in even worse $CV (>100\%)$ for some parameters and the predicted curves were not good fits for the plasma concentration versus time data. Since the parent HI-113 was so easy to be hydrolyzed, $Cl_{m1}$ was omitted from model 2 to obtain model 3, or omitted from model 1 to obtain model 4. However, curve fitting and CV for the various parameters did not improve after these changes. When we assumed no $Cl_{p}$, and also assumed that d4T was not formed directly from the parent HI-113 (no $Cl_{m2}$) (model 5), model fitting and CV for several parameters did not improve (Fig. 4B). Model 6 (neither $Cl_{p}$ nor $Cl_{m1}$) was found to better fit the observed data. The calculated $F$ value of 0.1 (comparison of model 1 and model 6) is smaller than the $F$ value (~4.0) found in the $F$ distribution table (McClave and Dietrich, 1979), suggesting that model 1 does not provide a better fit for the data than model 6. Therefore, model 6 depicted in Fig. 5A was used to describe the metabolite pharmacokinetics of ala-d4T-MP and d4T after i.v. injection of HI-113. According to this model, HI-113 is biotransformed to produce ala-d4T-MP ($Cl_{m1}$) and d4T ($Cl_{m2}$), respectively. Ala-d4T-MP derived from HI-113 can be further metabolized to form d4T ($Cl_{m12}$) or distributed to the extravascular compartment ($Cl_{m1a}$). D4T produced from either HI-113 or ala-d4T-MP is finally eliminated from the body ($Cl_{m22}$). The metabolic clearance of HI-113 and the formation clearance of the metabolites were determined to be 83.9 ml/min/kg for ala-d4T-MP and 87.4 ml/min/kg for d4T, respectively. The metabolic clearance of ala-d4T-MP and the formation clearance of its metabolite, d4T, were found to be 36.1 ml/min/kg. A small portion of ala-d4T-MP was distributed to the extravascular compartment with a $Cl_{m1d}$ of 47.1 ml/min/kg. Finally, d4T was eliminated with a $Cl_{m2}$ of 62.0 ml/min/kg.

The estimated pharmacokinetic parameter values are presented in Table 2. HI-113 had a $C_{max}$ of 211.6 μM and an AUC of 1071.8 μM·min. The CL of HI-113 was 174.5 ml/min/kg, which is approximately twice as fast as the rate of blood flow to the kidney or the liver (Davis and Morris, 1993). HI-113 had a steady-state volume of distribution ($V_{ss}$) of 920.0 ml/kg, which is roughly equal to the total volume of
water in the body (Davies and Morris, 1993). HI-113 had a short elimination half-life ($t_{1/2} = 3.6$ min).

The diastereoisomers of HI-113 can be separated using the HPLC method described above (the retention times are 28.7 and 28.9 min). The extinction coefficients were $8,686 \pm 96 \, \text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ for HI-113A and $10,802 \pm 81 \, \text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ for HI-113B. It is interesting to note that HI-113-A is metabolized more quickly than HI-113-B (Fig. 2C). The pharmacokinetic features of these two diastereoisomers are summarized in Table 2. HI-113-B had a slightly longer elimination half-life than the HI-113-A diastereoisomer ($4.3 \, \text{min}$) versus $2.6 \, \text{min}$), which may be due to the faster clearance of HI-113-A ($138.8 \, \text{min}$) and d4T ($17.4 \, \text{min}$; Table 2). The systemic clearance of ala-d4T-MP was $69.3 \, \text{ml/min/kg}$, which is equimolar to the 100-mg/kg dose of HI-113, the plasma concentration of d4T as a function of time can best be described by a one-compartment model (Fig. 7). The estimated pharmacokinetic parameter values are presented in Table 2. The estimated $C_{\text{max}}$ values for ala-d4T-MP and d4T were $11,761.5 \, \text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ and $1.03 \times 10^{5} \, \text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, respectively. The estimated AUC values were $318.9$ and $12,173.6 \, \text{mg} \cdot \text{h} \cdot \text{kg}^{-1}$, respectively. d4T had a short elimination half-life ($26.6 \, \text{min}$). The systemic clearance of ala-d4T-MP was slow with a CL of only $15.2 \, \text{ml/min/kg}$, which is much less than the blood flow to either the kidney or the liver (Davies and Morris, 1993). Ala-d4T-MP also had a small volume of distribution ($V_{\text{ss}}$) that is less than the total volume of water in the body (Davies and Morris, 1993). Nevertheless, the elimination half-life of ala-d4T-MP is biotransformed to form d4T.

**Pharmacokinetic Profile of d4T Following Intravenous Administration.** Following i.v. injection of ala-d4T-MP (75 mg/kg, a dose equivalent to the 100-mg/kg dose of HI-113 discussed above), the concentration versus time curves of its major metabolites ala-d4T-MP and d4T were bi- and monoeponential, respectively. The pharmacokinetic model depicted in Fig. 6A best described the pharmacokinetics of ala-d4T-MP after i.v. administration. According to this model, ala-d4T-MP can either be metabolized to form d4T ($\text{CL}_{\text{m1d}}$) or distributed to the extravascular compartment ($\text{CL}_{\text{m1d}}$). D4T derived from ala-d4T-MP is eliminated from the body ($\text{CL}_{\text{m1d}}$). When we assumed that both parent ala-d4T-MP and d4T can be eliminated from the body (addition of $\text{CL}_{\text{m1d}}$ in Fig. 6A), the predicted curves were not good fits for the observed plasma concentration versus time data. By simultaneous fitting of the parent ala-d4T-MP and d4T plasma concentration values versus time to the described model shown in Fig. 6A, the metabolic clearance of ala-d4T-MP and the formation clearance of d4T ($\text{CL}_{\text{m1d}}$) were estimated to be $15.6 \, \text{ml/min/kg}$ and $1.03 \times 10^{5} \, \text{ml/min/kg}$, respectively. The estimated AUC values were $318.9$ and $12,173.6 \, \text{mg} \cdot \text{h} \cdot \text{kg}^{-1}$, respectively. d4T had a short elimination half-life ($26.6 \, \text{min}$). The systemic clearance of d4T was slow with a CL of only $15.2 \, \text{ml/min/kg}$, which is slower than the blood flow to either the kidney or the liver (Davies and Morris, 1993). d4T had a moderately large volume of distribution ($V_{\text{ss}}$) that is approximately equal to the volume of water in the body (Davies and Morris, 1993).

**Pharmacokinetic Profile of HI-113 Following Oral Administration.** The pharmacokinetic behavior of orally administered HI-113
(100 mg/kg) was also examined. Both metabolites (ala-d4T-MP and d4T) were detected, but the concentration of the parent HI-113 was below the detection limit (0.25 \( \mu \)M). The T\(_{\text{max}}\) values were 9.3 min for ala-d4T-MP and 45.2 min for d4T. A one-compartment pharmacokinetic model was used to describe the time-dependent concentration changes for ala-d4T-MP and d4T (Fig. 8, A and B). The estimated values for the pharmacokinetic parameters are presented in Table 3. The maximum concentrations (C\(_{\text{max}}\)) for ala-d4T-MP and d4T are 15.6 and 29.5 \( \mu \)M, respectively. The elimination half-lives were 56.1 and 102.6 min for ala-d4T-MP and d4T, respectively.

Discussion

Our results presented herein provide unprecedented evidence that the novel anti-HIV agent HI-113 is quickly metabolized in vivo to form two major metabolites, ala-d4T-MP and d4T after i.v. injection (Fig. 5) as well as after oral administration (Fig. 8). Ala-d4T-MP can also be metabolized further to yield d4T (Fig. 8, A and B). The estimated values for the pharmacokinetic parameters are presented in Table 3. The maximum concentrations (C\(_{\text{max}}\)) for ala-d4T-MP and d4T are 15.6 and 29.5 \( \mu \)M, respectively. The elimination half-lives were 56.1 and 102.6 min for ala-d4T-MP and d4T, respectively.

Stability studies revealed that while HI-113 is readily metabolized in plasma to form ala-d4T-MP, only a small amount of d4T is formed. Furthermore, ala-d4T-MP was found to be stable in plasma. By comparison, a significant amount of d4T was formed after incubation of HI-113 with a liver homogenate (Fig. 3B). These findings are consistent with previous in vitro metabolic studies (Balzarini et al., 1996a,b; Venkatachalam et al., 1998; Saboulard et al., 1999).

Paraoxon, an inhibitor of both cholinesterase and carboxylesterase (Augustinsson, 1961; McCracken et al., 1993; Madhu et al., 1997), significantly prevented the hydrolysis of HI-113 to form ala-d4T-MP and d4T, suggesting that both cholinesterase and carboxylesterase are important for metabolism of HI-113. Physostigmine, an inhibitor of cholinesterase (Augustinsson, 1961; McCracken et al., 1993; Madhu et al., 1997), partially prevented the hydrolysis of HI-113, which further supports the importance of cholinesterase in hydrolysis of HI-113. EDTA, an inhibitor of arylesterase (Augustinsson, 1961; McCracken et al., 1993; Madhu et al., 1997), did not affect the hydrolysis of HI-113, indicating that arylesterase is probably not involved in the hydrolysis of HI-113. However, the importance of the hepatic P450 system in the metabolism of HI-113 is currently unknown.

In the present study, the elimination half-life of d4T following i.v. injection was 26.6 min, which is longer than the reported elimination half-life for d4T (17 min) in mice (Russell et al., 1990). The elimination half-life of intravenously administered d4T was similar to the elimination half-life of d4T derived from ala-d4T-MP (t\(_{1/2}\) of 32.4 min). In contrast, the elimination half-life for d4T derived from HI-113 was significantly prolonged (t\(_{1/2}\) of 116.2 min). Similarly, the elimination half-life for ala-d4T-MP derived from HI-113 was longer than the t\(_{1/2}\) for ala-d4T-MP administered intravenously (t\(_{1/2}\) of 138.8 versus 38.9 min).

Orally administered HI-113 also yielded ala-d4T-MP and d4T as the two major metabolites. No parent HI-113 was detectable in the blood after oral administration. This lack of HI-113 in the plasma may be attributed to several factors. First, while HI-113 was stable in gastric fluid and may be absorbed in the stomach, it was quickly hydrolyzed in blood. On the other hand, HI-113 decomposes readily in intestinal fluid to form ala-d4T-MP. This metabolite may be absorbed in the intestine and then further metabolized to yield d4T in the blood. The T\(_{\text{max}}\) and t\(_{1/2}\) values for d4T in mice were longer for orally administered HI-113 (45.2 and 102.6 min, respectively) than for orally administered d4T (5 and 18 min, respectively) (Russell et al., 1990). In comparison with the elimination half-lives measured following i.v.
TABLE 2

Estimated pharmacokinetic parameter values for HI-113 and its metabolites in BALB/c mice

Pharmacokinetic parameters in BALB/c mice (n = 4–5 mice per time point) are presented as the mean ± S.E. A correction factor of 1.24 was used when determining the concentrations of HI-113A and HI-113B because of their different extinction coefficients (10,802 for HI-113B versus 8,686 for HI-113A, ratio = 1.24:1).

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<th>Measured</th>
<th>Vss (ml/kg)</th>
<th>AUC (μM • min)</th>
<th>Cmax (μM)</th>
<th>t1/2 (min)</th>
<th>CL (ml/min/kg)</th>
<th>Tmax (min)</th>
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<td></td>
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<tr>
<td>Total HI-113</td>
<td></td>
<td>928.0 ± 127.4</td>
<td>1071.8 ± 81.8</td>
<td>211.6 ± 29.3</td>
<td>3.6 ± 0.3</td>
<td>174.5 ± 13.2</td>
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<td>1005.3 ± 134.0</td>
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<td>116.2 ± 11.9</td>
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<td>17.4 ± 2.6</td>
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<td>ala-d4T-MP</td>
<td></td>
<td>412.6 ± 126.3</td>
<td>11761.5 ± 447.2</td>
<td>16581 ± 544.9</td>
<td>38.9 ± 15.9</td>
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<td>32.4 ± 2.2</td>
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<td>d4T</td>
<td></td>
<td>581.8 ± 62.8</td>
<td>12173.6 ± 559.5</td>
<td>318.9 ± 15.7</td>
<td>26.6 ± 1.2</td>
<td>15.2 ± 0.7</td>
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</table>

N.D., not determined.

TABLE 3

Estimated pharmacokinetic parameter values following oral administration of HI-113 in BALB/c mice

Pharmacokinetic parameters in BALB/c mice (n = 4 mice per time point) are presented as the mean ± S.E.

<table>
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<tr>
<th></th>
<th>Measured</th>
<th>AUC (μM • min)</th>
<th>Cmax (μM)</th>
<th>t1/2 (min)</th>
<th>Tmax (min)</th>
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<td>Following i.v. injection of HI-113</td>
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<td>1355.4 ± 288.2</td>
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<td>d4T</td>
<td></td>
<td>5928.4 ± 294.6</td>
<td>29.5 ± 0.3</td>
<td>102.6 ± 3.8</td>
<td>45.2 ± 5.2</td>
</tr>
</tbody>
</table>

N.D., not determined.
injection, the $t_{1/2}$ values for both ala-d4T-MP and d4T were prolonged after oral administration of HI-113.

The exact mechanism for the observed longer elimination half-life of metabolites after administration of the parent compound HI-113 is not clear. Such a phenomenon was observed with other metabolites (Pang and Gillette, 1980; Pang, 1981; Houston and Taylor, 1984; Lin et al., 1984).

There was also a $10\%$ fold difference in distributional clearance of ala-d4T-MP ($4.7$ ml/min/kg) following i.v. injection of HI-113 versus ala-d4T-MP. Since the plasma concentration of ala-d4T-MP after i.v. injection of ala-d4T-MP is $10\%$-fold higher than that after i.v. injection of HI-113, this distributional process may be nonlinear.

In summary, HI-113 forms two active metabolites with favorable pharmacokinetics after both i.v. as well as oral administration. The intravenous administration of HI-113 results in more prolonged systemic exposure to ala-d4T-MP as well as d4T than the intravenous administration of an equimolar dose of ala-d4T-MP or d4T due to the apparently longer elimination half-lives of HI-113-derived metabolites. Similarly, the oral administration of HI-113 results in prolonged retention of ala-d4T-MP and d4T in the body. Ala-d4T-MP inhibits the replication of the HIV-1 strain HTLVIIIB in human T lymphocytes with an IC$_{50}$ value of $0.01$ $\mu M$ and a selectivity index of $>10,000$, whereas D4T inhibits HIV-1 replication with an IC$_{50}$ value of ~$0.01$ $\mu M$; selectivity index $>30,000$ may be attributed to the activity of both of its metabolites. These results also suggest that HI-113 may serve as a useful prodrug for these active metabolites (Mansuri et al., 1989; Horton et al., 1995; Balzarini et al., 1996a,b; Lea and Faulds, 1996). Furthermore, since i.v. administration of ala-d4T-MP results in formation of d4T, ala-d4T-MP may also find utility as a d4T prodrug.

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References


