Evaluation of the Pharmacokinetic Features and Tissue Distribution of the Potent Nonnucleoside Inhibitor of HIV-1 Reverse Transcriptase, N-[2-(2-fluorophenethyl)]-N′-[2-(5-bromopyridyl)]-thiourea (HI-240) with an Analytical HPLC Method

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Purpose. The purpose of the present study was to examine the pharmacokinetic features and tissue distribution of N-[2-(2-fluorophenethyl)]-N′-[2-(5-bromopyridyl)]-thiourea (HI-240), a novel non-nucleoside inhibitor of HIV reverse transcriptase with potent anti-viral activity against AZT-sensitive as well as multidrug-resistant HIV-1 strains.

Methods. A sensitive and accurate high performance liquid chromatography (HPLC)-based quantitative detection method was established to measure concentrations of HI-240 in pharmacokinetic studies. The plasma concentration-time data were modeled by using the WinNonlin program to estimate the pharmacokinetic parameter values.

Results. HI-240 had an elimination half-life of 78.3 ± 2.0 min after i.v. administration and 196.8 ± 3.1 min after i.p. administration. The systemic clearance of HI-240 was 2194 ± 61 ml/h/kg after i.v. administration and 9339 ± 1160 ml/h/kg after i.p. administration. Following i.v. injection, HI-240 rapidly distributed to and accumulated in multiple tissues with particularly high accumulation in adipose tissue, adrenal gland, and uterus+ovary. The concentration of HI-240 in brain tissue was comparable to that in the plasma, indicating that HI-240 easily crosses the blood-brain-barrier. Following i.p. injection, HI-240 was rapidly absorbed with a $t_{1/2ka}$ and a t_{max} values of less than 10 min. Following oral administration, HI-240 was absorbed with a $t_{1/2ka}$ of 4.2 ± 1.1 min and a t_{max} of 95.1 ± 25.1 min. The intraperitoneal bioavailability was estimated at 23.5%, while the oral bioavailability was only 1%.

Conclusions. The HPLC-based accurate and precise analytical detection method and pilot pharmacokinetic studies described herein provide the basis for advanced preclinical pharmacodynamic studies of HI-240. The ability of HI-240 to distribute rapidly and extensively into extravascular compartments and easily cross the blood-brain barrier represent significant pharmacokinetic advantages over AZT.

KEY WORDS: HI-240; nonnucleoside inhibitor; pharmacokinetics; HPLC.

INTRODUCTION

Several non-nucleoside inhibitors (NNIs) of HIV reverse transcriptase (RT) are currently under development as potential anti-AIDS drugs (1,2). NNIs inhibit HIV RT by altering either the conformation or mobility of RT through binding to a specific allosteric site near the polymerase site, thereby resulting in a noncompetitive inhibition of the enzyme (1,2). Recently, we designed a series of novel phenethyl thiazoyl-thiourea (PETT) compounds targeting the NNI binding site of HIV RT based on the structure of the NNI binding pocket (3–5). Our lead compound N-[2-(2-fluorophenethyl)]-N′-[2-(5-bromopyridyl)]-thiourea (HI-240) was noncytotoxic and elicited potent anti-HIV activity at subnanomolar concentrations with an unprecedented selectivity index of greater than 100,000 (3). HI-240 was more potent against HIV than AZT and trovirdine, another PETT derivative (3). The reported Wing 2 of the NNI binding pocket of RT is mostly hydrophobic except for the region near the ortho positions on both sides of the phenyl ring of PETT compounds where polar groups such as the ortho-F atom of HI-240 would be compatible. Unlike HI-240, trovirdine lacks such ring substitutents which could provide favorable interactions with these regions of the NNI binding site. Consequently, HI-240 was 7-fold more potent than trovirdine against the HIV-1 strain HTLVIIIB (3). Furthermore, HI-240 exhibited potent antiviral activity against AZT-resistant, NNI-resistant, as well as multidrug-resistant HIV-1 strains (4).

Further development of HI-240 will require detailed pharmacodynamic studies in preclinical animal models. Currently, there are no analytical methods available for detecting HI-240 in biological fluids, and therefore, no information is available regarding the pharmacokinetic features of this potent anti-HIV agent. Here, we describe a quantitative high performance liquid chromatography (HPLC)-based detection method which allows the measurement of HI-240 levels in plasma. The utility of this new assay was confirmed in a pilot pharmacokinetic study of HI-240 in mice, in which the pharmacokinetic features and tissue distribution of HI-240 were determined.

MATERIALS AND METHODS

Chemicals

Deionized distilled water was obtained from U.S. Filter (United States Filter Corporation, Cowell, MA). Methanol, acetonitrile, chloroform, glacial acetic acid were obtained from Fisher Chemicals (Fair Lawn, NJ). All other chemicals were analytical grade.

Synthesis and Characterization of PETT Derivatives

HI-240 {N-[2-(2-fluorophenethyl)]-N′-[2-(5-bromopyridyl)]-thiourea} and its internal standard HI-232 were synthesized as previously described (3). The structure (Fig. 1A) and physical as well as biological properties of HI-240 have been previously reported (3). Stock solutions of HI-240 and HI-232 were prepared in methanol and stored at −20°C. These stock solutions were diluted further in 50% methanol to yield appropriate working solutions for the preparation of standards to calibrate.

HPLC Determination of HI-240 Plasma Levels

Various combinations of acetonitrile (varying from 20% to 80%), and water containing 0.1% acetic acid (varying from

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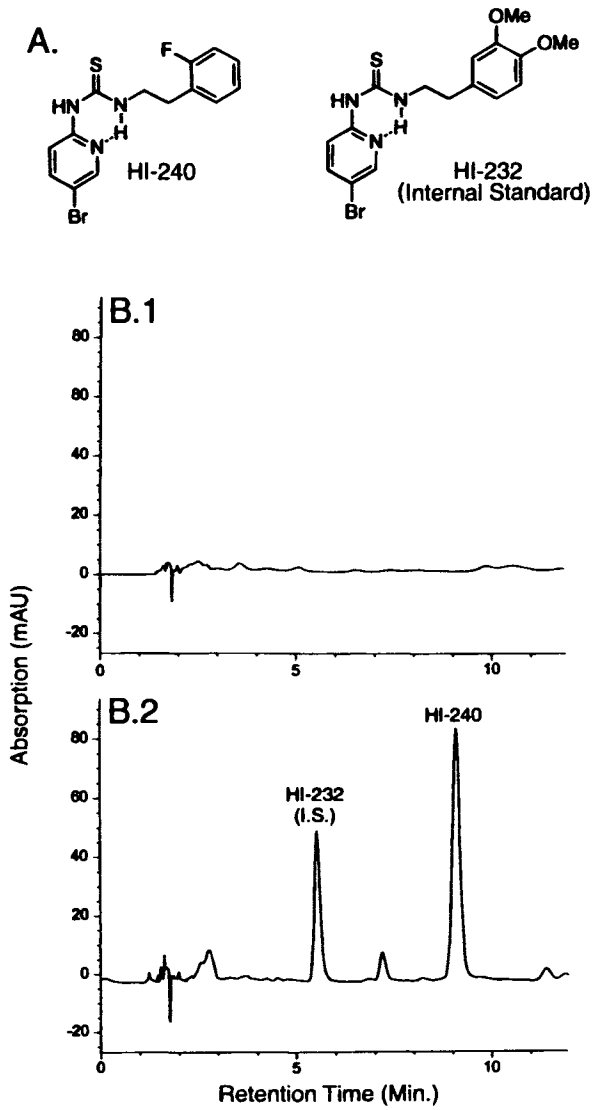

Fig. 1. (A) Chemical structures of HI-240 and HI-232 (internal standard). Molecular weight for HI-240 was 353. Representative chromatograms from (B.1.) blank plasma, (B.2.) plasma samples 15 min after i.p. injection of 40 mg/kg HI-240. I.S. = Internal Standard.

80% to 20%) were evaluated as mobile phases. It was determined that acetonitrile/water containing 0.1% of acetic acid (65/35, v/v) is a suitable mobile phase for separating HI-240 and its internal standard, HI-232. The mobile phase was degassed automatically by the electronic degasser system. The analytical column was equilibrated and eluted under isocratic conditions utilizing a flow rate of 1.0 ml/min at ambient temperature. The wavelength of detection was set at 275 nm. Peak width, response time and slit were set at >0.03 min, 0.5 s and 8 nm, respectively.

For determination of HI-240 levels, 10 μL of the internal standard HI-232 (100 μM) was added to a 100 μL plasma sample. For extraction, 7 mL chloroform was then added to the plasma sample, and the mixture was vortexed thoroughly for 3 min. Following centrifugation (300 × g, 5 min), the aqueous layer was frozen using acetone/dry ice and the organic phase was transferred into a clean test tube. The chloroform extracts were dried under a slow steady stream of nitrogen. The residue was reconstituted in 100 μL of methanol: water (9:1) and 50 μL aliquot of this solution was used for HPLC analysis using a previously reported HPLC method with a system comprised of a Hewlett Packard (HP) series 1100 equipped with an automated electronic degasser, a quaternary pump, an autosampler, an automatic thermostatic column compartment, diode array detector and a computer with a Chemostation software program for data analysis (6,7). All extraction procedures were carried out under room temperature.

For determination of extraction recovery, replicate (N = 5) plasma samples (100 μL/sample) were spiked with known amounts of HI-240 to yield a final concentration of 25 μM HI-240. The samples were extracted following the above extraction procedures. The extraction recovery (ER) was calculated using formula: %ER = {Peak Area $[HI\text{-}240]_{extracted}$/Peak Area $[HI\text{-}240]_{unextracted}$} × 100.

In order to generate a standard curve, HI-240 was added to plasma to yield final concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25 and 50 μM. Subsequently, 10 μL of the internal standard (HI-232, 100 μM) was added to each sample. The plasma samples with known amounts of HI-240 and its internal standard HI-232 were extracted as previously described, and the standard curves were generated by plotting the peak area ratios (HI-240/HI-232) against the drug concentrations tested. The unweighted linear regression analysis of the standard curves was performed by using the CA-Cricket Graph III computer program, Version 1.0 (Computer Association, Inc., Islandia, NY). The linearity was confirmed using the Instat Program V3.0 (GraphPad Software, San Diego, CA, USA).

To evaluate the intra-day accuracy and precision, HI-240 was added to drug-free plasma at concentrations of 2 μM or 20 μM. These standard samples were prepared and analyzed within 24 h. The concentrations were calculated using a standard curve. The ratio of the calculated concentration over the known concentration of HI-240 was used to determine the accuracy of the analytical method, and the coefficient of variation was used as an index of precision. The inter-day accuracy and precision were determined in 5 independent experiments over the course of one week. One-way analysis of variance (ANOVA) was performed using the Instat Program V3.0 to determine the statistical significance of differences between expected versus measured HI-240 levels in spiked samples.

Evaluation of Stability of HI-240 in Plasma

Plasma samples were spiked with HI-240 to yield a final HI-240 concentration of 100 μM. The samples were then divided into two aliquots. One of these aliquots was frozen at 20°C wheras the second aliquot was placed in a 37°C water bath. At a predetermined time, a 50 μl aliquot of HI-240-spiked plasma sample was extracted by adding 100 μl methanol for precipitation of plasma protein and the supernatant was then directly injected into a HPLC column to determine the absolute peak area. The absolute peak area was used to evaluate the stability of HI-240.

Evaluation of Blood Distribution of HI-240

Whole blood was obtained from CD-1 mice (N = 4) at 10 and 30 min after i.v. administration of a 40 mg/kg bolus dose of HI-240. The plasma was separated from the cell fraction

by centrifugation in a microcentrifuge for 10 min at 7000 × g. These fractions were individually extracted and analyzed for their HI-240 content by quantitative HPLC.

Evaluation of Pharmacokinetics of HI-240 in Mice

Female CD-1 mice from Charles River Laboratories (Wilmington, MA) were housed in a controlled environment (12-h light/12-h dark photoperiod, 22 ± 1°C, 60 ± 10% relative humidity), which is fully accredited by the USDA (United States Department of Agriculture). All mice were housed in microisolator cages (Lab Products, Inc., Maywood, NY) containing autoclaved bedding. The mice were allowed free access to autoclaved pellet food and tap water throughout the experiments. Animal studies were approved by Hughes Institute Animal Care and Use Committee and all animal care procedures conformed to the Principles of Laboratory Animal Care (NIH Publication #85-23, revised 1985).

In pharmacokinetic studies, mice were injected either intravenously (i.v.) via the tail vein or intraperitoneally (i.p.) with a bolus dose of 40 mg/kg of HI-240 dissolved in 100 μL DMSO:PBS (80:20, v/v). This amount of DMSO as vehicle has been shown to be well tolerated by mice when administrated by rapid i.v. or extravascular injection (8,9). The mice were anesthesized with methoxyflurane and blood samples (~200 μL) were obtained from the ocular venous plexus by retroorbital venipuncture at 0, 5, 10 (only in i.p. injection), 15, 30, 45 min and 1, 2, 4 and 6 h after i.v. or i.p. administration of HI-240. In order to determine the pharmacokinetics of HI-240 after oral administration, 12 h fasted mice were given a bolus dose of 400 mg/kg of HI-240 via gavage using a No. 21 stainless-steel ball-tipped feeding needle. Sampling time points were prior to and at 5, 10, 15, 30, 45 min and 1, 2, 4, 6 h following oral administration of HI-240.

All collected blood samples were heparinized and centrifuged at 7,000 × g for 10 min in a microcentrifuge to obtain plasma. The plasma samples were stored at −20°C until analysis. Aliquots of plasma were used for extraction and the HPLC analysis.

Tissue Distribution of HI-240 Following Intravenous Administration

CD-1 mice were injected i.v. with 40 mg/kg of HI-240. Following i.v. injection, the mice were sacrificed by cervical dislocation at 30 min or 2h. Selected tissues including the brain, heart, liver, lungs, kidneys, stomach, muscle, large intestine (without contents), small intestine (without contents), spleen, adipose tissue, skin, urinary bladder, adrenal glands, pancreas, uterus+ovary were excised. Tissues were rinsed with PBS, blotted, weighted and homogenized in at least 500 μL water with a Polytron (PT-MR2000) homogenizer (Kinematical AG Littau, Switzerland). Extraction of HI-240 from the tissue homogenates was done with chloroform following precipitation of tissue protein by methanol. The contents of HI-240 in various types of tissues were analyzed by HPLC as described above.

Pharmacokinetic Analysis

Pharmacokinetic modeling and pharmacokinetic parameter calculations were carried out using the pharmacokinetics software, WinNonlin Program, Standard Version 2.0 (Pharsight Inc., Mountain View, CA) with compartmental as well as non-compartmental methods. An appropriate pharmacokinetic model was chosen on the basis of lowest weighted squared residuals, lowest Schwartz Criterion (SC), lowest Akaike's Information Criterion (AIC) value, lowest standard errors of the fitted parameters, and dispersion of the residuals. 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 by the linear trapezoidal rule between first (0 h) and last sampling time plus C/k, where C is the concentration of last sampling and k is the elimination rate constant. The systemic clearance (CL) was determined by dividing the dose by the AUC. The mean residence time (MRT) was calculated by dividing the area under the first moment curve (AUMC) by AUC. Bioavailability (F) was estimated using the equation $F(\%) = AUC_{ip\ or\ po} \cdot Dose_{iv}/AUC_{iv} \cdot Dose_{ip\ or\ po}$. The mean absorption time (MAT) was calculated by the following equation: $MAT = MRT_{ip\ or\ po} - MRT_{iv}$ (10). Non-compartmental analysis was performed for the average plasma H1-240 concentration-time profiles following oral administration in order to estimate the plasma half-life and MRT values.

RESULTS AND DISCUSSION

Linearity and Sensitivity of HPLC-Based Detection Method

Under the chromatographic separation conditions described in the Materials and Methods section, the retention times for HI-240 and for HI-232 (a structurally similar PETT derivative which was used as an internal standard) were 5.5 minutes and 8.7 minutes, respectively. At the retention time of the HI-240 and HI-232, no interference peaks from blank plasma were observed (Fig. 1B1 and 1B2). With the described extraction conditions, the extraction recoveries from plasma were 75.1% for HI-240 and 76.6% for HI-232. Attempts for increasing the extraction recovery by adjustment of the pH in plasma with HCl or NaOH have failed. Therefore, plasma samples were used for extraction directly without adjustment of their pH. The standard curve was linear over the concentration-dose ranges tested and could be described by the regression equations: $Y = 10.622*X - 0.037$ ($r > 0.998$), in which Y is the agent recovered in μM in plasma, and X is the peak area ratio (HI-240/HI-232). The linearity was statistically confirmed using the Instat Program V3.0. The lowest limit of detection of HI-240 was 0.1 μM in 100 μL plasma at a signal-to-noise ratio of ~2. When drug levels were >50 μM, the assay was repeated using appropriately diluted samples in order to measure the drug levels within the range of linearity of the assay.

The results obtained also indicate that intra and inter-assay coefficients of variation (C.V.) in plasma were less than 6% (Table 1). The overall accuracy of this method was 97.2 ± 5.0% for plasma samples. There was no statistically significant difference by ANOVA analysis between expected and experimentally determined HI-240 levels in spiked samples. These results suggest that the HPLC method described above is satisfactory with respect to both accuracy and precision.

Stability of HI-240 in Plasma

The results shown in Fig. 2 demonstrated that HI-240 was very stable in plasma when stored at −20°C, but not when

Table 1. Intra-day and Inter-day Accuracy and Precision of the Determination of HI-240 Levels in Plasma Samples

Concentration		Accuracy	C.V.*
Added	Found	(%)	(%)
Intra-assay (n = 5)			
2 (μM)	1.90 ± 0.10	95.0 ± 4.9	5.2
20 (μM)	20.10 ± 1.16	100.5 ± 5.8	5.8
Inter-assay (n = 5)			
2 (μM)	1.92 ± 0.06	95.8 ± 3.0	3.1
20 (μM)	19.52 ± 0.54	97.6 ± 2.7	2.8

* C.V. = coefficient of variation

stored at 37°C. HI-240 was only stable for one day at 37°C, then it slowly decomposed. There were about 16%, 50% and 56% of HI-240 decomposed at Day 4, Day 6 and Day 11 if the plasma was stored at 37°C (Fig. 2).

Blood Distribution of HI-240

The measured HI-240 concentrations in the plasma fraction and in the cell fraction were 40.4 ± 11.7 μM and 15.8 ± 3.9 μM, respectively at 10 min, and 20.1 ± 6.6 μM and 8.1 ± 2.3 μM, respectively at 30 min following the i.v. administration of HI-240. The estimated plasma/whole blood ratio was 0.71:1 at both 10 min and 30 min following administration of HI-240.

Pharmacokinetic Features of HI-240 Following Intravenous Administration

The plasma concentration-time curves of HI-240 in CD-1 mice following i.v. injection of 40 mg/kg bolus dose are depicted in Fig. 3A. A two-compartment, first order pharmacokinetic model was best fit to the plasma concentration time curves obtained in CD-1 mice. The pharmacokinetic parameters are shown in Table 2. In CD-1 mice, HI-240 showed moderate

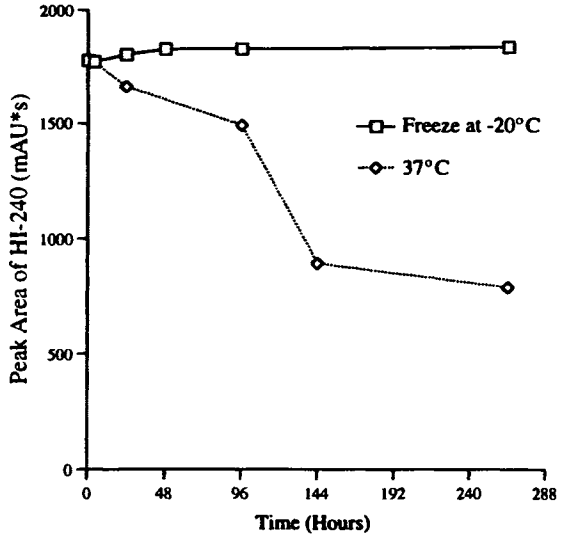

Fig. 2. Stability of HI-240 in plasma following stored at −20°C and 37°C. The data are presented as the mean of two determinations (N = 2).

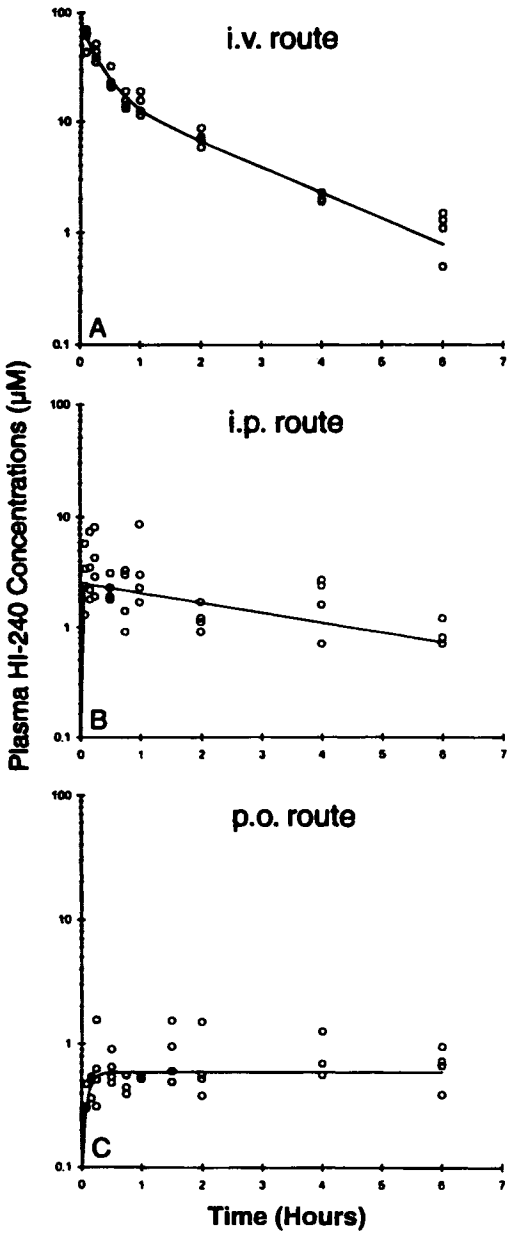

Fig. 3. Plasma concentration-time profiles of HI-240 in CD-1 mice (A) following i.v. bolus injection (40 mg/kg; N = 4); (B) following i.p. administration (40 mg/kg; N = 4); and (C) following oral administration (400 mg/kg N = 4).

to fast elimination ($t_{1/2}$ = 78.3 min and MRT = 84.6 min) and systemic clearance (CL = 2194 ml/h/kg). The values for AUC and C_{max} in CD-1 mice were 51.5 μM·h and 73.8 μM, respectively. The large volume of distribution at central compartment (1531 ml/kg) [~30-fold greater than the plasma volume (50 ml/kg)] or [~2-fold greater than the total body water (725 ml/kg)] and the large volume of distribution at steady state (3095 ml/kg) [~60-fold greater than the plasma volume (50 ml/kg)] or [~4-fold greater than the total body water (725 ml/kg)] (11) suggest that HI-240 is extensively distributed into extravascular compartments. The distribution half-life of 11.6 ± 2.8 min indicates that HI-240 is quickly distributed to all the tissues.

Table 2. Estimated Pharmacokinetic Parameter Values of HI-240 in CD-1 Mice

CD-1 mice	Dose (mg/kg)	Vc (ml/kg)	CL_s (ml/h/kg)	AUC (μM · h)	F (%)	C_{max} (μM)	$t_{1/2}$ (min)	$t_{1/2k\alpha}$ (min)	t_{max} (min)	MRT (min)
N = 4	i.v., 40	1531 ± 209	2194 ± 61	51.5 ± 1.5	ND	73.8 ± 9.2	78.3 ± 2.0	ND	ND	84.6 ± 2.6
N = 4	i.p., 40	10386 ± 1202[a]	9339 ± 1160[b]	12.1 ± 1.6	23.5%	2.5 ± 0.3	196.8 ± 3.1	1.0 ± 0.7	7.6 ± 4.2	334.4 ± 43.3
N = 4	p.o., 400	18657 ± 461[a]	ND	5.0 ± 0.3[c]	1 %	0.6 ± 0.02	16.7 h[d]	4.2 ± 1.1	95.1 ± 25.1	24.5 h[d]

Note: Pharmacokinetic parameters in CD-1 mice are present as estimates from pooled data (±SEM).
[a] Vc has been corrected by bioavailability.
[b] Apparant systemic CL without correction for bioavailability (F).
[c] AUC from 0–6 h.
[d] The parameters were calculated by non-compartmental modeling for average plasma HI-240 concentrations with time changes. Abbreviations: $t_{1/2}$ is terminal elimination half-life; $t_{1/2k\alpha}$ is absorption half-life; t_{max} is the time required to reach the maximum plasma drug concentration following i.p. or p.o. administration. ND, no determination.

Pharmacokinetic Features of HI-240 Following Intraperitoneal Administration

In CD-1 mice, a one-compartment, first order pharmacokinetic model was fit to the pharmacokinetic data obtained following the i.p. administration of a single 40 mg/kg bolus dose of HI-240 (Fig. 3B). The computer-estimated pharmacokinetic parameter values are shown in Table 2. In CD-1 mice, the estimated maximum plasma concentration (C_{max}) of HI-240 after i.p. administration was 2.5 μM, and its bioavailability was estimated to be 23.5%. HI-240 demonstrated rapid absorption after i.p. administration with an absorption half-life of 1.0 min, and the estimated time to reach maximum plasma HI-240 concentration (t_{max}) was 7.6 min. Following i.p. injection, HI-240 has a mean absorption time of 249.8 min, and a moderate elimination rate with an elimination half-life of 196.8 min and MRT of 334.4 min. The central volume of distribution of HI-240 was 10386 ml/kg [~200-fold greater than plasma volume (50 ml/kg)] or [~14-fold greater than the total body water (725 ml/kg)] (11) indicating that this agent has extensive extravascular distribution following i.p. administration.

Pharmacokinetic Features of HI-240 Following Oral Administration

We also examined the pharmacokinetics of HI-240 in mice after oral administration of a 400 mg/kg bolus dose. A one-compartment model was used to analyze the plasma HI-240 concentration changes over time (Fig. 3C). The calculated pharmacokinetic parameter values are presented in Table 2. The estimated oral bioavailability of HI-240 was only 1% with a predicted maximum concentration of 0.6 μM. HI-240 showed a very rapid absorption with an absorption half-life of 4.2 ± 1.1 min, and the time to reach maximum plasma HI-240 concentration was 95.1 ± 25.1 min. Similarly, trovirdine was reported to require approximately 60 min to reach its maximum plasma concentration after oral administration (12). By comparison, AZT requires only 15 min to reach its maximum plasma concentration after oral administration (13). By using non-compartmental modeling for the average plasma HI-240 concentration versus time change, the elimination half-life and MRT of HI-240 were estimated to be 16.7 h and 24.5 h, respectively. The >15-fold longer elimination half-life of HI-240 (16.7 h vs 1 h) represents a significant advantage over AZT and trovirdine (12,13). Notably, the drug absorption appeared to be continued with an estimated mean absorption time of 23.1 h, which provides the opportunity for achieving steady plasma concentrations and long-lasting biologic effects after oral administration of HI-240 despite its poor oral bioavailability.

Tissue Distribution

In mice receiving an i.v. bolus dose of HI-240, the apparent volume of distribution at central compartment (V_c) was 1531 ml/kg and the volume of distribution at steady state (Vss) was 3095 mL/kg, which are nearly 2-fold and 4-fold greater, respectively, than the total body water volume (725 ml/kg) (11). The large Vc and Vss values of HI-240 indicated that this anti-HIV agent is distributed rapidly and extensively into extravascular compartments.

In order to determine the in vivo tissue distribution profile of HI-240, multiple tissues were collected from CD-1 mice sacrificed at 30 min and 2 h after i.v. administration of a 40 mg/kg bolus dose of HI-240. HI-240 was extracted from homogenized tissue specimens with chloroform after methanol precipitation of tissue proteins. The HI-240 quantities of various tissue extracts were then determined using a quantitative HPLC method described above. The tissue distribution profile of HI-240 is shown in Fig. 4 and Table 3. At 30 min following i.v. injection of HI-240, tissue extracts from brain, liver, lung, kidney, adipose tissue, urinary bladder, adrenal gland and uterus+ovary contained large amounts (>10 μg/g tissue) of HI-240, whereas the tissue extracts from stomach, small and large intestine, spleen, skin, pancreas contained moderate amounts (5–10 μg/g tissue) of HI-240. In contrast, only trace amounts (<5 μg/g tissue) of HI-240 were detected in the heart, and muscle. There was 8.6 μg/ml of HI-240 detected in plasma at 30 min. At 2 h following i.v. administration, the HI-240 content was substantially reduced in most of the tissues. But there was a slight increase between 30 min and 2 h in HI-240 content of the heart, liver, muscle, and especially adipose tissue (Fig. 4). Thus, HI-240 shows an extensive distribution into multiple tissues followed by a rapid elimination from most tissues. The fair amount of HI-240 in the brain tissue extracts at 30 min suggests that HI-240 may be able to easily cross the blood brain barrier. Also shown in Table 3 are the tissue to plasma ratios (TPR) for HI-240 at different time points after administration. At 30 min, the TPR values were <1 for the heart, stomach, muscle, small and large intestine, and pancreas, indicating that HI-240 enters but does not accumulate in these tissues, wheras

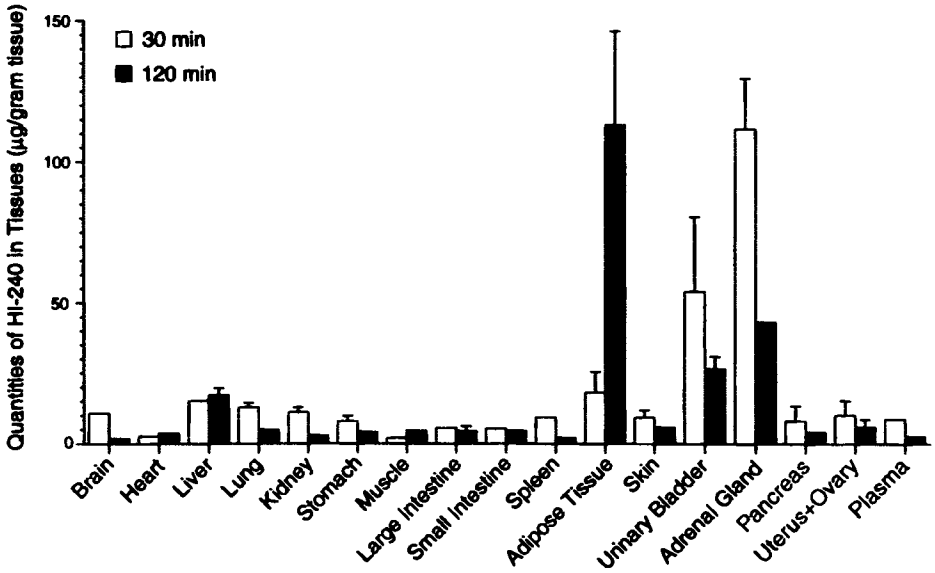

Fig. 4. Tissue distribution of HI-240 in CD-1 mice following i.v. administration of 40 mg/kg HI-240 (N = 3). The data are presented as mean (±SEM). The plasma samples were obtained from the mice following i.v. injection of 40 mg/kg HI-240 (N = 4). The unit in plasma was μg/ml.

the brain, liver, lung, kidney, spleen, adipose tissue, skin, urinary bladder, adrenal gland and uterus + ovary had TPR values of >1, suggesting that these organs may rapidly bind HI-240. At 2 h following the i.v. injection, the TPR increased to over 1 for most of the tissues except for brain and spleen, in which TPR decreased to below 1. These results indicate that HI-240 is capable of binding to and accumulating in most tissues, with especially high accumulation in adipose tissue, adrenal gland and uterus + ovary. The concentration of HI-240 in brain tissue is comparable to that in the plasma, indicating that HI-240 easily crosses the blood-brain-barrier, which we consider as a favorable pharmacokinetic property of this anti-HIV agent. This favorable pharmacokinetic property was also observed for trovirdine, another PETT nonnucleoside inhibitor of HIV-1 RT (12). In contrast, AZT has very poor penetration through the blood-brain barrier (13), which substantially limits its utility in AIDS patients with central nervous system involvement.

Table 3. Tissue Distribution of HI-240 in CD-1 Mice [Tissue to Plasma Ratio (ml Plasma/g Wet Tissue)]

Organs	30 min	120 min
Brain	1.3 ± 0.1	0.7 ± 0.03
Heart	0.3 ± 0.2	1.5 ± 0.05
Liver	1.8 ± 0.1	6.7 ± 1.0
Lung	1.5 ± 0.2	1.9 ± 0.1
Kidney	1.3 ± 0.2	1.2 ± 0.1
Stomach	0.9 ± 0.2	1.6 ± 0.3
Muscle	0.2 ± 0.1	1.8 ± 0.1
Large Intestine	0.6 ± 0.1	1.8 ± 0.7
Small Intestine	0.3 ± 0.1	1.8 ± 0.1
Spleen	1.1 ± 0.1	0.8 ± 0.05
Adipose Tissue	2.1 ± 0.9	43.9 ± 13.0
Skin	1.1 ± 0.3	2.3 ± 0.4
Urinary Bladder	6.2 ± 3.1	10.4 ± 1.7
Adrenal Gland	12.9 ± 2.1	16.8 ± 0.3
Pancreas	0.9 ± 0.6	1.6 ± 0.1
Uterus + Ovary	1.2 ± 0.6	2.3 ± 1.1
Plasma*	8.6 ± 0.8	2.6 ± 0.2

Note: All data were presented as mean (±SEM) (N = 3). *The plasma samples were obtained from the separated experiments with mice following iv injection of 40 mg/kg HI-240 (N = 4). The unit in plasma was μg/ml.

In conclusion, we have developed a highly sensitive and accurate analytical HPLC method for quantitative detection of the novel anti-HIV agent HI-240 in plasma. The availability of this assay will now permit more detailed pharmacokinetic studies of HI-240. Our findings presented herein indicate that HI-240 is quickly absorbed following intraperitoneal and oral administration and is distributed extensively in extravascular tissues. HI-240 also easily crosses the blood-brain-barrier. Furthermore, HI-240 has favorably long plasma elimination half lives of >1 h after i.v. administration, >3 h after i.p. administration, and 16.7 h after oral administration. These findings provide the basis for further evaluation of the clinical potential of HI-240 in preclinical studies.

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