IDX899 and IDX989 are new non-nucleoside reverse-transcriptase inhibitors (NNRTIs) that exhibit potent inhibition of HIV-1 replication, including NNRTI-resistant mutants. This microdose study investigates the pharmacokinetics and determined oral bioavailability. For each compound, 4 healthy male subjects are randomized to receive via a crossover design a single 100-µg oral and intravenous dose together with 100 nCi of [14C]-labeled drug. Plasma and urine samples are obtained over a period of 168 hours postdose and analyzed for total, unchanged drug and major metabolites using an accelerator mass spectrometry method. Based on total radioactivity, oral absorption is near complete. For the parent drug, mean absolute bioavailability is 61% and 65% for IDX899 and IDX989, respectively. Both compounds are extensively metabolized especially after oral dosing. Observed terminal phase half-lives after oral and intravenous doses range from 4 to 10 hours and are comparable for the 2 compounds. Urine excretion of radioactivity for both compounds is less than 10%. These data show for the first time that IDX899 and IDX989 possess favorable pharmacokinetic properties in humans, including high mean absolute bioavailability and long half-life. IDX899 has been selected based on these initial pharmacokinetic assessments and other criteria as the candidate for further clinical development.

Keywords: IDX899; NNRTI; HIV-1; microdose; pharmacokinetics

Journal of Clinical Pharmacology, XXXX;XX:1-9
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Microdose Pharmacokinetics of IDX899 and IDX989, Candidate HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors, Following Oral and Intravenous Administration in Healthy Male Subjects

Among drugs from the diverse classes of antiretrovirals for the treatment of human immunodeficiency virus type-1 (HIV-1) infection, only a few are recommended as components of initial combination therapy for treatment-naïve patients. Efavirenz and nevirapine are non-nucleoside reverse transcriptase inhibitors (NNRTIs) commonly used in such initial treatment regimens; however, their use is often limited by safety and emergence of resistance. The long-term nature of HIV management and the associated increase in drug resistance among HIV-infected patients provide a strong impetus to develop novel antiretrovirals with improved safety and resistance profiles.

IDX899 (2-Carbamoyl-5-chloro-1H-indol-3-yl)-[3-((E)-2-cyano-vinyl)-5-methyl-phenyl]-(R)-phosphinic acid methyl ester) and IDX989 (2-Carbamoyl-5-chloro-4-fluoro-1H-indol-3-yl)-[3-((E)-2-cyano-vinyl)-5-methyl-phenyl]-(R)-phosphinic acid methyl ester) are second-generation NNRTIs (Figure 1), candidates for development for the treatment of HIV-1 infection.
In in vitro cell-based serum-free assays, IDX899 and IDX989 exhibited comparable potencies against HIV-1 replication with mean 50% effective concentration (EC\textsubscript{50}) values of 1.0 nM and 1.2 nM, respectively. In cross-resistance testing using a variety of cell lines and HIV-1 strains, IDX899 and IDX989 retained marked activity against NNRTI-resistant viruses bearing single K103N or Y181C or double K103N/Y181C mutations with EC\textsubscript{50} values below 14.5 nM. Both compounds were also highly active against HIV-1 viruses bearing nucleoside reverse transcriptase inhibitor and/or protease inhibitor mutations. IDX899 and IDX989 were not inhibitory to human cellular DNA polymerase \(\alpha\), \(\beta\), or \(\gamma\) at clinically relevant concentrations with IC\textsubscript{50} of 60 \(\mu\)M or greater and had a favorable cytotoxicity profile in vitro with a selectivity index of 18,000 or greater based on the ratio of 50% cytotoxic concentration (CC\textsubscript{50}) to EC\textsubscript{50} determined in MT-4 cells.\textsuperscript{3,4}

Preclinical pharmacologic data showed that both compounds had good oral bioavailability in nonhuman primates. There are, however, differences in plasma exposure among species, with high levels observed in monkeys but low concentrations in rats and dogs following intravenous (IV) and oral administration (S. Good, unpublished data, January 2007). The species-dependent pharmacokinetics made it difficult to predict human exposure using animal models, providing the rationale for conducting a human microdose study.

The dose of the 2 NNRTIs selected for this study, 100 \(\mu\)g together with a tracer amount of 100 nCi of the corresponding \([^{14}\text{C}]\)-labeled compound, is comparable to routine environmental exposures and conformed to the current US Food and Drug Administration guidance of exploratory investigational new drug (IND) studies.\textsuperscript{5} Results from an extended single IV and oral dose toxicology study were fully supportive of the selected human microdose with a safety factor of approximately 1000-fold (J. Selden, unpublished data, February 2006).

The primary objective of this study was to evaluate the pharmacokinetics and determine the oral bioavailability of IDX899 and IDX989 after a single oral and IV microdose in healthy male subjects. Such early pharmacokinetic data in humans are of critical importance for further development of the chosen candidate.

**MATERIALS AND METHODS**

This study was conducted in accordance with Good Clinical Practice procedures, the principles of the Declaration of Helsinki, and US Food and Drug Administration regulations. Approval for the study was obtained from an independent Institutional Review Board (Lincoln, Nebraska).

The clinical study took place at MDS Pharma Services in Lincoln, Nebraska. The first subject was enrolled on January 22, 2007, and the last subject completed the study on February 6, 2007.

**Study Population**

All subjects gave written informed consent after the nature of the study was fully explained. Healthy adult nonsmoking men from the general population who had voluntarily consented to participate in the study were included if they met the following major inclusion criteria: subjects were aged 19 to 65 years with body weight within 15% of normal for their size and frame; subjects showed no evidence of clinically significant abnormalities on medical history, physical examination, 12-lead electrocardiogram (ECG), or clinical laboratory testing during screening; subjects used an acceptable double-barrier method of birth control 14 days before the first dose and continued to use an adequate method of birth control for at least 30 days after the last dose of the study drug; subjects discontinued use of chronic prescription medications within 3 months, acute prescription drugs within 14 days, and systemic over-the-counter medications (including aspirin, vitamins, and herbal supplements) or alcohol-containing beverages within 2 days of reporting to the clinic. Subjects were excluded if they had a history of clinically significant disease that, in the opinion of the investigator, might put them at risk; tested positive for HIV, hepatitis C virus, or hepatitis B virus; tested positive for drugs of abuse;
had a history of alcohol abuse; or had participated in a clinical study within 30 days prior to study drug administration.

**Study Design**

The trial was a single-center, randomized, single-dose study in healthy male subjects after oral and IV (as constant-rate IV infusion over 30 minutes) administration of IDX899 and IDX989. For each compound, 4 healthy adult male subjects were enrolled and randomized via a crossover design to receive a single microdose of 100 µg orally and intravenously together with a tracer amount of 100 nCi of [14C]-labeled drug. The oral and IV doses were separated by a washout period of 7 days.

For both drugs, a single dosing solution was prepared and used for both oral and IV administration. The dosing solution had a final concentration of 5 µg and 5 nCi/mL so that 20 mL of the resulting infusion solution contained the desired dose of 100 µg of cold drug plus 100 nCi of the [14C]-labeled drug. The specific activity was 2.307 and 2.159 dpm (disintegration per minute)/ng for IDX899 and IDX989, respectively.

Subjects received study drugs on an empty stomach after a fasting period of approximately 10 hours prior to dosing and for an additional 4 hours postdose. The oral dose was administered with 240 mL of water. During infusion of the IV dose, each subject was administered 300 mL of water.

**Safety Evaluation**

Routine safety assessments consisted of collecting all adverse events (AEs) and serious adverse events (SAEs), along with their severity and relationship to study drug. Safety assessments also included regular monitoring of hematology, blood chemistry, and urinalysis as well as vital signs, 12-lead ECG, and physical examination.

**Pharmacokinetic Sampling**

Intensive blood sampling was performed over a period of 168 hours. For the oral dose, blood samples were obtained prior to dosing (0 hours, predose) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 48, 72, 96, 120, 144, and 168 hours postdose. For the IV dose, blood samples were obtained prior to dosing (0 hours, predose), at 0.5 minutes (infusion midpoint) and 30 minutes (end of infusion), and then at 35 minutes, 40 minutes, 45 minutes, and 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 48, 72, 96, 120, 144, and 168 hours after the start of infusion. Blood samples were collected into Vacutainer tubes containing sodium heparin as anticoagulant at the time points specified above. Plasma was obtained by centrifugation at 2000 g for 15 minutes at 4°C and stored frozen at −20°C or below until analysis. Urine samples were collected for both oral and IV doses according to the following time intervals: −2 to 0 (predose), 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 36, 36 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 hours post oral dosing or the start of IV infusion. Urine samples were stored frozen at −20°C or below until analysis.

In human plasma and urine, the short-term stability of IDX899 has been documented when spiked samples were subject to 3 freeze-and-thaw cycles (−20°C to room temperature), storage at ambient temperature for 24 hours, and postpreparative storage for 57 hours for plasma samples and 101 hours for urine samples. Long-term storage stability was 86 days in plasma and 20 days in urine at −20°C or below.6 The stability of IDX989 during storage and preparation was inferred from in vivo animal data (S. Good, unpublished data, December 2006). Samples from the current study were analyzed without exceeding the respective long-term and short-term freeze–thaw stabilities.

**Sample Analysis**

Because of the extremely low amount of drug and radioactivity administered, accelerator mass spectrometry (AMS) was used to quantitate total [14C] and [14C] associated with parent drug and major metabolites for both IDX899 and IDX989 in plasma and urine samples. Radioactivity was then converted to amount and/or concentration using the respective specific activity. For total [14C] measurement, aliquots of plasma (60 µL) and urine (10 µL) samples were directly analyzed using AMS after specific sample preparation (see below).

Measurement for parent drugs and metabolite profiling required resolution of the analytes by high-performance liquid chromatography (HPLC). Eluent from the column was collected at 30-second intervals, and fractions across the peaks corresponding to the parent drug and metabolites were pooled, followed by AMS-specific preparation and final analysis by AMS (see below). Briefly, acetonitrile (600 µL) containing 3 µg/mL of nonradiolabeled IDX899 or IDX989 as internal standard (IS) was added to a 96-well protein precipitation plate. Plasma samples
(200 µL) were loaded into the wells, and the plate was sealed and agitated for approximately 5 minutes. A vacuum was applied to the plate until all the eluate was collected. The eluate was evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 40 µL of methanol followed by 160 µL of ammonium acetate solution (10 mM, pH 4.4). An aliquot of the reconstituted extract (typically 50 µL) was analyzed by HPLC followed by AMS (see below). Urine samples were directly analyzed by HPLC after adding the respective IS (0.5 µg) followed by AMS (see below).

Plasma extracts or urine samples were injected onto a Shimadzu HPLC system equipped with a UV detector. Chromatography was performed on a Synergi Polar RP column (4.6 × 250 mm, 4 µm, Phenomenex, Torrance, Calif), which was preceded by a Phenyl guard column (4.0 × 3.0 mm, Phenomenex). Elution was carried out using a linear gradient with 80% ammonium acetate (10 mM, pH 4.4, mobile phase A) and 20% acetonitrile vol/vol (mobile phase B) at time zero to 40% A and 60% B at 27 minutes, followed by isocratic elution with 20% A and 80% B from 27.1 to 30 minutes. Flow rate was maintained at 1 mL/min. Under these chromatographic conditions, the retention time for both parent drugs was about 26.5 minutes as ascertained by UV monitoring at 267 nm of the nonradiolabeled IS. Eluent was directed to a Shimadzu fraction collector as described above. For the quantitation of unchanged IDX899 and IDX989, fractions corresponding to the parent drug were pooled. For metabolite profiling, however, each 30-second fraction for a total of 60 per sample was individually analyzed by AMS to visualize chromatographically the metabolic profile.

Sample analysis using AMS was conducted at Xceleron Inc. (York, UK). Prior to analysis, aliquots of unknown samples, quality controls (QC), and standards (plasma, urine, and HPLC fractions) were subject to AMS-specific processing, that is, oxidation (combustion) followed by reduction (graphitization). Additional details of AMS procedures have previously been documented.7

The concentration of [14C]-related species in each sample was determined by way of a calibration curve.8 The x-axis ranged from 0.0135 to 12.6288 dpm/mL for IDX899 and from 0.0144 to 14.2029 dpm/mL for IDX989. The lower limit of quantitation taken at the lowest standard in the calibration curve was equivalent to 0.0059 ng/mL for IDX899 and 0.0067 ng/mL for IDX989, respectively. Based on QC samples from 0.7212 to 4.1271 dpm/mL, the assay precision and accuracy (percent deviation) were from 0.7% to 7.0% and from –0.9% to 28.5%, respectively.

Pharmacokinetic Analyses

The plasma concentration–time data were analyzed using noncompartmental methods for total and parent ID899 and IDX989. The maximum plasma drug concentration (Cmax) and time to Cmax (Tmax) and concentration at 24 hours after dosing (C24h) were directly obtained from the plasma concentration–time profiles. Area under the plasma concentration–time curve from time zero to t (AUC0-t), where t is the time of last measurable sample, was calculated according to the linear trapezoidal rule. The AUC from time zero to infinity (AUC0-∞) was estimated as AUC0-t + C/λz, where C is the plasma concentration of the last measurable sample and λz is the slope of the linear portion of the natural log-transformed post-peak plasma drug concentration–time curve estimated using linear regression. The observed terminal phase half-life (t1/2) was calculated as 0.693/λz.

Apparent total plasma clearance (CL) was calculated as dose/AUC0-∞ and apparent total volume of distribution (Vd) as CL/λz for parent drug following IV infusion only. Oral absorption was measured as (AUC0-∞oral/AUC0-∞IV) × (doseIV/doseoral) and expressed as percentage for both total radioactivity (extent of absorption) and parent drug (absolute oral bioavailability, F). The relative extent of biotransformation observed in plasma was assessed by calculating the ratio of exposure parameters (Cmax, Ka, and AUC) between parent and total radioactivity. Cumulative urine excretion (Ae) was calculated as sum of amount excreted during each interval and expressed as percentage of administered dose. Metabolic profiles were displayed chromatographically, and their relative abundance was calculated as percent of total radioactivity recovered from the column.

RESULTS

Subject Characteristics, Disposition, and Safety Evaluation

A total of 8 healthy male volunteers, most (6) of Caucasian ethnicity, were enrolled and completed the study with no significant deviations. Their mean ± standard deviation age and body weight at baseline were 41.3 ± 15.6 years and 76.6 ± 9.3 kg, respectively.

No SAEs were reported during the study. A total of 6 AEs of mild intensity were reported by 3 subjects.
following IV infusion. These AEs included eye pruritus, hordeolum, diarrhea, infusion site hematoma, and sinus congestion in two IDX899 recipients and fatigue in one IDX989 recipient. With the exception of fatigue, all AEs were considered unrelated to study drug. There were no treatment-related trends in the AEs, clinical laboratory, vital signs, physical examinations, or ECG findings.

**Plasma Pharmacokinetics**

The mean ± standard deviation plasma concentration versus time curves for the parent drug and total radioactivity following oral administration and intravenous infusion of a microdose (100 µg with 100 nCi [14C]-labeled drug) of IDX899 or IDX989 are depicted in Figure 2. After oral dosing, IDX899 and IDX989 were rapidly absorbed with C\text{max} attained at a median T\text{max} of 0.5 hours for the parent drug and about 1.0 hours for total radioactivity. The mean C\text{max} values for the parent drug and total [14C] were, respectively, 2.3 and 5.1 ng/mL for IDX899 and 1.6 and 4.7 ng/mL for IDX989. Following intravenous infusion, C\text{max} reached at the end of infusion (0.5 hours), was comparable between the parent drug and total [14C]: a mean of 4.7 versus 5.6 for IDX899 and 3.0 versus 5.3 ng/mL for IDX989. In addition, for both compounds, C\text{max} associated with total radioactivity was similar between the 2 routes of administration (Table I). For total exposure, the observed AUC\text{0-\infty} essentially represented AUC\text{0-\infty} (Table I). After oral administration, the mean AUC\text{0-\infty} values for the parent drug and total [14C] were, respectively, 7.6 and 53.4 ng h/mL for IDX899 and 7.0 and 54.2 ng h/mL for IDX989. Following intravenous infusion, the mean AUC\text{0-\infty} values for the parent drug and total [14C] were, respectively, 12.4 and 45.7 ng h/mL for IDX899 and 11.2 and 53.8 ng h/mL for IDX989. Based on total radioactivity, the overall absorption was complete with a mean extent of absorption of 115.9% for IDX899 and 105.9% for IDX989. For the parent drug, the absolute oral bioavailability (F) was also high with a mean of 61.0% for IDX899 and 65.4% for IDX989.

As displayed in Figure 2, regardless of route of dosing, both compounds exhibited multiphasic disposition for total plasma [14C] and biphasic disposition for the parent drug. The observed terminal phase half-life (t\text{1/2}) was therefore longer for total [14C] than for the parent drug. The mean t\text{1/2} for total [14C] was similar between oral and IV routes of administration: 47.5 and 43.4 hours for IDX899 and 20.8 and 23.6 hours for IDX989. For the parent drug, the mean t\text{1/2} was 4.4 and 6.5 hours for IDX899 oral and IV routes, respectively, and 4.4 and 7.4 hours for IDX989 oral and IV, respectively. Total plasma clearance (CL) and volume of distribution (V\text{d}) were comparable between the 2 compounds: mean CL was 8.1 and 10.5 L/h for IDX899 and IDX989, respectively, and mean V\text{d} was 76.0 and 102.8 L for IDX899 and IDX989, respectively.
Urine Excretion

Mean ± standard deviation cumulative urine excretion-time courses are illustrated in Figure 3. Urine excretion, evaluated for total radioactivity only, was low for both compounds. The mean cumulative percentage of dose excreted in urine (Ae) after IV administration was 8.3% and 6.5% for IDX899 and IDX989, respectively. Following oral administration, the mean cumulative percent of dose excreted was 7.0% and 6.6% for IDX899 and IDX989, respectively (Table I). Regardless of dosing route, more than 80% of the total radioactivity recovered in the 168-hour period was excreted within 24 to 36 hours postdose.

Metabolism

Both compounds underwent extensive biotransformation. As depicted in Figure 2, although time courses for the parent and total radioactivity were mostly overlapping within the first few hours after dosing, they quickly became separated, with the parent drug disappearing much faster and the difference getting larger with time. Quantitatively, the

**Table I** Pharmacokinetic Parameters of Total [14C] and Parent IDX899 and IDX989 After Oral Administration and Intravenous Infusion in Healthy Male Subjects

<table>
<thead>
<tr>
<th>Parametersa</th>
<th>Oral Administration</th>
<th>Intravenous Infusion</th>
<th>Oral Administration</th>
<th>Intravenous Infusion</th>
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<tr>
<td></td>
<td>Parent Drug</td>
<td>Total RA</td>
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<td></td>
<td>Parent Drug</td>
<td>Total RA</td>
</tr>
<tr>
<td>C_{max}, ng/mL</td>
<td>2.3 ± 0.1</td>
<td>5.1 ± 0.4</td>
<td>4.7 ± 0.8</td>
<td>5.6 ± 1.0</td>
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<td></td>
<td>(0.45 ± 0.01)</td>
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<td>(0.85 ± 0.10)</td>
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<tr>
<td>T_{max}, h</td>
<td>0.5</td>
<td>0.8</td>
<td>0.5</td>
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<td></td>
<td>(0.5-0.6)</td>
<td>(0.5-0.8)</td>
<td>(0.5-0.6)</td>
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<tr>
<td>C_{min}, mg/mL</td>
<td>0.03 ± 0.01</td>
<td>0.52 ± 0.08</td>
<td>0.03 ± 0.01</td>
<td>0.38 ± 0.02</td>
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<td>(0.06 ± 0.02)</td>
<td>(0.08 ± 0.03)</td>
<td>(0.08 ± 0.03)</td>
<td>(0.09 ± 0.05)</td>
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<tr>
<td>AUC_{0-t}, ng⋅h/mL</td>
<td>7.3 ± 1.6</td>
<td>49.9 ± 8.8</td>
<td>12.2 ± 1.4</td>
<td>43.9±3.1</td>
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<td>(0.15 ± 0.04)</td>
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<td>(0.28 ± 0.01)</td>
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<tr>
<td>AUC_{0-∞}, ng⋅h/mL</td>
<td>7.6 ± 1.4</td>
<td>53.4 ± 11.1</td>
<td>12.4 ± 1.3</td>
<td>45.7±5.7</td>
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<tr>
<td></td>
<td>(0.15 ± 0.03)</td>
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<td>(0.27 ± 0.01)</td>
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<tr>
<td>t_{1/2}, h</td>
<td>4.4 ± 0.3</td>
<td>47.5 ± 39.9</td>
<td>6.5 ± 0.6</td>
<td>43.4±26.2</td>
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<td></td>
<td>8.1 ± 0.9</td>
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<tr>
<td>V_{d}, L</td>
<td>76.0 ± 6.8</td>
<td>102.8 ± 41.8</td>
<td>105.9 ± 27.7b</td>
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<tr>
<td>Cmax, %</td>
<td>61.0 ± 6.1</td>
<td>115.9 ± 25.7b</td>
<td>65.4 ± 19.3</td>
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<tr>
<td></td>
<td>70.0 ± 2.0</td>
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<td>8.3±0.9</td>
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<td>Ae, %</td>
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</table>

*C_{max},* maximum plasma drug concentration; *T_{max},* time to *C_{max}; C_{min},* concentration at 24 hours after dosing; AUC_{0-t}, area under the plasma concentration–time curve from time zero to t; AUC_{0-∞}, area under the concentration–time curve from time zero to infinity; *t_{1/2},* terminal phase half-life; CL, total plasma clearance; V_{d}, apparent total volume of distribution; F, absolute oral bioavailability; Ae, cumulative urine excretion.

a. Parameters are presented as means ± standard deviations except for *T_{max},* where median (range) values are shown.

b. Extent of absorption based on total plasma radioactivity.
relative extent of biotransformation observed in plasma was assessed by calculating the ratio between parent and total radioactivity for the point (C_max and C_{24h}) and total ([AUC_0-24] and [AUC_0-∞]) exposure. The ratio was lower for oral administration, indicative of first-pass effects for both compounds. At T_max after oral dosing, less than half of plasma radioactivity was associated with the parent drug (mean ratio, 0.45 and 0.35 for IDX899 and IDX989, respectively), whereas for IV infusion, the parent drug represented on average 85% and 57% of total plasma [14C] for IDX899 and IDX989, respectively. Twenty-four hours after dosing for either route, the parent drug accounted only for 6% to 8% and 9% to 14% of total plasma [14C] for IDX899 and IDX989, respectively. Twenty-four hours after dosing for either route, the parent drug accounted only for 6% to 8% and 9% to 14% of total plasma [14C] for IDX899 and IDX989, respectively (Table I). The relative extent of biotransformation observed in plasma was similar between the 2 compounds. The parent drug represented approximately 15% of total plasma [14C] following oral dosing and approximately 21% to 28% after IV infusion (Table I).

Metabolic profiling was performed using pooled samples after oral dosing. For each compound, the 12-hour plasma samples and the 12- to 24-hour urine fractions from the 4 subjects were separately pooled and subject to extraction followed by AMS analysis as described in the methods. The metabolic profiles are shown in Figure 4. For both compounds, no significant radioactivity corresponding to the parent drug (elutes at a retention time of ~27 minutes) was detected in the plasma and urine pools, indicative of extensive biotransformation. The metabolic profiles of both the pooled plasma and urine samples derived from subjects administered [14C]-IDX899 consisted of 2 or 3 major peaks (defined as >10% of radioactivity recovered from column) eluted between 8 and 16 minutes, each representing 16% to 28% of total radioactivity (Figure 4). The metabolic profiles for [14C]-IDX989 consisted of a major peak at around 12 to 13 minutes that constituted 50% and 30% of the total radioactivity in plasma and urine, respectively (Figure 4). The structures of the metabolites were unknown. For both compounds, the presence of smaller metabolite peaks was also evident.

DISCUSSION

Since the publication of guidance documents initially by the EMEA and then the US FDA in response to the agency’s March 2004 Critical Path Initiative report, the role of human microdose studies under exploratory IND has become increasingly important in terms of accelerating early clinical drug development. In this context, the present study focused on determining the pharmacokinetics of 2 candidate HIV-1 NNRTIs in healthy male subjects, in an attempt to provide initial data on human bioavailability that is considered prerequisite to the successful development of oral antiretrovirals and to assist in selecting the most promising candidate for further clinical development.

With the support from an extended single-dose safety toxicology study in cynomolgus monkeys, 8 healthy male subjects were enrolled in this study and administered 100 µg fortified with 100 nCi of either...
[14C]-IDX899 or [14C]-IDX989 (4 subjects received each drug) orally and intravenously in a randomized, crossover design. The low dose of unlabelled drug with a tracer amount of [14C] label, considered comparable to routine environmental exposures, precluded the use of conventional bioanalytical technologies using mass spectrometry or liquid scintillation counting. In this study, the anticipated extremely low concentrations of the analytes necessitated the application of the ultrasensitive AMS technique. Samples were analyzed using AMS for total [14C] content, parent drug, and metabolic profiles. Pharmacokinetic parameters were calculated from these results.

Data from this study show for the first time that both IDX899 and IDX989 were rapidly and well absorbed after oral dosing in men. The extent of absorption relative to the IV route was virtually complete based on total radioactivity of parent drug and metabolites. For the parent drug, the absolute bioavailability was also high and was greater than 60% for both compounds.

The difference between total radioactivity and parent drug reflects metabolite formation. Both compounds underwent extensive biotransformation. A higher degree of metabolism was observed in plasma after oral dosing, where unchanged drug represented less than 10% of total radioactivity. This could be attributed to a first-pass effect because IDX899 and IDX989 are substrates of human intestinal and liver CYP450s as demonstrated with in vitro assays. The current study was not intended to profile metabolites in each sample, as 60 fractions per sample for a total of about 10 000 AMS runs would be prohibitive and unnecessary in such an early stage of development. Instead, only selected samples were analyzed for preliminary metabolic profile. The 12-hour plasma sample and the 12- to 24-hour urine fraction after oral dosing were chosen as they likely contained all metabolites formed and yet had sufficient radioactivity for AMS analysis. For each compound, the selected samples from the 4 subjects were pooled prior to analysis so that the obtained profile would be representative of the group. Two or 3 major metabolites were revealed for these compounds with a number of minor peaks. The complete metabolic profile, including structure identification, remains to be elucidated in future studies at pharmacologic doses.

Results from the present study further demonstrate that the human microdose pharmacokinetic properties were rather similar between these 2 structurally related compounds. Values of pharmacokinetic parameters describing absorption, distribution, extent of metabolism, and elimination, including cumulative urine excretion as summarized in Table I, are very close between IDX899 and IDX989. Therefore, although the favorable human microdose bioavailability data were a prerequisite to the overall development program, these data, within the scope of this study, were not directly instrumental in distinguishing the 2 candidates in terms of pharmacokinetic advantage. IDX899 was selected based on other criteria and is currently under clinical development.

The ability of microdose data to predict human pharmacokinetics at therapeutic doses remains uncertain.

Figure 4. Metabolic profiles of pooled plasma (12 hours) samples and pooled urine (12-24 hours) fractions of IDX899 and IDX989 after oral administration in healthy male subjects.
because of concerns about nonlinearity. Results from the recently published CREAM study comparing the pharmacokinetics of 5 drugs at microdoses and therapeutic doses have shed some light on the issue: the pharmacokinetics at therapeutic dose were predictable from microdose data with excellent concordance for 3 of the 5 tested drugs.\textsuperscript{6} For IDX899, a dose-escalation study at pharmacologic doses has recently been completed, allowing a retrospective assessment of the scalability of the microdose data. The dose-escalation study included single ascending doses of 200, 400, 800, and 1200 mg that were administered under fed conditions to cohorts of healthy male subjects. Results showed that IDX899 exhibited dose-proportional pharmacokinetics with respect to total exposure (AUC) and that food roughly doubled exposure.\textsuperscript{6} With a scaling factor of 2000- to 12 000-fold to these 4 doses from 100 µg, the extrapolated mean AUC\textsubscript{0-∞} from the microdose data compared with the actual data was 15.2 ± 2.8 versus 16.8 ± 7.8 µg·h/mL for the 200-mg dose, 30.4 ± 5.6 versus 26.5 ± 7.1 µg·h/mL for the 400-mg dose, 60.8 ± 11.2 versus 47.2±12.1 µg·h/mL for the 800-mg dose, and 91.2 ± 16.8 versus 106.4 ± 27.1 µg·h/mL for the 1200-mg dose, respectively. With a deviation of less than 30% between the microdose extrapolated and the actual data and considering the marked difference of up to 12 000-fold between the micro and pharmacologic doses, the predictivity of microdose data was excellent for IDX899. Such an agreement strongly suggests that the high bioavailability of IDX899 observed in the present study was preserved at pharmacologic doses when the drug was administered under fed conditions. In addition to plasma exposure, the microdose urine excretion data are also consistent with data at pharmacologic doses. In the present study, cumulative urine excretion measured as total radioactivity was 7%, which represented almost exclusively metabolites with no parent IDX899 detected in the 12- to 24-hour fraction despite the ultrasonesitive AMS. These data suggest that IDX899 underwent major hepatic clearance. After a single dose of 400 and 800 mg under fed conditions, parent IDX899 excreted in the urine was barely measurable and cumulative excretion was less than 0.001% of administered dose,\textsuperscript{6} confirming the microdose findings.

Finally, although safety assessment was not the primary focus of the microdose study, no SAEs or other safety concerns were reported in the course of the present study. In summary, data from the microdose study show that IDX899 and IDX989 were highly bioavailable upon oral administration in humans, an essential pharmacologic attribute that supports further clinical development of these experimental NNRTIs. Based on these initial pharmacokinetic assessments and other criteria, IDX899 was selected as the lead compound for further clinical development.

We thank the healthy volunteers and the staff of MDS Pharma Services and Xceleron Ltd.

Financial disclosure: This study was funded by Idenix Pharmaceuticals, Inc.

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