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EVALUATION OF MICRODOSING STRATEGIES FOR STUDIES IN PRECLINICAL DRUG DEVELOPMENT: DEMONSTRATION OF LINEAR PHARMACOKINETICS IN DOGS OF A NUCLEOSIDE ANALOG OVER A 50-FOLD DOSE RANGE

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ABSTRACT:

The technique of accelerator mass spectrometry (AMS) was validated successfully and used to study the pharmacokinetics and disposition in dogs of a preclinical drug candidate (7-deaza-2′-*C*-methyl-adenosine; Compound A), after oral and intravenous administration. The primary objective of this study was to examine whether Compound A displayed linear kinetics across subpharmacological (microdose) and pharmacological dose ranges in an animal model, before initiation of a human microdose study. The AMS-derived disposition properties of Compound A were comparable to data obtained via conventional techniques such as liquid chromatography-tandem mass spectrometry and liquid scintillation counting analyses. Compound A displayed multiphasic kinetics and exhibited low plasma clearance (5.8 ml/min/kg), a long terminal elimination half-life (17.5 h), and high oral bioavailability (103%). Currently, there are no published comparisons of the kinetics of a pharmaceutical compound at pharmacological versus subpharmacological doses using microdosing strategies. The present study thus provides the first description of the full pharmacokinetic profile of a drug candidate assessed under these two dosing regimens. The data demonstrated that the pharmacokinetic properties of Compound A following dosing at 0.02 mg/kg were similar to those at 1 mg/kg, indicating that in the case of Compound A, the pharmacokinetics in the dog appear to be linear across this 50-fold dose range. Moreover, the exceptional sensitivity of AMS provided a pharmacokinetic profile of Compound A, even after a microdose, which revealed aspects of the disposition of this agent that were inaccessible by conventional techniques.

The applications of accelerator mass spectrometry (AMS) are broad-ranging and vary from studying environmental and ecological issues such as the isotopic composition of the atmosphere, soil, and water (Hughen et al., 2000; Beck et al., 2001; Keith-Roach et al., 2001; Mironov et al., 2002), to archaeology and volcanology (Stafford et al., 1984; Vogel et al., 1990; Smith et al., 1999), to its use as a bioanalytical tool for nutritional research (Buchholz et al., 1999; Deuker et al., 2000; Weaver and Liebman, 2002). Biomedical applications of AMS and its use in the arena of pharmaceutical research also have been detailed in review articles (Barker and Garner, 1999; Garner, 2000; Turteltaub and Vogel, 2000). To date, most studies on the metabolism and disposition of xenobiotics by AMS have focused on the binding of carcinogens to DNA and proteins (Turteltaub et al., 1990, 1997; Frantz et al., 1995; Dingley et al., 1999; Li et al., 2003). These studies have demonstrated a linear relationship between dose and DNA adduct formation. Applications of AMS to the field of pharmaceutical sciences have been limited to only a few studies (Kaye et al., 1997; Young et al., 2001; Garner et al., 2002). However, the pharmaceutical industry is becoming increasingly aware of the potential benefits that may accrue from the ultra-high sensitivity afforded by AMS in terms of evaluating the pharmacokinetics of lead drug candidates in early development. Specifically, AMS allows administration of subpharmacological doses (microdoses) of carbon-14 or tritium-labeled investigational drugs to animals or humans at radiologically insignificant levels with the goal of obtaining preliminary information regarding the absorption, distribution, metabolism, and excretion of test compounds (Turteltaub and Vogel, 2000). An unre-

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ABBREVIATIONS: AMS, accelerator mass spectrometry; AUC, area under the plasma concentrations versus time curve; Compound A, 7-deaza-2′-*C*-methyl-adenosine; C_{max}, maximum concentration in plasma; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LSC, liquid scintillation counting; T_{max}, time of occurrence of maximum concentration in plasma; Vd_{ss}, steady-state volume of distribution.

solved issue, however, is whether the pharmacokinetics determined following a microdose are representative of those following a conventional (pharmacological) dose (Lappin and Garner, 2003).

This paper examines the linearity of kinetics of an antiviral nucleoside analog, 7-deaza-2′-*C*-methyl-adenosine (Compound A), across subpharmacological and pharmacological dose ranges in the dog before initiation of a human microdose study. The present study thus provides the first description of the full pharmacokinetic profile of a drug candidate assessed under these two dosing regimens.

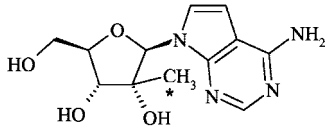
The specific objectives of this study, therefore, were 1) to assess the pharmacokinetics of Compound A in dogs by a conventional dosing approach using LC-MS/MS for sample analysis, 2) to assess the pharmacokinetics of Compound A in dogs by the microdose approach using AMS for sample analysis, 3) to compare the pharmacokinetics of Compound A at a microdose versus a pharmacological dose, and 4) to validate AMS for this application and to compare the sensitivity of AMS to that of LC-MS/MS.

Materials and Methods

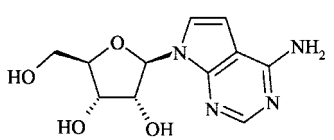
Chemicals. 7-Deaza-2′-*C*-[^{14}C]methyl-adenosine ([^{14}C]Compound A) (Fig. 1) was prepared by the Labeled Compound Synthesis Group (Merck Research Laboratories, Rahway, NJ). The specific activity of the material was 183.5 μCi/mg and the radiochemical purity was >98%. Unlabeled Compound A was synthesized by the Department of Process Research (Merck Research Laboratories, Rahway, NJ). Tubercidin was obtained from Sigma-Aldrich (St. Louis, MO). All other solvents and reagents were of either HPLC or analytical grade. All dosing solutions were prepared based on the molecular weight of the free base.

Animal Facility for the AMS Study. A room in the Merck Research Laboratories Drug Metabolism animal facility was designated for the purpose of conducting this AMS study in dogs. The room had not been used for any studies involving radiolabeled compounds in the past 6 years. The walls and dog cages were cleaned with detergent numerous times to remove any traces of ^{14}C from previous uses. Small glass fiber filters measuring 2.4 mm in diameter (Fisher Scientific, Pittsburgh, PA) were wet with alcohol and swiped over small areas of interest for monitoring background ^{14}C. Several such swipes were taken of the dog cages, food bowls, water supply, fume hood, air vents, door handles, and bench top surfaces. These were sent to Lawrence Livermore National Laboratory (Livermore, CA) for analysis to certify the absence of background ^{14}C contamination in the room and the environment in which the AMS study was to be performed. The results from these swipe test samples indicated that the environment was suitable for the conduct of an AMS study. No significant areas of contamination were identified.

All procedures for the study were carried out with utmost care to avoid contamination of samples with external sources of ^{14}C. Disposable supplies were used for all sample preparation and handling steps. In general, extreme care was exercised in setting up this facility to assure the successful conduct of the AMS component of the study (Buchholz et al., 2000). Moreover, access to this facility was limited to those researchers directly involved in the study.



Compound A



Tubercidin

FIG. 1. Structure of [^{14}C]Compound A and tubercidin. The asterisk in the structure of Compound A denotes the position of the ^{14}C radiolabel.

Pharmacokinetic Studies. All animal studies described in this paper were approved by the Merck Research Laboratories Institutional Animal Care and Use Committee. Male Beagle dogs from Marshall Farms (North Rose, NY) weighing 8.3 to 11.2 kg were used for the pharmacokinetic studies. Dogs were fasted overnight before drug administration and fed 4 h postdose. The oral dose solutions were prepared in 0.5% aqueous methylcellulose and the intravenous dose solutions were formulated in saline. Administration of the intravenous dose was via the cephalic vein as a slow bolus over 30 s at 0.1 ml/kg, and that of the oral dose solution was by gavage. Three groups of dogs (n = 2/group) were dosed with [^{14}C]Compound A. The first group of dogs was administered a 1 mg/kg oral dose (specific activity 0.017 μCi/mg). The second group of dogs was administered a 0.02 mg/kg oral dose (specific activity 0.546 μCi/mg), and the third group of dogs received a 0.02 mg/kg intravenous dose (specific activity 0.428 μCi/mg). Blood samples were collected from the jugular vein at predose and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 28, 32, 36, 48, 56, 72, and 80 h after drug administration. An additional sample at 0.083 h (5 min) was collected after intravenous administration of the compound.

In a separate pharmacokinetic study, dogs (n = 4, crossover study design) were dosed with unlabeled Compound A. For the first leg of this study, the dogs were administered a 0.4 mg/kg intravenous dose. Two weeks later, the dogs were administered a 1 mg/kg oral dose. Blood samples were collected from the jugular vein at predose and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 28, 32, 48, and 72 h after drug administration. An additional sample was collected at 0.083 h (5 min) after intravenous administration. All blood samples were collected in EDTA tubes and plasma was harvested by centrifugation. All samples were stored at −20°C or −70°C until analysis.

LC-MS/MS of Compound A in Plasma. To 50 μl of plasma was added 50 μl of methanol/water (50:50; v/v), 25 μl of internal standard (0.4 μg/ml tubercidin), and 1 ml of 0.1 M sodium phosphate solution (pH 2.0). The contents were briefly vortexed and the analytes (Compound A and internal standard) were extracted from plasma samples using an Oasis MCX (30-mg) extraction plate (Waters, Milford, MA). The plate was rinsed with 0.5% formic acid (0.5 ml) followed by acetonitrile (0.5 ml), and the analytes were eluted with 300 μl of acetonitrile/water/ammonium hydroxide (90:8:2; v/v). The eluent was neutralized with acetic acid and aliquots (20 μl) injected onto the LC-MS/MS system. Concentrations of parent (Compound A) were determined from a standard curve prepared daily by adding stock working standards of drug and internal standard into 50 μl of drug-free dog plasma. The validated assay demonstrated good linearity and reproducibility for Compound A over the concentration range 2 to 2000 ng/ml. The lower limit of quantitation was 2 ng/ml. The values for assay accuracy and precision (% CV) ranged from 95.5 to 102.3% and 2.1 to 7.2%, respectively. Compound A was shown to be stable in dog plasma during the time required for storage and analysis of the samples and for up to three freeze-thaw cycles (freezing at −20°C and thawing at room temperature).

The HPLC system consisted of a PerkinElmer LC200 pump (PerkinElmer Life and Analytical Sciences, Boston, MA) and a Varian Prostar 430 autosampler (Varian, Inc., Palo Alto, CA). The mass spectrometer was a PE Sciex API 4000 triple quadrupole system (PerkinElmerSciex Instruments, Boston, MA) equipped with a heated nebulizer interface (500°C) operated in the positive ion electrospray mode. Selected reaction monitoring of the precursor-to-product ion pairs m/z 281→135 for Compound A and m/z 267→135 for tubercidin was used for quantitation. PE Sciex Analyst Version 1.1 software was used to collect and process the data. The mobile phase consisted of acetonitrile/10 mM ammonium acetate (9:1; v/v), adjusted to pH 4.0 with acetic acid, at a flow rate of 400 μl/min. Chromatography was performed on a 2.0 × 100 mm, 5-μm Tosoh Biosep TSK-GEL Amide-80 column with a 2.0 × 10 mm guard cartridge of the same packing (Tosoh Bioscience LLC, Montgomeryville, PA).

AMS Measurement of Total ^{14}C in Plasma. All biological samples were shipped from Merck Research Laboratories to the Lawrence Livermore National Laboratory on dry ice. Upon receipt, samples were unpacked, inspected, and stored frozen (−20°C) pending analysis. Samples were converted to elemental carbon for AMS quantitation of ^{14}C in a two-stage process. First, samples were oxidized to CO_2 in individual sealed tubes containing cupric oxide, followed by reduction onto approximately 10 mg of iron catalyst in a second tube over zinc and titanium hydride (Vogel, 1992; Ognibene et al.,

2003). This process requires approximately 400 μg of carbon, corresponding to the amount available in approximately 10 μl of plasma. Aliquots were obtained from the defrosted plasma samples and placed in quartz combustion tubes. The exact sample mass in this process is not important, since AMS measures an isotope ratio that is converted to equivalent amounts of drug per milliliter or milligram of sample using the known isotopic content of the labeled compound and the average or individual carbon content of each sample (Vogel et al., 2001).

Reduced samples were pressed into aluminum holders, mounted in a wheel containing up to 58 samples of which 6 represented standards with well defined isotopic compositions, and analyzed by the 1-mV AMS spectrometer at Lawrence Livermore National Laboratory (Ognibene et al., 2002). Each sample was measured to >15,000 ^{14}C counts at least three times and, if necessary, up to seven times such that the last three measurements agreed to within 3% of each other without monotonic trend. The reported isotope ratio was derived by comparison of the measured isotope ratio to that of the known standards. Results were reported in the unit "Modern," which is equivalent to 97.8 attomole of ^{14}C per mg of carbon or 6.11 fCi of ^{14}C per mg of carbon or 0.0136 dpm per mg of carbon. "Modern" represents the expected natural level of ^{14}C in the biosphere and is a National Institute of Standards and Technology-traceable quantity (National Institute of Standards and Technology standard reference material 4990). AMS-derived ^{14}C concentrations, which include parent compound as well as metabolites, were converted to nanogram equivalents of Compound A per milliliter (ng Eq/ml) using the specific activity of the dosed compound.

Pharmacokinetic Analysis. Plasma concentrations of Compound A parent levels (measured by LC-MS/MS) and total ^{14}C levels (measured by AMS) were used to estimate pharmacokinetic parameters for each treatment group and subject. The areas under the plasma concentration-time curve ($AUC_{0\text{-}t}$) were calculated from the first time point up to the last time point with measurable drug concentration using the linear trapezoidal or linear/log-linear trapezoidal rule. The remaining area under the plasma concentration-time curve was estimated by dividing the observed concentration at the last time point by the elimination rate constant, and this value was used to estimate the $AUC_{0\text{-}inf}$. The percentage AUC extrapolated (% AUC Extrap) was a function of $(AUC_{0\text{-}inf} - AUC_{0\text{-}t}) \cdot 100/AUC_{0\text{-}inf}$. The intravenous plasma clearance was calculated by dividing the dose by $AUC_{0\text{-}inf}$. The alpha, beta, and gamma half-lives ($t_{1/2\alpha}$, $t_{1/2\beta}$, and $t_{1/2\gamma}$, respectively) were determined by fitting the plasma concentration-time data to a triexponential decay model with intravenous bolus input and linear first-order elimination from the central compartment using iterative unweighted nonlinear least-squares regression analysis. The regression lines through the plots of observed versus predicted concentrations did not differ from the line of identity, and no bias was observed. The volume of distribution at steady state (Vd_{ss}) was obtained from the product of the plasma clearance and the mean residence time. The maximum plasma concentration (C_{max}) and the time at which maximum concentration occurred (T_{max}) were obtained by inspection of the plasma concentration-time data. Absolute bioavailability was determined from dose-adjusted ($AUC_{0\text{-}inf}$) ratios.

Results

The pharmacokinetics of Compound A in dogs based on total ^{14}C levels determined by AMS and based on parent levels as determined by a LC-MS/MS assay were obtained following both intravenous and oral administration. The mean pharmacokinetic parameters derived from the LC-MS/MS study are summarized in Table 1. Compound A exhibited low plasma clearance (5.8 ± 0.7 ml/min/kg) and multiphasic kinetics with a long terminal elimination half-life ($t_{1/2\gamma}$) (17.5 ± 0.7 h). The Vd_{ss} was large (8.6 ± 2.4 l/kg) and exceeded total body water. After oral dosing, the absolute bioavailability of Compound A was high (103 ± 5%), with a T_{max} and C_{max} of 0.44 ± 0.38 h and 849 ± 168 ng/ml, respectively. For comparison, the average pharmacokinetic parameters of Compound A as determined by AMS and derived from a separate study in which radiolabeled compound was administered to dogs are presented in Table 2. These AMS-derived parameters when dose-normalized were comparable to the pharmacokinetic properties obtained for Compound A following analysis by LC-MS/MS (see Table 1).

TABLE 1

Mean pharmacokinetic parameters of Compound A in dogs after administration of a pharmacological dose as determined by LC-MS/MS analyses

Parameter	Intravenous Dose[a]	Oral Dose[a]
Dose (mg/kg)	0.4	1.0
$AUC_{0\text{-}t}$ (ng · h/ml)[b]	1057 ± 168	2727 ± 401
$AUC_{0\text{-}inf}$ (ng · h/ml)	1166 ± 154	3016 ± 395
% AUC extrapolated	9.8 ± 2.7	9.6 ± 3.4
CL_p (ml/min/kg)	5.8 ± 0.7	
$t_{1/2\alpha}$ (h)	0.1 ± 0.1	
$t_{1/2\beta}$ (h)	0.8 ± 0.2	
$t_{1/2\gamma}$ (h)	17.5 ± 0.7	
Vd_{ss} (l/kg)	8.6 ± 2.4	
C_{max} (ng/ml)		849 ± 168
T_{max} (h)		0.44 ± 0.38
Oral bioavailability (%)[c]		103 ± 5

CL_p, plasma clearance.
[a] Data are presented as mean ± S.D. (n = 4).
[b] The $AUC_{0\text{-}t}$ interval was from 0 to 72 h.
[c] This study was conducted as a crossover design. The oral bioavailability was determined from dose-adjusted ($AUC_{0\text{-}inf}$) ratios of the intravenous and oral legs of the study.

TABLE 2

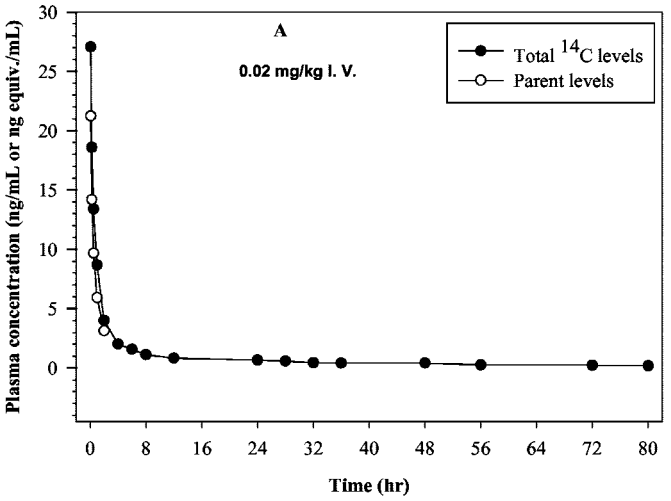
Pharmacokinetic parameters based on total ^{14}C levels as determined by accelerator mass spectrometry after administration of a microdose of [^{14}C]Compound A to dogs (n = 2)

Parameter	Intravenous Dose	Oral Dose
Dose (mg/kg)	0.02	0.02
$AUC_{0\text{-}t}$ (ng Eq · h/ml)[a]	66.5; 66.7	59.4; 49.5
$AUC_{0\text{-}inf}$ (ng Eq · h/ml)	78.4; 73.0	66.0; 57.3
% AUC extrapolated	15.0; 8.4	10.0; 14.0
$t_{1/2\alpha}$ (h)	0.13; 0.05	
$t_{1/2\beta}$ (h)	0.71; 0.71	
$t_{1/2\gamma}$ (h)	22.7; 16.0	
C_{max} (ng Eq/ml)		12.3; 13.0
T_{max} (h)		0.5; 0.25

[a] The $AUC_{0\text{-}t}$ interval was from 0 to 80 h.

Plasma concentration versus time profiles for Compound A after a single 0.02 mg/kg intravenous bolus or oral dose were determined by AMS (total ^{14}C levels) as well as by LC-MS/MS (parent levels) and are presented in Figs. 2 and 3. It should be noted that due to the small dose administered to dogs, it was not possible to quantify plasma levels beyond 2 h postdose by LC-MS/MS, because the levels had dropped below the lower limit of quantitation (2 ng/ml). The much more sensitive technique of AMS permitted monitoring of total ^{14}C plasma concentrations of Compound A down to 0.2 ng/ml, from which a pharmacokinetic profile at the 0.02 mg/kg microdose was obtained.

The plasma concentration-time profiles of total ^{14}C determined by AMS versus parent levels of Compound A determined by LC-MS/MS after a single 1 mg/kg oral dose are presented in Fig. 4, and the average pharmacokinetic parameters are summarized in Table 3 along with those of the 0.02 mg/kg oral dose for comparison. At a dose of 1 mg/kg, the $AUC_{0\text{-}t}$ and C_{max} ratios of parent Compound A to total ^{14}C levels were 0.77 and 0.76, respectively, indicating that the parent compound accounted for the majority of the ^{14}C in plasma. The $AUC_{0\text{-}t}$ and C_{max} at 1 mg/kg as determined by AMS were 2846 ng Eq · h/ml and 659 ng Eq/ml, respectively. At the 50-fold lower dose of 0.02 mg/kg, the $AUC_{0\text{-}t}$ and C_{max} as determined by AMS were proportionately lower (54.5 ng Eq · h/ml and 12.7 ng Eq/ml, respectively), i.e., 2725 ng Eq · h/ml and 635 ng Eq/ml, respectively, when dose-normalized for a dose of 1 mg/kg. Due to the low levels of radioactivity used in this study, it was not possible to detect total ^{14}C



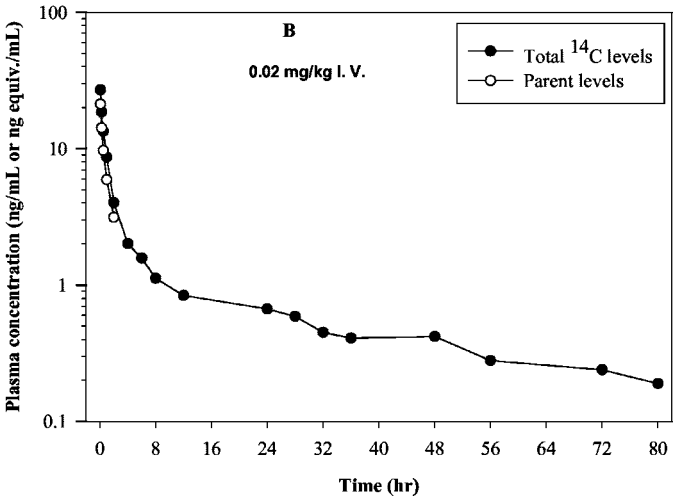


FIG. 2. Mean plasma concentration-time profiles of parent levels of Compound A (ng/ml; ○) and total ^{14}C levels (ng Eq/ml; ●) on a linear scale (A) and semilog scale (B) after intravenous administration of 0.02 mg/kg (~100 nCi) [^{14}C]Compound A to dogs (n = 2).

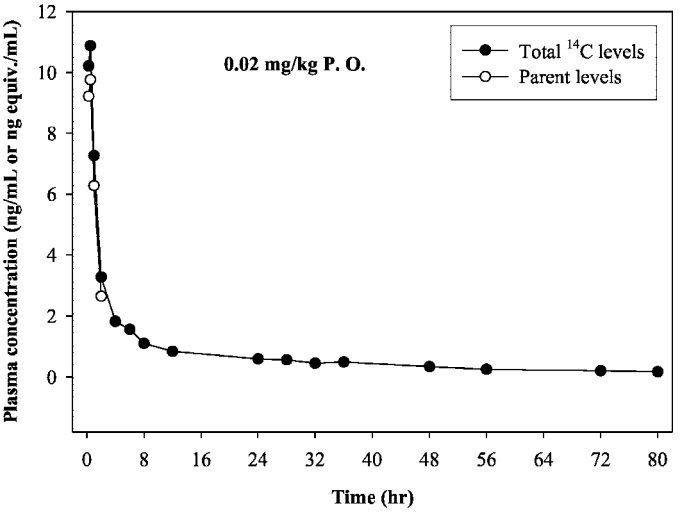


FIG. 3. Mean plasma concentration-time profiles of parent levels of Compound A (ng/ml; ○) and total ^{14}C levels (ng Eq/ml; ●) after oral administration of 0.02 mg/kg (~100 nCi) [^{14}C]Compound A to dogs (n = 2).

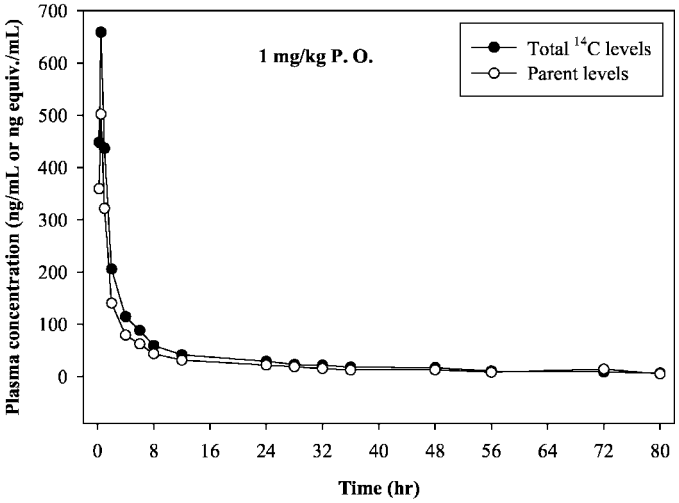


FIG. 4. Mean plasma concentration-time profiles of parent levels of Compound A (ng/ml; ○) and total ^{14}C levels (ng Eq/ml; ●) after oral administration of 1 mg/kg (~100 nCi) [^{14}C]Compound A to dogs (n = 2).

levels in plasma by the conventional technique of LSC. Once again, the enhanced sensitivity of AMS allowed monitoring of these plasma samples at both the pharmacological and microdose levels.

Discussion

Low-dose or microdose studies in humans potentially have an important place in the drug development process inasmuch as they can offer an early determination of the pharmacokinetic properties of a compound and thus assist in the selection of those drug candidates that possess optimal disposition properties for further evaluation in the clinic (Lappin and Garner, 2004). However, an underlying assumption with this approach is that the pharmacokinetics at the microdose (subpharmacological dose) reflect those at the pharmacological dose. This is especially important for compounds that are retained in a target tissue, such as anti-osteoporotics in the bone (Stepensky et al., 2003). In situations where the target tissue cannot be directly sampled, disposition properties of compounds can be estimated from the long-term kinetics of elimination through plasma or excreta (Gregory et al., 1998). Pharmacokinetic studies at subpharmacological doses, therefore, need to utilize analytical procedures that can define not only the initial absorption and distribution phases accurately, but also any slow elimination phase during which analyte concentrations may be very low. AMS has extremely high sensitivity for tracing isotopically labeled compounds, uses small sample sizes (5–10 μl), and can provide detailed kinetic profiles at low drug concentrations.

Preliminary pharmacokinetics of Compound A in dogs have been recently described by our colleagues (Olsen et al., 2004); however, the full pharmacokinetic profile outlining the multiphasic kinetics has not been previously reported. Furthermore, currently there are no published comparisons of the kinetics of a pharmaceutical compound at pharmacological versus subpharmacological doses using microdosing strategies. The present study thus provides the first full description of the pharmacokinetics of a development candidate assessed under these two dosing regimens and demonstrated that, in the case of Compound A, the pharmacokinetics in the dog appear to be linear over a 50-fold dose range. These pilot studies in dogs provide some reassurance that microdose studies with Compound A in humans would provide meaningful predictions of kinetics at higher (pharmacological) doses. From a methodological standpoint, microdose/AMS studies are labor-intensive with respect to sample collection and handling due to the need to prevent contamination by extraneous

TABLE 3

Average oral pharmacokinetic parameters of Compound A in dogs over a 50-fold dose range after administration of a pharmacological dose versus a microdose

The dose administered to dogs was 1 mg/kg for the pharmacological and 0.02 mg/kg for the microdose study. The microdose parameters in the table are presented as dose-normalized for a dose of 1 mg/kg. Data are presented as an average of two determinations.

Parameter	Pharmacological Dose		Microdose (AMS Analysis)
	LC-MS/MS Analysis[a]	AMS Analysis	
$AUC_{0\text{-}t}$ (ng or ng Eq · h/ml)[b]	2181	2846	2725
$AUC_{0\text{-}inf}$ (ng or ng Eq · h/ml)	2442	3113	3085
% AUC extrapolated	10.5	8.3	12
C_{max} (ng or ng Eq/ml)	502	659	635
T_{max} (h)	0.5	0.5	0.38
$AUC_{0\text{-}t}$ ratio at 1 mg/kg (parent levels/total ^{14}C levels)	0.77		NA
C_{max} ratio at 1 mg/kg (parent levels/total ^{14}C levels)	0.76		NA

NA, not applicable.

[a] These parameters were obtained in a group of dogs that were dosed with radiolabel in parallel to the dogs dosed for the AMS study. The 1 mg/kg oral data presented in Table 1 are from a separate study in which dogs were dosed with unlabeled compound. Parent levels of Compound A were determined by LC-MS/MS analysis and total ^{14}C levels were determined by AMS analysis.

[b] The $AUC_{0\text{-}t}$ interval was from 0 to 80 h.

sources of ^{14}C. However, when appropriate procedures are put in place, excellent concordance can be obtained between measurements conducted by AMS and LSC (Kaye et al., 1997; Garner et al., 2000; Young et al., 2001). In the present study, plasma levels of unchanged Compound A after a 1 mg/kg oral dose, measured by a specific LC-MS/MS assay, proved to be closely similar to those of total ^{14}C assessed by AMS. Therefore, it could be concluded that Compound A accounted for virtually all of the drug-related material in the circulation of dogs given a pharmacological dose of this agent. However, for compounds that are extensively metabolized, it would be necessary to fractionate the plasma samples to obtain a more accurate quantification of parent levels. Fractionation of samples by HPLC followed by AMS analysis allows the separation of various individual components that account for total radioactivity in the samples being analyzed (Garner et al., 2002). When the animals were treated with Compound A at a microdose (0.02 mg/kg), either orally or by the intravenous route, the limited sensitivity of the LC-MS/MS assay did not allow plasma concentrations of parent drug to be followed beyond the 2-h time point, whereas levels of total ^{14}C remained well above limits of detection by AMS through the final (80-h) time point. More importantly, the greatly enhanced sensitivity of AMS revealed the multiphasic kinetic profile of Compound A, which was not evident in the corresponding data set from the LC-MS/MS analyses, thereby reinforcing the need for AMS in microdose studies. The low levels of radioactivity used in this study precluded the use of LSC for determining total ^{14}C levels in plasma at all dose levels.

Previous reports on the use of AMS strategies in support of early drug development have focused largely on the benefit associated with exposure of human subjects to very low levels of radiation (Young et al., 2001; Garner et al., 2002.). According to regulations in the UK, exposure to such low levels of radioactive test material does not require regulatory approval since the level of exposure to ionizing radiation from such small doses is lower than that expected from the background environment. The dose of radioactivity used in the present dog study was at least 500- to 1000-fold lower than would be used for a conventional high-dose radiotracer study in which LSC was to be used for monitoring drug-related material in plasma and excreta. In the United States, radiological materials with specific activities less than 50 nCi/g can be disposed of by licensed radioactivity users as nonradioactive waste to a limit of 1 μCi per year (as stipulated in the Code of Federal Regulations 10-CFR-20.2005).

Currently, there are two published examples of clinical AMS studies in which the disposition of pharmaceuticals in humans was defined after administration of low levels of ^{14}C-labeled drugs (Young et al., 2001; Garner et al., 2002). The study reported in the present communication highlights a further benefit of AMS for pharmaceutical applications, namely, the definition of the full pharmacokinetic profile of a test compound, even after a microdose, that would not have been possible by conventional techniques due to limitations of sensitivity. Human pharmacokinetic measurements also have been determined over long time periods using AMS to quantify low levels of nutrients in a general population including healthy young adults (Dueker et al., 2000; Lemke et al., 2003).

In summary, the current study successfully validated the technique of accelerator mass spectrometry by investigating the disposition of Compound A in dogs, which afforded pharmacokinetic data in excellent agreement with those obtained via conventional techniques. The study also validated for the first time the use of a microdose strategy to assess the full pharmacokinetic profile of a drug candidate by demonstrating linearity across a 50-fold dose range. Investigations of this type in animal species should prove valuable in assessing the suitability of microdose/AMS approaches for exploratory pharmacokinetic studies in human subjects.

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