Analytical Methods for the Bioavailability Evaluation of Hydroxypyridinonate Actinide Decorporation Agents in Pre-Clinical Pharmacokinetic Studies

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Abstract

The hydroxypyridinonate ligands 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO) are two lead compounds under development for actinide chelation therapy. Methods to quantify these actinide decorporation agents in plasma are necessary to study their in vivo pharmacokinetic behavior. Such bioanalytical methods were developed with rat plasma, using liquid chromatography coupled with tandem mass spectrometry, and have a detection range of 0.05 to 2.5 µg/mL and 0.1 to 5 µg/mL for 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO), respectively. These methods were used to determine the in vivo plasma pharmacokinetics of the free acid and four salt forms of each ligand after a single intravenous or oral administration in rats. The different salt forms displayed similar pharmacokinetic profiles to those of the corresponding free acid, and the use of salt co-formers did not improve the oral bioavailability of the active pharmaceutical ingredients in rats. The described bioanalytical detection methods were successfully applied to the selection of solid-state forms of 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO) for future preclinical development activities, and will be adapted for use with plasma from other species.

Keywords: Hydroxypyridinone; Actinide Decorporation; Pharmacokinetics; Salt Screening

Abstract

The hydroxypyridinonate ligands 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO) are two lead compounds under development for actinide chelation therapy. Methods to quantify these actinide decorporation agents in plasma are necessary to study their in vivo pharmacokinetic behavior. Such bioanalytical methods were developed with rat plasma, using liquid chromatography coupled with tandem mass spectrometry, and have a detection range of 0.05 to 2.5 µg/mL and 0.1 to 5 µg/mL for 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO), respectively. These methods were used to determine the in vivo plasma pharmacokinetics of the free acid and four salt forms of each ligand after a single intravenous or oral administration in rats. The different salt forms displayed similar pharmacokinetic profiles to those of the corresponding free acid, and the use of salt co-formers did not improve the oral bioavailability of the active pharmaceutical ingredients in rats. The described bioanalytical detection methods were successfully applied to the selection of solid-state forms of 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO) for future preclinical development activities, and will be adapted for use with plasma from other species.

Introduction

The only practical therapy for internal contamination with radio nuclides such as the actinides is aggressive, and often protracted, treatment with chemical agents that can form excretable low molecular-weight chelates [1-3]. Two actinide decoration agents, 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO), are lead candidates for future actinide chelation therapy [4]. Both compounds contain hydroxypyridinonone metal-binding units linked to a polyamine scaffold through amide functionalities (Figure 1), resulting in multidentate ligand structures [tetradeinate for 5-LIO(Me-3,2-HOPO) and octadentate for 3,4,3-LI(1,2-HOPO)] with high selectivity and affinity for a broad range of actinide metal ions, including plutonium, americium, curium, neptunium and uranium [5,6]. Previous studies in several laboratory animal models, including mice, rats and dogs, have demonstrated the unrivaled actinide decoration efficacy of these ligands and suggest that, when appropriately formulated, they should have the advantage of oral delivery [2,4,7]. Broad efficacy and oral administration would be highly desirable from a logistical standpoint during a mass casualty radionuclide event and a vast improvement over the licensed diethylenetriamine pentaacetic acid (DTPA)-based products, DTPA being the only actinide decoration agent currently approved by the U.S. Food and Drug Administration (FDA) [8].

Although much is known about the in vivo efficacy and toxicology of 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO) in animal models [4,6,7], the pharmacological behavior of these ligands is still largely undefined. Only one previous study has addressed the pharmacokinetics of both ligands, and that study used 14C as a radioactive label [9]. While radioactive labels allow for the determination of the compound not only in the circulating fluids but also in different organs and excreta, they do not allow for discrimination between the active pharmaceutical ingredient (API) and potential metabolites or degradation products. Detection of non-degraded APIs can be obtained using spectroscopic...
Materials and Methods

General

All chemicals were obtained from commercial suppliers and used as received. The APIs 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO) were obtained from Albany Molecular Research, Inc. (Albany, NY), as described previously [4]. All nuclear magnetic resonance (NMR) spectra were acquired at ambient temperature on a Varian Unity INOVA 400 MHz spectrometer. X-ray powder diffraction patterns were collected with an Inel XRG-3000 diffractometer. An incident beam of Cu Kα radiation was produced using a fine-focus tube and a parabolically graded multilayer mirror. Elution solvents for plasma and sample analysis were purchased from Mallinkrodt Baker, Inc. (Phillipsburg, NJ), Sprague-Dawley rat plasma was purchased from Bioreclamation, Inc. (Hicksville, NY), and internal standards methyl nicotinate and ethyl nicotinate were purchased from Sigma Chemicals (St. Louis, MO). Blood was collected into MonojectTM Lavender Top Tubes containing 7.5% tripotassium ethylenediamine tetraacetic acid (K3EDTA) as the anticoagulant (Tyco Healthcare Group, Mansfield, MA). The general procedures for animal care and housing were conducted in accordance with the National Research Council for the Care and Use of Laboratory Animals and the Animal Welfare Standards Incorporated in 9 CFR Part 3, 1991 [13]. All procedures and protocols used in the described in vivo studies were reviewed and approved by the Institutional Animal Care and Use Committees of Lawrence Berkeley National Laboratory and SRI International, and were performed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Preparation of the salt forms

Four salt forms were prepared for each actinide-chelating agent. Salts of 5-LIO(Me-3,2-HOPO) were formed with citric acid, gentisic acid, N-methyl-γ-glucamine (meglumine), and tromethamine. Salts of 3,4,3-LI(1,2-HOPO) were formed with hippuric acid, succinic acid, meglumine, and l-lysine. All salt forms except [5-LIO(Me-3,2-HOPO);gentisic acid] were prepared similarly, on a 1-g scale with the co-former and free API combined in a 1:1 molar ratio and ethyl acetate (EtOAc) added until a slurry was achieved (~100 mg API per mL EtOAc). The sample was sealed, agitated, and warmed to ~55°C. After 48 h, the sample was slowly cooled to room temperature and vacuum filtered. The solids were then collected and dried under ambient conditions. For [5-LIO(Me-3,2-HOPO);gentisic acid], the slurry was obtained with a saturated solution of gentisic acid in methanol instead of EtOAc. The formation of amorphous salt powders was confirmed by X-ray powder diffraction. The API: co-former ratio in each salt was determined by comparing the resulting 1H NMR spectra to that of the corresponding free acid. Molecular weights for solution preparation were based on the API: co-former ratio. The purity of each salt form was verified by High Performance Liquid Chromatography (HPLC) following a previously described method [4].

Pharmacokinetic evaluation of the free acids and salt forms in rats

Ligand solutions were prepared in sterile water with the pH adjusted to 7.0-7.4 with 1 N NaOH. Dose volumes were 5 mL/kg for iv injection and either 5 mL/kg or 10 mL/kg for po administration. The tetradentate ligand 5-LIO(Me-3,2-HOPO) was first delivered at 100, 150, and 500 μmol/kg (equivalent to 40.7, 61, and 203 mg/kg) for iv, low po, and high po doses, respectively, and during a subsequent study at 100 μmol/kg for both iv and po administrations. The octadentate ligand 3,4,3-LI(1,2-HOPO) was first delivered at 30, 100, and 500 μmol/kg (equivalent to 22.5, 75, and 275 mg/kg) for iv, low po, and high po doses, respectively, and during a subsequent study at 100 μmol/kg for both iv and po administrations. Each of the salt forms was delivered at 100 μmol/kg (based on the molecular weight of the different forms to ensure similar API concentrations) by iv and po administrations. Groups containing three randomly assigned jugular vein catheterized (JVC) male Sprague-Dawley rats (11-12 weeks, 307-380 g) were given a single ligand dose by iv injection via the tail vein or by oral gavage. Blood samples (~0.3 mL) were collected from the JVC at various time points from 5 min to 24 h post dose. Rats were housed individually, given food and water ad libitum, and euthanized after the last blood collection.

Plasma sample preparation

Blood samples were mixed with K2EDTA anticoagulant, processed to plasma using standard methods, and stored frozen (~70°C) until analysis. Samples were then thawed and 50 μL aliquots were transferred to microfuge tubes containing acetonitrile (200 μL) to precipitate the plasma proteins. These mixtures were vortexed for 10 min and clarified by centrifugation (18,000 g for 5 min). The resulting supernatants were transferred to new tubes, evaporated under vacuum, and reconstituted with 50 μL of 10 mM NaEDTA in water containing 50 ng/mL of the internal standard for the assay [methyl nicotinate for 5-LIO(Me-3,2-HOPO), and ethyl nicotinate for 3,4,3-LI(1,2-HOPO)]. The reconstituted samples were clarified by centrifugation (18,000 g for 5 min), and transferred to HPLC vials fitted with glass inserts for Liquid Chromatography–tandem Mass Spectrometry (LC-MS/MS) analysis.

Calibration standards

Calibration standards [0.05, 0.2, 0.5, 0.75, 1, and 2.5 μg/mL for 5-LIO(Me-3,2-HOPO) and 0.1, 0.25, 0.5, 1, 2, 3, 4, and 5 μg/mL for 3,4,3-LI(1,2-HOPO)] were prepared in blank rat plasma that was processed in parallel with study samples. Primary stock solutions of 1 mg/mL 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO) were prepared in 50% (v/v) acetonitrile in water. The primary stocks were then used to prepare various spiking solutions by dilution with 10 mM NaEDTA in water. One volume of spiking solution was added to 99 volumes of blank rat plasma to obtain nominal concentrations of standards with a final non-plasma matrix of 1%. These calibration standards were stored at ~70°C until analysis. Calibration standards were analyzed in triplicate on each day of sample analysis.

Chromatographic system and conditions

Analysis was performed on a Waters 2795 Alliance Integrated...
System HPLC coupled with a Micromass Quattro LC spectrometer or on a Shimadzu LC-20AD HPLC pump coupled with an Applied Biosystems/MDS SCIEX 4000 Q TRAP system. Separation was achieved on a Synergi Fusion-RP column (Phenomenex, Torrance, CA; 4 µm, 50 x 2.0 mm) maintained at room temperature with two 0.1% formic acid mobile phases [(A) in distilled, deionized water and (B) in acetonitrile]. Samples were eluted using isocratic flow (100% A) over 1 min, followed by a gradient from 0% B to 95% B over 3.25 min at 0.3 mL/min, and an injection volume of 10 or 20 µL. Analytes and internal standards were detected by multiple reaction monitoring after electrospray ionization in the positive ion mode, using the following transitions: 429.3 → 278.2 for 5-LIO(Me-3,2-HOPO) plus 138.0 → 78.0 for methyl nicotinate and 775.3 → 195.0 for 3,4,3-LI(1,2-HOPO) plus 152.0 → 124.0 for ethyl nicotinate.

Data analysis

Peak responses of the analytes [peak area for 5-LIO(Me-3,2-HOPO) and peak height for 3,4,3-LI(1,2-HOPO)] were divided by the peak area or height of the internal standard for the assay to yield peak area or height ratios (PAR or PHR, respectively). Calibration standard curves were generated by performing weighted (1/y) quadratic regression of the PAR or PHR as the dependent variable (y-axis) and concentration as the independent variable (x-axis) using the Quanlynx System version 5.2 Professional. The equivalent API dose (mg/kg) was used to normalize the analysis for free acid and salt forms and avoid corrections for individual body weights. The following parameters and constants were determined: maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), area under the plasma concentration-time curve (AUC), volume of distribution (V), elimination clearance (Cl), and terminal elimination half-life (t_{1/2}). In addition, the percent oral bioavailability (F) was estimated, when possible, using the following formula: F = (AUC_{po}/AUC_{iv}) • (Dose_{po}/Dose_{iv}), where the mean AUC_{po} value and each individual AUC_{po} value were used.

Results and Discussion

Bioanalytical method

Separation of the two APIs, 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO), and their respective internal standards (methyl nicotinate and ethyl nicotinate) in plasma samples was achieved by LC-MS/MS. A Synergi Fusion-RP column with a polar embedded C18 phase was chosen to reduce the method run time and achieve the best peak shape for the targeted hydroxypyridinonate compounds. Liquid chromatography was performed with slightly acidic eluent (0.1% formic acid) to promote the formation of positive ions in the electrospray source of the mass spectrometer. The goodness of fit for the calibration standard curves was obtained from quadratic regressions, with r^2 varying from 0.953 to 0.996 for 5-LIO(Me-3,2-HOPO) and 0.963 to 0.990 for 3,4,3-LI(1,2-HOPO). The lower limit of quantification (LLOQ) values in rat plasma, defined as the lowest concentrations that can be measured yielding method precision values of coefficient of variation of 20% and % bias of ± 20% of the nominal value, were 50 ng/mL for 5-LIO(Me-3,2-HOPO) and 100 ng/mL for 3,4,3-LI(1,2-HOPO). The same bioanalytical methods were used to determine the ligand plasma concentrations in a pharmacokinetic evaluation of both APIs in rats and then in a follow-up pharmacokinetic evaluation of the free acids and four salt forms of both APIs.

Pharmacokinetic evaluation of 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO)

The objective of the initial study was to determine the plasma pharmacokinetics of 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO) in male rats after a single iv or po administration. One iv dose level [100 and 30 mol/kg for 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO), respectively] and two po dose levels [150 and 500 µmol/kg for 5-LIO(Me-3,2-HOPO) and 100 and 500 µmol/kg for 3,4,3-LI(1,2-HOPO)] were tested for both APIs in three rats per group. All rats survived until their scheduled euthanization, and most of the rats appeared normal at 4 and 24 h after dose administration. Red discolored urine and soft stool was observed in one of three rats 2 h after iv administration of 3,4,3-LI(1,2-HOPO), although this rat appeared normal 4 and 24 h post-dose. All three rats that were iv administered 5-LIO(Me-3,2-HOPO) had red or brown discolored urine 4 h post-dose and were normal 24 h post-dose. Discolored urine was not seen after po administration of either test article.

The mean API concentrations in the plasma 5 min to 8 h after iv and po administration are presented in (Figures 2 and 3) for 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO), respectively. The ligand 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) were detected in plasma samples, with peak areas normalized to the internal standards. The peak areas were used to calculate the plasma concentrations of each API, and a summary of the pharmacokinetic parameters is presented in Table 1.
HOPO) was not detected in plasma at the 100 µmol/kg po dose level and neither drug was measurable at 24 h. Both drugs showed log-linear decay in plasma after iv administration, but 5-LIO(Me-3,2-HOPO) had a higher peak concentration (197 ± 6 µg/mL) and total plasma exposure (AUC = 107 ± 9 h•µg/mL) (Table 1). There was a trend for faster Cl with 5-LIO(Me-3,2-HOPO), however, the V (1.60 ± 0.23 L/kg) and t½ (2.33 ± 0.44 h) values suggested there was greater tissue distribution than for 3,4,3-LI(1,2-HOPO). Oral administrations of the two drugs revealed that 5-LIO(Me-3,2-HOPO) had higher F values (a measure of bioavailability) than 3,4,3-LI(1,2-HOPO) (~3-5% versus 0.6 to 1.9% for most animals regardless of the salt form, except for the citric acid salt, the meglumine salt, and the tromethamine salt). The four outlying po animals had higher plasma levels than expected for 5-LIO(Me-3,2-HOPO) in vivo studies in rats was to determine the plasma pharmacokinetics and oral bioavailability of the different salt forms of 5-LIO(Me-3,2-HOPO) (with citric acid, gentisic acid, meglumine, and tromethamine) and 3,4,3-LI(1,2-HOPO) (with hippuric acid, succinic acid, meglumine, and L-lysine), in comparison to the corresponding free acid. The same influence of salt formation on pharmacokinetics and bioavailability

Salt/co-crystal screens were performed to potentially identify salt forms with improved bioavailability for each API [11]. Milling and reaction crystallization experiments, with thirty-one pharmaceutically acceptable co-formers [14] and salt attempts (Na, K, Ca and Mg) were carried out using a variety of solvent and pH conditions. Four different salt forms were selected for each API, based on the crystallinity and purity of the corresponding materials. The objective of this second set of in vivo studies in rats was to determine the plasma pharmacokinetics and oral bioavailability of the different salt forms of 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO) in male Sprague-Dawley rats (n = 3).

Plasma drug levels for the free acid and salt forms of 5-LIO(Me-3,2-HOPO) were >100 µg/g in 5 min after iv administration, and the log-linear decay of the concentration-time profiles was similar for all five compounds (Figure 4). Accordingly, AUC values (99-131 h•µg/ mL) were comparable among the five compounds and with the first iv administration (Table 2). Mean t½ values of 5-LIO(Me-3,2-HOPO) ranged from 0.65 to 0.76 h, and there was noticeable extravascular distribution (i.e., the majority of 5-LIO(Me-3,2-HOPO) did not stay in the plasma), based on V (0.27-0.48 L/kg). After po administration, plasma levels of 5-LIO(Me-3,2-HOPO) compounds were drastically lower than those from the iv group. 5-LIO(Me-3,2-HOPO) compounds were drastically lower than those from the iv group. Peak plasma levels (0.8-3.7 µg/mL) occurred 0.5 to 2 h post-dose and t½ (0.97-1.27 h) was somewhat slower than for iv administration. There was appreciable extravascular distribution based on estimates of V (0.47-0.71 L/kg). The range of individual F values was 0.6 to 1.9% for most animals regardless of the salt form, except for outlier animals (one in each of the groups administered with the free acid, the citric acid salt, the meglumine salt, and the tromethamine salt). The four outlying po animals had higher plasma levels than expected but the absorption and elimination patterns were not different, which suggests that this could be due to inter-animal variability. The effect was most obvious in the higher Cmax values in the early time points and AUC values for these four animals. The tmax, t½, V, and Cl values were

![Figure 4: Mean plasma time course of the free acid and four salt forms of 5-LIO(Me-3,2-HOPO) in male Sprague-Dawley rats after a single iv (100 µmol/kg) administration.](image-url)
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Intravenous Administration (iv)

| Compound | C max (µg/mL) | t max (h) | AUC last (h•µg/mL) | AUC cmax (h•µg/mL) | MRT last (h) | Cl (mL/h/kg) | V (L/kg) | t ½ (h) | F (%) |
|----------|---------------|-----------|-------------------|------------------|--------------|-------------|-----------|---------|-------|
| Free acid | 134 ± 29      | 0.14 ± 0.10 |102 ± 34          | 102 ± 34         | 0.89 ± 0.13  | 435 ± 165  | 0.48 ± 0.01| 0.76 ± 0.06|       |
| Citric acid salt | 177 ± 40 | 0.08 ± 0.00 |122 ± 24          | 122 ± 24         | 0.76 ± 0.06  | 340 ± 65   | 0.34 ± 0.10| 0.76 ± 0.06|       |
| Genticis acid salt | 116 ± 9 | 0.08 ± 0.00 |99 ± 23           | 99 ± 23          | 0.81 ± 0.11  | 424 ± 104  | 0.40 ± 0.08| 0.65 ± 0.06|       |
| Meglumine salt | 192 | 0.08 |149 | 149 | 0.60 | 273 | 0.27 | 0.69 |       |
| Tromethamine salt | 184 ± 54 | 0.08 ± 0.00 |131 ± 23          | 131 ± 23         | 0.74 ± 0.07  | 317 ± 52   | 0.34 ± 0.07| 0.75 ± 0.05|       |

Table 2: Mean pharmacokinetic parameters of 5-LIO(Me-3,2-HOPO) and four salt forms after a single 100 µmol/kg iv and po administration in male Sprague-Dawley rats (n = 3).

The mean plasma levels for all 3,4,3-LI(1,2-HOPO) test articles after iv administration (100 µmol/kg) showed a trend for two elimination phases, with the second, slower phase beginning approximately 3-4 h post-dose (Figure 5). The pharmacokinetic parameters after iv administration of 3,4,3-LI(1,2-HOPO) and the related salts showed the free ligand was never detected and the salt forms had one to three similar to the other replicate animals in each group, but F was higher.

For po administrations, V and Cl were calculated from V/F•F and Cl/F•F, respectively.

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Conclusion

The LC-MS/MS methods developed for the determination of the hydroxypyridinonate ligands 5-LIO(Me-3,2-HOPO) and 3,4,3-LI(1,2-HOPO) are sensitive with lower limits of detection of 0.05 and 0.1 µg/mL, respectively. They were successfully used to quantify the free acid forms and four salt forms of both APIs in rat plasma, after a single iv or oral administration. These pharmacokinetic studies revealed comparable ranges of plasma exposure (based on C p), however the octadentate ligand 3,4,3-LI(1,2-HOPO) had greater tissue distribution, a longer plasma t ½, and poorer oral bioavailability than the tetradentate ligand 5-LIO(Me-3,2-HOPO). The rate of 3,4,3-LI(1,2-HOPO) elimination (Cl) was higher for the 100 than the 30 µmol/kg iv dose level, which indicated there was dose-dependent pharmacokinetics. Salt formation with citric acid, gentisic acid, meglumine, and tromethamine for 5-LIO(Me-3,2-HOPO) and hippuric acid, succinic acid, meglumine, and L-lysine for 3,4,3-LI(1,2-HOPO), did not affect the pharmacokinetic profiles of the two APIs and did not enhance their oral bioavailability. The described bioanalytical methods will be used in further pharmacological studies to delineate the pharmacokinetics of these ligands in other animal species. The examination of pharmacokinetic profiles in different animal species will be instrumental in the determination of a dosing regimen for future clinical use [15,16]. Despite their low oral bioavailability, oral administration of these chelating agents still leads to exceptional actinide elimination rates [4,7]. Additional efforts are on-going to formulate optimized oral dosage forms.

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Table 3. Mean iv and individual po pharmacokinetic parameters of 3,4,3-LI (1,2-HOPO) and four salt forms after a single 100 µmol/kg administration in male Sprague-Dawley rats (n = 3).

| Compound          | Cmax (ng/mL) | tmax (h) | AUCmin (h•µg/ mL) | AUCinf (h•µg/ mL) | MRTlast (h) | CL (mL/h/kg) | V (L/kg) | t1/2 (h) |
|-------------------|--------------|----------|--------------------|--------------------|-------------|-------------|----------|----------|
| Free acid         | 121 ± 28     | 0.08 ± 0.00 | 98 ± 8             | 101 ± 67           | 1.2 ± 0.2   | 743 ± 48    | 2.6 ± 0.6 | 2.4 ± 0.4 |
| Hippuric acid salt| 229 ± 45     | 0.08 ± 0.00 | 154 ± 25           | 157 ± 25           | 1.1 ± 0.1   | 488 ± 83    | 1.3 ± 0.9 | 1.7 ± 0.9 |
| Succinic acid salt| 215 ± 16     | 0.08 ± 0.00 | 145 ± 31           | 146 ± 31           | 1.0 ± 0.1   | 527 ± 100   | 1.1 ± 0.6 | 1.4 ± 0.5 |
| Meglumine salt    | 184 ± 33     | 0.08 ± 0.00 | 110 ± 26           | 110 ± 26           | 0.8 ± 0.2   | 711 ± 188   | 1.2 ± 0.2 | 1.2 ± 0.1 |
| L-Lysine salt     | 185 ± 15     | 0.08 ± 0.00 | 121 ± 12           | 123 ± 11           | 1.0 ± 0.1   | 613 ± 54    | 1.4 ± 0.7 | 1.7 ± 1.0 |

* NC = not calculated; only one data point was available.
* ND = no data; the test article was never detected.
* Results derived from samples collected in one animal; no standard deviation is available.
* Results derived from samples collected in two animals; no standard deviation is available.

Intravenous Administration (iv)

| Compound | Cmax (g/mL) | tmax (h) | AUCinf (h•µg/ mL) | F (%) |
|----------|-------------|----------|-------------------|-------|
| Free acid | ND          | ND       | ND                | ND    |
| Hippuric acid salt | 227 | 1  | 122               | 0.05  |
| Succinic acid salt | 639 | 0.63  | 376               | 0.18  |
| Meglumine salt | 221 | 0.25  | NC†              | NC    |
| L-Lysine salt | 214 | 0.50  | 74               | 0.04  |

a Cmin is the first measurable concentration in plasma after dose administration.

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