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AI-700 pharmacokinetics, tissue distribution and exhaled elimination kinetics in rats

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Abstract

The purpose of these studies was to determine the pharmacokinetics, tissue distribution, and exhaled elimination kinetics in rats for intravenously administered AI-700, which consists of porous microspheres containing decafluorobutane (DFB), for use as an ultrasound contrast agent. [Pd]-AI-700 was administered intravenously to rats (10 mg microspheres/kg). Blood and tissue samples collected at specified times were analyzed for palladium by inductively coupled plasma-mass spectrometry (ICP-MS). AI-700 was also administered intravenously to rats (40 mg microspheres/kg) and expired air was collected over time. Expired air samples were analyzed for DFB by validated adsorbent trapping–thermal desorption–gas chromatography–mass spectrometry methodology.

Pd from [Pd]-AI-700 was cleared from blood with a ca. 50–85% decline from peak concentration within 5 min. At 1440 min post-dose, 52–72% of the Pd dose was recovered from organs of the reticuloendothelial system. Approximately 77% of the intravenously injected DFB was found in expired air within 3 h after dosing, with most of the DFB dose (61 ± 6%) expired within the first 10 min after dosing. As expected, the microspheres were cleared through the reticuloendothelial system, and the DFB was eliminated in expired air, with more than half of the DFB eliminated within the first 10 min after dosing.

Keywords: Ultrasound; Microsphere; Pharmacokinetics; Biodistribution; Perfluorocarbon

1. Introduction

AI-700 is an intravenous ultrasound contrast agent currently being studied clinically for evaluation of myocardial perfusion in the assessment of coronary artery disease (Hamilos and Patrianakos, 2005). The product consists of small (2 μm), porous microspheres filled with a perfluorocarbon gas, decafluorobutane (DFB), and formulated as a dry powder with mannitol and polysorbate 80 excipients (Straub et al., 2005). The primary component of the microsphere shell is the biodegradable and biocompatible synthetic polymer, poly(D,L-lactide-co-glycolide) (PLGA). The shell also contains the phospholipid 1,2-diarachidoyl-*sn*-glycero-3-phosphocholine (DAPC), which serves to minimize gas loss from the microspheres. Ammonium bicarbonate is used during the production of the microspheres (as a pore forming agent) to create the internal porosity of the microspheres. An ultrasound contrast agent should contain a gas for maximum compressibility and density differences between the agent and tissue, so that the contrast agent creates optimal acoustic response (de Jong and ten Cate, 1966), thus, the gas (DFB) is encapsulated within the porous microspheres of AI-700. For use in ultrasound imaging, AI-700 drug product is suspended in sterile water for injection, and administered by intravenous injection. Phases I and II clinical studies for the preliminary evaluation of the safety and efficacy of AI-700-enhanced ultrasound imaging have been completed (Walovitch et al., 2000; Grayburn et al., 2004; Weissman et al., 2004). AI-700 is currently being studied in Phase III trials for the evaluation of myocardial perfusion in the assessment of coronary artery disease.

Microspheres administered intravenously were expected to be cleared through the organs of the reticuloendothelial system (lung, liver, spleen), as has been described in literature for other

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microparticles (Arturson et al., 1983; Edman and Sjoholm, 1983; Davis and Illum, 1988; Ciftci et al., 1997; Marchal-Heussler et al., 1999; Lunsford et al., 2000; Panagi et al., 2001; Redhead et al., 2001). For such biodistribution studies for the PLGA microspheres of AI-700, no direct method for analysis of PLGA in tissues was available, and thus a method for labeling the microspheres with palladium was developed, which allowed for analysis of tissues for palladium content using inductively coupled plasma-mass spectrometry (ICP-MS).

The encapsulated gas was expected to be eliminated through expired air, as has been seen for other gas containing echocardiographic contrast agents (Hutter et al., 1999; Uran et al., 2005; Killam et al., 1999; Morel et al., 2000; Correas et al., 2001, 2005; Toft et al., 2006). The gas chromatography–mass spectrometry (GC–MS) method for analysis of exhaled decafluorobutane utilized was similar to a method recently described in literature (Uran et al., 2005).

This paper describes rat studies of the biodistribution and pharmacokinetics of the microspheres of AI-700, and includes both assessment of the clearance of the microspheres through the reticuloendothelial system, as well as the study of the kinetics of elimination of the encapsulated gas (DFB) through expired air.

2. Materials and methods

2.1. Materials

2.1.1. Production of AI-700 microspheres and AI-700 drug product

AI-700 microspheres were prepared by spray drying. The details of production have been described elsewhere (Straub et al., 1998, 2005; Walovitch et al., 2005). In brief, AI-700 was produced by spray drying a water-in-oil emulsion, with the oil phase being a methylene chloride solution containing PLGA and DAPC, and the water phase being a solution of ammonium bicarbonate in water. The resulting microspheres were dispersed in an aqueous vehicle containing mannitol and polysorbate 80, and the dispersion was sieved, filled into vials, frozen and lyophilized. The headspace of lyophilized vials was subsequently filled with DFB to produce AI-700 drug product.

AI-700 drug product was suspended in water for intravenous administration by venting the vial with a filtered vent needle, adding 5 mL of sterile water via a separate needle, removing the needles, and shaking the vial.

2.1.2. Production of [Pd]-AI-700 microspheres and [Pd]-AI-700 drug product

Palladium (Pd) was used as a label for the AI-700 microspheres. [Pd]-AI-700 was produced in a similar manner to AI-700 by spray drying a methylene chloride solution containing PLGA, DAPC, and $Pd(OAc)_2$. The resulting microspheres were dispersed in an aqueous vehicle containing mannitol and polysorbate 80, and the dispersion was sieved, filled into vials, frozen and lyophilized. The headspace of lyophilized vials was subsequently filled with DFB to produce [Pd]-AI-700 drug product. The Pd content of the [Pd]-AI-700 microspheres was determined by ICP-MS for Pd.

The particle size distribution of the reconstituted degassed [Pd]-AI-700 drug product diluted into an electrolyte solution was determined using a Coulter Multisizer II (Hialeah, FL) fitted with a 50-μm aperture, which uses an electrozone method for determination of particle size and particle concentration.

The rate of release of Pd from the reconstituted [Pd]-AI-700 drug product was evaluated by diluting 800 μL of the suspension (38.6 mg of microspheres) into 10 mL of bovine serum containing 0.03% w/v sodium azide (a surrogate for human blood) in tubes, and then rotating the tubes in a 37 °C incubator. At 6 and 24 h, samples were filtered to remove the microspheres, and the resulting solution was analyzed for soluble Pd by ICP-MS.

2.2. Methods

2.2.1. Biodistribution of [Pd]-AI-700 in rats after intravenous administration

This study adhered to the currently acceptable practices of good animal husbandry as per the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

For administration, [Pd]-AI-700 was reconstituted with 50:50 5% dextrose:sterile water (v/v) to a concentration of 21.4 mg microspheres/mL, which resulted in an iso-osmotic suspension. The test article suspension was administered intravenously into a tail vein of 9–13-week old Sprague–Dawley rats, via a catheter as a single dose of 10 mg microspheres/kg body weight (0.97 mg Pd/kg body weight).

Two cohorts (three rats/sex/cohort) were used in this study to allow for analysis of many time-points. In Cohort 1, blood samples were collected at 0, 1, 6, 11, 16, 30, 45, 60, 240, 480, and 1440 min post-dose. In Cohort 2, blood samples were collected at 0, 2, 7, 13, 20, 30, 45, 60, 120, 360, and 1440 min post-dose. Twenty-eight (28) tissues were collected from all rats following sacrifice after blood collection at the 1440 min sampling time.

Using a validated ICP-MS method, samples of the following tissues were analyzed for Pd content: blood (all sampling times), lung, liver, spleen, and the area of the tail containing the injection site. For analysis for Pd, the tissue samples were digested in aqua regia for 2 h at 120 °C, adjusted to a standard volume with water, and analyzed by ICP-MS for Pd. The ICP-MS method for analysis of Pd had an LOD of 0.01 μg/g. Spike-recovery experiments yielded acceptable precision and accuracy data, with the lowest levels tested being 0.048 μg Pd/g rat blood, 0.23 μg/g rat lung, 0.288 μg Pd/g rat tail, 0.35 μg Pd/g rat spleen, and 0.246 μg Pd/g rat liver.

The mean maximum blood concentration of Pd (C_{max}) and the time to maximum concentration (t_{max}) were determined from inspection of the individual Pd concentration–time data. The area under the mean blood concentration–time curve ($AUC_{0\text{-}1440}$) was estimated using the trapezoidal method.

2.2.2. Analysis of DFB in expired air from rats after intravenous administration of AI-700

This study adhered to the currently acceptable practices of good animal husbandry as per the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

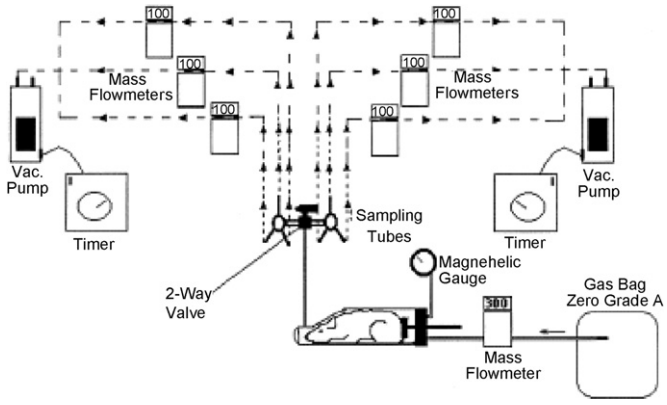

Fig. 1. Expired Air Collection System Using Tubes. Air flowed at a rate monitored using a mass flowmeter through the chamber containing the rat and then flowed into a valve to which was attached two sets of three sampling tubes containing adsorbants for capture of decafluorobutane. At select time intervals, the system passed all the air through one set of three adsorbant tubes. The amount of air that passed through each adsorbant tube was controlled using a vacuum pump and monitored using mass flow.

AI-700 was suspended in sterile water to a concentration of 40 mg microspheres/mL, which resulted in an iso-osmotic suspension. The rats were restrained in a custom designed, leak-tight, glass chamber. The test article suspension was administered intravenously into a tail vein to one group of five male and five female 8-week old Sprague–Dawley rats via a catheter as a single dose of 40 mg microspheres/kg (32.8 μL DFB/kg). An airstream of 300 mL/min was drawn past the animal's snout prior to, during and after dose administration. Upon initiation of dosing, and for the following 3 h, samples of the air flow were collected into absorbent traps (four males, four females; Fig. 1) or Tedlar gas sampling bags (one male, one female; Fig. 2). For absorbent tube sample collection, air samples were collected at frequent intervals during the initial 10 min after dosing (1 min intervals for the first 5 and 2.5 min intervals for the second 5 min), for 12.5 min intervals during the next 50 min after dosing, and for no more than 20 min intervals during the next 120 min after dosing. At approximately 6, 24 and 48 h after dosing, 20 min air samples were collected into absorbent tubes. For

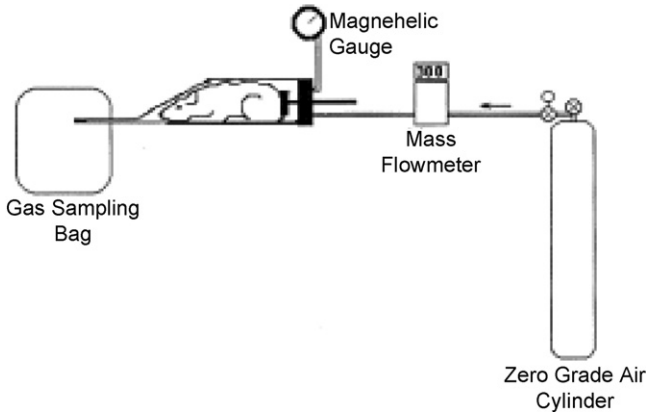

Fig. 2. Expired Air Collection System Using Bags. Air flowed from a cylinder at a rate monitored using a mass flowmeter through the chamber containing the rat and then flowed into a bag which captured all of the air flow.

gas bag sample collection, a single gas bag collected expired air for 180 min post-dosing. For the male rat studied using gas bag sample collection through 180 min, later samples (20 min air samples) were collected into tubes at approximately 6, 24 and 48 h after dosing. The collected expired air samples were analyzed by a validated assay involving thermal desorption–gas chromatography–mass spectrometry assay. The trapezoidal rule was used to estimate the amount of DFB expired over each interval post-dosing, with the cumulative amount of expired DFB through a given time being equal to the sum of the expired DFB from the intervals analyzed up to that time.

Thermal desorption–gas chromatography–mass spectrometry analysis method: The collected expired air samples were analyzed by a validated assay using thermal desorption–gas chromatography–mass spectrometry methodology. Known volumes of expiratory air from the dosed rats and/or volumes of gas from the Tedlar gas sampling bags were collected in adsorbent traps consisting of 500 mg of thermally preconditioned, Carboxen-569 adsorbent (Supelco) packed inside 10 cm length × 4 mm i.d. glass lined, stainless steel desorption tubes (Scientific Instrument Services, Inc., Ringoes, NJ). Adsorbent traps were injected with 1.0 μL of a 949 ng/μL stock solution of perfluorocyclohexane (PFCH, Aldrich Chemical Co.) in isooctane to serve as internal standard prior to analysis. Adsorbent traps were thermally desorbed into the GC–MS system using a Scientific Instrument Services model TD-2 Short Path Thermal Desorption System. Thermal desorption conditions were 10 s initial purge time, 30 s post-injection equilibration time and then thermal desorption at 275 °C for 60 s. The gas chromatograph was a Varian 3400 equipped with a 75 m × 0.45 mm i.d. DB-VRX wide bore capillary column containing a 2.55 μm film thickness (J&W Scientific). The injector temperature was 220 °C with a split ratio of 150:1. Helium was used as carrier gas with a column head pressure of 16 psi. The GC column was temperature programmed from 3 °C (hold 5 min) to 240 °C at a rate of 25 °C per minute with a 1.0 min hold at the upper limit. The GC was directly interfaced to a Finnigan MAT 8230 high resolution, double-focusing, magnetic sector mass spectrometer. The MS was operated in electron ionization (EI) mode and high resolution, selected ion monitoring of the *m/z* 69 (68.9952) base peak in the mass spectra of DFB and PFCH was performed. The analytical system was calibrated with certified reference standards of DFB in nitrogen. A linear (*r*-squared = 0.9977), 11-point DFB calibration with a dynamic range of 0.098–1000 nanoliters (nl) was established. The limit of detection (LOD) with a 2:1 signal to noise ratio (s/n) was 0.098 nl. The limit of quantification (LOQ) based on a 10:1 s/n was 0.49 nl. Under the stated analysis conditions DFB and PFCB eluted at 4.3 and 6.6 min retention time, respectively, as sharp, well-defined peaks. Analyses were conducted over an 11-day period and system suitability (chromatographic resolution) and between-batch analytical accuracy and precision standards were analyzed between groups of 20 study samples to verify system calibration and performance throughout the study. The mean analytical precision throughout the study was 10.84% R.S.D. ($n = 23$) and the mean backfit to calibration was (9.61%).

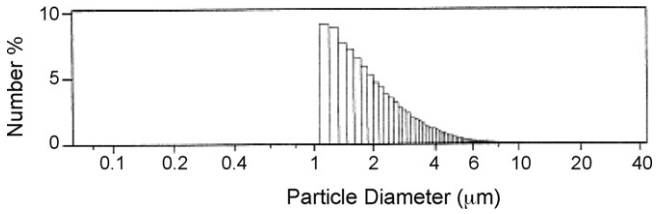

Fig. 3. Particle size distribution of [Pd]-AI-700.

3. Results

3.1. Characterization of microspheres

The Pd was 9.7% of the mass of the [Pd]-AI-700 microspheres as determined by ICP-MS for Pd. The mean particle diameter of the microspheres was 2.2 μm, with a concentration of 1.6×10^9 microspheres/mL. The particle size distribution for [Pd]-AI-700 is shown in Fig. 3. Analysis of the release of Pd from [Pd]-AI-700 in serum at 37 °C was performed to assess the degree of loss of label from the microspheres under conditions that simulated intravenous administration. The study revealed that only 7.9 and 11.5% of Pd was released in 6 and 24 h, respectively, which indicated that the label stayed associated with the microspheres.

Characterization of AI-700 has been previously described in literature (Straub et al., 2005). The ammonium bicarbonate used in the production of AI-700 microspheres functions as a pore forming agent to facility the production of porous particles that are ultimately filled with DFB.

3.2. Biodistribution of [Pd]-AI-700 in rats after intravenous administration

The blood Pd concentration data (μg Pd/g blood) after administration of [Pd]-AI-700 are shown in Fig. 4; data for Cohorts 1 and 2 have been combined to form one Pd concentration–time curve. Because no consistent gender differences in the blood concentrations were observed, data for both sexes were combined to calculate mean blood Pd concentrations for each cohort per time-point. Peak concentrations of Pd (C_{max}) were observed at the first sampling time (1–2 min post-dose). The C_{max} for Cohort 1 (first sampling time = 1 min) was 1.05 ± 0.665 μg/g, and C_{max} for Cohort 2 (first sampling time = 2 min) was 0.688 ± 0.214 μg/g. The concentration of Pd in blood decreased by approximately 50–85% during the 5-min interval immediately following C_{max}. Due to the small number of sampling times immediately post-dose, an accurate initial half-life could not be determined. At subsequent time-points the clearance of Pd was slower than initially seen, but blood Pd concentrations continued to decrease gradually. Measurable concentrations (0.024–0.034 μg/g blood) were observed at the 1440 min (24 h) sampling time.

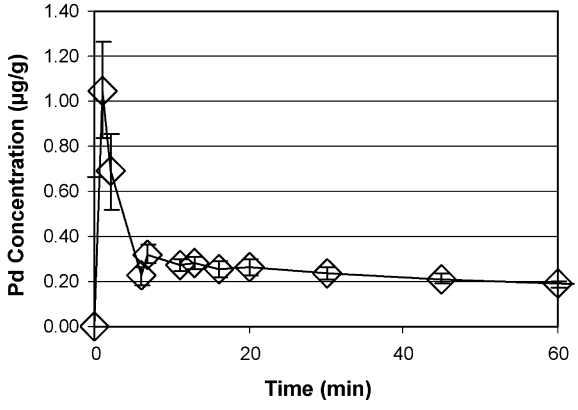

Fig. 4. Concentration of Pd in blood, following an intravenous dose of 0.97 mg Pd/kg body weight in [Pd]-AI-700. Combined mean values for Cohorts 1 and 2 are shown. Error bars are standard deviations.

The $AUC_{0\text{-}1440}$ values for the blood Pd concentration versus time curve were found to be 102 ± 8.28 μg min/g for Cohort 1 and 108 ± 14.7 μg min/g for Cohort 2.

At 24 h post-dose, tissue concentrations of palladium (μg Pd/tissue) were higher in the lung (37 ± 10 μg/g) and the spleen (25 ± 6 μg/g) than in the liver (13 ± 2 μg/g) (data from Cohorts 1 and 2 combined). Although the concentrations of Pd (on a μg/g basis) in the lung and the spleen were actually higher than those in the liver, a much higher percent of the Pd dose (higher total μg) was recovered in the liver (41.6 ± 6.6%) than in the lung (18.0 ± 4.1%) and spleen (5.2 ± 0.9%), due to the relatively large size of the liver. A total of 52–72% of the Pd dose was recovered in the lung, liver and spleen at 24 h post-dose.

3.3. Analysis of DFB in expired air from rats after intravenous administration of AI-700

Cumulative DFB in expired air after intravenous administration of AI-700 is presented in Table 1 (tube collection). The

Table 1
Expired DFB from rats using the tube collection method

Time (min)	Cumulative recovered dose of DFB (%) (mean ± S.D.)		
	Males ($n = 4$)	Females ($n = 4$)	Average ($n = 8$)
1.0	16.9 ± 1.3	22.9 ± 2.2	19.9 ± 3.6
2.0	31.5 ± 2.4	36.9 ± 2.1	34.2 ± 3.6
3.0	38.5 ± 3.2	45.6 ± 1.8	42.1 ± 4.5
4.0	43.3 ± 3.7	51.9 ± 1.6	47.6 ± 5.3
5.0	46.6 ± 3.9	56.0 ± 1.5	51.3 ± 5.7
7.5	52.8 ± 4.6	62.1 ± 1.1	57.4 ± 5.9
10.0	56.3 ± 4.6	66.1 ± 2.1	61.2 ± 6.2
22.5	61.9 ± 4.2	70.3 ± 3.4	66.1 ± 5.7
35.0	63.7 ± 4.3	71.5 ± 3.8	67.6 ± 5.6
47.5	64.5 ± 4.4	71.9 ± 4.0	68.2 ± 5.5
60.0	64.9 ± 4.4	72.2 ± 4.0	68.5 ± 5.5
80.0	65.3 ± 4.4	72.4 ± 4.1	68.9 ± 5.5
100.0	65.6 ± 4.4	72.6 ± 4.1	69.1 ± 5.5
120.0	65.8 ± 4.4	72.8 ± 4.2	69.3 ± 5.5
140.0	65.9 ± 4.4	72.9 ± 4.2	69.4 ± 5.4
160.0	66.0 ± 4.4	73.0 ± 4.2	69.5 ± 5.5
180.0	66.2 ± 4.4	73.1 ± 4.2	69.6 ± 5.4
360.0	69.6[a]	70.7[a]	70.2 ± 2.5[b]
1440.0	70.6[a]	71.3[a]	70.9 ± 2.5[b]
2880.0	71.0[a]	71.8[a]	71.4 ± 2.8[b]

[a] $n = 2$ rats/sex. S.D. not reported.
[b] $n = 4$ rats total.

Table 2
Comparison of gas elimination rates for ultrasound contrast agents

Agent	Gas[a]	Species	Gas elimination in expired air
AI-700	DFB	Rat	51.3 ± 5.7% of DFB was eliminated at 5 min
Optison™	OFP	Canine	OFP was rapidly cleared through the exhaled air, with an approximate 40-second mean residence time (Killam et al., 1999)
Optison™	OFP	Human	The half-life ranged from 30.6 to 83.7 s (Hutter et al., 1999)
Definity®	OFP	Human	OFP concentrations were not detected at 10 min in most subjects in expired air (Definity, 2004)
Sonovue™	SF_6	Rabbit	98% of the injected dose was recovered in exhaled air within 2 min (Sonovue, 2001)
Sonovue™	SF_6	Human	Approximately 40–50% of the injected dose of SF_6 was eliminated with the expired air during the first minute after dosing (Morel et al., 2000).

[a] OFP, octafluoropropane; SF_6, sulfur hexafluoride.

objective of the study was to evaluate the time-course of elimination of DFB in expired air, and thus the tube collection method was the primary method used. Due to an apriori concern that there might be leakage or incomplete collection of expired air with the tube collection method, a small number of animals ($n = 2$) had expired air over the first 3 h post-dose collected using a sampling bag technique. Because no consistent gender differences in the DFB concentrations were observed, data for males and females have been combined for analysis and discussion. The samples collected in tubes showed that most of the DFB (61 ± 6%) was expired within the first 10 min after dosing (Table 2, Fig. 5). However, additional samples collected up to 48 h after dosing indicated that DFB continued to be expired in small quantities. When results for samples collected at 6, 24 and 48 h were included and used to estimate total 48-h recovery, the DFB dose recovery was 71.4% (tube collection). The alternative method for collecting expired air samples (bag collection technique) was tested with only two animals. Analysis of air expired into a single gas sampling bag per animal showed that approximately 77% of the intravenously injected DFB was expired within 3 h after dosing. When results for samples collected at 6, 24 and 48 h were included and used to estimate total 48-h recovery, the DFB dose recovery was slightly higher, 78.6%, for the gas bag collection method. The data indicated that there was possibly some loss of DFB due to the tube collection technique relative to the bag collection technique. However, the slight increase in recovery for the bag technique was insufficient to warrant further testing using the bag collection technique.

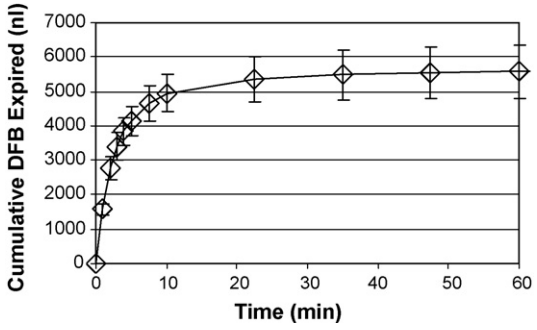

Fig. 5. Expired DFB, following an intravenous dose of 32.8 μL DFB/kg body weight dose in AI-700. Expired air samples were collected in tubes for four rats/sex/time point. Error bars are standard deviations.

4. Discussion

Porous PLGA microspheres were produced by spray drying an emulsion containing ammonium bicarbonate as the pore forming agent and filled with DFB for use as an ultrasound contrast agent. For biodistribution studies, the PLGA microspheres were labeled with Pd, and the Pd content in blood and tissues after intravenous administration of [Pd]-AI-700 to rats was determined by ICP-MS. For evaluation of DFB elimination after intravenous administration of AI-700 to rats, the expired air was captured and analyzed for DFB content by GC/MS/MS.

The results from the rat biodistribution study demonstrated that the majority of [Pd]-AI-700 microspheres were located, as expected, in the organs of the reticuloendothelial system (lung, liver, and spleen) at 24 h post-dose. In this study, the Pd was cleared from the blood with a ca. 50–85% decline from peak concentration during a 5 min interval immediately after C_{max}. This relatively rapid clearance of microspheres of [Pd]-AI-700 from the blood would be expected for intravenously administered microspheres with a size distribution as shown in Fig. 3, which have not been designed to have surface functionalities that would be seen with a stealth type of microparticle (Arturson et al., 1983; Edman and Sjoholm, 1983; Davis and Illum, 1988; Ciftci et al., 1997; Marchal-Heussler et al., 1999; Lunsford et al., 2000; Panagi et al., 2001; Redhead et al., 2001). Although, during the production of AI-700, the polysorbate 80 (which contains polyethylene glycol units, a type of functionality used in stealth microparticles) may have at least partially coated the AI-700 microspheres, some literature reports have indicated that polysorbates do not have a significant effect on the clearance of intravenously administered particles or emulsions (Lode et al., 2001; Ueda et al., 2002). The data for AI-700 were consistent with such earlier findings, indicating that the presence of a polysorbate in a formulation does not necessarily lead to the creation of a stealth-like microsphere.

DFB was eliminated in expired air, with more than half of the DFB dose eliminated within 10 min after dosing. As seen in Table 2, similar or faster rates of elimination via expired air of a fluorinated gas have been seen with other intravenously administered ultrasound contrast agents. Estimated DFB recovery of less than 100% for AI-700 in rats within 48 h post-dose may be due to at least one of the following: (1) use of the trapezoidal rule to estimate the amount of DFB excreted over 3–24 h intervals

may have resulted in an underestimate of the amount excreted or (2) less than 100% of expired air may have been collected due to gas leakage from the collection system. Consequently, the estimated DFB recovery is likely to indicate quantitative recovery of the administered dose over 48 h within the limits of cumulative experimental error.

In comparing the DFB elimination data to the microsphere biodistribution data, it was evident that the DFB was eliminated from the body through expired air faster than the microspheres themselves, which are detectable in significant quantities in the tissue at 24 h. The DFB dissolved into blood or tissue and then was eliminated through expired air, while the microsphere shell remained in the body. The main component of the shell (the PLGA) is biodegradable, and ultimately degrades to lactic acid and glycolic acid over a longer timeframe than that for the DFB elimination.

For imaging myocardial perfusion, a sufficient number of microspheres containing gas must be in circulation during the imaging time to provide for clinically relevant reflected ultrasound signal. Although, in the rat studies described herein, the microspheres were cleared from circulation with an approximately 50–85% decline from peak concentrations within 5 min, and $61 \pm 6\%$ of the DFB was eliminated within 10 min after dosing, clearance and elimination rates for rats may not be representative of those for humans (Ward and Smith, 2004). However, such studies in rats are useful in helping design similar studies in humans, and such rat studies are a necessary component of regulatory submissions such as INDs. Additionally, ultrasound is a highly sensitive detection system, and even individual microspheres may be detectable (Klibanov et al., 2004). Myocardial perfusion echocardiography studies in humans would be expected to be completed within approximately 2–3 min. Following completion of imaging, rapid clearance of the imaging agent is desirable. Thus, unlike liposomal based intravenous drug delivery systems such as those for amphotericin B and doxorubicin, a long circulating half-life is not required for an ultrasound contrast agent. The results from these rat studies suggest that the clearance rate of both the microspheres and the gas make AI-700 drug product appropriate for use in ultrasound imaging studies.

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