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● *Original Contribution*

PHARMACOKINETICS OF PERFLUOROBUTANE AFTER INTRA-VENOUS BOLUS INJECTION OF SONAZOID IN HEALTHY CHINESE VOLUNTEERS

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Abstract—Sonazoid is an ultrasound contrast agent based on microbubbles (MB) containing perfluorobutane (PFB) gas. Sonazoid is approved in Japan, Korea and Norway for contrast-enhanced ultrasonography of focal liver lesions and focal breast lesions (Japan only). The objective of this study was to determine the pharmacokinetics (PKs) and safety of Sonazoid in Chinese healthy volunteers (HVs) and to evaluate the potential for ethnic differences in PKs between Chinese and Caucasian HVs. Sonazoid was administered as an intra-venous bolus injection at the clinical dose of 0.12 μL or 0.60 μL MB/kg body weight to two groups of eight Chinese HVs. Expired air and blood samples were collected and analyzed using a validated gas chromatographic tandem mass spectrometry method, and the main PK parameters were calculated. The highest PFB concentrations in blood were observed shortly after intra-venous administration of Sonazoid, and elimination of PFB was rapid. In the 0.12 μL MB/kg body weight cohort, PFB concentrations above the limit of quantification were observed for only 10 to 15 min post-injection. In the 0.60 μL MB/kg body weight cohort, PFB concentrations above the limit of quantification were observed for 60 min post-injection, and the shape of the elimination curve suggested a biphasic elimination profile. The maximum observed concentration (C_{max}) values of PFB in blood were 2.3 ± 1.1 and 19.1 ± 9.2 ng/g for the 0.12 and 0.60 μL MB/kg body weight dose groups (mean ± standard deviation). Area under the curve values were 10.1 ± 2.7 and 90.1 ± 38.3 ng × min/g for the 0.12 and 0.60 μL MB/kg body weight dose groups. C_{max} values of PFB in exhaled air were 0.35 ± 0.2 and 2.4 ± 0.7 ng/mL for the 0.12 and 0.60 μL MB/kg body weight dose groups. Assessment of laboratory parameters, vital signs, oxygen saturation and electrocardiograms revealed no changes indicative of a concern. The PK profile and safety data generated in the Chinese HVs were comparable to previous data for Caucasian HVs. (E-mail: Susan.Hoppmann@ge.com) **© 2017 The Authors. Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).**

Key Words: **Ultrasound, Pharmacokinetics, Perfluorobutane, Sonazoid, Microbubbles, Microspheres.**

INTRODUCTION

Sonazoid (GE Healthcare, Amersham, UK) is a contrast agent for use in ultrasound imaging of focal liver lesions and focal breast lesions that has received marketing approval in Japan, Korea and Norway. An understanding of the pharmacokinetics of Sonazoid, including dose dependency and ethnic variability, is necessary for its safe and efficacious clinical use.

The drug product consists of an aqueous dispersion of lipid-stabilized perfluorobutane (PFB)-filled gas microbubbles (MB) with a median volume diameter of approximately 3 μm (Sontum et al. 1999) (Fig. 1). The microbubbles are stabilized with a monolayer of phospholipids obtained from hydrogenated egg phosphatidylserine, with palmitoyl-stearoyl-phosphatidylserine as the main constituent (Hvattum et al. 2006).

The currently approved clinical dose for liver and breast imaging is 0.12 μL MB/kg body weight (b.w.). After intra-venous injection, the microbubbles are restricted to the intra-vascular compartment, but have no molecular targets within the blood vessels. In the liver sinusoids,

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however, Sonazoid is taken up by Kupffer cells and eventually degraded. In the case of ultrasound imaging of the liver, contrast enhancement is initially based on the presence of intact MB in the luminal compartment of blood vessels (vascular imaging) (Forsberg et al. 2000; Moriyasu and Iijima 2002) and later in the Kupffer cells of the liver (Kupffer cell imaging) (Forsberg 2000, 2002; Kindberg 2003; Yanagisawa 2007). The phospholipids are metabolized in the liver, and the metabolites are most probably completely oxidized or incorporated into the endogenous lipid pool. No metabolic system for degradation or conjugation of PFB has been reported, and the PFB gas is expected to be released unchanged from the liver back into the circulation. PFB is very hydrophobic and has very low solubility in water, but because of the small doses administered, the PFB could theoretically be completely dissolved in the aqueous phase of the liver before it is released into the circulation. Alternatively, it may be released as naked PFB microbubbles (gas bubbles without a lipid covering) or encapsulated in lipoproteins together with other hydrophobic compounds such as triacylglycerols. PFB that has been released into circulation will then be expired *via* the lungs and, to some extent, taken up in fat tissues.

A gas chromatography tandem to mass spectrometry (GC-MS) analytical method for determination of PFB was developed and has been used in a previous pharmacokinetic (PK) study of Sonazoid performed by GE Healthcare. That study was a phase 1 study in Caucasian healthy volunteers (HVs) and patients with abnormal pulmonary diffusion capacity who received 0.6 μL MB/kg b.w. Sonazoid by bolus injection or infusion (Landmark et al. 2008). After bolus administration of 0.6 μL MB/kg b.w. Sonazoid to Caucasian HVs, blood concentrations of PFB declined rapidly and biphasically. The C_{max} value of PFB in blood after bolus administration of 0.6 μL MB/kg b.w. Sonazoid in Caucasian HVs was 28.9 ± 7.2 ng/g, and the area under the curve (AUC) value was 172.7 ± 28.0 ng × min/g (mean ± standard deviation [SD]). The time of the maximum observed concentration (t_{max}) generally occurred between 0.5 and 2 min after injection. The mean ± SD terminal blood elimination half-life in Caucasian HVs was 34.5 ± 5.7 min. After bolus administration, clearance and volume of distribution appeared to be independent of study population and gender. Concentrations of PFB in exhaled air declined biphasically after bolus administration of Sonazoid. The mean ± SD elimination half-life in HVs was 31.7 ± 4.3 min. The C_{max} value of PFB in exhaled air in Caucasian HVs was 3.5 ± 1.6 ng/g (mean ± SD), and the AUC value was 44.0 ± 11.4 ng × min/mL (mean ± SD).

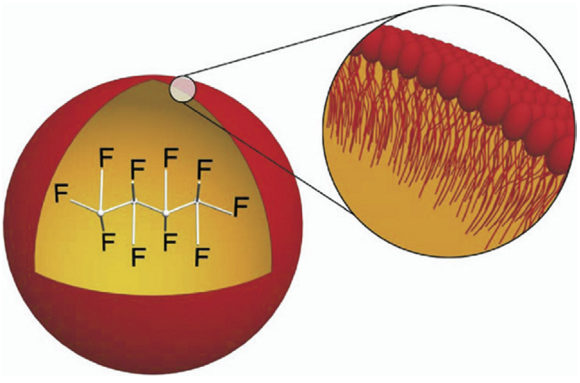

Fig. 1. Structure of a Sonazoid microbubble (MB). The Sonazoid drug product consists of an aqueous dispersion of lipid-stabilized perfluorobutane (PFB)-filled gas microbubbles with median volume diameter of approximately 3 μm (Sontum et al. 1999). The microbubbles are stabilized with a monolayer of phospholipids obtained from hydrogenated egg phosphatidylserine, with palmitoyl-stearoyl-phosphatidylserine as the main constituent (Hvattum et al. 2006).

The objective of the present study was to describe the PK properties and safety of Sonazoid in Chinese HVs after a bolus injection of Sonazoid at two dose levels: the clinical dose of 0.12 μL MB/kg b.w. and a high dose of 0.60 μL MB/kg b.w. The study results are discussed in comparison to the data obtained for Caucasian HVs (Landmark et al. 2008), with the focus on possible ethnicity-related variation of Sonazoid PK properties. As no metabolic system for degradation of PFB is known and no metabolites of PFB have been observed during the non-clinical and clinical testing of this agent, it was expected that the PKs of PFB in blood and exhaled air would not differ between different populations. The results obtained in the present study help to illustrate the extent of potential ethnic variability in the PKs of Sonazoid and other gas-filled microbubble-based ultrasound contrast agents.

METHODS

Materials

Sonazoid was produced by GE Healthcare, Oslo, Norway. Perfluoro-*n*-butane (PFB, C_4F_{10}) and perfluoro-*n*-pentane (PFP, C_5F_{12}) were purchased from Fluoromed (Round Rock, TX, USA). Tedlar bags (1000 mL) were purchased from SKC (Eighty Four, PA, USA), and headspace vials were purchased from Crawford Scientific (Strathaven, UK).

Clinical study

The study was designed to assess the PKs after intravenous bolus injection of Sonazoid in 16 Chinese HVs. A summary of the study population is given in Table 1. The study was conducted in full accordance with the 1996 revision of the Declaration of Helsinki, the Good Clinical

Practice: Consolidated Guidance (approved by the ICH) and applicable national and local laws and regulations. The study protocol was granted by the Chinese Food and Drug Administration (CFDA) and approved by the local ethics committee before the study commenced. Written informed consent was obtained from all participants. An overview of study procedures and timing is provided in Table 2. At the screening visit (up to 7 d before Sonazoid administration), patients had to satisfy all the inclusion criteria and none of the exclusion criteria defined by the protocol to be enrolled into the study. Demographic data (age, height, weight and body mass index) and medical history were recorded and a physical examination was performed. Blood samples for serum biochemistry and hematology were drawn, and urine for dipstick sample analysis was collected. A blood sample for hepatitis B, hepatitis C and human immunodeficiency virus screening was also obtained. Before administration of Sonazoid, concomitant medication and all pretreatment signs and symptoms were recorded. Vital signs were measured, pulse oximetry, physical examination and 12-lead ECG examinations were performed, and blood samples for serum biochemistry and hematology were drawn. Blood samples and exhaled air samples for PK analysis were collected.

Each subject received Sonazoid at a dose of 0.12 μL MB/kg b.w. (clinical dose, corresponding to 1.19 μg PFB/kg b.w.) or 0.60 μL MB/kg b.w. (corresponding to 5.94 μg PFB/kg b.w.) as a single intravenous bolus injection through a cannula, preferably in a forearm vein, with the subject in the supine position. Safety measurements, including monitoring for the occurrence of adverse events (AEs), were performed from the time of administration of Sonazoid until the last follow-up safety assessment. Blood samples and exhaled air samples were collected at various time points after dosing for the PK analyses. From 3 to 6 h after Sonazoid administration, before discharge from the study site, patients had a full safety follow-up and monitoring of AEs. At 24, 48 and 72 h after Sonazoid administration, patients were contacted by telephone to determine if any AEs had occurred or if any that were present when the subject left the study center had resolved or worsened.

Blood sample collection

Venous blood samples (approximately 5.0 mL per subject per time point) were collected with a heparinized Vacutainer tube from a Venflon in the arm contralateral to the one used to administer Sonazoid. Samples were obtained immediately before administration and at 0.5, 1, 2, 5, 10, 15 and 30 min and 1, 2, 4 and 24 h. For each time point and within 2 h, duplicate aliquots of approximately 2.0 mL of blood were transferred from the syringe to pre-weighed, capped, headspace vials containing 12 mL. The vials were weighed again to determine the weight of blood transferred to each vial, and the weights were recorded in the case report form. The vials were stored at 2°C to 8°C after sample collection until analysis of PFB gas was performed. Analysis was performed within 1 wk after sample collection.

Exhaled air sample collection

Exhaled air samples, approximately 500 mL, were collected in 1000-mL Tedlar bags immediately before Sonazoid administration and at 0.5, 1, 2, 5, 10, 15 and 30 min and 1, 2, 4 and 24 h afterward. Exhaled air samples were stored at room temperature until preparation for analysis. Duplicate 1.0-mL aliquots were removed from the Tedlar bags, with gastight syringes, and transferred to capped headspace vials containing 14 mL saline.

Table 1. Subject demographic characteristics and body measurements by dose group

Variable	0.12 μL MB/kg b.w. (N = 8)	0.60 μL MB/kg b.w. (N = 8)	Overall (N = 16)
Age (y)			
N	8	8	16
Mean ± SD	27.3 ± 5.3	25.4 ± 2.6	26.3 ± 4.14
Range	21–37	21–29	21–37
Gender, n (%)			
Male	4 (50.0)	4 (50.0)	8 (50.0)
Female	4 (50.0)	4 (50.0)	8 (50.0)
Race, n (%)			
Asian	8 (100.0)	8 (100.0)	16 (100.0)
Chinese	8 (100.0)	8 (100.0)	16 (100.0)
Weight (kg)			
Mean ± SD	58.4 ± 7.73	62.0 ± 6.07	60.2 ± 6.97
Range	50–69	54–70	50–70
Body mass index (kg/m^2)			
Mean ± SD	21.43 ± 1.60	21.42 ± 0.78	21.42 ± 1.21
Range	19.5–23.7	20.7–22.6	19.5–23.7

μL MB/kg b.w. = microliters of microbubbles per kilogram body weight; N = total number of patients in that dose group; n = number of patients with non-missing values for that variable; SD = standard deviation.

Table 2. Schedule of study procedures and timing

Variable	Screening (up to 7 d before Sonazoid™ injection)	Pre-administration	Bolus injection	Time after injection (min)															Time after injection (h)			
				0.5	1	2	3	5	6	9	10	15	16	30	31	60	61	120	121	4	24†	48†	72†
Inclusion and exclusion criteria	X																						
Informed consent	X																						
Demographic information	X																						
Medical history, smoking habits	X																						
Concomitant medication		X																					
Pregnancy test (fertile women)		X																					
Pre-treatment events		X																					
Physical examination	X	X*																		X			
Blood sample: Serum chemistry and hematology	X	X*																		X			
Urine dipstick	X																						
Injection site monitoring		X																		X			
Sonazoid administration			X																				
PK blood sampling		X		X	X	X		X			X	X		X		X		X		X			
Exhaled air collection		X			X		X		X	X			X		X		X		X	X			
Vital signs (blood pressure, heart rate)	X	X*			X			X			X	X		X		X				X			
ECG, 12-lead	X	X*																		X			
ECG, 3-lead					X			X			X	X		X		X				X			
Oxygen saturation		X			X			X			X	X		X		X				X			
Adverse events‡																							

ECG = electrocardiogram; PK = pharmacokinetic.

* If screening was performed on the same day as Sonazoid injection, screening samples/values could act as pre-administration baseline samples/values. At screening, a blood sample for hepatitis B, hepatitis C and human immunodeficiency virus (HIV) screening was also obtained.

† Telephone follow-up.

‡ Shading indicates continuous monitoring.

The vials were stored at 2°C to 8°C until analysis of PFB gas was performed. Analysis was performed within 1 wk of sample collection.

Perfluorobutane analyses of samples containing blood and exhaled air

Concentrations of PFB in blood and exhaled air were determined as described previously (Landmark et al. 2008). Briefly, headspace gas vials containing blood samples and exhaled air samples were allowed to warm to room temperature and spiked with an internal standard, perfluoro-*n*-pentane (PFP). The blood sample headspace gas vials were then sonicated to disrupt any remaining stabilized PFB microbubbles and placed in an automated headspace sampler. The calibration standard stocks and quality control stocks were made from gaseous PFB diluted in air in headspace gas vials containing 2 mL blood and 12 mL saline (blood analysis) or 14 mL saline (exhaled air analysis). Before analysis, the quality control samples and calibration standards were spiked with the internal standard and treated similarly to the blood or exhaled air samples.

The samples were analyzed together with calibration standard samples covering a range from 0.05 to 50 ng PFB/g blood or 0.10 to 100 ng PFB/mL exhaled air on a GC-2010 system (Shimadzu, Kyoto, Japan) comprising a Shimadzu AOC-5000 automatic headspace sampler coupled to a GCMS-QP2010 connected with a Shimadzu GC-MS detector. For analysis *via* GC-MS, the compounds were separated using a CP PoraBOND Q column, 25 m × 0.25 mm (Agilent Life Sciences). The calibration curves were obtained from the calibration standards by plotting the peak area ratio of PFB (*m/z* 69) to PFP (*m/z* 69) versus the theoretical concentration (in ng) of PFB in the calibration standards. The curve was fitted to a second-order polynomial equation, $Y = a + bx + cx^2$, with a weighting factor $1/x^2$. The amount of PFB in the samples was determined by calculating the same peak area ratio as above and correlating this ratio to nanograms of PFB/vial blood or exhaled air using the calibration curve.

A partial re-validation study of the headspace GC-MS method was carried out at Beijing Chao-Yang Hospital based on the full validation study performed previously (Landmark et al. 2008). The method as performed at Beijing Chao-Yang Hospital exhibited acceptable specificity, sensitivity, precision and accuracy with a calibration curve range of 0.05 to 50 ng PFB/g blood and 0.1 to 100 ng PFB/vial exhaled air. The calibration curve was fitted to the quadratic equation $y = ax^2 + bx + c$ with a weighting factor of $1/x^2$. A good fit of the calibration points to the calibration curve was obtained as illustrated by a high regression value of $r^2 > 0.99$.

This partial re-validation study confirmed that the previously developed method had been successfully transferred to the new site and the equipment was able to detect an analytical range similar to that developed in 2008. The LOQ defined in this partial re-validation study was 0.05 ng PFB/g for blood and 0.11 ng PFB/mL for exhaled air, which is similar that of the original analytical method. The results confirmed the reliability of the GC-MS method, and thus its suitability for concentration measurements of PFB in clinical samples in both phase I clinical studies.

Pharmacokinetic assessments

Blood and exhaled air concentration-time data were analysed using WinNonlin computer software (Pharsight 6.1, Mountain View, CA). A non-compartmental model was utilized to calculate the PK parameters. The maximum concentration (C_{max}) and time to C_{max} (t_{max}) after dosing were recorded as observed. Non-compartmental PK parameters were calculated as follows: $AUC_{0\text{-last}}$: area under the curve (AUC) estimated by linear-logarithmic method from time zero to the last time-point at which concentrations of PFB were above the LOQ of the assay. $AUC_{0\text{-inf}}$ = AUC from time zero to infinity, calculated from $AUC_{0\text{-last}} + (C_{last}/k_{el})$, where C_{last} is the observed concentration at the last measurable time point; $\%AUC_{ext}$ = percentage of the extrapolated area to total area calculated from $[(AUC_{0\text{-inf}} - AUC_{0\text{-last}})/AUC_{0\text{-inf}}] \times 100$; k_{el} = apparent terminal rate constant, calculated by linear least-squares regression analysis of the terminal linear portion of the semilogarithmic concentration-versus-time profile, using at least three concentration time points above LOQ; $t_{½}$ = apparent terminal half-life calculated from $\ln 2/k_{el}$; Clearance = total clearance, calculated from dose/$AUC_{0\text{-inf}}$ after bolus administration; V_d = apparent volume of distribution, calculated from clearance/k_{el} and normalized for

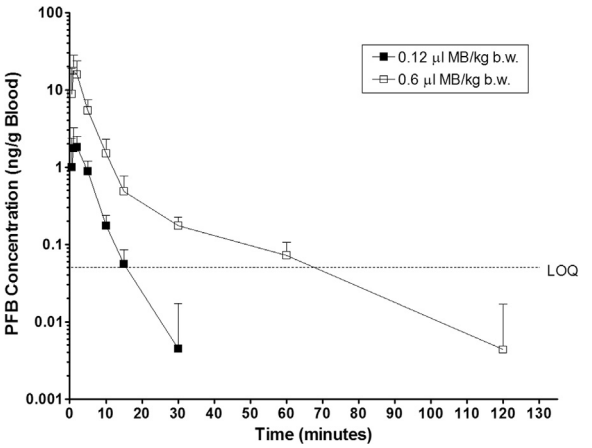

Fig. 2. Perfluorobutane (PFB) concentrations obtained from blood after administration of Sonazoid. Data are expressed as means ± standard deviations. LOQ = limit of quantification; MB = microbubbles.

Table 3. Pharmacokinetic parameters estimated from data obtained from Chinese HVs after intra-venous administration of Sonazoid

	Dose (μL MB/kg b.w.)	n	C_{max} (ng PFB/g or ng PFB/mL)	t_{max} (min)	$AUC_{0\text{-inf}}$ (ng PFB × min/g or ng PFB × min/mL)	$t_{½}$ (min)	V_d (g/kg)	Clearance (g/min/kg)
Blood	0.12	8	2.3 ± 1.1	1.5 ± 0.5	10.1 ± 2.7	2.7 ± 0.7	475.6 ± 155.5	124.8 ± 33.5
Blood	0.60	8	19.1 ± 9.2	1.9 ± 1.4	90.1 ± 38.3	17.0 ± 7.8	2146.0 ± 1912.7	78.5 ± 36.9
Exhaled air	0.12	8	0.4 ± 0.2	1.0 ± 0.0	7.1 ± 3.0	21.4 ± 8.0	na	na
Exhaled air	0.60	8	2.4 ± 0.7	1.0 ± 0.0	32.0 ± 6.5	22.0 ± 4.8	na	na

MB = microbubbles; b.w. = body weight; n = number of observations; PFB = perfluorobutane; C_{max} = maximum observed concentration; t_{max} = time at which C_{max} is reached; $AUC_{0\text{-inf}}$ = area under the concentration curve from time zero to infinity; $t_{½}$ = elimination half-life; V_d = volume of distribution; Clearance = dose/AUC.
All data are expressed as mean ± SD.

body weight. The clearance and V_d for PFB in exhaled air were not calculated.

Safety variables

Patients were monitored for the occurrence of AEs, serious AEs (SAEs), treatment-emergent AEs (TEAEs) and changes in serum biochemistry and hematology variables, vital signs, physical examination and injection-site status. Before, during and after administration of Sonazoid, the injection site was monitored for any abnormal findings. Safety monitoring was performed from before the time of administration of Sonazoid until the last follow-up safety assessment. From 3 to 6 h after Sonazoid administration, before discharge from the study site, patients had a full safety follow-up (vital signs, 12-lead ECG, physical examination, injection-site monitoring) and monitoring of AEs. Blood samples for serum biochemistry and hematology were drawn. At 24, 48 and 72 h after Sonazoid administration, patients were contacted by telephone to determine if any AEs had occurred or if any that were present when the subject left the study center had resolved or worsened.

Statistical analysis

Pharmacokinetic parameters, subject demographic characteristics and body measurements are reported as mean value, standard deviation, minimum, and maximum. Each PK parameter was estimated from a one-way analysis of variance (ANOVA) model with the treatment group as the factor. The 95% confidence interval (CI) for each PK parameter was calculated based on the *t*-distribution. To assess potential gender differences in PK parameters across dose groups, a two-way ANOVA model was used with

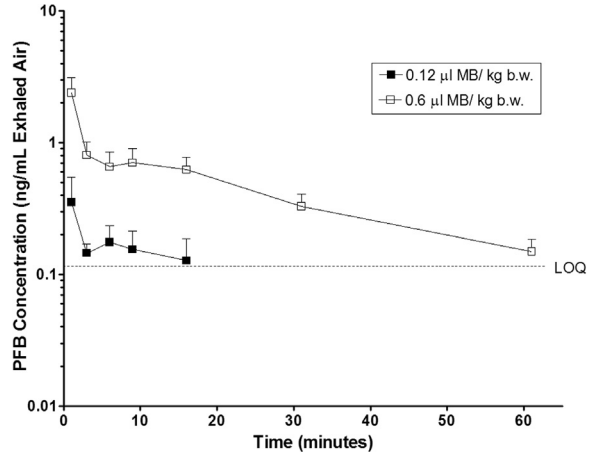

Fig. 3. Perfluorobutane (PFB) concentrations obtained from exhaled air after administration of Sonazoid. Data are expressed as means ± standard deviations. LOQ = limit of quantification; MB = microbubbles.

gender, treatment group and their interaction term as the factors.

RESULTS

Blood pharmacokinetic results

Mean blood concentrations of PFB are plotted versus time for both Sonazoid dose groups in Figure 2. As expected after intra-venous injection, the highest PFB concentrations were observed shortly after administration of Sonazoid. In both the 0.12 and 0.60 μL MB/kg b.w. dose groups, rapid initial elimination of PFB was observed. In the 0.12 μL MB/kg b.w. dose group, PFB concentrations above the LOQ (0.05 ng PFB/mL) were observed for the first 10 to 15 min post-injection. In the 0.60 μL MB/kg b.w. dose group, PFB concentrations above the LOQ were observed for 60 min post-injection, and the shape of the curve displayed a biphasic elimination profile.

Summary data for blood PK parameters after bolus injection are tabulated for males and females combined in Table 3. As expected, the blood PFB PK parameters $AUC_{0\text{-inf}}$ and C_{max} revealed that the systemic exposure to Sonazoid was significantly higher in the 0.60 μL MB/kg b.w. dose group than in the 0.12 μL MB/kg b.w. dose group ($p < 0.05$, based on the 95% upper and lower confidence limits) (Supplementary Table 1, online only, available at http://dx.doi.org/10.1016/j.ultrasmedbio.2017.01.003). For both dose groups, t_{max} was similar with mean values of 1.5 ± 0.5 and 1.9 ± 1.4 min post-injection for the 0.12 and 0.60 μL MB/kg b.w. dose groups, respectively. Mean C_{max} values for PFB in blood were 2.3 ± 1.1 and 19.1 ± 9.2 ng/g for the 0.12 and 0.60 μL MB/kg b.w. dose groups, respectively. Consistent with these findings, $AUC_{0\text{-inf}}$ values were 10.1 ± 2.7 ng × min/g and 90.1 ± 38.3 ng × min/g for the 0.12 and 0.60 μL MB/kg b.w. dose groups, respectively. A significant difference between dose groups was observed for $t_{½}$, with 2.7 ± 0.7 and 17.0 ± 7.7 min for the 0.12 and 0.60 μL MB/kg b.w. dose groups, respectively ($p < 0.05$, based on the 95% upper and lower confidence limits, Supplementary Table 1). However, in the 0.12 μl MB/kg b.w. dose group, PFB was only detected in blood up to 15 min post-injection, and the data points used for calculation of $t_{½}$ in this dose group represent predominantly the initial, rapid phase of elimination from the blood. The mean values for clearance were lower for the 0.60 μL MB/kg b.w. dose group compared with the 0.12 μL MB/kg b.w. dose group, whereas the reverse was seen for V_d. Based on inspection of the 95% confidence intervals, no significant gender differences were observed in blood PFB PK parameters (see Supplementary Table 1). However, the small sample size and extensive variability in the observed values limited the potential for finding significant gender differences.

Exhaled air pharmacokinetic results

Mean exhaled air concentrations of PFB are plotted versus time for both Sonazoid dose groups in Figure 3. The highest PFB concentrations were observed shortly after administration of Sonazoid. The concentrations thereafter decreased rapidly and biphasically, consistent with the decrease in blood PFB levels. The mean concentration of PFB fell below the LOQ after 16 min in the 0.12 μL MB/kg b.w. dose group and after 61 min in the 0.60 μL MB/kg b.w. dose group. Summary data for PK parameters of PFB in exhaled air are tabulated for male and female patients combined in Table 3. As anticipated, the exhaled air PK parameters $AUC_{0\text{-inf}}$ and C_{max} were significantly higher in the 0.60 μL MB/kg b.w. dose group compared with the 0.12 μL MB/kg b.w. dose group ($p < 0.05$, based on the 95% upper and lower confidence limits) (Supplementary Table 2, online only available at http://dx.doi.org/10.1016/j.ultrasmedbio.2017.01.003). The mean C_{max} value of PFB in exhaled air was 0.35 ± 0.2 and 2.4 ± 0.7 ng/mL for the 0.12 and 0.60 μL MB/kg b.w. dose groups, respectively. The $AUC_{0\text{-inf}}$ was 7.1 ± 3.0 and 32.0 ± 6.4 ng × min/mL for the 0.12 and 0.60 μL MB/kg b.w. dose groups, respectively. For both dose groups, $t_{½}$ values were similar, with 21.4 ± 8.0 and 22.0 ± 4.8 min for the 0.12 and 0.60 μL MB/kg b.w. dose groups, respectively. Based on inspection of the 95% confidence intervals, no significant gender differences were observed in the exhaled air PFB PK parameters (see Supplementary Table 2). However, the small sample size and extensive variability in the observed values limited the potential for finding significant gender differences.

Safety evaluation

No SAEs occurred, and no AEs leading to withdrawal of a subject occurred in this study. Eight patients (5 of 8 patients [62.5%] in the 0.12 μL MB/kg b.w. dose group and 3 of 8 patients [37.5%] in the 0.60 μL MB/kg b.w. dose group) experienced a total of 12 TEAEs, all of which were mild in intensity, and none was considered to be related to the study drug. Eleven of the 12 AEs were asymptomatic changes in blood pressure or heart rate. One subject in the 0.12 μL MB/kg b.w. dose group had a clinically significant change from baseline in 3- and 12-lead ECGs at 4 h post-injection. The subject was asymptomatic, but the investigator reported it as a mild, treatment-unrelated TEAE.

No trend toward dose-dependent changes was evident in the clinical laboratory data. No individual change in any clinical laboratory or vital sign variable was classified as clinically significant. Physical examination findings for all patients were normal at screening, baseline and 4 h post-injection. Injection sites were normal for all patients both pre- and post-Sonazoid injection.

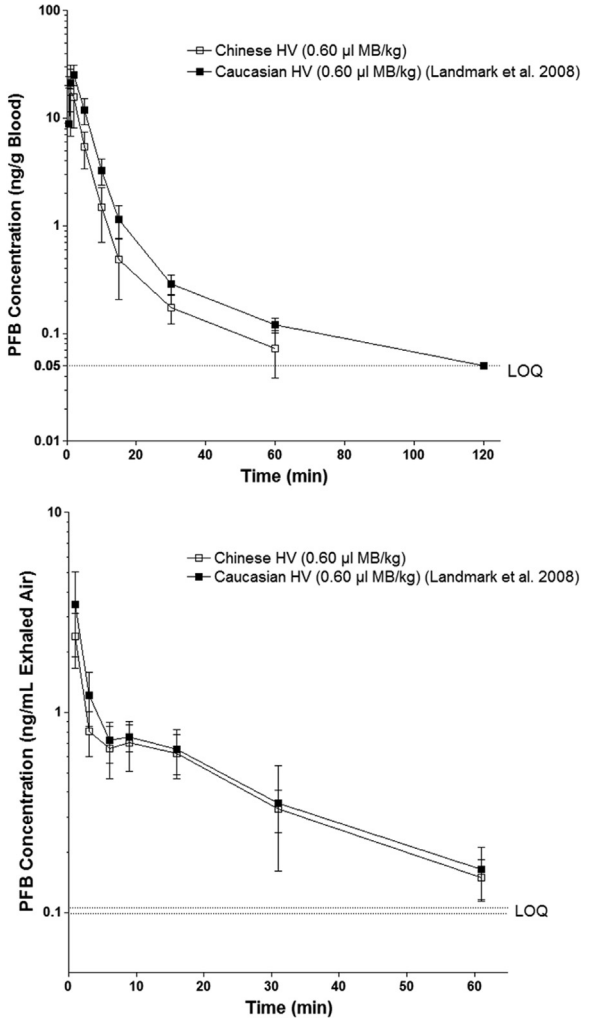

Fig. 4. Comparison of perfluorobutane (PFB) concentrations obtained from blood and exhaled air after administration of Sonazoid in Caucasian HVs and Chinese HVs. Data are expressed as means ± standard deviations. HVs = healthy volunteers; LOQ = limit of quantification; MB = microbubbles.

DISCUSSION

Intra-venous administration of doses of 0.12 and 0.6 µL MB/kg b.w. Sonazoid to healthy Chinese male and female patients was found to be safe and well tolerated. PFB was cleared from the blood rapidly after dosing, and the route of elimination of PFB was through the lungs. There were no apparent gender or dose dependencies in the PK parameters. This is consistent with the inert nature of PFB and its extremely rapid pulmonary elimination, which is governed predominantly by diffusion across the blood–air barrier.

After administration of Sonazoid to Chinese HVs, blood PK analysis revealed that for both dose groups, C_{max} was reached at 1 to 2 min post-injection and then PFB concentrations declined rapidly. The variability in C_{max} was attributed to stochastic variability in the time needed for complete mixing of the bolus volume with the total blood volume. In patients administered the high dose of 0.60 µL MB/kg b.w. Sonazoid, a biphasic blood concentration/time profile was observed, with a $t_{½}$ of 17 min. At 2 h post-injection, the PFB concentration was below the LOQ. In the 0.12 µL MB/kg b.w. dose group, the blood PFB concentration fell rapidly to the LOQ (between 10 to 30 min post-injection), resulting in incomplete and variable blood concentration/time profiles. A $t_{½}$ of 2.7 min was calculated for the 0.12 µL MB/kg b.w. dose group. PK parameters such as $t_{½}$ depend strongly on the number of data points with PFB blood concentration above LOQ, because the elimination rate constant is determined from the final three data points of the concentration profile. At the high dose level, these data points represent predominantly the elimination phase (up to 60 min). At the low dose level, $t_{½}$ was calculated from data obtained between 5 and 15 min post-injection, representing both the distribution and elimination phases. It is therefore difficult to compare $t_{½}$ at low dose levels with those at high levels.

The PFB concentration/time profiles for exhaled air were consistent with those for blood, confirming rapid pulmonary elimination of PFB after intra-venous administration of Sonazoid. In the 0.12 µL MB/kg b.w. dose group, PFB concentrations above the LOQ were observed up to 16 min post-injection, whereas in the 0.60 µL MB/kg b.w. dose group, PFB concentrations above the LOQ were observed up to 61 min post-injection. Because the volume of expired air was not measured, the pulmonary clearance could not be calculated.

The PK profile of Sonazoid generally is in line with that of other gas-filled microbubble-based ultrasound contrast agents, such as SonoVue (Morel et al. 2000), indicating that Sonazoid can be administered repeatedly to patients, with limited potential for long-term accumulation of PFB.

A central aim of this study was to investigate whether the drug exhibits ethnicity-related differences in pharmacokinetics. On the basis of the literature, it is known that perfluorocarbons are usually very stable toward all chemical reagents except alkaline metals, and the completely fluorinated alkanes used in ultrasound contrast agents (such as PFB) are essentially non-toxic (Clayton 1967). Perfluorocarbons have a high solubility in fatty tissue and a low solubility in blood (Weathersby and Homer 1980). They are known to be excreted from the body *via* the lungs without undergoing significant metabolism (Correas et al. 2001; Hutter et al. 1999; Killam et al. 1999; Landmark et al. 2008). In fact, no metabolic system for degradation of PFB is known, and no metabolites of PFB have been observed during the non-clinical and clinical testing of this agent. The main route of PFB elimination is diffusion across the blood/air interface in the alveoli, and the rate of elimination is

therefore governed predominantly by lung perfusion. Given these properties, we predicted a limited potential for ethnic differences in the PKs of PFB.

Indeed, plotting individual blood and exhaled air PFB concentration/time profiles from the Sonazoid PK study in Caucasian HVs (Landmark et al. 2008) and Chinese HVs reveals that the pharmacokinetics of Sonazoid are quite similar in the two studies (Fig. 4). PFB exhibits rapid, biphasic elimination as seen in the blood and exhaled air profiles. Exhaled air PFB concentration/time curves were for Chinese HVs and Caucasian HVs were very similar. For PFB in blood, the concentration/time profile was highly similar for Chinese HVs and Caucasian HVs. The measured blood PFB concentrations (absolute values) were slightly higher in Caucasian HVs. These differences in total blood PFB concentrations can be attributed to inter-study variation between the two study sites and the variability in PFB concentration between blood samples for each population. The higher concentrations of total PFB in blood in the Caucasian data set also contribute to the observed differences between studies in PK parameters, such as $t_{½}$ and systemic exposure (C_{max}, AUC). Non-compartmental analysis was used to estimate PK parameters in both studies, and differences in time points used for determining the slope of the terminal elimination phase partly explain these differences. The parallel slopes of both the early and late phases of the concentration/time curve, as well as the similar t_{max} values, strongly suggest that both the clearance and V_d of PFB are similar in Caucasian and Chinese patients. Because V_d and clearance independently determine the rate of disappearance of PFB from blood, differences in these parameters would be reflected in different slopes in the blood concentration/time profile. Furthermore, the exhaled air concentration/time profiles indicate that clearance is highly similar in the two studies (Fig. 4). In conclusion, PK data from studies performed in Caucasian and Chinese HVs reveal a high degree of similarity in blood and air concentration/time profile shapes. Considering the small differences in PFB concentrations measured in blood, this suggests that minor numeric differences in PK parameters are caused by systematic analytical variability across studies rather than ethnic variability.

CONCLUSIONS

Sonazoid at doses of 0.12 and 0.60 μL MB/kg b.w. was well tolerated by this study population of Chinese HVs. The elimination of PFB from blood and in exhaled air was biphasic, characterized by a rapid initial phase and a slower later phase. Pharmacokinetic data from studies performed in Caucasian and Chinese HVs indicate a high degree of similarity in blood and air concentration/time profiles, suggesting a limited potential for ethnic variability in the pharmacokinetics of Sonazoid.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.ultrasmedbio.2017.01.003.

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