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● *Original Contribution*

HUMAN PHARMACOKINETICS OF A PERFLUOROCARBON ULTRASOUND CONTRAST AGENT EVALUATED WITH GAS CHROMATOGRAPHY

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Abstract—The purpose of this study was to prospectively study the human pharmacokinetics of an ultrasound (US) contrast agent through its active ingredient, dodecafluoropentane (DDFP). Expired air and blood samples were collected from 24 volunteers after IV administration from 0.01 to 0.1 mL/kg. They were analyzed by a gas chromatographic method specially adapted to the study of DDFP. Blood data fitted to an open one-compartment model. Elimination half-life range was 1.8 to 2.5 min. The area under the curve was correlated to the dose (r^2 = 0.99). Mean blood clearance ranged from 30 to 49 mL/min kg. Blood apparent distribution volume ranged from 0.09 to 0.15 L/kg. In expired air, DDFP concentration exhibited a biexponential decay. The percentage of recovery was 98 ± 19% at 2 h. No extraneous peaks were observed, indicating no detectable DDFP metabolites. It was concluded that DDFP pharmacokinetics in blood fitted to an open one-compartment model with a fast elimination half-life. Recovery in expired air was almost complete 2 h after administration. (E-mail: jean-michel.correas@nck.ap-hop-paris.fr) **© 2001 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Contrast media, Ultrasound contrast agents, Pharmacokinetics, Fluorocarbon gas, Dodecafluoropentane, Gas chromatography.**

INTRODUCTION

The concept of contrast enhancement has been extended to ultrasound (US) imaging during the last decade. The microbubble persistence has been dramatically improved using two different approaches: external bubble encapsulation or stabilization and selection of low-solubility gases. Microbubbles were stabilized using sugar matrix (such as galactose (Schlief et al. 1990; Smith et al. 1984) or albumin microspheres (Feinstein et al. 1990), lipid (Unger et al. 1992) or polymers (Fritzsch et al. 1994; Schneider et al. 1992). Low-solubility gases also participated in the persistence improvement (Quay 1994). Most recent US contrast agents (USCA) combine several of these techniques to achieve prolonged enhancement of the Doppler signals and B-mode imaging (Correas and Quay 1996). This fluorocarbon emulsion (Sonus Pharmaceuticals, Bothell, WA) is the first USCA based on dodecafluoropentane (DDFP) and phase-shift technology. DDFP has a low water solubility (4×10^{-6} mol/L), a high molecular weight (288) and a low boiling point (29.3° C) (Correas et al.1997). The fluorocarbon emulsion is a liquid-in-liquid emulsion, where DDFP microdroplets phase shift to microbubbles after physical activation. The formulation evaluated in this study is slightly different from the approved product (EchoGen®, perflenapent emulsion) in terms of its composition and method of activation. The purpose of this phase 1 study was to study prospectively the human pharmacokinetics of DDFP in expired air and blood.

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MATERIALS AND METHODS

A total of 24 healthy volunteers (mean age = 27 ± 6 years, mean weight = 77 ± 8.5 kg) were enrolled after giving written informed consent in a phase 1 study, carried out in accordance with the Declaration of Helsinki. The fluorocarbon emulsion was administered at 0.01, 0.02, 0.05 and 0.1 mL/kg in a peripheral vein. The phase-shift transition was achieved with a bolus injection through a 1.2-μm filter (Sterile Acrodisc®, Gelman-

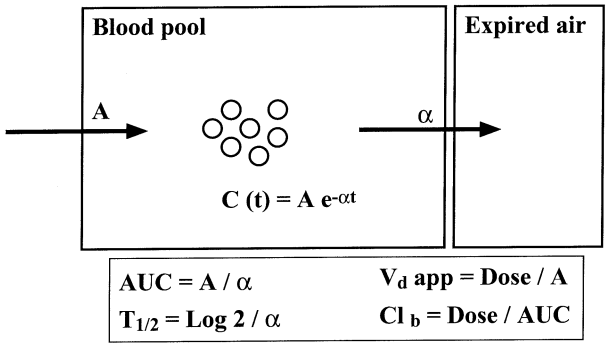

Fig. 1. One-compartment open model of DDFP elimination.

Sciences, Ann Arbor, MI) and flushed with saline. Each volunteer received a single injection after signing an approved informed consent form. Each dosage was given to 6 volunteers in an ascending dose-ranging protocol.

Blood data analysis

Blood samples were collected from a peripheral vein at predose and at 1, 2, 4, 6, 11, 16, 21, 31, 45, 60 and 90 min and 6 h after injection. A total of 3 mL were transferred to three sealed preweighted glass gas chromatographic vials containing 0.5 mL of isopropyl alcohol. Samples were stored at −20°C and shipped on dry ice to the laboratory.

DDFP was analyzed using a head space sampler attached to a gas chromatograph (model HP5890 Series II, Hewlett Packard, Palo Alto, CA). DDFP in the blood sample was vaporized by disrupting the emulsion with isopropyl alcohol and heating the sample at 70°C. A head space sampler (HP 7694, Hewlett Packard) was used to introduce the vaporized sample into the chromatograph. Volatiles were extracted by displacing the head-space above the blood sample with a carrier gas. Within the chromatograph, DDFP and isopropyl alcohol traveled down the column (DB5, 1.5-μm film thickness, 60-m length) at different rates. Separation was achieved with a flame ionization detector. The retention times of DDFP and isopropyl alcohol were 4.5 and 7 min, respectively. The method was validated using DDFP standards (1 to 1000 μg) prepared with isopropyl alcohol and a matrix of 1 mL of human blood. The limit of detection of the method was 20 ng/L and the limit of quantification was 100 ng/L. The values measured from the three vials were averaged at each time point for evaluation.

Blood data fitted to a one-compartment open model with bolus input and a first-order output (Fig. 1).

The function that defines this model is $C(t) = A\ e^{-\alpha t}$, where $C(t)$ represents the drug concentration at time t, A is the calculated 0 time y-intercept and α is the first-order rate constant. Constants A and α were calculated using a nonlinear curve-fitting algorithm with Deltagraph® 3.1 Pro (Deltapoint, Inc, Monterey, CA). The correlation coefficient (r) was calculated for each curve fitting to verify the adequacy of the model.

The following parameters were calculated: AUC (area under the curve) = A/α; T1/2 (distribution phase half-life) = $0.693/\alpha$; Vd app (apparent volume of distribution) = Dose/A; and Cl_b (total body clearance) = Dose/AUC. Pharmacokinetics parameters were determined separately for each volunteer and averaged per dosage group.

Expired air data analysis

Expired air samples were collected in polyvinyl fluoride (Tedlar®) bags at baseline and at 1, 2, 4, 6, 11, 16, 21, 31, 45, 60, 90, 120 and 240 min after injection, except for the 0.01 mL/kg group, where the 1-min sample was not collected. The 1-L Tedlar® bags were equipped with a port for inflation, a locking mechanism to reseal the port and a gasket sample port for removing samples for gas chromatographic analysis.

The expired air was analyzed on site, using a portable gas chromatography unit (model MTI P-200, Microsensor Technology, Fremont, CA), equipped with an MTI PoraPlot Q-4 m gas chromatographic module and a thermal conductivity detector. DDFP concentration in the Tedlar® bags was analyzed by direct injection within the gas chromatograph through the injection valve equipped with an 8-μL sample loop. Standards were prepared by accurately pumping 1 L of air into the bag and adding DDFP through the septum, to construct the calibration curve. The limit of detection was 25 μg/L and the limit of quantification was 40 μg/L. The accuracy of the method was determined by analyzing air samples to which known amounts of DDFP had been added, and was 96.5 to 103% recovery level from 150 μg/L to 100 mg/L DDFP concentrations. At each time point, 10 values were averaged for evaluation after discarding the first three values for carryover reasons. All samples were analyzed for DDFP concentration, until three consecutive samples were below the limit of quantification. Timed and cumulated recovery level from the DDFP amounts administered were calculated for each volunteer and averaged per dosage group. The amount of DDFP excreted in the expired air was calculated with several steps: compensation for the DDFP dilution in the Tedlar® bag due to the dead space, curve fitting of DDFP concentration, calculation of DDFP amount excreted per breath with compensation due to the exchanging space (alveolar volume), and addition of the amount exhaled to fill the 1-L bag.

The determination of DDFP amount expired in air was corrected from the DDFP concentration measured by gas chromatography as follows: DDFP amount =

Table 1. Pharmacokinetics of the DDFP emulsion in blood

Dosage groups (ml/kg)	DDFP administered (mg)*	Blood half life (T1/2) (min)	AUC (mg·min/L)*	Vd (L/kg)*	Cl (mL/min kg)
Group 0.01	14.9 ± 1.2 (13.4; 16.6)	2.5 ± 2.1 (0.9; 6.5)	8.3 ± 3.7 (3.4; 12.4)	0.09 ± 0.05 (0.03; 0.16)	30.1 ± 16.5 (16.3; 57.9)
Group 0.02	31.6 ± 5.0 (25.4; 39.6)	2.1 ± 0.8 (1.3; 3.3)	10.0 ± 2.1 (6.5; 12.6)	0.14 ± 0.09 (0.07; 0.30)	41.7 ± 10.5 (31.7, 61.8)
Group 0.05	75.1 ± 7.4 (66.6; 87.2)	1.8 ± 0.4 (1.4; 2.4)	21.8 ± 5.5 (16.6; 30.9)	0.13 ± 0.05 (0.07; 0.21)	48.2 ± 11.2 (32.3 60.3)
Group 0.1	160.2 ± 13.8 (141.0; 182.8)	2.3 ± 1.1 (1.2; 3.5)	42.6 ± 7.6 (28.9; 49.6)	0.15 ± 0.06 (0.08; 0.21)	48.6 ± 10.8 (40.3, 69.2)

Average ± SD (min; max); AUC = Area under the curve; Vd = volume of distriution; Cl = total body clearance.

DDFP concentration × 1/0.85. This factor was applied due to the dilution of DDFP expulsed by the alveoles with the dead space (such as trachea and large bronchia) within the Tedlar® bag. In the normal population, average dead space volume is 0.15 L (Wright 1971). Then, the DDFP amount was fitted to a biexponential function to calculate the amount excreted per breath. The respiration rate was known prior and at 1.5, 3.5, 5.5, 10.5, 15.5 and 30 min, and 1, 2 and 4 h after administration. The volume of air exchanging DDFP is the alveolar volume and is approximately 0.35 L in the normal population (Wright 1971). Using the fitting equation and the respiration rate, the amount of DDFP excreted per breath was calculated and multiplied by the alveolar volume. The amount of DDFP excreted to fill the 1-L Tedlar® bags was calculated separately because, during deep expiration, the alveolar volume changes to 0.85 L. The percentage of DDFP recovered from the amount administered was calculated at 1, 2, 5, 10, 15, 30, 60, 120 and 240 min by adding the amount excreted per breath to the amount recovered in each bag.

The lack of a 1-min datum value induced some error in the biexponential fitting accuracy for the lowest dosage group. A 1-min datum was calculated in this group from the 2-min measurement by using a coefficient of 2.5 determined as the ratio of the 1-min and 2-min data points from the three other groups.

The overall percentage of DDFP recovery was calculated for each group and for the overall study.

RESULTS

Blood pharmacokinetic results

Raw data and curve-fitting in blood. A large variation in the DDFP concentration was observed between the volunteers even within the same dosage group(Table 1). At 1 min, the DDFP concentration ranged from 0.86 to 3.07 μg/L for the 0.01 mL/kg group, from 0.32 to 1.21 μg/L for the 0.02 mL/kg group, from 1.15 to 7.31 μg/L for the 0.05 mL/kg group, and from 0.67 to 14.58 μg/L for the 0.1 mL/kg group. However, the DDFP concentration in blood exhibited a linear relationship with the amount of DDFP administered at each time point 1, 3 and 5 min (respectively r = 0.98, 0.99 and 0.98). In the two lowest dosage groups, a monoexponential curve fitting was difficult to achieve, due to the lack of data points attributed to the very small amount of DDFP administered. Despite this limitation, a monoexponential decay curve fitting was applied with a good correlation coefficient, ranging from 0.93 to 0.99 in all groups (0.95 at 0.01 mL/kg, 0.98 at 0.02 mL/kg, 0.99 at 0.05 mL/kg and 0.97 at 0.1 mL/kg) (Fig. 2). In 9 cases, the 3-min DDFP concentration value was higher than the 1-min DDFP concentration value. In these cases, curve fitting did not include the first concentration value. DDFP concentrations below the limit of quantification were not taken into consideration for blood pharmacokinetics.

Pharmacokinetic parameters in blood. Blood pharmacokinetic parameters are summarized in Table 1.

- DDFP half-life in blood ranged from 0.9 to 6.5 min and was independent of the amount of DDFP administered (r = 0.06). Averaged half-life was 2.2 ± 1.2 min for all groups. The 6.5-min datum point was obtained from a volunteer of the lowest dosage group, whose data set was limited.
- Averaged half-life excluding this volunteer was 2.0 ± 0.8 min.

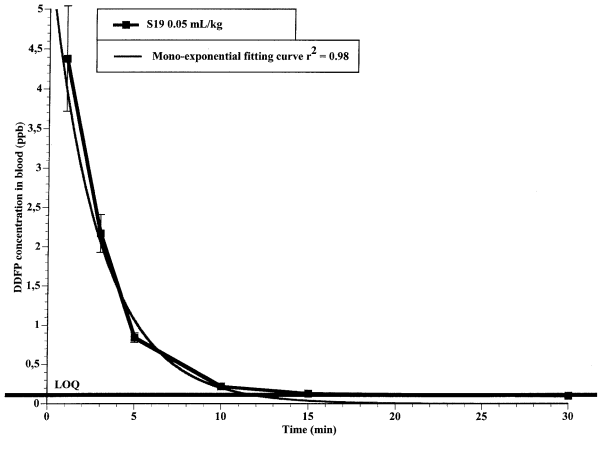

Fig. 2. DDFP concentration in blood for subject 19, following IV administration of theDDFP emulsion at 0.05 mL/kg. LOQ = limit of quantification.

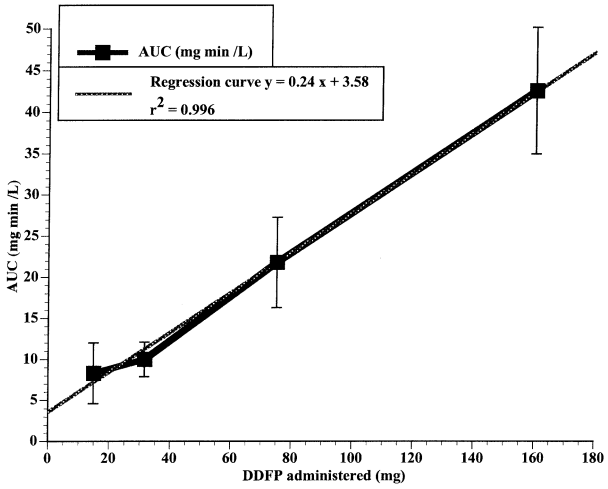

Fig. 3. Relationship between the area under the curve (AUC) and the amount of DDFP administered.

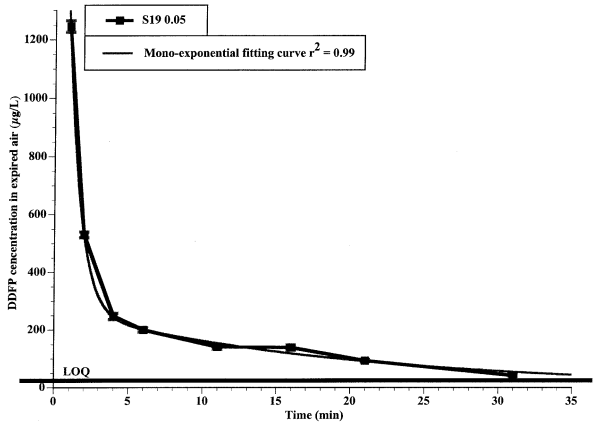

Fig. 4. DDFP concentration in expired air for subject 19, following IV administration of the fluorocarbon emulsion at 0.05 mL/kg. LOQ = limit of quantification.

- Area under the curve (AUC) averaged, ranged from 8.3 to 42.6 mg min/L depending on the amount of DDFP administered. Despite some variations, AUC exhibited a linear relationship with the amount of DDFP administered, with a regression coefficient of 0.99 (Fig. 3).
- Blood apparent distribution volume (Vd) ranged from 0.09 ± 0.05 L/kg at 0.01 mL/kg to 0.15 ± 0.06 L/kg at 0.1 mL/kg. The large standard deviation was due to volunteers with small data set values. There was a limited linear relationship between Vd and the amount of DDFP administered (r = 0.88).
- Mean blood clearance (Cl) ranged from 30.1 ± 16.5 mL/min kg at 0.01 mL/kg to 48.6 ± 10.8 mL/min kg at 0.1 mL/kg. A linear relationship was found between Cl and the amount of DDFP administered (r^2 = 0.75), despite some variations in mean blood clearance values, particularly in the lowest dosage group.

Expired air pharmacokinetic results

Raw data and curve fitting. Variations in the raw DDFP concentrations measured from the Tedlar® bags were found between volunteers, even within the same dosage group (Table 2). At 2 min, DDFP concentration ranged from 93 to 159 µg/L for the 0.01 mL/kg group, from 135 to 404 µg/L for the 0.02 mL/kg group, from 369 to 839 µg/L for the 0.05 mL/kg group, and from 440 to 3415 µg/L for the 0.1 mL/kg group. Despite these variations, there was an excellent correlation between DDFP concentration and the dose administered for each time point measurement (r = 0.98 to 0.99). The biexponential curve fitting was accurately applied to all groups (r = 0.98 ± 0.03) (Fig. 4). In the lowest dosage group, the estimated 1-min DDFP concentration correlated well with the higher doses, and allowed an accurate biexponential curve fitting (r = 0.99).

Pharmacokinetic parameters. At 2 h after administration, the average DDFP recovery in all dosage groups ranged from 95 to 103% (average 98 ± 19%) (Fig. 5).

A larger variation in DDFP recovery was found in the lowest dosage groups, due to the small amount of DDFP detectable and to the approximation of the 1-min datum point. In the highest dosage group (0.1 mL/kg), the DDFP recovery at 2 h ranged from 92 to 120% (average 103 ± 12%), with a lower standard deviation compared to the other groups.

Table 2. Pharmacokinetics of the DDFP emulsion in expired air

Dosage groups (mL/kg)	DDFP administered (mg)	Time to 50% recovery (min)	Average recovery at 120 min (% of the dose administered)
Group 0.01	14.9 ± 1.2 (13.4; 16.6)	5.8 ± 3.8 (1.4; 10.6)	95 ± 23 (57; 116)
Group 0.02	31.6 ± 5.0 (25.4; 39.6)	13.3 ± 8.6 (4.4; 25.6)	97 ± 19 (86;135)
Group 0.05	75.1 ± 7.4 (66.6; 87.2)	11.1 ± 8.8 (1.4; 23.6)	98 ± 25 (66; 133)
Group 0.1	160.2 ± 13.8 (141.0; 182.8)	3.7 ± 1.2 (1.6; 4.8)	103 ± 12 (92; 120)
All dosages	70.4 ± 58.0 (13.4; 182.8)	8.1 ± 6.9 (1.4; 25.6)	98 ± 19 (57; 135)

Average ± SD (min; max).

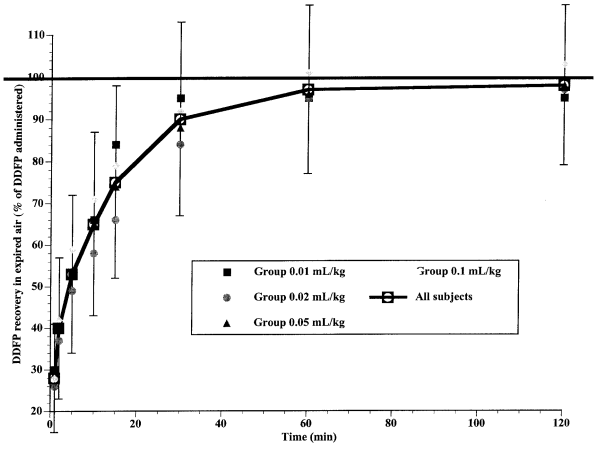

Fig. 5. DDFP recovery in expired air expressed as a percentage of the amount administered IV.

Over- and underestimation of DDFP recovery (respectively 135% to 133% and 65% to 70%) were related to high and low respiration rates (respectively, 30 to 24 breaths per min and 12 to 16 breaths per min, compared to an average of 20 breaths per min for the remaining volunteers).

DISCUSSION

The pharmacokinetics of microbubble USCA is still a challenge, because of the small amount of gas administered and the difficulty in quantifying the dose in the plasma and/or in the expired air. Initially, these pharmacokinetics were studied through the metabolism of the USCA ingredients only. Levovist® (Schering SA, Berlin, Germany) is a US contrast agent based on air microbubbles stabilized within a galactose matrix and palmitic acid. Its pharmacokinetics were studied through the metabolism and excretion of its stabilizing ingredients, the galactose and the palmitic acid (Schlief 1997). However, the active component of USCA is represented by the microbubbles themselves, and not by the stabilizing ingredients. It is likely that the pharmacokinetics of these ingredients follow the microbubble gas component pharmacokinetics. Air microbubbles in the blood pool might be detected by introducing a physicochemical change in their composition (such as radioactive oxygen or carbon). But the change in the gas composition of the microbubbles may alter their physicochemical properties and, potentially, their stability in the plasma and, therefore, modify the pharmacokinetics.

The physicochemical properties of perfluorocarbon gases differ from those of air components. They can be detected using gas chromatography with specific columns. The specificity of this detection is excellent and relies on the properties of the gas chromatography columns. This technique is also very sensitive for the detection of a small amount of perfluorocarbon gas. It appears to be appropriate for USCA pharmacokinetic measurements because the total amount of gas administered within the entire blood pool is about a few milligrams.

Therefore, a new gas chromatography method was validated to detect dodecafluoropentane in blood and expired air. Theoretically, DDFP could be found under three different forms in the plasma: microdroplets, microbubbles and free dissolved gas. Heating and disrupting emulsion within the blood sample should allow the recovery of DDFP under any physical form. USCA pharmacokinetics are different from those of iodinated or magnetic resonance imaging (MRI) contrast agents, because the active ingredient is eliminated through the lungs, without any excretion in the urine. Their pharmacokinetics might also be different from inhaled fluorocarbon gases, such as 1,1,2-trichloro-1,2,2-trifluoroethane and chlorodifluoromethane used as dry cleaning or refrigerant agents (Woolen et al. 1990, 1992).

In our study, pharmacokinetic parameters in expired air and in blood were acquired simultaneously with safety data. Other techniques have been evaluated for quantification of expired gas pharmacokinetics, such as closed chambers or a snorkel-type mouthpiece connected to a gas chromatography system. Unfortunately, they require large and heavy instruments (Wilson et al. 1983). The size of the exposure chamber ($2.5 \times 2.5 \times 2.5$ m) is not suitable to dose USCA gases, due to the very small amount administered IV. Because of the chamber size, it does not allow simultaneous evaluation of safety information in good conditions.

A chromatography technique was also used to measure the pharmacokinetics of octafluoropropane (Hutter et al. 1999). Octafluoropropane is the active ingredient of another USCA, Optison® (FS069, Molecular Biosystems, San Diego, CA). This perfluorocarbon gas is encapsulated in human serum albumin microspheres. In a phase 1 study, octafluoropropane was dosed only in expired air samples collected in 20-mL syringes. However, healthy volunteers were equipped with a noseclip and mouthpiece connected to a respirometer. This setting may limit the collection of adverse events and the patient mobilization for simultaneous US scanning.

The method we developed offers the possibility of getting all information required for the correct evaluation of a new drug: pharmacokinetics in expired air and blood and safety data. The limit of quantification was reached very quickly in the blood samples, particularly in the low-dosage groups, due to the very small amount of DDFP administered and to the preliminary lung extraction. DDFP concentration fitted to an open one-compart-

ment model in blood, with a linear relationship between the area under the curve and the dose administered. Modeling was less accurate in the low-dosage group, due to fewer time points and the limit of quantification. Perflenapent emulsion pharmacokinetics exhibited a rapid blood clearance rate that was consistent with the imaging findings and the safety profile.

In expired air, DDFP concentration fitted to a biexponential model, with accuracy for all groups, due to a higher number of measurements above the limit of quantification. It was possible to calculate the DDFP percentage of recovery from the dose injected in all cases. The value close to 100% was consistent with the physicochemical properties of DDFP that are not metabolized in humans. No extraneous peak was observed in chromatographic analysis, indicating no detectable metabolite with this technique.

A more complex pharmacokinetic model was used for Optison®. This "physiologically based pharmacokinetic model" is a six-compartment model with two lung compartments (alveolar and dead volume), the heart, slowly and richly perfused tissues and the gastrointestinal tract. This model allows control of multiple independent pharmacokinetic parameters, such as distribution coefficients, in and out flow for each compartment. However, most of the parameters have very little impact on the pharmacokinetics (tissue distribution coefficients, fecal elimination, cardiac output, absorption in tissues). A 100% change in the tissue distribution coefficients leads to less than a 0.1% change in octafluoropropane exhalation rate (Hutter et al. 1999). The fluorocarbon gas has never been found in feces or urine (Hutter et al. 1999). In our study, a simple biexponential model was found to fit the expired air experimental data with accuracy. The major parameter for estimation of the lung pharmacokinetics remains the ventilation rate in both phase 1 studies.

This evaluation of USCA pharmacokinetics using gas chromatography can be extended to other compounds with perfluorocarbon gases. Our preliminary approach with Tedlar® bags, allowing intermittent measurement of gas elimination in expired air, should improve with continuous sampling. An increased knowledge of USCA pharmacokinetics might become a useful tool for safety and efficacy evaluation.

SUMMARY

The feasibility of the pharmacokinetic study of perfluorocarbon gas emulsion in humans was demonstrated by a method using gas chromatography of its active ingredient. The technique was easy to perform using a portable gas chromatograph. The accuracy of the method was acceptable. DDFP pharmacokinetics in blood fitted to an open one-compartment model, with a fast elimination half-life attributed to the high first-pass extraction ratio of the lungs. Mean recovery in expired air was 100 ± 13% 2 h after injection. No extraneous peak was observed in chromatographic analysis, indicating no detectable metabolism.

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