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Emulsified Perfluorochemicals as Respiratory Gas Carriers: Recovery of Perfluorodecalin Emulsion Droplets from Rat Tissues

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Abstract—To further understand the in-vivo biokinetic behaviour of perfluorochemical (PFC) emulsions, male rats were injected (10 mL kg^{-1}) with 30% (w/v) emulsified perfluorodecalin (FDC) and uptake into tissues assessed. At 72 h after injection, the mean (± s.e.m.) diameters of FDC droplets recovered from liver and spleen were 2·24 ± 0·04 and 2·78 ± 0·10 μm, respectively; droplets recovered from lung after 72 h (mean: 1·73 ± 0·13 μm) were significantly smaller ($P < 0·01$). After 7 days, FDC droplet diameters in liver had increased to 3·31 ± 0·13 μm ($P < 0·01$) and those in lung to 2·71 ± 0·14 μm ($P < 0·01$); droplets in spleen after 7 days (2·22 ± 0·09 μm) were similar to those at 72 h. These data support the hypothesis that significant initial coalescence of FDC droplets occurs in the rat liver and spleen, with further coalescence in the liver up to 7 days. The mean percentage of the injected FDC dose recovered from the liver after 72 h was 2·2 ± 0·4%, and after 7 days was 0·07 ± 0·05% ($P < 0·01$). A smaller decrease in the percent injected FDC in spleen also occurred over the same period (72 h: 1·9 ± 0·3%; 7 days: 0·8 ± 0·5%; $P < 0·01$). The percent injected FDC in lung was similar at 72 h (0·007 ± 0·004%) and 7 days (0·005 ± 0·001%). FDC was undetectable (< 0·001%) in all blood samples. The greater rate of FDC elimination from the liver than from the spleen may be related to differences in the rates of reticuloendothelial system processing between these organs.

Perfluorochemical (PFC) emulsions are being widely used as intravascular oxygen carriers and diagnostic imaging agents, and the perfluorodecalin (FDC)-based commercial emulsion, Fluosol (Alpha Therapeutic, Thetford, UK) has been clinically approved for oxygenating the myocardium during coronary balloon angioplasty (Lowe 1991, 1992). Other applications for PFCs and their emulsions include uses as adjuncts to cancer therapy, as perfusates for isolated organs, and as respiratory tract infiltrates for the treatment of neonatal pulmonary disorders (Lowe 1991, 1992).

Following injection into the vascular compartment or intraperitoneal space, PFC emulsion droplets accumulate in lymphoid tissues, causing an increase in organ weight generally in proportion with dose administered. The tissue responses to emulsified PFCs also depend upon the composition of the injected formulation, and the species studied (Lowe 1988). PFC droplets are detectable in the liver and spleen as clusters, often termed 'foamy vesicles', owing to their electron microscopical appearance (Caiazza et al 1984; Nanney et al 1984). Marked coalescence of PFC droplets occurs in these tissues (Bentley et al 1993) before their release back into the blood from which they are eventually excreted, primarily by expiration through the lungs (Tsuda et al 1988).

To further understand the in-vivo biokinetic behaviour of PFCs, we have recovered emulsion droplets from tissues and determined their size following injection of emulsified FDC into rats. A simple biokinetic model of PFC excretion has been used to estimate the mean residence time of FDC droplets in the lung. Some of these results have already been published in a preliminary form (Lowe & Washington 1993).

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Materials and Methods

Preparation of emulsion

The emulsion consisted of 30% (w/v) FDC (Flutec PP5; Rhône-Poulenc, ISC Division, UK) and 4% (w/v) silica gel-purified Pluronic F-68 (ICI-Atochem, UK; (Bentley et al 1989)) in an aqueous phase containing 0·9% (w/v) NaCl. The emulsion was prepared using a Microfluidizer (Microfluidics, Newton, USA) as described previously (Johnson et al 1990a,b). The mean droplet diameter of the emulsion, as measured by photon correlation spectroscopy (Malvern Instruments K7025, Malvern Instruments, UK), was 226 nm, with a polydispersity of 0·23.

Care of animals and experimental procedures

Mature male Wistar rats (283–321 g; n = 13) were used. They were maintained in the laboratory animal house under controlled conditions (13 h light, 11 h dark; temperature 24 ± 1°C) with free access to a standard diet (Rat and Mouse Breeding Diet, Haygates, UK).

Animals were lightly anaesthetized with ether and then injected intraperitoneally with 10 mL kg^{-1} of either FDC emulsion or sterile isotonic saline solution (0·9% w/v NaCl; Viaflex; Travenol, UK; controls). At 72 h or 7 days after injection, animals were killed by cervical dislocation and their livers, spleens and lungs removed, weighed, rinsed with saline, and frozen (−20°C). Blood samples were also obtained by cardiac puncture, placed in plastic tubes containing heparin (Sarstedt, Leicester, UK), and assayed immediately.

Extraction and sizing of PFC droplets

The procedure for extraction of PFCs was modified from that described previously (Bentley et al 1993). Briefly, tissue or blood samples (1–5 g or 1·0 mL) were homogenized for

1 min in 30 mL of distilled water using a Silverson homogenizer at 2000 rev min^{-1}. Two millilitres of sodium hypochlorite (35%; Rhône-Poulenc, UK) was added and the homogenization repeated. Samples were then centrifuged at 3000 *g* for 10 min, the supernatant discarded, and residual tissue fragments rinsed from the pellet of PFC droplets with 4% (w/v) Pluronic F-68 solution. Droplets were then counted and their size distribution measured using a Coulter Multisizer with a 50 μm aperture tube (size analysis range 1–20 μm). The presence of PFC droplets and absence of cellular debris was confirmed by light microscopy (Nikon Ultraphot; 400 ×). Sizes measured by light diffraction were in approximate agreement with those estimated by microscopy.

Biokinetic modelling

A simplified biokinetic model was created using the software package STELLA (Fig. 1; Washington et al 1990). This model represents the clearance of PFC droplets from the peritoneal cavity via the vascular compartment to the lymphoid tissues (liver and spleen) and lungs. The equations generated in this model are listed in Fig. 1b. Peritoneal/blood and lymphoid clearance parameters were estimated from

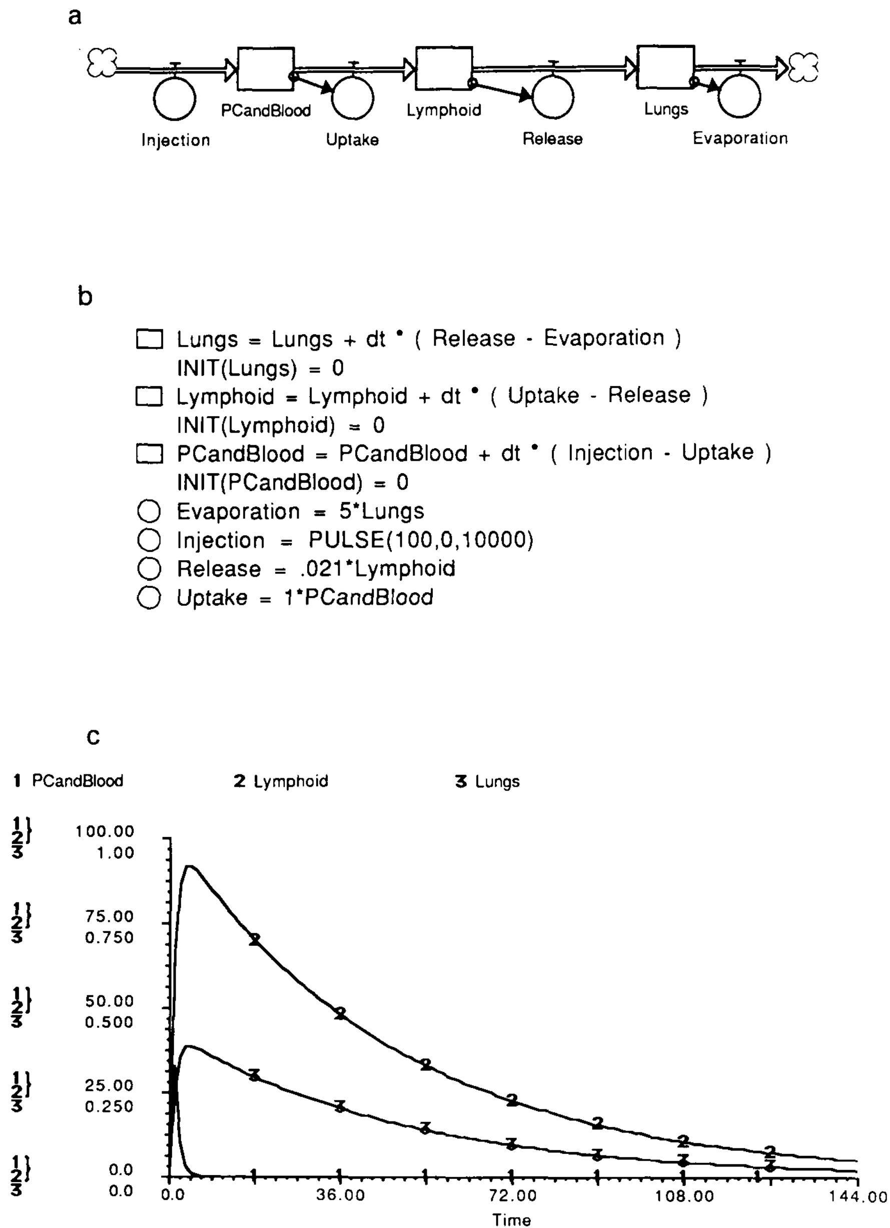

FIG. 1. Biokinetic compartmental model of the distribution and excretion of FDC from the peritoneal cavity, lymphoid tissues and lungs of rats using STELLA. a. Compartmental model. b. Model equations. The rate constant values of evaporation and uptake represent a single point on the sensitivity analysis, while the rate constant for lymphoid release is derived from fitting published data to the model as described in the text. c. Typical simulation of FDC content in compartments. The vertical axis for Fig. 1c represents the percentage dose in the compartment.

previous measurements of PFC concentrations in these compartments (Yokoyama et al 1982). The dependence of PFC droplet concentrations in the lung on the pulmonary residence time was then computed by sensitivity analysis.

Statistical analyses

Statistical analyses were performed according to the methods of Snedecor & Cochran (1989). Means ± s.e.m. have been used throughout unless stated otherwise. Statistical significance between mean values was assessed using a conventional Student's *t*-test; a probability of $P<0.05$ was considered significant.

Results

Tissue weights

Injection of PFC emulsion produced a significant ($P<0.05$) increase in mean liver weight after 72 h and 7 days (Table 1). In contrast, no corresponding changes in spleen weight were observed. Furthermore, there were no significant changes in lung weights between the two experimental treatments.

Table 1. Mean liver and spleen weights in male rats at 72 h or 7 days following injection of either FDC emulsion or saline (controls).

		Liver weight (% body weight)		Spleen weight (% body weight)	
Treatment	n	72 h	7 days	72 h	7 days
Controls	7	4·40 ± 0·03	4·39 ± 0·01	0·29 ± 0·01	0·29 ± 0·01
FDC emulsion	6	5·80 ± 0·08*	5·50 ± 0·91*	0·33 ± 0·04	0·31 ± 0·03

Values are mean ± s.e.m. *$P<0.05$ compared to control mean value.

Recovery of PFCs from tissues

At 72 h after emulsion injection, the mean (± s.e.m.; n = 3 throughout) diameters of droplets recovered from the liver and spleen were 2·24 ± 0·04 and 2·78 ± 0·10 μm, respectively (Table 2). Droplets recovered from the lung after 72 h were significantly smaller ($P<0.01$) than those extracted from the liver or spleen.

At 7 days after injection, the mean diameter of droplets recovered from the liver had risen to 3·31 ± 0·13 μm ($P<0.01$) and those in the lung to 2·71 ± 0·14 μm ($P<0.01$). In contrast, the mean diameter of droplets extracted from the spleen after 7 days was similar to that measured after 72 h (Table 2).

Table 2. Mean volume diameter (μm) ± s.e.m. of FDC droplets recovered from the lung, liver and spleen of rats at 72 h and 7 days after injection of FDC emulsion.

	Mean volume diameter (μm)	
	72 h	7 days
Liver	2·24 ± 0·04	3·31 ± 0·13
Spleen	2·78 ± 0·10	2·22 ± 0·09
Lung	1·73 ± 0·13	2·71 ± 0·14

The mean percentage of the injected FDC dose recovered from the liver after 72 h was 2·2 ± 0·4% and this had fallen to 0·08 ± 0·05% ($P<0.01$) after 7 days (Table 3). A smaller decrease in the percentage of the injected FDC in the spleen also occurred over the same period ($P<0.01$; Table 3). The percentage of the FDC in the lung was considerably smaller than that in the liver or spleen and did not change significantly between 72 h and 7 days (Table 3). FDC was undetectable (< 0·001%) in all blood samples, as determined by the droplet recovery technique.

Table 3. Mean percentage ± s.e.m. of FDC recovered from the lung, liver and spleen of rats at 72 h and 7 days after injection of FDC emulsion.

	FDC dose in tissue (% total)	
	72 h	7 days
Liver	2·2 ± 0·4	0·08 ± 0·05
Spleen	1·9 ± 0·3	0·8 ± 0·5
Lung	0·007 ± 0·004	0·005 ± 0·001

Biokinetic modelling

Fig. 2 shows the dependence of 72-h and 7-day FDC concentrations in the lung on the elimination rate constant from this compartment. The predicted FDC concentrations decreased with increasing elimination rate, from 0·5% at $k_{el} = 1\ h^{-1}$ to 0·005% at $k_{el} = 100\ h^{-1}$ (72 h), and from 0·15% at $k_{el} = 1\ h^{-1}$ to 0·0008% at $k_{el} = 100\ h^{-1}$ (7 days).

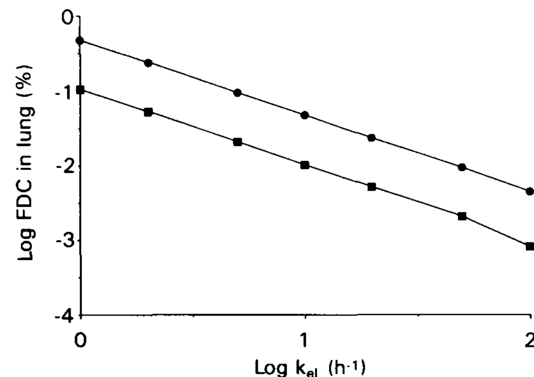

FIG. 2. Computed pulmonary FDC concentration (log % FDC in lung) vs pulmonary elimination rate constant ($k_{el}\ h^{-1}$) at 72 h (●) and 7 days (■) after injection of FDC emulsion.

Discussion

The observed increase in liver weight was consistent with that seen in rats in response to injection of an identical dose of a similar FDC emulsion (Bentley et al 1993), thus confirming that PFCs are transiently stored in reticuloendothelial (RES) tissues following their uptake from the blood (Lowe 1988).

The present results extend previous preliminary findings (Bentley et al 1993) that coalescence of FDC droplets occurs in the liver between 72 h and 7 days following injection. Additionally, coalescence occurred in both organs during the first 72 h of the experiment, since a maximum increase in droplet diameter of ~10 times that at preparation was observed. The small quantity of FDC retained in the lung suggests that, in contrast to liver or spleen, the excretion rate from this organ is relatively high. This accords with previous findings (Yamanouchi et al 1975) that PFCs are rapidly eliminated from the body by expiration. The absence of detectable levels of FDC in the blood at 72 h or 7 days after injection supports previous findings that the vascular half-life of this compound is ~26 h (Yamanouchi et al 1975).

The greater rate of FDC elimination from the liver than from the spleen may be related to differences in the rates of RES processing between these organs. In this regard, the spleen has a higher density and greater accessibility of phagocytic cells (unit mass of tissue)$^{-1}$ compared to the liver (Wisse & Knook 1977) and would therefore be expected to retain PFC droplets for longer periods. In addition, the marginally lower degree of droplet coalescence in the spleen after 7 days may reflect a slower metabolism of FDC emulsion droplets, involving adsorption of surface proteins and breakdown of the Pluronic F-68 surfactant film.

Comparison of the biokinetic model with the observed lung FDC concentrations suggests that the elimination of FDC from this tissue proceeds with a rate constant of the order of 20-60 h^{-1}, and also that a typical FDC droplet has a residence time of approximately 1-3 min in the lung. These results may also be influenced by the possibility that adipose tissues act as a parallel compartment to the vasculature for PFC distribution (Tsuda et al 1988). If this were the case, the observed FDC levels would be lower than those computed. Additional loss mechanisms (such as adipose partitioning) would cause these estimates to be increased; thus they represent a minimum residence time in this compartment.

A limitation of the present model is that it utilizes PFC excretion parameters based on data obtained using the mixed proprietary FDC/perfluorotripropylamine emulsion, Fluosol (Green Cross, Japan; Yokoyama et al (1982)), and we are aware of possible differences in excretion rates of different PFCs. Indeed, there is a scarcity of reliable pharmacokinetic data on PFC emulsions, and consequently, the droplet residence times suggested by the present model should only be taken to indicate an order of magnitude, rather than precise values. Nevertheless, it is apparent that the residence time of FDC droplets in the lung is relatively short. This is not surprising since the vapour pressure of FDC is 16·5 mBar at 37°C and thus, micron-sized droplets would be expected to evaporate rapidly. This contrasts sharply with droplets of involatile triglycerides (such as those used for parenteral feeding) which are suspected to have much longer local residence, causing pulmonary microemboli (Appelgren & Rossner 1980; Allen & Murray 1985). The shorter residence times of FDC droplets predicted in the present experiments may reduce the risk of pulmonary damage by this mechanism, compared to less volatile compounds (Riess & Le Blanc 1988) which are not as attractive for intravascular applications.

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