affected markedly by morphine (Leander 1983b). This may be explained by the fact that proxorphan and bremazocine, but not ketazocine or U-50488H,* have μ-opioid receptor antagonist activity. These results may indicate that tifluadom also lacks antagonist activity at μ-receptors.

The present results further support the in-vitro observation that tifluadom is a fairly specific κ-opioid agonist without actions at the μ-opioid receptor. Tifluadom should be a useful tool in the further study of κ-receptor functions.

The author thanks Dr Romer of Sandoz, Basle, for providing the tifluadom and Mr John Hart for his technical assistance.

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J. Pharm. Pharmacol. 1984, 36: 556–559
Communicated January 30, 1984

Fluosol 43 intravascular persistence in mice measured by ^{19}F nmr

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Quantitative determination of the intravascular persistence of F-tri-n-butylamine (FC 43 as Fluosol 43) in mice was carried out using ^{19}F nmr spectroscopy. The method allows the direct study of whole blood, neither separation nor extraction of the sample being required. Accuracy (reproducibility) is better than ±3%, and is comparable to that of the gas chromatographic (gc) method. The sensitivity of detection is less than that of the gc method but is sufficient for this biological study. It was observed that the intravascular elimination of F-tri-n-butylamine follows a non-linear kinetic and becomes faster about 40 h after the injection. This phenomenon may be explained by the size-increase of the FC 43 droplets in the emulsion. Indeed, at about 40 h after injection, the level of Pluronic F-68 in the bloodstream was no longer sufficient to maintain the stability of the FC-43 droplets. They therefore tended to coalesce forming larger droplets that were phagocytosed more rapidly by the histiocytes of the reticuloendothelial system.

Highly fluorinated organic compounds are excellent solvents for oxygen and carbon dioxide and they can be emulsified to form particles small enough to circulate in the micro blood vessels. Hence, perfluorochemical particles can effect the main function of erythrocytes. The other functions of blood, such as transport of ions and nutrients and holding osmotic and oncotic pressures, are maintained by the aqueous phase which is formulated to simulate the plasma (Clark & Gollan 1966; Sloviter & Kamimoto 1967; Geyer et al 1968; Le Blanc & Riess 1982).

Perfluorochemical emulsions must be non-toxic and should meet the two following requirements: (i) they must remain in the circulating blood for a reasonable time (i.e. they must offer good in-vivo stability), (ii) they must be rapidly eliminated from the body to avoid side effects.

It is therefore necessary to know the residence time in the bloodstream and the distribution throughout the body of perfluorochemicals and also whether long-term retention of these compounds occurs in various organs.

The amounts of perfluorocompounds in body fluids and organs have been determined by specific gravity (Clark et al 1974), by sodium biphenyl reagent combustion followed by determination of the fluoride ion released (Clark et al 1974; Stein et al 1975) or by gas chromatography (gc) after extraction with various solvents (Yamanouchi et al 1975; Cao et al 1981).

These methods lack simplicity and/or accuracy: the measurement of specific gravity only indicates the overall effect; the recovery of fluoride ion after sodium biphenyl combustion is not complete; the gc method needs extraction which is time-consuming and limits its accuracy: when a known amount of perfluorochemical in emulsified form is added to blood, the recovery is over 94% with a standard deviation (s.d.) of ±3·5% (Yamanouchi et al 1975) or over 91% with s.d. ±5% (Cao et al 1981).

We now report a new and direct assay method for the determination of perfluorochemicals in whole blood using fluorine-19 nmr (^{19}F nmr), with which we have investigated the intravascular persistence of Fluosol 43 in mice.

Fluosol 43 is one of the three commercial emulsions manufactured by the Green Cross Corporation (the other two are Fluosol-DA 20% and 35% which are mixtures of two perfluorochemicals (F-tri-n-propyl-

* Correspondence.

amine and F-decaline) which must be stored frozen (Naito & Yokoyama 1978)). We chose to study Fluosol 43 since it was felt preferable to develop the ^{19}F nmr assay using a stable emulsion (therefore easier to handle) composed of a single perfluorinated compound: F-tri-n-butylamine.

Table 1. Composition of Fluosol 43.

FC 43 $(C_4F_9)_3N$	20 w/v%
Pluronic F-68	2·56
NaCl	0·60
KCl	0·034
$CaCl_2$	0·028
$MgCl_2$	0·020
$NaHCO_3$	0·210
Glucose	0·180
Hydroxyethylstarch	3·0

Materials and methods

Perfluorochemical emulsion. The Fluosol 43 stem emulsion (F-tri-n-butylamine: FC 43, emulsified with Pluronic F-68 in water) and annex solutions A and B (furnishing the emulsion with physiological osmolarity, oncotic pressure and buffer capacity) were products of The Green Cross Corporation, Osaka, Japan. The emulsion was lot n°. 024 GE, the annex A solution was lot no°. 013 GS, the annex solution B was lot n°. 018 GE. The final mixture was prepared before each series of experiments and used immediately to avoid salt precipitation or enlargement of the emulsion particles.

The final composition of Fluosol 43 is given in Table 1 (Naito & Yokoyama 1978).

Animal experiments. CDF_1 strain female mice, 22–26 g were injected with the perfluorochemical at 8 g kg^{-1} body weight (i.e. 1 ml emulsion for a 25 g mouse) through the tail vein at a rate of 1 ml min^{-1}. The mice behaved normally after the injection.

At various intervals after injection, blood samples (0·4–0·8 ml) were drawn by cardiac puncture in heparinized syringes (20 µl) of 10% heparin solution for each sample) and were collected directly in 5 mm diameter nmr tubes. Most of the animals lived after the cardiac puncture.

Nmr determination of F-tri-n-butylamine (FC 43). ^{19}F nmr spectra were recorded at 84·6 MHz with a Perkin Elmer R 32 nmr spectrometer. The ambient probe temperature was 35·5 °C. The ^{19}F peaks areas of the blood sample were compared with those of a calibration emulsion of known concentration (in the range 7·46 to 59·7 mM) of F-tri-n-butylamine, prepared by dilution in water of Fluosol 43 stem emulsion. The concentration of the standard solution was chosen to be close to that of the blood sample to be analysed.

Its spectrum was recorded immediately before and after that of the blood sample in rigorously identical conditions (Fig. 1). The assay consisted of excising and weighing the surface areas covered by the 4 signals; the final value was the average of 2 such operations. The error with this method (resulting from the spectrum recording, the cutting out and the weighing) when determined using the calibration emulsions, was always lower than ±3%.

Results

The blood elimination of F-tri-n-butylamine was followed from 5 min to 102 h after injection. The results are in Table 2 and Fig. 2.

The variation of the FC-43 concentration with time presented several characteristic regions: (i) from 5 min to 3 h, there was little change in the perfluorochemical concentrations, these were reduced at 7 min, 36 min and 1 h due to haemodilution caused by the injection of about 1 ml of emulsion (3 h after injection the blood

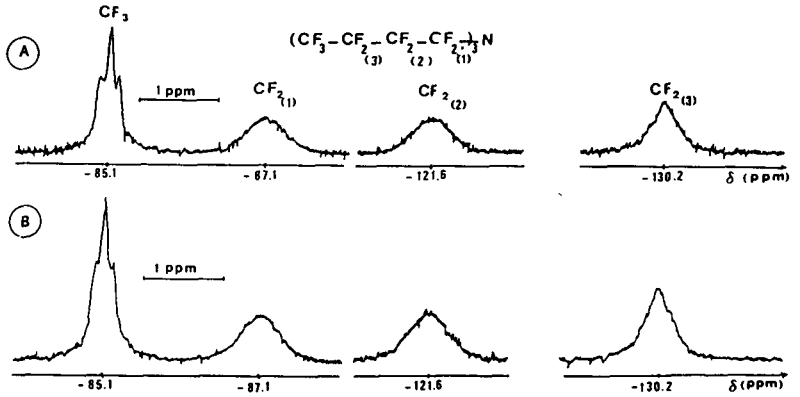

FIG. 1. Nmr spectra of F-tri-n-butylamine: A: in standard emulsion (59·7 mM); B: in whole blood (480 min after injection). Chemical shifts are expressed in ppm and are related to $CFCl_3$. Negative numbers indicate upfield direction from the reference. Measurements are made using CF_3COOH (0·5% aqueous solution) as an external standard (CF_3COOH at 77 ppm upfield from $CFCl_3$). The following spectra recording conditions are used: sweep range: 10 ppm; sweep time: 600 s; filter: 2; H_1 level; 14: sensitivity: 2.

The four distinct resonance lines were assigned from the peak intensities and from the literature data of chemical shifts observed for analogous compounds (Battais et al 1978). Spin-spin splittings are not resolved for the all CF_2 groups.

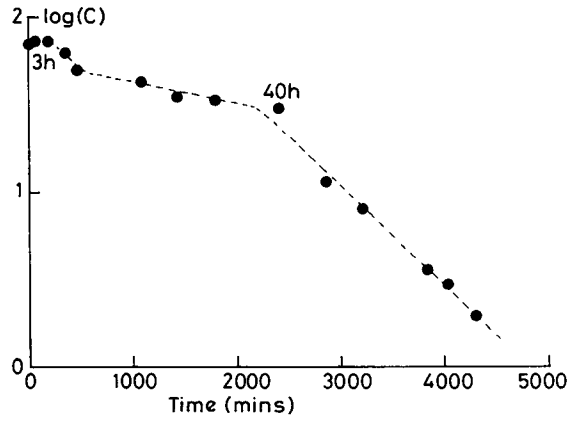

FIG. 2. Semi-logarithmic plot of blood F-tri-n-butylamine concentrations versus time (min).

volume returns to normal). (ii) from 3 h to 40 h, there was a non-linear decrease phase; (iii) from 40 to 96 h (limit of method sensitivity) there was a linear decrease phase in which the FC 43 half life span ($t_{1/2}$ = 9·6 h) is lower than that from 3 to 40 h.

Table 2. Blood F-tri-n-butylamine concentrations, mg ml^{-1} (s.d.), in mice after a single intravenous dose of 8 g kg^{-1}. Results are given after correction due to heparin dilution. The value of each point is obtained from 4 to 11 mice.

Time (min)	Blood FC 43 concn
6·9	71·3 (8·3)
35·6	71·9 (5·6)
61·7	73·9 (2·3)
190·6	73·6 (2·3)
363·7	63·0 (4·8)
474·0	50·4 (4·1)
1095·4	43·1 (4·55)
1439·0	35·2 (2·6)
1802·3	33·7 (7·2)
2423·1	30·1 (7·5)
2867·0	11·4 (3·1)
3219·2	8·0 (1·4)
3839·0	3·6 (2·9)
4040·4	2·9 (2·5)
4311·4	1·9 (2·0)
5782·0	ND
6149·5	ND

ND: not detected.

Discussion

Nmr was the analytical method chosen because (i) biological samples such as blood can be analysed without the need of separation or extraction; (ii) it is possible to follow quantitatively the evolution of any fluorinated compound (and/or its metabolites) without any confusion with biological components; (iii) fluorine-19 is an attractive nucleus for biological nmr work. This isotope, occurring at 100% natural abundance, has spin 1/2 and a relative sensitivity of 0·833 that of proton. It shows a great chemical shift sensitivity to changes in its environment and a large chemical shift range (960 ppm). This latter property allows the various fluorinated groups in one molecule to be clearly distinguished in spite of signal line broadening due to the biological system. The reproductibility of the ^{19}F nmr assay method (±3%) is comparable to that of the gc method which was also ±3% (Yamanouchi et al 1975).

The main limitation of nmr applied to biological problems is its low intrinsic sensitivity with respect to the possibilities of gc or hplc. With our continuous wave spectrometer, the smallest detectable concentration of F-tri-n-butylamine is approximately $2 \cdot 10^{-3}$ M. (The gc method allows lower concentrations of FC 43 to be detected: $2 \cdot 10^{-4}$ M, Yamanouchi et al 1975, and $6 \cdot 10^{-5}$ M, Cao et al 1981.) These concentrations can be reached with ^{19}F nmr Fourier transform spectrometry.

The above advantages for ^{19}F nmr make it useful to study the fate of perfluorinated compounds in biological media.

Perfluorochemicals used as blood substitutes are biologically inert and are not catabolized in-vivo (Clark et al 1974; Yamanouchi & Yokoyama 1975; Yokoyama et al 1978; Naito & Yokoyama 1978). When injected intravascularly, they are excreted almost exclusively by exhalation and transpiration (Clark et al 1974; Yokoyama et al 1975, 1978). Biliary excretion is a minor route of elimination; urinary elimination was not detected (Yokoyama et al 1975, 1978; Naito & Yokoyama 1978).

The perfluorochemical particles are phagocytosed by the histiocytes of the reticuloendothelial system (liver, spleen, bone marrow . . .) where they are retained for a long time (Okamoto et al 1975a; Kitazawa & Ohnishi 1982).

F-tri-n-butylamine is excreted from the body mainly through expiration (<0·1% via the biliary route). Its expiration rate is slow (0·173 mg h^{-1} kg^{-1} in rats given 4 g FC 43 per kg) and no change is found during the first week after the injection. The expiratory excretion rate of F-tri-n-butylamine is not related to its blood level as it is no longer detected in the blood one week after injection whereas only about 0·7% of the dose is eliminated by expiration during the first week (Yokoyama et al 1975).

Considering the time over which the blood perfluorochemical concentration was followed (about 100 h), it can be assumed that elimination via the lungs is also negligible.

The only possibility of FC43 elimination is therefore its storage in various tissues, this is practically irreversible over the period of time used in our study (at a dose of 4 g kg^{-1} in rats, the half-life of F-tri-n-butylamine in the whole body is 900 days, Naito & Yokoyama 1978).

We tried to describe the FC 43 blood concentration-time profile (Fig. 2) with a linear decomposition curve, but this was not possible. So, the F-tri-n-butylamine elimination from mouse circulation seems to follow a non-linear kinetic that can be either time-dependent

or/and dose-dependent. The dose dependence would mean that a saturation process in storage organs is involved. In spite of the high dose we used (8 g kg^{-1}), we think that such a process is unlikely since it was shown that as the dose of injected perfluorochemical is increased, the amount stored in the liver and the time required to reach the maximal level are also increased (12 h for 2 g FC 43 kg^{-1} and about 2 days for 4 g kg^{-1}) (Okamoto et al 1975a; Yamanouchi et al 1975; Naito & Yokoyama 1978). By analogy with the observations made with F-decalin (Naito & Yokoyama 1978), we assume that for FC 43, the time required to reach this maximal level is longer than 2 days.

The time dependence would result from the particle size-increase phenomenon. It is known that, in the bloodstream, the average size of FC 43 particles increases with time (this is due to coalescence) (Okamoto et al 1975b) and that larger particles are eliminated more rapidly than smaller ones: for example, the half-life span of FC 43 in the circulation of rabbits given 12 g FC 43 kg^{-1} in fine (weight average diameter 0·08 μm) and coarse (weight average diameter 0·15 μm) emulsions is about 71 and 43 h respectively (Okamoto et al 1975b; Naito & Yokoyama 1978).

The elimination rate of Pluronic F-68 is rather higher than that of FC-43 when a FC-43/Pluronic F-68 emulsion is injected into rabbits: 48 h after injection, the surfactant concentration was only 0·5% i.e. the minimal concentration required for the stability of the particles in the emulsion (Okamoto et al 1975b). So, we assume that the non-linearity of our pharmacokinetics is mainly due to its time dependence.

The rate increase of F-tri-n-butylamine blood elimination at about 40 h may be attributed to an increase in the emulsion particles size. We suppose that this phenomenon, which starts at injection time, is sharply accelerated around 40 h when the level of Pluronic F-68 is most probably no longer sufficient to stabilize the perfluorochemical particles. The enlarged particles are therefore deposited more rapidly into the reticuloendothelial system and are more easily eliminated from the bloodstream.

The authors are gratefully indebted to Pfrimmer and Co. (Erlangen, RFA) for generously providing experimental materials; further they acknowledge their appreciation to Dr C. Picard (Sanofi) for helpful discussions about the pharmacokinetics.

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