Pergamon

0731-7085(94)00104-9

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 12, pp. 1489–1493, 1994

0731-7085/94 $7.00 + 0.00

The minimal metabolism of inhaled 1,1,1,2-tetrafluoroethane to trifluoroacetic acid in man as determined by high sensitivity ^{19}F nuclear magnetic resonance spectroscopy of urine samples

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Abstract: In this work, oxidative metabolism of the new propellant, 1,1,1,2-tetrafluoroethane to trifluoroacetic acid in man is shown to be minimal. Alternative propellants and refrigerants are under development to replace the currently used chlorofluorocarbons which lead to stratospheric ozone depletion. One potentially useful replacement is the hydrofluorocarbon, 1,1,1,2-tetrafluoroethane (HFA-134a). Before it can be used, however, particularly as a propellant in an aerosol pharmaceutical formulation whereby the compound is in effect dosed to people, it is important that the safety of this compound is established. As a part of this safety evaluation it is necessary to understand the metabolism of HFA-134a. In this work the production of the potential oxidative metabolite of HFA-134a, trifluoroacetic acid (TFA) has been studied in human urine following inhalation dosing with HFA-134a. The concentrations of TFA in urine have been measured using a highly sensitive ^{19}F nuclear magnetic resonance procedure with a limit of detection of 10 ng ml^{-1} based on an acquisition time of only 2.25 h per sample. TFA is the only fluorinated species observed in the urine samples and only at very low levels, indicating that the oxidative route of metabolism can occur *in vivo* in man, but this metabolism is minimal in terms of percentage of administered dose.

Keywords: *1,1,1,2-tetrafluoroethane; trifluoroacetic acid; urine; metabolism; ^{19}F-NMR.*

Introduction

Recent years have seen ever-increasing concern over the widespread usage of chemicals that are believed to destroy atmospheric ozone. Chief among these chemicals are the chlorofluorocarbons (CFCs) which have been extensively used as refrigerants, as blowing agents in foam plastic manufacture, as cleaning fluids and as aerosol propellants, including use in medicinal products where the propellants convey drugs to the respiratory tract. These CFCs have been shown to lead to raised stratospheric chlorine radical levels which in turn catalyse the depletion of ozone [1]. Subsequently, as a result of governmental accords aimed at protection of the atmosphere through the eventual cessation of CFC production and use, much effort and money has been expended on developing suitable alternatives.

One potentially useful alternative to CFCs is the hydrofluorocarbon, 1,1,1,2-tetrafluoroethane (HFA-134a), which has suitable physical properties for use as a refrigerant or a propellant. For any compound to which the public may become exposed, and particularly where that compound is in effect dosed to people, as in the case of an excipient in aerosol medicinal products, it is important that the safety of the compound is established. The full toxicological evaluation of HFA-134a is still under investigation.

As a part of this process, the metabolic fate of HFA-134a in man needs to be understood. Olson *et al.* have studied extensively the *in vitro* metabolism of HFA-134a [2–4] using rat, rabbit and human hepatic microsomes and have demonstrated that, in these systems, HFA-134a is metabolized in an oxidative manner by cytochrome P-450, predominantly by the CYP 2E1 isozyme. The product of metabolism in this way would ultimately be expected to be trifluoroacetic acid (TFA) according to the pathway shown in Fig. 1. They found no evidence of any reductive metabolism of HFA-134a.

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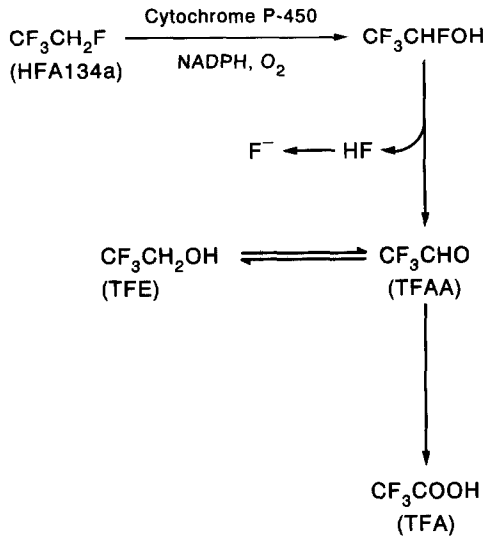

Figure 1
The probable route of cytochrome P-450 mediated oxidative metabolism of HFA-134a. TFE = trifluoroethanol; TFAA = trifluoroacetaldehyde.

It is important to know if this metabolic route also applies *in vivo*. In the *in vivo* situation, evidence for the presence of an oxidative metabolic pathway would be provided by the detection of TFA, or other related compounds in urine samples following inhalation of HFA-134a. It has previously been shown that HFA-134a does exhibit some oxidative metabolism to TFA *in vivo* in rats, and that this TFA is eliminated in the urine [5]. Further, in studies of the metabolism of halocarbon anaesthetics (a similar class of structure of HFA-134a), TFA has been shown to be produced in humans and subsequently measured in significant amounts in the urine [6, 7].

Most previous assays for TFA have been based on gas chromatography [e.g. 8, 9] and ion chromatography [10]. There are also examples of the use of ^{19}F nuclear magnetic resonance spectrometry (^{19}F-NMR) [e.g. 11]. For a highly inert and volatile compound such as HFA-134a, the vast majority of any administered dose would be eliminated unchanged by exhalation. Therefore, these existing methods do not have the low ng ml^{-1} sensitivity required for our application. The use of ^{19}F-NMR as a bioanalytical tool has grown over recent years [e.g. 12–15] and is ideally suited to the analysis of urine samples for TFA content, or other organofluorine metabolites of HFA-134a, as there is little requirement for sample pre-treatment. This is due to the matrix containing no endogenous fluorine containing substances, other than possibly inorganic fluorine from environmental sources such as toothpaste which will be unlikely to interfere with the ^{19}F-NMR signal from a fluoro-organic molecule like TFA.

In this study, a highly sensitive ^{19}F-NMR procedure has been developed for the analysis of TFA in urine and has been used to study the metabolism of HFA-134a in man.

Experimental

Clinical

Four healthy male volunteers (aged 33–56) weighing 71.3–80.5 kg each received a single 1200-mg dose of HFA-134a administered as 16 actuations of a metered dose inhaler delivering 75 mg HFA-134a per actuation. Each inhalation was performed approximately 30 s after the start of the previous inhalation. The inhalers were supplied by the Clinical Supplies Unit, Glaxo Research and Development Ltd. Urine samples were collected over the following 24 h in the periods 0–3, 3–6, 6–12 and 12–24 h. The volunteers were allowed to consume a light breakfast (containing no more than 200 ml of fluid) at least 1 h prior to dosing. Fluid intake was also restricted until the completion of the 3–6 h urine collection, to minimize urine volume and, therefore, maximize the concentration of any TFA present.

Urine sample preparation

Aliquots (0.5 ml) of the urine samples for ^{19}F-NMR investigation were placed in 5 mm silanized glass nuclear magnetic resonance tubes. The tubes were silanized by immersion in toluene (Specified Lab Reagent, Fisons, Loughborough, UK) containing 10% dichlorodimethylsilane (Sigma, Poole, UK) for 1 h, washed with methanol and dried prior to use. Two hundred and twenty μl of 10 M sodium hydroxide, prepared by dissolving 40 g (Analar grade, BDH, Poole, UK) in 100 ml distilled water, was added to basify the samples, together with 80 μl deuterated water (Aldrich, Gillingham, Dorset, UK) for the purpose of locking the spectrometer. Quantification was enabled by the use of calibration standards over the range 10–125 ng ml^{-1} prepared by the addition of small volumes of an accurately prepared dilution of TFA in distilled water to

control human urine. Further confidence in the data was obtained by the analysis of quality control samples at concentrations chosen to cover the expected range of TFA concentrations in study samples. These were prepared in the same manner, but from a completely independent set of TFA dilutions.

^{19}F-nuclear magnetic resonance spectrometry conditions

^{19}F-NMR spectra were obtained on a Varian Unity 400 MHz spectrometer, operating at 376.289 MHz using a 5 mm probe, covering a spectral width of 7782 Hz with 10176 data points. A pulse width of 30° was used with no delay between pulses. Twelve thousand transients were acquired for each spectrum at 25°C. The method was linear over the calibration range used. A blank control human urine sample was also run under the conditions given above except that 5800 transients were acquired with a 4 s delay. Spectra were collected for the calibration and quality control samples and for the 0–3 and 3–6 h samples, these being the samples most likely to contain any TFA produced. The 6–12 and 12–24 h samples were retained but not analysed. The ^{19}F-NMR signal corresponding to TFA was integrated for all samples and the integral height for the calibration standards plotted against the known concentration of TFA in the standards, with linear regression, to provide a calibration line. The integral heights for the quality control samples and the study samples were interpolated against this line to calculate the TFA concentrations present. A spectrum was also collected for an aqueous mixture of TFA, TFE (the Sigma Chemical Co., Poole, UK) and fluoride anion (sodium fluoride) to ensure that the signals from these latter two did not interfere with the signal due to TFA. The other intermediate in the metabolic pathway, TFAA, is unstable and was not available. However, this instability makes it extremely unlikely that any TFAA would be eliminated unchanged in urine.

Results and Discussion

It was established during the development of the ^{19}F-NMR procedure that TFA was being lost from samples, possibly by adsorption. Given that each sample result required 2.25 h to acquire, this presented a stability issue. It was found that basifying the samples with 220 μl of 10 M sodium hydroxide removed this problem. As an additional precaution the samples were basified immediately on collection and all ^{19}F-NMR spectra were recorded within a total of 48 h of sample collection.

The developed ^{19}F-NMR method was found to be suitable for the determination of TFA in human urine samples following no other sample pre-treatment than that described above and has a limit of detection of 10 ng ml^{-1}. A typical calibration graph obtained showing the linearity and sensitivity of the procedure over the range 10–125 ng ml^{-1} is shown in Fig. 2. The procedure also provides a

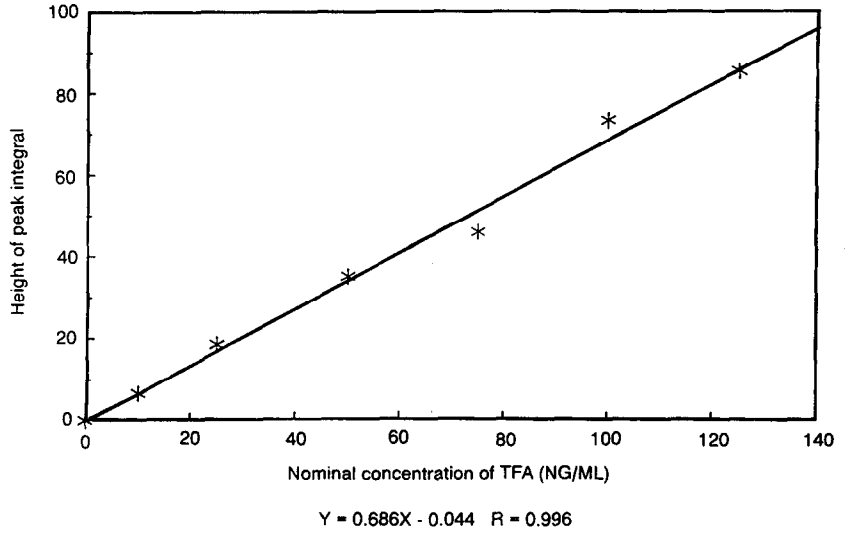

Figure 2
A typical calibration line of the ^{19}F-NMR analysis of TFA in human urine over the range 10–125 ng ml^{-1}.

screen for other fluorinated metabolites of HFA-134a, such as the intermediate TFE, as measurement of a standard of this compound showed a response at a similar but different chemical shift. The concentrations of TFA found in human urine and the calculated percentages of administered HFA-134a dose represented by these concentrations are shown in Table 1. It was decided not to analyse the 6–12 and 12–24 h samples as the amounts of TFA observed in the 0–3 and 3–6 h samples were insignificant related to the HFA-134a dose administered. An example of the ^{19}F-NMR spectrum obtained for a human urine sample following administration of a 1200 mg inhaled dose of HFA-134a is shown in Fig. 3. No fluorinated components other than TFA were observed in any of the spectra. Any fluoride ion which would be expected to be present would not be observed in the range scanned. Furthermore, TFA was only detectable in three of the samples and in all cases representing less than 0.0005% of the dose. These results suggest that an oxidative metabolic pathway does exist in man *in vivo* for HFA-134a, producing TFA, but it only represents a very small component of the elimination of HFA-134a from the body, as would be expected for a compound which is naturally a gas and rapidly eliminated unchanged by exhalation. The presence of this oxidative metabolism is in good agreement with the results obtained in human hepatic microsomal studies by Surbrook and Olson [4].

To date, the use of ^{19}F-NMR at sub μg ml^{-1} concentrations has tended to need long acquisition times, effectively prohibiting its use for monitoring more than a very limited number of samples. Alternatively, spectra can be collected over a short period of time but at the cost of reducing sensitivity. Our procedure uses a relatively short acquisition time and shows extremely high sensitivity, allowing the application of ^{19}F-NMR to greater numbers of low concentration samples within an acceptably short period of time. The accuracy of the

Table 1
The concentrations of trifluoroacetic acid in urine following administration of 1200 mg HFA-134a to healthy volunteers by inhalation

Volunteer no.	Sample time (h)	Trifluoroacetic acid concentration (ng ml^{-1})	Urine volume (ml)	Total amount of trifluoroacetic acid (ng)	% of dose*
1313	0–3	<10	188.7	—	—
	3–6	<10	346.8	—	—
1436	0–3	<10	122.1	—	—
	3–6	15	113.6	1704	0.00013
1022	0–3	13	160.0	2080	0.00016
	3–6	<10	134.3	—	—
1072	0–3	14	370.4	5186	0.00039
	3–6	<10	207.6	—	—

*These values have been corrected for the molecular weight difference between TFA and HFA-134a.

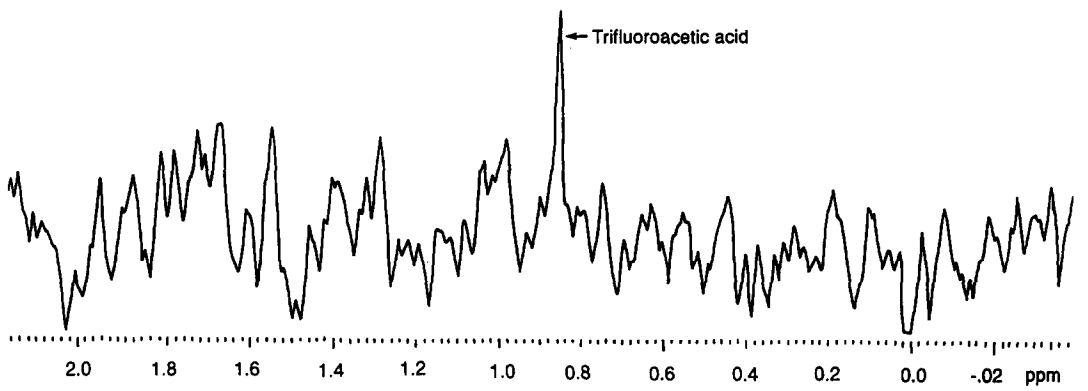

Figure 3
^{19}F-NMR spectrum for a urine sample containing 15 ng ml^{-1} TFA following inhalation dosing of 1200 mg HFA-134a to a volunteer.

method at these very low concentrations was shown by the quality control results (deviation ~15–30% from nominal) to be less than would be normally accepted for, say, chromatographic analysis, but still sufficient to demonstrate the key result that the metabolism of HFA-134a to TFA accounts for a minute percentage of the administered dose in man.

Conclusions

A highly sensitive ^{19}F-NMR method has been developed and used, but not formally validated, for the analysis of TFA in urine with little sample pre-treatment. The method has a limit of detection of 10 ng TFA/ml of urine.

This procedure has been applied to the determination of TFA in urine following the administration of an inhaled dose of the propellant HFA-134a to human volunteers. TFA is found in human urine, but at very low levels corresponding to an extremely low percentage of the administered HFA-134a dose. No other fluorinated components were observed, suggesting that the metabolism of HFA-134a in man is minimal and that which does occur follows the oxidative pathway previously observed *in vitro* leading to the formation of TFA.

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[Received for review 28 March 1994; revised manuscript received 20 July 1994]