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RAPID COMMUNICATION

MEASUREMENTS OF IN VIVO HEPATIC HALOTHANE METABOLISM IN RATS USING ^{19}F NMR SPECTROSCOPY

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Applications of in vivo nuclear magnetic resonance (NMR) spectroscopy to the study of cellular and organ metabolism have increased steadily since the development of this approach. Studies have been carried out describing the use of ^{31}P and ^{13}C NMR to analyze both normal and stressed metabolism in the heart [1], liver [2,3], and brain [4]. In contrast, relatively few studies have been carried out which describe the application of in vivo NMR techniques to the study of the metabolism of xenobiotics in perfused organs or laboratory animals. ^{19}F NMR is particularly well suited for such applications as a consequence of the high intrinsic sensitivity for detection and the lack of significant ^{19}F background. In vivo ^{19}F NMR studies of the metabolism of 5-fluorouracil [5] and 2-fluorodeoxyglucose [6] have been reported recently.

Any attempt to understand the metabolism of xenobiotics in living systems must deal with the hepatic metabolism of such chemicals in general, and with the effects of biotransformation by the cytochrome P-450 enzyme system in particular. Despite the central role of this enzyme system in the detoxification and clearance of most xenobiotics, there has been as yet no attempt to determine whether the effects of cytochrome P-450 induction on drug metabolism can be directly monitored in vivo using NMR spectroscopy. As a model for studying the role of P-450 induction, we have carried out ^{19}F NMR studies using a surface coil positioned over the liver of anesthetized rats. Halothane (2-chloro-2-bromo-1,1,1-trifluoroethane) was selected as a model xenobiotic because (1) it accumulates in high concentration in the liver, (2) its metabolism is cytochrome P-450 dependent [7] and (3) its metabolism has been implicated as a cause of liver damage in some patients given halothane as a surgical anesthetic [7]. This report demonstrates that cytochrome P-450 induction can be monitored in vivo using ^{19}F surface coil NMR, and that hepatic halothane metabolism is altered by this induction.

METHODS

Rat preparation and treatment. Young male Fisher 344 rats (Charles River, 200-300 g body weight) were used in this study. Rats were surgically herniated 48-72 hr before NMR observation to remove peritoneal muscle from above the liver which would interfere with the NMR measurement [8]. Ketamine/xylazine (Vetalar, 100 mg/ml ketamine, Parke Davis, supplemented with Rompun, 30 mg/ml; 1 ml/kg, i.p.) was used to anesthetize the rats during surgery. Rats were fed with standard rat chow and water ad lib. until halothane administration. In

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some experiments, rats were fed ad lib. with a diet of rat chow to which was added 0.05% phenobarbital for 1 week prior to surgery to induce liver cytochrome P-450 enzymes.

Halothane (Halocarbon Laboratories, Hackensack, NJ, 2% for 5 min, 1% for an additional 55 min) was administered by ventilation under 99% oxygen in a rebreathing small animal anesthesia machine (Summit Hill Laboratories, Navesink, NJ). Rats were allowed to revive from halothane anesthesia for 15-60 min, after which inactin (Byk, 100 mg/ml in isotonic saline, 1 ml/kg rat, i.p.) was administered. Inactin is a long-acting anesthetic which allows continual NMR measurements of up to 12 hr [9]. We are presently limited in that we cannot anesthetize rats which are positioned in the bore of our NMR magnet; this limitation imposes a delay time of 1-3 hr between the end of halothane exposure and the beginning of the NMR experiment.

Nuclear magnetic resonance. ^{19}F NMR measurements were carried out at 339.7 MHz on a Nicolet 8.45T wide bore spectrometer. Upfield chemical shifts are in the negative direction. A "homebuilt" rat probe consisting of two concentric coils tuned for ^{19}F and ^{31}P was used in all studies. Spectra were accumulated using a single 90° pulse of 60 μsec calibrated to maximize fluorine signal from the liver, and a 1- or 2-sec recycle time. Sweep width was ± 15,000 Hz using 4K data points. A 20 Hz exponential line broadening was applied to the free induction decays before Fourier transformation. Other details regarding spectral acquisition may be found in the figure legends.

RESULTS AND DISCUSSION

Figure 1 shows a series of ^{19}F NMR spectra which follows the in vivo clearance and metabolism of halothane from the rat liver. As discussed in Methods, these measurements were made on rats previously dosed with halothane but anesthetized during the NMR studies with a non-fluorinated anesthetic in order to allow determination of the rate of halothane clearance. From analysis of Fig. 1, the ^{19}F resonance corresponding to halothane decreased exponentially in intensity with time over the period of the observation. The loss of halothane signal can thus be modeled by a first-order decay, with a half-life in liver of 3.5 hr. As shown in the inset to Fig. 1, the data fit quite well to a single first-order decay. Since we are unable to observe early times following halothane exposure (see Methods), it is possible that a second quickly-eliminated population of halothane exists which escaped detection. In addition to the loss of intensity corresponding to the halothane resonance, at times greater than approximately 8 hr post-administration, a second resonance is visible approximately 0.6 ppm downfield from the halothane peak. On the basis of studies with liver extracts, model compounds, and the known metabolic transformations of halothane [7], the downfield resonance was assigned to trifluoroacetate anion. Although we have observed other halothane metabolites in vitro [10], only the above two compounds have been observed in vivo under these experimental conditions.

In rats subjected to prior administration of phenobarbital to induce cytochrome P-450, the same two metabolites, assigned to halothane and to trifluoroacetate, were observed, but the time course of the metabolism differed dramatically from the non-induced controls (Fig. 2). Halothane is observed to clear much more rapidly from the liver of the induced rats ($T_{1/2}$ = 2.5 hr), and the appearance of the trifluoroacetate resonance can be discerned at much shorter time periods post-exposure to the anesthetic.

Based on a comparison of Figures 1 and 2, it is apparent that phenobarbital induction led to a more rapid clearance of halothane from the liver, together with a more rapid appearance and a higher mean level of trifluoroacetate. Despite the latter observation, integration of the ^{19}F resonances in the spectra shows that the trifluoroacetate con-

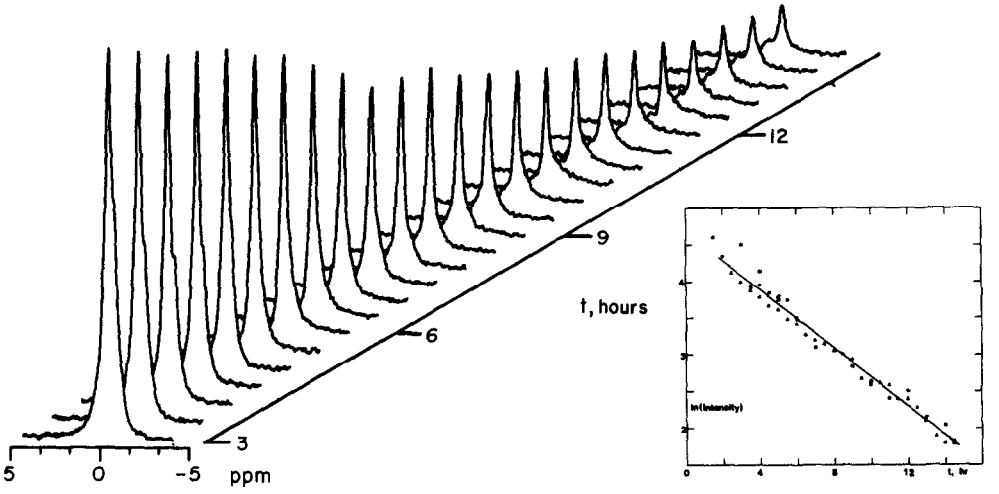

Fig. 1. Stack plot of ^{19}F NMR spectra of rat liver following a 1 hr exposure to halothane. The spectra shown are the time averages of 1600 scans accumulated over 30 min. The first spectrum was begun 150 min following the end of halothane exposure. The decay of halothane signal with time can be modeled as a single exponential first-order decay process, with a rate constant of 0.20 ± 0.01 hr^{-1} (± denotes one standard deviation, N = 3, r^2 curve-fitting values ranged from 0.89 to 0.99). Inset. Plot of the natural logarithm of halothane intensity versus time. Intensities of the ^{19}F NMR resonance corresponding to halothane were determined by the "cut and weigh" method. Separation of the halothane and trifluoroacetate resonances was determined by eye. The six-hour time point of the data sets was normalized to equal intensity to allow comparison of all experiments. The different symbols correspond to data collected from three separate experiments. The solid line represents the best fit of a first-order decay process to the experimental data. The slope of this fit, -0.20 hr^{-1}, is equal to the decay constant for halothane. The r^2 curve fitting constant of the experimental data to the calculated line is 0.98.

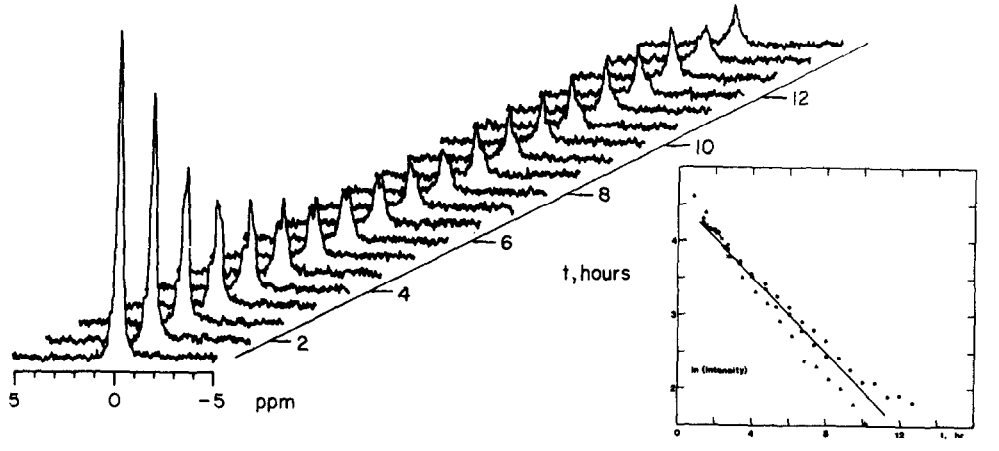

Fig. 2. Stack plot of ^{19}F NMR spectra of the liver of a phenobarbital-induced rat following a 1 hr exposure to halothane. The spectra shown are the time averages of 540 scans accumulated for 20 min, with a 20-min delay between spectra. The first-order rate constant describing loss of halothane signal is calculated in induced rats to be 0.28 ± 0.04 hr^{-1} (± denotes one standard deviation, N = 4, r^2 curve-fitting values ranged from 0.99 to 1.00). The clearance rate for halothane from phenobarbital-induced rats was significantly different from the clearance rate in noninduced rats using Student's t-test ($P < 0.05$). Inset. Plot of the natural logarithm of normalized halothane intensity versus time. The data were analyzed as described in the inset to Figure 1, except that the measured intensities were normalized to the 2 hr time point. The different symbols correspond to data collected in 4 separate experiments. The rate constant determined is -0.26 hr^{-1}, with an r^2 curve fitting constant of 0.95.

centration in the liver of the induced rats increased rapidly to a steady-state level but remained fairly constant over the time course of the study. In the study shown in Fig. 2, the steady-state value is reached after only 2 hr. This result presumably reflects the dynamic nature of the system under study, such that the rates of trifluoroacetate production and clearance become sufficiently close to lead to a fairly constant level of the metabolite in the tissue.

The studies reported here demonstrate that cytochrome P-450 induction, and hence P-450-dependent metabolic transformation, can alter significantly the rate of clearance of halothane from the liver. Since trifluoroacetate levels increased more rapidly but remained at an elevated steady-state level relative to the non-induced controls, the data suggest that much of the observed clearance occurred via the transformation of halothane to trifluoroacetate followed by the excretion of this polar metabolite. This conclusion is consistent with the observation of large amounts of trifluoroacetate in the urine of halothane-dosed experimental animals [11].

The phenobarbital-induced Fisher 344 rat has been used as a model system for halothane hepatitis [12]. Since as noted above, both the reductive and oxidative metabolic pathways for halothane metabolism involve cytochrome P-450, it is not possible from these induction studies to determine which pathway may play a greater role in halothane-related pathology. Moreover, studies carried out under hypoxic conditions evidence a greater degree of toxicity, implicating reductive metabolism in the pathological response [7]. However, the observation of such large levels of tissue trifluoroacetate ion, which most probably reflects the existence of an extremely reactive acid chloride intermediate, suggests a significant role for oxidative metabolism in the production of toxic symptoms. Further studies are necessary to determine the contributions of oxidative and reductive metabolism of halothane to the development of halothane hepatitis.

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