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RAPID COMMUNICATION

IN VIVO METABOLISM OF THE HYDROCHLOROFLUOROCARBON 1,1-DICHLORO-1-FLUOROETHANE (HCFC-141b)

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Hydrochlorofluorocarbons (HCFCs) are being developed as substitutes for chlorofluorocarbons (CFCs) that deplete stratospheric ozone (1). 1,1-Dichloro-1-fluoroethane (HCFC-141b) is a potential substitute for CFC-11 in foam-blowing operations (2), and a mixture of HCFC-141b and HCFC-123 is an effective substitute for CFC-113 which is used as a cleaning agent in the computer industry (3). U.S. production of CFC-11 and CFC-113 in 1986 amounted to 90,000 and 79,000 metric tons, respectively. [^{36}Cl]HCFC-141b undergoes dechlorination when incubated with rat hepatic microsomal fractions in the presence of NADPH and oxygen (4), but no organic metabolites have been identified. Because of the potential commercial importance of HCFC-141b, the accompanying potential for human exposure, and the paucity of published information about its metabolism, we have studied its in vivo metabolic fate. Also, knowledge about the metabolism of HCFC-141b may have predictive value for the metabolism of other 1,1,1-trihaloethanes that are promising CFC substitutes.

MATERIALS AND METHODS

Instrumental analyses. NMR spectra were acquired with a Bruker WP-270 instrument equipped with a dedicated 5-mm ^{19}F probe and operating at 254.18 MHz for fluorine. The pulse width was 3 μsec, and the interpulse time was 0.7 sec for covalent-binding assays (spectral width = 50 KHz) or 1.7 sec for metabolite quantification (spectral width = 5 KHz). The method of Hull (5) was used for quantification of metabolites by ^{19}F NMR. Spectra were acquired at room temperature with sample spinning. For the assay of covalent binding a minimum of 40,000 transients were acquired. Chemical shifts and signal integrals were referenced to a 5.0 mM solution of trifluoroacetamide in D_2O (δ = 0 ppm). Mass spectra were recorded with a Hewlett-Packard 5880A GC equipped with an HP-1 (dimethylpolysiloxane gum) capillary column and coupled to an HP-5970 mass selective detector (70 eV, electron impact).

Chemicals. HCFC-141b was purchased from PCR Inc. (Gainesville, FL) and contained 1% of an unidentified, non-chlorinated impurity. Dichlorofluoroacetic acid was purchased from Fluorochem Ltd. (Derbyshire, U.K.). 2,2-Dichloro-2-fluoroethanol was synthesized from dichlorofluoroacetic acid by the slow addition over 2 hr of lithium aluminum hydride (0.5 equiv., equal to 2.0 equiv. of hydride ion) dissolved in dry tetrahydrofuran in a closed vessel at -5°. One volume of cold water was added, and the solution was filtered and then extracted three times with 2 vol. of ethyl ether. The ether was evaporated yielding a mixture of 2,2-dichloro-2-fluoroethanol [MS: m/z (relative intensity) 136 (0.2), 134 (0.8), 132 (1.2), 105 (2.4), 103 (15), 101 (23), 99

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(11), 97 (40), 79 (39), 77 (100); ^{19}F NMR (δ, D_2O) 10.77 (t, $^3J_{HF}$ = 14.3 Hz)] and dichlorofluoroacetaldehyde; this mixture was suitable as a reference standard without further purification of the haloethanol because the NMR resonances of the two components are well separated.

Animal treatment. Adult, male Fischer 344 rats (Charles River, 300-320 g) were exposed by inhalation to 1.15 ± 0.04% HCFC-141b in air (v/v) for 2 hr. The inhalation chamber used has been described (6), except that $CaCl_2$ was used to absorb water and that consumed oxygen was replaced via an attached spirometer. Chamber HCFC-141b concentrations were measured during exposure by GC-MS. The treated animals were immediately placed in metabolism cages and kept on a 12-hr light/dark cycle. Urine was collected for 15 hr into vessels maintained at -78° and was stored frozen until analyzed.

NMR analysis of covalent binding. After 15 hr, livers were removed from ether-anesthetized rats, and microsomal and cytosolic fractions were isolated by differential centrifugation: the supernatant from a 10,000 *g*, 20-min centrifugation of the liver homogenate was centrifuged for 60 min at 100,000 *g*, yielding the cytosolic fraction as the supernatant and the microsomal fraction as the pellet. These fractions were dialyzed (Spectrapor 3 tubing, Spectrum Medical Industries, 3500 mol. wt. cutoff) in 10 mM phosphate buffer, pH 7.0, containing 0.1% sodium dodecyl sulfate (SDS) for at least 48 hr at 4° to remove weakly bound metabolites (7). Dialyzed protein was lyophilized, dissolved in D_2O, and analyzed by ^{19}F NMR. Protein concentrations in the NMR tube were kept as high as possible consistent with maintaining a liquid, albeit viscous, solution; a minimum of 75 mg protein/mL was used. Hepatic fractions from rats treated with 1.3 ± 0.1% halothane for 2 hr were prepared similarly.

β-Glucuronidase incubations. The formation of 2,2-dichloro-2-fluoroethyl glucuronide as a metabolite of HCFC-141b was investigated by incubating urine from HCFC-141b-treated rats (urine was pooled, filtered, lyophilized, dissolved in D_2O, and divided into 0.6-mL aliquots) with 10 mg β-glucuronidase (Sigma, type B-3) in an NMR tube at 37° (pH 5). ^{19}F NMR spectra were taken at various times to follow the course of the reaction. Control incubations were conducted either by deleting β-glucuronidase from the incubation mixture or by adding saccharic acid 1,4-lactone (10 mM final concentration), a competitive inhibitor of β-glucuronidase (8), to the incubation mixture prior to the addition of the enzyme. Filtration of the incubation mixtures through a 0.45 μm membrane cartridge markedly reduced the observed NMR line widths.

RESULTS AND DISCUSSION

No covalent binding of fluorinated metabolites of HCFC-141b to liver proteins was detected by ^{19}F NMR (data not shown). The oxidative metabolism of 1,1,1-trihaloethanes does not result in the production of reactive electrophiles (9), thus accounting for the failure to detect HCFC-141b metabolites attached to hepatic protein. A covalent adduct was detected, however, in livers of rats exposed to 2,2-dichloro-1,1,1-trifluoroethane (HCFC-123) and halothane, which are metabolized to the electrophile trifluoroacetyl chloride (10). The resulting amino acid adduct has been identified as N^{ϵ}-trifluoroacetyllysine (10).

Urine collected from each animal was analyzed by ^{19}F NMR, and a single resonance of low intensity was seen in each case. The spectral pattern of this fluorine-containing metabolite was studied in spectra that were acquired from urine that had been pooled, filtered, and lyophilized. The NMR spectra of the concentrated urine contained the same single resonance, a triplet at 12.15 ppm with $^3J_{HF}$ = 14.3 Hz. No other NMR resonances were found in a chemical shift range (350 ppm) encompassing organofluorine compounds (11), indicating that rats inhaling 1% HCFC-141b for 2 hr produce a single fluorinated urinary metabolite.

The resonance centered at 12.15 ppm was identified as 2,2-dichloro-2-fluoroethyl glucuronide. Incubation

of concentrated urine from HCFC-141b-treated rats with β-glucuronidase resulted in the loss of the ^{19}F NMR resonance at 12.15 ppm and the appearance of a triplet at 10.77 ppm that was identical with synthetic 2,2-dichloro-2-fluoroethanol (Fig. 1). No 2,2-dichloro-2-fluoroethanol formation was observed when β-glucuronidase was omitted from the reaction mixture or when 10 mM saccharic acid 1,4-lactone (8) was added prior to the addition of β-glucuronidase (data not shown).

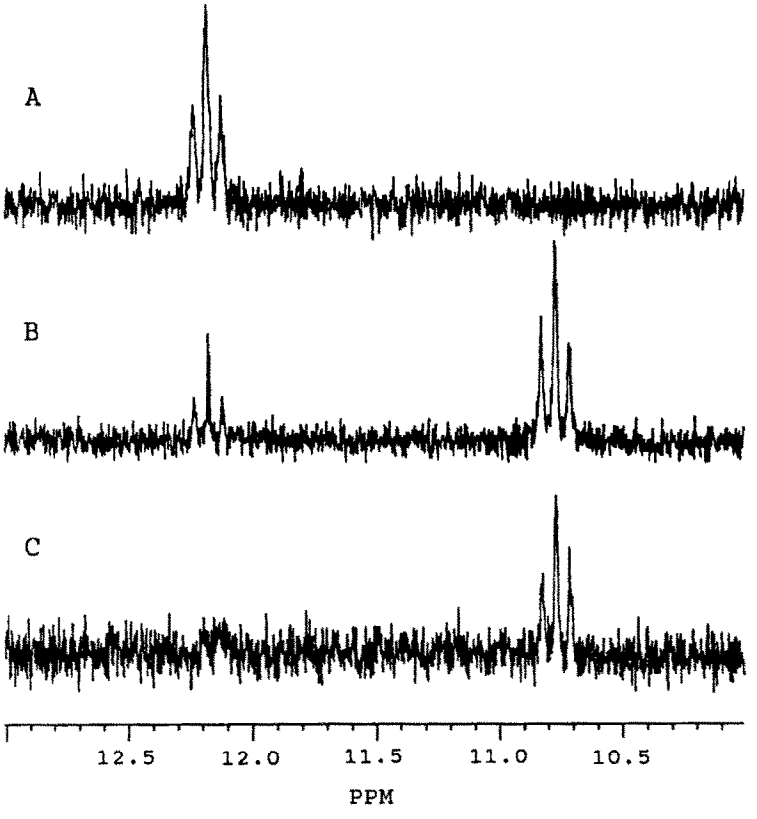

Fig. 1. ^{19}F NMR spectra of incubation mixtures containing concentrated urine from HCFC-141b-treated rats and β-glucuronidase. Spectra were taken after (A) 0, (B) 2, and (C) 4 hr of incubation at 37°. The chemical shift is shown as ppm downfield from trifluoroacetamide (δ = 0). Data shown are representative of three experiments.

The average urinary excretion of 2,2-dichloro-2-fluoroethyl glucuronide during 15 hr after a 2-hr exposure of rats to 1.1% HCFC-141b was 1.0 μmol (N = 3). Unconjugated 2,2-dichloro-2-fluoroethanol was not detected in the urine of treated rats.

The route of metabolism reported here for HCFC-141b is consistent with that reported for the chlorinated analog 1,1,1-trichloroethane. 1,1,1-Trichloroethane is metabolized to 2,2,2-trichloroethanol, which is excreted as its glucuronide conjugate (12), and to a lesser extent to trichloroacetic acid (13) in rats. The oxidation of 1,1,1-trichloroethane to 2,2,2-trichloroethanol is catalyzed by hepatic cytochromes P450 (14). Although dichlorofluoroacetic acid was not detected as a urinary metabolite of HCFC-141b in these studies, dichlorofluoroacetic acid was detected in the urine of rats exposed by inhalation to 4% HCFC-141b in air for 4 hr,* indicating that the oxidation of 2,2-dichloro-2-fluoroethanol may become important at higher exposure concentrations or after repeated exposure.

Although no toxic effects of HCFC-141b have been reported, 2,2,2-trifluoroethanol, an analog of 2,2-dichloro-2-fluoroethanol, is toxic in rats (15). The in vivo toxicity of 2,2,2-trifluoroethanol is characterized by

*Loizou G, Harris JW, and Anders MW, unpublished experiments.

decreased white blood cell counts, intestinal damage, necrosis of lymphatic tissue, and lethality (16). Further studies to investigate the formation and toxicity of 2,2-dichloro-2-fluoroethanol as a metabolite of HCFC-141b are warranted.

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