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DOMINANT ROLE OF CYTOCHROME P-450 2E1 IN HUMAN HEPATIC MICROSOMAL OXIDATION OF THE CFC-SUBSTITUTE 1,1,1,2-TETRAFLUOROETHANE

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ABSTRACT:

The chlorofluorocarbon substitute 1,1,1,2-tetrafluoroethane (HFC-134a) is subject to metabolism by cytochrome P-450 in hepatic microsomes from rat, rabbit, and human. In rat and rabbit, the P-450 form 2E1 is a predominant low-K_M, high-rate catalyst of HFC-134a biotransformation and is prominently involved in the metabolism of other tetrahaloalkanes of greater toxicity than HFC-134a [*e.g.* 1,2-dichloro-1,1-difluoroethane (HCFC-132b)]. In this study, we determined that the human ortholog of P-450 2E1 plays a role of similar importance in the metabolism of HFC-134a. In human hepatic microsomes from 12 individuals, preparations from subjects with relatively high P-450 2E1 levels were shown to metabolize HFC-134a at rates 5- to 10-fold greater than microsomes of individuals with lower levels of this enzyme; the increased rate of metabolism of HFC-134a was specifically linked to increased expression of P-450 2E1. The primary evidence for this conclusion is drawn from studies using mechanism-based inactivation of P-450 2E1 by diethyldithiocarbamate, competitive inhibition of HFC-134a oxidation by *p*-nitrophenol (a high-affinity substrate for P-450 2E1), strong positive correlation of rates of HFC-134a defluorination with *p*-nitrophenol hydroxylation in the study population, and correlation of P-450 2E1 levels with rates of halocarbon oxidation. Thus, our findings support the conclusion that human metabolism of HFC-134a is qualitatively similar to that of the species (rat and rabbit) used for toxicological assessment of this halocarbon. Although hazard from HFC-134a exposure is not anticipated in most humans (based on toxicological evaluation in laboratory animals), our results suggest that HFC-134a exposure should be minimized for individuals with chemical exposure histories commensurate with elevation of P-450 2E1 (*i.e.* frequent contact with agents such as ethanol, trichloroethylene, or pyridine). Furthermore, these findings suggest that toxicity assessments of certain other haloethanes currently under consideration as replacements for chlorofluorocarbons should be considered in animals with elevated P-450 2E1.

HFC-134a,[1] an alternative to CFC-12 in applications as an air-conditioning refrigerant and aerosol propellant, has no propensity for stratospheric ozone depletion and possesses very little toxic potential (reviewed in ref. 1). It is, however, understood that the biological impact of many chemicals is inextricably linked to their metabolism. This strongly suggests that a metabolic profile be developed for any chemical likely to be released into the human environment. Furthermore, the common use of laboratory animals as surrogates for humans in evaluating the toxicity of chemicals assumes implicitly that interspecies differences in metabolism are minimal or nonexistent. This assumption, as applied to HFC-134a and related haloalkane CFC substitutes, has not been tested.

Acute and repetitive exposures to HFC-134a produce little adverse effect in rats, rabbits, and dogs (1) and, consistent with these observations, studies using animal tissue homogenates and other *in vitro* preparations have generally shown low rates of HFC-134a metabolism (2–4). The metabolism of HFC-134a is catalyzed by cytochrome P-450 in an oxygen-dependent reaction, with F^- and TFA evolved as the primary products (3, 4). This is also presumed to be the case for humans based on *in vitro* evidence, although the rate of hepatic HFC-134a oxidation in tissues of human origin appears to be less than that in rats (5). However, the toxicity of certain gaseous anesthetics (*e.g.* methoxyflurane and fluroxene) and solvent hydrocarbons (*e.g.* HCFC-132b and 1,1,1,2-tetrachloroethane) related to HFC-134a is known to result from metabolism yielding F^-, TFA, and TFE (6–12). Therefore, circumstances that may facilitate accelerated metabolism of HFC-134a in humans are of toxicological interest.

Cytochrome P-450, an enzyme existing in multiple closely related forms, catalyzes the oxidative metabolism of a wide variety of both natural and man-made chemicals (13). Numerous forms of P-450 have been identified and isolated, and many of these enzymes are known to be relatively specific for the metabolism of certain chemicals. For example, P-450 2E1[2] is known to be prominently involved in the metabolism of HFC-134a in rats and rabbits (4). It is also known that exposure of rats to a variety of compounds, including ethanol, acetone, isopropanol, pyridine, trichloroethylene, and isoniazid, greatly increases hepatic expression of P-450 2E1 (14–19). Rates of HFC-134a metabolism *in vitro* are substantially elevated in microsomes from pyridine-treated rats and rabbits (4). This observation as-

[1] Abbreviations used are: HFC-134a, 1,1,1,2-tetrafluoroethane; CFC-12, difluorodichloromethane; CFC, chlorofluorocarbon; P-450, cytochrome P-450; F^-, fluoride ion; TFA, trifluoroacetic acid; HCFC-132b, 1,1-difluoro-1,2-dichloroethane; HCFC-133a, 1,1,1-trifluoro-2-chloroethane; HCFC-124, 1,1,1,2-tetrafluoro-2-chloroethane; TFE, trifluoroethanol; P-450 2E1, cytochrome P-450 2E1; GMRES, General Motors Research and Environmental Staff; TISAB, total ionic strength adjusting buffer, PNP, *p*-nitrophenol; 4-NC, 4-nitrocatechol (1,2-dihydroxy-4-nitrobenzene); DEDTC, diethyldithiocarbamate.

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[2] The nomenclature used to identify cytochromes P-450 in this study is that recommended in ref. 13.

sumes additional importance, considering that human liver also expresses an inducible P-450 2E1 ortholog (17). Humans with a history of chronic alcoholism and individuals undergoing pharmacotherapy with isoniazid may have increased levels of P-450 2E1 (17). However, the role of human P-450 2E1 *vs.* other P-450 isoforms in metabolism of HFC-134a, and other tetrahaloethanes, is unknown. In this study, we present an assessment of the contribution of P-450 2E1 to the overall rate of human microsomal HFC-134a metabolism.

Materials and Methods

Chemical. HFC-134a (Genetron® 134a, lot 335, Allied Chemical, Genetron Division, Morristown, NJ) was supplied as a biphasic mixture (liquid/gas). The gas-phase material was determined to be 97% pure by GC/MS; 1,1,2,2-tetrafluoro-1,2-dichloroethane (0.9%), 1,1,1-TFE (0.8%), and air (0.9%) were the major impurities identified (20). All other chemicals and reagents were supplied by Sigma Chemical Co. (St. Louis, MO), except as specified.

Laboratory Animals. The livers of adult (225–275 g body weight) male Fischer 344 rats [CDF^R (F-344)Crl BR, VAF/Plus®, Charles River Laboratories, Kingston, NY] were used to prepare microsomes. Rats were housed in the vivarium of the Biomedical Science Department, GMRES, in conditions conforming to AAALAC recommendations.[3] Purina 5012 rat chow (Ralston Purina Co., St. Louis, MO) and filtered tap water were available for consumption *ad libitum*. Pyridine-treated rats (100 mg/kg body weight) received an ip injection of an aqueous solution of pyridine (10%, v/v) once daily for four consecutive days.

Human Liver Specimens. Human liver tissues from subjects 1–10 were obtained from the human tissue repository program administered by Stanford Research Institute (Menlo Park, CA). Liver specimens from donor organs (subject age and additional information in ref. 5) were stored at −135°C, shipped frozen, and maintained at −70°C until homogenized for microsome preparation. Other human liver tissue specimens (subjects 11 and 12) were obtained from Dr. F. P. Guengerich (Vanderbilt University, Nashville, TN). Tissues from the latter two subjects were selected based on elevated expression of P-450 2E1 relative to other samples collected by Dr. Guengerich (personal communication). All human tissues were screened for hepatitis B and human immunodeficiency viruses (HIV), and only those specimens that were uniformly negative for the presence of these agents were used in our work. All tissues were initially obtained by institutionally approved procedures, and a research proposal describing this project was reviewed by GMRES' institutional Human Research Committee.

Microsome Preparation. Rat liver microsomes were prepared as described by Lake (21) after brief *in situ* liver perfusion with cold 0.154 M KCl:0.05 M Tris-HCl (pH 7.4). For each preparation, the entire livers of three rats were combined. Human liver microsomes were prepared by the same method, with the exception that portions of liver (*ca.* 5–20 g) rather than entire organs were used. Human liver tissues were not pooled, and the resultant metabolism data are expressed for each individual organ donor. Protein content of the microsomal suspensions was determined by the bicinchonic acid method (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as the standard. Aliquots of the microsomal preparations in 0.154 M KCl:0.05 M Tris-HCl (pH 7.4) were quick-frozen in liquid N_2 and stored at −70°C.

[3] The Research Biomedical Laboratory of GMRES is accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). The rationale and experimental protocol for use of an animal model in this project have been reviewed by GMRES' institutional Animal Research Committee. This research follows procedures outlined in the "Guide for Care and Use of Laboratory Animals." U.S. Department of Health and Human Services, the Public Health Service, the National Institutes of Health (NIH Publication No. 85-23, Rev. 1985), and "Policy on Humane Care and Use of Laboratory Animals by Awardee Institutions" (NIH Guide for Grants and Contracts, vol. 14, no. 8, 1985), and complies with provisions of the Animal Welfare Act of 1966 (P.L. 89-544) as amended in 1970 (P.L. 91-579) and 1976 (P.L. 94-270), and the Food Security Act of 1985 (P.L. 99-158).

Electrophoresis of Microsomal Proteins. SDS-PAGE of SDS-solubilized hepatic proteins was performed as described by Laemmli (22), with 2.5 and 7.5% acrylamide stacking and resolving zones, respectively. Separation was achieved at 50 mA constant current.

Western Blot Analysis of P-450 2E1. Following SDS-PAGE, proteins were electrophoretically transferred from unfixed gels to nitrocellulose membranes using a semidry transfer apparatus (Bio-Rad, Rockville Centre, NY). The membranes were blocked against nonspecific antibody binding, and immunospecific detection of P-450 2E1 was accomplished using a polyclonal rabbit antirat liver P-450 2E1 IgG (OXYgene Dallas, Dallas, TX). Binding was visualized using biotinylated goat antirabbit IgG and an avidin–horseradish peroxidase complex with 4-chloro-1-napthol as chromophore (23). Quantitation of antibody binding to P-450 2E1 in immunoblots of microsomal proteins was accomplished by scanning blots with a soft laser densitometer (SLR-504-XL, Biomed Instruments, Inc., Fullerton, CA). The peak heights obtained from scans of immunoblots of microsomes were expressed on a relative scale in which the value for subject 9 (least intense blot) was set as 1. Preliminary experiments, in which multiple dilutions of human microsomes were immunoblotted for P-450 2E1, showed that protein loadings of 20 μg per lane produced immunoblots for which optical density was reproducible and in the linear range.

Hepatic Microsomal Biotransformation of HFC-134a. Microsomal incubations were conducted in 13 × 100 mm screw-top tubes with silicon-teflon septa containing 1 mg microsomal protein suspended in 1 ml 0.1 M Tris-HCl buffer (pH 7.6) with 10 μmol glucose-6-phosphate, 1 μmol NADP, and 1 unit glucose-6-phosphate dehydrogenase. After addition of the reaction components, the tubes were sealed and held on ice until incubation commenced. A 15 min preincubation in the absence of HFC-134a was conducted at 37°C and 160 oscillations/min in a rack slanted at 22° from horizontal (to increase the gas-liquid interface area). All tubes were immediately placed on ice. The head-space of each tube was then purged for at least 45 sec with a gas mixture containing the desired concentration of HFC-134a. This mixture was introduced at a rate of 0.8–1.0 liter/min *via* a syringe needle passed through the septum while a separate needle provided pressure relief. Atmospheres containing HFC-134a (balance of gas mixture 21% O_2 and variable amounts of N_2) were prepared by simultaneously mixing the halocarbon and diluting gases with multiple gas-flow controllers (Dwyer Instruments, Michigan City, IN) that were calibrated with a bubble chamber flow-cell (Gilian Instruments, Wayne, NJ). The microsomal preparations were then incubated at 37°C and 160 oscillations/min for 15 min. Tubes were then transferred to a 60°C water bath for 5 min, chilled for 10 min on ice, uncapped, and the entire contents transferred to conical bottom centrifuge tubes. Following 10-min centrifugation at 1750*g*, liquid supernatants were used for F^- determinations as described later.

Preliminary experiments verified that, under the conditions described for microsomal incubations, catalytic activity was linear as either the duration of incubation or microsomal protein concentration was altered.

Potentiometric Detection of F^-. The supernatants from microsomal incubations were combined with an equal volume of TISAB (1 M acetic acid, 1 M sodium chloride, and 0.012 M *trans*-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid in deionized water, pH 5.5) (24). While constantly stirred, samples were analyzed with a F^- ion activity electrode (Orion Research, Cambridge, MA) and an Orion 940 ion analyzer. Values for each sample were read only after a 5–10 min equilibration period. The electrode was calibrated immediately before use with solutions of known F^- content; blanks used to establish the zero potential were 0.1 M Tris-HCl (pH 7.6) mixed 1:1 with TISAB. The slopes of the resulting calibration curves averaged about −59 mV/10-fold change in F^- concentration. Rates of HFC-134a defluorination were corrected for F^- found in method blanks (complete incubations with 0% HFC-134a).

Microsomal Metabolism of PNP. PNP metabolism was assessed in reaction mixtures prepared and incubated as already described (see *Hepatic Microsomal Biotransformation of HFC-134a*) and containing 5–750 μM PNP. At the conclusion of an incubation, sufficient 0.6 M perchloric acid was added to each tube to achieve a final concentration of 0.2 M perchloric acid. Tubes were transferred to ice, held for 15 min,

and the contents were centrifuged for 10 min at 1750*g*. The liquid supernatants were analyzed for 4-NC as described by Koop *et al.* (25). Metabolite concentrations were determined by reference to absorbance calibration curves prepared with authentic 4-NC. Under the conditions described for microsomal incubations, formation of 4-NC was linear during the entire course of the incubation (data not shown).

DEDTC Inhibition of Microsomal Metabolism. In some experiments, HFC-134a or PNP metabolism by human microsomes was assessed in the presence of DEDTC that was added to the reaction components just before a 15-min preincubation period. DEDTC concentrations that resulted in 90% inhibition of catalytic activity were determined in incubations conducted with liver microsomes from control and pyridine-treated rats (see *Laboratory Animals*). DEDTC (300 μM) inhibited the rate of PNP metabolism by 90%. DEDTC (180 μM) was used in assays of HFC-134a biotransformation. All reactions proceeded as previously described.

Inhibition of HFC-134a Defluorination by PNP in Human Hepatic Microsomes. Reactions were conducted using microsomes from subject 11. HFC-134a (7.3%) was added to one series of incubation tubes with varying PNP content (5–750 μM), while the tubes were held on ice. In another series, each tube received 45.1% HFC-134a (as described in *Hepatic Microsomal Biotransformation of HFC-134a*). All mixtures were then incubated at 37°C and 160 oscillations/min for 15 min. Reactions were terminated, and F^- was quantitated as previously described.

Results

Substrate-Saturation Kinetics for HFC-134a Defluorination by Human Microsomes. Rat hepatic microsomes produce F^- *via* oxidative, but not reductive, P-450-catalyzed biotransformation, and this metabolite serves as an index of HFC-134a biotransformation (3). Previously, we reported that the rate of defluorination of HFC-134a by hepatic microsomes was significantly increased following treatment of rats with pyridine, an inducer of P-450 2E1 (4, 19). In assessing HFC-134a metabolism by human microsomes, it was of interest that 1 subject (no. 11) showed a marked increase in the relative capacity to defluorinate this hydrohalocarbon in the range of concentrations tested (fig. 1*A*). At saturating concentrations of HFC-134a (*i.e.* 45.1% HFC-134a), rates of HFC-134a metabolism by subject 11, and also subject 12 for which complete substrate saturation kinetics were not evaluated, were approximately 5- to 10-fold greater than those of all other subjects (table 1). Similarly, rates of hydroxylation of PNP, a known high-affinity substrate of P-450 2E1 (19, 25), by microsomes from subject 11 were increased over the entire range of substrate concentrations (fig. 1*B*). Maximal rates of PNP hydroxylation by subjects 11 and 12 varied from 3- to 15-fold greater than that observed for all other individuals (table 1). The calculated apparent maximal rates of metabolism (V_{max} normalized for microsomal protein content) for subject 11 were 13.8-fold and 6.5-fold greater than the average V_{max} for defluorination and hydroxylation, respectively, obtained from other subjects (fig. 1 legend). Expressed as a function of cytochrome P-450 content, V_{max} for defluorination and hydroxylation by subject 11 was 9.9-fold and 4.4-fold greater, respectively, than the average for other subjects.

Characterization of DEDTC as an Inhibitor of P-450 2E1. Microsomes from pyridine-treated rats were determined to be high in P-450 2E1 content not only by their increased capacity to hydroxylate PNP (table 2), but also by immunochemical staining of Western blots (4, 19). Almost 88% of the PNP hydroxylase activity of pyridine-induced rat liver microsomes was lost after 15 min preincubation with 300 μM DEDTC, while addition of 180 μM DEDTC abolished nearly all of the capacity to defluorinate HFC-134a (table 2, fig. 2). At the same DEDTC concentrations, PNP hydroxylation and HFC-134a defluorination were 61 and 100% inhibited, respectively, in microsomes from untreated rats (table 2). Varying the duration of the preincubation with DEDTC significantly affected the resulting degree of inhibition; at least 15 min of preincubation was required to reduce rates of metabolism (PNP or HFC-134a) maximally (not shown).

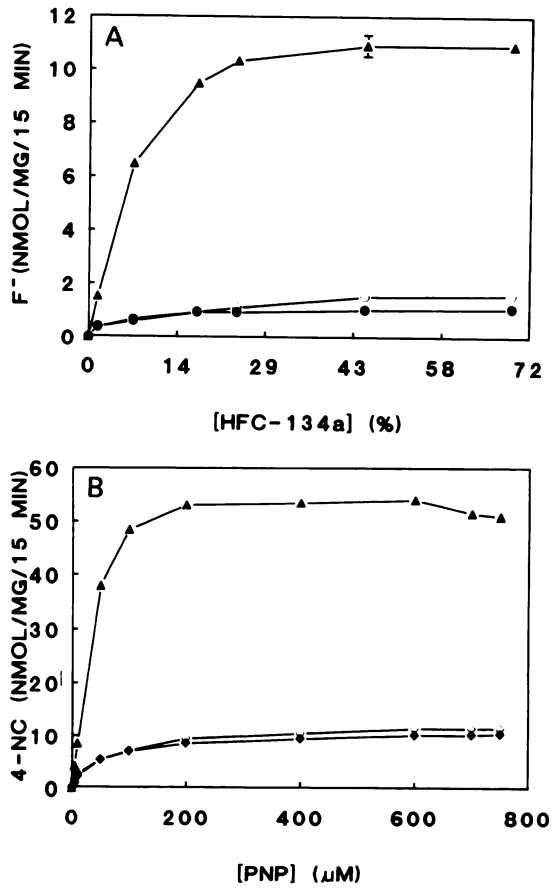

FIG. 1. *Substrate-saturation kinetics of HFC-134a defluorination (A) or PNP hydroxylation (B) by human hepatic microsomes.*

Data are from microsomes of five individuals (subjects 3, ◆; 6, ●; 8, ◇; 10, O; and 11, ▲) and represent means ± SD of triplicate determinations. The apparent V_{max} for defluorination by subject 11 and the average of subjects 6 and 10 is 16.6 ± 0.2 and 1.1 ± 0.1 nmol F^-/mg protein/15 min, respectively, or 60.3 ± 0.3 and 6.1 ± 0.4 nmol F^-/nmol P450/15 min. V_{max} for hydroxylation by subject 11 and subjects 3 and 8 combined is 74.3 ± 2.0 and 11.4 ± 0.4 nmol 4-NC/mg protein/15 min, respectively, or 270.1 ± 6.6 and 61.4 ± 2.5 nmol 4-NC/nmol P450/15 min.

These findings agree quantitatively with published results regarding the efficacy of DEDTC as a specific inhibitor of P-450 2E1-catalyzed functions in human liver microsomes (26). In addition, the failure of a relatively low concentration of DEDTC to completely inhibit PNP hydroxylation in microsomes from control or pyridine-induced rat liver is consistent with the minor involvement of P-450s other than 2E1 in this metabolic transformation (25).

Inhibition of HFC-134a Defluorination by DEDTC in Human Hepatic Microsomes. Of hepatic microsomal samples from 11 humans, the capacity to hydroxylate PNP in 10 subjects was inhibited between 51 and 81% (mean inhibition of 63%) following a 15-min preincubation with 300 μM DEDTC (table 1). However, PNP hydroxylation by microsomes of subject 11 was

TABLE 1

Metabolism of HFC-134a and PNP by human hepatic microsomes

Subject	Rate of Product Formation (nmol/mg protein/15 min)			
	F^-[a]		4-NC[b]	
	−DEDTC	+DEDTC[c]	−DEDTC	+DEDTC[d]
1	1.1 ± 0.0	0.0	8.1 ± 0.1	3.4 ± 0.1
2	1.1 ± 0.1	0.0	10.1 ± 0.3	5.3 ± 0.1
3	1.3 ± 0.1	0.0	9.6 ± 0.3	3.1 ± 0.2
4	1.8 ± 0.0	0.0	16.4 ± 0.3	6.4 ± 0.1
5	1.5 ± 0.1	0.0	13.8 ± 0.3	5.9 ± 0.0
6	1.1 ± 0.0	0.0	8.8 ± 0.1	4.3 ± 0.3
7	2.1 ± 0.1	0.0	12.0 ± 0.1	2.3 ± 0.2
8	1.1 ± 0.1	0.0	9.2 ± 0.1	2.9 ± 0.1
9	0.1 ± 0.0	0.0	3.8 ± 0.1	1.1 ± 0.0
10	1.1 ± 0.1	0.0	7.6 ± 0.2	2.5 ± 0.1
11	10.5 ± 0.2	0.0	55.3 ± 1.5	4.9 ± 0.1
12	9.5 ± 1.3	0.1	37.8 ± 0.4	ND[e]

Values are means ± SD of three determinations. Assay conditions are as described in *Materials and Methods.*

[a] From HFC-134a (45.1% gas-phase HFC, 21% O_2, balance N_2), corrected for F^- in incubations with 0% HFC-134a.

[b] From 600 μM PNP.

[c] 180 μM DEDTC included during preincubation as described in *Materials and Methods.*

[d] 300 μM DEDTC included during preincubation.

[e] ND, not determined.

TABLE 2

Effect of DEDTC on metabolism of HFC-134a and PNP by rat hepatic microsomes

Treatment	Rate of Product Formation (nmol/mg protein/15 min)			
	F^-[a]		4-NC[b]	
	−DEDTC	+DEDTC[c]	−DEDTC	+DEDTC[d]
None	2.6 ± 0.0	0.0	9.2 ± 0.4	3.6 ± 0.3
Pyridine	21.0 ± 2.0	0.6 ± 0.0	63.7 ± 1.0	8.8 ± 0.1

Values are means ± SD of three determinations. Assay conditions and treatment are as described in *Materials and Methods.*

[a] From HFC-134a (45.1% gas-phase HFC, 21% O_2, balance N_2), corrected for F^- in incubations with 0% HFC-134a.

[b] From 600 μM PNP.

[c] 180 μM DEDTC included during preincubation as described in *Materials and Methods.*

[d] 300 μM DEDTC included during preincubation.

found to be 91% inhibited under the same conditions. These results duplicate almost exactly those obtained for pyridine-treated rats and untreated rats (*vide supra*). Defluorination of HFC-134a was entirely abolished by DEDTC, a P-450 2E1-specific inhibitor (26, 27), in human hepatic microsomes regardless of the level of P-450 2E1 expression (table 1).

Effect of PNP on HFC-134a Defluorination in Human Liver Microsomes. In an earlier publication, we reported that substrates that are preferentially metabolized by P-450 2E1 (*e.g.* PNP and aniline) decrease the rate of rat liver microsomal HFC-134a defluorination in a concentration-dependent manner (4). Coincubation of various PNP concentrations and the HFC with liver microsomes from subject 11 revealed that PNP is equally effective in decreasing the rate of HFC-134a defluorination by human microsomes (fig. 3). Plotting the inverse of the rate of HFC-134a defluorination *vs.* PNP concentration (*i.e.* Dixon plot) suggests that (as in the case of pyridine-induced rat microsomes), HFC-134a metabolism by P-450 2E1-enhanced human microsomes is competitively inhibited by PNP. Assuming this to be

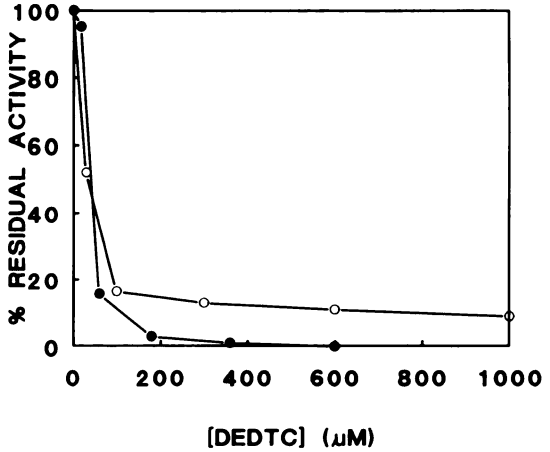

FIG. 2. *Inhibition of HFC-134a defluorination (●) and PNP hydroxylation (○) by DEDTC in pyridine-induced rat liver microsome.*

Data are expressed as means ± SD of triplicate determinations. 100% activity corresponds to 21.0 and 63.7 nmol product/mg protein/15 min for HFC-134a defluorination and PNP hydroxylation, respectively.

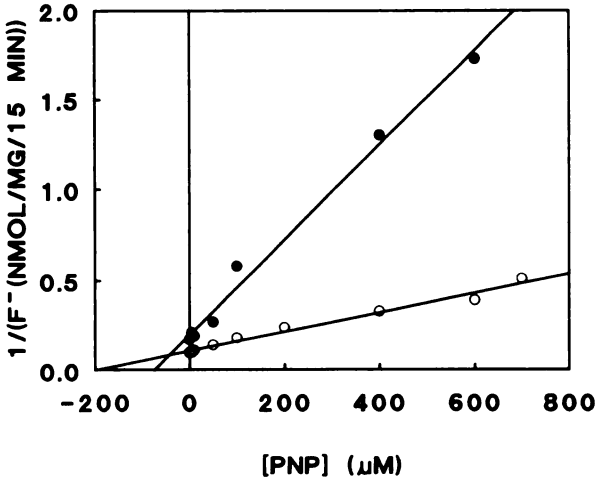

FIG. 3. *Inhibition of human liver microsomal HFC-134a defluorination by PN.*

Reciprocal rates of HFC-134a (7.3%, ●; 45.1%, ○) defluorination [(nmol/mg protein/15 min)$^{-1}$] are plotted against PNP concentration (Dixon plot). The results suggest competitive inhibition by PNP (calculated K_i: 49 μM PNP).

the case, the calculated K_i for competitive inhibition of HFC-134a defluorination by PNP is 49 μM PNP. PNP has previously been shown to inhibit competitively rat hepatic microsomal HFC-134a metabolism with a very similar K_i *(36 μM) (4).*

Correlation of Rates of HFC-134a Defluorination with P-450 2E1 Content in Human Hepatic Microsomes. A rabbit antirat P-450 2E1 IgG showed immunochemical reactivity against a single band (M_r *ca.* 52 kDa) of SDS-PAGE-separated human liver microsomal proteins in samples 1–11 (fig. 4). This is consistent with the range of molecular weights (51–54 kDa) reported for P-450 2E1 from multiple species, including humans (15–17). Densitometric quantitation of the blots revealed that microsomes of subject 11 contained comparatively more (almost 2- to 14-fold) P-450 2E1 than the other 10 subjects probed in this manner. Linear regression analysis of the relationship between HFC-134a defluorination and the relative amount of P-450 2E1 for subjects 1–11 yielded a correlation coefficient (r) of 0.90 (fig. 5*A*). Similar assessment of microsomal P-450 2E1 content and rates of PNP

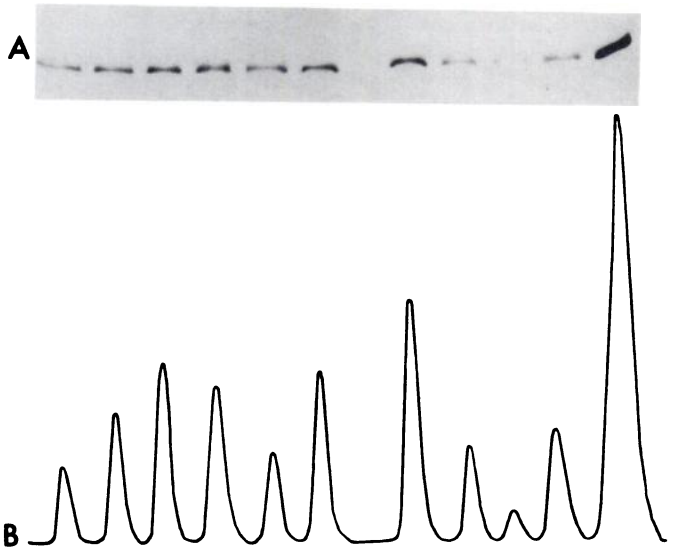

FIG. 4. *Immunostaining (Western blotting) of human liver microsomal proteins with a rabbit antirat P-450 2E1 antibody (A).*

All lanes contain 20 μg of microsomal protein and range from subject 1 (*left*) to subject 11 (*right*). Results from densitometric scan of the immunoblot (*B*).

hydroxylation produced a correlation coefficient of 0.89 (not shown). Conversely, plotting of the residual catalytic activity in the presence of DEDTC *vs.* relative P-450 2E1 content resulted in a correlation coefficient of 0 for HFC-134a defluorination (data not shown). A plot of rates of PNP hydroxylation *vs.* HFC-134a defluorination by human hepatic microsomes (subjects 1–12) yielded an *r* value of 0.98 for correlation of these two catalytic functions (fig. 5*B*).

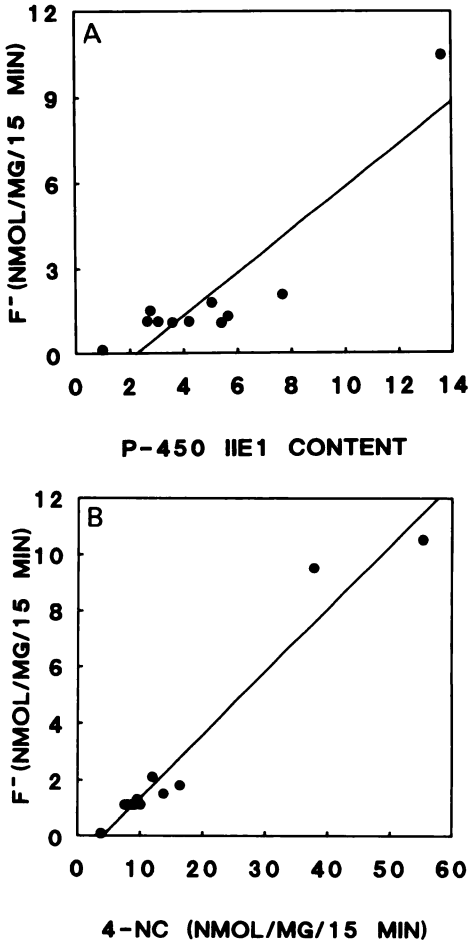

FIG. 5. *Correlation of P-450 2E1 content with HFC-134a defluorination [(A) subjects 1–11] and correlation of metabolism of HFC-134a with that of PNP [(B) subjects 1–12] in human liver microsomes.*

Determination of P-450 2E1 content is as described in fig. 4 and *Materials and Methods.* All data points represent means ± SD of triplicate determinations for each enzymatic activity.

Discussion

Certain provisions of the Clean Air Act Amendments of 1990 (28) were devised primarily to help ameliorate the depletion of stratospheric ozone attributed to continued elevation of atmospheric levels of CFCs (29). In compliance with this mandate, which bans CFC-12 production by the year 2000 (28), HFC-134a has been selected as an alternate to CFC-12 for use in automotive air-conditioning systems and as a propellant for pharmaceutical aerosols. It is therefore necessary to gain an in-depth appreciation for any possible adverse implications for human health associated with use of this chemical.

A priori consideration of the nature of the halogen substituents of HFC-134a suggests that this chemical will be metabolized to only a limited extent. This hypothesis is supported by the low rates of microsomal defluorination reported in this publication, as well as similar data derived from hepatic microsomes of rat and rabbit (3–5) and intact rat hepatocytes (2). Furthermore, the high vapor pressure and very low coefficients for HFC-134a partitioning into water and lipids (30) suggest that absorption and retention, and thus probable toxicity, of this halocarbon *in vivo* will be quite limited. However, no comparative data describing the rates of metabolism of HFC-134a in relation to other congeners in the series of tetrahaloethanes exist. This fact, coupled with the knowledge that metabolism of certain tetrahaloethanes similar to HFC-134a (*e.g.* HCFC-132b and HCFC-133a) has been implicated in the toxicity of these agents (1, 8–11) raises some concern whether exposure of humans to agents that elevate HFC-134a metabolism might render certain individuals susceptible to toxicity from exposure to high concentrations of this chemical. The notion that conditions might exist in which metabolism of HFC-134a is elevated is based on an understanding of the role of cytochrome P-450, and especially P-450 2E1, in hydroxylation (and subsequent defluorination) of HFC-134a in hepatic tissue of rats and rabbits (4).

Cytochrome P-450 exists in multiple forms in liver tissue. P-450 2E1 is important because: *1*) it is expressed in human liver; *2*) its catalytic activity in humans may be increased by exposure to certain chemicals, or by conditions such as fasting or diabetes; and *3*) it is primarily responsible for HFC-134a metabolism in animals used as surrogates for humans in toxicology testing. Thus, we sought to determine the isozyme specificity of HFC-134a defluorination in human liver, a phenomenon recently attributed to cytochromes P-450 (5). The results of experiments described here strongly support the conclusion that cytochrome P-450 2E1 of human liver is a predominant catalyst of the metabolism of HFC-134a. This finding is concordant with our earlier observations that oxidation of HFC-134a by microsomes of rat or rabbit liver is largely a function of P-450 2E1 (4). Thus, our findings augment the general notion that the functions and

substrate specificities of P-450 2E1 are similar from species to species.

Analysis of substrate saturation kinetics in microsome preparations from subjects 6, 9, and 11 reveals that microsomes from these individuals produced F^- from HFC-134a at substantially different maximal rates, yet the calculated K_M values were similar (*ca.* 9% gas phase HFC-134a). Thus, the oxidation of HFC-134a is apparently monophasic, which argues against the involvement of P-450s with higher K_M's for HFC-134a. This conclusion is further supported by results from experiments in which P-450 2E1 was presumably selectively inhibited by inclusion of DEDTC. Low concentrations of DEDTC and other organosulfur compounds, such as diallyl sulfide and phenethyl isothiocyanate, have been shown to irreversibly inhibit the catalytic activity of both rat and human P-450 2E1 toward a variety of substrates, both *in vitro* and *in vivo* (26, 27, 31, 32). Such effects are probably attributable to the NADPH-dependent DEDTC-mediated conversion of P-450 to P-420 (33). The efficiency of DEDTC as an inhibitor of HFC-134a in human hepatic microsomes suggests further that P-450s other than 2E1, but with K_M values for HFC-134a similar to P-450 2E1, are unlikely to be important in metabolism of this halocarbon. Further, it is known that the microsomal oxidative metabolism of HFC-134a, as well as that of the related pentahaloethane HCFC-124, is not induced by phenobarbital or aroclor 1254, except for a slight increase in the rate of metabolism at very high (≥25% gas-phase HFC) substrate concentrations (3, 34). On the other hand, pyridine administration to rats and rabbits, which greatly increases the expression of P-450 2E1 (19), results in a pronounced increase in microsomal F^- production from even low concentrations of HFC-134a (4).

In addition to utility of the foregoing information with regard to understanding HFC-134a metabolism in humans, we propose that cytochrome P-450 2E1 is prominently involved in the initial oxidation of other tetrahaloethanes, some of which, unlike HFC-134a, have previously been demonstrated to have significant toxicity (8, 10). This speculation has been substantiated in the case of HCFC-132b (9). In addition, the oxidative metabolism of several 1,1,1-trifluoro-2,2-dihaloethanes, which are metabolized to biologically reactive intermediates, has been shown to be dependent on the level of expression of cytochrome P-450 2E1 (34, 35). Thus, careful evaluation of the rates of metabolism of haloethanes (and presumably halopropanes) with proposed uses as substitutes for CFCs is warranted. Of equal importance is the conclusion that assessment of toxicity of such materials should be made under circumstances including elevated levels of cytochrome P-450 2E1, because it is not possible at present to predict what proportion of the human population expresses higher than average levels of P-450 2E1.

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