Anesthesiology
79:795–807, 1993

J. B. Lippincott Company, Philadelphia

Identification of Cytochrome P450 2E1 as the Predominant Enzyme Catalyzing Human Liver Microsomal Defluorination of Sevoflurane, Isoflurane, and Methoxyflurane

Evan D. Kharasch, M.D., Ph.D., Kenneth E. Thummel, Ph.D.†*

Background: **Renal and hepatic toxicity of the fluorinated ether volatile anesthetics is caused by biotransformation to toxic metabolites. Metabolism also contributes significantly to the elimination pharmacokinetics of some volatile agents. Although innumerable studies have explored anesthetic metabolism in animals, there is little information on human volatile anesthetic metabolism with respect to comparative rates or the identity of the enzymes responsible for defluorination. The first purpose of this investigation was to compare the metabolism of the fluorinated ether anesthetics by human liver microsomes. The second purpose was to test the hypothesis that cytochrome P450 2E1 is the specific P450 isoform responsible for volatile anesthetic defluorination in humans.**

Methods: **Microsomes were prepared from human livers. Anesthetic metabolism in microsomal incubations was measured by fluoride production. The strategy for evaluating the role of P450 2E1 in anesthetic defluorination involved three approaches: for a series of 12 human livers, correlation of microsomal defluorination rate with microsomal P450 2E1 content (measured by Western blot analysis), correlation of defluorination rate with microsomal P450 2E1 catalytic activity using marker substrates (para-nitrophenol hydroxylation and chlorzoxazone 6-hydroxylation), and chemical inhibition by P450 isoform-selective inhibitors.**

Results: **The rank order of anesthetic metabolism, assessed by fluoride production at saturating substrate concentrations, was methoxyflurane > sevoflurane > enflurane > isoflurane > desflurane > 0. There was a significant linear correlation of sevoflurane and methoxyflurane defluorination with antigenic P450 2E1 content ($r = 0.98$ and $r = 0.72$, respectively), but not with either P450 1A2 or P450 3A3/4. Comparison of anesthetic defluorination with either para-nitrophenol or chlorzoxazone hydroxylation showed a significant correlation for sevoflurane ($r = 0.93$, $r = 0.95$) and methoxyflurane ($r = 0.78$, $r = 0.66$). Sevoflurane defluorination was also highly correlated with that of enflurane ($r = 0.93$), which is known to be metabolized by human P450 2E1. Diethyldithiocarbamate, a selective inhibitor of P450 2E1, produced a concentration-dependent inhibition of sevoflurane, methoxyflurane, and isoflurane defluorination. No other isoform-selective inhibitor diminished the defluorination of sevoflurane, whereas methoxyflurane defluorination was inhibited by the selective P450 inhibitors furafylline (P450 1A2), sulfaphenazole (P450 2C9/10), and quinidine (P450 2D6) but to a much lesser extent than by diethyldithiocarbamate.**

Conclusions: **These results demonstrate that cytochrome P450 2E1 is the principal, if not sole human liver microsomal enzyme catalyzing the defluorination of sevoflurane. P450 2E1 is the principal, but not exclusive enzyme responsible for the metabolism of methoxyflurane, which also appears to be catalyzed by P450s 1A2, 2C9/10, and 2D6. The data also suggest that P450 2E1 is responsible for a significant fraction of isoflurane metabolism. Identification of P450 2E1 as the major anesthetic metabolizing enzyme in humans provides a mechanistic understanding of clinical fluorinated ether anesthetic metabolism and toxicity. (Key words: Anesthetics, volatile: desflurane; enflurane; isoflurane; methoxyflurane; sevoflurane. Biotransformation: fluorometabolites. Enzyme: cytochrome P450; cytochrome P450 2E1; CYP2E1. Ions: fluoride. Interactions: drug. Metabolism: drug.)**

BIOTRANSFORMATION of the fluorinated ether volatile anesthetics results in the production of fluoride metabolites, capable of producing hepatic or renal toxicity. Inorganic fluoride ions, liberated during the metabolism of certain agents, may cause subclinical nephrotoxicity or overt renal insufficiency at excessive concentrations.[1] Excessive inorganic fluoride ion production and nephrotoxicity have limited the clinical use of methoxyflurane,[1] have been the subject of considerable scrutiny in the development of sevoflurane,[2–4] and are of concern in the development of any

* Associate Professor of Anesthesiology; Adjunct Associate Professor of Medicinal Chemistry.

† Assistant Professor of Pharmaceutics.

Received from the Departments of Anesthesiology, Medicinal Chemistry, and Pharmaceutics, University of Washington, Seattle, Washington. Accepted for publication June 15, 1993. Supported by NIH R01 GM48712, Maruishi Pharmaceutical Co., a Faculty Development Award in Clinical Pharmacology from the Pharmaceutical Manufacturers Association (E.D.K.), and NIH R29 GM48394 (K.E.T.). Presented in part at the annual meeting of the American Society of Anesthesiologists, San Francisco, California, October 26–30, 1991.

Address reprint requests to Dr. Kharasch: Department of Anesthesiology, RN-10, University of Washington, Seattle, Washington 98195.

new fluorinated anesthetic. Organic fluoride metabolites of methoxyflurane, enflurane, and isoflurane are capable of binding to liver proteins and triggering an idiosyncratic hepatic necrosis similar to that caused by halothane.[5,6]

Despite the significant role of metabolism in volatile anesthetic toxicity, as well as in the total body clearance of certain volatile agents,[7] quantitative aspects of hepatic anesthetic metabolism in humans are incompletely understood. Metabolism of halothane, enflurane, isoflurane, desflurane, and sevoflurane in humans has been estimated indirectly, either by recovery of urinary metabolites[2,8-11] or by mass balance studies.[7,12,13] Estimates of metabolism provided by these two techniques are frequently different and sometimes controversial.[14,15] Although there are innumerable animal studies measuring hepatic anesthetic metabolism directly using *in vitro* assays, there are no data comparing the metabolism of volatile anesthetics by human tissues. Thus, the first purpose of this investigation was to determine the rates of fluorinated ether anesthetic metabolism catalyzed by human liver microsomes. We then tested the hypothesis that the rates of hepatic anesthetic metabolism (estimated from the rates measured *in vitro*) predict clinical peak plasma fluoride concentrations *in vivo*.

Volatile anesthetic metabolism is catalyzed by microsomal cytochrome P450, actually a family of enzyme isoforms with discreet but overlapping substrate specificities. To date, approximately 14 human hepatic cytochrome P450 isoforms have been identified.[16] Some of these isoforms have animal orthologs with similar substrate specificities, while other animal forms may have no identifiable human equivalent. Animal studies have demonstrated the involvement of ethanol- and isoniazid-inducible cytochrome P450 2E1‡ in the metabolism of enflurane, methoxyflurane, and sevoflurane.[18,19] We recently reported the involvement of P450 2E1 in the metabolism of enflurane by human liver microsomes.[20] The second purpose of this investigation, therefore, was to test the hypothesis that cytochrome P450 2E1 is also responsible for the defluorination of methoxyflurane, sevoflurane, and isoflurane in humans.

‡ The cytochrome P450 isozymes are named here in accordance with accepted convention.[17] Cytochrome P450 2E1 previously was known as human $P450_j$. The orthologous P450 2E1 enzyme in rats and rabbits previously was designated $P450_j$ and $P450_{LM3a}$, respectively.

Materials and Methods

Chemicals

Sevoflurane was supplied by Maruishi Pharmaceutical Co. (Osaka, Japan). Methoxyflurane was the gift of Abbott Laboratories (North Chicago, IL). Enflurane and isoflurane were purchased from Anaquest (Madison, WI). Furafylline and 6-hydroxychlorzoxazone were the gift of Dr. Kent L. Kunze, and sulfaphenazole was kindly provided by Dr. William F. Trager, University of Washington. Unless specified, all other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

Protein Preparation and Immunochemical Techniques

All animal experiments were approved by the Institutional Animal Care and Use Committee. All human tissue experiments were approved by the Institutional Human Subjects Review Committee.

Human livers were obtained from kidney donors and stored at −80° C until used. Tissue was thawed in 10 mM potassium phosphate buffer (*p*H 7.4) containing 0.9% NaCl. Microsomes were prepared by homogenizing the thawed tissue in five volumes of 10 mM potassium phosphate buffer (*p*H 7.4)/10 mM EDTA/1.15% KCl in a Waring blender, followed by five strokes with a glass-Teflon homogenizer. The homogenate was centrifuged at 9,000 g for 25 min, and the supernatant centrifuged at 110,000 g for 70 min. Microsomal protein was carefully separated from the glycogen pellet, resuspended in 100 mM sodium pyrophosphate buffer (*p*H 7.4)/1 mM EDTA, and again centrifuged at 110,000 g for 70 min. The washed pellet was resuspended in either 50 mM potassium phosphate buffer (*p*H 7.4) or Tris HCl buffer (*p*H 7.4) and stored at −80° C. Glycerol, conventionally used in microsome preparation, was omitted from the buffers to prevent interference with cytochrome P450 2E1 activity.[21] Microsomal protein concentrations were determined by the method of Lowry *et al.* using bovine serum albumin as the standard.[22] Total microsomal cytochrome P450 content was determined from the reduced minus oxidized carbon monoxide difference spectrum.[23]

Specific anti-rat cytochrome P450 2E1 IgG antibody was prepared as described previously.[20] Briefly, rat cytochrome P450 2E1 was purified from hepatic microsomes of adult males made diabetic by a single intravenous injection of streptozotocin, according to the method of Favreau *et al.*[24] IgG was purified from the serum of rabbits immunized with the purified rat P450

2E1. The specificity of the purified polyclonal antibody toward human liver P450 2E1 was improved by immunopurification, which removed minor cross-reactivity to a single lower molecular weight band.[20] Antibody specificity for human P450 2E1 was determined by Ouchterlony immunodiffusion analysis and Western blot analysis.

Microsomal P450 2E1 content was determined by Western blot analysis using anti-rat P450 2E1 as the primary antibody. The analysis was conducted as described by Favreau *et al.*[24] Immunopositive protein bands were quantified by scanning densitometry (BioImage, Millipore, Ann Arbor, MI) using purified human P450 2E1 as the reference standard. Microsomal P450 1A2 and 3A3/4 proteins were quantified analogously using immunoselective rabbit antibodies against human cytochrome P450 3A3/4/5 and rabbit P450 1A2, except that relative blot intensities were obtained for P450 1A2 rather than absolute P450 content.[25] Table 1 provides the microsomal content of cytochrome P450 and P450 isoforms in the liver microsomes used.

Table 1. Cytochrome P450 Contents of Microsomes from Human Livers

Liver Code	Specific Content*	P450 Isoform Content		
		P450 1A2†	P450 2E1‡	P450 3A3/4†
HL-101	0.44	18.8	73	88
HL-103	0.45	38.1	49	54
HL-104	0.40	2.7	288	52
HL-105	0.61	30.4	61	81
HL-106	0.42	39.4	157	152
HL-107	0.32	34.5	372	46
HL-108	0.49	7.9	137	53
HL-109	0.33	7.7	182	143
HL-110	0.66	31.7	95	35
HL-111	0.55	18.9	69	33
HL-113	0.49	14.9	55	49
HL-118	0.42	4.2	339	33
HL-119	0.50	16.4	108	115

* Nanomoles total P450 per milligram protein.

† Relative band density per nanomole total P450.

‡ Picomole per nanomole total P450.

Enzyme Assays

Volatile Anesthetics. Anesthetic defluorination was determined in screw-capped polyethylene vials in a reaction mixture (1.0 ml) containing 2–5 mg microsomal protein, 2 mM nicotinamide adenine dinucleotide phosphate (NADPH), and 1 μl anesthetic in 100 mM Tris HCl buffer (*p*H 7.4). Reactions (37° C with reciprocal shaking) were initiated by the addition of anesthetic agent after a 5-min preincubation period and terminated after 30 min by immersion in a boiling water bath for 5 min. Preliminary experiments were conducted to ensure that all anesthetics were present at saturating concentrations (*i.e.* , no additional defluorination with 2 or 3 μl of anesthetic)§ and that fluoride production was linear throughout the 30-min incubation period. Terminated reaction mixtures were centrifuged at 13,000 g for 5 min, an aliquot of the supernatant transferred to a clean polyethylene vial, and 45 μl of high ionic strength acetate buffer (5 M acetic acid, 2.5 M NaOH) added. Fluoride concentrations were measured with an Orion (Boston, MA) fluoride-specific electrode.[26] Standard curves were prepared each day by adding known amounts of sodium fluoride to microsomes and analyzing as described.

Experiments using isoform-selective chemical P450 inhibitors were conducted by replacing NADPH with an NADPH-regenerating system (1 mM NADPH, 10 mM glucose-6-phosphate, 1 IU/ml glucose-6-phosphate dehydrogenase, and 5 mM $MgCl_2$). Inhibitors were present at the following final concentrations: 20 μM furafylline, 0.5 μM sulfaphenazole, 5 μM quinidine, 100 μM (except where indicated in figure legends) diethyldithiocarbamate, and 100 μM troleandomycin. All inhibitors were added in Tris HCl buffer except troleandomycin, which was added in methanol (1 μl of 100-mM stock, final methanol concentration 0.1%). Reaction mixtures containing sulfaphenazole and quinidine were preincubated at 37° C for 5 min without NADPH, and the defluorination reaction was initiated by simultaneous addition of the regenerating system and anesthetic agent (1 μl). Reaction mixtures containing furafylline, diethyldithiocarbamate, and troleandomycin were preincubated at 37° C for 10 min (except where indicated) with the NADPH-regenerating system, and the defluorination reaction was initiated by adding anesthetic agent. The defluorination reactions were terminated after 30 min, and fluoride production was determined as described above. Defluorination rates in the presence of inhibitor were compared with appropriate controls (no preincubation, 10 min preincubation, or 10 min preincubation with 0.1% methanol

§ Anesthetics also were present at saturating concentrations in the aqueous phase based on theoretical calculations. These calculations were confirmed, for example, for isoflurane, which was present at 2.2 mM as determined by gas chromatography.

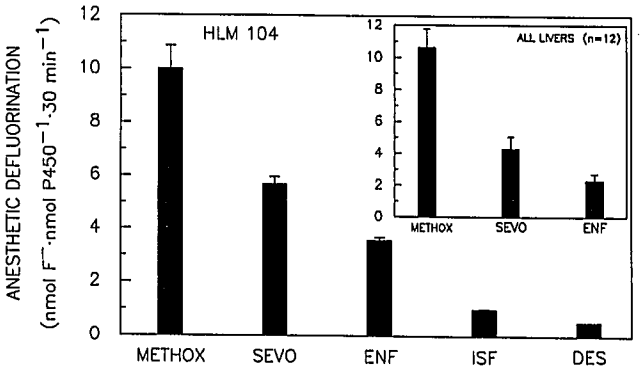

Fig. 1. Human liver microsomal volatile anesthetic defluorination. The main figure shows the defluorination (mean ± SD of triplicate determinations) of methoxyflurane, sevoflurane, enflurane, isoflurane, and desflurane by one human liver (HL-104). Fluoride formation from desflurane was less than the limit of quantitation (0.5 nanomoles), but greater than zero, and thus is shown as the limit of quantitation for this liver. The inset compares the metabolism (mean ± SE) of methoxyflurane, sevoflurane, and enflurane in 12 human livers. Fluoride formation from isoflurane and desflurane was less than the limit of quantitation for several livers, and mean values, therefore, are not shown.

in the absence of inhibitor) and results expressed as a percentage of the uninhibited rate.

Chlorzoxazone. The 6-hydroxylation of chlorzoxazone was measured in a reaction mixture containing 1 mM chlorzoxazone, 0.1 nanomoles microsomal P450, 1 mM NADPH, and 100 mM potassium phosphate buffer (*p*H 7.4) in a final volume of 1.0 ml. Reactions (37° C, 10 min) were terminated with the addition of 5 ml methylene chloride. The internal standard pentoxifylline (100 ng) was added, and extractions were performed in screw-capped culture tubes with shaking for 15 min. After centrifugation, the organic layer was transferred to a clean test tube and evaporated to dryness under a nitrogen stream, and the residue was redissolved in 100 μl mobile phase for high-performance liquid chromatography analysis. The high-performance liquid chromatography system consisted of a Hewlett Packard (Avondale, PA) series 1050 chromatograph with variable-wavelength detector and 30 μl sample loop and an Econosphere reverse-phase C18 column (4.6 × 250 mm, 5 μm) with C18 guard column (Alltech, Deerfield, IL). The mobile phase was 80% (vol/vol) 0.15% acetic acid (*p* H 4.7) and 20% acetonitrile, at a flow rate of 1.0 ml/min. Retention times of 6-hydroxychlorzoxazone and pentoxifylline were 10.2 and 13.2 min, respectively, monitored by ultraviolet absorbance at 282 nm. Quantitation of 6-hydroxychlorzoxazone was accomplished using a standard curve of peak area ratios (6-hydroxychlorzoxazone/pentoxifylline) *versus* 6-hydroxychlorzoxazone (250–5,000 ng/ml), prepared using blank microsomes.

Para-nitrophenol. Microsomal para-nitrophenol hydroxylation was determined essentially as described by Reinke and Meyer.[27] Incubations (37° C, 10 min) contained 0.5 nanomoles microsomal P450, 200 μM para-nitrophenol, and 1 mM NADPH in 100 mM potassium phosphate buffer (*p*H 7.4). Para-nitrocatechol formation was measured spectrophotometrically at 546 nm and quantitated using a standard curve.

Coumarin. Microsomal coumarin 7-hydroxylase activity was measured according to the method of Miles *et al.*[28] Incubations (37° C, 15 min) contained 50 μg microsomal protein, 50 μM coumarin, and 1 mM NADPH in 25 mM potassium phosphate buffer (*p*H 7.2). 7-Hydroxycoumarin formation was measured fluorimetrically with excitation at 376 nm and emission at 460 nm.

Analysis

Rates of metabolism are expressed as nanomoles product formed per nanomoles total spectral P450 per unit time and are presented as the mean ± SD unless indicated otherwise. The relationships between microsomal defluorination rate and various indices of microsomal cytochrome P450 2E1 activity or protein content were examined by linear least squares correlation analysis. Anesthetic fluoride production *in vitro* and *in vivo* was compared using Spearman's rank correlation.

Results

Comparative Anesthetic Metabolism

The limit of fluoride quantitation in our assay was 0.5 nanomoles/ml. Defluorination of methoxyflurane, the currently used volatile anesthetics, and those agents currently in clinical trials, was compared in microsomes from one typical human liver (HL-104) in which metabolism of all anesthetics could be quantitated (fig. 1). The rank order of activity, measured by fluoride production at saturating substrate concentrations, was methoxyflurane > sevoflurane > enflurane > isoflurane > desflurane > 0. Fluoride formation from desflurane was greater than zero but less than the limit of quantitation and is therefore given as the limit of quantitation. Defluorination of methoxyflurane, sevoflurane, and enflurane was also compared in a population of 12

human livers (fig. 1, inset). The mean rate of fluoride formation from methoxyflurane markedly exceeded that from sevoflurane, which in turn was almost twice that from enflurane. Mean data were not obtained for isoflurane and desflurane because fluoride production from several livers was below the limit of quantitation.

Role of P450 2E1 in Anesthetic Defluorination

The strategy for evaluating the role of P450 2E1 in anesthetic defluorination involved three approaches using a population of human livers. First, correlation of defluorination rates with microsomal P450 2E1 protein content. Second, correlation of defluorination rates with microsomal P450 2E1 catalytic activity using marker substrates. Third, use of chemical inhibitors selective for certain P450 isoforms, to assess the contribution of those individual P450 isoforms to total metabolism.

Rates of sevoflurane and methoxyflurane defluorination were compared first with microsomal P450 isoform content in the 12 human livers studied. Microsomal P450 2E1 protein content was determined by Western immunoblot experiments using anti-rat P450 2E1 antibody, which cross-reacted specifically with the orthologous human P450 2E1. There was a significant linear correlation between sevoflurane defluorination and antigenic P450 2E1 content ($r = 0.98$, $P < 0.001$; fig. 2, top). The regression line passes near the origin, suggesting that no other enzyme present in the microsomes had appreciable activity toward sevoflurane. Anesthetic defluorination also was compared with the microsomal content of two other human cytochrome P450 isoforms, P450 1A2 and P450 3A3/4. Defluorination of sevoflurane did not correlate with the immunodetectable content of either P450 1A2 or P450 3A3/4 ($r = 0.34$ and $r = 0.27$, respectively, both nonsignificant; data not shown). In contrast to sevoflurane, methoxyflurane defluorination and P450 2E1 content were positively correlated, but less strongly (fig. 2, bottom). The correlation coefficient was significant ($r = 0.72$, $P < 0.01$), suggesting a role for P450 2E1, but there was considerable scatter about the line of regression. Furthermore, the regression was displaced along the ordinate, suggesting catalytic activity by other cytochrome P450 isoforms present in the livers. Methoxyflurane defluorination did not correlate significantly, however, with the immunodetectable content of either P450 1A2 or P450 3A3/4 ($r = 0.51$ and $r = 0.06$, respectively, both nonsignificant; data not shown).

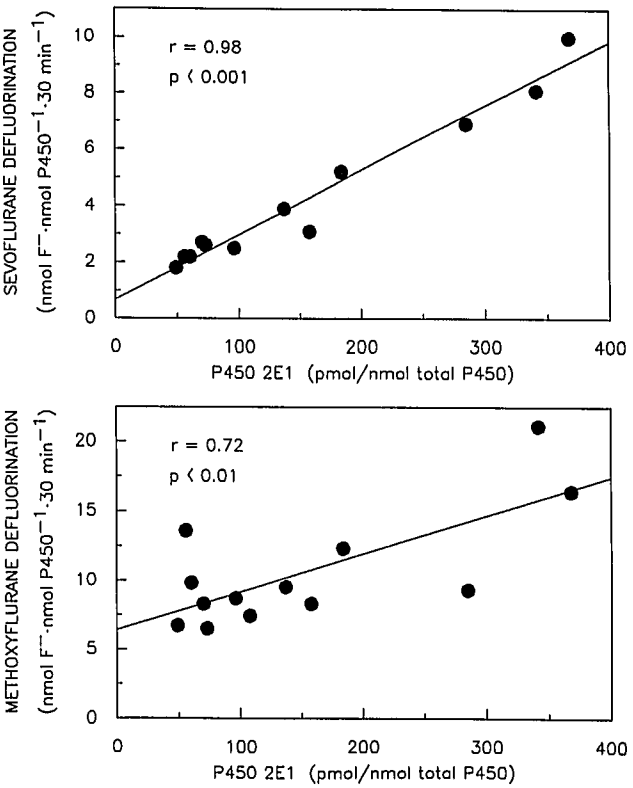

Fig. 2. Correlation of sevoflurane and methoxyflurane defluorination with microsomal cytochrome P450 2E1 content. Results are presented for sevoflurane (*top*) and methoxyflurane (*bottom*). P450 2E1 content was determined by Western blot analysis. Each data point represents the mean of duplicate determinations from one human liver. For methoxyflurane the y-intercept was significantly different from zero ($P < 0.05$), and the 95% confidence interval excluded zero.

If two drug metabolism reactions are catalyzed by the same cytochrome P450 isoform, then in a liver population with a range of isoform activities, the two biotransformation rates should correlate positively. Metabolism of para-nitrophenol is catalyzed predominantly by cytochrome P450 2E1 and thus has been proposed as a specific metabolic marker for microsomal P450 2E1 activity.[29] Comparison of anesthetic defluorination and para-nitrophenol hydroxylation rates in the series of livers studied showed a significant correlation for both sevoflurane ($r = 0.93$, $P < 0.001$) and methoxyflurane ($r = 0.78$, $P < 0.002$; fig. 3). Microsomal P450 2E1 activity also was measured with a second marker substrate with unambiguous P450 2E1 selectivity, chlorzoxazone 6-hydroxylation.[30] There was a significant correlation between chlorzoxazone 6-hydroxylation and defluorination of sevoflurane ($r = 0.95$,

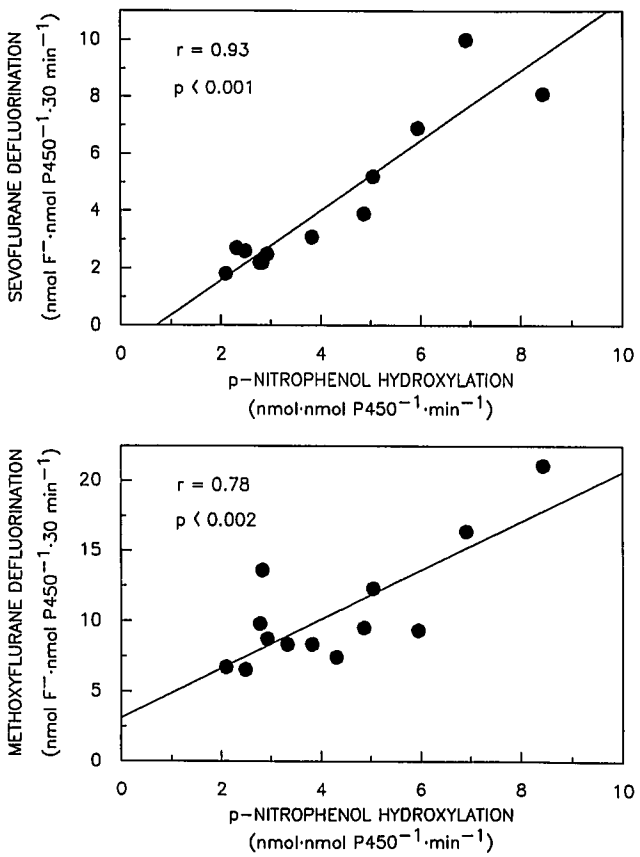

Fig. 3. Correlation of sevoflurane and methoxyflurane defluorination with microsomal cytochrome P450 2E1 catalytic activity, measured by hydroxylation of para-nitrophenol. Results are presented for sevoflurane (*top*) and methoxyflurane (*bottom*). Each data point represents the mean of duplicate determinations from one human liver.

$P < 0.001$) and methoxyflurane (r = 0.66, $P < 0.02$; fig. 4). These data suggest that metabolism of sevoflurane, methoxyflurane, para-nitrophenol, and chlorzoxazone are catalyzed by the same enzyme, namely cytochrome P450 2E1.

We have shown previously that human liver microsomal defluorination of enflurane is catalyzed predominantly by P450 2E1.[20] Sevoflurane defluorination, therefore, was compared with that of enflurane. Rates of sevoflurane defluorination were highly correlated with those of enflurane (r = 0.93, $P < 0.001$), suggesting that these two anesthetics are metabolized by the same enzyme (fig. 5). Cytochrome P450 2A6 has been shown to metabolize some P450 2E1 substrates.[16] Therefore, we compared rates of sevoflurane defluorination with P450 2A6 activity, measured as the hydroxylation of coumarin. There was no significant correlation (r = 0.52, $P > 0.05$; data not shown) between sevoflurane and coumarin metabolism, suggesting that P450 2A6 is not responsible for a significant portion of sevoflurane metabolism.

Previous investigations in animals have shown that isoflurane can inhibit the metabolism of other volatile anesthetics.[31] If two volatile anesthetics are defluorinated by the same enzyme, then they are candidates to mutually inhibit each other's metabolism in a competitive manner. We tested the hypothesis that volatile anesthetics can influence each other's metabolism by human liver microsomes. Because the measured metabolite, fluoride, is common to both reactions, the more slowly metabolized anesthetic (*e.g.*, isoflurane) would be seen to retard the metabolism of the more

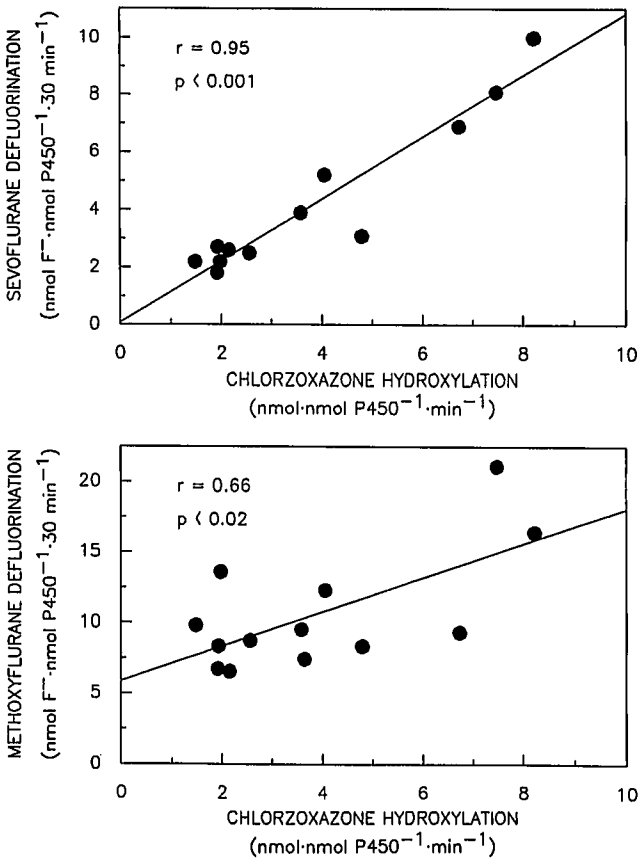

Fig. 4. Correlation of sevoflurane and methoxyflurane defluorination with microsomal cytochrome P450 2E1 catalytic activity, measured by chlorzoxazone 6-hydroxylation. Results are presented for sevoflurane (*top*) and methoxyflurane (*bottom*). Each data point represents the mean of duplicate determinations from one human liver.

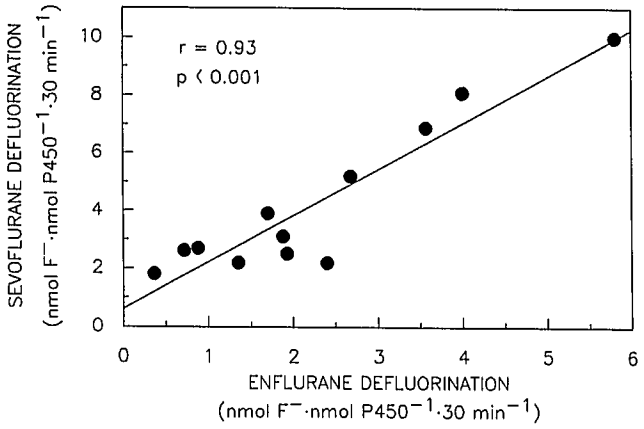

Fig. 5. Correlation of enflurane and sevoflurane defluorination by human liver microsomes. Each data point represents the mean of duplicate determinations from one human liver.

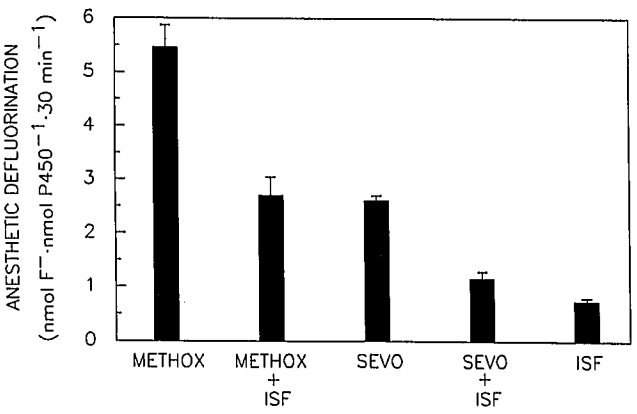

Fig. 6. Influence of isoflurane on methoxyflurane and sevoflurane metabolism. All anesthetics were incubated at saturating concentrations using microsomes from a representative human liver (HL-121). Results are the mean ± SD of three determinations.

rapidly metabolized agent (sevoflurane or methoxyflurane) if the hypothesis is correct. Figure 6 shows that isoflurane diminished the defluorination of both methoxyflurane and sevoflurane. This result suggests that isoflurane is defluorinated by the same enzyme as sevoflurane and methoxyflurane (P450 2E1), although isoflurane theoretically could bind to but not be metabolized by that enzyme.

Chemical inhibitors that are selective for a particular P450 isoform may be used to probe the involvement of that isoform in drug metabolism. Diethyldithiocarbamate is known to be a mechanism-based inhibitor of cytochrome P450 2E1.[32] At concentrations less than 300 μM, diethyldithiocarbamate is metabolized selectively by P450 2E1, which in turn is inactivated as a consequence of the catalytic cycle. When microsomes from a representative human liver were preincubated with diethyldithiocarbamate and NADPH for 10 min before anesthetic addition, there was a concentration-dependent inhibition of sevoflurane, isoflurane, and methoxyflurane defluorination (fig. 7). This experiment was repeated at 100 μM diethyldithiocarbamate using four additional livers. Mean (±SD) rates of anesthetic defluorination in the presence of 100 μM diethyldithiocarbamate for five livers studied were 33 ± 11%, 31 ± 13%, and 44 ± 6% of control for sevoflurane, methoxyflurane, and isoflurane, respectively. Inhibition of desflurane metabolism could not be studied because of the low basal rate of defluorination.

To assess the possible participation of other cytochrome P450 isoforms in anesthetic defluorination, microsomal defluorination activity was probed using a panel of selective inhibitors (fig. 8). The inhibitors and isoforms included furafylline (P450 1A2), sulfaphenazole (P450 2C9 and 2C10), quinidine (P450 2D6), and troleandomycin (P450 3A).[33] Inhibitor concentrations and incubation conditions were chosen to suppress >80% of isoform activity.[20] None of these se-

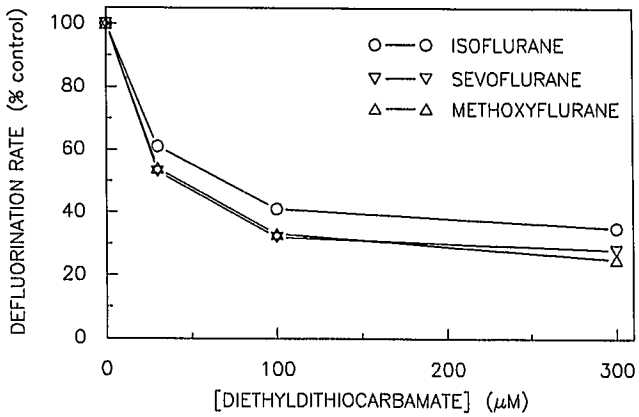

Fig. 7. Inhibition of anesthetic defluorination by diethyldithiocarbamate. Microsomes from a representative liver (HL-111) were preincubated with a nicotinamide adenine dinucleotide phosphate-regenerating system and indicated concentrations of diethyldithiocarbamate for 10 min. Anesthetic was added to initiate the defluorination reaction, and fluoride formation was determined after an additional 30 min as described in methods. Each data point represents the mean of duplicate determinations. The uninhibited rates of fluoride production were 4.1, 2.0, and 0.73 nanomoles·nanomoles $P450^{-1}\cdot 30\ min^{-1}$, respectively, for methoxyflurane, sevoflurane, and isoflurane. Longer preincubation times increased slightly, but not significantly, the degree of inhibition (data not shown).

lective inhibitors, except diethyldithiocarbamate, diminished the defluorination of sevoflurane. This further suggests that none of these constitutive P450 isoforms, other than P450 2E1, contributes significantly to the defluorination of this anesthetic agent. In contrast, methoxyflurane defluorination was inhibited by furafylline, quinidine, and sulfaphenazole, but to a much lesser extent than by diethyldithiocarbamate. Thus, members of the P450 2C family, P450 1A2, and P450 2D6 appear to catalyze methoxyflurane defluorination in addition to the predominant enzyme, P450 2E1.

Discussion

Comparative Anesthetic Metabolism

There are no studies comparing the rates of volatile anesthetic defluorination in humans. Rates have only been inferred indirectly from the extent of anesthetic metabolism. It is important, however, to distinguish between the rate and extent of anesthetic metabolism, because both may bear on anesthetic toxicity. The extent of anesthetic metabolism has been measured both by recovery of urinary metabolites[2,8-11] and by mass balance studies.[7,12,13] The mass balance technique, which defines metabolism as the difference between anesthetic taken up and that recovered from exhaled gases, is subject to inaccuracy because of extrapulmonary anesthetic elimination, insufficient sensitivity to detect modest metabolism, and normalization of results to that of isoflurane, which is assumed not to be metabolized.[7,12] Metabolism assessments made by urinary fluoride recovery may underestimate the extent of metabolism because of incomplete metabolite recovery (fluoride retention in bone, formation of multiple metabolites, or nonrenal metabolite elimination) and are usually less than those obtained by mass balance techniques.[7] Furthermore, the extent of metabolism will be governed not only by the rate of hepatic metabolism but also by the metabolite excretion rate and by anesthetic solubility (which determines the rate of anesthetic washout).

Application of these available techniques to sevoflurane and desflurane has produced conflicting estimates of metabolism. Shiraishi and Ikeda, using metabolite recovery, found that sevoflurane metabolism was five or six times greater than that of isoflurane,[34] whereas Yasuda *et al.*, using mass balance techniques, concluded that the metabolism of sevoflurane did not differ from that of isoflurane.[12] Holaday and Smith[2]

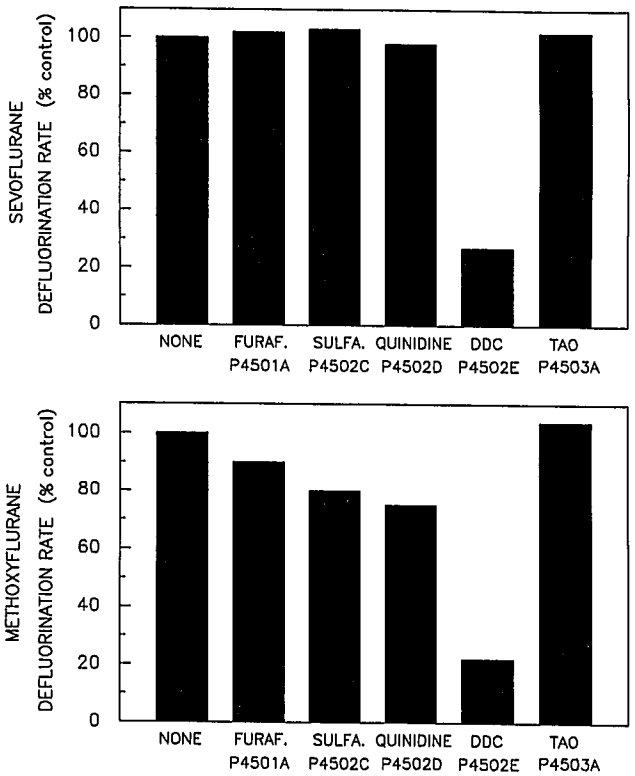

Fig. 8. Effect of P450 inhibitors on anesthetic metabolism. A panel of inhibitors, each selective for a particular isoform of human P450, was used at optimal concentrations. The inhibitor and the isoform that it inhibits are shown on the bottom of the graph. These included furafylline (P450 1A2), sulfaphenazole (P450 2C9/10), quinidine (P450 2D6), diethyldithiocarbamate (P450 2E1), and troleandomycin (P450 3A3/4). Shown are results for sevoflurane (*top*) and methoxyflurane (*bottom*). One hundred percent activity for methoxyflurane and sevoflurane defluorination was 8.4 and 4.5 nanomoles · nanomoles $P450^{-1} \cdot 30\ min^{-1}$, respectively.

suggested that sevoflurane and enflurane were similar with respect to biotransformation, while others found that sevoflurane metabolism was two to five times greater than that of enflurane.[34] Desflurane appeared not to undergo metabolism based on mass balance studies[13] and measurements of serum and urine fluoride concentrations,[11,35] but increased urinary trifluoroacetic acid levels demonstrated a small degree of metabolism.[35]

Our results obtained by direct *in vitro* measurement of human liver metabolism clearly demonstrate that the rank order of anesthetic defluorination rates is methoxyflurane > sevoflurane > enflurane > isoflurane > desflurane. The rate of isoflurane defluorination was similar to that reported previously for human liver mi-

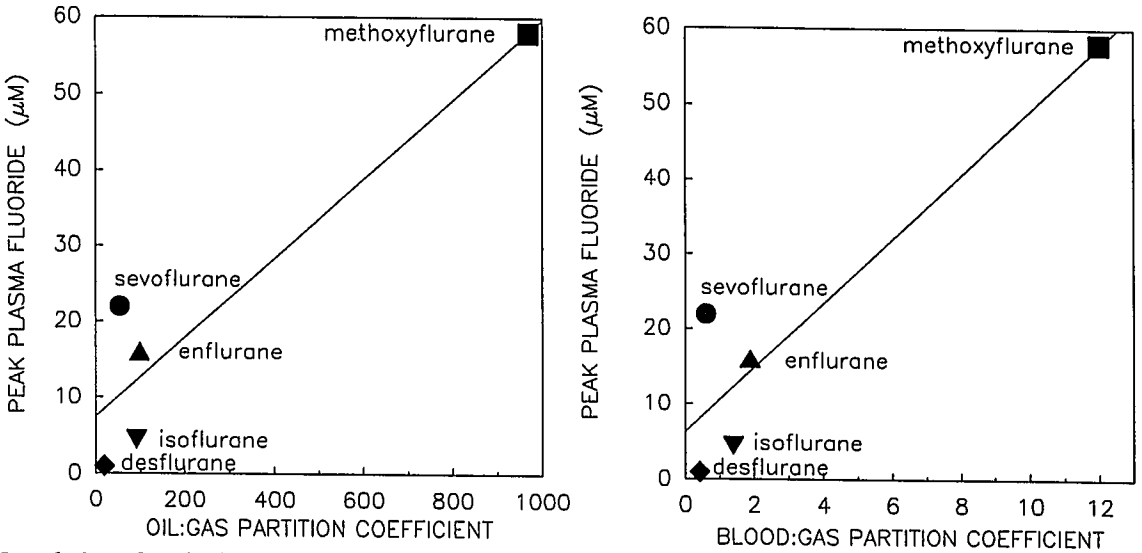

Fig. 9. Correlation of peak plasma fluoride concentration with anesthetic solubility (oil:gas or blood:gas partition coefficient). Shown are literature values for measured peak plasma fluoride concentrations following 3 MAC h of anesthesia.[1,3,35,41-43] Spearman's rank correlation coefficient for each relationship was 0.70 (not significant).

crosomes,[36] and the lowest rates we observed for enflurane metabolism were comparable to the rate reported by Hitt *et al.*[37] Because both inorganic and organic fluoride metabolites are produced in parallel,[38] the rank order for organic fluoride formation likely resembles that for inorganic fluoride formation. It is clear that desflurane undergoes metabolism, but at a rate less than that of isoflurane. The small degree of metabolism observed clinically after desflurane anesthesia thus is due to the low rate of hepatic biotransformation rather than to sequestration or loss of metabolites, in agreement with previous suggestions.[35] Sevoflurane defluorination occurred at twice the rate of enflurane defluorination and five or six times that of isoflurane. Estimates of sevoflurane metabolism derived from mass balance or metabolite recovery studies may reflect the extent of metabolism but underestimate the rate of hepatic metabolism.

Most recent estimates of clinical anesthetic metabolism were obtained during simultaneous administration of multiple volatile agents. Simultaneous administration was thought not to influence the pharmacokinetic behavior and metabolism of individual agents.[7] However, isoflurane decreases the metabolism of methoxyflurane and sevoflurane (fig. 6). All anesthetics metabolized by the same enzyme may potentially inhibit each other's metabolism at clinically relevant, saturating concentrations. Thus, estimates of biotransformation obtained from mass balance studies during multiple agent administration may underestimate the extent of anesthetic metabolism, as suggested.[7]

Fluoride Formation, Anesthetic Metabolism, and Solubility

It commonly has been accepted that solubility in blood governs anesthetic metabolism.[8,38-40] We tested this hypothesis that peak plasma fluoride concentrations after comparable MAC-hours of anesthesia are proportional to anesthetic solubility. Peak plasma fluoride concentrations, reported previously in comparable clinical studies of anesthetic defluorination,[1,3,35,41-43] were compared with anesthetic solubility (fig. 9). The correlation between peak plasma fluoride concentration and either oil-gas or blood-gas partition coefficients was not statistically significant. In contrast, there was a highly significant correlation between clinical peak plasma fluoride concentrations and the rate of *in vitro* human liver microsomal defluorination ($r = 0.99$, $P < 0.001$; fig. 10). Although the duration and extent of anesthetic metabolism are influenced by anesthetic solubility,[44] peak plasma fluoride concentrations achieved clinically appear largely unrelated to anesthetic solubility. Peak plasma fluoride concentrations, however, do appear proportional to the rate of hepatic metabolism. These comparisons demonstrate the role of hepatic metabolism in determining *in vivo* anesthetic defluorination and illustrate the

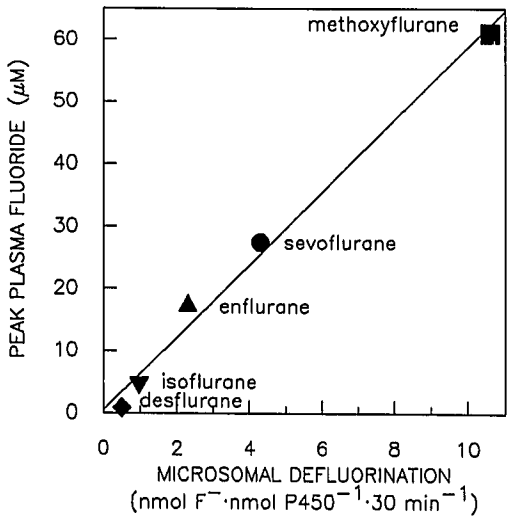

Fig. 10. Correlation of peak plasma fluoride concentration with anesthetic metabolism. *In vivo* peak plasma fluoride concentrations are the same as those in figure 9. Liver microsomal defluorination data are from figure 1. Mean values are shown for methoxyflurane, sevoflurane, and enflurane, and single liver values are shown for isoflurane and desflurane. Spearman's rank correlation coefficient was 1.00 ($P < 0.05$). The correlation coefficient determined by linear regression analysis was 0.99 ($P < 0.001$).

utility of human hepatic microsomal anesthetic metabolism *in vitro* as a model for defluorination *in vivo*.

Role of P450 2E1 in Anesthetic Defluorination

Results of this investigation demonstrate that cytochrome P450 2E1 is the principal, if not sole human liver microsomal enzyme catalyzing the defluorination of sevoflurane. Cytochrome P450 2E1 also participates prominently in the defluorination of isoflurane, and probably desflurane as well. In addition, P450 2E1 is the principal, although not exclusive enzyme responsible for the metabolism of methoxyflurane. Selective inhibitor data suggest that methoxyflurane metabolism also is catalyzed by P450s 1A2, 2C9/10, and 2D6. These results, in conjunction with previously demonstrated P450 2E1 participation in human liver enflurane metabolism,[20] suggest that cytochrome P450 2E1 is the predominant catalyst of human liver microsomal biotransformation of fluorinated ether anesthetics.

These conclusions are based on the correlation of defluorinase activity with P450 2E1 protein contents and with the catalytic activities toward known P450 2E1 substrates, as well as the effects of isoform-selective chemical inhibitors. For example, sevoflurane metabolism could only be attributed to P450 2E1, based on: (1) the high correlation of anesthetic defluorination with the antigenic content of P450 2E1 but not two other major constitutive P450 isoforms, (2) the high correlation of sevoflurane defluorination with P450 2E1 catalytic activity (para-nitrophenol and chlorzoxazone hydroxylation), and (3) exclusive chemical inhibition by diethyldithiocarbamate.

The alternative explanation for the correlations observed, that the various reactions are catalyzed by different P450 isoforms, would require coordinate expression of the different enzymes in the heterogenous population of livers studied, which is highly unlikely. Although metabolism of para-nitrophenol has been proposed as a specific metabolic marker for microsomal P450 2E1 activity,[29] more recent evidence suggests that P450 2E1 is the predominant, but not necessarily the exclusive catalyst of para-nitrophenol metabolism in human tissues.[45] A contribution to para-nitrophenol metabolism by a non-2E1 species of P450 would account for the slight displacement of the sevoflurane regression line along the axis of para-nitrophenol metabolism (fig. 3, top).

Anesthetic Metabolism by P450 2E1: Clinical Implications

Identification of cytochrome P450 2E1 as the major enzyme responsible for defluorination of methoxyflurane, sevoflurane, isoflurane, and enflurane in humans permits reconciliation of several clinical and experimental observations. These pertain to anesthetic metabolism, anesthetic toxicity, and enzyme induction.

Anesthetic Metabolism. Peak plasma fluoride concentrations are markedly increased in obese patients anesthetized with methoxyflurane,[46] enflurane,[47,48] or isoflurane[48] as a result of enhanced anesthetic biotransformation. Although it has been suggested that fatty liver infiltration increases hepatic anesthetic uptake,[46] most authors agree that the mechanism of increased hepatic anesthetic metabolism in obesity remains unclear.[47,48] Obesity increases two- to threefold the hepatic content and catalytic activity of P450 2E1,[45] as well as the microsomal metabolism of enflurane.[49] From this recent observation, together with our data, we conclude that increased cytochrome P450 2E1 content and activity may account for enhanced anesthetic defluorination and elevated plasma fluoride concentrations in obesity.

Anesthetic Toxicity. Identification of multiple P450 isoforms contributing to methoxyflurane de-

fluorination may help explain the extensive biotransformation that occurs *in vitro* and *in vivo,* and thus, in part, methoxyflurane nephrotoxicity. Clinically, methoxyflurane is biotransformed to a greater degree than other volatile agents. Extensive methoxyflurane metabolism has been attributed either to sustained methoxyflurane residence in the liver[8] or to rapid rates of hepatic metabolism.[7] Results of the present investigation, demonstrating a significantly greater rate of methoxyflurane defluorination relative to that of the other agents, supports this latter hypothesis. The rate of P450 2E1-catalyzed defluorination was faster for methoxyflurane than for any other agent we studied. Furthermore, methoxyflurane was the only agent for which evidence was obtained of metabolism by constitutive P450s in addition to P450 2E1. Finally, an appreciable portion of methoxyflurane defluorination may occur extrahepatically.[50] P450s that appear to defluorinate methoxyflurane are found in human kidney and lung (P450 1A), and intestine (P450 2C and 2D6),[51,52] as well as in the liver.

Differences in methoxyflurane and sevoflurane susceptibility to metabolism by P450 isoforms may influence the relative nephrotoxic potential of these agents. Methoxyflurane nephrotoxicity is classically associated with plasma inorganic fluoride concentrations exceeding 50 μM .[1] Despite plasma fluoride concentrations often exceeding 50 μM , sevoflurane anesthesia, to date, has not been associated with nephrotoxicity.[3,4] These differences may relate to sustained *versus* brief elevations of plasma fluoride concentrations seen with methoxyflurane compared to sevoflurane, due in part to more rapid methoxyflurane metabolism by P450 2E1 and metabolism by multiple P450 isoforms. We also raise the hypothesis that organ selectivity in anesthetic metabolism may influence anesthetic toxicity. Methoxyflurane, metabolized by several P450 isoforms, undergoes extensive renal defluorination.[50] In contrast, sevoflurane metabolism is catalyzed predominantly, if not exclusively, by P450 2E1. Enflurane, which, like sevoflurane, is defluorinated exclusively by P450 2E1, undergoes dramatically less renal defluorination.[50] Because significant amounts of human renal P450 2E1 have not been found,[52] meaningful human renal metabolism may be associated uniquely with methoxyflurane. This intriguing hypothesis that renal defluorination underlies the nephrotoxicity of methoxyflurane, but not sevoflurane, requires further investigation.

Enzyme Induction. Chronic isoniazid therapy in humans induces the metabolism of enflurane and isoflurane, markedly increasing peak plasma fluoride concentrations.[42,53] The finding that isoniazid induction in humans increases cytochrome P450 2E1,[16] in conjunction with our data, now provides a mechanistic basis for this clinical observation. In contrast with isoniazid, enzyme induction with barbiturates or phenytoin does not increase anesthetic defluorination in humans,[54] because these agents do not induce the major hepatic defluorinating enzyme, P450 2E1. However, barbiturates induce the metabolism of methoxyflurane,[1] which is catalyzed by a broad spectrum of P450 isoforms including the barbiturate-inducible P450 2C isoform.[55,56]

Anesthetic Metabolism: Human Versus *Animal Models*

For anesthetic agents metabolized primarily by P450 2E1, there appears to be a high concordance between inducer and inhibitor effects in animals and humans, due to the structural and catalytic homology between human and animal P450 2E1.[16] For example, in rats, like in humans, isoniazid induces P450 2E1,[16] the isoform responsible for anesthetic defluorination in this species,[18,19] and stimulates methoxyflurane, enflurane, sevoflurane, and isoflurane defluorination *in vivo.*[42,53,57]

In contrast, data obtained from phenobarbital- or phenytoin-induced animals cannot be extrapolated reliably to humans because of the dissimilarity in the identity and intrinsic activity of enzymes induced. P450 2B, the major family of cytochrome P450 induced by phenobarbital in rats, rabbits, and guinea pigs, is expressed minimally, if at all, in human liver.[58] Other cytochrome families that are inducible in humans by phenobarbital, P450 3A, and P450 2C,[16,55,56] appeared not to metabolize sevoflurane. Thus, although phenobarbital and phenytoin induce enflurane, sevoflurane, and isoflurane metabolism in rats and rabbits,[38,57,59] they would be expected to have negligible influence on the metabolism of these agents in humans.[54] Thus, human microsomal anesthetic metabolism *in vitro* models more closely human anesthetic metabolism *in vivo* and appears a preferable model compared to rat or rabbit *in vitro* anesthetic metabolism.

In summary, we have demonstrated that cytochrome P450 2E1 is the predominant, if not singular human enzyme responsible for the defluorination of sevoflurane. Several human cytochrome P450 enzymes metabolize methoxyflurane, in addition to the major enzyme P450 2E1. P450 2E1 also appears to catalyze a major

portion of isoflurane metabolism. In addition, we have shown that peak plasma fluoride concentrations in the perianesthetic period correlate best with the rate of anesthetic metabolism, rather than lipid solubility.

The authors thank Jim Lillibridge for the analysis of chlorzoxazone metabolism.

References

1. Cousins MJ, Mazze RI: Methoxyflurane nephrotoxicity: A study of dose response in man. JAMA 225:1611–1616, 1973

2. Holaday DA, Smith FR: Clinical characteristics and biotransformation of sevoflurane in healthy human volunteers. ANESTHESIOLOGY 54:100–106, 1981

3. Frink EJ Jr., Ghantous H, Malan TP, Morgan S, Fernando J, Gandolfi AJ, Brown BR Jr: Plasma inorganic fluoride with sevoflurane anesthesia: Correlation with indices of hepatic and renal function. Anesth Analg 74:231–235, 1992

4. Kobayashi Y, Ochiai R, Takeda J, Sekiguchi H, Fukushima K: Serum and urinary inorganic fluoride concentrations after prolonged inhalation of sevoflurane in humans. Anesth Analg 74:753–757, 1992

5. Christ DD, Kenna JG, Kammerer W, Satoh H, Pohl LR: Enflurane metabolism produces covalently bound liver adducts recognized by antibodies from patients with halothane hepatitis. ANESTHESIOLOGY 69:833–838, 1988

6. Christ DD, Satoh H, Kenna JG, Pohl LR: Potential metabolic basis for enflurane hepatitis and the apparent cross-sensitization between enflurane and halothane. Drug Metab Disp 16:135–140, 1988

7. Carpenter RL, Eger EI II, Johnson BH, Unadkat JD, Sheiner LB: The extent of metabolism of inhaled anesthetics in humans. ANESTHESIOLOGY 65:201–205, 1986

8. Holaday DA, Rudofsky S, Treuhaft PS: The metabolic degradation of methoxyflurane in humans. ANESTHESIOLOGY 33:579–593, 1970

9. Chase RE, Holaday DA, Fiserova-Bergerova V, Saidman LJ, Mack FE: The biotransformation of Ethrane in humans. ANESTHESIOLOGY 35: 262–267, 1971

10. Holaday DA, Fiserova-Bergerova V, Latto IP, Zumbiel MA: Resistance of isoflurane to biotransformation in humans. ANESTHESIOLOGY 43:325–332, 1975

11. Jones RM, Koblin DD, Cashman JN, Eger EI II, Johnson BH, Damask MC: Biotransformation and hepato-renal function in volunteers after exposure to desflurane (I-653). Br J Anaesth 64:482–487, 1990

12. Yasuda N, Lockhart SH, Eger EI II, Weiskopf RB, Liu J, Laster M, Taheri S, Peterson NA: Comparison of kinetics of sevoflurane and isoflurane in humans. Anesth Analg 72:316–324, 1991

13. Yasuda N, Lockhart SH, Eger EI II, Weiskopf RB, Johnson BH, Freire BA, Fassoulaki A: Kinetics of desflurane, isoflurane, and halothane in humans. ANESTHESIOLOGY 74:489–498, 1991

14. Brown BR, Frink EJ Jr: Metabolism of sevoflurane (letter). Anesth Analg 73:671, 1991

15. Eger EI II: Metabolism of sevoflurane (letter). Anesth Analg 73:668, 1991

16. Wrighton SA, Stevens JC: The human hepatic cytochromes P450 involved in drug metabolism. Crit Rev Toxicol 22:1–21, 1992

17. Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gunzalez FJ, Coon MJ, Gunsalus IC, Gotoh O, Okuda K, Nebert DW: The P450 superfamily: Update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. DNA Cell Biol 12:1–51, 1993

18. Hoffman J, Konopka K, Buckhorn C, Koop DR, Waskell L: Ethanol-inducible cytochrome P450 in rabbits metabolizes enflurane. Br J Anaesth 63:103–108, 1989

19. Tsutsumi R, Leo MA, Kim C, Tsutsumi M, Lasker J, Lowe N, Lieber CS: Interaction of ethanol with enflurane metabolism and toxicity: Role of P450IIE1. Alcoholism Clin Exp Res 14:174–179, 1990

20. Thummel KE, Kharasch ED, Podoll T, Kunze K: Human liver microsomal enflurane defluorination catalyzed by cytochrome P-450 2E1. Drug Metab Dispos 21:350–357, 1993

21. Yoo JSH, Cheung RJ, Patten CJ, Wade D, Yang CS: Nature of N-nitrosodimethylamine demethylase and its inhibitors. Cancer Res 47:3378–3383, 1987

22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951

23. Estabrook RW, Peterson J, Baron J, Hildebrandt J: The spectrophotometric measurement of turbid suspensions of cytochromes associated with drug metabolism. Methods Pharmacol 2:303–350, 1972

24. Favreau LV, Malchoff DM, Mole JE, Schenkman JB: Responses to insulin by two forms of rat hepatic microsomal cytochrome P-450 that undergo major (RLM6) and minor (RLM5b) elevations in diabetes. J Biol Chem 262:14319–14326, 1987

25. Kharasch ED, Thummel K: Human alfentanil metabolism by cytochrome P450 3A3/4: An explanation for the interindividual variability in alfentanil clearance? Anesth Analg 76:1033–1039, 1993

26. Fry BW, Taves DR: Serum fluoride analysis with the fluoride electrode. J Lab Clin Med 75:1020–1025, 1970

27. Reinke LA, Moyer MJ: A microsomal oxidation which is highly inducible by ethanol. Drug Metab Disp 13:548–552, 1985

28. Miles JS, McLaren AW, Forrester LM, Glancey MJ, Lang MA, Wolf CR: Identification of the human liver cytochrome P-450 responsible for coumarin 7-hydroxylase activity. Biochem J 267:365–371, 1990

29. Koop DR: Hydroxylation of p-nitrophenol by rabbit ethanol-inducible cytochrome P-450 isozyme 3a. Mol Pharmacol 29:399–404, 1986

30. Peter R, Bocker R, Beaune PH, Iwasaki M, Guengerich FP, Yang CS: Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome P-450IIE1. Chem Res Toxicol 3:565–573, 1990

31. Fiserova-Bergerova V: Inhibitory effect of isoflurane upon oxidative metabolism of halothane. Anesth Analg 63:399–404, 1984

32. Guengerich FP, Kim D-H, Iwasaki M: Role of human P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. Chem Res Toxicol 4:168–179, 1991

33. Guengerich FP, Shimada T: Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. Chem Res Toxicol 4:391–407, 1991

34. Shiraishi Y, Ikeda K: Uptake and biotransformation of sevoflurane in humans: A comparative study of sevoflurane with halothane, enflurane, and isoflurane. J Clin Anesth 2:381–386, 1990

35. Sutton TS, Koblin DD, Gruenke LD, Weiskopf RB, Rampil IJ, Waskell L, Eger EI II: Fluoride metabolites after prolonged exposure of volunteers and patients to desflurane. Anesth Analg 73:180–185, 1991

36. Bradshaw JJ, Ivanetich KM: Isoflurane: A comparison of its metabolism by human and rat hepatic cytochrome P-450. Anesth Analg 63:805–813, 1984

37. Hitt BA, Mazze RI, Beppu WJ, Stevens WC, Eger EI II: Enflurane metabolism in rats and man. J Pharmacol Exp Ther 203:193–202, 1977

38. Mazze RI: Metabolism of the inhaled anaesthetics: Implications of enzyme induction. Br J Anaesth 56:27S–41S, 1984

39. Greenstein LR, Hitt BA, Mazze RI: Metabolism *in vitro* of enflurane, isoflurane, and methoxyflurane. ANESTHESIOLOGY 42:420–424, 1975

40. Berman ML, Holaday DA: Inhalation anesthetic metabolism and toxicity, Clinical Anesthesia. Edited by Barash PG, Cullen BF, Stoelting RK. New York, JB Lippincott, 1989, pp 323–338

41. Mazze RI, Cousins MJ, Barr GA: Renal effects and metabolism of isoflurane in humans. ANESTHESIOLOGY 40:536–542, 1974

42. Mazze RI, Woodruff RE, Heerdt ME: Isoniazid-induced enflurane defluorination in humans. ANESTHESIOLOGY 57:5–8, 1982

43. Smith I, Ding Y, White PF: Comparison of induction, maintenance, and recovery characteristics of sevoflurane-N_2O and propofol-sevoflurane-N_2O with propofol-isoflurane-N_2O anesthesia. Anesth Analg 74:253–259, 1992

44. Feingold A, Holaday DA: The pharmacokinetics of metabolism of inhalation anaesthetics: A simulation study. Br J Anaesth 49:155–162, 1977

45. Raucy JL, Lasker JM, Kraner JC, Salazar DE, Lieber CS, Corcoran GB: Induction of cytochrome-P450IIE1 in the obese overfed rat. Mol Pharmacol 39:275–280, 1991

46. Young SR, Stoelting RK, Peterson C, Madura JA: Anesthetic biotransformation and renal function in obese patients during and after methoxyflurane or halothane anesthesia. ANESTHESIOLOGY 42: 451–457, 1975

47. Miller MS, Gandolfi AJ, Vaughan RW, Bentley JB: Disposition of enflurane in obese patients. J Pharmacol Exp Ther 215:292–296, 1980

48. Strube PJ, Hulands GH, Halsey MJ: Serum fluoride levels in morbidly obese patients: Enflurane compared with isoflurane anesthesia. Anaesthesia 42:685–689, 1987

49. Yoo JSH, Ning SM, Pantuck CB, Pantuck EJ, Yang CS: Regulation of hepatic microsomal cytochrome-P450IIE1 level by dietary lipids and carbohydrates. J Nutr 121:959–965, 1991

50. Blitt CD, Gandolfi AJ, Soltis JJ, Brown BR Jr: Extrahepatic biotransformation of halothane and enflurane. Anesth Analg 60:129–132, 1981

51. Pacifici GM, Viani A, Franchi M, Gervasi PG, Longo V, Di Simplicio P, Temellini A, Romiti P, Santerini S, Vannucci L, Mosca F: Profile of drug-metabolizing enzymes in the cortex and medulla of the human kidney. Pharmacology 39:299–308, 1989

52. de Waziers I, Cugnenc PH, Yang CS, Leroux JP, Beaune PH: Cytochrome P-450 isoenzymes, epoxide hydrolase and glutathione transferases in rat and human hepatic and extrahepatic tissues. J Pharmacol Exp Ther 253:387–394, 1990

53. Gauntlett IS, Koblin DD, Fahey MR, Konopka K, Greunke LD, Waskell L, Eger EI II: Metabolism of isoflurane in patients receiving isoniazid. Anesth Analg 69:245–249, 1989

54. Dooley JR, Mazze RI, Rice SA, Borel JD: Is enflurane defluorination inducible in humans? ANESTHESIOLOGY 50:213–217, 1979

55. Perrot N, Nalpas B, Yang CS, Beaune P: Modulation of cytochrome P450 isozymes in human liver by ethanol and drug intake. Eur J Clin Invest 19:549–555, 1989

56. Morel F, Beaune P, Ratanasavanh D, Flinois J-P, Guengerich FP, Guillouzo A: Effects of various inducers on the expression of cytochromes P-450 IIC8, 9, 10 and IIIA in cultured adult human hepatocytes. Toxicol In Vitro 4:458–460, 1990

57. Rice SA, Talcott RE: Effects of isoniazid treatment on selected hepatic mixed-function oxidases. Drug Metab Disp 7:260–262, 1979

58. Yamano S, Nhamburo PT, Aoyama T, Meyer U, Inaba T, Kalow W, Gelboin HV, McBride OW, Gonzalez FJ: cDNA cloning and sequence and cDNA-directed expression of human P450 IIB1: Identification of a normal and two variant cDNAs derived from the CYP2B locus on chromosome 19 and differential expression of the IIB mRNAs in human liver. Biochemistry 28:7340–7348, 1989

59. Waskell L, Canova-Davis E, Philpot R, Parandoush Z, Chiang JYL: Identification of the enzymes catalyzing metabolism of methoxyflurane. Drug Metab Disp 14:643–648, 1986