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Clinical Sevoflurane Metabolism and Disposition

II. The Role of Cytochrome P450 2E1 in Fluoride and Hexafluoroisopropanol Formation

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Background: **Sevoflurane is metabolized to free fluoride and hexafluoroisopropanol (HFIP). Cytochrome P450 2E1 is the major isoform responsible for sevoflurane metabolism by human liver microsomes *in vitro*. This investigation tested the hypothesis that P450 2E1 is predominantly responsible for sevoflurane metabolism *in vivo*. Disulfiram, which is converted *in vivo* to a selective inhibitor of P450 2E1, was used as a metabolic probe for P450 2E1.**

Methods: **Twenty-one patients within 30% of ideal body weight, who provided institutional review board-approved informed consent and were randomized to receive disulfiram (500 mg oral, n = 11) or nothing (control, n = 10) the night before surgery, were evaluated. All patients received sevoflurane (2.7% end-tidal, 1.3 MAC) in oxygen for 3 h after propofol induction. Thereafter, sevoflurane was discontinued, and anesthesia was maintained with propofol, fentanyl, and nitrous oxide. Blood sevoflurane concentrations during anesthesia and for 8 h thereafter were measured by gas chromatography. Plasma and urine fluoride and total (unconjugated plus glucuronidated) HFIP concentrations were measured by an ion-selective electrode and by gas chromatography, respectively, during anesthesia and for 96 h postoperatively.**

Results: **Patient groups were similar with respect to age, weight, sex, case duration, and intraoperative blood loss. The total sevoflurane dose, measured by cumulative end-tidal sevoflurane concentrations (3.7 ± 0.1 MAC-h; mean ± SE), total pulmonary uptake, and blood sevoflurane concentrations, was similar in both groups. In control patients, plasma fluoride and HFIP concentrations were increased compared to baseline values intraoperatively and postoperatively for the first 48 and 60 h, respectively. Disulfiram treatment significantly diminished this increase. Plasma fluoride concentrations increased from 2.1 ± 0.3 μM (baseline) to 36.2 ± 3.9 μM (peak) in control patients, but only from 1.7 ± 0.2 to 17.0 ± 1.6 μM in disulfiram-treated patients ($P < 0.05$ compared with control patients). Peak plasma HFIP concentrations were 39.8 ± 2.6 and 14.4 ± 1.1 μM in control and disulfiram-treated patients ($P < 0.05$), respectively. Areas under the plasma fluoride- and HFIP-time curves also were diminished significantly to 22% and 20% of control patients, respectively, by disulfiram treatment. Urinary excretion of fluoride and HFIP was similarly significantly diminished in disulfiram-treated patients. Cumulative 96-h fluoride and HFIP excretion in disulfiram-treated patients was 1,080 ± 210 and 960 ± 240 μmol, respectively, compared to 3,950 ± 560 and 4,300 ± 540 μmol in control patients ($P < 0.05$).**

Conclusions: **Disulfiram, an effective P450 2E1 inhibitor, substantially decreased fluoride ion and HFIP production during and after sevoflurane anesthesia. These results suggest that P450 2E1 is a predominant P450 isoform responsible for human sevoflurane metabolism *in vivo*. (Key words: Anesthetics, volatile: sevoflurane. CYP2E1. Cytochrome P450 2E1. Ions: fluoride. Kidney, urine. Liver, metabolism. Metabolism. Metabolites: fluoride; hexafluoroisopropanol. Pharmacokinetics. Toxicity: renal.)**

SEVOFLURANE, like other fluorinated anesthetics, undergoes oxidative defluorination with the liberation of free fluoride ion.[1,2] Unlike other fluorinated anesthetics, the additional major metabolites of sevoflurane have been well characterized.[1,3,4] These investigations indicate that inorganic fluoride and hexafluoroisopropanol (HFIP) are the major products of human sevo-

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flurane metabolism. HFIP circulates in blood primarily as the glucuronide conjugate and is excreted in urine.[4]

Clinical evaluations of sevoflurane have shown wide variability in metabolism, as monitored by plasma fluoride concentrations. Average peak plasma fluoride concentrations ranged from 15 to 30 μM after 1–2 MAC-h sevoflurane.[1,5–7] However there was considerable variability in the individual values, for example, 12–35 μM in one investigation[7] and 10–50 μM in another study.[6] Higher plasma fluoride concentrations have been associated with longer sevoflurane exposures.[6–10] No characteristic factors have been identified in patients with the highest plasma fluoride concentrations in these investigations. Higuchi *et al.* reported that plasma fluoride concentrations and urinary fluoride excretion were greater in obese than nonobese patients.[11] Frink *et al.,* however, found no effect of obesity on sevoflurane metabolism.[12]

Thus, the mechanism of variability in sevoflurane metabolism is unknown. There is speculation that generalized enzyme induction (most commonly by drugs such as barbiturates) could lead to increased fluoride production.[6] The role of enzyme induction (by drugs such as phenobarbital, diphenylhydantoin, or isoniazid) in sevoflurane production in humans is unresolved.

In the absence of human data, animal investigations on sevoflurane fluoride production have been cited in attempts to predict the effects of hepatic enzyme activity on sevoflurane metabolism in man. Enzyme induction by treatment with phenobarbital,[13–15] phenytoin,[14] isoniazid,[16,17] and chronic ethanol administration[18,#] has been shown to increase sevoflurane defluorination in rat liver microsomes *in vitro.* In contrast, Baker *et al.* found no effect of phenobarbital on sevoflurane metabolism.[17] *In vivo,* enzyme induction by phenobarbital pretreatment resulted in a two- to fivefold increase in plasma fluoride concentrations after sevoflurane administration in rats.[19,20] Animal pretreatment with polycyclic aromatic hydrocarbons, which induce different P450 isoforms than phenobarbital, had no effect on sevoflurane defluorination in rat liver microsomes *in vitro*[13,15] but significantly increased sevoflurane defluorination in rats *in vivo.*[20]

Thus, animal data regarding sevoflurane metabolism are contradictory. Furthermore, animal models of sevoflurane metabolism are not applicable to humans,[21] because some important sevoflurane defluorinating enzymes present in animals frequently are not present in humans. Our laboratory has studied the defluorination of sevoflurane by human liver microsomes *in vitro.*[21] We identified the isoniazid- and ethanol-inducible cytochrome P450 isoform P450 2E1 as the predominant human hepatic enzyme responsible for sevoflurane defluorination *in vitro.* Clinical investigations are required to identify the enzyme responsible for sevoflurane metabolism *in vivo.*

The purpose of this investigation was to identify the human enzyme responsible for anesthetic defluorination in patients undergoing sevoflurane anesthesia. We tested the hypothesis that cytochrome P450 2E1 catalyzes sevoflurane defluorination in humans *in vivo.* The hypothesis was tested by administering sevoflurane to patients in whom P450 2E1 activity was inhibited by prior treatment with a single dose of disulfiram and comparing sevoflurane metabolism with that in control patients receiving no pretreatment. Single-dose disulfiram inhibition of human P450 2E1 activity has been demonstrated previously as an effective probe for *in vivo* participation of this isoform in drug metabolism.[22,23]

Materials and Methods

Patient Selection and Clinical Protocol

Twenty-two nonsmoking ASA physical status 1 or 2 patients undergoing anesthesia for elective surgery with anticipated duration of 3–5 h were studied. The investigational protocol was approved by the Institutional Human Subjects Committee, and all patients provided written informed consent. Eligibility and exclusion criteria are described in the accompanying article.[4] Most patients were evaluated and enrolled before the day of surgery, randomized to control or disulfiram groups at that time, and admitted to the hospital the morning of surgery. A few subjects were inpatients. Patients were randomized by blocks to receive 500 mg disulfiram orally at bedtime on the evening before surgery (n = 12) or nothing (control, n = 10). Patients in the control group also are described in the accompanying article.[4] Patients in the disulfiram group were provided disulfiram at the time of enrollment and instructed to ingest the drug at 10 PM the evening before surgery.

All patients received a standardized general anesthetic designed to minimize potential drug interactions other

Masaki E, Kondou T, Kobayashi K: Relationship between serum F- and cytochrome P-450 after sevoflurane anesthesia in ethanol treated rats. ANESTHESIOLOGY 73:A396, 1990.

than the desired test interaction. An indwelling venous catheter, used for blood sampling, was placed in the arm contralateral to the arm used for intravenous fluid administration. Patients received midazolam (1 mg) intravenously after completion of the disulfiram breath test (see below). Anesthesia was induced with propofol (1–2.5 mg/kg) and fentanyl (50–100 μg), and succinylcholine was administered to facilitate tracheal intubation. Immediately after the trachea was intubated, anesthesia was maintained with sevoflurane (2.7% end-tidal concentration) in oxygen (2–5 l/min). Inspired and end-tidal sevoflurane concentrations were monitored continuously (Capnomac, Datex Medical Instrumentation, Tewksbury, MA). The inspired sevoflurane percentage was titrated to maintain the desired end-tidal sevoflurane concentration of 2.7% (1.3 MAC). Sevoflurane MAC (2.05 end-tidal percent[24]) was not adjusted for age. Use of muscle relaxants was avoided when possible. When muscle relaxation was necessary, patients received atracurium. No neuraxial local anesthetics or opioids were used intraoperatively. Sevoflurane 2.7% end-tidal concentration was maintained for 3 h, unless the duration of surgery was less than 3 h. For operations lasting longer than 3 h, the sevoflurane was discontinued after 3 h, total gas flows were increased to 6 l/min, and anesthesia was maintained with propofol, nitrous oxide, and fentanyl. Nitrous oxide was not started until the end-tidal sevoflurane concentration had decreased to less than 0.2%. Inspired and end-tidal sevoflurane concentrations were recorded at 15-min intervals while patients were intubated.

Venous blood samples for determination of blood sevoflurane concentration and plasma fluoride and HFIP concentrations were obtained at the times described previously.[4] Urine for determination of fluoride concentration was obtained before induction and for the following consecutive intervals beginning at the start of sevoflurane anesthesia: 0–12, 12–24, 24–36, 36–48, 48–72, and 72–96 h (or until the time of hospital discharge). Urine was thoroughly mixed, the volume was measured, and an aliquot was frozen at −20°C for later analysis.

Clinical evaluation included intraoperative and recovery room hemodynamics and laboratory evaluation (hematology, clinical chemistry, and urinalysis) performed before the study, baseline before anesthesia, 24 h after sevoflurane, and 96 h after sevoflurane (or before hospital discharge). Clinical evaluations also included three recovery indexes: emergence, command response, and orientation time. Emergence was defined as the time from the end of surgery to eye-opening in response to verbal command. Command response was the time from the end of surgery until the patient squeezed the observer's hand in response to command or demonstrated purposeful movement. Orientation time was recorded when the patient stated their name, birth date, and age. Clinical recovery indexes were judged by an independent observer who was not an investigator.

Analytical Methods

Compliance with disulfiram ingestion was assessed preoperatively before midazolam administration using a breath test for exhaled carbon disulfide.[25,26] Disulfiram is rapidly metabolized after absorption. After a single dose, major metabolites include carbon disulfide, diethyldithiocarbamate, and diethylamine.[27] Carbon disulfide is detectable in plasma, urine, and exhaled breath, and diethylamine is detected in plasma and urine. Concentrations of these metabolites peak approximately 12 h after disulfiram administration, permitting assessment of compliance 10 h after disulfiram dosing. Patients exhaled into two gas washing bottles connected in series. The first bottle contained phenolphthalein and 15 ml of 75 mM NaOH to trap carbon dioxide, and the second bottle contained 15 ml of modified McKees solution ((w/v) 5% diethylamine, 5% triethanolamine, 0.002% copper (II) acetate, and 10% isooctane in ethanol) to trap carbon disulfide. Patients exhaled until 1.1 mEq of expired carbon dioxide had been collected, indicated by a change in the first solution from pink to colorless. An unequivocal yellow color in the second bottle, due to copper diethyldithiocarbamate, was indicative of disulfiram ingestion and adequate absorption.

Eleven patients taking disulfiram had a positive breath test, and one disulfiram-treated patient had a negative breath test. Subsequent urine and blood tests for disulfiram metabolites were negative in this patient, who had severe diarrhea and, therefore, was excluded from the data analysis. No control patient exhibited a positive disulfiram breath test. Thus, data from 10 control and 11 disulfiram-treated patients were analyzed.

Analytical methods are described in the accompanying article.[4] Briefly, total HFIP (unconjugated HFIP and HFIP-glucuronide) in plasma and urine and blood sevoflurane concentrations were determined by gas chromatography with headspace sampling and flame ionization detection. Plasma and urine fluoride con-

Table 1. Patient Demographics

	Control (n = 10)	Disulfiram-treated (n = 11)
Age (yr)	44 ± 4 (23–68)	48 ± 4 (29–67)
Gender (M:F)	3:7	4:7
Weight (kg)	73 ± 5 (55–102)	79 ± 8 (45–118)
Body mass index*	26 ± 2 (22–35)	27 ± 1 (18–34)
Duration of surgery (h)	4.6 ± 0.5 (2.7–7.8)	4.5 ± 0.5 (2.7–8.1)
Blood loss (ml)	320 ± 140 (100–1,500)	230 ± 80 (50–1,000)

Values are mean ± SE (range). No significant differences were found between groups for any patient characteristic.

* Weight (kg)/height^2 (m^2).

centrations were measured using a fluoride-selective electrode.

Data Analysis

Anesthetic dose was calculated as the product of end-tidal sevoflurane concentration (expressed in MAC, uncorrected for age, where MAC = 2.05%) and time, determined in 2-min intervals until 10 min after incision and every 15 min thereafter, with total exposure expressed in MAC-hours. Sevoflurane dose also was calculated from total pulmonary anesthetic uptake and expressed in millimoles.[4]

Patients' demographic data, recovery indexes, and peak plasma fluoride and HFIP concentrations were analyzed by Student's unpaired *t* test. Blood sevoflurane concentrations, plasma fluoride, and HFIP concentrations and urine fluoride and HFIP excretion in the two groups were compared by two-way repeated-measures analysis of variance. Net plasma fluoride and HFIP concentrations were obtained by subtracting the preanesthetic baseline value from all subsequent values. Results are expressed as the mean ± SE.

Results

Patient demographic data are provided in table 1. Control and disulfiram-treated groups were similar with respect to age, weight, sex, duration of surgery, and surgical blood loss. Propofol doses were similar in both groups (table 2). Three methods were used to determine the dose of sevoflurane delivered: end-tidal sevoflurane concentrations, calculated total pulmonary uptake, and blood sevoflurane concentrations (table 2). All three methods showed that the sevoflurane dose was similar in both groups. Total sevoflurane dose was 3.7 ± 0.1 MAC-h in both control and disulfiram-treated patients, respectively ($P > 0.05$). Total pulmonary uptake was 88.8 ± 9.1 mmol in control patients and 98.8 ± 8.1 mmol in disulfiram-treated patients, respectively ($P > 0.05$). Blood sevoflurane concentrations in control and disulfiram-treated patients were not significantly different at any time, and areas under the curves were not significantly different (fig. 1).

Disulfiram treatment significantly diminished fluoride production, as assessed by plasma fluoride concentrations and urinary fluoride excretion. Measured plasma

Table 2. Anesthetic Exposure and Dose

	Control (n = 10)	Disulfiram-treated (n = 11)
Sevoflurane		
Exposure (min)	174 ± 4 (135–184)	171 ± 5 (136–180)
Dose (MAC-h)	3.7 ± 0.1 (3.0–4.2)	3.7 ± 0.1 (3.0–4.2)
Dose (mmol)	88.8 ± 9.1 (55.6–141.3)	98.9 ± 8.1 (46.7–136.2)
Propofol		
Induction dose (mg)	200 ± 30 (100–400)	202 ± 26 (100–400)
Infusion dose (mg)*	1,353 ± 286 (225–2,390)	2,220 ± 705 (393–4,358)
Infusion duration (min)*	109 ± 26 (15–240)	155 ± 33 (45–285)

Values are mean ± SE (range). No significant differences were found between groups for any variable.

* Propofol infusions were used after 3 h of sevoflurane exposure if the surgical duration exceeded 3 h (control, n = 7; disulfiram, n = 6).

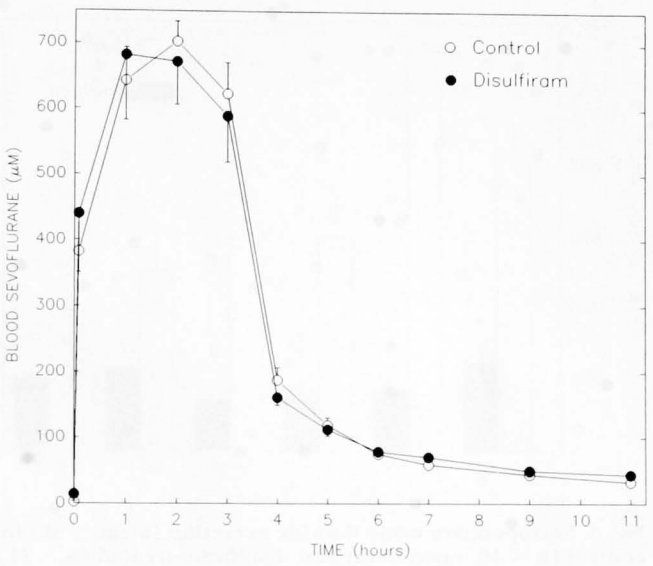

Fig. 1. Blood sevoflurane concentration (mean ± SE) in control (n = 10, open circles) and disulfiram-treated (n = 11, closed circles) patients. Sevoflurane was administered from 0 to 3 h, unless the surgical procedure ended before 3 h. Anesthetic concentrations in the two groups were not significantly different at any time. Areas under the curves were 2,790 ± 130 and 2,580 ± 280 μM · h, respectively, in control and disulfiram-treated patients ($P > 0.05$).

fluoride concentrations increased from a baseline of 2.1 ± 0.3 μM before anesthetic induction to a peak concentration of 36.2 ± 3.9 μM (range 23.3–61.5) in control patients (fig. 2). In disulfiram-treated patients, fluoride concentrations increased from 1.7 ± 0.2 μM (baseline) to a peak concentration of 17.0 ± 1.6 μM, which was significantly different from control patients ($P < 0.05$). Net peak plasma fluoride production, measured as the difference between peak and preanesthetic fluoride concentrations, was 34.0 ± 3.9 μM in control patients but significantly less (15.4 ± 1.5 μM) in disulfiram-treated patients ($P < 0.05$). Areas under the net plasma fluoride concentration-time curves were decreased to 22% of control patients by disulfiram pretreatment, from 1,110 ± 160 to 241 ± 72 μM · h. The time to peak plasma fluoride concentration was significantly shortened by disulfiram pretreatment (fig. 2). In contrast to control patients, in whom peak plasma fluoride concentrations occurred 2.0 ± 0.4 h after the end of sevoflurane administration, plasma fluoride concentrations in disulfiram-treated patients peaked almost immediately (0.1 ± 0.1 h) after cessation of sevoflurane ($P < 0.05$).

Urine fluoride excretion was similarly significantly diminished in disulfiram-treated patients. Urine fluoride excretion by disulfiram-treated patients was significantly less than that by control patients during all four 12-h collection periods (*i.e.*, days 1 and 2) after sevoflurane administration (fig. 3). There was a trend toward similarly diminished urine fluoride excretion in disulfiram-treated patients on days 3 and 4. However, the differences were not statistically significant. Cumulative 96-h fluoride excretion in disulfiram-treated patients was 1,080 ± 210 μmol, compared to 3,950 ± 560 μmol in control patients ($P < 0.05$). The mean decrease in urinary fluoride excretion (73%) in disulfiram-treated patients was similar to the decrease in area under the curve of net plasma fluoride concentration *versus* time (78%).

Disulfiram treatment also significantly attenuated production of HFIP, the other major metabolite of sevoflurane, as measured by plasma HFIP concentrations and urinary HFIP excretion. HFIP was undetectable in preanesthetic baseline samples in both patient groups.

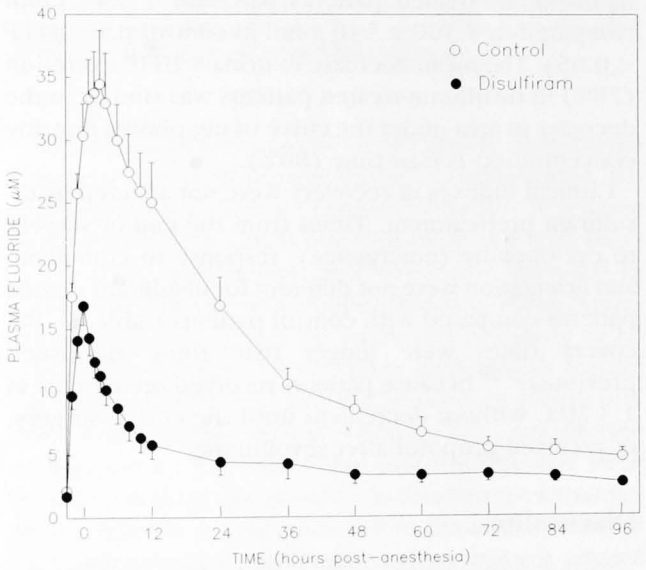

Fig. 2. Measured plasma fluoride concentrations (mean ± SE) in control (n = 10, open circles) and disulfiram-treated (n = 11, closed circles) patients. Sevoflurane was administered from 0 to 3 h, unless the surgical procedure ended before 3 h. Mean fluoride concentrations in control patients were significantly different from preanesthetic values at all times during sevoflurane exposure and through 48 h after exposure ($P < 0.05$). Mean fluoride concentrations in disulfiram-treated patients were significantly different from preanesthetic values at all times during sevoflurane and through 8 h after exposure ($P < 0.05$). Mean fluoride concentrations in disulfiram-treated patients were significantly different from those of control patients at all times during sevoflurane through 24 h after the end of anesthesia ($P < 0.05$). The number of patients remaining after day 2 decreased because of hospital discharge.

Whereas peak total plasma HFIP concentration was 39.8 ± 2.6 μM in control patients, peak plasma HFIP concentration was decreased to only 14.4 ± 1.1 μM by disulfiram pretreatment (fig. 4). Areas under the plasma HFIP concentration-time curves were decreased to 20% of control patients by disulfiram pretreatment, from 1,370 ± 110 to 268 ± 88 μM · h. Whereas HFIP concentrations peaked almost immediately (0.6 ± 0.4 h) after the end of sevoflurane anesthesia in disulfiram-treated patients, peak HFIP concentrations occurred 5.6 ± 0.8 h after sevoflurane exposure in control patients.

Urine HFIP excretion also was markedly reduced by disulfiram pretreatment. Urine HFIP excretion (fig. 5) by disulfiram-treated patients was significantly different from that by control patients for the first four 12-h collection periods. There was a trend toward similarly diminished urine HFIP excretion in disulfiram-treated patients on days 3 and 4, but the differences were not statistically significant. Cumulative 96-h HFIP excretion in disulfiram-treated patients was 960 ± 240 μmol compared to 4,300 ± 540 μmol in control patients ($P < 0.05$). The mean decrease in urinary HFIP excretion (78%) in disulfiram-treated patients was similar to the decrease in area under the curve of net plasma fluoride concentration *versus* time (80%).

Clinical indexes of recovery were not affected by disulfiram pretreatment. Times from the end of surgery to eye-opening (emergence), response to command, and orientation were not different for disulfiram-treated patients compared with control patients (table 3). Recovery times were longer than those published previously[7,28] because patients received sevoflurane at 1.3 MAC without decrement until the end of surgery, or received propofol after sevoflurane.

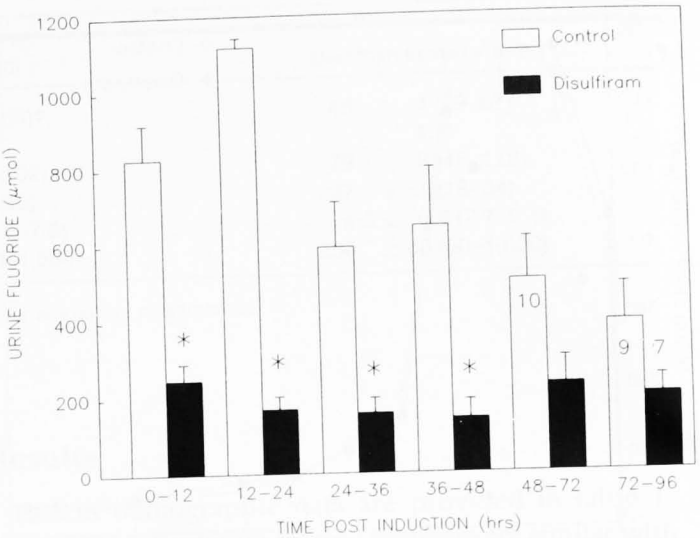

Fig. 3. Postoperative urine fluoride excretion (mean ± SE) in control (n = 10, open bars) and disulfiram-treated (n = 11, closed bars) patients. *Significantly different urine fluoride excretion in disulfiram-treated patients compared to control patients ($P < 0.05$). Control and disulfiram groups initially consisted of 10 and 11 patients, respectively, but the number of patients remaining (shown in or above the bar) after day 2 decreased because of hospital discharge.

Discussion

Patient pretreatment with a single dose of disulfiram before anesthesia resulted in significant inhibition of sevoflurane metabolism, evidenced by substantial (73–80%) reductions in plasma fluoride and HFIP concentrations and urinary fluoride and HFIP excretion. This effect of disulfiram could not be attributed to differences in sevoflurane dose, which was similar in both groups, as indicated by end-tidal sevoflurane concentrations, total pulmonary sevoflurane uptake, and blood sevoflurane concentrations. Rather, the data demonstrate that differences between groups were due to disulfiram inhibition of sevoflurane metabolism. Disulfiram inhibition of sevoflurane metabolism suggests that cytochrome P450 2E1 is a predominant P450 isoform catalyzing human sevoflurane metabolism *in vivo*.

We have shown previously that disulfiram is an effective inhibitor of human P450 2E1 activity *in vivo*.[22] A single oral dose of disulfiram (500 mg) administered 10 h before ingestion of chlorzoxazone, used as a specific noninvasive probe of hepatic P450 2E1 activity,[29] significantly diminished P450 2E1 activity *in vivo*, evidenced by an 85% decrease in chlorzoxazone 6-hydroxylation. Single-dose disulfiram administered the evening before surgery also significantly inhibited enflurane defluorination in patients receiving 3.9–4.1 MAC-h enflurane, as assessed by changes in plasma fluoride concentrations and urinary fluoride excretion.[23] Peak plasma fluoride concentrations were 24.3 ± 3.8 μM in untreated patients, whereas disulfiram treatment abolished the rise in plasma fluoride concentration. Fluoride excretion in disulfiram-treated patients was 62 ± 10 and 61 ± 12 μmol on days 1 and 2, respectively, compared to 1,094 ± 185 and 1,196 ± 223 μmol, respectively, in control patients.

Although the effectiveness of P450 2E1 inhibition *in vivo* by single-dose disulfiram has been established[22] and the selectivity of disulfiram toward P450 2E1 has been shown *in vitro*,[30] the absolute specificity of single-

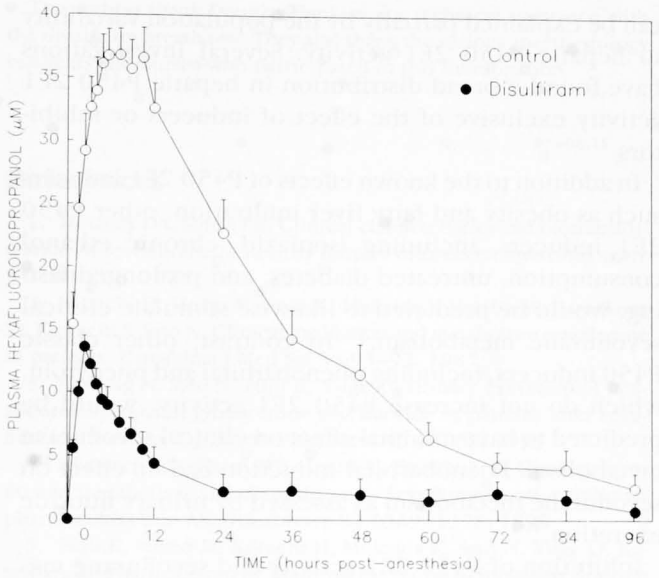

Fig. 4. Plasma total hexafluoroisopropanol (HFIP; unconjugated HFIP and HFIP-glucuronide) HFIP concentrations (mean ± SE) in control (n = 10, open circles) and disulfiram-treated (n = 11, closed circles) patients. Sevoflurane was administered from 0 to 3 h, unless the surgical procedure ended before 3 h. Mean total HFIP concentrations in control patients were significantly different from preanesthetic values at all times during sevoflurane anesthesia through 60 h after the end of anesthesia ($P < 0.05$). Mean total HFIP concentrations in disulfiram-treated patients were significantly different from preanesthetic values between 2 h after the start of anesthesia through 8 h after the end of anesthesia ($P < 0.05$). Mean HFIP concentrations in disulfiram-treated patients were significantly different from those of control patients at all times during sevoflurane anesthesia through 48 h after the end of sevoflurane exposure ($P < 0.05$). The number of patients remaining after day 2 decreased because of hospital discharge.

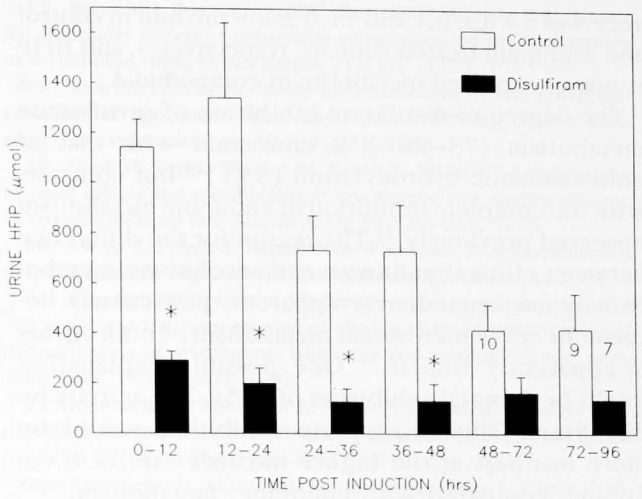

Fig. 5. Postoperative urine total hexafluoroisopropanol (HFIP; unconjugated HFIP and HFIP-glucuronide) excretion (mean ± SE) in control (n = 10, open bars) and disulfiram-treated (n = 11, closed bars) patients. *Significantly different urine fluoride excretion in disulfiram-treated patients compared to control patients ($P < 0.05$). Control and disulfiram groups initially consisted of 10 and 11 patients, respectively, but the number of patients remaining (shown in or above the bar) after day 2 decreased because of hospital discharge.

dose disulfiram toward P450 2E1 *in vivo* has not been demonstrated. Diethyldithiocarbamate, the active inhibitory metabolite of disulfiram, can inhibit *in vitro* human liver microsomal coumarin 7-hydroxylation, which is catalyzed by P450 2A6.[31] The influence, however, of disulfiram on human coumarin 7-hydroxylation *in vivo* has not been established. Furthermore, P450 2E1 is a major component of the P450s in the liver, whereas P450 2A6 represents less than 1% of total hepatic P450.[32] Thus, disulfiram-inhibitable metabolism is attributed predominantly but not necessarily exclusively to P450 2E1. Disulfiram inhibition of sevoflurane metabolism *in vivo* strongly supports a role for P450 2E1 but does not exclude the possible participation of other P450 isoforms.

Disulfiram inhibition of P450 2E1 activity diminished but did not abolish the metabolism of sevoflurane. Disulfiram-treated patients showed increases in plasma fluoride and HFIP concentrations compared with baseline values, and fluoride and HFIP were excreted in urine. The fluoride production observed in disulfiram-treated patients cannot be attributed primarily to alternative pathways of sevoflurane metabolism, further metabolism of HFIP to fluoride, or fluoride production from sources other than sevoflurane (*i.e.*, sevoflurane compound A).[33,34] This is because fluoride and HFIP production were comparable in disulfiram-treated patients, HFIP is not metabolized to fluoride by human liver microsomes,** HFIP elimination was not altered significantly by disulfiram pretreatment (HFIP clear-

Table 3. Patient Recovery Data

	Control (n = 10)	Disulfiram-treated (n = 11)
Emergence (min)	25 ± 6	16 ± 3
Command response (min)	25 ± 6	19 ± 4
Orientation (min)	37 ± 8	24 ± 4

Values are mean ± SE. No significant differences were found between groups for any recovery parameter.

** Kharasch ED: Unpublished data. 1995.

ance was 52.6 ± 6.1 and 71.0 ± 8.9 ml/min in control and disulfiram-treated patients, respectively), and HFIP is not an expected metabolite of compound A.

The degree of disulfiram inhibition of sevoflurane metabolism (73–80%) is consistent with that of chlorzoxazone hydroxylation (85%)[22] but contrasts with the complete inhibition of enflurane metabolism observed previously.[23] The reason for the difference between clinical enflurane and sevoflurane metabolism is not immediately apparent, particularly because *in vitro* microsomal metabolism of both agents is apparently similar.[21] One possible explanation might be subtotal inhibition of P450 2E1 activity by disulfiram. This incomplete inhibition would be more manifest at the higher intrinsic rate of sevoflurane, compared with enflurane, metabolism.[21,35] Alternatively, P450 isoforms other than P450 2E1 that are not inhibited by disulfiram may contribute to sevoflurane but not enflurane metabolism. Nevertheless, it appears that P450 2E1 is a predominant P450 isoform catalyzing human sevoflurane metabolism *in vivo*.

P450 2E1 participation in human sevoflurane metabolism *in vivo* mirrors the role of P450 2E1 in human liver microsomal sevoflurane defluorination *in vitro*.[21] These data provide validation for human microsomal sevoflurane metabolism *in vitro* as a model for sevoflurane biotransformation *in vivo*.

In addition to providing *in vitro-in vivo* correlations of metabolism, the current identification of P450 2E1 participation in human sevoflurane metabolism provides a mechanistic basis for several clinical observations regarding sevoflurane metabolism.[21] For example, Higuchi *et al.* reported that sevoflurane defluorination was significantly greater in obese than nonobese patients,[11] whereas Frink *et al.* found no relationship between obesity and sevoflurane metabolism.[12] Higuchi *et al.* suggested that the high incidence of hepatic fatty infiltration in their obese patients may account for the observed difference. Identification of the role of P450 2E1 in clinical sevoflurane metabolism corroborates this contention. Hepatic P450 2E1 content and P450 2E1-dependent anesthetic metabolism are substantially higher in livers with fatty infiltration compared to normal livers.†† The individual heterogeneity in sevoflurane metabolism observed currently and previously[1,5–10] can be explained partially by the population variability in hepatic P450 2E1 activity. Several investigations have found a broad distribution in hepatic P450 2E1 activity exclusive of the effect of inducers or inhibitors.[21,36–38]

In addition to the known effects of P450 2E1 inducers such as obesity and fatty liver infiltration, other P450 2E1 inducers, including isoniazid, chronic ethanol consumption, untreated diabetes, and prolonged fasting, would be predicted to likewise stimulate clinical sevoflurane metabolism.[39] In contrast, other classic P450 inducers, including phenobarbital and phenytoin, which do not increase P450 2E1 activity, would be predicted to have minimal effect on clinical sevoflurane metabolism. Phenobarbital induction had no effect on sevoflurane metabolism as assessed by urinary fluoride excretion.[40]

Inhibition of P450 2E1 activity and sevoflurane metabolism would not be of expected clinical consequence. We observed that clinical indexes of recovery were not influenced by the rate or extent of sevoflurane metabolism. This is consistent with the small extent of sevoflurane metabolism[1,4,41] and demonstrates that metabolism does not play a significant role in terminating the clinical effect of sevoflurane. Furthermore, no alternate pathways of sevoflurane metabolism have been identified toward which sevoflurane might be "switched" if the primary P450 2E1-dependent pathway was inactive.

Thus, even in patients with minimal or no hepatic metabolic capacity, such as those with diminished enzyme activity or with intrinsic liver disease, recovery from sevoflurane anesthesia should not be affected significantly. This has been confirmed clinically, whereby sevoflurane recovery was similar in healthy patients and those with hepatic disease.‡‡

Clinical consequences of P450 2E1 induction of sevoflurane metabolism and increased metabolite formation have not been fuily characterized. Obese patients demonstrated significantly greater sevoflurane metabolism than normal patients, with 11 of 15 obese patients exhibiting peak serum fluoride concentrations greater than 50 μM, but there were no abnormalities of renal function.[11] Other investigations have similarly shown no link between sevoflurane-dependent elevations in plasma fluoride concentration and renal dysfunction.[10,42]

In summary, we have shown that P450 2E1 is a predominant cytochrome P450 isoform responsible for clinical sevoflurane metabolism in humans.

†† Thummel KE: Unpublished observations. 1994.

‡‡ Frink E Jr: Personal communication. 1994.

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Effect on Left Anesth

Axel W. Goertz
Uwe Schirmer,

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* Staff Anesth

† Resident in

‡ Professor o

§ Professor o
Anesthesiology.

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