Compound A Uptake and Metabolism to Mercapturic Acids and 3,3,3-Trifluoro-2-fluoromethoxypropanoic Acid during Low-flow Sevoflurane Anesthesia

Biomarkers for Exposure, Risk Assessment, and Interspecies Comparison

Evan D. Kharasch, M.D., Ph.D.,* Carole Jubert, Ph.D.†

Background: Sevoflurane is degraded during low-flow anesthesia to fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether ("compound A"), which causes renal necrosis in rats but is not known to cause nephrotoxicity in surgical patients. Compound A is metabolized to glutathione S-conjugates and then to cysteine S-conjugates, which are N-acetylated to mercapturic acids (detoxication pathway), or metabolized by renal ß-lyase to reactive intermediates (toxification pathway) and excreted as 3,3,3-trifluoro-2-fluoromethoxypropanoic acid. This investigation quantified compound A metabolites in urine after low-flow sevoflurane administration, to assess relative flux via these two pathways.

Methods: Patients (n = 21) with normal renal function underwent low-flow (1 L/min) sevoflurane anesthesia designed to maximize compound A formation. Inspiratory, expiratory, and alveolar compound A concentrations were quantified. Urine mercapturic acids and 3,3,3-trifluoro-2-fluoromethoxypropanoic acid concentrations were measured by gas chromatography and mass spectrometry.

Results: Sevoflurane exposure was 3.7 ± 2.0 MAC-h. Inspired compound A maximum was 29 ± 14 ppm; area under the inspired concentration versus time curve (AUC_{\text{insp}}) was 78 ± 58 ppm·h. Compound A dose, calculated from pulmonary uptake, was 0.39 ± 0.35 mmol (4.8 ± 4.0 μmol/kg) and correlated with AUC_{\text{insp}} (r² = 0.84, P < 0.001). Mercapturic acid excretion was complete after 2 days, whereas 3,3,3-trifluoro-2-fluoromethoxypropanoic acid excretion continued for 3 days in some patients. Total (3-day) mercapturates and fluoromethoxypropanoic acid excretion was 95 ± 49 and 294 ± 416 μmol, respectively (1.2 ± 0.6 and 3.6 ± 5.0 μmol/kg).

Conclusion: Compound A doses during 3.7 MAC-h, low-flow sevoflurane administration in humans are substantially less than the threshold for renal toxicity in rats (200 μmol/kg). Compound A metabolites quantification may provide a biomarker for compound A exposure and relative metabolism via toxification and detoxication pathways. Compared with previous investigations, relative metabolic flux (fluoromethoxypropanoic acid/mercapturates) through the toxification pathway was sixfold greater in rats than in humans. Species differences in dose and metabolism may influence compound A renal effects. (Key words: Anesthetic degradation; haloalkene; ß-lyase; glutathione; nephrotoxicity.)

SEVOFLURANE undergoes base-catalyzed degradation in carbon dioxide absorbents to the haloalkene fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether ("compound A"), which occurs in higher amounts during low-flow anesthesia. Typically of numerous haloalkenes, compound A causes nephrotoxicity in rats, specifically corticomedullary proximal tubular necrosis, accompanied by proteinuria, glucosuria, and enzurminia. The threshold for renal tubular necrosis in rats (250 g) is generally accepted to be 290-340 ppm·h. There are, however, species differences in the threshold for compound A nephrotoxicity. Monkeys showed histologic and biochemical evidence of renal toxicity at 800 ppm·h but not 600 ppm·h. Swine showed no renal tubular cell necrosis after 612 ppm·h, and a threshold for toxicity has not been established. In humans, numerous investigations have evaluated renal function after surgical anesthesia with low-flow sevoflurane, in which maximum inspired compound A concentrations averaged 8-24 and 20-32 ppm with soda lime and barium hydroxide lime, respectively, and exposures were as high as 400 ppm·h. Using standard clinical (creatinine, blood urea nitrogen, serum electrolytes) and validated biomarkers (N-acetyl-proline, mercapturates, urinary kynurenine, and 2-phenylacetate), we found no evidence of renal toxicity with low-flow sevoflurane.
Glutathione-dependent metabolism of compound A (FDVE). Identities of reactive intermediates are postulated, but the thionoacyl fluoride is the most likely intermediate formed by β-lyase-catalyzed S-conjugates metabolism.
Materials and Methods

Clinical Protocol
Twenty-one patients who were American Society of Anesthesiologists physical status I-III, without history of hepatic or renal disease, and undergoing anesthesia for elective noncardiac and nonaortic surgery with planned duration > 2 h were studied. Patients (5 men, 16 women) aged 49 ± 12 yr (range, 30-69 yr) and weighing 76 ± 12 kg (range, 52-97) were studied. The investigation was approved by the Institutional Review Board, and all patients provided written informed consent. The anesthetic protocol was designed to maximize compound A formation (fresh barium hydroxide lime, total gas flow rate 1 l/min, and high sevoflurane concentrations, achieved by precluding opioids [except 50-150 µg fentanyl for induction], nitrous oxide, and intraoperative neuraxial opioids and local anesthetics). End-tidal anesthetic concentrations were monitored continuously (Capnomac, Datex Medical Instrumentation, Tewksbury, MA). Respiratory gas samples were obtained using gastight syringes, from the inspiratory (F\textsubscript{i}) and expiratory (F\textsubscript{e}) limbs of the anesthesia circuit adjacent to the respective valves, and from a catheter positioned coaxially in the endotracheal tube with the tip protruding just beyond the tube orifice after ventilation was held for 30 s (alveolar samples, F\textsubscript{A}). Samples were obtained after intubation, 5, 10, 15, 30, 60, 90, and 120 min after the start of low-flow anesthesia, hourly thereafter, and at the end of low-flow anesthesia. Urine was collected in 24-h analysis. Additional details regarding the experimental protocol, and results of renal function evaluations in a multicenter superset of these patients, have been published previously.

Data in this investigation represent additional evaluations performed at the time of the original investigation, and subsequent laboratory studies, on the University of Washington cohort from the previous investigation.

Analytical Methods
Compound A concentrations in respiratory gas samples were determined by gas chromatography with flame ionization detection, as described previously.

Urine concentrations of N-acetyl-S-(1,1,3,3,3-pentafluoro-2-fluoromethoxypropyl)-L-cysteine (6% and 5% at 0.9 and 46 µg/ml), N-acetyl-S-(1-fluoro-2-fluoromethoxy)-2-(trifluoromethyl)-vinyl)-L-cysteine (6% and 2% at 1.1 and 54 µg/ml), 3,3,3-trifluoro-2-(fluoromethoxy)propanoic acid, and trifluoroacetic acid were determined by GC/MS as described previously. Briefly, the internal standards N-acetyl-S(2,2-difluoro-vinyl)-L-cysteine (1.25 µg) or dichloroacetic acid (159 ng) were added to urine (0.1-1 ml) that was acidified and extracted with diethyl ether. Samples were derivatized with diazomethane or diphenyl-diazomethane for mercapturic acid or fluoropropionic acid analysis, respectively, and analyzed by GC/MS. Analyses were performed on a Hewlett-Packard 5890 Series II GC-5972 mass selective detector, using a DB-17 column (30 m x 0.52 mm x 0.5 µ) (J & W Scientific, Folsom, CA). The injector and detector temperatures were 250°C and 300°C, respectively, and the column head pressure was 5 psi for all assays. For mercapturates analysis, the oven was at 35°C for 5 min, increased 5°C/min to 210°C, then at 20°C/min to 280°C and held for 7 min. Selected-ion monitoring was used to quantify the methyl esters of N-acetyl-S-(1-fluoro-2-fluoromethoxy-2-(trifluoromethoxymethyl)vinyl)-L-cysteine (m/z 236), N-acetyl-S-(1,1,3,3,3-pentafluoro-2-fluoromethoxypropyl)-L-cysteine (m/z 256), and N-acetyl-S-(2,2-difluoro-vinyl)-L-cysteine (m/z 196). For analysis of 3,3,3-trifluoro-2-fluoromethoxypropanoic acid and trifluoroacetic acid, the oven was at 35°C for 5 min, increased 20°C/min to 250°C, then at 10°C/min to 280°C, and held for 10 min. Selected-ion monitoring was used for diphenylmethy1 esters of 3,3,3-trifluoro-2-fluoromethoxypropanoic (m/z 342), trifluoroacetic (m/z 310), and dichloroacetic (m/z 294) acids.

Calibration standards containing N-acetyl-S-(1,1,3,3,3-pentafluoro-2-fluoromethoxypropyl)-L-cysteine (0.5-116 µg/ml), N-acetyl-S-(1-fluoro-2-fluoromethoxy)-2-(trifluoromethyl)-vinyl)-L-cysteine (0.5-134 µg/ml), 3,3,3-trifluoro-2-(fluoromethoxy)propanoic acid (1-50 µg/ml), and trifluoroacetic acid (0.1-10 µg/ml) were prepared daily using blank urine. Standard curves of peak area ratios were linear over the concentrations used (r² > 0.99 for all analytes), and the limit of quantification was defined as the lowest point on the standard curve. Interday coefficients of variation were: N-acetyl-S-(1,1,3,3,3-pentafluoro-2-fluoromethoxypropyl)-L-cysteine (5% and 5% at 0.9 and 46 µg/ml), N-acetyl-S-(1-fluoro-2-fluoromethoxy)-2-(trifluoromethyl)-vinyl)-L-cysteine (6% and 2% at 1.1 and 54 µg/ml), 3,3,3-trifluoro-2-(fluoromethoxy)propanoic acid (6% at 2.5 and 25 µg/ml), and trifluoroacetic acid (6% and 4% at 0.5 and 25 µg/ml).

Data Analysis
Sevoflurane exposure was calculated as the area under the end-tidal concentration versus time curve, determined in 15-min intervals. Compound A exposure was similarly calculated as the product of inspiratory concentration and
time. To permit comparison with the previously published data, the 5-, 10-, and 15-min samples were not used for this calculation. The dose of compound A actually administered was taken as compound A uptake, calculated as described previously for sevoflurane and other anesthetics. Uptake rate was calculated as

$$V_\text{pul} = V_\text{E} \cdot (F_i - F_m)$$

where $V_\text{pul}$ is the total pulmonary uptake rate (L compound A vapor/min), $V_\text{E}$ was the minute ventilation (l/min), and $F_i$ and $F_m$ were the inspired and mixed expired compound A concentrations, respectively, determined at each measurement interval. $F_m$ was calculated according to

$$F_m = (f_A \cdot F_A) + (f_{D} \cdot F_D)$$

where $f_A$ and $f_D$ represent the fraction of alveolar and dead space ventilation, respectively. Values for $f_A$ and $f_D$ during mechanical ventilation were taken as 0.5 each. Mixed-expired gas samples are conventionally obtained by placing a mixing box in the expiratory limb of the anesthesia circuit. Preliminary studies showed that sevoflurane concentrations in mixed expired samples obtained distal to a 3-l mixing box were not substantially different than those measured at the end of the circuit adjacent to the expiratory valve without the box (not shown); thus, the mixing box was not subsequently used to obtain mixed-expired compound A samples. $V_\text{pul}$ was therefore also calculated using $F_i$ for $F_m$, which provided an upper bound (more conservative estimate) of $V_\text{pul}$. Values for total pulmonary compound A uptake (l/min) were converted to mol/min by application of the general gas equation. The sum of the products of pulmonary compound A uptake rate and exposure time for each interval gave the total dose in moles.

Results are expressed as the mean ± SD. Correlations were analyzed by linear regression analysis. Statistical significance was assigned at $P < 0.05$.

**Results**

*Compound A Concentrations*

Compound A was readily detected at the first sampling time, 5 min after the start of low-flow anesthesia. Mean concentrations are shown in figure 2A. Concentrations in most patients plateaued after approximately 30 min (shown previously). They continued to increase in three patients, two of whom underwent laparoscopic procedures with $CO_2$ insufflation and who also received much higher sevoflurane concentrations (3.0%, 3.1%, and 2.6%) compared with all others (mean, 1.9%), which influenced the mean values. Expired and alveolar compound A concentrations were significantly correlated (slope = 0.77, $r^2 = 0.75$, $P < 0.001$, data not shown), although there was greater variability in the latter measurement (fig. 2A), possibly reflecting the fact that mechanical ventilation, but not fresh gas flow, was interrupted during alveolar sampling. Compound A uptake was approximated from the $F_i/F_m$ ratio (fig. 2B). The ratio rapidly reached equilibrium; 5-min values were 91% and 87% of those at 30 and 60 min, respectively.
Although the equilibrium ratio was only 0.64 at 2 and 3 h. Similar results were obtained using the more conventional $F_A/F_I$ ratio, although variability was greater because of the greater variability in $F_A$ (not shown).

Compound A exposure and estimated dose are provided in Table 1. Exposure, measured as the inspired AUC (AUCinsp), was $78 \pm 58$ ppm·h (range, 10–223). Total dose, estimated from the pulmonary uptake rate, and conventionally calculated using $F_A$ and $F_I$ to determine $F_M$, was $0.23 \pm 0.22$ mmol. Uptake was also calculated using $F_K$ as the value for $F_K$ (0.39 ± 0.35 mmol; 4.8 ± 4.0 μmol/kg). This method avoids potential sampling confounders, makes no assumptions about the proportions of alveolar and dead space ventilation, does not consider any compound A adsorption by the anesthesia circuit, and provides a more conservative (higher) estimate for dose. There was a significant correlation ($r^2 = 0.84, P < 0.001$) between this dose and compound A exposure measured as AUCinsp (Fig. 3A) or as AUCinsp · exp ($r^2 = 0.78, P < 0.001$, data not shown). A similar correlation was also observed ($r^2 = 0.84$) using the lower estimate for compound A dose and AUCinsp (not shown). The higher estimate for compound A dose (0.39 ± 0.35 mmol) is used in the remainder of this report. There was also a significant correlation ($r^2 = 0.76, P < 0.001$) between compound A dose and sevoflurane exposure (MAC-h; Fig. 3B).

**Compound A Metabolites Excretion**

Excretion of the mercapturic acids N-acetyl-S-(1-fluoro-2-fluoromethoxy-2-(trifluoromethyl)vinyl)-L-cysteine and N-acetyl-S-(1-fluoro-2-fluoromethoxy-2-(trifluoromethyl)vinyl)-L-cysteine in the urine of patients exposed to compound A during low-flow sevoflurane was identified and quantified. An extracted, methylated urine sample (Fig. 4A) showed peaks at 35.2 and 35.5 min that were not present in preanesthesia urine (not shown). Mass spectra of these

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**Table 1. Patient Demographics and Sevoflurane Exposure**

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Value</th>
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<tr>
<td>Duration of anesthesia (h)</td>
<td>3.5 ± 1.3 (1.4–7.3)</td>
</tr>
<tr>
<td>Sevoflurane exposure (MAC-h)</td>
<td>3.7 ± 2.0 (1.2–8.5)</td>
</tr>
<tr>
<td>Compound A inspired maximum (ppm)</td>
<td>29 ± 14 (10–67)</td>
</tr>
<tr>
<td>Compound A inspired AUC ($\text{ppm} \cdot \text{h}$)</td>
<td>78 ± 58 (10–223)</td>
</tr>
<tr>
<td>Compound A inspired–expired AUC ($\text{ppm} \cdot \text{h}$)</td>
<td>26 ± 16 (4–64)</td>
</tr>
<tr>
<td>Compound A uptake ($\text{mmol}$)†</td>
<td>0.39 ± 0.35 (0.06–1.66)</td>
</tr>
<tr>
<td>Compound A uptake ($\mu\text{mol/kg}$)</td>
<td>4.8 ± 4.0 (0.8–19.0)</td>
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</table>

Values are mean ± SD (range).

MAC = minimum alveolar concentration; AUC = area under the curve.

† Calculated using $F_K$ for $F_M$. Uptake conventionally calculated using $F_A$ and $F_I$ to determine $F_M$ was $0.23 \pm 0.22$ mmol.

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Fig. 3. Relationship between compound A dose, and exposure to compound A (A) or sevoflurane (B). Compound A dose was estimated from pulmonary uptake and exposure from the area under the curve of inspired compound A concentration versus time (AUCinsp). Sevoflurane exposure was determined from end-tidal MAC-h. Each data point represents one patient. The patient with the highest compound A exposure underwent head-up laparoscopic cholecystectomy with intraabdominal CO2 insufflation. The high measured tidal volumes (8–11 l/min, uncorrected for circuit compliance) likely overestimated effective alveolar ventilation, thereby overestimating compound A dose. Correlations without data from this patient are shown by the dotted line ($r^2 = 0.92$ for compound A exposure; $r^2 = 0.77$ for sevoflurane exposure).
peaks are shown in figures 4B and 4C. Diagnostic ions and corresponding fragments were m/z 278 ([M-COOCX₃J]⁺) and 256 ([M-COOCX₃COCH₃]⁺) for the alkene; m/z 298 ([M-COOCX₂COCH₃]⁺), 256 ([M-COOCX₂COCH₃]⁺) and 176 ([C₆H₁₅NO₃S]⁺) for the alkane. Characteristic mercapturate fragments (m/z 144, C₆H₁₁NO₂S and 88 C₆H₁₅NO₂) were also observed. Based on similarities of retention time and mass spectra to those of the synthetic compound A mercapturic acids, the excreted compounds were identified as N-acetyl-S-(l,1,3,3,3-pentafluoro-2-fluoromethoxypropyl)-L-cysteine and N-acetyl-S-(l-fluoro-2-fluoromethoxy-2-(trifluoromethyl)vinyl)-L-cysteine. No attempts were made to resolve and separately quantify the (E)- and (Z)-isomers of N-acetyl-S-(l-fluoro-2-fluoromethoxy-2-(trifluoromethyl)vinyl)-L-cysteine.

Excretion of 3,3,3-trifluoro-2-fluoromethoxypropanoic acid, which results from renal β-lyase-mediated metabolism of compound A cysteine S-conjugates, was also identified and quantified. An extracted, derivatized urine sample (fig. 5A) showed a peak at 16.4 min that was not present in urine obtained before anesthesia (not shown). The mass spectrum of this peak (fig. 5B) is identical to that of the synthetic, derivatized acid (not shown). Concentrations ranged from 0.5 to 79 µg/ml. 3,3,3-Trifluoro-2-fluoromethoxypropanoic acid has been reported to decompose to trifluorolactic acid in vitro and in vivo, and excretion of this acid would also represent compound A cysteine S-conjugates metabolism by β-lyase. Trifluorolactic acid was detected in urine (fig. 5A, 16.2 min), based on selected-ion monitoring, although concentrations were low (0–1.1 µg/ml), highly variable, and insufficient to obtain full scan mass spectra of the excreted compound. Identification, therefore, was based on selected-ion monitoring of diagnostic ions, as described previously.

Daily excretion of mercapturic acids and β-lyase-de-
CLINICAL UPTAKE AND METABOLISM OF COMPOUND A

Fig. 5. GC/MS analysis of organic fluoroacids, derived from β-lyase-catalyzed metabolism of compound A S-conjugates, in human urine. (A) Selected-ion mode chromatograph (m/z 342, 310, 294) of a diphenyldiazomethane-derivatized extract of urine from a patient undergoing low-flow sevoflurane. The 3,3,3-trifluoro-2-(fluoromethoxy)propanoic acid and trifluorolactic acid peaks at 16.4 and 16.2 min, respectively, were not present in urine obtained before anesthesia (not shown). (B) Mass spectrum of the 16.4-min peak in (A), which was identical to synthetic 3,3,3-trifluoro-2-(fluoromethoxy)propanoic acid diphenylmethyl ester.

Table 2. Daily Excretion of Mercapturic Acids and β-Lyase-derived Fluoroacid Metabolites in Urine after Low-flow Sevoflurane

<table>
<thead>
<tr>
<th>Excretion (μg/day)</th>
<th>0–24 h</th>
<th>24–48 h</th>
<th>48–72 h</th>
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<tbody>
<tr>
<td>Alkane mercapturic acid</td>
<td>49.8 ± 35.2</td>
<td>3.6 ± 9.4</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Alkane mercapturic acid</td>
<td>39.6 ± 22.8</td>
<td>2.2 ± 4.8</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>3,3,3-Trifluoro-2-(fluoromethoxy)propanoic acid</td>
<td>167 ± 232</td>
<td>97 ± 219</td>
<td>36 ± 58</td>
</tr>
<tr>
<td>Trifluorolactic acid</td>
<td>1.2 ± 3.2</td>
<td>0.3 ± 0.9</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>
greater than would have been predicted by extrapolating linearly from time zero to the first 0.5–2 h measurement. One other investigation also detected compound A immediately after the onset of low-flow anesthesia. These results show that many previous investigations have somewhat underestimated actual compound A exposure, with the underestimate greater for those measuring compound A beginning at 2 h.

Compound A uptake, estimated from the FE/FI ratio, was rapid. The FE/FI ratio of 0.64 observed presently is similar to that previously described by Frink et al. It is also consistent with the FET/FI ratio of 0.8 reported to be achieved after 1–2 h, in which end-tidal gas samples were used to estimate alveolar compound A concentrations. The rapid increase in FET/FI ratio is consistent with the low solubility of compound A (blood:gas partition coefficient of 0.31). However, the equilibrium value is lower than the solubility would predict. As identified earlier, this may relate to compound A reactivity with blood and plasma constituents, most likely protein thiols, and possibly also reactivity with tissue thiols.

The dose of compound A taken up can be compared with exposure to compound A and to sevoflurane. Compound A exposure (measured as AUC0→∞) was an excellent predictor of compound A dose (measured from inspired and expired compound A concentrations and minute ventilation). Low-flow sevoflurane exposure (end-tidal MAC-h) was also an excellent predictor of the compound A dose (approximately 0.1 mmol compound A per MAC-h low-flow [1 l/min] sevoflurane). These relationships can be used to estimate compound A doses in other investigations in which it is not measured directly.

Low-flow sevoflurane in this investigation (3.7 ± 0.2 MAC-h) resulted in a compound A dose of 0.39 ± 0.35 mmol. Previously, a similar sevoflurane exposure (3.7 ± 0.3 MAC-h) resulted in a sevoflurane dose of 88.8 ± 28.8 mmol. Assuming that the same sevoflurane exposure in the present investigation resulted in similar sevoflurane uptake, the compound A dose was approximately 1/200 that of sevoflurane. This finding is consistent with several investigations showing that inspired compound A concentrations are approximately 1/500–1/1,000 those of sevoflurane, and with the solubility and FE/FI ratio for compound A.

Compound A doses administered during low-flow anesthesia in humans can be compared with those causing nephrotoxicity in rats. Although animal investigations using inhalation exposure do not permit assessment of the actual compound A dose administered, doses are known when intraperitoneal injection is used. The nephrotoxic threshold in rats was 200 μmol/kg intraperitoneal compound A, which produced histologic and biochemical alterations similar to those elicited by the inhaled threshold of 290-340 ppm · h. The compound A dose received by patients undergoing 3.7 ± 0.2 MAC-h low-flow sevoflurane (4.8 ± 4.0 μmol/kg) is substantially less than the nephrotoxic threshold in rats (200 μmol/kg).

**Compound A Metabolism**

Results of this investigation provide clear GC/MS identification and quantitation of compound A alkane and alkene mercapturic acids and 3,3,3-trifluoro-2-fluoromethoxypropanoic acid excretion in urine. They confirm qualitative NMR and selected-ion mode GC/MS identifications published previously. These results demonstrate that compound A undergoes metabolism in humans *via* a complex bioactivation scheme involving the concerted participation of several organs and enzyme systems, collectively referred to as the β-lysase pathway. Specifically, identification of the mercapturic acids N-acetyl-S-(1,1,3,3,3-pentafluoro-2-fluoromethoxypropyl)-l-cysteine and N-acetyl-S-(1-fluoro-2-fluoromethoxy-2-(trifluoromethyl)vinyl)-l-cysteine demonstrates that compound A undergoes metabolism to the

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**Fig. 6. Cumulative daily postoperative excretion of mercapturic acids and β-lysase-derived fluoroacid metabolites in urine after low-flow sevoflurane.** Open bars represent the alkane mercapturate N-acetyl-S-(1,1,3,3,3-pentafluoro-2-fluoromethoxypropyl)-l-cysteine, striped bars represent the alkene mercapturate N-acetyl-S-(1-fluoro-2-fluoromethoxy-2-(trifluoromethyl)vinyl)-l-cysteine, cross-hatched bars represent 3,3,3-trifluoro-2-(fluoromethoxy)propanoic acid, and solid bars represent trifluorolactic acid.
glutathione S-conjugates S-[1,1-difluoro-2-fluoromethoxy-2-(trifluoromethyl)ethyl]glutathione and S-[1-fluoro-2-fluoromethoxy-2-(trifluoromethyl)vinyl]glutathione, which in turn are cleaved to the corresponding cysteine S-conjugates S(1,1,3,3,3-pentafluoro-2-fluoromethoxypropyl)-cysteine and S(1-fluoro-2-fluoromethoxy-2-(trifluoromethyl)vinyl)-cysteine, which undergo subsequent N-acetylation. Slightly greater amounts of N-acetyl-S(1,1,3,3,3-pentafluoro-2-fluoromethoxypropyl)-cysteine compared with N-acetyl-S(1-fluoro-2-fluoromethoxy-2-(trifluoromethyl)vinyl)-cysteine were excreted. It is not known, at present, whether this represents differences in the biosynthesis of the alkane and alkene glutathione S-conjugates, or in their subsequent interorgan transport and processing. Identification of 3,3,3-trifluoro-2-fluoromethoxypropanoic acid demonstrates that the cysteine S-conjugates undergo metabolism by renal cysteine conjugate β-lyase in vivo to reactive intermediates, which are subsequently hydrolyzed. Because 3,3,3-trifluoro-2-fluoromethoxypropanoic acid is a common product of both S-conjugates metabolism, the present results do not indicate whether one, or both, cysteine S-conjugates are metabolized by β-lyase. 3,3,3-Trifluoro-2-fluoromethoxypropanoic acid has been reported to be unstable, degrading to trifluorolactic acid. 24 Quantification of this degradation product in vivo might be important, because it would also represent cysteine S-conjugates metabolism by β-lyase. Trifluorolactic acid was rarely observed, however, and never accounted for more than a small fraction of the 3,3,3-trifluoro-2-fluoromethoxypropanoic acid excreted in human urine. Assay sensitivity does not appear to explain this result, as recovery averaged 95% and 0.1 μg/ml was readily detectable. Trifluorolactic acid excretion in human urine after low-flow sevoflurane was identified previously. 22 Excretion was not quantified, but qualitatively appeared small relative to that of the mercapturates and 3,3,3-trifluoro-2-fluoromethoxypropanoic acid. 22 Thus, although 3,3,3-trifluoro-2-fluoromethoxypropanoic acid decomposition to trifluorolactic acid readily occurred in vivo, this finding does not appear quantitatively significant in humans in vivo.

Relative cysteine S-conjugates metabolism by β-lyase versus N-acetylation can be assessed by comparing cumulative excretion of β-lyase-dependent fluorocarbons (3,3,3-trifluoro-2-(fluoromethoxy)propanoic acid plus trifluorolactic acid) and mercapturic acids (alkane plus alkene). The ratio of fluorocarbons to mercapturates was approximately 1.5:1 after 1 day (determined to permit comparison with animal data, see below) and 3:1 after 5 days.

Although haloalkene metabolism and toxification by the glutathione-dependent β-lyase pathway has been amply demonstrated in animals, relatively few haloalkenes have been shown to undergo metabolism by this route in humans, and even less quantitative information is available. Urinary excretion of the mercapturic acid metabolites of trichloroethene 30-32 and tetrachloroethene 33-35 was recently shown in humans receiving occupational or deliberate exposure, demonstrating the biosynthesis of glutathione- and cysteine S-conjugates. Urinary excretion of chloroacetic acid after trichloroethene 36 and dichloroacetic acid after tetrachloroethene 35 showed that β-lyase-dependent cysteine S-conjugates metabolism can also occur in humans. Compound A appears to be the first fluoroalkene shown to undergo metabolism in humans by the glutathione-dependent β-lyase pathway, the first fluoroalkene in which metabolism by N-acetylation and renal β-lyase has been quantified and may be an excellent probe to explore the toxicologic significance of these pathways in humans.

Limited comparisons of quantitative metabolism of compound A and other haloalkenes in humans are available. After occupational exposure to 200-400 ppm · h tetrachloroethene, mercapturic acid excretion in spot urine samples was 0.010-0.015 nmol/mg creatinine. 42 In volunteers exposed to 60, 120, or 240 ppm · h tetrachloroethene, cumulative 35-h mercapturic acid excretion was 45 ± 12, 142 ± 14, and 211 ± 46 nmol, or approximately 0.045, 0.14, and 0.21 nmol/mg creatinine (assuming 1 g/day creatinine excretion), and appeared essentially complete after 35 h. 13 After 240, 480, and 960 ppm · h volunteer exposure to trichloroethene, cumulative 48-h excretion of mercapturic acids was 250 ± 40, 370 ± 30, and 430 ± 10 nmol, or approximately 0.25, 0.37, and 0.43 nmol/mg creatinine. 40 After trichloroethene intoxication, mercapturic acid excretion on days 1-3 was 0.26, 0.42, and 1.25 nmol/mg creatinine. 41 In the present investigation after 78 ± 58 ppm · h compound A, mercapturates excretion was 59 ± 34, 3.5 ± 8.2, and 0 nmol/mg creatinine on days 1-3. Thus mercapturates of compound A were excreted as fast or faster than those of other haloalkenes. Quantitatively, excretion of compound A mercapturates (nmol/kg) also appeared greater than that of other haloalkenes, likely reflecting the fact that these other haloalkenes also undergo considerable cytochrome P450-mediated metabolism, whereas compound A undergoes comparably less P450-mediated metabolism. 58 We find only one investigation that measured urinary excretion of β-lyase-dependent haloacid metabolites after haloalkene exposure in humans. Vökel et al. found only traces of...
These in vivo results are consistent with previous in vitro data showing that β-lyase-catalyzed metabolism of compound A cysteine-S-conjugates was 8- to 30-times greater in rat compared with human kidneys.23

In summary, this investigation quantified human exposure and dose of compound A, as well as compound A S-conjugates metabolism by N-acetylation (detoxication) and β-lyase (toxication), and compared them to previous results in rats, which appear more susceptible to compound A nephrotoxicity. Humans, compared with rats, receive markedly lower doses of compound A and metabolize a lower fraction by renal β-lyase than by N-acetylation. These species differences may influence compound A renal effects.

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