Anesthesiology
1998; 89:1174–83

Fate and Toxicity of 2-(Fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (Compound A)-derived Mercapturates in Male, Fischer 344 Rats

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Background: **2-(Fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (compound A) is formed in the anesthesia circuit by the degradation of sevoflurane. Compound A is nephrotoxic in rats and undergoes metabolism by the mercapturic acid pathway in rats and humans to yield the mercapturates *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine (compound 3) and *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine (compound 5). These experiments were designed to examine the fate and nephrotoxicity of compound A–derived mercapturates in rats.**

Methods: **The deacetylation of compounds 3 and 5 by human and rat kidney cytosol and with purified acylases I and III was measured, and their nephrotoxicity was studied in male Fischer 344 rats. The metabolism of the deuterated analogs of compounds 3 and 5, [*acetyl*-$^2H_3$]*S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine (compound 3-d_3) and [*acetyl*-$^2H_3$]*S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine (compound 5-d_3), respectively, was measured.**

Results: **Compound 5, but not compound 3, was hydrolyzed by human and rat kidney cytosols and by acylases I and III. ^{19}F nuclear magnetic resonance spectroscopic analysis showed no urinary metabolites of compound 3, but unchanged compound 5 and its metabolites 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid and 2-[1-(fluoromethoxy)-2,2,2-trifluoroethyl]-4,5-dihydro-1,3-thiazole-4-carboxylic acid were detected in urine. Compound 5 (250 μM/kg) produced clinical chemical and morphologic evidence of renal injury in two of three animals studied.**

Conclusions: **Compounds 3 and 5 underwent little metabolism. Compound 5, but not compound 3, was mildly nephrotoxic. These results indicate that compound A–derived mercapturate formation constitutes a detoxication pathway for compound A. (Key words: Acylase; β-lyase; nephrotoxicity.)**

2-(Fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (compound A; fig. 1, compound 1) is the major degradation product of sevoflurane formed in anesthesia circuits in the presence of soda lime or Baralyme (Chemetum, St. Louis, MO).[1–3] Compound A is nephrotoxic when given by inhalation or intraperitoneally to rats.[4–9] It undergoes glutathione-dependent metabolism to yield glutathione *S*-conjugates, which are hydrolyzed by γ-glutamyltransferase and dipeptidases to give the cysteine *S*-conjugates *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine (fig. 1, compound 2) and *S*-[2-fluoromethoxy-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine (fig. 1, compound 4).[10] Compounds 2 and 4 undergo β-lyase–dependent biotransformation to nephrotoxic metabolites.[9–12] The compound A–derived mercapturates *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine (fig. 1, compound 3) and *S*-[2-fluoromethoxy-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine (fig. 1, compound 5) are excreted in the urine of rats given compound A and in the urine of humans anesthetized with sevoflurane.[10,12]

Mercapturates of several haloalkenes that are structurally similar to compound A are nephrotoxic *in vivo* and cytotoxic in kidney cells.[13–18] The β-lyase inhibitor (aminooxy)acetic acid reduces the nephrotoxicity of these haloalkene-derived mercapturates, indicating hydrolysis of the mercapturates and β-lyase–dependent bioactivation of the released cysteine *S*-conjugates.

The deacylation of *N*-acyl-L-amino acids is catalyzed by

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Received from the University of Rochester, Rochester, New York. Submitted for publication March 20, 1998. Accepted for publication July 7, 1998. Supported by Abbott Laboratories and the National Institute of Environmental Health Sciences, grant ES03127. A preliminary report of this work was presented at the 37th Annual Meeting of the Society of Toxicology, Seattle, Washington, March 2–5, 1998. Dr. Anders is a consultant to Abbott Laboratories.

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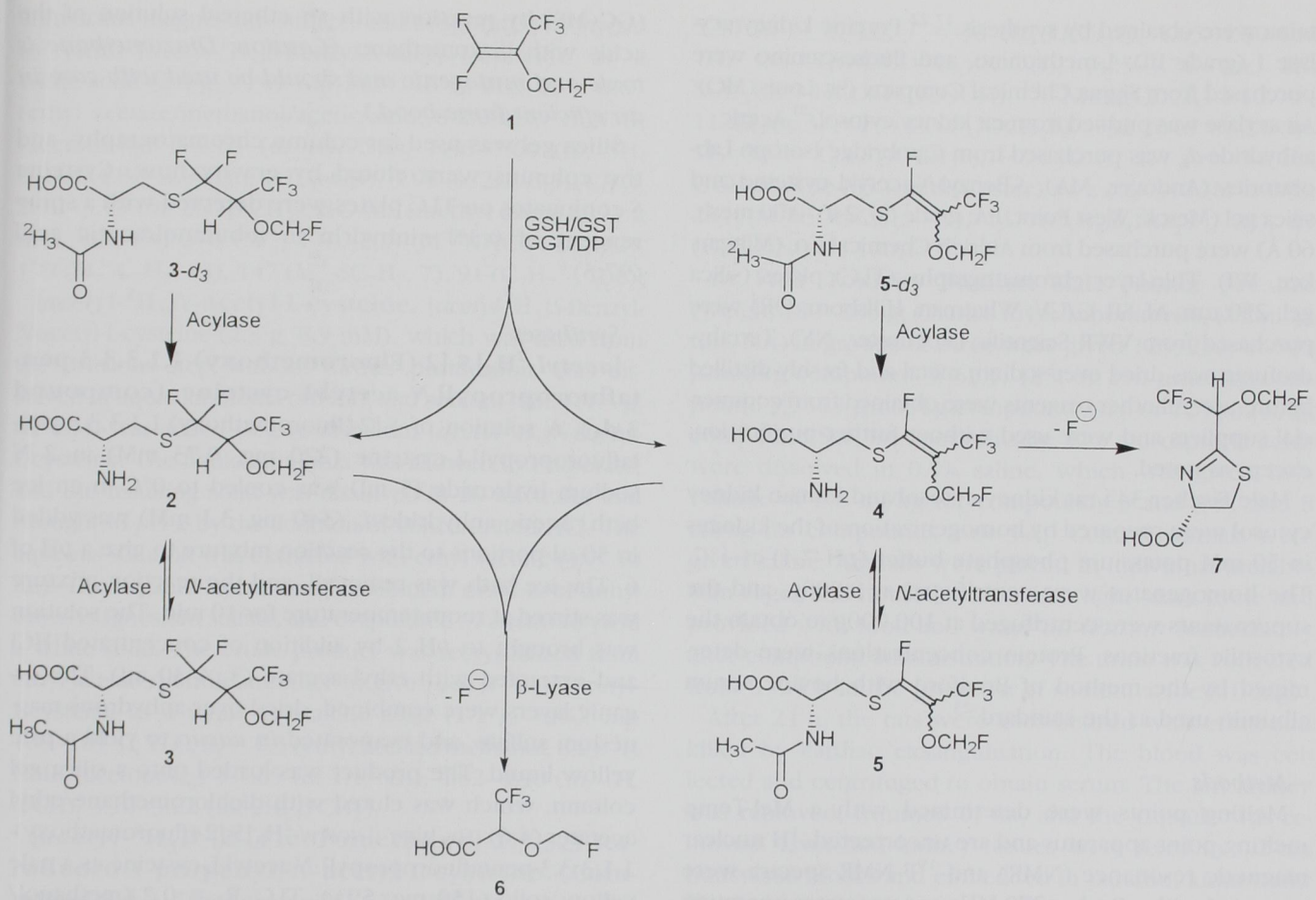

Fig. 1. The proposed pathway for biosynthesis and hydrolysis of compound A–derived mercapturates. Compound 1, 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (compound A); compound 2, *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine; compound 3, *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine; compound 3-d_3, [*acetyl*-$^2H_3$]*S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine; compound 4, *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine; compound 5, *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine; compound 5-d_3, [*acetyl*-$^2H_3$]*S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine; compound 6, 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid; compound 7, 2-[1-(fluoromethoxy)-2,2,2-trifluoroethyl]-4,5-dihydro-1,3-thiazole-4-carboxylic acid; GST, glutathione *S*-transferase; GSH, glutathione; GGT, γ-glutamyltransferase; DP, dipeptidase; β-lyase, cysteine conjugate β-lyase.

acylases.[19] Acylases I and III catalyze the deacetylation of several haloalkene-derived mercapturates that are nephrotoxic, cytotoxic, and mutagenic cysteine *S*-conjugates.[20] Thus acylases play important roles in the β-lyase–dependent bioactivation of haloalkenes and, therefore, contribute to the conjugation-dependent toxicity of haloalkenes.

The current experiments were designed to study the role of acylases in the β-lyase–dependent metabolism and the nephrotoxicity of compound A–derived mercapturates. The nephrotoxicity of compound A–derived mercapturates 3 and 5 was studied in male Fischer 344 rats. In addition, the acylase-catalyzed deacetylation of mercapturates 3 and 5 was studied in rat and human kidney cytosol and with acylase I and purified rat kidney acylase III.

Materials and Methods

Materials

2-(Fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (fig. 1, compound 1) was provided by Abbott Laboratories (Abbott Park, IL). *S*-[2-(Fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine and *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine were synthesized as described previously.[21] *S*-(1,2-Dichlorovinyl)-*N*-acetyl-L-cysteine and *S*-(2-chloro-1,1,2-trifluoroethyl)-*N*-acetyl-L-cys-

teine were obtained by synthesis.[17,22] Porcine kidney acylase I (grade III), L-methionine, and fluorescamine were purchased from Sigma Chemical Company (St. Louis, MO). An acylase was purified from rat kidney cytosol.[20] Acetic anhydride-d_6 was purchased from Cambridge Isotope Laboratories (Andover, MA). *S*-Benzyl-*N*-acetyl-L-cysteine and silica gel (Merck, West Point, PA; grade 60, 240–400 mesh, 60 Å) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Thin-layer chromatography (TLC) plates (silica gel, 250 μm, AL SIL G/UV; Whatman, Hillsboro, OR) were purchased from VWR Scientific (Rochester, NY). Tetrahydrofuran was dried over sodium metal and freshly distilled before use. All other reagents were obtained from commercial suppliers and were used without further purification, except as noted.

Male Fischer 344 rat kidney cytosol and human kidney cytosol were prepared by homogenization of the kidneys in 50 mM potassium phosphate buffer (*p*H 7.4) at 4°C. The homogenates were centrifuged at 9,000*g*, and the supernatants were centrifuged at 100,000*g* to obtain the cytosolic fractions. Protein concentrations were determined by the method of Bradford with bovine serum albumin used as the standard.[23]

Methods

Melting points were determined with a Mel-Temp melting point apparatus and are uncorrected. ^{1}H nuclear magnetic resonance (NMR) and ^{19}F NMR spectra were recorded with a Bruker 270 MHz spectrometer operating at 270 MHz for ^{1}H and 254 MHz for ^{19}F. Chemical shifts, δ, are reported in parts per million (ppm). The internal standard (δ = 0.0 ppm) for ^{1}H NMR with $CDCl_3$ as the solvent was tetramethylsilane. The solvent resonance peak at 2.1 ppm was used as the internal standard for ^{1}H NMR spectra when acetone-d_6 was the solvent. Trifluoroacetamide (δ = 0.0 ppm) was used as the external standard for ^{19}F NMR spectra.

Mass spectra were recorded with a Hewlett-Packard 5890 series II gas chromatograph (25 m × 0.2 mm, 0.5-μm film thickness, HP-1 crosslinked methyl siloxane column; Hewlett-Packard, Wilmington, DE) coupled to a Hewlett-Packard 5972 series II mass selective detector; the injector and transfer-line temperatures were 200°C and 240°C, respectively. The methyl esters of compounds 3, 5, 3-d_3, 5-d_3, or [*acetyl*-^{2}H$_3$]*S*-benzyl-*N*-acetyl-L-cysteine were analyzed with a temperature program of 50°C for 1 min followed by a linear gradient of 10°C/min to 240°C. Methyl esters of compounds 3 and 5 and [*acetyl*-^{2}H$_3$]*S*-benzyl-*N*-acetyl-L-cysteine were prepared for analysis by gas chromatography–mass spectrometry (GC–MS) by reaction with an ethereal solution of the acids with diazomethane. (*Caution: Diazomethane is toxic and mutagenic and should be used with care in an efficient fume hood.*)

Silica gel was used for column chromatography, and the columns were eluted by gravity flow. Cysteine *S*-conjugates on TLC plates were detected with a spray reagent of 0.3% ninhydrin in *n*-butanol/acetic acid (97:3).

Syntheses

[*acetyl*-^{2}H$_3$]*S*-[2-(Fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine (compound 3-d_3). A solution of *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine (220 mg, 0.73 mM) in 2 N sodium hydroxide (5 ml) was cooled to 0°C in an ice bath. Acetic anhydride-d_6 (340 mg, 3.1 mM) was added in 50-μl portions to the reaction mixture to give a *p*H of 6. The ice bath was removed, and the reaction mixture was stirred at room temperature for 10 min. The solution was brought to *p*H 2 by addition of concentrated HCl and extracted with ethyl acetate (3 × 30 ml). The organic layers were combined, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to yield a pale yellow liquid. The product was loaded onto a silica gel column, which was eluted with dichloromethane/ethyl acetate (4:6) to give [*acetyl*-^{2}H$_3$]*S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine as a pale yellow solid (150 mg, 59%): TLC, R_f = 0.2 (methanol/ethyl acetate/acetic acid, 1:9:0.01); ^{1}H NMR ($CDCl_3$) δ 8.5 (bs, 1H, COOH), 6.90 (d, 1H, NH), 5.5 (d of d, 2H, OCH_2F), 4.90–5.10 (m, 1H, $CF_2CH(CF_3)OCH_2F$), 4.42–4.57 (m, 1H, $CH_2CH(NH_2)COOH$), 3.37–3.50 and 3.52–3.72 (d of m, 2H, $SCH_2CH(NH_2)COOH$); ^{19}F NMR ($CDCl_3$) δ 3.77–3.89 (m, 3F, $CF_2CH(CF_3)OCH_2F$), −5.90 to −0.90 (m, 2F, $CF_2CH(CF_3)OCH_2F$), −77.20 (t, 1F, J = 54 Hz, OCH_2F); GC–MS (methyl ester) t_R 17.4 min, *m/z* (%) 360 (M^+, 0.3), 301 (M^+-$COOCH_3$, 6.5%), 179 ($C_6H_7D_3NO_3S^+$, 38), 147 ($C_5H_3D_3NO_2S^+$, 25), 89 ($C_3H_5DNO_2^+$, 100).

[*acetyl*-^{2}H$_3$]*S*-Benzyl-*N*-acetyl-L-cysteine. A solution of *S*-benzyl-L-cysteine (2.11 g, 10 mM) in water (20 ml) containing sodium hydroxide (2.2 g, 55 mM) was cooled to 0°C in an ice bath. Acetic anhydride-d_6 (2.3 g, 21 mM) was added in 100-μl portions to the reaction mixture to give a *p*H of 6. The ice bath was removed, and the reaction mixture was stirred at room temperature for 10 min. The solution was brought to *p*H 2 by the addition of concentrated HCl and extracted with ethyl acetate (3 × 75 ml). The organic layers were combined, dried over

anhydrous magnesium sulfate, and evaporated *in vacuo* to yield [*acetyl*-$^2H_3$]*S*-benzyl-*N*-acetyl-L-cysteine as a white solid (2.4 g, 94%): mp 142–146°C; TLC, R_f = 0.67 (ethyl acetate/methanol/acetic acid, 8:2:0.01); ^{1}H NMR (acetone-d_6) δ 7.65 (d, 1H, NH), 7.36–7.60 (m, 5H, C_6H_5), 4.82–4.94 (m, 1H, α-CH), 3.95 (s, 2H, $C_6H_5CH_2$), 2.90–3.15 (m, 2H, β-CH_2); GC–MS (methyl ester) t_R 21.2 min, *m/z* (%) 270 (M^+, 1.8), 208 (M^+-$NHCOCD_3$, 12), 179 (M^+-C_7H_7, 13), 147 (M^+-SC_7H_7, 7), 91 ($C_7H_7^+$, 100).

[*acetyl*-$^2H_3$]*N*-Acetyl-L-cysteine. [*acetyl*-$^2H_3$]*S*-Benzyl-*N*-acetyl-L-cysteine (2.3 g, 8.9 mM), which was used from the previous step without further purification, was dissolved in liquid ammonia (50 ml), and sodium metal (1.5 g, 62 mM) was added to give disodium [*acetyl*-$^2H_3$]*N*-acetyl-L-cysteine. The liquid ammonia was allowed to evaporate, and the resulting solid was dissolved in 20 ml water and brought to *p*H 2 by the addition of concentrated HCl. The aqueous solution was extracted with ethyl acetate (3 × 50 ml). The organic layers were combined, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to yield a white solid. The crude product was recrystallized from ethyl acetate/petroleum ether to give [*acetyl*-$^2H_3$]*N*-acetyl-L-cysteine as a white crystalline solid (1.1 g, 74%): mp 108–109°C; TLC, R_f = 0.3 (ethyl acetate/methanol, 8:2); ^{1}H NMR (acetone-d_6) δ 7.65 (d, 1H, NH), 4.82–4.90 (m, 1H, α-CH), 3.10–3.22 (m, 2H, β-CH_2).

[*acetyl*-$^2H_3$]*S*-[2-(Fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine (Compound 5-d_3). Triethylamine (1.51 g, 15 mM) was added to a stirred solution of [*acetyl*-$^2H_3$]*N*-acetyl-L-cysteine (830 mg, 5 mM) in THF (25 ml) in a flask that was purged with nitrogen, and the solution was cooled to 0°C. 2-(Fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (compound 1, 1.08 g, 6 mM) in 5 ml THF was added by drops to the reaction mixture over 10 min, and the reaction was stirred for 1.5 h at 0°C. After addition of 30 ml water, the reaction was brought to *p*H 2 with 5N HCl, and the mixture was extracted with ethyl acetate (3 × 50 ml). The organic layers were combined, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to yield a yellow viscous oil. The product was loaded onto a silica gel column, which was eluted with dichloromethane/ethyl acetate (6:4) to give [*acetyl*-$^2H_3$]*S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine as a yellow solid (389 mg, 25%): TLC, R_f = 0.21 (methanol/ethyl acetate/acetic acid, 1:9:0.01); ^{1}H NMR ($CDCl_3$) δ 6.77 (d, 1H, $CHNHCOCH_3$), 5.30–5.50 (d of d, 2H, *J* = 54 Hz, OCH_2F), 4.65–4.80 (m, 1H, $CH_2CH(NHCOCH_3)COOH$), 3.25–3.70 (m, 2H, $SCH_2CH(NHCOCH_3)COOH$); ^{19}F NMR ($CDCl_3$) δ 10.90–11.20 (m, 3F, $CF = C(CF_3)OCH_2F$), −32.20 to −32.0 (m, 1F, $CF = C(CF_3)OCH_2F$), −74.90 (t, 1F, *J* = 54 Hz, OCH_2F); GC–MS (methyl ester) *m/z* (%) 340 (M^+, 0.3), 281 (M^+-$COOCH_3$, 7.7), 147 ($C_5H_3D_3NO_2S^+$, 88), 89 ($C_3H_5DNO_2^+$, 100).

***In Vivo* Toxicity Studies.** Male Fischer 344 rats (weigh, 180–200 g; Charles River Laboratories, Wilmington, MA) in groups of three were given 125, 250, or 500 μmol/kg compound 3; 62.5, 125, or 250 μmol/kg compound 5‖; 125 μmol/kg compound 3-d_3; or 125 μmol/kg compound 5-d_3 intraperitoneally. The mercapturic acids were dissolved in 0.9% saline, which was given in a volume of 6.6 ml/kg for compounds 3 and 3-d_3 and 8 ml/kg for compounds 5 or 5-d_3. Control animals were given saline. Animals were placed in individual metabolism cages and housed with a 12-h light–dark cycle and provided with food and water *ad libitum* immediately after compound administration. The urine was collected from 0–24 h in the presence of sodium azide (10 mg).

After 24 h, the rats were anesthetized with ether and killed by cardiac exsanguination. The blood was collected and centrifuged to obtain serum. The left kidney was removed, trimmed of fat, and the capsule was removed. Liver and kidney tissues were fixed in 0.2-cm transverse blocks and embedded in paraffin; kidney and liver tissue were sectioned at 3 and 5 μm, respectively. The sections were stained with hematoxylin and eosin. The entire nephron was examined microscopically with specific severity scoring of the proximal convoluted tubules, differentiated by location into juxtamedullary, paracortical, or cortical regions. Lesions from each region were given scores of 0 (no significant lesions), 0.5, 1.0, or 1.5 to 4+ (maximal severity). The amount of proteinaceous material in the collecting ducts was also scored from 0 (no protein casts) to 4+ (abundant protein casts). The results are reported as the sum of the individual scores for each region, including protein casts; thus scores can range from 0 to 16. In the liver, the extent of inflammation of the portal triads, together with the severity of necrosis, was evaluated. All slides were read by a pathologist (R.B.B.) and were coded as to the experimental treatment.

Urine and serum glucose concentrations, serum glutamate-pyruvate transaminase activities, and blood urea nitrogen concentrations were measured with Sigma Kits 115, 505, and 535, respectively (Sigma Chemical Co.). Urine protein concentrations were measured by the

‖ The solubility of compound 5 prevented administration of doses > 250 μmol/kg.

method of Bradford (Bio-Rad Protein Assay Dye Reagent Concentrate; Bio-Rad, Richmond, CA) with bovine serum albumin as the standard.[23]

Results were evaluated statistically by analysis of variance with Dunnett's multiple comparison test (InStat; GraphPad Software, San Diego, CA). A level of $P < .05$ was chosen for acceptance or rejection of the null hypothesis.

Analysis of Rat Urine by ^{19}F NMR Spectroscopy and Gas Chromatography–Mass Spectrometry. For ^{19}F NMR spectroscopic analysis, rat urine (0.6 ml) was centrifuged to remove insoluble material, and the supernatant was mixed with 100 μl deuterium oxide and placed in a 5-mm NMR tube. The urine was then analyzed by ^{19}F NMR spectroscopy.

For GC–MS analysis, *S*-(2,2-dibromo-1,1-difluoroethyl)-*N*-acetyl-L-cysteine (0.1 mg) was added to each urine sample (2 ml) as an internal standard, and the samples were brought to *p*H 1.4 with 5 N hydrochloric acid. The samples were extracted with ethyl acetate (3 × 2 ml); the organic layers were separated and combined, dried over anhydrous sodium sulfate, and evaporated to dryness under a stream of nitrogen. The dried samples were treated with excess diazomethane in ether, evaporated to dryness, and dissolved in 200 μl dichloromethane. To prepare the standard curves, a mixture of synthetically prepared compounds 3 and 3-d_3 or 5 and 5-d_3 were added to control urine samples and subjected to the extraction procedure described above. Samples (1 μl) were analyzed by GC–MS, as described in Materials and Methods. The mercapturic acids in urine were analyzed by selective ion monitoring of ions characteristic for unlabeled mercapturic acids (*m/z* 144, $C_5H_6NO_2S^+$, or 176, $C_6H_{10}NO_3S^+$) and deuterium labeled (*m/z* 147, $C_5H_3{}^2H_3NO_2S^+$, or 179, $C_6H_7{}^2H_3NO_3S^+$).[24]

Acylase Assays. Acylase activity with compound 3 as the substrate was determined by measuring the amount of the deacetylated product, *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine (fig. 1, compound 2) formed. Compound 2 concentrations were determined by reaction with fluorescamine.[25] The limit of detection of deacetylated mercapturates was 1 pM. The reaction mixtures (1 ml) contained human or rat kidney cytosol (2 or 3 mg protein), acylase I or purified rat kidney acylase (2 μg protein), and 4 μmol substrate in 50 mM potassium phosphate buffer (*p*H 7.4). The reaction mixtures were incubated at 37°C for 30 min, and the reaction was stopped by the addition of 0.2 ml 20% trichloroacetic acid. The mixtures were allowed to stand for 10 min in an ice bath and were then centrifuged (at 500*g* for 10 min). A sample (40 μl) of the supernatant was added to 3.6 ml of 50 mM potassium phosphate buffer (*p*H 7.4), and the volume was brought to 4 ml by the addition of water. Fluorescamine (300 μl of a solution containing 10 mg fluorescamine dissolved in 33 ml acetone) was added to the sample, and the fluorescence intensity (390 nm excitation, 475 emission) was measured with a Perkin-Elmer LS-5 Fluorescence Spectrophotometer (Norwalk, CT). Acylase activity with *S*-(2-chloro-1,1,2-trifluoroethyl)-*N*-acetyl-L-cysteine and *S*-(1,2-dichlorovinyl)*N*-acetyl-L-cysteine, which are known acylase substrates,[16,26] was determined similarly.

Previous studies show that compound 4 undergoes rapid cyclization with the stoichiometric release of fluoride at *p*H 7.4.[21] Thus the deacetylation of compound 5 was determined by quantifying the fluoride ion release. The reaction mixtures were prepared as described before for compound 3. Fluoride ion concentrations were measured with a fluoride-specific electrode (ATI Orion, Boston, MA) and a Corning *p*H 3meter (Corning Glass Works, Medfield, MA). A standard curve was prepared with sodium fluoride.

Results

Compound 3 given intraperitoneally to male Fischer 344 rats at doses of 125, 250, and 500 μmol/kg did not cause significant changes in serum glucose concentrations, blood urea nitrogen concentrations, serum glutamate-pyruvate transaminase activities, in urine volumes, or in urine glucose and protein concentrations compared with control animals (data not shown).

Intraperitoneal administration of compound 5 to male Fischer 344 rats at 62.5 or 125 μmol/kg did not produce significant changes in blood or urine clinical chemical parameters compared with control animals (data not shown). Two of the three rats given 250 μmol/kg compound 5 showed increases in urine glucose concentrations of 114 and 76 mg at 0–24 h (control urine glucose, 6.54 ± 1.38; mean ±SD) but showed no significant increases in urine volumes and protein concentrations. In addition, no significant increases in blood urea nitrogen concentrations and serum glutamate-pyruvate transaminase activities were observed in rats given any dose of compound 5 compared with controls (data not shown).

Histopathologic studies of kidneys from rats given compound 3 showed no significant increase in necrosis at the corticomedullary junction compared with control animals (fig. 2): the histopathologic scores for rats given 250 and 500 μmol/kg compound 3 were 0.66 ± 0.57

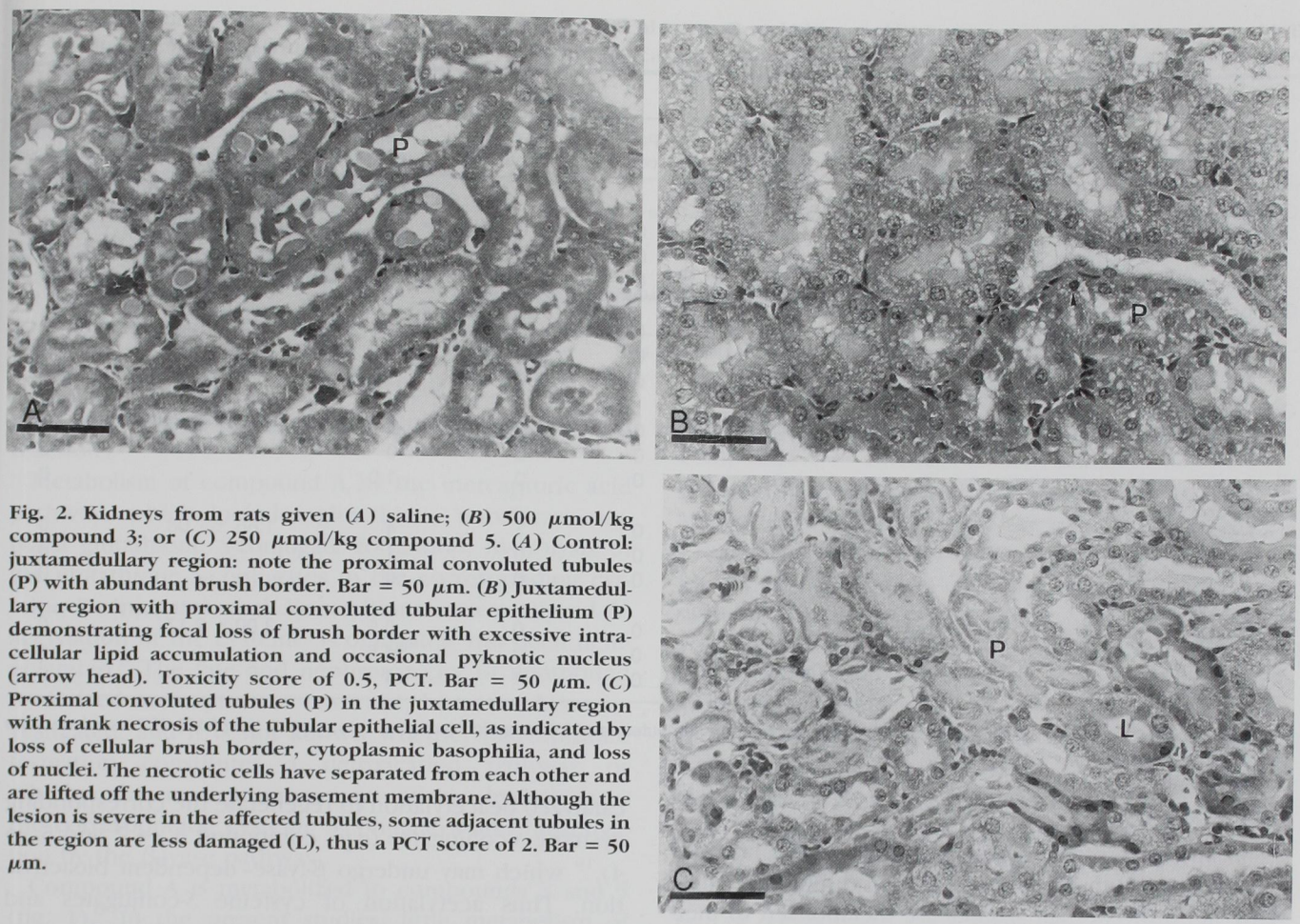

Fig. 2. Kidneys from rats given (*A*) saline; (*B*) 500 μmol/kg compound 3; or (*C*) 250 μmol/kg compound 5. (*A*) Control: juxtamedullary region: note the proximal convoluted tubules (P) with abundant brush border. Bar = 50 μm. (*B*) Juxtamedullary region with proximal convoluted tubular epithelium (P) demonstrating focal loss of brush border with excessive intracellular lipid accumulation and occasional pyknotic nucleus (arrow head). Toxicity score of 0.5, PCT. Bar = 50 μm. (*C*) Proximal convoluted tubules (P) in the juxtamedullary region with frank necrosis of the tubular epithelial cell, as indicated by loss of cellular brush border, cytoplasmic basophilia, and loss of nuclei. The necrotic cells have separated from each other and are lifted off the underlying basement membrane. Although the lesion is severe in the affected tubules, some adjacent tubules in the region are less damaged (L), thus a PCT score of 2. Bar = 50 μm.

and 1.33 ± 1.44, which were not significantly different from those of control animals (control = 0; table 1).[3] Histopathologic studies of the kidneys from two of three rats given 250 μmol/kg compound 5 showed necrosis at the corticomedullary junction (scores = 4.0 and 4.5). With the exception of injury attributed to an apparent intrahepatic injection of 250 μmol/kg compound 5, which produced hepatic necrosis that was accompanied by maximal renal juxtamedullary necrosis, no morphologic evidence of hepatic injury was observed in rats given compounds 3 or 5.

The ^{19}F NMR spectroscopic analysis of urine from rats given 250 μmol/kg compound 3 showed resonances assigned only to unchanged compound 3 (fig. 3). Although ^{19}F NMR spectroscopic analysis of urine from rats given 250 μmol/kg compound 5 showed resonances assigned mainly to unchanged compound 5, resonances assigned to 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid (fig. 1, compound 6), 2-[1-(fluoromethoxy)-2,2,2-trifluoroethyl]-4,5-dihydro-1,3-thiazole-4-carboxylic acid (the cyclization product of compound 4; fig. 1, compound 7), and inorganic fluoride were also observed (fig. 4).

To study the *in vivo* hydrolysis and reacetylation of compound A–derived mercapturates, rats were given 125 μmol/kg compounds 3-d_3 or 5-d_3, and the amounts of compounds 3 and 3-d_3 or 5 and 5-d_3, respectively, in urine were quantified by GC–MS analysis. Unchanged compounds 3-d_3 and 5-d_3 accounted for about 60% of the administered dose, whereas compounds 3 and 5 accounted for about 14% and 5%, respectively, of the administered dose (fig. 5).

No hydrolysis of compound 3 was detected with human or rat kidney cytosol or with acylase I and purified rat kidney acylase (table 2). Hydrolysis of compound 5 was observed with human or rat kidney cytosol or with acylase I and purified rat kidney acylase, but the rates were much lower than with *S*-(2-chloro-1,1,2-trifluoroethyl)-*N*-acetyl-L-cysteine or *S*-(1,2–dichlorovinyl)-*N*-acetyl-L-cysteine, which are known acylase substrates.[16,26]

Table 1. Morphological Assessment of Kidney and Liver Damage in Rats Given *S*-[2-(Fluoromethoxy)]1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine (Compound 3) and *S*-[2-(Fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine (Compound 5)

Mercapturate (μmol/kg)	Animal Number E162-	Kidney						Liver Necrosis
		PCT juxtamed	PCT paracort	PCT cort	Protein	Sum	Mean ± SD	
Compound 3 (250)	250	1	0	0	0	1	0.66 ± 0.57	0
	251	0	0	0	0	0		0
	252	1	0	0	0	1		0
Compound 3 (500)	202	0	0	0	0.5	0.5	1.33 ± 1.44	0
	203	3	0	0	0	3.0		0
	204	0.5	0	0	0	0.5		0
Compound 5 (62.5)	259	0	0	0	0	0	0.33 ± 0.57	0
	260	0	0	0	0	0		0
	261	1	0	0	0	1.0		0
Compound 5 (125)	262	3	0	0	1	4	1.66 ± 2.08	0
	263	1	0	0	0	1		0
	264	0	0	0	0	0		0
Compound 5 (250)	256	0.5	0	0	0	0.5	3.00 ± 2.17	0
	257	4	0.5	0	0	4.5		4
	258	2	1	0	1	4		0

* The high scores for renal juxtamedullary necrosis and liver necrosis are attributed to an intrahepatic injection of compound 5. If rat E162-257 is excluded, the mean score for renal injury would be 2.25.

Discussion

The objective of this work was to study the fate and nephrotoxicity of compound A–derived mercapturates. Many nephrotoxic haloalkenes are metabolized to mercapturates (*S*-substituted *N*-acetyl-L-cysteines), which cannot undergo cysteine conjugate β-lyase–catalyzed bioactivation because substrates for the pyridoxal phosphate-dependent β-lyase must possess a primary amino group.[27] Mercapturates may, however, undergo acylase-catalyzed deacetylation to regenerate the corresponding cysteine *S*-conjugates (*e.g.*, compound 3 → 2 and 5 → 4),[19] which may undergo β-lyase–dependent bioactivation. Thus acetylation of cysteine *S*-conjugates and deacetylation of mercapturates may constitute detoxication or bioactivation reactions for nephrotoxic haloalkenes, depending on the relative rates of acetylation and deacetylation. Many haloalkene-derived mercapturates are nephrotoxic or cytotoxic in kidney cells, indicating

Table 2. Biotransformation of *S*-[2-(Fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine (Compound 3), *S*-[2-(Fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine (Compound 5), *S*-(2-Chloro-1,1,2-trifluoroethyl)-*N*-acetyl-L-cysteine, and *S*-(1,2-dichlorovinyl)-*N*-acetyl-L-cysteine by Rat and Human Kidney Cytosol, Acylase I, and Acylase

Substrate	Specific Activity (μmol/mg protein/min)			
	Human kidney cytosol	Rat kidney cytosol	Acylase I	Purified rat kidney acylase
S-[2-(Fluoromethoxy)-1,3,3,3-pentafluoro-propyl]-*N*-acetyl-L-cysteine (compound 3)	ND	ND	ND	ND
S-[2-(Fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine (compound 5)	0.002 ± 0.001	0.005 ± 0.001	1.3 ± 0.4	1.3 ± 0.2
S-(2-Chloro-1,1,2-trifluoroethyl)-*N*-acetyl-L-cysteine	0.150 ± 0.02	0.7 ± 0.05	68.2 ± 2.3	ND
S-(1,2-Dichlorovinyl)-*N*-acetyl-L-cysteine	0.05 ± 0.01	0.06 ± 0.02	ND	15.8 ± 5.0

Data are shown as mean ± SE (n = 3). Acylase activity was determined by incubating human or rat kidney cytosol or acylase I and purified rat kidney acylase with the substrates and measuring product formation.

ND = no detectable activity.

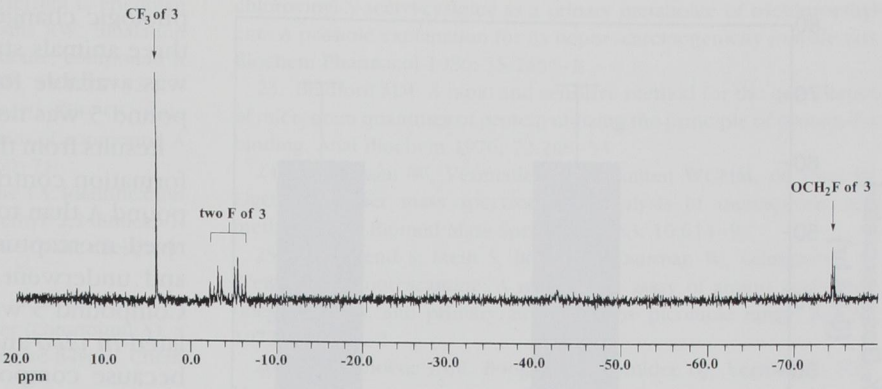

Fig. 3. ^{19}F NMR of the urine of a rat given 500 μmol/kg *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine (compound 3) intraperitoneally. The urine samples were analyzed as described in the Materials and Methods section. The spectrum shown is typical of the spectra recorded in three rats.

that deacetylation reactions do serve as bioactivation reactions.[13–18,28,29]

Metabolism of compound A by the mercapturic acid pathway, which includes glutathione transferase-catalyzed glutathione *S*-conjugate formation, hydrolysis of the glutathione *S*-conjugates to the corresponding cysteine *S*-conjugates, and *N*-acetyltransferase-catalyzed *N*-acetylation of the cysteine *S*-conjugates, has been demonstrated.[6] The fate and nephrotoxicity of compound A–derived mercapturates have not, however, been investigated, and it is not known whether mercapturate formation constitutes a detoxication pathway or whether the mercapturates may undergo hydrolysis to the corresponding cysteine *S*-conjugates and bioactivation by the β-lyase pathway.

Compound A is metabolized to compounds 3 and 5 (fig. 1).[6] In the present studies, little metabolism of compound 3 was detected: ^{19}F NMR spectra of the urine of rats given compound 3 showed excretion of only unchanged mercapturate (fig. 3). Compound 3-d_3 was, however, deacetylated and reacetylated *in vivo* as shown by the presence of compound 3 in the urine of rats given compound 3-d_3 (fig. 5), indicating that compound 3-d_3 was hydrolyzed to compound 2, which was acetylated to give compound 3 (fig. 1, compound 3-d_3 → compound 2 → compound 3). No acylase-catalyzed hydrolysis of compound 3 was observed (table 1). Previous studies also show deacetylation and reacetylation of mercapturates: About 60% of the administered dose of [*acetyl*-$^2H_3$]*S*-(2,2-dichloro-1,1-difluoroethyl)-*N*-acetyl-L-cysteine and [*acetyl*-$^2H_3$]*S*-(2,2-dibromo-1,1-difluoroethyl)-*N*-acetyl-L-cysteine is excreted in the urine, and 17% and 31% is excreted as the unlabeled mercapturate.[28] The observation of deacetylation of compound 3 *in vivo*, but not *in vitro*, may indicate that its hydrolysis is catalyzed by hydrolases in intestinal bacteria.[30] Finally, compound 3 was not nephrotoxic or hepatotoxic, as demonstrated by clinical chemical studies and by morphologic examination of the kidneys and livers of rats given compound 3 (fig. 2). These data show that formation of compound 3 constitutes a detoxication pathway for compound A.

Compound 5 was also deacetylated and reacetylated *in vivo*, as shown by the presence of compound 5 in the urine of rats given compound 5-d_3 (fig. 5) (fig. 1, compound 5-d_3 → compound 4 → compound 5). In addition, the acylase-catalyzed hydrolysis of compound 5 was detected (table 1), but at low rates

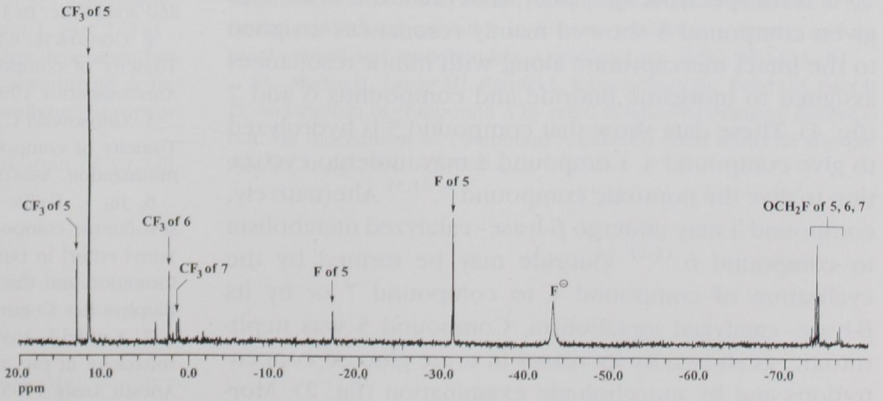

Fig. 4. ^{19}F NMR of the urine of a rat given 250 μmol/kg *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine (compound 5) intraperitoneally. The urine samples were analyzed as described in the Materials and Methods section. The spectrum shown is typical of the spectra recorded in three rats.

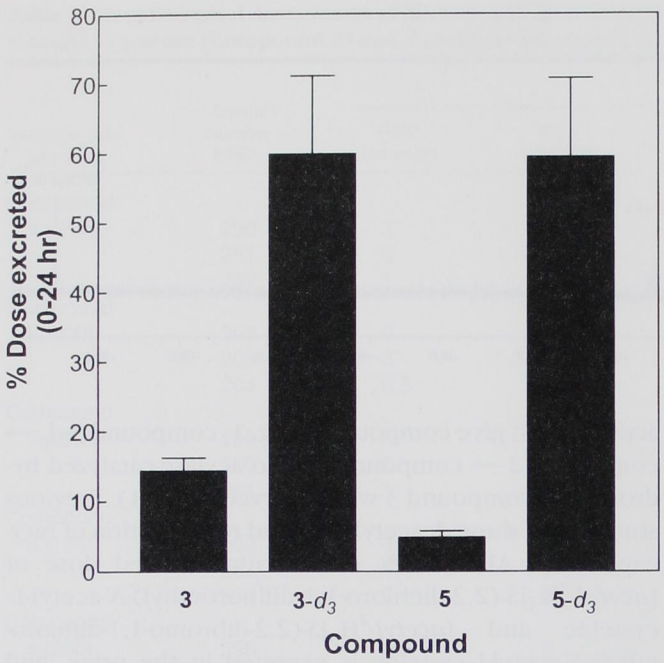

Fig. 5. Excretion of *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine (compound 3) and [*acetyl*-$^2H_3$]*S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine (compound 3-d_3) in rats given [*acetyl*-$^2H_3$]*S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine and of *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine (compound 5) and [*acetyl*-$^2H_3$]*S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine (compound 5-d_3) in rats given [*acetyl*-$^2H_3$]*S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine. Rats were given 125 μmol/kg compound 3-d_3 or 5-d_3 intraperitoneally, and the fraction of the administered dose excreted from 0–24 h as compounds 3 and 3-d_3 or compounds 5 and 5-d_3, respectively, was quantified by gas chromatography–mass spectrometry, as described in the Materials and Methods section. Data are shown as mean ± SEM (n = 3).

compared with *S*-(1,2-dichlorovinyl)-L-cysteine and *S*-(2-chloro-1,1,2-trifluoroethyl)-L-cysteine, which are known acylase substrates.[16,26]

^{19}F NMR spectroscopic analysis of the urine of animals given compound 5 showed mainly resonances assigned to the intact mercapturate along with minor resonances assigned to inorganic fluoride and compounds 6 and 7 (fig. 4). These data show that compound 5 is hydrolyzed to give compound 4. Compound 4 may undergo cyclization to give the nontoxic compound 7.[21,31] Alternatively, compound 4 may undergo β-lyase–catalyzed metabolism to compound 6.[11,21] Fluoride may be formed by the cyclization of compound 4 to compound 7 or by its β-lyase–catalyzed metabolism. Compound 5 was nephrotoxic, as shown by increases in urine glucose concentrations and by morphologic examination (fig. 2). Morphologic changes were, however, seen in only two of three animals studied, indicating that little compound 4 was available for β-lyase–catalyzed bioactivation. Compound 5 was not hepatotoxic.

Results from the present study show that mercapturate formation contributes more to the detoxication of compound A than to its bioactivation. The compound A–derived mercapturate compound 3 was not nephrotoxic and underwent little metabolism *in vivo* or *in vitro*. Compound 5 was mildly nephrotoxic and was deacetylated *in vivo* and *in vitro*. These findings are significant because compounds 3 and 5 are metabolites of compound A in rats and in humans exposed to sevoflurane and thereby exposed to compound A.[6,12] Thus the formation of mercapturates of compound A would reduce its bioactivation through the β-lyase pathway. Compound A is nephrotoxic in rats,[4–9,32,33] and evidence supporting a role for the β-lyase–dependent bioactivation of compound A has been presented.[6,9–11,21,31,34] Although considerable evidence implicates the β-lyase pathway in the nephrotoxicity of compound A, reports that purport to show that the β-lyase pathway is not involved have been presented.[33,35]

The authors thank Sandra E. Morgan for her assistance in preparing the manuscript.

References

1. Frink EJ Jr, Malan TP, Morgan SE, Brown EA, Malcomson M, Brown BR Jr: Quantification of the degradation products of sevoflurane in two CO_2 absorbants during low-flow anesthesia in surgical patients. ANESTHESIOLOGY 1992; 77:1064–9

2. Fang ZX, Eger EI II: Factors affecting the concentration of compound A resulting from the degradation of sevoflurane by soda lime and Baralyme® in a standard anesthetic circuit. Anesth Analg 1995; 81:564–8

3. Bito H, Ikeda K: Effect of total flow rate on the concentration of degradation products generated by the reaction between sevoflurane and soda lime. Br J Anaesth 1995; 74:667–9

4. Gonsowski CT, Laster MJ, Eger II EI, Ferrell LD, Kerschmann RL: Toxicity of compound A in rats. Effect of a 3-hour administration. ANESTHESIOLOGY 1994; 80:556–65

5. Gonsowski CT, Laster MJ, Eger II EI, Ferrell LD, Kerschmann RL: Toxicity of compound A in rats. Effect of increasing duration of administration. ANESTHESIOLOGY 1994; 80:566–73

6. Jin L, Baillie TA, Davis MR, Kharasch ED: Nephrotoxicity of sevoflurane compound A [fluoromethyl-2,2-difluoro-1-(trifluoromethyl-)vinyl ether] in rats: Evidence for glutathione and cysteine conjugate formation and the role of renal cysteine conjugate β-lyase. Biochem Biophys Res Commun 1995; 210:498–506

7. Kandel L, Laster MJ, Eger EI II, Kerschmann RL, Martin J: Nephrotoxicity in rats undergoing a one-hour exposure to compound A. Anesth Analg 1995; 81:559–63

8. Keller KA, Callan C, Prokocimer P, Delgado-Herrera L, Friedman MB, Hoffman GM, Wooding WL, Cusick PK, Krasula RW: Inhalation toxicity study of a haloalkene degradant of sevoflurane, compound A (PIFE), in Sprague-Dawley rats. ANESTHESIOLOGY 1995; 83:1220–32

9. Kharasch ED, Thorning D, Garton K, Hankins DC, Kilty GC: Role of renal cysteine conjugate β-lyase in the mechanism of compound A nephrotoxicity in rats. ANESTHESIOLOGY 1997; 86:160–71

10. Jin L, Davis MR, Kharasch ED, Doss GA, Baillie TA: Identification in rat bile of glutathione conjugates of fluoromethyl 2,2-difluoro-1-(trifluoromethyl)vinyl ether, a nephrotoxic degradate of the anesthetic agent sevoflurane. Chem Res Toxicol 1996; 9:555–61

11. Spracklin DK, Kharasch ED: Evidence for metabolism of fluoromethyl 2,2-difluoro-1-(trifluoromethyl)vinyl ether (compound A), a sevoflurane degradation product, by cysteine conjugate β-lyase. Chem Res Toxicol 1996; 9:696–702

12. Iyer RA, Frink EJ Jr, Ebert TJ, Anders MW: Cysteine conjugate β-lyase-dependent metabolism of compound A (2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene) in human subjects anesthetized with sevoflurane and in rats given compound A. ANESTHESIOLOGY 1998; 88: 611–8

13. Pratt IS, Lock EA: Deacetylation and further metabolism of the mercapturic acid of hexachloro-1,3-butadiene by rat kidney cytosol in vitro. Arch Toxicol 1988; 62:341–5

14. Vamvakas S, Dekant W, Berthold K, Schmidt S, Wild D, Henschler D: Enzymatic transformation of mercapturic acids derived from halogenated alkenes to reactive and mutagenic intermediates. Biochem Pharmacol 1987; 36:2741–8

15. Ishmael J, Lock EA: Nephrotoxicity of hexachlorobutadiene and its glutathione-derived conjugates. Toxicol Pathol 1986; 14:258–62

16. Boogaard PJ, Commandeur JNM, Mulder GJ, Vermeulen NPE, Nagelkerke JF: Toxicity of the cysteine-*S*-conjugates and mercapturic acids of four structurally related difluoroethylenes in isolated proximal tubular cells from rat kidney. Uptake of the conjugates and activation to toxic metabolites. Biochem Pharmacol 1989; 38:3731–41

17. Commandeur JNM, Brakenhoff JPG, De Kanter FJJ, Vermeulen NPE: Nephrotoxicity of mercapturic acids of three structurally related 2,2-difluoroethylenes in the rat. Biochem Pharmacol 1988; 37:4495–504

18. Wolfgang GHI, Gandolfi AJ, Stevens JL, Brendel K: *N*-Acetyl *S*-(1,2-dichlorovinyl)-L-cysteine produces a similar toxicity to *S*-(1,2-dichlorovinyl)-L-cysteine in rabbit renal slices: Differential transport and metabolism. Toxicol Appl Pharmacol 1989; 101:205–19

19. Anders MW, Dekant W: Aminoacylases. Adv Pharmacol 1994; 27:433–50

20. Uttamsingh V, Anders MW: Purification and characterization of an aminoacylase catalyzing the hydrolysis of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine. Fundam Appl Toxicol 1996; 30(Suppl 1, Part 2):314

21. Iyer RA, Anders MW: Cysteine conjugate β-lyase-dependent biotransformation of the cysteine *S*-conjugates of the sevoflurane degradation product 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (compound A). Chem Res Toxicol 1997; 10:811–9

22. Dekant W, Metzler M, Henschler D: Identification of *S*-1,2-dichlorovinyl-N-acetyl-cysteine as a urinary metabolite of trichloroethylene: A possible explanation for its nephrocarcinogenicity in male rats. Biochem Pharmacol 1986; 35:2455–8

23. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248–54

24. Onkenhout W, Vermeulen NPE, Luijten WCMM, de Jong HJ: Electron impact mass spectrometric analysis of mercapturic acid methyl esters. Biomed Mass Spectrom 1983; 10:614–9

25. Udenfriend S, Stein S, Böhlen P, Dairman W, Leimgruber W, Weigele M: Fluorescamine: A reagent for assay of amino acids, peptides, proteins, and primary amines in the picomole range. Science 1972; 178:871–2

26. Commandeur JNM, Boogaard PJ, Mulder GJ, Vermeulen NPE: Mutagenicity and cytotoxicity of two regioisomeric mercapturic acids and cysteine S-conjugates of trichloroethylene. Arch Toxicol 1991; 65:373–80

27. Cooper AJL: Enzymology of cysteine *S*-conjugate β-lyases. Adv Pharmacol 1994; 27:73–115

28. Commandeur JNM, Stijntjes GJ, Wijngaard J, Vermeulen NPE: Metabolism of L-cysteine *S*-conjugates and *N*-(trideuteroacetyl)-L-cysteine *S*-conjugates of four fluoroethylenes in the rat. Role of balance of deacetylation and acetylation in relation to the nephrotoxicity of mercapturic acids. Biochem Pharmacol 1991; 42:31–8

29. Stijntjes GJ, Commandeur JNM, te Koppele JM, McGuinness S, Gandolfi AJ, Vermeulen NPE: Examination of structure-toxicity relationships of L-cysteine-*S*-conjugates of halogenated alkenes and their corresponding mercapturic acids in rat renal tissue slices. Toxicology 1993; 79:67–79

30. Larsen GL, Bakke JE: Metabolism of mercapturic acid-pathway metabolites of 2-chloro-*N*-isopropylacetanilide (propachlor) by gastrointestinal bacteria. Xenobiotica 1983; 13:115–26

31. Iyer RA, Baggs RB, Anders MW: Nephrotoxicity of the glutathione and cysteine conjugates of the sevoflurane degradation product 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (compound A) in male, Fischer 344 rats. J Pharmacol Exp Ther 1997; 283:1544–51

32. Morio M, Fujii K, Satoh N, Imai M, Kawakami T, Negishi A, Kumagai Y, Kawai T: Reaction of sevoflurane and its degradation products with soda lime. Toxicity of the byproducts. ANESTHESIOLOGY 1992; 77:1155–64

33. Martin JL, Kandel L, Laster MJ, Kerschmann RL, Eger EI II: Studies on the mechanism of nephrotoxicity of compound A in rats. J Anesthesiol 1997; 11:32–7

34. Iyer RA, Anders MW: Cysteine conjugate β-lyase-dependent biotransformation of the cysteine *S*-conjugates of the sevoflurane degradation product compound A in human, nonhuman primate, and rat renal cytosol and mitochondria. ANESTHESIOLOGY 1996; 85:1454–61

35. Martin JL, Laster MJ, Kandel L, Kerschmann RL, Reed GF, Eger II EI: Metabolism of compound A by renal cysteine-S-conjugate β-lyase is not the mechanism of compound A-induced renal injury in the rat. Anesth Analg 1996; 82:770–4