Nephrotoxicity of the Glutathione and Cysteine S-Conjugates of the Sevoflurane Degradation Product 2-(Fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (Compound A) in Male Fischer 344 Rats

RAMASWAMY A. IYER, RAYMOND B. BAGGS and M. W. ANDERS

Departments of Pharmacology and Physiology (R.A.I., M.W.A.) and of Laboratory Animal Medicine (R.B.B.), University of Rochester, Rochester, New York

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ABSTRACT

2-(Fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (Compound A) is a halogenated alkene that is nephrotoxic in rats when administered by inhalation or intraperitoneally. Compound A undergoes glutathione-dependent metabolism: Compound A-derived glutathione S-conjugates and mercapturates are excreted in the bile and urine, respectively, of rats given Compound A. The present experiments were designed to study the nephrotoxicity of the Compound A-derived glutathione and cysteine S-conjugates, S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]glutathione (compound 2), S-[2-(fluoromethoxy)-1,1,3,3,3-tetrafluoro-1-propenyl]glutathione (3), S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine (4) and S-[2-(fluoromethoxy)-1,3,3,3-pentafluoro-1-propenyl]-L-cysteine (5). Conjugates 2, 3 and 4 given intraperitoneally produced dose-dependent nephrotoxicity that was characterized by diuresis, increased excretion of glucose and protein, elevated blood urea nitrogen concentrations and severe morphological changes in the kidneys, particularly in the proximal tubules. Glutathione S-conjugate 2, at a dose of 500 \( \mu \)mol/kg, was hepatotoxic. Cysteine S-conjugate 5 was not nephrotoxic, apparently because of its facile cyclization to the thiazolone 2-{[1-(fluoromethoxy)-2,2,2-trifluoroethyl]-4,5-dihydro-1,3-thiazole-4-carboxylic acid, which is not a \( \beta \)-lyase substrate. Also, the \( \alpha \)-methyl analog of cysteine S-conjugate 4 S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-DL-\( \alpha \)-methylcysteine, which cannot undergo \( \beta \)-lyase-dependent bioactivation, was not nephrotoxic. These in vivo data show that Compound A-derived S-conjugates are nephrotoxic and that the toxicity is associated with \( \beta \)-lyase-dependent bioactivation.

2-(Fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (Compound A) is the major degradation product of sevoflurane formed in anesthesia circuits in the presence of soda lime or Baralyme (Bito and Ikeda, 1994c; Fang and Eger, 1995; Frink et al., 1992b). Compound A is nephrotoxic when administered to rats by inhalation or intraperitoneally (Gonsowski et al., 1994a,b; Jin et al., 1995; Kandel et al., 1995; Keller et al., 1995; Kharasch et al., 1997). Compound A is a structural analog of several nephrotoxic fluoroalkenes that undergo glutathione- and \( \beta \)-lyase-dependent bioactivation. The \( \beta \)-lyase pathway involves hepatic glutathione S-conjugate formation, \( \gamma \)-glutamyltransferase- and dipeptidase-catalyzed hydrolysis to the corresponding cysteine S-conjugates, active uptake of the cysteine S-conjugates by the kidney and bioactivation by renal cysteine conjugate \( \beta \)-lyase (for review, see Dekant et al., 1994). Halothane undergoes base-catalyzed degradation to 2-bromo-2-chloro-1,1,fluoroethene (Sharpe et al., 1979), which is nephrotoxic (Raventós and Lemon, 1965) and is metabolized by the mercapturic acid pathway in humans (Wark et al., 1990). Glutathione and cysteine S-conjugates of 2-bromo-2-chloro-1,1-difluoroethene are nephrotoxic in rat and undergo \( \beta \)-lyase-dependent bioactivation (Finkelstein et al., 1992, 1994, 1995, 1996).

The available data indicate that Compound A is metabolized via the \( \beta \)-lyase pathway (fig. 1). The glutathione S-transferase-catalyzed reaction of Compound A 1 with glutathione gives diastereomeric S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]glutathione (compound 2) and (E)- and (Z)-S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]glutathione (3), which have been identified in the bile of rats given Compound A (Jin et al., 1995, 1996) and are formed in vitro by the glutathione S-transferase-catalyzed reaction of Compound A with glutathione (Jin et al., 1996). The \( \gamma \)-glutamyltransferase- and dipeptidase-catalyzed hydrolysis of glutathione S-conjugates 2 and 3 would give the cysteine S-conjugates S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine (4) and S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine (5), respectively. Cysteine S-conjugates 4 and 5 are substrates for rat, human and nonhuman primate renal mi-
thochondrial and cytosolic β-lyase (Iyer and Anders, 1996). Cysteine S-conjugates 4 and 5 are converted to the corresponding mercapturic acids, which are excreted in the urine of rats given Compound A (Jin et al., 1995) and in the urine of human subjects anesthetized with sevoflurane (Iyer et al., 1997). Also, cysteine S-conjugate 5 undergoes rapid cyclization to give 2-[1-(fluoromethoxy)-2,2,2-trifluoroethyl]-4,5-dihydro-1,3-thiazole-4-carboxylic acid 10 (Iyer and Anders, 1977). β-Lyase-catalyzed β-elimination reactions of cysteine S-conjugates 4 and 5 would be expected to afford 2-(fluoromethoxy)-1,3,3,3-pentafluoropropene and 2-(fluoromethoxy)-1,3,3,3-pentafluoropropanethiolate 6 and 2-(fluoromethoxy)-1,3,3,3-pentafluoropropanethiol 7, respectively. Thiolates 6 and 7 may give 2-(fluoromethoxy)-3,3,3-trifluoroethionopropyl fluoride 8, which may undergo hydrolysis to give 2-(fluoromethoxy)-3,3,3-trifluoroethionopropyl fluoride, which has been identified in the urine of rats given Compound A (Spracklin and Kharasch, 1996) and the urine of human subjects anesthetized with sevoflurane (Iyer et al., 1997). Thiaoctyl fluoride 8 may also react with tissue nucleo-

To study the nephrotoxicity of the Compound A-derived glutathione and cysteine S-conjugates S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]glutathione 2, S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]glutathione 3, S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-l-cysteine 4, and S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-l-cysteine 5, conjugates 2, 3, and 4 were nephrotoxic in male Fischer 344 rats, but cysteine S-conjugate 5 and S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-dl-a-methylcysteine were not nephrotoxic.

Experimental Procedures

Materials

2-(Fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (Compound A, 1) was provided by Abbott Laboratories (Abbott Park, IL). BAKER-BOND octadecyl (C18, 40 μm Prep LC packing) reverse-phase chromatographic column packing was obtained from J. T. Baker, Inc. (Phillipsburg, NJ). TLC plates (Whatman, silica gel, 250 μm, AL SIL G/UV with a fluorescent indicator) were purchased from VWR Scientific (Rochester, NY). N,N-Dimethylformamide was dried over calcium oxide and freshly distilled before use. All other reagents were obtained from commercial suppliers and were used without further purification, except as noted. N-Formylglutathione was synthesized by the procedure of Anderson et al. (1985). S-Benzyl-a-methyl-l-cysteine was synthesized by the procedure of Potts (1955).

Analytical Methods

Melting points were determined with a Buerk 270-MHz spectrometer operating at 270 MHz for 1H NMR and 19F NMR spectra were recorded with a Bruker 270-MHz spectrometer operating at 270 MHz for 1H and 254 MHz for 19F. Chemical shifts, δ, are reported in parts per million (ppm). The HOD resonance at 4.7 ppm was used as the internal standard for 1H NMR spectra when D2O was the solvent. The solvent resonance peak at 2.47 ppm was used as the internal standard for 19F NMR spectra when dimethylsulfoxide-d6 was the solvent. Trifluoroacetamide (0.0 ppm) was used as the external standard for 19F NMR spectra. Electrospray mass spectra of the glutathione conjugates 2 and 3 were obtained on a Sciex 300 electrospray and VG AutoSpec mass spectrometer at the University of California, San Francisco, Mass Spectrometry Facility. Elemental analyses were determined by Midwest Microlab (Indianapolis, IN). Silica gel or BAKERBOND were used for column chromatography. Glutathione and cysteine S-conjugates on TLC plates were detected with a spray reagent of 0.3% ninhydrin solution in n-butanol/acetic acid (97:3).

Syntheses

S-[2-(Fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]glutathione (2). Solid potassium hydroxide was added to a stirred suspension of glutathione (6.14 g, 20 mmol) and ethylenediaminetetraacetic acid disodium salt (72 mg, 0.2 mmol) in water (10 ml) to a pH of 9.6, when the suspension became a clear solution. A solution of butylated hydroxytoluene (42 mg, 0.2 mmol) in ethanol (10 ml) was added followed by 5 ml of water. 2-(Fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (1, 3.96 g, 22.0 mmol) in 5 ml of ethanol was added dropwise during 50 min to the stirred reaction mixture. The reaction mixture was stirred at room temperature for 3 h. Ethanol/H2O (1:1, 20 ml) was added, and the solution was brought to pH 2 by addition of trifluoroacetic acid. Evaporation of the solvent in vacuo gave a yellow oil. 19F NMR spectroscopic analysis of the crude reaction mixture showed the formation of S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]glutathione 2 and S-[2-(fluoromethoxy)-1,3,3,3-
tetrafluoro-1-propenylglutathione 3 in an approximate ratio of 7:3. The yellow viscous oil was dissolved in aqueous 0.1% acetic acid solution and loaded onto a C18 reverse-phase column. The column was eluted with acetonitrile/water/acidic acid (5:94:1 and then 10: 89:1). The initial fractions were pooled and analyzed by TLC. (Glutathione S-conjugate 2 is not UV-active, whereas conjugate 3 is UV-active on TLC plates with a fluorescent indicator.) Evaporation of the solvent gave S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyrl]glutathione 2 as a white solid (0.2% g, 232%): m.p. 185–187°C (dec); TLC, Rf = 0.25 (n-butanol/water/acidic acid, 6:1:1); 1H NMR (DMSO-D6) δ 8.92 (t, 1H, NH of Gly), 8.70 (d, 1H, NH of Cys), 5.60–5.80 (m, 2H, CF3CH2COOH and OCH2F), 5.56 (d of d, 1H, OCH2F), 4.50–4.68 (m, 1H, α-CH of Cys), 3.78 (2H, α-CH of Gly), 3.56–3.70 (m, 2H, α-CH of Gly and β-CH of Cys), 3.08–3.20 (m, 1H, β-CH of Cys), 2.35–2.50 (m, 2H, –CH2 of Gly), 1.80–2.15 (m, 2H, –CH2 of Glu); 4F NMR (DMSO-D6) δ 3.45–3.80 (m, 3F, CF3CH2COOH and OCH2F), –5.90 to –4.00 (m, 1F, CF3CH2COOH and OCH2F), –8.20 to –7.00 (m, 1F, CF3CH2COOH and OCH2F), –74.80 (t, 1F, J = 54 Hz, OCH2F); MS (electrospray) m/z (%) 488 ([M+H]+, 100), 413 ([M+H-Gly]+), 2, 359 ([M+H-NH2]+), 5, 319 [259–266, 2], 299 (H3NOCC(=NH)CH2SCF2CH2OCH2F)+, 2), 256 ([NH2=CH2CH2SCF2CH2OCH2F]+, 3).

S-[2-(fluoromethoxy)-1,1,3,3,3-tetrafluoro-1-propenylglutathione 3. S-[2-(fluoromethoxy)-1,1,3,3,3-tetrafluoro-1-propenyl-N-formylglutathione. Diisopropylethylamine (1.0 g, 7.8 mmol) was added to a stirred solution of N-formylglutathione (1.0 g, 2.98 mmol) in N,N-dimethylformamide (15 ml) under a nitrogen atmosphere, and the solution was cooled to 0°C. 2-(Fluoromethoxy)-1,1,3,3,3-tetrafluoro-1-propene (590 mg, 3.30 mmol) in 5 ml of N,N-dimethylformamide was added dropwise to the reaction mixture over 10 min, and the reaction was stirred for 45 min at 0°C. After addition of 30 ml of water, the pH of the solution was brought to pH 4.5 with conc. HCl. The solution was concentrated to about 10 ml and loaded onto a C18 reverse-phase column. Elution with acetonitrile/water/acidic acid (20:79:1) gave a waxy pale yellow solid (1.3 g, 94%): TLC, Rf = 0.62 (n-butanol/water/acidic acid, 6:1:1); 1H NMR (DMSO-D6) δ 8.38–8.58 (m, 2H, NH of Gly and NH of Cys), 8.15 (s, 1H, N-formyl), 5.65 (d of d, 2H, J = 54 Hz, OCH2F), 4.30–4.40 (m, 1H, α-CH of Cys), 3.82 (d, 2H, α-CH of Gly), 3.60–3.75 (m, 1H, α-CH of Gly), 3.40–3.52 (m, 1H, β-CH of Cys), 3.15–3.28 (m, 1H, β-CH of Cys), 2.25–2.40 (m, 2H, –CH2 of Gly), 1.80–2.15 (m, 2H, –CH2 of Glu); 19F NMR (DMSO-D6) δ 11.00–11.70 (m, 3F, CF3C(CF3)OCH2F), –33.50 to –32.20 (m, 1F, CF3C(CF3)OCH2F), –74.90 (t, 1F, J = 54 Hz, OCH2F), –75.70 (t, 1F, J = 54 Hz, OCH2F); elemental analysis for C8H11NO3F6S, calcd, C, 30.48; H, 3.52; N, 4.44; F, 36.16; found, C, 30.18; H, 3.48; N, 4.37; F, 36.43.

In Vivo Toxicity Studies

Male Fisher 344 rats (200–250 g, Charles River Laboratories, Inc., Wilmington, MA) were given 125, 250 or 500 μmol/kg of conjugates 2 or 4, 62.5, 125 or 250 μmol/kg conjugate 3 or 31.25, 62.5 or 125 μmol/kg conjugate 5 i.p. The conjugates were dissolved in 0.9% saline, which was given in a volume of 6.6 ml/kg. Control animals received saline. All rats were housed individually in metabolism cages with a 12-h light/dark cycle and were provided with food and water ad libitum. Urine was collected at 24-h intervals in the presence of sodium azide (10 mg).

After 48 h, the rats were anesthetized with ether and sacrificed by cardiac exsanguination. The blood was collected and centrifuged to obtain serum. The left kidney was removed and trimmed of fat; the capsule was removed and weighed. Liver and kidney tissues were fixed in 0.2% 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 scored from 0 (no protein casts) 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; hence, 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 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, St. Louis, MO). Urine protein concentrations were measured by the method of Bradford (1976) (Bio-Rad Protein Assay Dye Reagent Concentrate; Bio-Rad, Richmond, CA) with bovine serum albumin as the standard.

Results

Syntheses

S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]glutathione 2 and S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl-
animals given 500 μmol/kg glutathione S-conjugate 2 could be separated from conjugate 3 by C18 reverse-phase column chromatography, conjugate 3, free of conjugate 2, could not be obtained in satisfactory yield. To improve the yield and purity of conjugate 3, the γ-glutamyl amino group of glutathione was protected as the N-formyl group (Anderson et al., 1985) to increase its solubility in polar aprotic solvents. Reaction of a solution of N-formylglutathione in anhydrous N,N-dimethylformamide with fluoroalkene 1 gave S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-N-formylglutathione as the major product. Hydrolysis of the N-formyl group under mildly acidic condition gave glutathione S-conjugate 3.

**Toxicity Studies**

Intraperitoneal administration of glutathione S-conjugates 2 or 3 or cysteine S-conjugate 4 to male Fischer 344 rats caused changes in blood and urine chemistry indicative of kidney damage. Urine glucose excretion was markedly increased in rats given 250 μmol/kg conjugate 2 (fig. 2), 125 μmol/kg conjugate 3 (fig. 3) or 125, 250 or 500 μmol/kg conjugate 4 (fig. 4) after 24 and 48 h. Urine glucose concentrations were lower in rats given 500 μmol/kg conjugate 2 (fig. 2) or 250 μmol/kg conjugate 3 (fig. 3) than in rats given 250 μmol/kg conjugate 2 or 125 μmol/kg conjugate 3, respectively. Serum glucose concentrations were not altered in rats given conjugates 2, 3 or 4 (data not shown). Urine protein excretion rates were elevated after 24 and 48 h in rats administered 250 μmol/kg conjugate 2 (fig. 2), 125 μmol/kg conjugate 3 (fig. 3) or 125, 250 or 500 μmol/kg conjugate 4 (fig. 4). Increases in urine volumes were seen in rats given 250 μmol/kg conjugate 2 (fig. 2) or 125 μmol/kg conjugate 3 (fig. 3) after 24 h, whereas in rats given conjugate 4 increases in urine volumes were seen after giving doses of 125, 250 or 500 μmol/kg after 24 and 48 h (fig. 4). Blood urea nitrogen concentrations were elevated in rats given 250 μmol/kg conjugate 2 (fig. 2), 125 or 250 μmol/kg conjugate 3 (fig. 3) or 250 or 500 μmol/kg conjugate 4 after 48 h (fig. 4). Blood urea nitrogen concentrations were increased after 24 h in rats given 500 μmol/kg conjugate 2 (fig. 2). Serum glutamate-pyruvate transaminase activities were elevated after 24 h in animals given 500 μmol/kg conjugate 2 (fig. 2).

Hepatic lesions were seen only in rats given 500 μmol/kg glutathione S-conjugate 2, which was lethal after 24 h (fig. 5A). Hepatic lesions were not observed in rats given 250 μmol/kg glutathione S-conjugate 3 or 500 μmol/kg cysteine S-conjugate 4 or in control animals. Kidney/body weight percentages were increased after 24 h in animals given 500 μmol/kg conjugate 2 or after 48 h in animals given 250 or 500 μmol/kg conjugate 4 (table 1). Morphological changes indicative of kidney damage were seen in rats treated with conjugates 2, 3 or 4, but no significant lesions were observed in control animals. Conjugate-induced kidney damage was dose-dependent, and the results are reported as the summary damage score (table 1), which is the sum of the individual severity scores for each region of the kidney and provides an integrated index of the severity of the damage to the nephron. Damage was most severe in the juxtamedullary proximal convoluted tubules, and less damage was seen in the paracortical and cortical nephrons, particularly at lower doses (fig. 5C).

Cysteine S-conjugate 5 was not nephrotoxic, as indicated by the lack of changes in urine or blood chemistry, when given to rats at doses of 31.25, 62.5 or 125 μmol/kg (data not shown). Similarly, S-[2-(fluoromethoxy)-1,1,3,3,3-pentaflu-
oropropyl]-DL-a-methylcysteine produced no changes in blood and urine chemistry compared with control animals when given to rats at doses of 500 or 750 \( \mumol/kg \) (data not shown).

**Discussion**

The objective of this work was to study the nephrotoxicity of glutathione and cysteine S-conjugates of Compound A in male Fischer 344 rats. The data presented herein show that S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]glutathione 2, S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]glutathione 3 and S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine 4 were nephrotoxic when given to rats i.p. In contrast, S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine 5 was not nephrotoxic (see below). The nephrotoxicity of these conjugates was characterized by increases in urine glucose and protein excretion rates, in urine volumes, in kidney/body weight percentages and in blood urea nitrogen concentrations (figs. 2–4).

The morphological assessment of kidney damage after ad-
ministration of conjugates 2, 3 or 4 was consistent with the clinical-chemistry data (table 1). Selective damage to the proximal convoluted tubules, which is the site of glucose reabsorption (von Baeyer, 1981), is expected to cause increases in glucose excretion. Also, the lack of changes in serum glucose concentrations in S-conjugate-treated rats indicates that the increased glucose excretion was associated with decreased tubular reabsorption. The decrease in glucose excretion rates in rats given 500 or 250 μmol/kg glutathione S-conjugate 2 or 3 (fig. 2 or 3), respectively, can be explained by decreases in glomerular filtration associated with tubular damage (van Liew et al., 1967). The increases in urinary protein excretion rates reflect the protein casts that were seen in the collecting ducts in S-conjugate-treated rats.

Glutathione S-conjugate 2 at a dose of 500 μmol/kg was hepatotoxic, as indicated by increases in serum glutamate-pyruvate transaminase activities. Morphological examination of the livers of rats given conjugate 2 showed hepatocellular necrosis. Also, conjugate 2 given at a dose 500 μmol/kg dose was lethal after 24 h, but the analogous cysteine S-conjugate 4 was not hepatotoxic at a dose of 500 μmol/kg.

<table>
<thead>
<tr>
<th>Conjugate (μmol/kg)</th>
<th>Kidney/Body Weight (× 100)</th>
<th>Morphological Assessment of Kidney Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.34 ± 0.01</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td>Conjugate 2 (125)</td>
<td>0.38 ± 0.03</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td>Conjugate 2 (250)</td>
<td>0.39 ± 0.03</td>
<td>12.3 ± 2.62*</td>
</tr>
<tr>
<td>Conjugate 2 (500)</td>
<td>0.44 ± 0.03*</td>
<td>16.0 ± 0.00*</td>
</tr>
<tr>
<td>Conjugate 3 (62.5)</td>
<td>0.37 ± 0.02</td>
<td>2.3 ± 0.47*</td>
</tr>
<tr>
<td>Conjugate 3 (125)</td>
<td>0.37 ± 0.03</td>
<td>12.3 ± 2.86*</td>
</tr>
<tr>
<td>Conjugate 3 (250)</td>
<td>0.37 ± 0.03</td>
<td>15.3 ± 0.47*</td>
</tr>
<tr>
<td>Conjugate 4 (125)</td>
<td>0.39 ± 0.01*</td>
<td>5.7 ± 1.69*</td>
</tr>
<tr>
<td>Conjugate 4 (250)</td>
<td>0.41 ± 0.01*</td>
<td>8.0 ± 0.81*</td>
</tr>
<tr>
<td>Conjugate 4 (500)</td>
<td>0.39 ± 0.02*</td>
<td>10.0 ± 1.63*</td>
</tr>
</tbody>
</table>

* Significantly different (P ≤ .05) from control rats.

Fig. 5. (A) Liver from rat given 500 μmol/kg S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]glutathione 2. Note the pronounced centrilobular necrosis (n), with a border composed of cells with pyknotic nuclei (arrow head) between normal periatrial (t) hepatocytes and those about the central vein (c). Bar = 100 μm. (B) Normal liver. Triad (t) and central vein (c). Bar = 100 μm. (C) Kidney from rat given 500 μmol/kg S-[2-(fluoromethoxy)-1,3,3,3-pentafluoropropyl]glutathione 2. The proximal convoluted tubules (p) are lined by necrotic epithelium. The distal convoluted tubules (d) and glomeruli (g) are spared. Bar = 100 μm. (D) Normal kidney. Proximal (p) and distal (d) convoluted tubules are normal. Bar = 100 μm. Although sections from all treated or control animals were examined morphologically, only sections from individual rats are shown in the figure.
Hepatotoxicity is not a common feature of S-conjugate-induced toxicity. Increases in serum transaminase activities and morphological evidence of liver damage have been reported in rats given 500 μmol/kg of S-(2-bromo-2-chloro-1,1-difluoroethyl)glutathione or S-(2-bromo-2-chloro-1,1-difluoroethyl)-l-cysteine, the glutathione and cysteine S-conjugates, respectively, of 2-bromo-2-chloro-1,1-difluoroethylene, a degradation product of halothane (Finkelstein et al., 1992). In addition, liver damage was observed in some rats given 450 μmol/kg of S-(2,2-dichloro-1,1-difluoroethyl)-N-acetyl-l-cysteine (Commandeur et al., 1987).

The available data indicate that Compound A undergoes β-lyase-dependent metabolism, as shown in figure 1. The data presented herein also indicate a role for the β-lyase pathway in the observed nephrotoxicity of Compound A in rats: Compound A is nephrotoxic in rats (Gonsowski et al., 1994a, b; Jin et al., 1995; Kandel et al., 1995; Keller et al., 1995; Kharasch et al., 1997). Similarly, several fluoroalkenes are nephrotoxic and undergo β-lyase-dependent bioactivation (Dekant et al., 1994). Compound A-derived glutathione S-conjugates 2 and 3 and cysteine S-conjugate 4 were nephrotoxic in rats (fig. 2–5). Cysteine S-conjugate 4 is a substrate for renal mitochondrial and cytosolic β-lases (Iyer and Anders, 1996). The finding that S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-DL-lysine is not nephrotoxic also indicates a role for β-lyase-dependent bioactivation. The catalytic cycle of the pyridoxal phosphate-dependent β-lyase requires abstraction of the α-hydrogen. Hence, the lack of an α-hydrogen in the α-methyl analog of cysteine S-conjugate 4 prevents the critical β-lyase-catalyzed β-elimination reaction required for toxicity. The α-methylcysteine S-conjugates S-(2-chloro-1,1,2-trifluoroethyl)-DL-α-methylcysteine (Dohn et al., 1985a) and S-(1,2-dichlorovinyl)-DL-α-methylcysteine (Elfarra et al., 1986) are not nephrotoxic. Finally, the nephrotoxicity of Compound A is partially inhibited by the β-lyase inhibitor (aminooxy)acetic acid (Jin et al., 1995; Kharasch et al., 1997), which also indicates a role for β-lyase-dependent bioactivation. Although considerable evidence implicates the β-lyase pathway in the bioactivation of Compound A, evidence purporting to show that the β-lyase pathway is not involved has been presented (Martin et al., 1996). Preliminary studies show that acvinil (aminooxy)acetic acid partially block the nephrotoxicity of glutathione S-conjugates 2 and 3 (R. A. Iyer and M. W. Anders, unpublished results). Hence, further studies are warranted to provide insight into the role of the β-lyase pathway in Compound A-induced nephrotoxicity in rats.

Although Compound A is clearly nephrotoxic in rats, Compound A-associated nephrotoxicity has not been observed in the clinical use of sevoflurane as an anesthetic agent in humans (Bito and Ikeda, 1994a, c; Bito and Ikeda, 1996; Conzen et al., 1995; Frink et al., 1992a; Higuchi et al., 1995; Mori et al., 1996; Nishiyama et al., 1996; Tsukamoto et al., 1996). Transient renal injury has, however, been reported in human volunteers anesthetized with sevoflurane and thereby exposed to Compound A (Eger et al., 1997). Others have failed to detect renal injury in human volunteers anesthetized with sevoflurane (Ebert et al., 1997). The concentration of Compound A found in the anesthetic circuit ranges from 14 to 30 ppm (Bito and Ikeda, 1994b). The threshold for Compound A-induced nephrotoxicity in rats ranges from 50 to 114 ppm for a 3-h exposure (Gonsowski et al., 1994b; Keller et al., 1995). The lack of Compound A-induced nephrotoxicity in humans may be related to both the relatively low concentrations of Compound A in the anesthetic circuit and to the low renal β-lyase activities in humans (Iyer and Anders, 1996; Lash et al., 1990).

The relative roles of glutathione S-conjugates 2 and 3 and of cysteine S-conjugates 4 and 5 in the nephrotoxicity of Compound A are not clear. Glutathione S-conjugate 3 was more nephrotoxic than conjugate 2 (figs. 2 and 3). Although glutathione S-conjugate 3 was nephrotoxic, the corresponding cysteine S-conjugate 5 was not nephrotoxic. This indicates that cysteine S-conjugate 5 obtained by the hydrolysis glutathione S-conjugate 3 is delivered to the kidney and undergoes β-lyase-catalyzed bioactivation. In contrast, administered cysteine S-conjugate 5 was not nephrotoxic, perhaps because it undergoes rapid cyclization to thiazoline 10, which cannot undergo β-lyase-dependent bioactivation (Iyer and Anders, in press). Although plasma concentrations of Compound A-derived S-conjugates have not been reported, nearly equal fractions (about 15%) of a dose of Compound A are excreted in the bile as glutathione S-conjugates 2 and 3 (Jin et al., 1996).

In summary, glutathione and cysteine S-conjugates of Compound A are nephrotoxic in rats and are bioactivated by the β-lyase pathway. Hence, the observed nephrotoxicity of Compound A in rats may be associated with glutathione S-conjugate formation and β-lyase-dependent bioactivation of the corresponding cysteine S-conjugates. Further studies exploring the role of the β-lyase pathway in the nephrotoxicity of Compound A are warranted.

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