BIOTRANSFORMATION OF L-CYSTEINE S-CONJUGATES AND N-ACETYL-L-CYSTEINE S-CONJUGATES OF THE SEVOFLURANE DEGRADATION PRODUCT FLUOROMETHYL-2,2-DIFLUORO-1-(TRIFLUOROMETHYL)VINYL ETHER (COMPOUND A) IN HUMAN KIDNEY IN VITRO: INTERINDIVIDUAL VARIABILITY IN N-ACETYLATION, N-DEACETYLATION, AND \( \beta \)-LYASE-CATALYZED METABOLISM

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ABSTRACT:

Fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether (FDVE; 1) is a fluoroalkene formed by the base-catalyzed degradation of the anesthetic sevoflurane. FDVE is nephrotoxic in rats. In both rats and humans, FDVE undergoes glutathione-dependent conjugation, cleavage to cysteine \( S \)-conjugates, and renal \( \beta \)-lyase-catalyzed metabolism to reactive intermediates, which may cause nephrotoxicity. Interindividual variability in renal metabolism of FDVE is unknown. Therefore, this investigation quantified \( \beta \)-lyase-catalyzed bioactivation and \( N \)-acetylation of FDVE cysteine \( S \)-conjugates and reactivation of mercapturates by \( N \)-deacetylase in cytosol and microsomes from 20 human kidneys. In cytosol, \( N \)-acetylation ranged from 0.008 to 0.045 (0.024 ± 0.01) nmol of mercapturate/mg/min and from 0.001 to 0.07 (0.024 ± 0.02) nmol of mercapturate/mg/min for alkane and alkene cysteine \( S \)-conjugates, respectively. Similar results for microsomal \( N \)-acetylation were obtained; \( N \)-acetylation ranged from 0.005 to 0.055 (0.025 ± 0.02) nmol of mercapturate/mg/min and from 0.001 to 0.06 (0.030 ± 0.02) nmol of mercapturate/mg/min for alkane and alkene cysteine \( S \)-conjugates, respectively. \( \beta \)-Lyase-catalyzed metabolism to pyruvate varied from 0.004 to 0.14 (0.051 ± 0.04) nmol/mg/min and from 0.10 to 0.40 (0.26 ± 0.08) nmol/mg/min for alkane and alkene cysteine \( S \)-conjugates, respectively. \( N \)-deacetylation of mercapturates ranged from 0.8 to 2.5 (1.25 ± 0.57) nmol of cysteine \( S \)-conjugate formed/mg/min and 0.05 to 0.37 (0.17 ± 0.10) nmol of cysteine \( S \)-conjugate formed/mg/min for alkane and alkene FDVE mercapturates. Cytosolic cysteine \( S \)-conjugates metabolism by renal \( \beta \)-lyase predominated over \( N \)-acetylation (ratio of activities was 0.2–6 and 3–146 for the alkane and alkene cysteine \( S \)-conjugates). \( N \)-deacetylation predominated over \( N \)-acetylation (ratio of activities was 20–205 and 2–54 for the alkane and alkene \( S \)-conjugates). There was considerable (up to 50-fold) interindividual variability in rates of FDVE toxification (\( \beta \)-lyase metabolism and \( N \)-deacetylation) and detoxification. This interindividual variability may effect individual susceptibility to the nephrotoxicity of FDVE and other haloalkenes.

Sevoflurane (fluoromethyl 2,2,2-trifluoro-1-[trifluoromethyl]ethyl ether) is an inhalational anesthetic agent that is used worldwide. When used in anesthesia machines equipped with carbon dioxide adsorbents containing strong base, sevoflurane undergoes base-catalyzed elimination of hydrogen fluoride to form fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether (FDVE; 1), trivially referred to as compound A) as a major degradation product (Hanaki et al., 1987; Frink et al., 1992). Patients may be exposed to 20 to 50 ppm of FDVE under these conditions. Like several other haloalkenes, FDVE is nephrotoxic in rats. In both rats and humans, FDVE undergoes glutathione-dependent conjugation, cleavage to cysteine \( S \)-conjugates, and renal \( \beta \)-lyase-catalyzed metabolism to reactive intermediates, which may cause nephrotoxicity. Interindividual variability in renal metabolism of FDVE is unknown. Therefore, this investigation quantified \( \beta \)-lyase-catalyzed bioactivation and \( N \)-acetylation of FDVE cysteine \( S \)-conjugates and reactivation of mercapturates by \( N \)-deacetylase in cytosol and microsomes from 20 human kidneys. In cytosol, \( N \)-acetylation ranged from 0.008 to 0.045 (0.024 ± 0.01) nmol of mercapturate/mg/min and from 0.001 to 0.07 (0.024 ± 0.02) nmol of mercapturate/mg/min for alkane and alkene cysteine \( S \)-conjugates, respectively. Similar results for microsomal \( N \)-acetylation were obtained; \( N \)-acetylation ranged from 0.005 to 0.055 (0.025 ± 0.02) nmol of mercapturate/mg/min and from 0.001 to 0.06 (0.030 ± 0.02) nmol of mercapturate/mg/min for alkane and alkene cysteine \( S \)-conjugates, respectively. \( \beta \)-Lyase-catalyzed metabolism to pyruvate varied from 0.004 to 0.14 (0.051 ± 0.04) nmol/mg/min and from 0.10 to 0.40 (0.26 ± 0.08) nmol/mg/min for alkane and alkene cysteine \( S \)-conjugates, respectively. \( N \)-deacetylation of mercapturates ranged from 0.8 to 2.5 (1.25 ± 0.57) nmol of cysteine \( S \)-conjugate formed/mg/min and 0.05 to 0.37 (0.17 ± 0.10) nmol of cysteine \( S \)-conjugate formed/mg/min for alkane and alkene FDVE mercapturates. Cytosolic cysteine \( S \)-conjugates metabolism by renal \( \beta \)-lyase predominated over \( N \)-acetylation (ratio of activities was 0.2–6 and 3–146 for the alkane and alkene cysteine \( S \)-conjugates). \( N \)-deacetylation predominated over \( N \)-acetylation (ratio of activities was 20–205 and 2–54 for the alkane and alkene \( S \)-conjugates). There was considerable (up to 50-fold) interindividual variability in rates of FDVE toxification (\( \beta \)-lyase metabolism and \( N \)-deacetylation) and detoxification. This interindividual variability may effect individual susceptibility to the nephrotoxicity of FDVE and other haloalkenes.

The formation of cysteine \( S \)-conjugates is thought to play an important role in the nephrotoxicity of haloalkenes. Chlorinated and fluorinated alkenes are nephrotoxic, and their nephrotoxicity is associated with a multistep pathway that includes hepatic glutathione \( S \)-conjugate formation, enzymatic hydrolysis of the glutathione \( S \)-conjugates to cysteine \( S \)-conjugates, renal uptake of cysteine \( S \)-conjugates, and bioactivation by renal cysteine \( S \)-conjugate \( \beta \)-lyase to reactive species, in which the reaction with cellular proteins is associated with cell damage and death (Dekant et al., 1987, 1991; Pähler et al., 1999). The equilibrium between cysteine \( S \)-conjugate and mercapturic acid concentrations thus influences the extent of \( \beta \)-lyase-dependent bioactivation and subsequent nephrotoxicity (Commandeur et al., 1988; Boogaard et al., 1989; Iyer et al., 1998; Kharasch et al., 1999). In rats, differences between compounds in the rates of \( N \)-acetylation and \( N \)-deacetylation activities and differences in the specific activities of \( \beta \)-lyase-catalyzed metabolism were thought to explain the respective differences in the toxicity of various haloalkenes (Commandeur et al., 1991). FDVE metabolism via glutathione conjugation and the \( \beta \)-lyase pathway has been established in rats and humans. In rats, in vivo

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1 Abbreviations used are: FDVE, fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether; GSH, glutathione; LC/MS, liquid chromatography/mass spectrometry; GS/MS, gas chromatography/mass spectrometry; HKC, human kidney cytosol.

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INTERINDIVIDUAL VARIABILITY IN HUMAN RENAL HALOALKENE METABOLISM

FDVE undergoes a reaction with GSH to form two alkane diastereomers S-[1,1-difluoro-2-fluoromethoxy-2-(trifluoromethyl)ethyl]glutathione (Fig. 1, 2) and two alkene (E)- and (Z)-S-[1,1-difluoro-2-fluoromethoxy-2-(trifluoromethyl)vinyl]glutathione conjugates (Fig. 1, 3), which undergo cleavage to the corresponding cysteine S-conjugates (Fig. 1, 4 and 5) (Jin et al., 1995, 1996; Spracklin and Kharasch, 1996). In rats, N-acetylation forms the mercapturates, two alkane S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-N-acetyl-L-cysteine diastereomers (Fig. 1, 6) and (E)- and (Z)-S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-N-acetyl-L-cysteine (Fig. 1, 7), which are excreted in urine, as identified by ion-spray LC-tandem mass spectrometry, 19F NMR, and selected-ion mode GC/MS (Jin et al., 1995; Spracklin and Kharasch, 1996; Iyer et al., 1998). The cysteine S-conjugates are also metabolized by rat renal β-lyase in vitro and in vivo to reactive intermediates, which may bind to cellular macromolecules or undergo hydrolysis to 3,3,3-trifluoro-2-(fluoromethoxy)propanoic acid (Fig. 1, 8) (Iyer and Anders, 1996, 1997; Spracklin and Kharasch, 1996; Iyer et al., 1998; Kharasch et al., 1999). The latter has been identified in rat urine by 19F NMR and GC/MS (Spracklin and Kharasch, 1996; Iyer and Anders, 1997). Cysteine S-conjugates are metabolized by human kidney cytosol and mitochondria in vitro (Iyer and Anders, 1996). FDVE mercapturates (6 and 7) and 3,3,3-trifluoro-2-(fluoromethoxy)propanoic acid (8) were recently identified by 19F NMR and GC/MS in the urine of patients exposed to FDVE while undergoing sevoflurane anesthesia (Iyer et al., 1998; Kharasch et al., 1999). It has been reported that 3,3,3-trifluoro-2-(fluoromethoxy)propanoic acid (8) was unstable and decomposed to trifluoroacetic acid (9) (Iyer and Anders, 1997), which also was variably found in vivo by 19F NMR and GC/MS in rat and human urine (Iyer et al., 1998; Kharasch et al., 1999).

Although bioactivation (N-deacetylation, β-lyase metabolism) and inactivation (N-acetylation) of cysteine S-conjugates and mercapturates of FDVE clearly occurs in humans, interindividual variability has not been reported. Such variability may suggest individuals at potentially higher risk for FDVE toxicity. More generally, interindividual human variability in haloalkane and haloalkane S-conjugates metabolism is not well characterized. FDVE may thus serve as a model compound for characterizing this variability. Since relative bioactivation and inactivation of S-conjugates may affect the relative nephrotoxicity of different haloalkanes and haloalkanes (Commandeur et al., 1991; Birner et al., 1993, 1997; Bernauer et al., 1996; Brüning et al., 1998; Völkel et al., 1998), relative bioactivation and inactivation may similarly affect the relative susceptibility of different individuals. Therefore, in the present study, cystosolic and microsomal fractions from 20 human kidneys were used to determine the interindividual variability in relation to N-acetylation/N-deacetylation balance and β-lyase-catalyzed metabolism of cysteine S-conjugates and mercapturates of FDVE.

**Experimental Procedures**

**Materials.** FDVE (99.92% purity) was provided by Abbott Laboratories (Abbott Park, IL). L-Cysteine and N-acety-L-cysteine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). All other reagents were obtained from commercial suppliers and used without further purification. S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine, S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine, S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-N-acetyl-L-cysteine, and S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-N-acetyl-L-cysteine were synthesized as described previously (Iyer and Anders, 1997).

The cysteine conjugates of FDVE (yellow oily product) were purified by semipreparative high-performance liquid chromatography (Hewlett-Packard 1050 LC system; Waldbronn, Germany) using an Ultrasphere ODS column (25-cm × 10-mm i.d., 5-μm particle size; Beckman Coulter, Inc., Fullerton, CA). The mobile phase of 10% methanol in water (adjusted with trifluoroacetic acid), pH 3.0, was delivered at a flow rate of 5 ml min−1. The products were detected by UV absorbance at 215 nm. The cysteine S-conjugates of FDVE were eluted with retention times of 13.5 min and 14.6 min (two alkane diastereomers), 18.9 min (mixture of E- and Z-alkenes). The fractions were collected and evaporated in vacuo to yield white crystals of S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine (4) and E- and Z-S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine (Fig. 1, 5).

The N-acetylated-L-cysteine S-conjugates of FDVE (yellow oily product) were also purified as described above. The mobile phase composition was 40% methanol in water, pH 3.0. The mercapturic acid conjugates were eluted with retention times of 12.5 min (mixture of two alkane diastereomers) and 17.2 min. The products were detected by UV absorbance at 215 nm. The cysteine S-conjugates of FDVE were eluted with retention times of 13.5 min and 14.6 min (two alkane diastereomers), 18.9 min (mixture of E- and Z-alkenes). The fractions were collected and evaporated in vacuo to yield white crystals of S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine (4) and E- and Z-S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine (Fig. 1, 5).

**In Vitro Studies.** Human kidneys were obtained from the National Disease Research Interchange (Philadelphia, PA). Cadaveric kidneys were retrieved within 4 h of asystole, frozen in liquid nitrogen, and shipped on dry ice to our laboratory. Donor kidneys were obtained from organ donors, perfused with University of Wisconsin solution, shipped on wet ice to our laboratory, and frozen in liquid nitrogen. Tissue was stored at −80°C until used. Previous experiments showed similar rates of anesthetic metabolism from cadaveric or donor kidneys (Kharasch et al., 1995). Cytosolic and microsomal fractions from 20 human kidneys were prepared as described by Kharasch and Thummel (1993). Cytosol was dialyzed in Slide-A-Lyzer dialysis cassettes (3.5-kDa cut-off; Pierce Co., Rockford, IL) for 24 h at 4°C in 0.1 M phosphate buffer, pH 7.4, and stored at −80°C until required. Protein concentrations of cytosolic and microsomal fractions were measured by the method of Lowry et al. (1951), with bovine serum albumin as the standard. Heat-inactivated cytosol and microsomes were prepared by boiling for 3 to 4 min.

**β-Lyase-Dependent Biotransformation of Cysteine S-Conjugates.** Incubation mixtures contained cysteine S-conjugates (2 mM), human kidney cytosolic protein (2 mg ml−1), and potassium phosphate buffer (100 mM), pH 7.4, in a final volume of 0.25 ml. The first chuting (13.5 min) alkane conjugate diastereomer, and a mixture of E- and Z-alkene cysteine conjugates were...
studied. The reaction mixtures were incubated for 30 min at 37°C with constant shaking and terminated by adding 30% (v/v) trichloroacetic acid in water (to pH 2.0). Precipitated proteins were removed by centrifugation for 10 min at 13,000g. Pyruvate concentrations in the supernatant were quantified enzymatically (Pyruvate kit 726; Sigma Chemical Co., St. Louis, MO). The pyruvate released was converted to lactate by lactate dehydrogenase in the presence of NADH. The progress of the reaction was monitored by measuring the decrease in absorbance at 340 nm in a spectrophotometer (Molecular Devices Corp., Sunnyvale, CA). Heat-inactivated proteins were used as a negative control. Standard curves were prepared with pyruvic acid. The limit of detection was 50 pmol, with a linear response obtained with samples containing 0.05 to 5 nmol.

**N-Acetylation of Cysteine S-Conjugates of FDVE.** The assay was carried out as described previously (Aigner et al., 1994). Briefly, cysteine S-conjugates (2 mM) were incubated with human kidney cytosolic and microsomal protein (2.5 mg ml⁻¹), acetyl-CoA (1.6 mM), and potassium phosphate buffer (150 mM), pH 7.0, in a final volume of 0.25 ml. The first eluting (13.5 min) alkane conjugate diastereomer and a mixture of E- and Z-alkene cysteine conjugates were studied. The above mixture was incubated at 37°C for 30 min, and the reaction was terminated by heating at 100°C for 2 min. The proteins were precipitated on ice for 2 min and separated by centrifugation for 30 min at 13,000g. The supernatant (10 μl) was analyzed by LC/MS to determine the amount of N-acetylated conjugates present. Heat-inactivated human kidney cytosol and microsomes were used as a negative control. Calibration curves were prepared with synthetic N-acetyl-l-cysteine conjugates (0.01–1.5 nmol) and were linear over the concentration range used (r² > 0.99).

**N-Deacetylation of N-Acetyl-l-Cysteine Conjugates of FDVE.** The reaction mixture (0.25 ml) contained 2.5 mg ml⁻¹ human kidney cytosolic protein and mercapturic acid conjugates of FDVE (2 mM) in 50 mM potassium phosphate buffer, pH 7.4. The mixture of alkane diastereomers and the Z-alkene mercapturate was studied. The mixture was incubated at 37°C for 30 min, and the reaction was stopped by adding 20 μl of 20% trichloroacetic acid. The mixture was allowed to stand for 10 min on ice and then centrifuged. A sample (10 μl) of supernatant was analyzed by LC/MS. Control experiments with heat-inactivated human kidney cytosol were performed. Samples for calibration curves were prepared by adding known amounts of synthetic cysteine S-conjugates (1.0–40 nmol). Calibration curves were linear over the concentration range used (r² > 0.99).

**Analysis.** The LC/MS system consisted of a membrane degasser, a binary solvent delivery system, and an auto sampler (1100 Series; Agilent Technologies, Palo Alto, CA) fitted with a Supelcosil LC-18-DB (15-cm × 3-mm i.d., 3 μm; Supelco Co., Bellefonte, PA) reverse-phase analytical column with an isotropic mobile phase of 40% methanol in ammonium acetate buffer (5 mM), pH 3.0, delivered at 0.5 ml min⁻¹. The quadrupole mass spectrometer (Agilent Technologies) was equipped with an electrospray interface and operated in the positive ionization mode. The interface was maintained at 325°C with a nitrogen nebulization pressure of 20 psi, resulting in a flow of 6.0 liters min⁻¹. Quantitative analysis was performed in the selected ion-monitoring mode with a TechnoMAT (MDS-Sciex, Sunnyvale, CA). Heat-inactivated proteins were used as a negative control.

**Results.** Analysis of rates of the β-lyase-catalyzed reaction of S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-l-cysteine and E- and Z-S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-l-cysteine in kidney cytosolic fractions of 20 humans revealed considerable variation among individuals (Fig. 2, A and B). Overall, rates of β-lyase-catalyzed metabolism of cysteine S-conjugates (4 and 5) varied from 0.004 to 0.14 (0.051 ± 0.04, mean ± S.D.; n = 20), and from 0.10 to 0.40 (0.26 ± 0.08) nmol of pyruvate formed/mg of protein/min for alkane and alkene cysteine S-conjugates, respectively. When data were analyzed statistically, pyruvate formation from an alkane cysteine S-conjugate showed a unimodal and positively skewed distribution (Fig. 2C); however, a unimodal and approximately symmetrical distribution was obtained from alkane cysteine S-conjugates (Fig. 2D).

Cysteine S-conjugates (4 and 5) were biotransformed to corresponding mercapturates (6 and 7) by human kidney cytosol (Fig. 3, A and B). Overall, rates of N-acetylation exhibited a range of 0.008 to 0.045 (0.024 ± 0.01) and 0.001 to 0.07 (0.024 ± 0.02) nmol of mercapturate formed/mg of protein/min for alkane and alkene cysteine S-conjugates, respectively. N-Acetylation of an alkane cysteine S-conjugate showed a unimodal and approximately symmetrical distribution (Fig. 3C). In contrast, N-acetylation of the alkene cysteine S-conjugates showed a unimodal and positively skewed distribution (Fig. 3D).

In microsomes, similar N-acetylation results were obtained (Fig. 4, A and B). The rates of N-acetylation ranged from 0.005 to 0.055 (0.025 ± 0.02) and 0.001 to 0.06 (0.030 ± 0.02) nmol of mercapturate formed/mg of protein/min for alkane and alkene cysteine S-conjugates, respectively. Compared with cysteolic N-acetylation, rates of microsomal N-acetylation were evenly distributed (Fig. 4, C and D).

The specific activities of N-deacetylase toward the N-acetyl-l-cysteine conjugates (6 and 7) of FDVE in human renal cytosol are shown in Fig. 5, A and B. Overall, rates of N-deacetylation of compounds 6 and 7 varied from 0.8 to 2.5 (1.25 ± 0.57) and 0.05 to 0.37 (0.17 ± 0.10) nmol of cysteine S-conjugate formed/mg of protein/min for alkane and alkene mercapturates, respectively. N-deacetylation of an alkane mercapturate showed a unimodal and positively skewed distribution, whereas unimodal and approximately symmetrical distribution was obtained with the alkene mercapturates (Fig. 5, C and D).

When comparing the ratio of β-lyase-catalyzed metabolism and N-acetylation of both cysteine S-conjugates, the reaction usually occurred in favor of the β-lyase pathway (Fig. 6, A and B). In addition, the ratio of β-lyase-catalyzed metabolism to N-acetylation for the alkene cysteine S-conjugate (3–146) (32 ± 37) was substantially greater than that of the alkane cysteine S-conjugate (0.2–6) (2.3 ± 4.7).

**Fig. 2.** Pyruvate formation from alkane and alkene cysteine S-conjugates. Pyruvate formation from S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-l-cysteine (A) and S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-l-cysteine (B). β-Lyase activity was determined by incubating human kidney cytosol (2 mg of protein ml⁻¹) in potassium phosphate buffer (150 mM), pH 7.0, for 30 min at 37°C with cysteine S-conjugates (2 mM) and by measuring the amount of pyruvate formed; data are expressed as nanomoles of pyruvate per milligram of protein per min and are shown as mean (n = 20). Population distribution of pyruvate formation from S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-l-cysteine (C); and S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-l-cysteine (D).
1998). N-acetylation of S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine (A) and S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine in renal cytosol (B). Cysteine S-conjugates (2 mM) were incubated in potassium phosphate buffer (150 mM), pH 7.4, at 37°C for 30 min in the presence of 1.6 mM acetyl-CoA and human renal cytosol (2.5 mg ml⁻¹). N-acetyltransferase activity was determined by measuring the amount of mercapturic acid formed (nanomoles per milligram per minute) (n = 20). Population distribution of mercapturic acid formation from S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine (C) and S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine (D).

Discussion

The nephrotoxicity in rats of several haloalkenes is most likely mediated by the biosynthesis and renal processing of glutathione S-conjugates. Cysteine S-conjugates play a central role as penultimate metabolites in this pathway, as substrates for renal cysteine conjugate β-lyase, which forms the ultimate toxic metabolites (Finkelstein et al., 1992, 1996; Dekant et al., 1994; Green et al., 1997; Völkel et al., 1998). N-Acetylation to the mercapturic acid, which is not a substrate for β-lyase, is therefore an important deactivation mechanism, and differences in the rate of N-acetylation may be determinants for the relative toxicity of different cysteine S-conjugates. Although haloalkene metabolism and toxification in animals by GSH- and β-lyase-catalyzed metabolism has been demonstrated in detail, relatively few haloalkenes have been shown to undergo metabolism by this route in
humans. Urinary excretion of the mercapturic acid metabolites of trichloroethene and tetrachloroethene was recently shown in humans receiving occupational or deliberate exposure, demonstrating the bio-

synthesis of glutathione- and cysteine $S$-conjugates and subsequent cleavage to halo acid via $\beta$-lyase-catalyzed metabolism (Birner et al., 1993, 1996; Bernauer et al., 1996). Although FDVE undergoes metabolism in humans by the GSH- and $\beta$-lyase-catalyzed pathway, interindividual variability has not been reported. Furthermore, because relative bioactivation and inactivation may affect the susceptibility of different individuals. FDVE may be an excellent probe to explore this variability. The objective of this work was to investigate the interindividual variability in $N$-acetylation, $N$-deacetylation, and $\beta$-lyase-catalyzed metabolism of cysteine $S$-conjugates and mercapturates of FDVE in 20 human renal fractions.

It has been shown that FDVE undergoes GSH- and $\beta$-lyase-dependent biotransformation in rats in vitro and in vivo (Jin et al., 1995, 1996; Iyer and Anders, 1996, 1997; Spracklin and Kharasch, 1996; Kharasch et al., 1999) and in humans in vitro and in vivo (Iyer et al., 1998; Uttamsingh et al., 1998; Kharasch and Jubert, 1999). The cysteine $S$-conjugates were biotransformed to pyruvate, a known product of the $\beta$-lyase-catalyzed $\beta$-elimination reaction of cysteine $S$-conjugates (Iyer and Anders, 1996, 1997). In the present investigation, there was up to 35-fold interindividual variability in rates of the $\beta$-lyase-catalyzed reaction of FDVE-cysteine $S$-conjugate by renal cytosol from 20 donors. Pyruvate formation from the alkene cysteine $S$-conjugate was 5-fold greater than from the alkane cysteine $S$-conjugate; however, there was less variability (4- versus 35-fold). An asymmetrical, unimodal and positively skewed distribution of pyruvate formation from alkane cysteine $S$-conjugate clearly showed that most of the individuals in the population had lower pyruvate formation, whereas a few subjects had higher $\beta$-lyase activity. Thus, a relatively small number of subjects might be at greater risk from
β-lyase-catalyzed metabolism of FDVE or other halocarbons. In contrast, pyruvate formation from the alkene cysteine S-conjugate had an approximately symmetrical distribution. Individual variation in rates of GSH conjugation of trichloroethylene in human liver cytosol and microsomes has been reported (Lash et al., 1999). For example, S-[1,2-dichlorovinyl]glutathione formation in liver cytosol and microsomes from 20 individual donors exhibited a 6.5-fold variation in microsomes but only a 2.4-fold variation in cytosol. The present results appear to be the first characterization of interindividual variability in β-lyase activity in human kidneys.

In the present experiments, the N-acetylation of both S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine, and S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine with acetyl-CoA by human kidney cytosolic and microsomal fractions was observed. In contrast, it was reported that N-acetylation of S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine with acetyl-CoA by the porcine kidney microsomal fraction was not observed (Kraus et al., 2000). We found that rates (per milligram of protein) of N-acetylation of the alkane and alkene cysteine S-conjugates were comparable in cytosol and microsomes; however, the variation in N-acetylation of the alkane cysteine S-conjugate was 6-fold in cytosol and 11-fold in microsomes compared with 70-fold in cytosol and 60-fold in microsomes for that of the alkene cysteine S-conjugate. When the population distribution for N-acetylation was analyzed, a unimodal and approximately symmetrical distribution and a unimodal but positively skewed distribution was observed in cytosol for the alkane and alkene, respectively (Fig. 3, C and D). But, the distribution of N-acetylation activity in microsomes for the alkane and alkene conjugates was more uniformly distributed.

The bioactivation reaction via β-lyase was greater with the alkene than the alkane cysteine S-conjugate, whereas N-acetylation occurred equally for both alkane and alkene cysteine S-conjugates. In the population distribution analysis, the ratio of β-lyase-catalyzed metabolism to N-acetylation showed unimodal and positively skewed distribution for both alkane and alkene S-conjugates. If the ratio of β-lyase to N-acetylation activity determines susceptibility to toxicity between different humans in the same way that the ratio determines susceptibility to different compounds in rats, then a small number of subjects might be at greater risk for halocarbon toxicity.

Metabolism of FDVE by the mercapturic acid pathway has been demonstrated in vivo and in vitro (Jin et al., 1996; Uttamsingh et al., 1998; Kharasch et al., 1999). Mercapturate formation constitutes a detoxication pathway, whereas mercapturates may undergo hydrolysis back to the corresponding cysteine S-conjugates and bioactivation by the β-lyase pathway. It was previously reported that S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-N-acetyl-L-cysteine but not S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-N-acetyl-L-cysteine was deacetylated in vivo and in vitro by rat and human kidney cytosol (Uttamsingh et al., 1998). In contrast, in the present investigation, both alkane and alkene mercapturates of FDVE were deacetylated to corresponding cysteine S-conjugates by human kidney cytosol. The rates of N-deacetylation of S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-N-acetyl-L-cysteine and S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-N-acetyl-L-cysteine were substantially greater than N-acetylation of S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine and S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine. It is apparent that N-deacetylation predominates over N-acetylation; thus, N-deacetylation appears to be an important route of metabolism of mercapturic acids of FDVE. It may result in a higher availability of the corresponding cysteine S-conjugates to the bioactivating enzyme cysteine S-conjugate β-lyase in the kidney.

The β-lyase activity versus N-acetylasone of cysteine S-conjugates of FDVE in humans in vitro and in vivo can be compared. In the present investigation, the ratio of summed activity of β-lyase versus N-acetylation of cysteine S-conjugates (alkane plus alkene) in vitro ranged from 2 to 20. This result was consistent with in vivo observations in humans. The ratio of cumulative excretion of β-lyase-dependent fluorocarboxy [3,3,3-trifluoro-2-(fluoromethoxy)propanoic acid plus trifluorolactic acid versus mercapturic acids (alkane plus alkene) in urine after anesthesia with sevoflurane was 1.5:1 after 1 day and 3:1 after 3 days (Kharasch and Jubert, 1999). Interspecies comparisons are also possible. In vivo, renal β-lyase activity and β-lyase metabolism of FDVE-cysteine conjugates were approximately 8 to 30 times greater in rat versus human kidneys (Iyer et al., 1997; Uttamsingh et al., 1998). In vivo, the relative metabolic flux of FDVE through toxification (β-lyase) versus detoxification (mercapturate formation) pathways was 6-fold greater in rats than humans (Kharasch and Jubert, 1999).

Relative flux through activating and detoxifying pathways may determine toxicity of the cysteine S-conjugates. The greatest risk of toxicity would result from the combination of both high N-deacetylation/N-acetylation and high pyruvate formation/N-acetylation. In rats, the toxicity of the GSH and mercapturic acid conjugates of FDVE was greater for the alkene than the alkane (Iyer et al., 1997; Uttamsingh et al., 1998). The mechanism for this difference is unknown. In the present investigation, there was a linear correlation (r = 0.707; P < 0.001) between both ratios reflecting concerted bioactivation (N-deacetylation/N-acetylation and pyruvate formation/N-acetylation) (Fig. 8A). In contrast, for the alkane cysteine S-conjugate, no correlation was observed (r = 0.103, not significant) (not shown). This may explain the greater toxicity of the alkene compared with the alkane conjugates in rats.

**Fig. 8.** Relative ratios of bioactivation for alkane and alkene S-conjugates.

The relationship between pyruvate formation/N-acetylation and N-deacetylation/N-acetylation for alkene S-conjugate (A); the correlation of relative ratios of pyruvate formation/N-acetylation versus N-deacetylation/N-acetylation for both alkane and alkene S-conjugates (B); ratio of alkane S-conjugates (pyruvate formation/N-acetylation) versus (N-deacetylation/N-acetylation); ratio of alkene S-conjugates (pyruvate formation/N-acetylation) versus (N-deacetylation/N-acetylation).
Humans, compared with rats, have not shown evidence of nephrotoxicity from FDVE (Bito and Ikeda, 1994; Kharasch and Jubert, 1999). This difference has been attributed to species differences in FDVE exposure, β-lyase activity, and relative toxicity versus de-toxication (Kharasch and Jubert, 1999; Kharasch et al., 1999). The present results suggest another possible explanation. Although the relative ratio of β-lyase-catalyzed metabolism was higher for the allene S-conjugate, the ratio of N-deacetylation was much lower (Fig. 8B). Conversely, although the relative ratio of N-deacetylation was greater for the allene S-conjugate, the ratios of β-lyase-catalyzed metabolism were low. Thus, neither compound fell into the highest risk range with human cytosol. This could afford relative protection in humans and could also, in part, explain the difference between rats and humans with respect to FDVE nephrotoxicity.

References


