Inactivation of Glutathione Transferase Zeta by Dichloroacetic Acid and Other Fluorine-Lacking α-Haloalkanoic Acids

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Dichloroacetic acid (DCA) is a contaminant of chlorinated drinking water supplies, is carcinogenic in rats and mice, and is a therapeutic agent used for the treatment of congenital lactic acidosis. The biotransformation of DCA to glyoxylic acid is catalyzed by glutathione transferase zeta (GSTZ). Treatment of rats and human subjects with DCA increases its plasma elimination half-life and reduces the extent of DCA biotransformation in rat hepatic cytosol. In the investigation presented here, the kinetics of the DCA-induced inactivation of GSTZ, the turnover of GSTZ, and the susceptibility of GSTZ to inactivation by a panel of α-haloacids were studied. DCA rapidly inactivated GSTZ in both rat hepatic cytosol and intact Fischer 344 rats. The time course of inactivation in vivo was mirrored by a concomitant loss of immunoreactive GSTZ protein. The turnover of GSTZ in rat liver was 0.21 day^{-1}, which corresponded to a half-life of 3.3 days. The degree of GSTZ inactivation after daily administration of DCA could be predicted from the amount of inactivation after a single treatment. Other fluorine-lacking dihaloacetic acids also inactivated GSTZ, whereas α-monohaloacids and fluorine-containing dihaloacetic acids failed to inactivate GSTZ. These data show that the observed DCA-induced decrease in the level of DCA metabolism is caused by the inactivation of GSTZ.

Introduction

Trihalomethanes and haloacetic acids, including dichloroacetic acid (DCA)[1] and bromochloroacetic acid, are the most abundant disinfection byproducts found in chlorinated drinking water supplies in the United States (*1*, *2*). DCA is also formed as a metabolite of tetrachloroethene, trichloroethene, trichloroacetic acid, and chloral hydrate (*3*−*6*).

DCA is hepatocarcinogenic in male B6C3F1 mice (*7*) and in male Fischer 344 rats (*8*), which are estimated to be about 10 times more sensitive than male B6C3F1 mice to the induction of hepatocellular neoplasia by DCA (*8*). The mechanism by which DCA induces liver tumor formation has not been determined.

DCA, which activates pyruvate dehydrogenase secondary to its inhibition of pyruvate dehydrogenase kinase, is used clinically in the management of congenital lactic acidosis (*9*). DCA has been proposed for use as a neuroprotective agent (*10*) and in the management of other disease-associated lactic acidoses.

DCA is metabolized to chloroacetic acid, glyoxylic acid, glycolic acid, oxalic acid, glycine, hippuric acid, phenylacetylglycine, and carbon dioxide (*11*−*13*). The biotransformation of DCA to glyoxylic acid is catalyzed by glutathione-dependent cytosolic enzymes (*14*, *15*). Recent studies show that GSTZ catalyzes the biotransformation of DCA to glyoxylic acid (*16*) and that glyoxylic acid is the sole product of the GSTZ-catalyzed biotransformation of DCA (*17*). The formation of glycolic acid and oxalic acid can be explained by the lactate dehydrogenase-catalyzed reduction and oxidation, respectively, of glyoxylic acid. α-Ketoglutarate:glyoxylate carboligase catalyzes the conversion of glyoxylic acid to carbon dioxide, and the transamination of glyoxylic acid affords glycine, which is metabolized to hippuric acid. GSTZ is identical with maleylacetoacetate isomerase, which catalyzes the isomerization of maleylacetoacetate to fumarylacetoacetate, the penultimate step in the tyrosine degradation pathway (*18*).

Pharmacokinetic studies show that repeated treatment with DCA increases its plasma elimination half-life in both rats and humans (*13*, *19*−*22*). Furthermore, the extent of glutathione-dependent biotransformation of DCA to glyoxylic acid is reduced in hepatic cytosol from rats given DCA (*15*).

The objective of the experiments described here was to explore further the mechanisms of the apparent DCA-induced reduction in the level of DCA metabolism. We report herein the kinetics of the DCA-induced inactivation of GSTZ, the turnover of GSTZ, and the susceptibility of GSTZ to inactivation by a panel of α-haloacids. These data provide the first direct evidence that GSTZ is irreversibly inactivated by DCA.

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[1] Abbreviations: DCA, dichloroacetic acid; GSTZ, glutathione transferase zeta.

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Materials and Methods

Chemicals and Reagents. Dichloroacetic acid, difluoroacetic acid, 2-chloropropionic acid, 2,2-dichloropropionic acid, and bromofluoroacetic acid ethyl ester were obtained from Aldrich Chemical Co. (Milwaukee, WI). Dibromoacetic acid was purchased from Janssen Chimica (Beerse, Belgium). Chlorofluoroacetic acid ethyl ester was obtained from Lancaster Synthesis, Inc. (Windham, NH). Bromochloroacetic acid was kindly provided by A. B. DeAngelo (U.S. Environmental Protection Agency, Research Triangle Park, NC). Phenylhydrazine, potassium ferricyanide, phenylmethanesulfonyl fluoride, and mono- and dibasic potassium phosphates were purchased from Sigma Chemical Co. (St. Louis, MO). Dithiothreitol was purchased from Eastman Kodak (Rochester, NY). Rabbit anti-human GSTZ1-1 polyclonal antibody was prepared as described previously (*23*). Reagents for SDS−PAGE, Western analysis, and protein quantification were obtained from Bio-Rad Laboratories (Hercules, CA). Other reagents were purchased from commercial suppliers.

Syntheses. The method of Tong et al. (*17*) was used with slight modification to prepare bromofluoro- and chlorofluoroacetic acids; 2 g of the ethyl esters was heated with stirring at 50 °C in 40 mL of 1 N H_2SO_4 until the immiscible ester disappeared (3−4 h). The reaction mixture was then extracted four times with ethyl acetate, and the combined extracts were concentrated under vacuum. The crude product was purified by Kugelrohr distillation (*24*). ^{1}H NMR spectroscopy indicated that the acids were >99% pure.

Animal Treatments and Preparation of Liver Cytosol. Male Fischer 344 rats (200−225 g, Charles River Laboratories, Wilmington, MA) were kept on a 12 h light/dark cycle and were provided with food and water ad libitum. Rats were given α-haloalkanoic acids as neutralized solutions in 0.9% NaCl by ip injection between 9 a.m. and noon. Control animals were given 0.9% NaCl. At the indicated times, the rats were anesthetized with diethyl ether and killed by decapitation. The livers were homogenized in 4 volumes (w/v) of 20 mM potassium phosphate buffer (pH 7.4) containing 1.15% KCl, 2 mM EDTA, 2 mM DTT, and 100 μM phenylmethanesulfonyl fluoride. The cytosolic fraction was prepared by differential ultracentrifugation, as previously described (*25*), and dialyzed overnight with two buffer changes against 30 volumes of homogenization buffer (without 1.15% KCl). Dialyzed cytosol was stored at −80 °C until it was used. No reduction in GSTZ activity was observed after storage for several months under these conditions. Protein concentrations were determined by the method of Bradford (*26*) with bovine serum albumin as the standard.

Activity Assay. GSTZ activity was determined by measuring the extent of product formation with a discontinuous assay, as previously described (*16*). Reaction mixtures contained rat liver cytosol (0.5 mg of protein/mL) and glutathione (1 mM) in a final volume of 1 mL of 0.1 M phosphate buffer (pH 7.4). Reaction mixtures were incubated for 3 min at 37 °C; the reaction was initiated by addition of 0.5 mM DCA. At the indicated times, or routinely after 20 min, reactions were quenched by addition of 50 μL of trifluoroacetic acid. The precipitated proteins were removed by centrifugation, and the supernatant was analyzed for glyoxylic acid by the method of Vogels and Van Der Drift (*27*).

Western Analysis and Quantification of Immunoreactive GSTZ. For quantification of immunoreactive GSTZ, rat liver cytosol (16 μg of protein) from all rats at all time points was resolved by electrophoresis on 12% gels according to the method of Laemmli (*28*) and then electrophoretically transferred to 0.45 μm nitrocellulose membranes. For the data shown in Figure 2B, 15% gels were used, and 20 μg of liver cytosolic protein from rats at each time point with median enzyme activity was analyzed. Western analysis was performed according to instructions provided with the Bio-Rad goat anti-rabbit IgG−alkaline phosphatase Immun-Blot assay kit. Membranes were incubated for 1 h with a 1:1000 dilution of rabbit anti-human GSTZ1-1, which cross-reacts with rat GSTZ (*16*), and for 1 h with a 1:3000 dilution of goat anti-rabbit IgG−alkaline phosphatase conjugate. The alkaline phosphatase substrates 5-bromo-4-chloro-3-indoyl phosphate and nitroblue tetrazolium were employed for detection. Developed blots were scanned as gray-scale images, and the density in each band was determined with the GelPlot2 macro of ScionImage β3b software (www.scioncorp.com). The linearity of the response was confirmed by analyzing 3.2−16 μg samples of protein from control rat liver cytosol. Only samples from the same blot were compared when determining relative immunoreactivities.

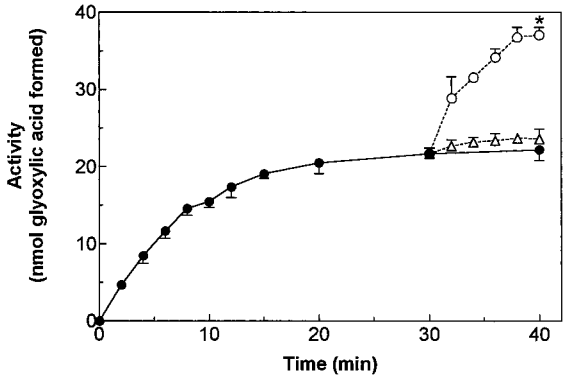


Figure 1. DCA-induced inactivation of GSTZ in vitro. Reaction mixtures contained rat liver cytosol (0.5 mg of protein/mL), 1 mM glutathione, and 100 mM phosphate buffer (pH 7.4) and were incubated for 3 min at 37 °C, and reactions were then initiated by addition of 0.5 mM DCA. At the indicated times, the reactions were quenched by addition of trifluoroacetic acid, and glyoxylic acid formation (●) was quantified as described in Materials and Methods. Where indicated (dashed lines), the reaction mixtures were incubated and analyzed for glyoxylic acid as described above except that after incubation for 30 min a second addition of either rat liver cytosol (○) or DCA (△) was made, and the reaction mixtures were incubated for 0−10 min. The reactions were quenched, and glyoxylic acid formation was quantified, as described above. Data are shown as means ± SD ($n = 3$). The asterisk indicates a significant difference ($p < 0.05$) from the complete system (●) or after the second addition of DCA (△).

Estimation of Protein Turnover. The turnover rate of GSTZ in rat liver was estimated from the time course of inactivation (see Figure 2A) by the method of Price et al. (*29*). [Discussions of this and other methods for determining the rate of protein turnover are available (*30*, *31*).] Briefly, after administration of an irreversible inhibitor, the rate at which an enzyme recovers activity is a function of two competing processes: synthesis and degradation. This relationship is described in eq 1, where e is defined as enzyme activity:

$$\frac{de}{dt} = k_{\text{synthesis}} - k_{\text{degradation}} \times e \qquad (1)$$

The rates of synthesis and degradation are zero- and first-order processes, respectively. At steady state,

$$\frac{de}{dt} = 0 \text{ and } k_{\text{synthesis}} = k_{\text{degradation}} \times e_{\text{ss}} \qquad (2)$$

Substitution of eq 2 into eq 1 and rearrangement yield the first-order relationship

$$\frac{de}{dt} = k_{\text{degradation}}(e_{\text{ss}} - e) \qquad (3)$$

Integration of eq 3 gives

$$\ln(e_{\text{ss}} - e) = -k_{\text{degradation}} \times t + c \qquad (4)$$

The degradation rate of the enzyme is obtained from the slope of a plot of the natural logarithm of the difference between the enzyme activity at steady state and the enzyme activity at several times during the course of recovery versus time. The

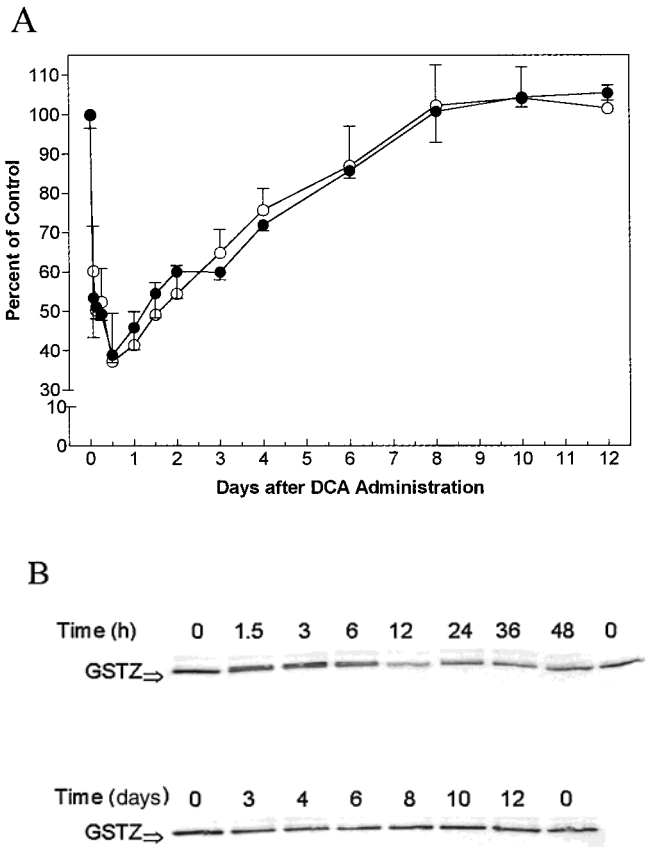


Figure 2. DCA-induced inactivation of GSTZ in vivo. (A) Male Fischer 344 rats ($n = 3$/time point) were given 0.3 mmol of DCA/kg ip and were killed at the indicated times. GSTZ activity (●) and the amount of immunoreactive GSTZ protein (○) were measured in liver cytosol as described in Materials and Methods and are expressed as the percent of the 0 h value. Data are shown as means ± SD ($n = 3$). (B) Representative immunoblots of rat liver cytosol (20 μg of protein/lane) collected at the indicated times after DCA administration.

half-life for protein turnover is related to the degradation rate constant by the expression

$$t_{1/2} = \ln 2/k_{\text{degradation}} = 0.693/k_{\text{degradation}} \quad (5)$$

For this study, steady-state activity was taken as the activity determined 12 days after giving DCA.

Statistical Analysis. Statistical analyses were carried out with Microsoft Excel 97. Data are expressed as means ± SD. Some data were analyzed with a two-tailed Student's *t* test. A *p* value of ≤0.05 was chosen for acceptance or rejection of the null hypothesis.

Results

Inactivation of GSTZ in Vitro by DCA. To investigate whether the observed decrease in the extent of biotransformation of DCA seen in hepatic cytosol from rats treated with DCA was due to the inactivation of GSTZ, the kinetics of the biotransformation of DCA to glyoxylic acid by rat liver cytosol was studied. The extent of biotransformation of DCA to glyoxylic acid was not linear with time and showed no further increase after incubation for 20−30 min (Figure 1), after which time an estimated 5% of the DCA had been converted to glyoxylic acid. The nonlinearity of the biotransformation of DCA could be due to the establishment of an equilibrium between substrate and product, competitive inhibition, or inactivation of GSTZ. To determine the mechanism, a second addition of either DCA or cytosol was made after the reaction reached a plateau. After addition of cytosol, the reaction proceeded at the initial rate, whereas the addition of DCA did not result in further glyoxylic acid formation, indicating the enzyme was inactivated (Figure 1). To determine whether GSTZ was stable under the incubation conditions, rat liver cytosol was incubated for 30 min before the addition of DCA. No reduction in the initial rate of reaction or change in the overall time course of formation of glyoxylic acid from DCA was observed (data not shown).

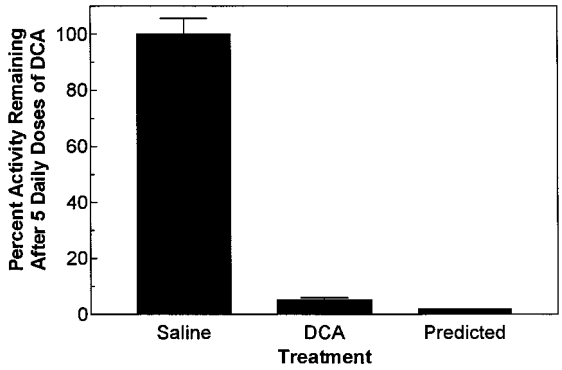


Figure 3. DCA-induced inactivation of GSTZ after five daily treatments. Male Fischer 344 rats ($n = 3$/treatment group) were given five daily doses of 0.3 mmol of DCA/kg or saline ip. Animals were killed 24 h after the last dose of DCA. GSTZ activity was measured in liver cytosol, as described in Materials and Methods. Data are expressed as the percent of control (means ± SD, $n = 3$). Activity remaining 24 h after giving a single dose of 0.3 mmol of DCA/kg (Figure 2) was used to predict that GSTZ activity would be reduced to ~2% [$(\%A_{24h})^5$] after five daily doses.

Inactivation of GSTZ in Vivo by DCA. The dose dependency and kinetics of the DCA-induced inactivation of GSTZ in rats were studied. Treatment of rats with 0.15, 0.30, and 0.60 mmol of DCA/kg reduced GSTZ activity 24 h after treatment to 79 ± 3%, 55 ± 4%, and 24 ± 3%, respectively, compared with saline-treated rats.

The kinetics of GSTZ inactivation was investigated after giving rats 0.30 mmol of DCA/kg. Administration of DCA resulted in a rapid loss of both enzymatic activity and immunoreactive GSTZ protein (panels A and B of Figure 2). The nadir of both activity and the amount of immunoreactive GSTZ protein occurred 12 h after treatment, and GSTZ activity and the amount of immunoreactive GSTZ protein did not return to initial values until 10−12 days after treatment (Figure 2A). Furthermore, administration of five daily doses of 0.30 mmol of DCA/kg to Fischer 344 rats reduced GSTZ activity to about 5% of control values (Figure 3) and immunoreactive GSTZ protein concentrations to less than 10% of control values (data not shown). The observed degree of inactivation was similar to that predicted by extrapolating the activity remaining 24 h after a single administration to 5 days [$(\text{activity}_{24h})^5$] (Figure 3).

Turnover of GSTZ. The data presented above indicated that GSTZ was irreversibly inactivated by DCA and targeted for proteolysis in vivo. It was, therefore, important to determine the time course of recovery of activity. The method of Price et al. (*29*) was used to determine the rate of turnover of GSTZ in vivo. GSTZ was degraded in rat liver at the rate of -0.21 day^{-1}, which corresponded to a $t_{1/2}$ of 3.3 days (Figure 4).

Inactivation of GSTZ by DCA Analogues. GSTZ catalyzes the biotransformation of a range of α-haloal-

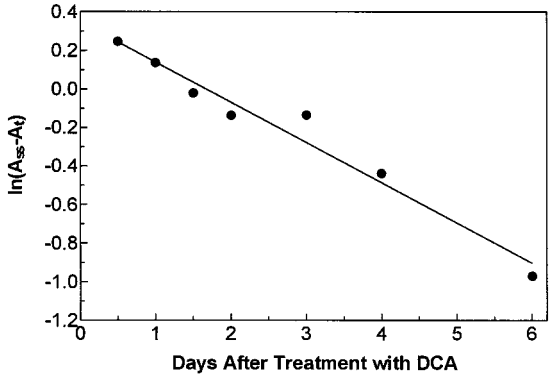


Figure 4. Determination of the rate of GSTZ turnover in rat liver. The activity data from the time course of inactivation experiment (Figure 2) were used to determine the degradation rate constant, as described in Materials and Methods. The degradation rate constant for GSTZ was -0.21 day^{-1} ($r^2 = 0.96$), which corresponded to a half-life of 3.3 days.

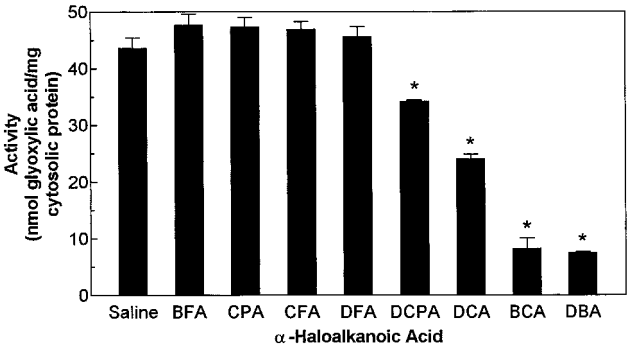


Figure 5. Inactivation of GSTZ in vivo by α-haloalkanoic acids. Male Fischer 344 rats ($n = 3$/treatment group) were given 0.3 mmol of α-haloalkanoic acids/kg ip and were killed 12 h after treatment. GSTZ activity was measured in liver cytosol, as described in Materials and Methods. Data are expressed as means ± SD ($n = 3$). The asterisk indicates a significant difference ($p < 0.05$) from rats given saline. BFA, bromofluoroacetic acid; CPA, (*R*,*S*)-2-chloropropionic acid; CFA, chlorofluoroacetic acid; DFA, difluoroacetic acid; DCPA, 2,2-dichloropropionic acid; DCA, dichloroacetic acid; BCA, bromochloroacetic acid; DBA, dibromoacetic acid.

kanoic acids (*17*). Hence, the inactivation of GSTZ by α-haloalkanoic acids was studied in vivo. Treatment of rats with 2,2-dichloropropionic acid, DCA, bromochloroacetic acid, and dibromoacetic acid reduced GSTZ activity 12 h after treatment to 78 ± 0%, 55 ± 2%, 19 ± 4%, and 17 ± 0% ($n = 3$ rats/treatment group), respectively, compared with saline-treated animals. Administration of bromofluoroacetic acid, (*R*,*S*)-2-chloropropionic acid, chlorofluoroacetic acid, and difluoroacetic acid had no effect on GSTZ activity (Figure 5).

Discussion

The study presented here provides the first direct evidence that the DCA-induced reduction in its extent of metabolism is caused by the irreversible inactivation of GSTZ.

In vitro biotransformation experiments showed that the rate of biotransformation of DCA to glyoxylic acid by rat liver cytosol was linear with time for about 10 min and then reached a plateau. The reason for the decrease in the rate of GSTZ-catalyzed biotransformation of DCA was investigated by making a second addition of either enzyme or DCA to the stalled reaction. The observation that a second addition of enzyme, but not of DCA, could restart the reaction indicated that the enzyme was irreversibly inactivated. If the reaction had stalled because of the establishment of an equilibrium between substrate and product or competitive inhibition, a second addition of DCA would have been expected to restart the reaction. Also, dialysis of DCA-inactivated recombinant GSTZ1-1 did not result in significant recovery of activity,[2] which also indicates that DCA irreversibly inactivates GSTZ. Gonzalez-Leon et al. (*32*) recently reported that the V_{max} for the biotransformation of dibromoacetic acid, bromochloroacetic acid, and DCA was decreased after their administration in the drinking water, but did not alter the K_m of the enzyme for their biotransformation, which is consistent with the inactivation of GSTZ.

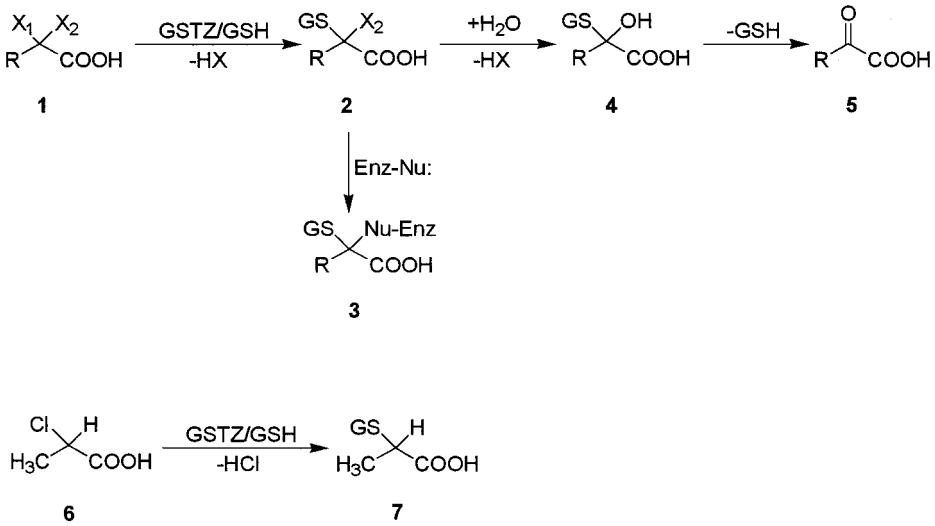
DCA-induced inactivation of GSTZ in vivo was rapid and dose-dependent. The maximal reduction of GSTZ activity occurred 12 h after the administration of a single dose of 0.3 mmol of DCA/kg. This dose is similar to the therapeutic doses used in the management of lactic acidosis (*33*), but is much larger than the estimated human intake from drinking water containing DCA, which amounts to about 4 μg kg^{-1} day^{-1} (*33*). The elimination $t_{1/2}$ of DCA in Fischer 344 rats is 2.4 h (*22*); hence, after 5 $t_{1/2}$ (or 12 h), more than 95% of the DCA would be eliminated and unavailable to inactivate GSTZ. Quantitative Western analysis revealed that the decrease in GSTZ activity paralleled the decrease in the amount of immunoreactive GSTZ protein during the time course of enzyme inactivation, indicating that DCA-inactivated GSTZ was rapidly degraded. The rapidity of the loss of GSTZ activity and protein in vivo complements the in vitro observation that DCA inactivates GSTZ directly. Hence, other mechanisms of downregulation, e.g., decreased levels of transcription or translation or decreased mRNA stability, are not involved in the observed reduction in the level of DCA metabolism. Furthermore, the finding that the GSTZ activity remaining 5 days after the daily administration of DCA could be predicted from the activity remaining 24 h after a single treatment with DCA indicates that GSTZ activity is not induced by repeated DCA treatment.

Inspection of the immunoblots (Figure 2B) showed the appearance of an immunoreactive protein with a molecular mass slightly higher than that of GSTZ in samples taken 1.5, 3, and 6 h after DCA administration, which may indicate that GSTZ is inactivated through a covalent modification involving DCA or glutathione, or both. The higher-molecular mass, immunoreactive GSTZ was not present in naïve rats or at any time during the course of GSTZ recovery, which further implicates this as an inactivated form of the enzyme.

The rate of turnover of hepatic GSTZ was 0.21 day^{-1} in Fischer 344 rats. The determination of the turnover rate is important for understanding DCA metabolism in vivo, because it allows prediction of the time required for restoration of GSTZ activity after any level of GSTZ inactivation. Curry et al. (*20*) showed that increased plasma DCA elimination half-lives can persist for as long as 3 months after DCA exposure in humans, indicating a different turnover rate in humans and rats. Also, Gonzalez-Leon et al. (*32*) reported that DCA metabolic activity recovers much faster in mice than in rats after continuous DCA treatment for 2 weeks.

[2] H.-F. Tzeng and M. W. Anders, unpublished observations.

Scheme 1



Tong et al. (*17*) recently reported that GSTZ catalyzes the biotransformation of a range of dihaloacetic acids and α-halopropionic acids. In the studies presented here, fluorine-lacking dihaloacetic acids (DCA, bromochloroacetic acid, and dibromoacetic acid) and 2,2-dichloropropionic acid inactivated GSTZ, but no inactivation was observed after giving fluorine-containing dihaloacetic acids (bromofluoroacetic acid, chlorofluoroacetic acid, and difluoroacetic acid) or (*R*,*S*)-2-chloropropionic acid. The inactivation of GSTZ by fluorine-lacking dihaloacetic acids (Scheme 1, **1**, R = H, $X_1 = X_2$ = Br or Cl) may be explained by the formation of an *S*-(α-halocarboxymethyl)glutathione intermediate (Scheme 1, **2**, R = H, X_2 = Br or Cl), which may react with a nucleophilic site on the enzyme to give a covalently modified, inactivated enzyme (Scheme 1, **3**, R = H). The hydrolysis of the *S*-(α-halocarboxymethyl)glutathione intermediate would afford the hemithioacetal *S*-(α-hydroxycarboxymethyl)-glutathione (Scheme 1, **4**, R = H), which may eliminate glutathione to give glyoxylic acid (Scheme 1, **5**, R = H). Hence, hydrolysis of *S*-(α-halocarboxymethyl)glutathione (Scheme 1, **2**) would compete with inactivation of the enzyme. Similarly, GSTZ-catalyzed reaction of 2,2-dichloropropionic acid (Scheme 1, **1**, R = CH_3, $X_1 = X_2$ = Cl) with glutathione may afford an *S*-(α-methyl-α-halocarboxymethyl)glutathione intermediate (Scheme 1, **2**, R = CH_3, X_2 = Cl), which may react with a nucleophilic site on the enzyme (Scheme 1, **3**, R = CH_3) or may undergo hydrolysis to pyruvic acid (Scheme 1, **5**, R = CH_3). The reason for the failure of monofluorine-containing dihaloacetic acids, which are substrates for GSTZ, to inactivate the enzyme is not fully understood. It is possible to speculate that the *S*-(α-fluorocarboxymethyl)glutathione intermediate (Scheme 1, **2**, R = H, X_2 = F) cannot form a resonance-stabilized zwitterionic intermediate because fluoride is a strong base and, therefore, a poor nucleofuge (*34*). Hence, this fluorine-containing intermediate may be more stable than the bromine- or chlorine-containing analogues and may undergo hydrolysis to give product rather than producing detectable inactivation. The failure of (*R*,*S*)-2-chloropropionic acid (Scheme 1, **6**) to inactivate GSTZ is consistent with the formation of the stable glutathione conjugate *S*-(α-methylcarboxymethyl)glutathione (Scheme 1, **7**) (*17*). Difluoroacetic acid is not a substrate for GSTZ (*17*). Hence, neither (*R*,*S*)-2-chloropropionic acid nor difluoroacetic acid forms a reactive intermediate that inactivates GSTZ.

The development of isozyme-selective inhibitors of glutathione transferases that are effective in vivo has received much recent attention (for a review, see ref *35*). Presently, few isozyme-selective, irreversible inhibitors of glutathione transferases have been identified; haloenol lactone (*36*), *N*-ethylmaleimide (*37*), and derivatives of ethacrynic acid (*38*) are isozyme-selective, irreversible inhibitors of glutathione transferase π. In addition, the in vivo effectiveness of glutathione transferase inhibitors has only been demonstrated for ethanol (*39*) and (*R*)-5-(ethyloxycarbonyl)-2-γ-(*S*)-(glutamylamino)-*N*-2-heptylpentamide (*40*). The findings presented here demonstrate that DCA is an effective in vivo inactivator of GSTZ. Also, DCA is expected to exhibit isozyme selectivity since, unlike the other irreversible inhibitors that are active site-directed, DCA is apparently a mechanism-based or k_{cat} inhibitor of GSTZ.[3] In addition, the biotransformation of DCA to glyoxylic acid does not consume or oxidize glutathione (*17*), indicating that DCA, unlike ethacrynic acid and other inhibitors that are substrates for glutathione transferases, is unlikely to deplete cellular glutathione concentrations and perturb cellular redox status. Further investigations into the isozyme selectivity and mechanism of inactivation of GSTZ by α-haloalkanoic acids are warranted.

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