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Glutathione Carbamoylation with *S*-Methyl *N*,*N*-diethylthiolcarbamate Sulfoxide and Sulfone

MITOCHONDRIAL LOW K_m ALDEHYDE DEHYDROGENASE INHIBITION AND IMPLICATIONS FOR ITS ALCOHOL-DETERRENT ACTION

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ABSTRACT. *S*-Methyl *N*,*N*-diethylthiolcarbamate sulfoxide (DETC-MeSO) and sulfone (DETC-$MeSO_2$) both inhibit rat liver low K_m aldehyde dehydrogenase ($ALDH_2$) *in vitro* and *in vivo* (Nagendra *et al.*, *Biochem Pharmacol* **47:** 1465–1467, 1994). DETC-MeSO has been shown to be a metabolite of disulfiram, but DETC-$MeSO_2$ has not. Studies were carried out to further investigate the inhibition of $ALDH_2$ by DETC-MeSO and DETC-$MeSO_2$. In an *in vitro* system containing hydrogen peroxide and horseradish peroxidase, the rate of DETC-MeSO oxidation corresponded to the rate of DETC-$MeSO_2$ formation. Carbamoylation of GSH by both DETC-MeSO and DETC-$MeSO_2$ was observed in a rat liver S_9 fraction. Carbamoylation of GSH was not observed in the presence of *N*-methylmaleimide. In *in vitro* studies, DETC-MeSO and DETC-$MeSO_2$ were equipotent $ALDH_2$ inhibitors when solubilized mitochondria were used, but DETC-MeSO was approximately four times more potent than DETC-$MeSO_2$ in intact mitochondria. In studies with rats, the dose (i.p. or oral) required to inhibit 50% $ALDH_2$ (ED_{50}) was 3.5 mg/kg for DETC-MeSO and approximately 35 mg/kg for DETC-$MeSO_2$, approximately a 10-fold difference. Furthermore, maximum $ALDH_2$ inhibition occurred 1 hr after DETC-MeSO administration, whereas maximal $ALDH_2$ inhibition occurred 8 hr after DETC-$MeSO_2$ dosing. DETC-MeSO is, therefore, not only a more potent $ALDH_2$ inhibitor than DETC-$MeSO_2$ *in vivo*, but also *in vitro* when intact mitochondria are utilized. The *in vitro* results thus support the *in vivo* findings. Since oxidation of DETC-MeSO can occur both enzymatically and non-enzymatically, it is possible that DETC-$MeSO_2$ is formed *in vivo*. DETC-$MeSO_2$, however, is not as effective as DETC-MeSO in inhibiting $ALDH_2$, probably because it has difficulty penetrating the mitochondrial membrane. Thus, even if DETC-$MeSO_2$ is formed *in vivo* from DETC-MeSO, it is the metabolite DETC-MeSO that is most likely responsible for the inhibition of $ALDH_2$ after disulfiram administration. BIOCHEM PHARMACOL **55**;6:749–756, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. disulfiram; aldehyde dehydrogenase; *S*-methyl *N*,*N*-diethylthiolcarbamate sulfoxide; carbamoylation

The basis for the clinical use of disulfiram in the treatment of alcohol abuse is its inhibition of liver mitochondrial $ALDH_2$.§ Although disulfiram has been used for almost 50 years, the seminal studies by Yourick and Faiman [1, 2] first showed that bioactivation was required in order for disulfiram to inhibit $ALDH_2$ *in vivo*. Subsequent studies provided detailed evidence that disulfiram undergoes a series of oxidations ultimately forming DETC-MeSO, the chemical species proposed to be responsible for the inhibition of $ALDH_2$ *in vivo* [3]. The metabolic scheme comprising the bioactivation process [4–6] and the cytochrome P450 (CYP P_{450}) drug-metabolizing enzymes in both rat and human liver required for this bioactivation [7] have been identified and reported.

DETC-MeSO is readily oxidized to DETC-$MeSO_2$ during synthesis if care is not taken [3]. Since oxidative processes are available *in vivo*, the possibility exists that DETC-$MeSO_2$ can be formed *in vivo* either enzymatically or non-enzymatically. This likelihood is further supported from the observation that dimethyl sulfone is found in urine in both rats and humans given dimethyl sulfoxide [8]. Therefore, inhibition of $ALDH_2$ by DETC-$MeSO_2$ was investigated, and it too inhibits $ALDH_2$ both *in vitro* and *in vivo*, although DETC-$MeSO_2$ is not as potent an inhibitor of $ALDH_2$ as is DETC-MeSO [9]. In subsequent studies by Mays *et al.* [10], those investigators confirmed the inhibi-

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§ *Abbreviations:* $ALDH_2$, low K_m aldehyde dehydrogenase; DETC-MeSO, *S*-methyl *N*,*N*-diethylthiolcarbamate sulfoxide; DETC-$MeSO_2$, *S*-methyl *N*,*N*-diethylthiolcarbamate sulfone; DETC-GS, *S*-(*N*,*N*-diethylcarbamoyl) glutathione; GST, glutathione *S*-transferase; NEM, *N*-ethylmaleimide; CID, collision-induced dissociation; and HRP, horseradish peroxidase.

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tion of $ALDH_2$ by DETC-$MeSO_2$ *in vitro*, but no *in vivo* studies were carried out. Contrary to our earlier studies [9], Mays *et al.* [10] found DETC-$MeSO_2$ to be more potent than DETC-MeSO *in vitro*, and they suggested that DETC-$MeSO_2$ may be the ultimate metabolite responsible for $ALDH_2$ inhibition. However, those authors employed solubilized mitochondria rather than the intact mitochondria that we used [9]. In more recent studies, however, Mays *et al.* [11] found DETC-MeSO to be a more potent inhibitor of $ALDH_2$ than is DETC-$MeSO_2$ when intact mitochondria are employed.

DETC-MeSO can be detected in plasma from rats treated with either disulfiram or DETC-MeSO [12], but DETC-$MeSO_2$ has never been detected *in vivo* even after DETC-$MeSO_2$ administration [9]. GSH, an important substrate in detoxification mechanisms [13], is found in high concentrations *in vivo*. Since electrophilic *S*-oxides and sulfones carbamoylate cellular nucleophiles, for example GSH [14], DETC-MeSO and DETC-$MeSO_2$ can react with either $ALDH_2$ or GSH, subsequently forming protein–drug or GSH–drug adducts. Several explanations for the difference in potency between DETC-MeSO and DETC-$MeSO_2$ *in vivo* are possible. For example, carbamoylation of GSH may be more rapid with DETC-$MeSO_2$ than with DETC-MeSO, or DETC-$MeSO_2$ is not readily accessible to the $ALDH_2$ enzyme. Potential carbamoylation is supported by others [15, 16]. Hubbell and Casida [15] in studies with several thiocarbamate herbicides found the respective sulfoxides to be rapidly carbamoylated, while Jin *et al.* [16], employing mass-spectroscopic techniques, found the DETC-GS conjugate in bile from rats treated with disulfiram (the parent of DETC-MeSO). However, Jin *et al.* [16] did not differentiate whether the GSH conjugate formed originated from either DETC-MeSO or DETC-$MeSO_2$.

In the present investigations, the carbamoylation of GSH by DETC-MeSO and DETC-$MeSO_2$ was studied further in order to provide a possible explanation for the difference in potency that existed between DETC-MeSO and DETC-$MeSO_2$ *in vivo*, or when intact mitochondria were used in *in vitro* studies. Results from these investigations showed that carbamoylation of GSH with DETC-$MeSO_2$ was more rapid than with DETC-MeSO in forming DETC-GS. DETC-$MeSO_2$ was less potent than DETC-MeSO as an inhibitor of $ALDH_2$ *in vitro* when intact mitochondria were used, but was of equal potency when solubilized mitochondria were employed. DETC-$MeSO_2$ also was less effective than DETC-MeSO as an $ALDH_2$ inhibitor after either i.p. or oral administration to rats.

MATERIALS AND METHODS

Chemicals

DETC-MeSO [3] and DETC-$MeSO_2$ [9] were synthesized as described previously. All other chemicals, including purified rat liver GST (EC 2.5.1.18), were purchased from the Sigma Chemical Co. C-18 Sep-Pak extraction columns were purchased from Alltech, and Centricon ultrafilters were obtained from Amicon, Inc.

In Vitro *Studies*

PEROXIDASE-MEDIATED OXIDATION OF DETC-MeSO. DETC-MeSO (20–100 μM) was incubated with HRP (5 units/mL) and H_2O_2 (2 mM) in 100 mM phosphate buffer, pH 7.0, at 37° in a water bath for 1, 5, 10, 15, 20, and 30 min. The incubation volume was 1 mL. The reaction was stopped by the addition of 10 mM $CaCl_2$. After centrifugation, the unreacted DETC-MeSO, or the DETC-$MeSO_2$ formed, was removed by solid phase extraction in acetonitrile, and the concentration was determined by reverse-phase HPLC, as described previously [17].

DETC-GS. DETC-MeSO- and DETC-$MeSO_2$–GSH conjugates were synthesized as described previously [16], and verified by MS/MS (CID of MH^+ at m/z 407). DETC-MeSO or DETC-$MeSO_2$ (100 μM) was incubated for 30 min at 37° with GSH (1 mM) with or without GST (5 units/mL) in 0.1 M potassium phosphate buffer, pH 7.0, containing 1 mM EDTA. The incubation volume was 1 mL. The reaction was stopped by centrifuging the contents of the incubation at 4° through Centricon membrane filters. The filtrate was evaporated to dryness, and the residue obtained was subjected to mass spectral analysis.

KINETICS OF DETC-MeSO/DETC-$MeSO_2$ CARBAMOYLATION. Either 50 μM DETC-MeSO or DETC-$MeSO_2$ was reacted with 100 μM GSH in 50 mM potassium phosphate, pH 7.0, at 37°. The reaction was terminated at different time intervals by adding 5 mM 5,5′-bis-dithionitrobenzoic acid (DTNB). DTNB reacts with unreacted GSH, and the yellow chromogen that developed was measured at 412 nm. Rate constants for DETC-MeSO and DETC-$MeSO_2$ reaction with GSH were determined using the extinction coefficient of 13,600 M^{-1} cm^{-1} for thionitrobenzoic acid.

CARBAMOYLATION OF ENDOGENOUS LIVER GSH BY DETC-MeSO OR DETC-$MeSO_2$. The S_9 (9000 *g* for 15 min) fraction, prepared from liver obtained from an untreated rat, was used as an alternate source of GST and GSH. The S_9 fraction (1 mg protein/mL) was incubated with 10 mM NEM at 37° in 0.1 M potassium phosphate, pH 7.0. NEM alkylates GSH, and unreacted NEM was removed by passing the solution through a Sep-Pak C-18 cartridge. The eluate, which contained no GSH, was collected under negative pressure and incubated with DETC-MeSO or DETC-$MeSO_2$ (100 μM) for 30 min at 37°, after which the mixture was filtered through a Centricon membrane filter. DETC-MeSO or DETC-$MeSO_2$ contained in the filtrate was extracted by solid-phase extraction and quantitatively determined by reverse-phase HPLC (Beckman/Altex, C-18 column, 250 × 4.6 mm, 5 μm particle size; mobile phase, 50% acetonitrile/50% water; flow rate, 1 mL/min; detection, 215 nm) as described in detail previously [18]. Car-

bamoylated GSH in the filtrate was identified by FAB MS/MS. Control experiments were identical except that NEM was not included.

MASS SPECTRAL ANALYSIS. Mass spectra of DETC-GS conjugates were obtained employing an AUTOSPEC-Q tandem hybrid mass spectrometer (Fiscons/VG Analytical Ltd.) equipped with an OPUS data system. Fast atom bombardment (FAB) experiments were performed using a cesium gun operated at 20 KeV energy and 2 μA emission. Samples derived from Centricon filtration were further concentrated using Speed-Vac and added to the thioglycerol/glycerol (3:1) matrix. The GSH conjugate was analyzed directly by FAB MS/MS. CID experiments were performed with precursor ions attenuated 50% with Xenon in the collision quadropole at 50 eV energy. The analysis of DETC-GS was based on the characteristic formation of MH^+ ion 407. Similar results were obtained from the DETC-$MeSO_2$ incubation with GSH.

INHIBITION OF RAT LIVER $ALDH_2$ BY DETC-MESO OR DETC-$MESO_2$. Studies were carried out with liver mitochondria isolated from untreated rats as described [3, 9]. The incubation mixture contained intact (no deoxycholate) or solubilized (0.5 mg deoxycholate/mg protein) mitochondria (equivalent of 2 mg protein), phosphate buffer, 1 mM EDTA, and various concentrations of either DETC-MeSO or DETC-$MeSO_2$ (dissolved in 15 μL ethanol) in a final volume of 1.5 mL. Incubations were carried out for 1 hr at 37° in a metabolic shaking water bath. Mitochondria were isolated by centrifugation, and $ALDH_2$ activity was determined spectrophotometrically by monitoring the formation of NADH at 340 nm [19]. Protein content was determined by the method of Lowry *et al.* [20], using bovine serum albumin as the standard.

In Vivo *Studies*

DETC-MeSO (2.6, 5.2, 10.2, and 20.4 mg/kg) or DETC-$MeSO_2$ (2.6, 10.2, 20.4, and 44.8 mg/kg) was dissolved in PEG 200 and administered either i.p. or orally (1 mL/kg). The rats were anesthetized with CO_2 and decapitated 8 hr after dosing, the livers were removed, and $ALDH_2$ was determined. For determination of the time–course for $ALDH_2$ inhibition, DETC-MeSO (5.2 mg/kg) was administered i.p. or orally to rats, and the rats were killed 30 min, 1, 4, 8, 24, or 48 hr after drug administration. DETC-$MeSO_2$ (44.8 mg/kg) was administered in a similar way to rats, and the rats were killed 1, 4, 8, 12, 24, or 48 hr after drug administration. The rats were decapitated after CO_2 anesthesia.

$ALDH_2$ ASSAY. Liver from drug-treated or control rats was homogenized in 0.25 M sucrose, the mitochondrial fraction was isolated by differential centrifugation, and $ALDH_2$ activity was determined as described earlier for *in vitro* study [19].

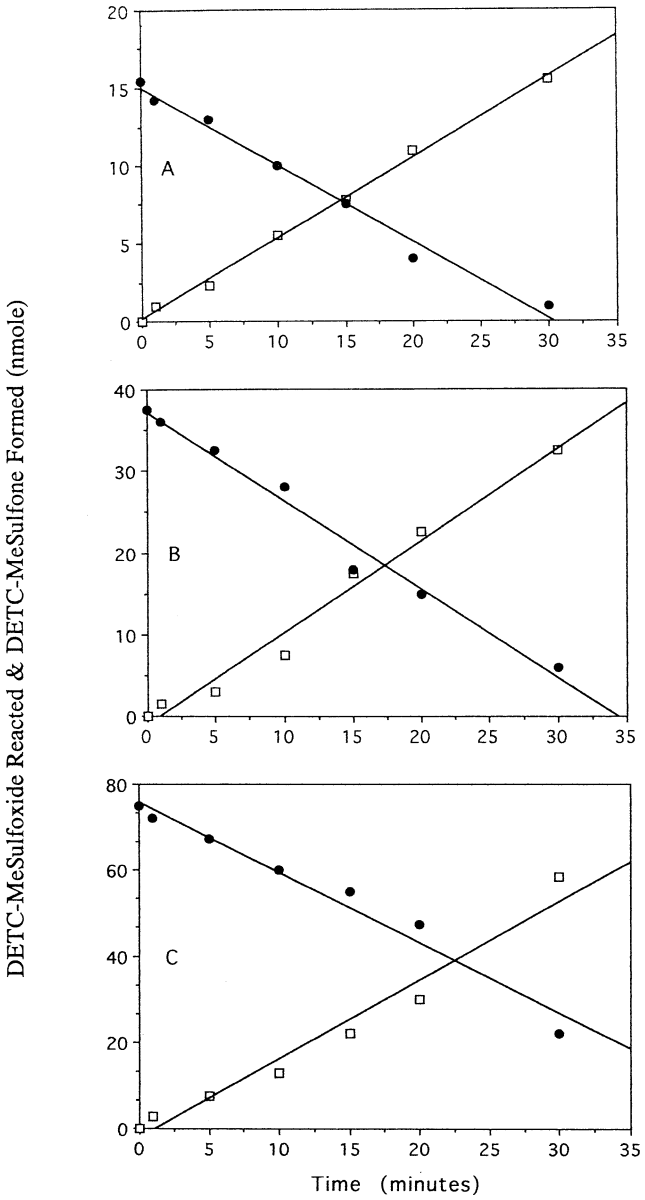


FIG. 1. *In vitro* oxidation of DETC-MeSO to DETC-$MeSO_2$. DETC-MeSO 20 μM (A), 50 μM (B), and 100 μM (C), HRP (5 units/mL) and H_2O_2 (2 mM) were incubated in phosphate buffer at various times. Incubations were carried out in duplicate. Unreacted DETC-MeSO (●) and DETC-$MeSO_2$ (□) formed (nmol) were determined by HPLC (see Materials and Methods). Each value is the mean of triplicate readings.

RESULTS

HRP and hydrogen peroxide were employed to study the oxidation of DETC-MeSO. DETC-MeSO was readily oxidized to DETC-$MeSO_2$ in the presence of the heme-containing enzyme HRP and hydrogen peroxide. A 1:1 mass balance was found between the loss of DETC-MeSO and the formation of DETC-$MeSO_2$, regardless of the initial concentration of DETC-MeSO employed (Fig. 1). When the incubation contained either HRP or hydrogen peroxide but not both, oxidation of DETC-MeSO was not

TABLE 1. Role of endogenous GSH in the conjugation of DETC-MeSO

Incubation	DETC-MeSO remaining (μM)	% DETC-MeSO reacted with GSH	DETC-$MeSO_2$ formed (μM)
SO alone (100 μM)	77 ± 1.0	0	ND*
S_9 + SO	24 ± 1.0	69.0	ND
S_9 + GST + SO	16 ± 1.0	79.0	ND
S_9 + NEM + SO + GST	70 ± 3.5	10.0	ND
S_9 + NEM + SO	70 ± 1.5	9.0	ND

DETC-MeSO (SO, 100 μM), GST (5 units/mL), and rat liver supernatant (S_9, 9000 *g* fraction equivalent of 1 mg protein) were incubated with or without NEM (10 mM) at 37° for 30 min. Ethiolate sulfoxide was the internal standard. Recovery of SO was about 77%. The remaining SO or SO_2 formed was determined by HPLC. Values are means ± SEM of triplicates.

* ND = not detected.

observed (data not shown), suggesting that both must be present for DETC-MeSO oxidation to occur.

Carbamoylation of GSH can be expected to occur with sulfoxides or sulfones, in general. The kinetics of conjugation of DETC-MeSO or DETC-$MeSO_2$ with GSH, although suggested from the studies by others [16], have never been studied in detail. Therefore, studies were carried out to examine the carbamoylation of GSH, employing an S_9 liver fraction (Tables 1 and 2). DETC-MeSO and DETC-$MeSO_2$ were incubated separately with the S_9 fraction of rat liver homogenate with or without GST added. Incubation of DETC-MeSO for 30 min with the S_9 rat liver fraction resulted in an approximately 70% loss of the DETC-MeSO (Table 1), compared with a loss of approximately 90% for DETC-$MeSO_2$ when DETC-$MeSO_2$ was incubated with the S_9 fraction (Table 2). The addition of GST to the incubation did not increase the loss of either DETC-MeSO or DETC-$MeSO_2$ markedly (Tables 1 and 2). This observation is probably due to the extremely rapid reaction rate for GSH carbamoylation by either DETC-MeSO or DETC-$MeSO_2$, and thus any effect GST may have on the reaction rate is insignificant at the concentration employed. Mass spectrometry (Fig. 2, A and B, respectively) confirmed the formation of the GSH conjugate (DETC-GS; MH^+ at $m/z = 407$) that can be formed by either DETC-MeSO or DETC-$MeSO_2$ (Scheme 1). The GSH conjugate of DETC-MeSO or DETC-$MeSO_2$ was not formed when tissue GSH was depleted by the addition of NEM to the S_9 fraction (Tables 1 and 2), and also confirmed by mass spectroscopy (spectra not shown).

Carbamoylation studies also were carried out in a buffer system in which 0.1 mM GSH was incubated with either DETC-MeSO or DETC-$MeSO_2$, and the rate of the reaction was determined spectrophotometrically by measuring the residual thiol by use of Ellman's reagent. In addition, the rate of carbamoylation also was determined by HPLC. Employing both of these techniques, the rate of reaction was more rapid with DETC-$MeSO_2$. The second order rate constants for DETC-MeSO and DETC-$MeSO_2$ were 10 and 14 M^{-1} sec^{-1}, respectively, by monitoring residual thiol, and 3.4 and 7.0 M^{-1} sec^{-1}, respectively, when the disappearance of DETC-MeSO or DETC-$MeSO_2$ was determined by the HPLC method.

In previous studies [9], DETC-MeSO was found to be more potent than DETC-$MeSO_2$ as an inhibitor of $ALDH_2$, both *in vitro* and *in vivo*. However, others [10] observed that in *in vitro* studies, DETC-$MeSO_2$ was a more potent inhibitor than DETC-MeSO. In those studies [10], a solubilized mitochondrial preparation was used, whereas we [9] utilized intact mitochondria. No *in vivo* studies were carried out by those investigators [10]. We examined the effect of solubilization of mitochondria on $ALDH_2$ inhibition, and the data are given in Table 3. In intact mitochondria, DETC-MeSO was approximately four times more potent than DETC-$MeSO_2$, whereas with solubilized rat liver mitochondria both DETC-MeSO and DETC-$MeSO_2$

TABLE 2. Role of endogenous GSH in the conjugation of DETC-$MeSO_2$

Incubation	DETC-$MeSO_2$ remaining (μM)	% DETC-$MeSO_2$ reacted with GSH	DETC-MeSO formed (μM)
SO_2 alone (100 μM)	76 ± 2.5	0	ND*
S_9 + SO_2	7 ± 2.0	90.0	ND
S_9 + SO_2 + GST	5 ± 0.5	94.0	ND
S_9 + NEM + SO_2	75 ± 2.0	3.0	ND
S_9 + NEM + SO_2 + GST	74 ± 2.5	3.0	ND

DETC-$MeSO_2$ (SO_2, 100 μM), GST (5 units/mL), and rat liver supernatant (S_9, 9000 *g* fraction equivalent of 1 mg protein) were incubated with or without NEM (10 mM) at 37° for 30 min. The remaining SO_2 or SO formed, if any, was determined by HPLC. Ethiolate sulfoxide was the internal standard. Recovery of sulfone was about 76%. Values are means ± SEM of triplicates.

* ND = not detected.

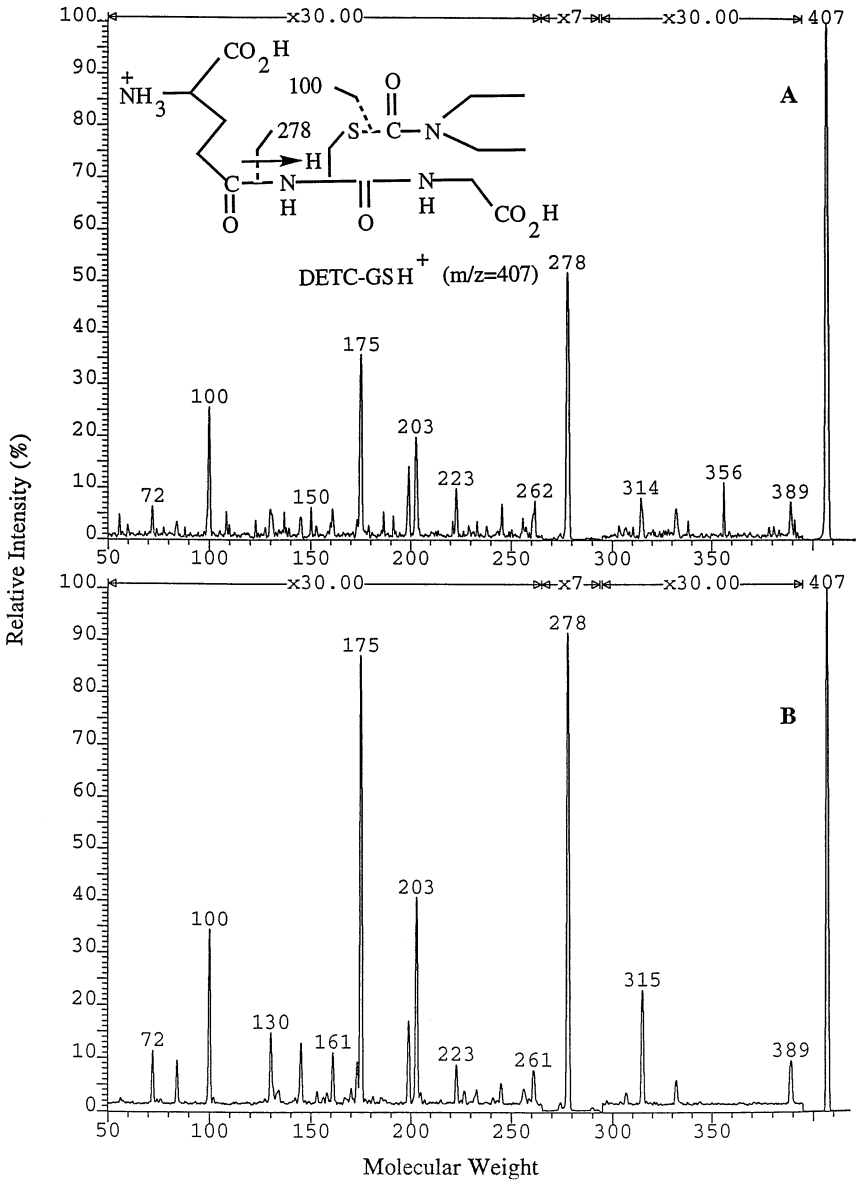
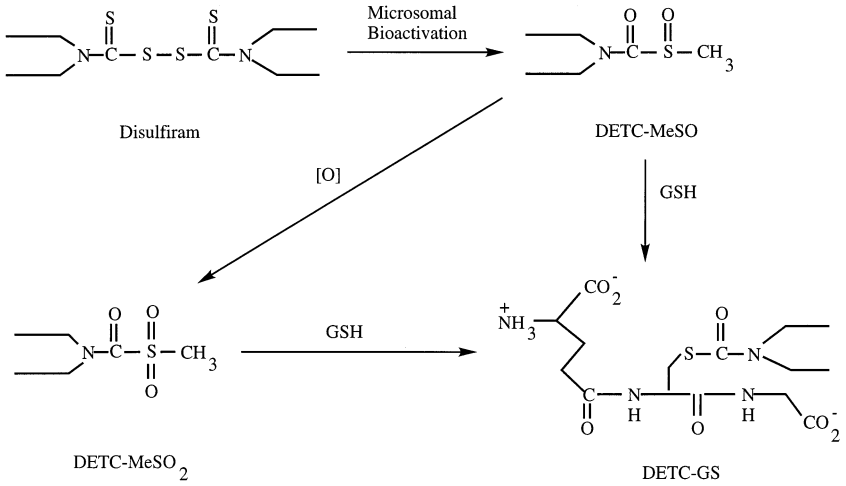


FIG. 2. Spectrum of product ions obtained by FAB MS/MS of the MH^+ ion (m/z = 407) of (A) DETC-MeSO–GSH conjugate; and (B) DETC-$MeSO_2$–GSH conjugate, using rat liver S_9 fraction as the source for GSH. The origins of the structurally diagnostic product ions at m/z 278 and 100 [$(Et_2NCO)^+$] are shown.

were equipotent. These *in vitro* studies were extended to *in vivo* studies. Dose–response data were generated after the oral administration of DETC-MeSO and DETC-$MeSO_2$ to rats. For comparative purposes, the data generated from previous studies in our laboratory after the i.p. administration of DETC-MeSO [3] and DETC-$MeSO_2$ [9] were used. The dose–response relationship for both DETC-MeSO and DETC-$MeSO_2$ was found to be similar for both the i.p. and oral route of administration. Regardless of whether DETC-MeSO was administered i.p. or orally, it was approximately 10-fold more potent than DETC-$MeSO_2$ (Fig. 3). The time–course for the inhibition of rat liver $ALDH_2$ *in vivo* also was examined (Fig. 4). Inhibition of $ALDH_2$ occurred more rapidly after DETC-MeSO administration. $ALDH_2$ was inhibited maximally 1 hr after DETC-MeSO administration, whereas maximal $ALDH_2$ inhibition for DETC-$MeSO_2$ occurred approximately 8 hr after drug dosing.

DISCUSSION

DETC-MeSO was oxidized readily to DETC-$MeSO_2$ by the heme-containing enzyme HRP and hydrogen peroxide (Fig. 1). The rate of DETC-MeSO oxidation corresponded to the rate of DETC-$MeSO_2$ formation. The half-time for the disappearance of DETC-MeSO was similar for all three concentrations employed and consistent with these concentrations being lower than the Michaelis constant for DETC-MeSO. Furthermore, HRP and hydrogen peroxide were both required for DETC-MeSO oxidation. This result is similar to the reaction between hemoprotein peroxidases and hydrogen peroxide in which HRP reacts with hydrogen peroxide [21]. It seems likely that DETC-MeSO oxidation also occurs *in vivo*, since both peroxidase and H_2O_2 are readily available. The possibility of DETC-MeSO oxidation *in vivo* also is supported by studies of others. For example,



SCHEME 1. GSH carbamoylation with DETC-MeSO and DETC-$MeSO_2$. The intermediates formed and the cytochrome P450 enzymes required are described in Refs. 4–7. DETC-MeSO and DETC-$MeSO_2$ both carbamoylate GSH, but it is DETC-MeSO which is believed to be the metabolite responsible for $ALDH_2$ inhibition *in vivo*.

Hucker *et al.* [8] found dimethyl sulfone in the urine of both rats and humans after dimethyl sulfoxide administration. The thioester eptam also has been shown to be converted to eptam sulfoxide and eptam sulfone by lipid peroxyl radicals in striped bass [22]. In those studies [22], both the sulfoxide and the sulfone were formed when stronger oxidizing agents were used. Thus, it seems likely that DETC-$MeSO_2$ *in vivo* is formed from DETC-MeSO.

Studies with the rat liver S_9 fraction clearly showed carbamoylation of GSH by DETC-MeSO and DETC-$MeSO_2$ (Tables 1 and 2). When NEM was added to the incubation containing the S_9 fraction, no carbamoylation by either DETC-MeSO or DETC-$MeSO_2$ was observed (Tables 1 and 2). Incubation of the NEM-pretreated S_9 fraction with DETC-MeSO or DETC-$MeSO_2$ did not produce the DETC-GS conjugate, as reflected by lack of the characteristic m/z value for the MH^+ ion of the DETC-GS at 407 (spectra not shown). Addition of GST to the incubation did not increase the rate of loss of either DETC-MeSO or DETC-$MeSO_2$. One explanation may be that carbamoylation is extremely rapid, and, therefore, GST has no further effect on the reaction rate. Furthermore, carbamoylation of GSH in 0.1 M potassium phosphate buffer, pH 7.0, showed similar amounts of loss of sulfoxide and sulfone, as observed in the studies with the S_9 fraction with or without GST added (data not shown).

TABLE 3. *In vitro* inhibition of rat liver $ALDH_2$ by DETC-MeSO and DETC-$MeSO_2$

	IC_{50} (μM)	
Inhibitor	Intact mitochondria	Solubilized mitochondria
DETC-MeSO	0.554 ± 0.07	0.488 ± 0.159
DETC-$MeSO_2$	2.275 ± 0.06	0.524 ± 0.134

Intact and solubilized (with deoxycholate; 0.5 mg/mg protein) mitochondria isolated from rat liver were incubated with various concentrations of DETC-MeSO or DETC-$MeSO_2$ at 37° for 1 hr in a water bath. Deoxycholate was added later to intact mitochondria before the $ALDH_2$ assay. The IC_{50} values were determined by plotting concentrations versus percent inhibition. The points were analyzed by nonlinear regression. Values are means ± SEM of triplicate measurements.

The finding of particular significance was that DETC-MeSO and DETC-$MeSO_2$ were equipotent $ALDH_2$ inhibitors when solubilized mitochondria were employed, but that DETC-MeSO was approximately four times more potent than DETC-$MeSO_2$ when intact mitochondria were used. Mays *et al.* [11] also found DETC-MeSO to be more potent in intact mitochondria. Whereas we found a 4-fold difference, Mays *et al.* [11] found DETC-MeSO to be approximately ten times more potent than DETC-$MeSO_2$ in intact mitochondria. Employing solubilized mitochondria, Mays *et al.* [11] found DETC-$MeSO_2$ to be approximately twice as potent as DETC-MeSO, whereas in our studies (Table 3) the two were almost equipotent. These differences are not great, and the studies from both laboratories are supportive of each other.

More importantly, comparative differences in $ALDH_2$ inhibition between DETC-MeSO and DETC-$MeSO_2$, employing either intact or solubilized mitochondria, are reflected by the *in vivo* studies. In the dose–response studies in rats, regardless of the route of administration, oral or i.p., DETC-MeSO was always more potent than DETC-$MeSO_2$ (Fig. 3). After either i.p. or oral administration, the dose which inhibited $ALDH_2$ by 50% (ID_{50}) for DETC-MeSO was 3.5 mg/kg, whereas the ID_{50} for DETC-$MeSO_2$ was approximately 35 mg/kg, almost a 10-fold potency difference. The time–course for $ALDH_2$ inhibition also favored DETC-MeSO (Fig. 4). Peak inhibition of $ALDH_2$ by DETC-MeSO was observed after 1 hr, whereas for DETC-$MeSO_2$, peak inhibition of $ALDH_2$ occurred 8 hr after i.p. administration. Of significant interest is the difference in toxicity between DETC-MeSO and DETC-$MeSO_2$. In the original studies that first reported on the inhibition of $ALDH_2$ by DETC $MeSO_2$ [9], dose–response studies with DETC-$MeSO_2$ could not be completed because of its

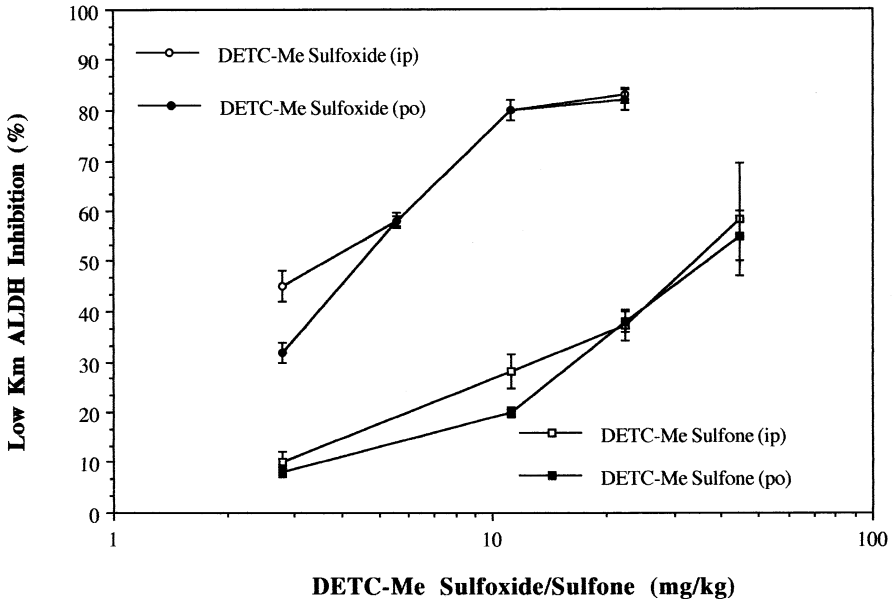


FIG. 3. Effect of DETC-MeSO or DETC-$MeSO_2$ on rat liver $ALDH_2$ activity *in vivo*. Various concentrations of DETC-MeSO and DETC-$MeSO_2$ were administered either i.p. or orally, and 8 hr later $ALDH_2$ activity was determined in isolated mitochondria as described in Materials and Methods. The results are presented as means ± SEM of single observations from each of four rats. Control (uninhibited) $ALDH_2$ activity = 18.5 ± 2.0 nmol/min/mg protein.

extreme toxicity. Toxicity by sulfones also has been reported by others. For example, molinate sulfone has been found to be extremely toxic to fish [23], while *S*-ethyl *N*,*N*-dipropylthiocarbamate (EPTC) sulfone is toxic to mice [15]. Although it seems quite clear that thiocarbamate sulfones are toxic, the mechanism for this toxicity remains to be delineated.

The present studies showed that DETC-MeSO and DETC-$MeSO_2$ inhibited $ALDH_2$ both *in vitro* and *in vivo*. The findings that DETC-$MeSO_2$ was less potent than DETC-MeSO *in vitro* when intact mitochondria were used, and also less potent than DETC-MeSO in animals, suggest that DETC-$MeSO_2$ has difficulty gaining access to $ALDH_2$ in the mitochondria. This also has been suggested recently by others [11]. Thus, even if DETC-$MeSO_2$ is formed *in vivo*, it is DETC-MeSO that is most likely responsible for the inhibition of $ALDH_2$, since DETC-$MeSO_2$ has difficulty penetrating cell membranes. Furthermore, in a recent study [24] we have shown that GSH carbamoylation mediates the effect by which DETC-MeSO produces $ALDH_2$ inhibition *in vivo*. Intravenous administration of an equimolar concentration of DETC-GS or DETC-MeSO to mice produced a comparable degree of $ALDH_2$ inhibition. In light of the several cytochrome P450 enzymes responsible for disulfiram bioactivation [7], and the possibility of inefficient bioactivation, it is suggested that DETC-MeSO may be a better clinical candidate than disulfiram for the treatment for alcohol abuse and alcoholism. DETC-

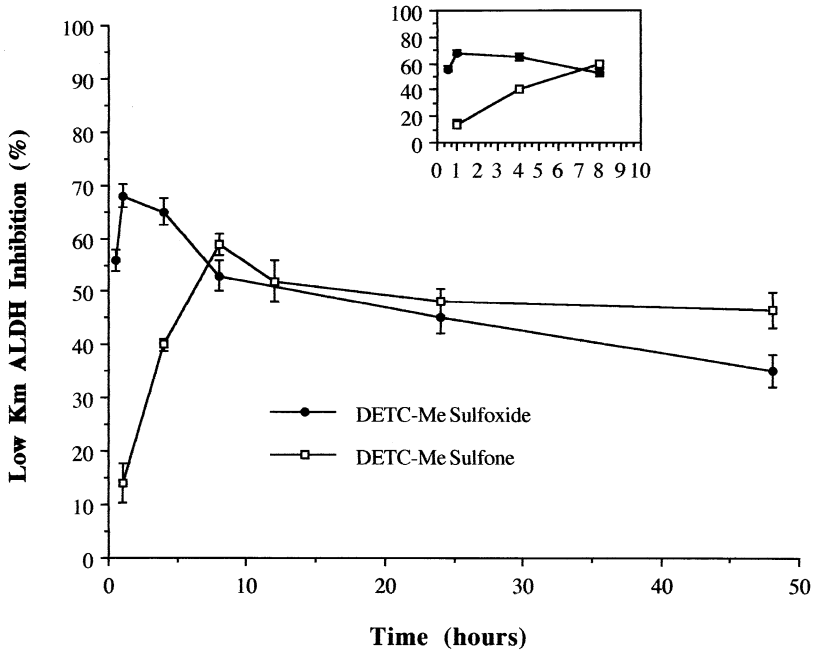


FIG. 4. Onset (inset) and recovery of rat liver $ALDH_2$ inhibition after treatment with DETC-MeSO or DETC-$MeSO_2$. Rats were administered i.p. either DETC-MeSO (5.2 mg/kg) or DETC-$MeSO_2$ (44.8 mg/kg). At the times indicated, liver $ALDH_2$ activity was determined. Results are presented as means ± SEM of single observations from each of four rats. Control (uninhibited) $ALDH_2$ activity = 18.5 ± 2.0 nmol/min/mg protein.

$MeSO_2$, because of its difficulty in gaining access to the mitochondrial membrane-bound $ALDH_2$, and its extreme toxicity, would not be a viable clinical candidate.

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