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S-Methyl *N*,*N*-diethylthiolcarbamate sulfone, an *in vitro* and *in vivo* inhibitor of rat liver mitochondrial low K_m aldehyde dehydrogenase

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Abstract—*S*-Methyl *N*,*N*-diethylthiolcarbamate sulfone (DETC-Me sulfone) was investigated for its rat liver mitochondrial low K_m aldehyde dehydrogenase ($ALDH_2$) inhibitory properties. DETC-Me sulfone inhibited $ALDH_2$ *in vitro* (IC_{50} = 3.8 μM) and *in vivo* (ID_{50} = 170 μmol/kg; 31 mg/kg). Maximum inhibition (60%) of $ALDH_2$ was observed 8 hr after DETC-Me sulfone administration. In addition, incubation of *S*-methyl *N*,*N*-diethylthiolcarbamate (DETC-Me) or *S*-methyl *N*,*N*-diethylthiolcarbamate sulfoxide (DETC-Me sulfoxide) with rat liver microsomes and an NADPH-generating system failed to produce DETC-Me sulfone. Furthermore, DETC-Me sulfone could not be detected in plasma from rats treated with either DETC-Me sulfoxide or DETC-Me sulfone. In conclusion, DETC-Me sulfone inhibited $ALDH_2$ *in vitro* and *in vivo*. However, there was no evidence suggesting that DETC-Me sulfoxide was metabolized to DETC-Me sulfone.

Key words: aldehyde dehydrogenase; disulfiram; *S*-methyl *N*,*N*-diethylthiolcarbamate sulfone; *S*-methyl *N*,*N*-diethylthiolcarbamate sulfoxide; aldehyde dehydrogenase inhibition

Recent evidence indicates that disulfiram is bioactivated through a series of intermediates, ultimately forming DETC-Me sulfoxide,* the metabolite proposed to be responsible for inhibition of rat liver mitochondrial low K_m $ALDH_2$ [1–5]. The proposed metabolic sequence is disulfiram → diethyldithiocarbamate → diethyldithiocarbamate methyl ester → DETC-Me → DETC-Me sulfoxide [3]. The formation of DETC-Me sulfoxide is mediated by CYP [5]. During the chemical synthesis of DETC-Me sulfoxide [2], over-oxidation of the precursor DETC-Me with sodium metaperiodate produced a small quantity of DETC-Me sulfone. Studies were carried out, therefore, to determine if DETC-Me sulfone inhibited rat liver $ALDH_2$ both *in vitro* and *in vivo*. Studies were also conducted to investigate if DETC-Me sulfoxide could be oxidized to DETC-Me sulfone *in vitro* and *in vivo*.

Materials and Methods

Synthesis of DETC-Me sulfone. DETC-Me and DETC-Me sulfoxide were synthesized as described [2, 6]. DETC-Me sulfone was synthesized [7] by oxidizing 3.5 mmol of DETC-Me with 6.75 mmol 3-chloroperoxybenzoic acid and purified [2] as described previously. ^{1}H-NMR (300 MHz, $CdCl_3$), δ = 3.75 (q, 2H, *J* = 7.1 Hz), 3.41 (q, 2H, *J* = 7.2 Hz), 3.13 (s, 3H), 1.3 (t, 3H, *J* = 7.1 Hz), 1.23 (t, 3H, *J* = 7.2 Hz)]. Mass spectra (chemical ionization/NH_3) *m*/*z* (relative intensity): 197 ($M + NH_4^+$,65), 180 (MH^+, 20), 100 (100), 74 (30).

Rat liver $ALDH_2$ determination. In the *in vivo* studies, rats were killed by decapitation, and the livers were removed and homogenized in 0.25 M sucrose buffer. The mitochondria were isolated from the homogenates by differential centrifugation and solubilized in sodium deoxycholate; $ALDH_2$ activity was determined by monitoring the conversion of NAD to NADH as described by Tottmar *et al.* [8]. For the *in vitro* inhibition studies, isolated mitochondria from liver were incubated with DETC-Me sulfone (0.01 to 1000 μM) for 60 min at 37°, the incubation was terminated by centrifugation, the mitochondria were solubilized in sodium deoxycholate, and the $ALDH_2$ activity was determined [8].

In vivo *metabolism studies.* DETC-Me sulfoxide (10.3 mg/kg) or DETC-Me sulfone (44.8 mg/kg) was administered to fasted, mature male rats (3 rats/group). The rats were killed 30 min later, the blood was collected, and the plasma was separated. The rat plasma was then analyzed for the presence of DETC-Me sulfone by HPLC [2].

In vitro *metabolism studies.* DETC-Me (1 mM) or DETC-Me sulfoxide (1 mM) was incubated with mature male rat liver microsomes (0.67 mg/mL), 0.67 mM $NADP^+$, 6.7 mM glucose-6-phosphate, 0.67 U/mL glucose-6-phosphate dehydrogenase, and 1 mM EDTA in potassium phosphate buffer (0.1 M, pH 7.4) in a final volume of 1.5 mL. After 30 min of incubation at 37°, the reaction was stopped and the amount of DETC-Me sulfone formed was determined by HPLC [5].

Results and Discussion

The concentration of DETC-Me sulfone that inhibited rat liver $ALDH_2$ in isolated mitochondria by 50% (IC_{50}) was calculated to be 3.8 μM. $ALDH_2$ was inhibited 100% *in vitro* by 100 μM DETC-Me sulfone. For comparative purposes, DETC-Me is a poor inhibitor of rat liver $ALDH_2$ *in vitro* [2, 6], while the IC_{50} for DETC-Me sulfoxide is 0.75 μM [2]. In rats, the ID_{50} for DETC-Me sulfone was approximately 170 μmol/kg (31 mg/kg) i.p. (Fig. 1A), whereas the ID_{50} for DETC-Me and DETC-Me sulfoxide is 44.2 μmol/kg (6.5 mg/kg) and 21.5 μmol/kg (3.5 mg/kg) i.p. respectively [2]. The dose of DETC-Me sulfone that produced maximum inhibition of $ALDH_2$ could not be determined because at doses higher than 44.8 mg/kg (i.p.), DETC-Me sulfone was lethal to the rats. The time course of inhibition of $ALDH_2$ in rats was also studied. Eight hours after DETC-Me sulfone administration (44.8 mg/kg), rat liver $ALDH_2$ was inhibited 60%, with $ALDH_2$ still inhibited approximately 50% after 48 hrs (Fig. 1B). Recovery of $ALDH_2$ activity after DETC-Me sulfone administration could not be determined because the rats did not survive past 48 hr.

The metabolic formation of DETC-Me sulfone both *in vitro* and *in vivo* was also investigated. In the *in vitro* studies, DETC-Me or DETC-Me sulfoxide was incubated

* Abbreviations: DETC-Me, *S*-methyl *N*,*N*-diethylthiolcarbamate; DETC-Me sulfoxide, *S*-methyl *N*,*N*-diethylthiolcarbamate sulfoxide; DETC-Me sulfone, *S*-methyl *N*,*N*-diethylthiolcarbamate sulfone; $ALDH_2$ mitochondrial low K_m aldehyde dehydrogenase; and CYP, cytochrome P450.

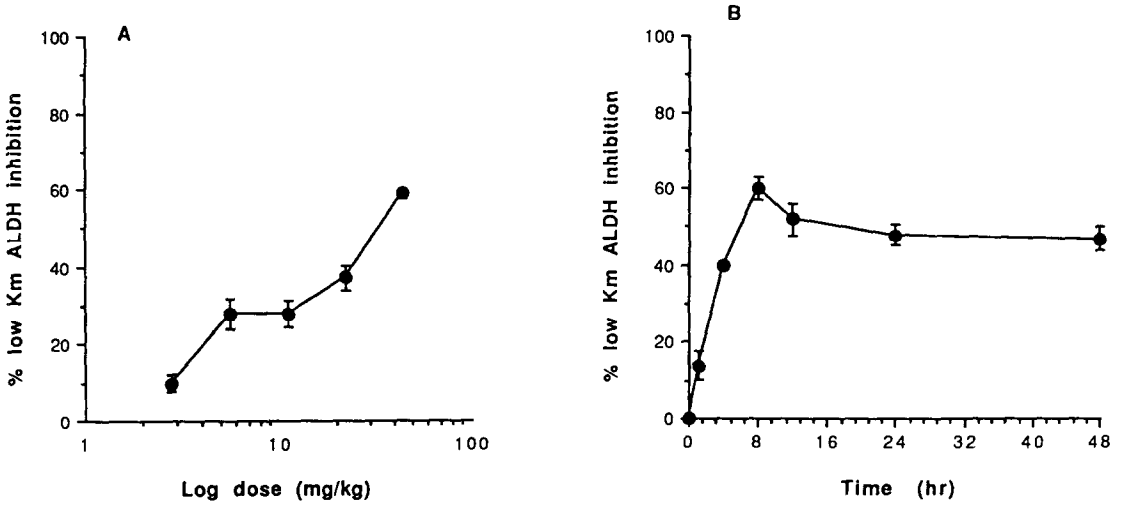

Fig. 1. (A) Dose–response for inhibition of rat liver $ALDH_2$: DETC-Me sulfone was dissolved in corn oil and the doses given were 2.8, 5.6, 11.2, 22.4 and 44.8 mg/kg, i.p. The rats were killed 8 hr after DETC-Me sulfone administration and $ALDH_2$ activity was determined [8]. (B) Time-course inhibition of rat liver $ALDH_2$. DETC-Me sulfone was administered at a dose of 44.8 mg/kg i.p., the rats were killed 1, 4, 8, 12, 24, and 48 hrs later, and $ALDH_2$ activity was determined [8]. Each data point is the mean ± SEM of four rats. A representative control value for the enzyme activity was 22.8 ± 1.8 nmol NADH/min/mg protein.

with rat liver microsomes in the presence of an NADPH-generating system. Although incubation of DETC-Me with microsomes results in the formation of DETC-Me sulfoxide [3], DETC-Me sulfone could not be detected in this incubation (data not shown). Similarly, DETC-Me sulfone was not detected in the microsomal incubation when DETC-Me sulfoxide was employed as the substrate (data not shown). However, DETC-Me sulfone could be detected and quantified in the incubation in which a known amount of DETC-Me sulfone was added (data not shown). It thus appears that CYP and flavin monooxygenase do not play a role in the oxidation of DETC-Me sulfoxide to DETC-Me sulfone. This conclusion is also supported by two additional observations: (1) the CYP inhibitor 1-benzylimidazole blocks the formation of DETC-Me sulfoxide from DETC-Me and subsequent inhibition of $ALDH_2$, both *in vivo* and *in vitro*, and (2) in contrast, treatment of rats with 1-benzylimidazole prior to DETC-Me sulfoxide administration does not block DETC-Me sulfoxide-mediated $ALDH_2$ inhibition *in vivo* [3]. If DETC-Me sulfoxide was oxidized to DETC-Me sulfone by CYP, then 1-benzylimidazole should have blocked the inhibition of $ALDH_2$ if DETC-Me sulfone was the active metabolite.

DETC-Me sulfone was not detected in plasma obtained from rats treated with either DETC-Me sulfoxide (10.3 mg/kg, i.p.) or DETC-Me sulfone (44.8 mg/kg, i.p.) (data not shown). Furthermore, DETC-Me sulfone was not detected in rat plasma in which a known standard of DETC-Me sulfone (final concentration 2 μg/mL) was added (data not shown). The inability to detect DETC-Me sulfone in rat plasma could be the result of its rapid conjugation with either protein or non-protein thiols similar to that observed with other thiocarbamates [7].

In conclusion, DETC-Me sulfone inhibited $ALDH_2$ both *in vivo* and *in vitro*. At the present time, it is not known whether DETC-Me sulfone can be formed *in vivo*. Even if DETC-Me sulfone was formed *in vivo*, its formation does not appear to be mediated by CYP or flavin-containing monooxygenases. Non-enzymatic oxidation of DETC-Me sulfoxide by molecular oxygen, hydrogen peroxide, or enzymatic oxidation by peroxisomes *in vivo* cannot be ruled out. Even if DETC-Me sulfone was formed *in vivo* by these mechanisms, its concentration is probably extremely low, and it is unlikely that DETC-Me sulfone contributes to the inhibition of $ALDH_2$ *in vivo*. This is supported by the findings that DETC-Me sulfoxide is approximately 10 times more potent than DETC-Me sulfone *in vivo* ([2] (Fig. 1A), and, in addition, DETC-Me sulfoxide can be readily detected in plasma after disulfiram or DETC-Me sulfoxide administration to rats [4]. Thus, DETC-Me sulfoxide appears to be the disulfiram metabolite responsible for $ALDH_2$ inhibition as previously proposed [2–4]. The mechanism by which DETC-Me sulfone produces its toxicity *in vivo* is unknown at this time, although conjugation with critical proteins could be one explanation.

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