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RAPID COMMUNICATION

IN VITRO AND *IN VIVO* INHIBITION OF RAT LIVER ALDEHYDE DEHYDROGENASE BY *S*-METHYL *N,N*-DIETHYLTHIOLCARBAMATE SULFOXIDE, A NEW METABOLITE OF DISULFIRAM

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The basis for the clinical use of disulfiram in the treatment of ethanol abuse is its inhibition of liver aldehyde dehydrogenase (E.C. 1.2.1.3, ALDH). The reactions of disulfiram with ALDH have recently been reviewed [1]. Although disulfiram has been studied for over 40 years, it is still not clear whether disulfiram or a metabolite is the active chemical species responsible for ALDH inhibition *in vivo*. In seminal studies, Yourick and Faiman [2] found that administration of the disulfiram metabolite diethyldithiocarbamate-methyl ester (DDTC-Me) to rats inhibited rat liver mitochondrial low K_m ALDH. In subsequent comparative studies in rats, disulfiram, diethyldithiocarbamate (DDTC), and DDTC-Me exhibited similar rat liver mitochondrial low K_m ALDH inhibitory profiles, and all produced a disulfiram-ethanol reaction (DER) after an ethanol challenge [3]. Because DDTC-Me was relatively inactive as a rat liver mitochondrial low K_m ALDH inhibitor *in vitro*, this suggested that *in vivo*, another chemical species was responsible for the liver ALDH inhibition. This led to the discovery of *S*-methyl *N,N*-diethylthiolcarbamate (DETC-Me), first reported by Hart *et al.* [4] and later described in detail [5]. The formation of DETC-Me also was confirmed by Johansson *et al.* [6]. Because DETC-Me is inactive *in vitro* [5], unless long incubation periods and high concentrations are employed, this did not suggest that DETC-Me was the active chemical species as proposed by Johansson *et al.* [6].

The role of bioactivation in disulfiram's action as a rat liver mitochondrial low K_m ALDH inhibitor *in vivo* has been described recently by Yourick and Faiman [7]. In those studies, the cytochrome P450 (P450) inhibitor *N*-octylimidazole blocked the rat liver mitochondrial low K_m ALDH inhibition by disulfiram, DDTC, DDTC-Me and DETC-Me. In addition, in *in vitro* incubation studies with rat liver mitochondria, the addition of liver microsomes to the incubation markedly increased the inhibition of rat liver mitochondrial low K_m ALDH by DDTC and DDTC-Me. Data are now given which identify for the first time the formation of *S*-methyl *N,N*-diethylthiolcarbamate sulfoxide (DETC-MeSO) (Fig. 1), a natural metabolite of disulfiram and a potent inhibitor of rat liver mitochondrial low K_m ALDH both *in vitro* and *in vivo*.

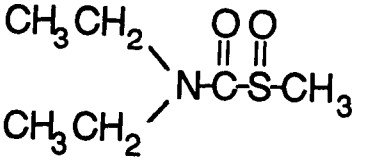

Fig. 1. Chemical structure of *S*-methyl *N,N*-diethylthiolcarbamate sulfoxide (DETC-MeSO).

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MATERIALS AND METHODS

Synthesis of DETC-MeSO. DETC-Me was synthesized as described by Hart *et al.* [5]. One equivalent of DETC-Me was reacted with one equivalent of sodium metaperiodate ($NaIO_4$) in 50:50 methanol:water for 48 hr, and the reaction mixture was extracted with methylene chloride and concentrated under reduced pressure. The resulting yellow oil was purified by reverse-phase medium-pressure liquid chromatography utilizing a Sepralyte C_{18} stationary phase and 20:80 acetonitrile:water mobile phase to remove any unreacted DETC-Me. Fractions containing DETC-MeSO were pooled, extracted into methylene chloride, and concentrated under reduced pressure; DETC-MeSO was confirmed by mass spectral analysis, IR and NMR. [^{1}H NMR (500 MHz, $CDCl_3$) 3.57-3.47 (m, 2H), 3.44-3.38 (m, 2H), 2.71 (s, 3H), 1.22 (t, 3H, J = 7.12 Hz), 1.17 (t, 3H, J = 7.09 Hz); mass spectroscopy: CIMS (NH_3) m/z (relative intensity), 164 (M^+, 13), 148 (3), 100 (100), 72 (86), 44 (82); IR (neat: 2980, 1690, 1420, 1255, 1210, 1065, 1035 cm^{-1})].

In vivo inhibition of rat liver mitochondrial low K_m ALDH. Charles River-derived Sprague Dawley male rats (250–350 g) were maintained on a 12-hr light-dark cycle with access to lab chow and water *ad lib.* Animals were fasted overnight prior to drug administration, with water allowed *ad lib.* The rats were treated with different doses of DETC-MeSO, and 8 hr later the animals were anesthetized with carbon dioxide and decapitated. The liver was quickly removed and homogenized in 0.25 M sucrose, the mitochondria were isolated, and rat liver mitochondrial low K_m ALDH activity was determined [8].

In vitro inhibition of rat liver mitochondrial low K_m ALDH. Mitochondria were isolated from the livers of untreated male rats by differential centrifugation. The mitochondria were incubated with various concentrations of DETC-MeSO for 60 min at 37°, and then isolated by centrifugation, solubilized, and assayed for rat liver mitochondrial low K_m ALDH activity [8].

Plasma DETC-MeSO. Plasma DETC-MeSO was determined by the method described for DETC-Me [5] except that a 20:80 acetonitrile:water mobile phase was used.

RESULTS AND DISCUSSION

DETC-MeSO was found to be a potent inhibitor of rat liver mitochondrial low K_m ALDH both *in vitro* and *in vivo*. The concentration of DETC-MeSO which inhibited 50% of the rat liver mitochondrial low K_m ALDH (IC_{50}) *in vitro* after a 60 min incubation was calculated to be 0.75 μM (Fig. 2), whereas 200 μM DETC-Me, the presumed immediate precursor of the sulfoxide, only inhibited rat liver mitochondrial low K_m

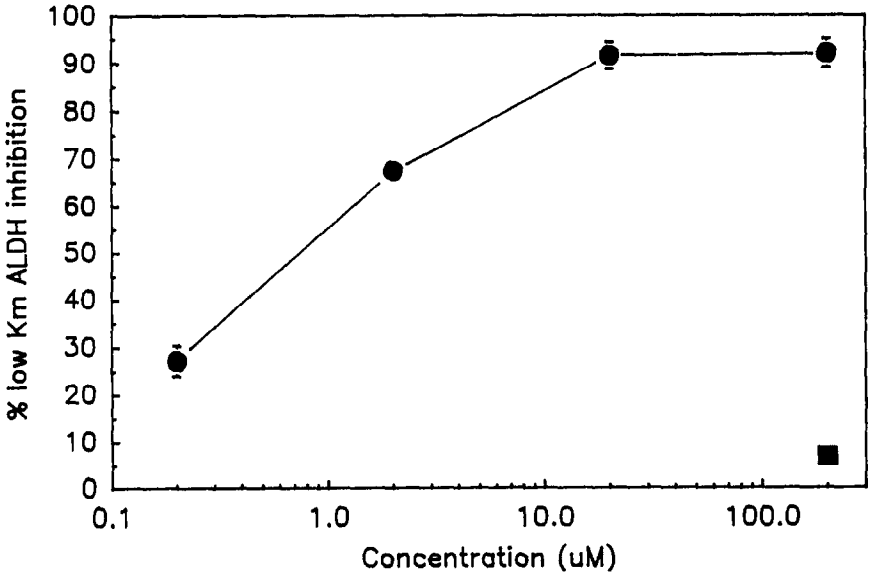

Fig. 2. *In vitro* inhibition of rat liver mitochondrial low K_m ALDH by DETC-MeSO. Isolated rat liver mitochondria were incubated with 0.2, 2.0, 20, or 200 μM DETC-MeSO (●) or 200 μM *S*-methyl *N,N*-diethylthiolcarbamate (DETC-Me) (■). After incubation for 1 hr, mitochondria were isolated and low K_m ALDH activity was determined. Results (mean ± SEM, N = 4) are expressed as percent low K_m ALDH inhibition compared to control incubations (vehicle only). Controls = 9.1 ± 0.21 nmol NADH/min/mg protein.

ALDH 5%. The i.p. dose of DETC-MeSO which inhibited rat liver mitochondrial low K_m ALDH 50% (ID_{50}) *in vivo* was determined to be 3.5 mg/kg (Fig. 3). For comparative purposes, the ID_{50} values for disulfiram, DDTC, DDTC-Me and DETC-Me also administered 8 hr before rat liver mitochondrial low K_m ALDH determination were 56.2, 15.5, 15.5, and 6.5 mg/kg, respectively [5]. Thus, as disulfiram is metabolized, the several

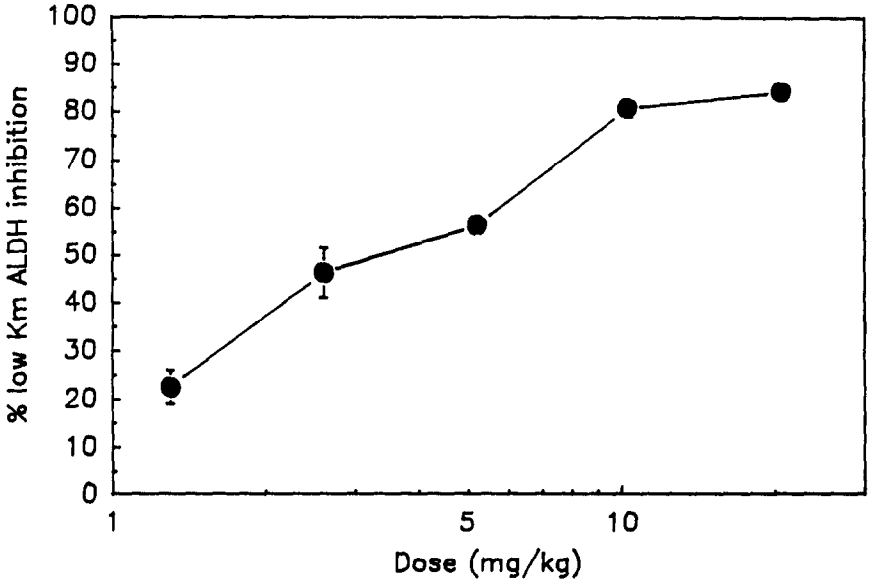

Fig. 3. *In vivo* inhibition of rat liver mitochondrial low K_m ALDH by DETC-MeSO. Rats were treated with 1.3, 2.6, 5.2, 10.3 and 20.6 mg/kg DETC-MeSO i.p. After 8 hr, the rats were killed, livers were removed and rat liver mitochondrial low K_m ALDH activity was determined. Results (mean ± SEM, $N = 4$) are expressed as percent low K_m ALDH inhibition compared to control incubations (vehicle only). Where the SEM is not shown, the SEM is on the data point. Controls = 25.7 ± 1.24 nmol NADH/min/mg protein.

sequential intermediates become more potent as the active chemical species is approached. Because maximal rat liver mitochondrial low K_m ALDH inhibition by disulfiram and its metabolites occurs between 2 and 8 hr after drug treatment [3], similar ID_{50} values for DDTC and DDTC-Me would be expected since DDTC is rapidly methylated to DDTC-Me [9,10]. To confirm the formation of DETC-MeSO *in vivo*, four rats were treated with disulfiram (75 mg/kg i.p.) and killed 4 hr later; the concentration (mean ± SEM) of plasma DETC-MeSO was determined to be 116 ± 15 ng/mL. Furthermore, in preliminary experiments, DETC-MeSO also was found in plasma of rats treated with the disulfiram metabolites DDTC, DDTC-Me, and DETC-Me. Thus, after the administration of either disulfiram or its metabolites to rats, rat liver mitochondrial low K_m ALDH was always inhibited and, in addition, DETC-MeSO was found in plasma. Rats also were treated with 10.3 mg/kg i.p. of DETC-MeSO, challenged with ethanol (20%, w/v) 8 hr later, and killed 30 min after ethanol. No acetaldehyde was detected in control rats, whereas in DETC-MeSO-treated rats challenged with ethanol the concentration (mean ± SEM) of plasma acetaldehyde was 433 ± 56 μM. Acetaldehyde was determined as described previously [3].

Several pieces of evidence suggest that DETC-MeSO may be the metabolite to which disulfiram must be bioactivated *in vivo* in order for liver ALDH to be inhibited. These include dose-response data *in vivo* which show that disulfiram is less potent than DDTC < DDTC-Me < DETC-Me < DETC-MeSO [5] (Fig. 3). Also, DETC-MeSO is a potent inhibitor both *in vitro* and *in vivo*. Although disulfiram is also an effective rat liver mitochondrial low K_m ALDH inhibitor both *in vitro* and *in vivo*, the administration of a P450 inhibitor to rats prior to disulfiram administration blocks the ALDH inhibitory action of disulfiram [7]. While it is not yet clear at what stage P450 is important, data to date indicate that P450 plays an important role in the conversion of DETC-Me to DETC-MeSO (unpublished results). For example, we have found that inhibition of P450 in rats not only blocked the inhibition of rat liver mitochondrial low K_m ALDH by DETC-Me, but also no DETC-MeSO was detected in plasma. Furthermore, the administration of a P450 inhibitor

prior to the administration of DETC-MeSO did not block the inhibition of rat liver mitochondrial low K_m ALDH.

In summary, these data provide the first evidence that DETC-MeSO is a natural metabolite of disulfiram, and a potent inhibitor of rat liver mitochondrial low K_m ALDH both *in vitro* and *in vivo*. It is therefore proposed that, based upon evidence to date, DETC-MeSO appears to be the chemical species to which disulfiram must be bioactivated, and is the metabolite most likely responsible for disulfiram's inhibition of rat liver mitochondrial low K_m ALDH *in vivo*. Characterization of the properties of DETC-MeSO as the metabolite responsible for disulfiram's action as an ALDH inhibitor is presently in the process of being completed.

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