Biochemical Pharmacology, Vol. 38, No. 3, pp. 413–421, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

COMPARATIVE ASPECTS OF DISULFIRAM AND ITS METABOLITES IN THE DISULFIRAM–ETHANOL REACTION IN THE RAT

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(*Received* 20 *April* 1988; *accepted* 27 *June* 1988)

Abstract—Diethyldithiocarbamate-methyl ester (DDTC-Me), a metabolite of disulfiram, has been shown recently to produce a disulfiram–ethanol reaction (DER). Studies were carried out to compare the ethanol-sensitizing properties of DDTC-Me with those of disulfiram and diethyldithiocarbamate (DDTC) in the rat. All three drugs inhibited liver mitochondrial low K_m aldehyde dehydrogenase (ALDH) *in vivo*, with maximal ALDH inhibition occurring 8 hr after drug administration. The onset of ALDH inhibition was most rapid after DDTC-Me administration. ALDH was inhibited approximately 50% 0.5 hr after DDTC-Me, whereas ALDH was inhibited only 5 and 10%, respectively, after disulfiram and DDTC. Not until 8 hr after drug treatment was ALDH inhibition the same for disulfiram, DDTC and DDTC-Me. The degree of ALDH inhibition from 8 to 172 hr after dosing was the same for all three drugs. An ethanol (1 g/kg, 20% v/v) challenge administered to rats treated with disulfiram (75 mg/kg), DDTC (114 mg/kg), or DDTC-Me (41.2 mg/kg) for 8 hr produced similar blood acetaldehyde/ethanol concentration–time profiles. In addition, all three agents produced a DER (hypotension, tachycardia). No DER occurred if ethanol was administered more than 24 hr after drug pretreatment. The hypotension associated with the DER correlated with the increased blood acetaldehyde but not blood ethanol. A threshold blood acetaldehyde of 110 μM appeared to be required for hypotension to occur, and this was related to ALDH inhibition of approximately 40%. The tachycardia associated with the DER correlated more with blood ethanol. After DDTC-Me administration, no disulfiram or DDTC could be detected in the plasma. Furthermore, no DDTC-Me was found in the plasma 8 hr after DDTC-Me administration, suggesting that no correlation exists between the DER and plasma concentration of DDTC-Me and most likely disulfiram. These data suggest that the alcohol-sensitizing properties of DDTC-Me are similar to those observed with disulfiram and DDTC. Since DDTC-Me is an active metabolite and more potent than disulfiram and DDTC in producing a DER, disulfiram metabolism is an important consideration in the disulfiram–ethanol reaction.

The most widely accepted mechanism explaining the alcohol-sensitizing properties of disulfiram [disulfiram–ethanol reaction (DER)] is that of low K_m mitochondrial aldehyde dehydrogenase (ALDH) inhibition, and a subsequent increase in acetaldehyde upon ethanol ingestion. Although the role of acetaldehyde in producing the DER is controversial, recent studies in humans suggest that the peak in plasma acetaldehyde may be a major determinant in predicting the severity of hypotension during the DER [1].

In vivo, disulfiram is metabolized to diethyldithiocarbamate (DDTC), diethyldithiocarbamate-methyl ester (DDTC-Me), carbon disulfide, and diethylamine, and these metabolites have been found in biological fluids and tissues. It is generally believed that disulfiram is the chemical species responsible for producing the DER. Disulfiram inhibits ALDH both *in vivo* and *in vitro*, whereas DDTC, an effective *in vivo* inhibitor, is approximately 100-fold less potent as an inhibitor of ALDH *in vitro* [2]. For this reason it has been suggested that DDTC is effective *in vivo* because it is reoxidized to disulfiram [3, 4]. Carbon disulfide is not known to inhibit ALDH, and while diethylamine has been reported to inhibit mitochondrial ALDH *in vitro*, the concentrations of diethylamine used were relatively high [5]. Gessner and Jakubowski [6] first reported that DDTC-Me is formed *in vivo* after the administration of disulfiram. DDTC-Me has been found in plasma and tissue after the administration of disulfiram in many species including rats [6, 7], mice [8], dogs [8], and humans [9, 10]. *In vitro*, DDTC-Me does not inhibit the low K_m ALDH [11]. In more recent *in vivo* studies [12], DDTC-Me was found to inhibit the liver mitochondrial low K_m ALDH in rats. The marked inhibitory action of DDTC-Me on the low K_m ALDH *in vivo* is similar to that of disulfiram, whereas the lack of an effect by DDTC-Me *in vitro* is consistent with that observed with DDTC [2]. In addition, in DDTC-Me-treated rats challenged with ethanol, a DER-like reaction characterized by a drop in mean arterial pressure (MAP) and tachycardia is produced [12].

The focus of the present study was to compare the recently recognized alcohol-sensitizing properties of DDTC-Me with those of disulfiram and DDTC so that the chemical species responsible for producing the DER could be better ascertained. This could provide not only an understanding of the relationship

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between mitochondrial low K_m ALDH inhibition, plasma acetaldehyde and ethanol and their respective role in the DER, but also help in delineating the mechanism of the DER.

METHODS

Drug administration. Disulfiram (Sigma Chemical Co., St. Louis, MO) was recrystallized from 100% ethanol and homogenized in 0.5% methylcellulose in saline. This was then administered in a dose of 75 mg/kg. Diethyldithiocarbamate (trihydrate sodium salt, Sigma Chemical Co.) was dissolved in saline for injection and given in a dose of 114 mg/kg. Diethyldithiocarbamate-methyl ester was synthesized in the laboratory using a modified method of Koch [13] as previously described by Faiman *et al.* [14]. The dose of DDTC was calculated on the basis that one molecule of disulfiram forms two molecules of DDTC. The dose of DDTC-Me was calculated in a similar manner, except that, because of cardiovascular toxicity, one-half of the equivalent dose of disulfiram was used. Ethanol (1 g/kg) was given as a 20% (v/v) solution in saline. All drugs were administered intraperitoneally (i.p.) in the ALDH activity studies, or via an implanted intraperitoneal catheter (i.i.c.) in the DER studies.

Animals. Sprague–Dawley derived female rats (200–300 g) were used throughout the study. The animals were bred from a resident colony maintained in the Animal Care Unit at the University of Kansas. Rats were maintained on a 12-hr light–dark cycle with access to food and water *ad lib.* The night before an experiment, all food was removed.

In vivo *aldehyde dehydrogenase activity.* Rats were treated with disulfiram, DDTC, or DDTC-Me and killed by cervical dislocation. Portions of the liver were removed for enzyme analysis. The liver was homogenized, and the subcellular nuclear, mitochondrial, lysosomal, microsomal and cytosolic fractions were isolated according to the method of Tottmar *et al.* [15]. The low and total ALDH activities were determined, with the high K_m activity calculated by subtracting the low K_m activity from the total activity [15]. In reference cuvettes containing all reaction components except acetaldehyde, no background activity was detected.

Mean arterial pressure and heart rate. Polyethylene tubing (PE-50) was inserted into the femoral artery. The catheter then was passed subcutaneously over the leg and along the spine, and exited at the midscapula region of the back. The rats were allowed to recover from surgery for 1 day before use. All measurements were performed on conscious, unrestrained rats. This procedure has been described previously in detail [12].

Blood ethanol and acetaldehyde. Blood acetaldehyde and ethanol were measured simultaneously using the head-space chromatographic method of Eriksson *et al.* [16], and modified for hemolyzed rat blood. Rats were anesthetized with pentobarbital (35 mg/kg, i.p.), and blood was drawn from the abdominal aorta into a heparinized syringe. In a 30-ml serum bottle, 0.5 ml of whole blood was mixed with 7.0 ml of distilled water to hemolyze the blood. The serum bottle was sealed with a rubber stopper and placed into a 65° water bath for 5 min. A Hamilton gas-tight syringe was used to remove 1.0 ml of head-space gas which was then injected into the gas chromatograph (Shimadzu GC-9A, flame ionization detector). The stainless steel column was a 60/80 Carbopack B/5% Carbowax 20M, 6 ft × 1/8 in (Supelco, Inc.). Temperatures: column oven, 85°; injector, 120°. Gas flow rates: hydrogen, 35 ml/min; air, 450 ml/min; carrier gas (nitrogen), 30 ml/min. Retention times: acetaldehyde, 0.9 min; ethanol, 2.2 min. Samples were quantitated from daily standard curves prepared from freshly distilled acetaldehyde and ethanol. The sensitivity for acetaldehyde was 5 μM. No spontaneously formed acetaldehyde could be detected in blood to which known amounts of ethanol were added.

Plasma DDTC-Me. Plasma DDTC-Me was measured using the HPLC procedure previously described by Jensen and Faiman [17].

Statistical analysis. Results were analyzed using a one-way analysis of variance. If significance was detected, a Student–Newman–Keuls *á posteriori* test was used to determine differences between group means.

RESULTS

Enzyme activities for low and high K_m ALDH in the nuclear, mitochondrial, lysosomal, cytosolic and microsomal fractions in control, and disulfiram, DDTC and DDTC-Me treated rats are shown in Table 1. In the rat liver, the greatest concentration of the low K_m ALDH was found in the mitochondrial fraction. This is consistent with the findings of others [15]. The degree of inhibition of the low K_m mitochondrial ALDH with respect to time after a single dose of disulfiram, DDTC, or DDTC-Me is shown in Fig. 1. The degree of ALDH inhibition for these three drugs was identical between 8 and 168 hr after dosing (Fig. 1b). However, during the first 8 hr after drug administration (Fig. 1a), low K_m ALDH was inhibited more rapidly with DDTC-Me than by disulfiram or DDTC. For example, maximal low K_m ALDH inhibition occurred 2 hr after DDTC-Me administration, 4 hr after disulfiram administration, and 8 hr after DDTC dosing. No inhibition of the high K_m mitochondrial ALDH by disulfiram, DDTC or DDTC-Me was found at any of the time periods (data not shown).

The degree of low K_m ALDH inhibition was similar for disulfiram, DDTC and DDTC-Me 8 hr after dosing, and for this reason, an 8-hr pretreatment time was selected for the following studies. An ethanol (1 g/kg, i.i.c.) challenge given 8 hr after disulfiram and DDTC-Me administration to rats produced a rapid decrease in MAP, which began to return to normal within 3–4 hr. Disulfiram and DDTC-Me decreased MAP to a similar degree and for a similar duration, whereas the hypotensive effect associated with DDTC was less marked. The increase in tachycardia at various times after the ethanol challenge was greater only in DDTC-Me and DDTC-pretreated rats when compared to ethanol alone (Fig. 2).

Blood acetaldehyde in rats given disulfiram, DDTC or DDTC-Me 8 hr before an ethanol chal-

Table 1. Effects of disulfiram, DDTC and DDTC-Me on low and high K_m ALDH activity in rat liver subcellular fractions

	Nuclear	Mitochondrial	Lysosomal	Cytosolic	Microsomal
			High K_m ALDH		
			(nmol NADH formed/min/mg protein)		
Control	11.9 ± 1.1	12.8 ± 1.1	20.2 ± 2.2	2.5 ± 0.5	28.6 ± 2.9
Disulfiram	11.1 ± 1.6	14.7 ± 1.2	22.8 ± 1.5	1.3 ± 0.1*	25.6 ± 0.5
DDTC	12.5 ± 0.9	15.1 ± 0.7	23.4 ± 2.0	1.1 ± 0.2*	30.3 ± 1.6
DDTC-Me	12.2 ± 0.3	16.1 ± 0.8	24.4 ± 2.3	1.1 ± 0.1*	28.9 ± 2.3
			Low K_m ALDH		
			(nmol NADH formed/min/mg protein)		
Control	12.1 ± 0.7	19.4 ± 1.7	3.4 ± 0.3	1.1 ± 0.03	2.2 ± 0.2
Disulfiram	3.8 ± 0.7*	7.3 ± 0.2*	2.5 ± 0.4*	0.6 ± 0.09*	2.0 ± 0.2
DDTC	5.1 ± 0.9*	7.5 ± 1.4*	2.5 ± 0.3*	0.6 ± 0.1*	2.2 ± 0.4
DDTC-Me	3.9 ± 0.4*	7.6 ± 0.9*	2.8 ± 0.3*	0.5 ± 0.06*	2.0 ± 0.1

Disulfiram (75 mg/kg), DDTC (114 mg/kg) and DDTC-Me (41.2 mg/kg) were administered i.p. Eight hours later, the rats were killed and the livers were removed for ALDH determination. Data represent the mean ± SE for four rats.

* $P < 0.05$, when compared to untreated control rats.

lenge peaked 30 min post-ethanol (Fig. 3). DDTC-Me pretreatment produced a 30% greater accumulation of acetaldehyde when compared to disulfiram and DDTC 30 min after ethanol administration. At 1, 3 and 6 hr post-ethanol no differences in blood acetaldehyde were found among all three drug treatment groups. No acetaldehyde could be detected in rats receiving ethanol alone. The blood ethanol concentration–time profile in rats pretreated with disulfiram, DDTC or DDTC-Me 8 hr before ethanol administration is shown in Fig. 4. Blood ethanol peaked 0.5 hr after an ethanol challenge, remained constant for up to 1 hr and then fell sharply and was barely detectable after 6 hr. The blood ethanol profiles for the drug-pretreated groups were not different from the vehicle-treated ethanol-challenged group, except at the 1 hr post-ethanol time point, where the DDTC-Me-pretreated group had a significantly higher blood ethanol concentration when compared to the other groups.

Studies were carried out to determine when the decrease in MAP (Fig. 5) or the increase in heart rate (Fig. 6) was lost in drug-treated (disulfiram, DDTC or DDTC-Me) rats challenged with ethanol. Disulfiram, DDTC and DDTC-Me given 0.5 to 24 hr before an ethanol challenge produced a significant drop in MAP, with the drop in MAP more variable after DDTC administration. The decrease in MAP 48, 72, 120 and 168 hr after drug administration was not different from that of the ethanol control. Ethanol alone produced an increase in heart rate of approximately 40 beats/min. Except for some

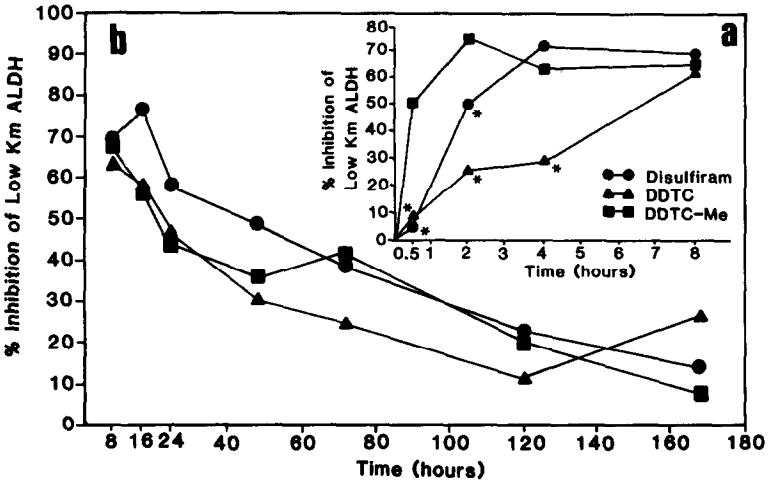

Fig. 1. Panels a and b: Relationship between the time after a single dose of disulfiram, DDTC, or DDTC-Me and inhibition of low K_m ALDH. Disulfiram (75 mg/kg), DDTC (114 mg/kg) and DDTC-Me (41.2 mg/kg) were given i.p. Data are given as percent inhibition and represent the mean of four rats. Key: (*) $P < 0.05$, compared to DDTC-Me.

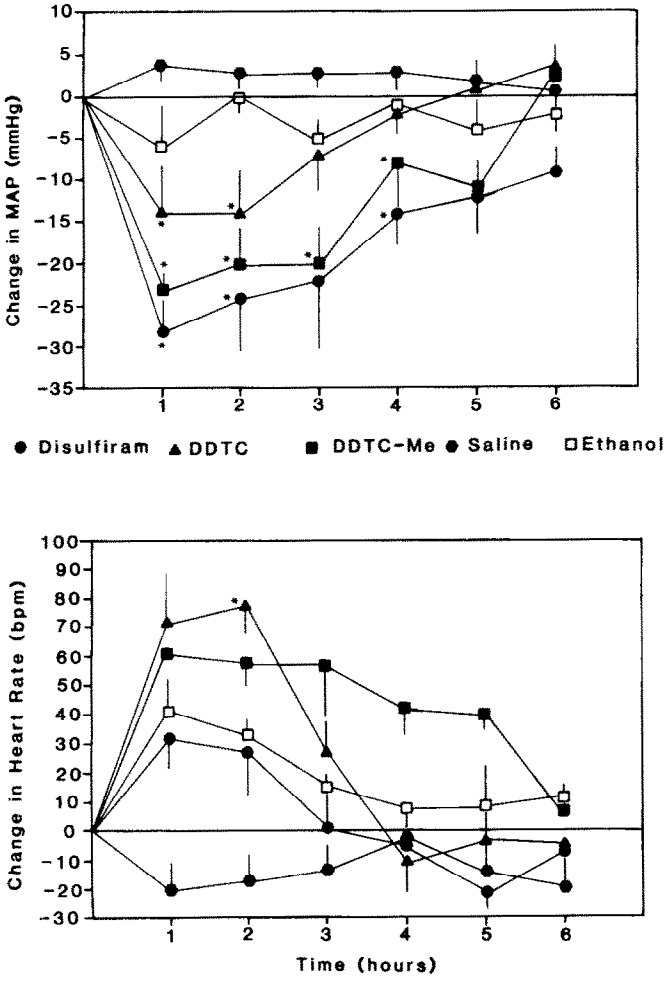

Fig. 2. Hypotension and tachycardia after ethanol challenge to rats pretreated with disulfiram, DDTC or DDTC-Me. Disulfiram (75 mg/kg), DDTC (114 mg/kg), DDTC-Me (41.2 mg/kg) and saline were given i.i.c. 8 hr before an ethanol (1 g/kg, 20% v/v, i.i.c.) challenge. Corn oil vehicle was given 8 hr before the ethanol challenge (□). Results are means ± SE of at least four rats. Key: (*) $P < 0.05$, compared to untreated controls.

selected time periods (16-hr pretreatment with all drugs, 2-hr DDTC-Me and 72-hr DDTC pretreatment), the drug-treated and ethanol-challenged groups did not exhibit any greater degree of tachycardia than the group receiving ethanol alone (Fig. 6).

Blood acetaldehyde (Fig. 7) and ethanol (Fig. 8) were determined in disulfiram-, DDTC- and DDTC-Me-treated rats challenged with ethanol 2, 8, 24, 48, 72 and 120 hr after drug administration. DDTC-Me administered 2 hr before an ethanol challenge produced a larger increase in blood acetaldehyde when compared to the disulfiram and DDTC groups. This was consistent with the degree of low K_m ALDH inhibition observed 2 hr after drug pretreatment, that is DDTC-ME > disulfiram > DDTC (Fig. 1a). Eight and 24 hr after pretreatment with disulfiram, DDTC or DDTC-Me, a significant decrease in MAP was detected (Fig. 5) which corresponded with the increased blood acetaldehyde (Fig. 7). Seventy-two and 120 hr after drug administration, no statistically significant decreases in MAP during the DER were found (Fig. 5). This was consistent with the low concentration of acetaldehyde detected in blood at 72 hr, whereas no blood acetaldehyde was found if ethanol was given 120 hr after drug dosing (Fig. 7). Blood ethanol concentrations measured during the DER over the entire drug pretreatment time profile are shown in Fig. 8. No differences between the drug-treated groups and the controls (ethanol only) were found. From these results it appears that the initiation and extent of the hypotension associated with the DER is dependent upon blood acetaldehyde.

The decrease in mean arterial pressure correlated with both ALDH inhibition and blood acetaldehyde, but not with either blood ethanol or heart rate. Acetaldehyde accumulation, as expected, was a function of the degree of low K_m ALDH inhibition. In general, the increase in heart rate observed during the DER was not different than the tachycardia observed in rats treated with ethanol alone.

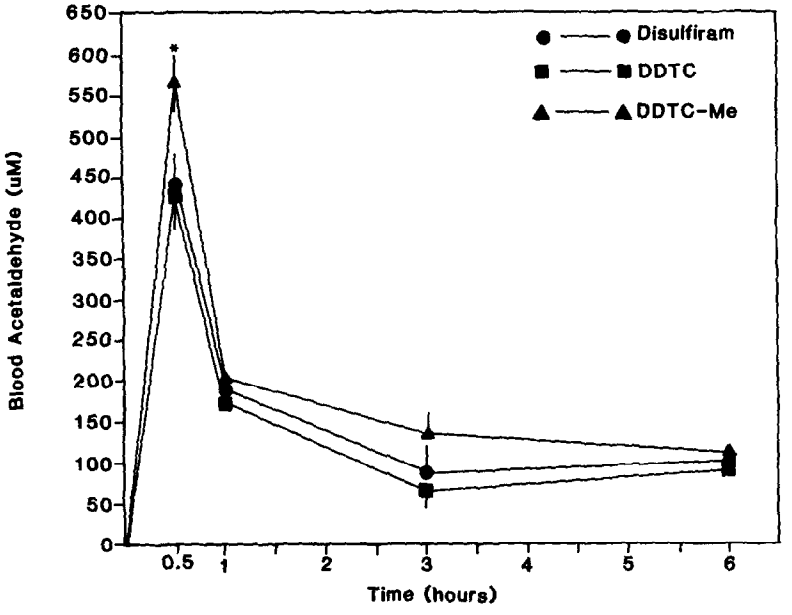

Fig. 3. Blood acetaldehyde concentration–time profile after an ethanol challenge to rats pretreated with disulfiram, DDTC or DDTC-Me. Disulfiram (75 mg/kg), DDTC (114 mg/kg) and DDTC-Me (41.2 mg/kg) were given i.p. 8 hr before an ethanol (1 g/kg, 20% v/v, i.p.) challenge, and blood acetaldehyde was determined. No acetaldehyde could be detected in rats receiving ethanol alone. Results are means ± SE of four rats. Key: (*) $P < 0.05$, compared to disulfiram and DDTC.

DISCUSSION

Disulfiram inhibits the rat liver mitochondrial low K_m ALDH both *in vivo* and *in vitro*, whereas DDTC, although exhibiting limited inhibitory activity *in vitro*, is an effective inhibitor *in vivo*. It has therefore been suggested that ALDH inhibition by DDTC *in vivo* is the result of its reoxidation to the disulfide [4, 18, 19]. Although Faiman *et al.* [8] found [^{35}S]disulfiram in plasma after i.v. [^{35}S]DDTC administration to a dog, the amount of [^{35}S]disulfiram detected was extremely small and could have been due to disulfiram contamination of the administered [^{35}S]DDTC. The recent finding that DDTC-Me is a

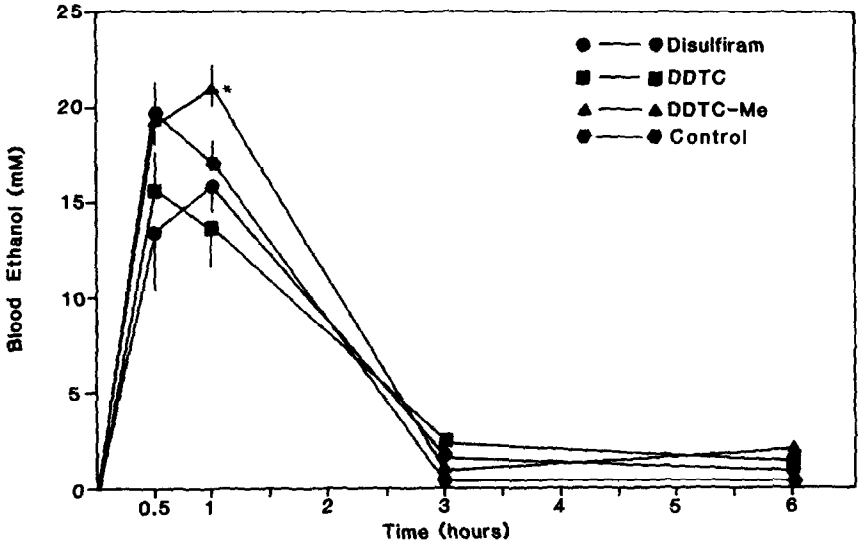

Fig. 4. Blood ethanol concentration–time profile after ethanol challenge to rats pretreated with disulfiram, DDTC or DDTC-Me. Disulfiram (75 mg/kg), DDTC (114 mg/kg) and DDTC-Me (41.2 mg/kg) were given i.p. 8 hr before an ethanol (1 g/kg, 20% v/v, i.p.) challenge. Control represents vehicle-treated ethanol-challenged rats. Results are means ± SE of four rats. Key: (*) $P < 0.05$, compared to other groups.

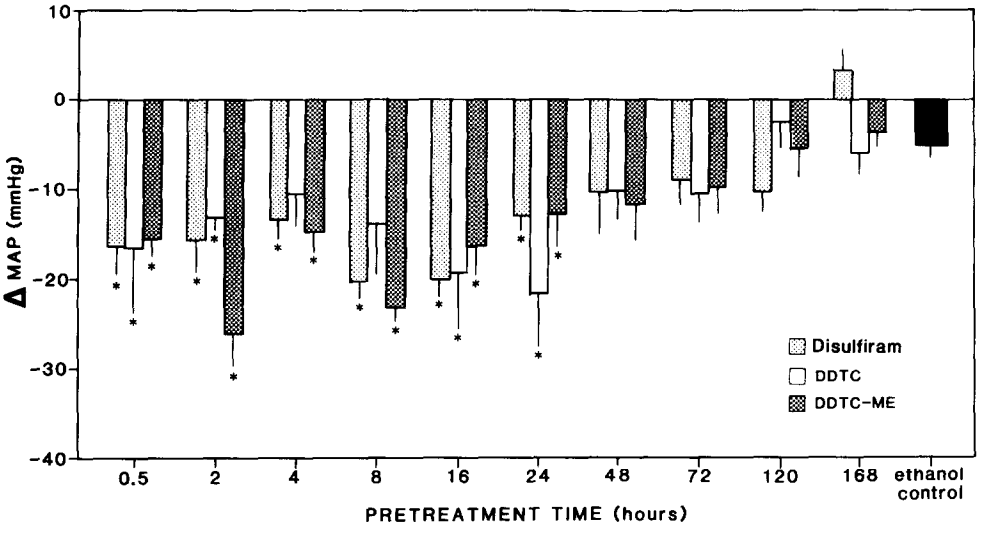

Fig. 5. Relationship between mean arterial pressure (MAP) and time after treatment with a single dose of disulfiram, DDTC, or DDTC-Me and an ethanol challenge. Disulfiram (75 mg/kg), DDTC (114 mg/kg) and DDTC-Me (41.2 mg/kg) were given i.p. 0.5 to 168 hr before ethanol (1 g/kg, 20% v/v, i.p.), and the mean arterial pressure (MAP) was determined 1 hr after ethanol administration. Results are means ± SE of four to eight rats for drug-treated, and for ethanol control, N = 14. Key: (*) $P < 0.05$ compared to ethanol control.

potent inhibitor of low K_m ALDH *in vivo* [12] could explain the low K_m ALDH inhibition *in vivo* by DDTC, rather than its reoxidation to disulfiram. The lack of ALDH inhibition by DDTC *in vitro* but effectiveness *in vivo* further suggests that a metabolite of DDTC may be the active chemical species causing ALDH inhibition.

Maximal low K_m ALDH inhibition occurred approximately 8 hr after the administration of disulfiram, DDTC, or DDTC-Me, with ALDH activity

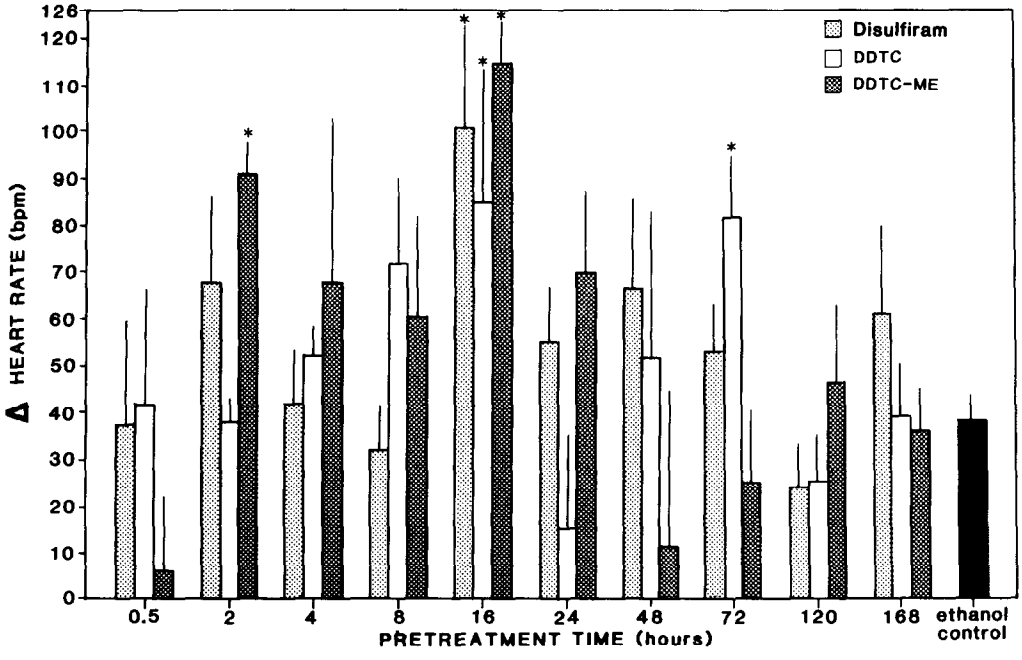

Fig. 6. Relationship between change in heart rate and time after treatment with a single dose of disulfiram, DDTC, or DDTC-Me and an ethanol challenge. Disulfiram (75 mg/kg), DDTC (114 mg/kg) and DDTC-Me (41.2 mg/kg) were given i.p. 0.5 to 168 hr before an ethanol challenge, and the heart rate was determined 1 hr after ethanol (1 g/kg, 20% v/v, i.p.) administration. Results are means ± SE of four to eight rats for drug-treated, and for ethanol control, N = 14. Key: (*) $P < 0.05$ compared to ethanol control.

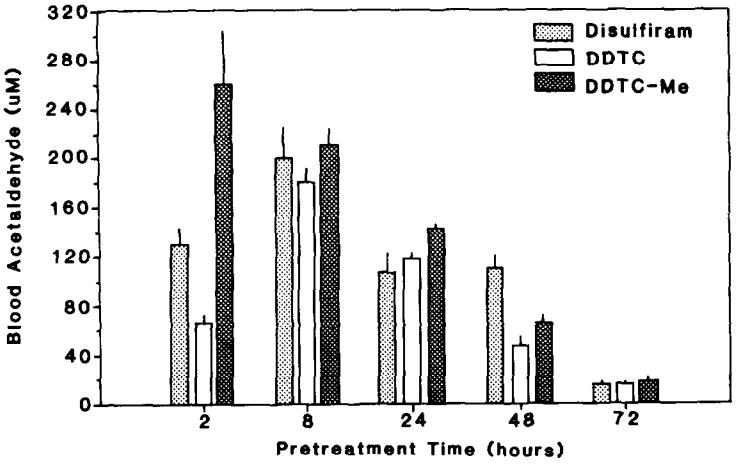

Fig. 7. Blood acetaldehyde during the DER. Disulfiram (75 mg/kg), DDTC (114 mg/kg) and DDTC-Me (41.2 mg/kg) were given i.p. 2–72 hr before an ethanol (1 g/kg, 20% v/v, i.p.) challenge. Blood acetaldehyde was determined 1 hr after ethanol administration. No acetaldehyde was detected in non-drug pretreated rats. Results are means ± SE of four rats.

gradually returning to normal after 168 hr (7 days) (Fig. 1). These data are similar to the findings of Deitrich and Erwin [4] who reported that maximal inhibition of ALDH occurs approximately 12 hr after dosing with disulfiram and DDTC, returning to normal after 140 hr. Of greater interest, however, was the rate at which the low K_m ALDH was inhibited (Fig. 1b). The most rapid inhibition occurred after DDTC-Me. Since disulfiram and DDTC first require bioactivation to DDTC-Me, this would account for the initial time lag of ALDH inhibition for disulfiram and DDTC. Not only was inhibition of ALDH rapid after DDTC-Me, but the DDTC-Me dose-equivalent used was one-half that of disulfiram. Furthermore, in previous studies, even when DDTC-Me was given in a dose of 1.7 mg/kg i.p., the low K_m ALDH was inhibited 10% [12], whereas the dose of disulfiram required to obtain this degree of inhibition was approximately 5–10 mg/kg i.p. (unpublished results). Another reason for the slower rate of ALDH inhibition by DDTC is probably due to the hydrophilic property of DDTC and slow distribution

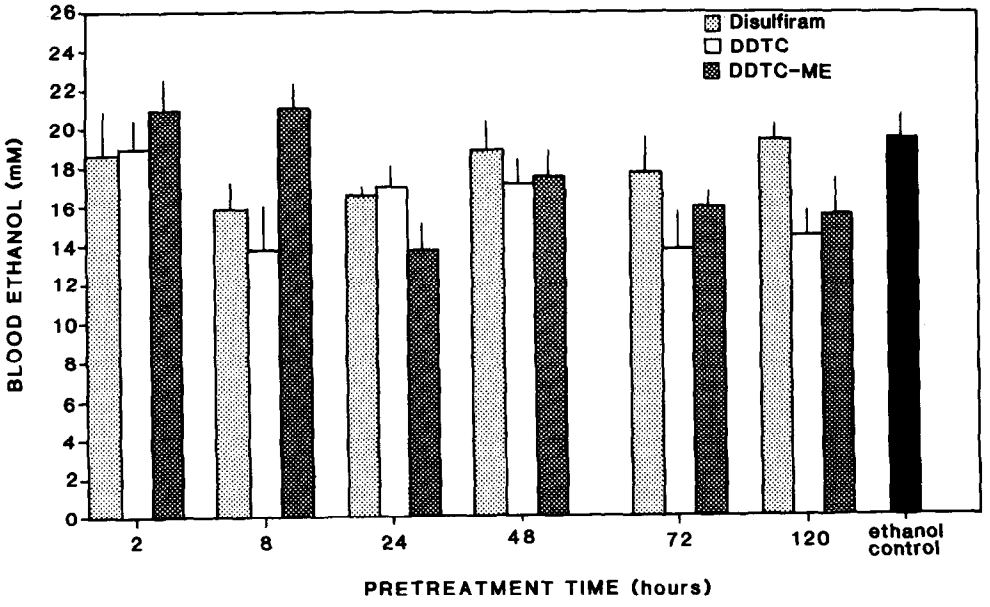

Fig. 8. Blood ethanol concentration during the DER. Disulfiram (75 mg/kg), DDTC (114 mg/kg) and DDTC-Me (41.2 mg/kg) were given i.p. 2–72 hr before an ethanol (1 g/kg, 20% v/v, i.p.) challenge, and blood ethanol was determined 1 hr after ethanol administration. Results are means ± SE of four rats.

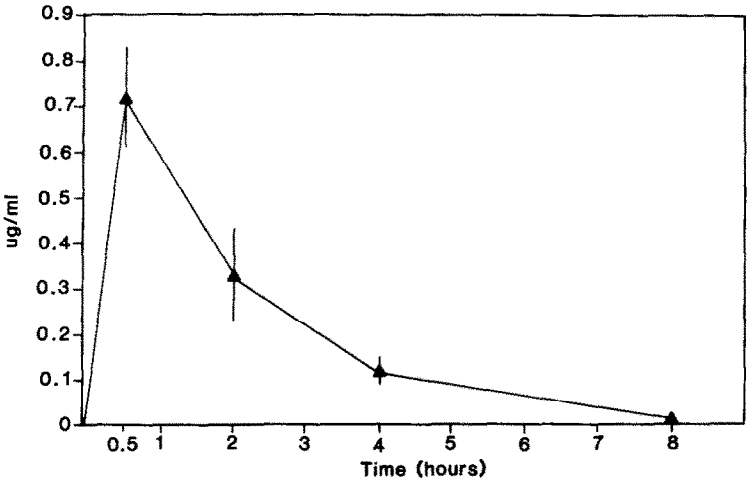

Fig. 9. Plasma DDTC-Me concentration–time profile. DDTC-Me (41.2 mg/kg) was administered as a single i.p. dose. Data are means ± SE of four rats.

compared to the lipophilic characteristics of disulfiram.

The primary mechanism proposed for the alcohol-sensitizing properties of disulfiram is that of low K_m ALDH inhibition and the subsequent increase in plasma acetaldehyde after ethanol [20]. In rats treated with disulfiram, DDTC, or DDTC-Me and then challenged with ethanol within 24 hr, a significant decrease in MAP was observed. When these drugs were given 48 hr or longer before an ethanol challenge, no statistically significant decreases in MAP during the DER were found (Fig. 5). Iber and Chowdhury [21] similarly found no DER in human volunteers with normal liver function and given ethanol more than 24 hr after disulfiram administration. Thus, animal and human data appear to be consistent with each other. The relationship between the decrease in MAP and plasma acetaldehyde during the DER also was examined. Plasma acetaldehyde in rats challenged with ethanol 72 hr after disulfiram, DDTC, or DDTC-Me was lower than that found in rats challenged with ethanol 24 hr after these drug treatments (Fig. 7). The decrease in MAP during the first 24 hr but not at later times (Fig. 5) appears to correlate with plasma acetaldehyde (Fig. 7). No acetaldehyde was detected in rats treated with ethanol alone. Significant decreases in MAP were generally noted when blood acetaldehyde concentrations were above 110 μM. Since the blood ethanol concentrations during the DER were constant regardless of drug pretreatment time (Fig. 8), the only factor which correlated with the hypotensive response during the DER was blood acetaldehyde. It is therefore proposed that the intensity of hypotension during the DER is dependent upon a critical degree of low K_m ALDH inhibition, approximately 40%, which is necessary to reach a "threshold" concentration of blood acetaldehyde (110 μM) after an ethanol challenge to produce the hypotension. In contrast, the tachycardia associated with the DER appears to be ethanol related (Fig. 6), with only a small additive effect attributable to acetaldehyde. This is consistent with the findings of Hellström and Tottmar [22], in which ethanol (1 g/kg, i.p.) caused slight tachycardia while the MAP did not change from control levels. These data provide additional evidence that the symptoms, collectively referred to as the DER and their intensities, are due to the interaction of ethanol, acetaldehyde, dose of ethanol and disulfiram, and the disulfiram pretreatment time.

In previous studies in rats [14], neither DDTC nor disulfiram were found in plasma after [^{35}S]DDTC-Me administration. In light of the small dose of DDTC-Me required to produce a disulfiram-like ethanol reaction, the rapid onset of low K_m ALDH inhibition, and the knowledge that DDTC-Me *in vivo* does not form DDTC or disulfiram, it is proposed that either DDTC-Me or some other metabolite may be the active chemical species responsible for the alcohol-sensitizing effect of disulfiram. Furthermore, since DDTC-Me in plasma is virtually nonexistent 8 hr after DDTC-Me administration (Fig. 9), a time at which maximal ALDH inhibition occurred (Fig. 1), the alcohol-sensitizing properties of DDTC-Me are not related to the plasma drug concentration of DDTC-Me. This also suggests that, in all probability, no correlation exists between plasma disulfiram and the DER.

Disulfiram is rapidly reduced in both humans and animals to DDTC. The DDTC formed can either be degraded nonenzymatically producing diethylamine and carbon disulfide, glucuronidated and excreted by the kidney, or methylated forming DDTC-Me with *S*-adenosyl methionine transmethylase catalyzing the reaction [23]. Although there may be other metabolites, these have yet to be identified and quantitated. Previously this metabolic scheme has not been seriously considered in the DER, since disulfiram always has been assumed to be the chemical species responsible for the DER. The present studies suggest that this is not the case since DDTC-Me can not only produce a DER, but does so at a fraction of the dose of disulfiram (Fig. 2). The importance of DDTC-Me formation may possibly explain why many patients receiving disulfiram do not experience a DER when challenged with ethanol

[24]. Finally, the data from this study suggest that disulfiram, DDTC and DDTC-Me all produce similar effects on low K_m mitochondrial ALDH inhibition, blood acetaldehyde, blood ethanol, hypotension and tachycardia. All three of these agents are capable of producing the DER, with DDTC-Me being the most potent. It is therefore proposed that DDTC-Me or some other metabolite derived therefrom and not disulfiram, is the chemical species responsible for producing the DER.

Acknowledgements—This research was supported by Grant AA03577 from the National Institute on Alcohol Abuse and Alcoholism.

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