Biochemical Pharmacology, Vol. 38, No. 7, pp. 1053–1059, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

DIETHYLTHIOCARBAMIC ACID METHYL ESTER

A POTENT INHIBITOR OF ALDEHYDE DEHYDROGENASE FOUND IN RATS TREATED WITH DISULFIRAM OR DIETHYLDITHIOCARBAMIC ACID METHYL ESTER

BENNY JOHANSSON, ERLING N. PETERSEN* and ELISABETH ARNOLD*

Toxicology Section, Department of Clinical Chemistry, General Hospital, S-214 01 Malmö, Sweden; and * A/S Dumex, Copenhagen, Denmark

(*Received* 31 *May* 1988; *accepted* 28 *September* 1988)

Abstract—Rats were treated with disulfiram (Antabuse®, DSF) or its metabolite diethyldithiocarbamic acid methyl ester (Me-DDC) and challenged with ethanol. The blood pressure response to ethanol was followed and blood was analyzed for DSF, Me-DDC and diethyldithiocarbamic acid (DDC). The rat liver aldehyde dehydrogenase (ALDH) isozyme activities were measured 2 hr after the ethanol challenge.

Both treatments produced a significant fall in the blood pressure when challenged with ethanol, probably caused by a marked decrease in hepatocyte low K_m and high K_m activities.

The mean plasma concentration ranges of Me-DDC and DDC were found to be 49–1241 nmol/l and 182–841 nmol/l, respectively, whereas DSF was undetectable.

In addition, it was found that inactivation of hepatocyte low K_m ALDH activity was dependent on preoxidation of Me-DDC by the microsomal cytochrome P-450 mixed function oxidases. Me-DDC was found to be oxidized under aerobic conditions in the presence of NADP to form diethylthiocarbamic acid methyl ester (Me-DTC). The structure was confirmed from its MS/EI fragmentation spectrum. Me-DTC was found to be a potent inhibitor of low K_m ALDH when added to rat liver homogenate. The compound was also identified as a metabolite in rat blood collected from the DSF and Me-DDC treated rats, and in blood from human alcoholics on DSF treatment.

Me-DTC appears to be more selective for the low K_m isozymes whereas the opposite seems to be the case for the hydrolytic product, DTC.

Disulfiram (DSF) is a drug used in the treatment of alcoholism [1]. Despite more than 35 years of clinical experience, its basic mechanism of action remains unknown, whether it is due to DSF or its metabolites. It has been suggested that the rapidly formed monomer of DSF, DDC [2], is implicated in the *in vivo* inactivation of the DSF sensitive hepatocyte low K_m ALDH [3, 4], by covalent interaction with a cysteine residue located close to the active site of the isozyme [5, 6], or by formation of an intramolecular disulphide link via a DDC adduct [7]. However, it has been clearly demonstrated [7, 8] that, unlike the *in vivo* inactivated enzyme, the enzyme inhibited *in vitro* is reversed when treated with various reducing agents, e.g. glutathione, 2-mercaptoethanol and dithiothreitol.

A significant relationship between measurable plasma concentrations of Me-DDC, a completely inhibited DSF-susceptible ALDH isozyme in erythrocytes, and a decrease in the diastolic blood pressure has been demonstrated after ethanol challenge in human volunteers treated with increasing doses of DSF [9].

These findings prompted an investigation of other possible inhibition routes of ALDH, beyond those caused by intact DSF, with special attention to one of its metabolites. The aim of this investigation was to elucidate whether the Me-DDC or any other metabolite is responsible for any of the effects observed in rats and humans receiving DSF.

MATERIALS AND METHODS

Me-DDC, diethyldithiocarbamic acid ethyl ester (Et-DDC) and diethyldithiocarbamic acid propyl ester (prop-DDC) were kindly supplied by A/S Dumex (Copenhagen, Denmark). NAD and NADP were obtained from Boehringer Mannheim (Mannheim, F.R.G.), sodium deoxycholate, sucrose, diethylamine and acetaldehyde from E. Merck (Darmstadt, F.R.G.), Pyrazole from Janssen (Beerse, Belgium), magnesium chloride, ethyl iodide and methyl iodide from BDH Chemicals (Poole, U.K.), glutathione and 2-mercaptoethanol from Sigma Chemical Co. (St. Louis, MO) and carbonyl sulphide from Alfax AB (Malmö, Sweden). All other chemicals were of analytical grade and obtained from E. Merck.

Diethylthiocarbamic acid (DTC) was synthesized by bubbling pure COS gas through an ethanolic solution (3 ml) containing 1.0 ml of diethylamine at room temperature for 1.5 hr. The DTC formed was methylated to form Me-DTC, by addition of a two molar excess of methyl iodide at 37° for 1 hr. The internal standard diethylthiocarbamic acid ethyl ester (Et-DTC) was synthesized in the same way, except that methyl iodide was exchanged for ethyl iodide in the alkylation step. After evaporation and

repeated washing with water, the compounds were dried over sodium sulphate and the purities and structures were confirmed from their UV spectrum, gas chromatographic characteristics and MS/EI fragmentation patterns, respectively.

The GC/MS analysis was performed with a JEOL DMS 300 mass spectrometer interfaced to a Varian 3700 gas chromatograph. The MS conditions were as previously described [11]. Chromatography was performed on a 2 m × 2 mm i.d. glass column packed with SE 30, 80–100 mesh, operating at a temperature of 120°, with a helium carrier gas flow rate of 25 ml/min. The injector temperature was set to 180°.

In the first part of this experiment, four rats (male, Sprague–Dawley, weighing 400 g) each received a 100 mg/kg i.p. and 30 mg/kg i.p. dose of DSF, 21 hr and 5 hr before sacrifice, respectively. DSF was made up as a suspension in water, with a few drops of the tenside Duphasol-X added. Three hours before sacrifice the rats were anaesthetized with urethane 1.25 g/kg i.p. and the blood pressure in the carotid artery was recorded. The body temperature was kept at 37° by a heating pad. The heart rate was obtained from the ECG. After stabilisation of the blood pressure for about 30 min, ethanol was injected i.p. in a dose of 1 g/kg (10 ml/kg of a 10% v/v solution). The cardiovascular reaction was followed for 2 hr at which time blood was collected and centrifuged at 3000 rpm for 10 min. The liver was removed and stored at −70° together with the plasma until assayed. In the second part, ten rats were divided into two groups of five. The first group was given Me-DDC 60 mg/kg + 30 mg/kg as above. The rats in the second group were each given 60 mg/kg i.p. one hour before the ethanol challenge (3 hr before sacrifice).

Fractionation of fresh homogenate from rat livers was performed as previously described [12]. The total and low K_m ALDH activities in the homogenates were measured spectrophotometrically by following the reduction of NAD at 37° and at 340 nm in 50 μl homogenate aliquots. The reaction mixture contained 50 mmol/l sodium pyrophosphate buffer, pH 9.0, 0.25% (w/v) sodium deoxycholate, 0.5 mmol/l NAD and 0.1 mmol/l pyrazole. The substrate acetaldehyde was added at two concentrations; 5.0 mmol/l (total activity) and 0.025 mmol/l (low K_m activity), respectively [13].

The cytochrome P-450 catalyzed oxidation of Me-DDC to yield Me-DTC in rat hepatocytes was investigated according to the method of Axelrod [14]. The biotransformation of metabolites and inactivation of low K_m and high K_m ALDH activities after addition of Me-DDC to the homogenates were analyzed simultaneously. Five hundred microlitres of the homogenate [12] was incubated in 4 ml septum cap vials (Alltech Assoc. CA) with 5 μmol NAD, 0.1 μmol NADP, 5 μmol $MgCl_2$, 0.6 μmol Me-DDC, and 2.0 ml 0.2 mol/l phosphate buffer, pH 7.4. Incubation was done aerobically or under nitrogen at 37° with gentle shaking for 2 hr. The reaction was terminated by incubation at 4° for 15 min. Two hundred and fifty microlitre aliquots of the homogenate mixture were solubilized with sodium deoxycholate and analyzed for the ALDH activities [13].

The biotransformation of Me-DDC to yield Me-DTC was assessed by an on-line pre-column enrichment HPLC assay [15]. The reaction mixture was extracted with 5 ml of chloroform, containing 80 μmol of the internal standard, propyl-DDC, at ambient temperature for 15 min. The organic phase was separated and evaporated to dryness, and the residue was dissolved in 50 μl of methanol. Five microlitre sample volumes were injected and analyzed at 254 nm (Me-DDC) and 214 nm (Me-DTC), respectively.

The inactivation of ALDH activity by Me-DTC and DTC was studied in 500 μl rat liver homogenates [12] added to 2.0 ml of 0.2 mol/l phosphate buffer, pH 7.4, in three concentrations of Me-DTC, 2760 μmol/l, 276 μmol/l and 27.6 μmol/l, and at a DTC concentration of 34.1 μmol/l, respectively. Samples were incubated at 37° for 1 hr with and without addition of oxidative factors [14], and then analyzed for the ALDH activities.

To elucidate the reversibility of the inactivation of ALDH by Me-DTC, 2-mercaptoethanol or glutathione was added to the reaction mixture at a concentration of 200 μmol/l, which was previously found adequate to restore *in vitro* inactivated ALDH from various species [4, 8, 10]. The mixture was incubated at 37° for 5 min immediately before the addition of acetaldehyde and NAD for determination of the ALDH activity.

Protein concentrations in the homogenates were determined by the method of Lowry [16].

The concentration of Me-DTC in plasma collected from rats treated with DSF or Me-DDC and in human plasma collected from five alcoholics 2 hr after ingestion of a single oral dose of 400 mg DSF was analyzed by a modification of an HPLC assay [15]. The blood was anticoagulated with sodium heparin. The plasma was separated by centrifugation at 2500 g at 4° for 15 min. Two hundred microlitres of plasma containing the internal standard Et-DTC in a concentration of 500 nmol/l was injected directly on the enrichment precolumn. The enriched compounds were separated on a reversed phase column (250 mm × 4 mm i.d.) packed with Lichrosorb RP-18, 7 μm (E. Merck). The mobile phase consisted of 10 mmol/l phosphate buffer, pH 7.5, acetonitrile 45/55 (v/v) % and the compounds were detected at 214 nm. The concentrations were determined from a standard graph covering the range of 0.05–4.00 μmol/l.

Plasma was analyzed for the presence of DTC to investigate the occurrence of metabolic hydrolytic formation of DTC in rats treated with DSF or Me-DCC [15].

RESULTS

Synthesis of DTC, Me-DTC and Et-DTC

All three substances were obtained in a high purity grade with less than 0.5% of impurities. The gas chromatographic retention time of Me-DTC was estimated to be 1.15 min and that of Et-DTC to be 3.20 min. From the Me-DTC MS/EI fragmentation pattern (Fig. 1), it could be seen that the compound decomposes with a molecular ion at *m/e* 147, a base peak at *m/e* 100 and with a typical fragment at *m/e* 72. The ethyl derivative showed a similar spectrum

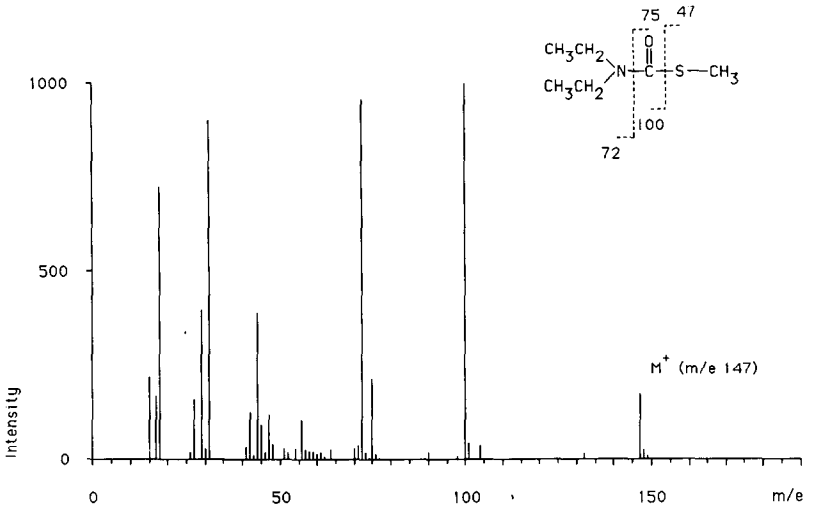

Fig. 1. EI mass spectrum of Me-DTC. The fragmentation pattern shows the M^+ ion at *m/e* 147, the loss of S-CH_3 (*m/e* 47), leaving the base peak at *m/e* 100, and loss of CO (*m/e* 28), giving a chief fragment at *m/e* 72.

Table 1A. Rat hepatocyte ALDH activity and concentrations of Me-DDC, DDC and Me-DTC in rat plasma after intraperitoneal administration of DSF and Me-DDC

		ALDH activity			Plasma concentration		
Homogenate*	Dosage mg/kg	nmol of NADH formed/min/mg protein Total	Low K_m	High K_m	Me-DDC (nmol/l)	DDC	Me-DTC (μmol/l)
Control	—	2.59 ± 0.24	0.26 ± 0.04	2.33 ± 0.22	—	—	—
DSF	100 + 30	1.58 ± 0.49	0.20 ± 0.05	1.38 ± 0.46	49 ± 36	841 ± 581	1.3 ± 0.5
Me-DDC†	60 + 30	1.46 ± 0.28	0.18 ± 0.06	1.28 ± 0.24	249 ± 219	182 ± 169	2.1 ± 1.1
Me-DDC‡	60	1.58 ± 0.48	0.22 ± 0.04	1.36 ± 0.52	1241 ± 653	259 ± 147	10.1 ± 7.1

* The values are the mean ± SD from four controls, four DSF treated rats, and ten Me-DDC treated rats, respectively.
† The values are the mean ± SD from five Me-DCC treated rats examined 5 hr after the last drug administration.
‡ The values are the mean ± SD from five Me-DDC treated rats examined 3 hr after drug administration.
The ALDH activity values from each rat are an average of two determinations.

except that the molecular ion was found at *m/e* 161. The UV spectrum showed an absorption maximum at 206 nm for Me-DTC and at 195 nm for DTC.

Blood pressure, hepatocyte ALDH activities, plasma Me-DDC, DDC and Me-DTC concentrations in DSF or Me-DDC treated rats

Table 1B shows that ethanol 1 g/kg did not produce any significant effect on the cardiovascular parameters. The DSF or Me-DDC treatment produced a significant fall in both systolic ($P < 0.05$–0.01) and diastolic pressure ($P < 0.01$–0.001) at 30 min with partial recovery at 120 min.

Rats treated with one high dose of Me-DDC 1 hr before ethanol challenge showed the same effect as with two doses.

The homogenates were prepared and analyzed 5 hr after the last dose was given. The mean (±SD) ALDH activities are shown in Table 1A. The rats treated with DSF demonstrate a 23% decrease in the low K_m activity, whereas the high K_m activity was reduced by 41% ($P < 0.01$). In the Me-DDC groups, the inactivation of the low K_m activity was more pronounced, giving a decrease of 31%, whereas the high K_m activity was diminished by 45% ($P < 0.001$).

The values were 15% and 42% ($P < 0.01$), respectively, when a single dose of Me-DDC was studied.

Comparison of the mean plasma concentration of Me-DDC between the groups treated twice (Table 1A), shows a five-fold increase in the rats treated with Me-DDC. In samples collected 3 hr after the single treatment, this divergence was even higher (25-fold) ($P < 0.01$), and is probably explained by the shorter time between ingestion of the drug and collection of the sample for analysis.

The reverse was found in the plasma concentration of DDC and in the two groups. The mean DCC concentration in the DSF group was estimated to be 841 nmol/l, whereas that in the Me-DDC group was as low as 182 nmol/l.

To investigate whether Me-DTC and DTC could be detected as metabolites in blood after adminis-

Table 1B. Blood pressure and heart rate in rats treated with DSF and Me-DDC

Treatment	Dosage mg/kg i.p.	Time (hr) from dosing 1st	2nd	Heart rate % of control	Systolic BP % of control	Diastolic BP % of control
Saline	—	1	—	101.8 ± 5.5	95.0 ± 2.9	92.5 ± 5.2
DSF	100 + 30	19	3	107.8 ± 19.8	85.0 ± 6.7*	65.5 ± 8.9†
Me-DDC	60 + 30	19	3	105.8 ± 11.7	75.2 ± 7.4†	61.2 ± 9.2‡
Me-DDC	60	1	—	96.8 ± 4.3	77.6 ± 8.5†	61.8 ± 12.0†

The cardiovascular response to ethanol 1 g/kg i.p. challenge after treatments with disulfiram and Me-DDC. The responses are given at 30 min where the maximal effects were observed. The responses were slightly less at 120 min. The data were analyzed by Student's *t*-test.

* $P < 0.05$; † $P < 0.01$ and ‡ $P < 0.001$.

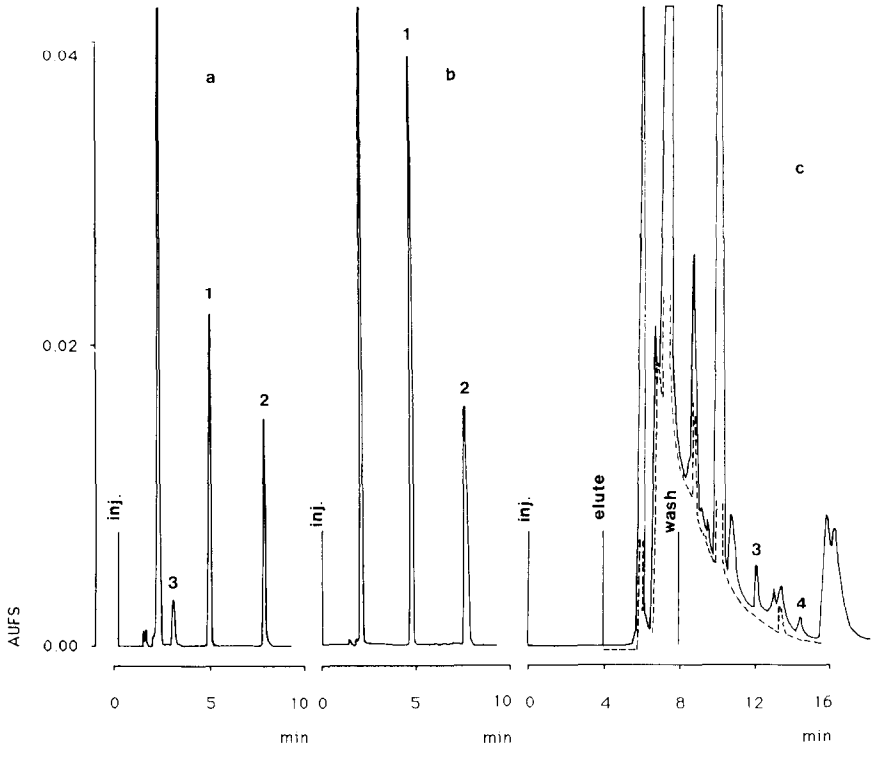

Fig. 2. HPLC profiles obtained from chloroform extract of rat liver homogenates, (a) aerobic conditions, (b) under nitrogen, and (c) in rat plasma. Dashed line represents a control plasma. The peaks show Me-DDC (1), propyl-DDC (2), Me-DTC (3) and ethyl-DTC (4). The analysis was performed by a modified HPLC system (15) and the compounds were separated on a reversed phase Lichrosorb RP 18 (Merck) column, equilibrated with the mobile phase, consisting of 10 mmol/l phosphate buffer, pH 7.5/ acetonitrile 45/55 v/v % (c) and 25/75 v/v % (a) and (b), respectively.

tration of DSF or Me-DDC to rats, plasma chloroform extracts were analyzed by GC/MS. The formation and distribution of Me-DTC in blood was confirmed from its MS/EI spectrum. The fragmentation patterns were identical with the spectrum obtained from pure Me-DTC (Fig. 1). However, the formation of DTC in the liver, and its distribution to the blood could not be demonstrated.

When analyzed by HPLC, the chromatogram (Fig. 2c) confirmed the presence of an Me-DTC peak with a retention time of 12.1 min. The mean plasma concentration of Me-DTC in the DSF treated rats was found to be 1.3 μmol/l and in the Me-DDC groups 2.1 μmol/l (21 hr + 5 hr) and 10.1 μmol/l (3 hr), respectively (Table 1A). When the ALDH activities were compared with the Me-DTC concentrations in plasma of the 21 hr + 5 hr Me-DDC and DSF groups, the former clearly tended to be more inactivated, as was also shown by the higher Me-DTC level.

Microsomal oxidative formation of Me-DTC in vitro

The inactivation of low K_m activity in rat hepatocytes, is achieved aerobically (Table 2). The low K_m activity was decreased by 46% ($P < 0.01$) in the presence of NADP. Hence, no inactivation was

Table 2. Oxidative formation of Me-DTC from Me-DDC in rat hepatocytes *in vitro*

Homogenate	ALDH activity nmol of NADH formed/min/mg protein Total	Low K_m	High K_m	Elimination of Me-DDC μmol/l	Formation of Me-DTC
Control	2.47 ± 0.05	0.28 ± 0.01	2.19 ± 0.06	—	—
Me-DDC, air	1.18 ± 0.66	0.15 ± 0.04	1.03 ± 0.68	105 ± 32	Positive
Me-DDC, N_2	1.66 ± 0.64	0.28 ± 0.06	1.38 ± 0.63	41 ± 18	Negative

Rat liver homogenates were incubated at 37° for 2 hr with 240 μmol/l Me-DDC and oxidative factors (see text), and analyzed for ALDH activity. The values are the mean ± SD of duplicate determinations in homogenates from three control rats.

Table 3. The inhibitory effect of Me-DTC and DTC on rat hepatocyte low and high K_m ALDH activities *in vitro*

Homogenates Me-DTC/DTC μmol/l	Oxidative factors	ALDH activity nmol NADH formed/min/mg protein Total	Low K_m	High K_m	Elimination of Me-DTC μmol/l
Control	–	1.14 ± 0.11	0.25 ± 0.06	0.89 ± 0.17	—
Me-DTC, 2760	–	0.45 ± 0.05	0.03 ± 0.02	0.41 ± 0.03	1253 ± 442
Me-DTC, 2760	+	0.55 ± 0.07	0.10 ± 0.03	0.45 ± 0.05	1074 ± 657
Me-DTC, 276	–	0.67 ± 0.11	0.01 ± 0.01	0.66 ± 0.12	—
Me-DTC, 27.6	–	0.86 ± 0.39	0.06 ± 0.06	0.75 ± 0.26	—
DTC, 34.1	–	0.60 ± 0.03	0.22 ± 0.02	0.41 ± 0.04	—

Rat liver homogenates were incubated at 37° for 1 hr in air and analyzed for the ALDH activity. The values are the mean ± SD of duplicate determinations in homogenates from three control rats.

observed with incubation under nitrogen. The remaining activity of the high K_m isozymes in aerobic conditions was 47% ($P < 0.05$) and with nitrogen 63%. Thus, these isozymes were apparently inhibited by Me-DDC under both aerobic and anaerobic conditions. However, inhibition was more pronounced in the presence of oxygen. Furthermore, the metabolic elimination of Me-DDC was also increased, by a value of 44% ($P < 0.01$), compared to 17% ($P < 0.05$) under nitrogen.

In the HPLC chromatogram derived from a chloroform extract incubated aerobically, a peak appeared, which had a shorter retention time than that of Me-DDC, suggesting the formation of a compound with more hydrophilic properties than the parent drug (Fig. 2, a and b). The compound was identified as Me-DTC from its chromatographic characteristics and MS/EI fragmentation pattern, and comparable to the spectrum in Fig. 1.

Inactivation of ALDH activity in rat hepatocytes in vitro

It is evident (Table 3) that low K_m and high K_m isozymes are inhibited by Me-DTC. The inactivation is independent of oxidative metabolism, which suggests that the parent compound acts as the inhibitor. The low K_m isozymes are almost completely inhibited in the absence of oxidative factors, even when the concentration of inhibitor is decreased 100-fold from 2760 μmol/l to 27.6 μmol/l ($P < 0.01$). The high K_m ALDH, however, showed reduced inactivation from 54% ($P < 0.01$) to 16%. In the presence of oxidative factors, inactivation was less pronounced and could probably be explained by a simultaneous competitive oxidative metabolic elimination of the inhibitor.

At concentrations approximately similar to those of Me-DTC, the free acid, DTC, shows a reversed selectivity for the isozymes as compared to Me-DTC, with a 54% ($P < 0.01$) decrease in the high K_m activity, but only 12% in the low K_m activity.

The inhibition of ALDH caused by Me-DTC could not be reversed when glutathione or 2-mercaptoethanol was added, nor did an increase of the concentration of the latter compound up to 20 mmol/l regain enzyme activity (unpublished observation).

Concentrations of Me-DTC in human plasma

In chloroform extracts from five human plasma samples, Me-DTC was identified as a metabolite by its MS/EI spectrum, similar to that shown in Fig. 1.

DISCUSSION

The main object of this study was to elucidate whether Me-DDC, after its administration to rats, shows inhibitory action on the low K_m and high K_m ALDH isozymes in hepatocytes. The significant decrease (Table 1, A and B) in the blood pressure and inactivation of both low K_m and high K_m activities agreed with observations in rats treated with DSF. This, confirms the importance of Me-DDC as a metabolite involved in the irreversible inactivation of these enzymes. The more pronounced inactivation of the low K_m isozymes after Me-DDC treatment is probably explained by the higher concentration of drug found in the blood. The compound is highly lipid soluble [17], and readily penetrates cell membranes and is thus extensively distributed within the

cell, where it becomes available for further biotransformation.

The unexpected formation of DDC in eight of the Me-DDC treated rats indicates the presence of a liver enzyme with a thio ester hydrolytic activity. This metabolic pathway enables the formation of other previously reported [12, 18] metabolites, which have been found in blood after ordinary DSF treatment.

Metabolic formation of Me-DTC occurs both in DSF treated rats and in rats given Me-DDC (Table 1). Increased transformation is demonstrated in the latter group. This divergence may be an expression of increased oxidation, owing to the higher concentrations of Me-DDC in plasma.

In rats challenged with ethanol 1 hr after ingestion of Me-DDC, the decrease in blood pressure and the remaining ALDH activity resemble those in the 21 hr + 5 hr group, indicating a rapid onset of the DSF/alcohol reaction (DAR), which is also confirmed by the higher concentrations of Me-DTC in plasma.

Me-DDC was reported inactive as an *in vitro* inhibitor of ALDH [19]. This makes it unlikely that Me-DDC is active *in vivo*. The parent compound is apparently transformed along two different biotransformation pathways. The formation of an inhibitor with high affinity for the low K_m isozymes is achieved by an oxidative and NADP dependent reaction, whereas the inactivation of the high K_m isozymes is also demonstrated in anaerobic conditions. A metabolic pathway independent of oxygen is the thioesterase dependent hydrolytic reaction proposed above. However, no such metabolic pathway was found in rat liver homogenates incubated with Me-DDC and analyzed for the presence of DDC. This observation does not exclude it as an anaerobic elimination pathway *in vivo*.

In rat hepatocytes exposed to increasing Me-DTC, the inactivation of the low K_m isozymes seems to be independent of the concentration of inhibitor in the range studied, compared to the progressive inactivation of the high K_m isozymes. The reversed inactivation profile shown by DTC demonstrates a higher affinity for the high K_m isozymes [19].

It has been reported previously that ALDH enzymes that are inhibited *in vivo* are unaffected when exposed to reducing agents [7, 8]. This was confirmed in the present study. The irreversibility is due to a non-reducible stable covalent binding between inhibitor and enzyme. However, this binding is not to be regarded as a normal covalent disulphide interchange reaction as it is resistant to low molecular weight thiols.

The irreversible binding is achieved by oxidation of Me-DDC, forming a metabolite structurally related to the normal substrates. The sulphur atom is exchanged for oxygen in the Me-DTC metabolite, hence creating an intra-molecular binding polarity with the electrons displaced towards the oxygen, which facilitates a nucleophilic attack on the exposed electrophilic centre. Thus, it is tempting to speculate about an inhibitory mechanism of the reaction, similar to that suggested for the irreversible inactivation of acetylcholinesterase [20].

DTC, a possible hydrolytic metabolite of Me-DTC, has properties resembling those of DDC, in that it is a water-soluble chelating compound, which forms an extremely stable and water-insoluble complex with cupric ions (unpublished observations). However, unlike DDC, it is inadequate as an *in vitro* inhibitor: the inhibitory action exerted by DTC is probably attributable to the enhanced nucleophilic character on the sulfhydryl group.

Preliminary results obtained in a subsequent study confirm the hypothesis that Me-DTC acts as the inhibitor of ALDH *in vivo* after DSF treatment. Rats treated with a single intraperitoneal 30 mg/kg dose of the drug and examined 1 hr after administration demonstrated a positive DAR after ethanol challenge, which was valid as a significant decrease in blood pressure [21].

The finding of Me-DTC as a metabolite in human blood after DSF treatment, suggests a similar inactivation of ALDH in human and rat hepatocytes.

In summary, these results conclude that the Me-DTC metabolite formed from Me-DDC by microsomal oxidative metabolism is implicated in the inhibitory action of ALDH isozymes in rat hepatocytes *in vivo*. Furthermore, it is also suggested that this may be the inhibitor *in vivo* in rats and humans on DSF treatment.

REFERENCES

1. Hald J and Jacobsen E, The formation of acetaldehyde in the organism after ingestion of Antabuse (tetraethylthiuramdisulphide) and alcohol. *Acta Pharmacol* **4**: 305–310, 1948.
2. Strömme JH, Interaction of disulfiram and diethyldithiocarbamate with serum proteins studied by means of a gel-filtration technique. *Biochem Pharmacol* **14**: 381–391, 1965.
3. Deitrich RA and Erwin VG, Mechanism of the inhibition of aldehyde dehydrogenase *in vivo* by disulfiram and diethyldithiocarbamate. *Mol Pharmacol* **7**: 301–307, 1971.
4. Kitson TM, The effect of disulfiram on aldehyde dehydrogenase of sheep liver. *Biochem J* **151**: 407–412, 1975.
5. von Bahr-Lindström H, Jeck R, Woenckhaus C, Sohn S, Hempel J and Jörnvall H, Characterization of coenzyme binding site of liver aldehyde dehydrogenase: differential reactivity of coenzyme analogues. *Biochemistry* **24**: 5847–5851, 1985.
6. Hempel J, von Bahr-Lindström H and Jörnvall H, Aldehyde dehydrogenase from human liver. Primary structure of cytoplasmatic isozyme. *Eur J Biochem* **141**: 21–35, 1984.
7. Vallari RC and Pietruszko R, Human aldehyde dehydrogenase: mechanism of inhibition by disulfiram. *Science* **216**: 637–639, 1982.
8. Tottmar O and Hellström E, Aldehyde dehydrogenase in blood: a sensitive assay and inhibition by disulfiram. *Pharmacol Biochem Behav* **18**: 103–107, 1983.
9. Johansson B, Angelo H, Christensen JK, Möller IW and Rönsted P, Dose/effect relationship of disulfiram in human volunteers. II Biochemical studies. *Alcoholism* submitted.
10. Kitson TM, The inactivation of aldehyde dehydrogenase by disulfiram in the presence of glutathione. *Biochem J* **199**: 255–258, 1981.
11. Johansson B, Carbonsylsulphide: a copper chelating metabolite of disulfiram. *Drug Metab Disp* accepted 1988.
12. Tottmar O, Pettersson H and Kiessling K-H, The sub-

cellular distribution and properties of aldehyde dehydrogenases in rat liver. *Biochem J* **135**: 577–586, 1973.
13. Tottmar O and Marchner H, Disulfiram as a tool in the studies on metabolism of acetaldehyde in rats. *Acta Pharmacol Toxicol* **38**: 366–375, 1976.
14. Axelrod J, The enzymatic deamination of amphetamine (Benzedrine). *J Biol Chem* **214**: 753–763, 1955.
16. Lowry OH, Rosebrough NJ and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
17. Johansson B, Inhibition of erythrocyte aldehyde dehydrogenase activity and elimination kinetics of diethyldithiocarbamic acid methyl ester and its monothio analogue after the administration of single and repeated doses of disulfiram in man. *Eur J Clin Pharmacol* in press.
18. Kaslander J, Formation of an *S*-glucuronide from tetraethylthiuramdisulfide (Antabuse) in man. *Biochim Biophys Acta* **71**: 730–732, 1963.
19. Kitson M, The effect of some analogues of disulfiram on aldehyde dehydrogenases of sheep liver. *Biochem J* **155**: 445–448, 1976.
20. Ariens EJ, Simonis AM and Offermeier J, *Introduction to General Toxicology*. Academic Press, New York, 1976.
21. Petersen EN, Pharmacological effects of diethylthiocarbamic acid methyl ester: the active metabolite of disulfiram. *Eur J Clin Pharmacol* submitted.