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THE EFFECTS OF CEPHEM ANTIBIOTICS AND RELATED COMPOUNDS ON THE ALDEHYDE DEHYDROGENASE IN RAT LIVER MITOCHONDRIA

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Abstract—The effects of cephem antibiotics and their related compounds on aldehyde dehydrogenase obtained from rat liver mitochondria were studied. A pH of 8.8 and reaction temperature 24° were the conditions for measurement of enzyme activity. The apparent Michaelis constant K_m values for NAD, acetaldehyde and propionaldehyde were 3.8×10^{-5} M, 4.0×10^{-5} M and 2.5×10^{-5} M, respectively. Cefamandole, cefoperazone and cefmetazole, having a 1-methyl-5-thiotetrazol group at position 3 of the cephem ring, caused a relatively potent inhibition of aldehyde dehydrogenase. Cefmetazole and cefoperazone also showed a significant inhibition on highly purified yeast aldehyde dehydrogenase; the extent of inhibition on yeast enzyme was almost the same as that on rat mitochondrial aldehyde dehydrogenase. The decrease in enzyme activity effected by 1-methyl-1H-tetrazol-5-thiol (MTT) was greater than those of 1H-tetrazol (TZ), 1H-tetrazol-5-thiol and 1-(2-dimethylaminoethyl)-1H-tetrazol-5-thiol, but was, of course, less than that of disulfiram. Cefamandole, cefmetazole and MTT showed competitive inhibition with NAD, while TZ was uncompetitive inhibitor with respect to both NAD and acetaldehyde. Enzyme inhibition caused by disulfiram, cefmetazole and MTT increased time-dependently and the addition of 2-mercaptoethanol into the medium effectively and completely restored enzyme inhibition. These results suggest that thiol group at position 5 of 1H-tetrazol ring is responsible for the type of inhibition with NAD, and methyl group at position 1 of 1H-tetrazol ring is important to exhibit a potent inhibition on aldehyde dehydrogenase.

We have recently reported that cephem antibiotics containing a 1-methyl-5-thiotetrazol side group at position 3 or a 1H-tetrazol group at position 7 of the cephem ring caused a significant increase in the blood acetaldehyde level in rats after ethanol administration [1]. In addition, 1-methyl-1H-tetrazol-5-thiol and 1H-tetrazol displayed potent disulfiram-like activity in *in vivo* experiments [1].

It is well known that disulfiram inhibits aldehyde dehydrogenase and causes an accumulation of acetaldehyde in the blood after ethanol administration [2–4]. Previous research has shown that disulfiram is a competitive inhibitor of aldehyde dehydrogenase with respect to NAD and uncompetitive with aldehyde substrates [5, 6]. Though cephem antibiotics having the above-described side chain groups display disulfiram-like activity, their chemical structures are similar to neither that of disulfiram nor those of aldehyde compounds.

Taking into account these observations, the present study was undertaken in order to clarify the effects of certain cephem antibiotics on aldehyde dehydrogenase obtained from rat liver mitochondria. Whether the test drugs displayed competitive or uncompetitive inhibition with these substrates, and the relationship between inhibition of aldehyde dehydrogenase and the chemical structure of 1H-tetrazol analogues, which are similar to the side chain of cephem antibiotics, were also investigated.

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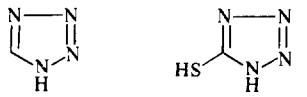
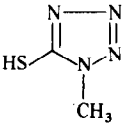
MATERIALS AND METHODS

Male Wistar-strain rats weighing 300–400 g were used in these experiments. The drugs tested were disulfiram (Tokyo Kasei), cefotaxime sodium (Hoechst, Japan), cefazolin sodium (Fujisawa), ceftezole sodium (Chugai), cefamandole sodium (Shionogi), cefoperazone sodium (Toyama), cefotiam dihydrochloride (Takeda), latamoxef sodium (Shionogi), cefmetazole sodium (Sankyo), cefotetan (Yamanouchi) and cefbuperazone sodium (Toyama). The following compounds which have a structural resemblance to the side chain of cephem antibiotics, were also used: 1H-tetrazol (TZ, Tokyo Kasei), 1H-tetrazol-5-thiol (TT, provided by Lilly), 1-methyl-1H-tetrazol-5-thiol (MTT, provided by Lederle, Japan), and 1-(2-dimethylaminoethyl)-1H-tetrazol-5-thiol (DTT, provided by Takeda). In the case of MTT, sodium salt was used in addition to the base (sodium MTT was provided by Hoechst). The chemical structures of these compounds are shown in Fig. 1. All drugs used were dissolved in water, except disulfiram, which was dissolved in propylene glycol (Katayama). The reagents used were rotenone (Aldrich), nicotinamide-adenine dinucleotide (NAD, Boehringer Mannheim), acetaldehyde (Merck), propionaldehyde (Tokyo Kasei), 2-mercaptoethanol (Nakarai), pyrazole (Tokyo Kasei) and sodium deoxycholate (Sigma).

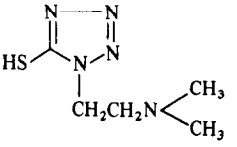
Aldehyde dehydrogenase activity was measured according to the method of Tottmar *et al.* [7] or



1H-tetrazol (TZ) 1H-tetrazol-5-thiol (TT)



1-methyl-1H-tetrazol-5-thiol (MTT)



1-(2-dimethylaminoethyl)-1H-tetrazol-5-thiol (DTT)

Fig. 1. Chemical structures of 1H-tetrazol related compounds.

Bostian and Betta [8]. Partially-purified rat liver mitochondria, used as enzyme source, was prepared as follows. A portion (10 g) of liver was homogenized in 50 ml of 0.25 M sucrose–5 mM Tris–HCl buffer (pH 7.2), after which homogenate was centrifuged for 10 min at 700 *g*; subsequently, the supernatant was centrifuged for 15 min at 10,000 *g*. All steps were carried out at 4°. The pellet was resuspended in the same buffer, and sodium deoxycholate at a concentration of 0.25 mg/mg of protein was added to the samples in order to attain clear solutions for spectrophotometric measurement.

The assay mixture contained sodium pyrophosphate buffer (50 mM; pH 8.8), NAD (0.015–0.5 mM), pyrazole (0.1 mM), rotenone (2 μM), acetaldehyde (40–150 μM) and rat mitochondria protein (0.8 mg); the total volume was 3.02 ml. Pyrazole and rotenone were added to inhibit alcohol dehydrogenase and mitochondrial NAD oxidase, respectively [7]. Protein concentrations were measured using the method of Lowry *et al.* [9]. Acetaldehyde was added to initiate the reaction. Unless otherwise noted, the assay was performed at 24°, and reduction of NAD to NADH was measured spectrophotometrically at 340 nm (Hitachi, Model 101).

In some experiments, the reversibility of enzyme activity inhibited by test drugs such as disulfiram, cefmetazole, MTT was tested by means of dialysis [10, 11]. The reaction mixtures consisted of 6.6 ml of 50 mM pyrophosphate buffer (pH 8.8), 0.3 ml of mitochondrial enzyme, and 0.03 ml of test drug solution. The final concentration of disulfiram was 100 μM, while those of the other test drugs were 10 mM, respectively. In control solutions, 0.03 ml of either propylene glycol or water was added instead of the test drug solution. After 4 hr of dialysis against N_2-saturated phosphate buffer at 4°, the aldehyde dehydrogenase activity of the reaction mixtures was assayed as described previously.

In another experiment, purified aldehyde dehydrogenase obtained from Bakers yeast (potassium-activated from Bakers yeast, Sigma) was used. The enzyme activity and the purity were determined by a slight modification of the method of Bostian and Betta [8]. Briefly, enzyme sample and marker proteins were prepared for SDS/polyacrylamide–gel electrophoresis by dissociation in a boiling-water bath for 3 min in 0.01 M Tris–HCl buffer (pH 6.8), 1% (w/v) SDS and 5% (v/v) mercaptoethanol, at a protein concentration of 1.6 mg/ml; 25–50 μg of protein was used for loading 7% (w/v) polyacrylamide gels, and electrophoresis was performed after the method of Bostian and Betta [8]. Densitometric determination was performed by means of a high speed TLC scanner (Shimazu, CS-90). Molecular weight of aldehyde dehydrogenase used in this experiment was estimated as *ca.* 62,500, while those of BSA and egg albumin were 67,500 and 45,000, respectively. The purity of the enzyme was 95.9%. The assay mixture used for the yeast enzyme consisted of 0.1 M Tris–HCl buffer (pH 8.0), 0.5 mM NAD, 50 μM acetaldehyde, 10 mM 2-mercaptoethanol and 0.1 M KCl, and the final volume was 2.5 ml.

RESULTS

Changes in enzyme activity of aldehyde dehydrogenase prepared from rat liver mitochondria

In preliminary experiments, it was found that the optimal pH for measuring rat liver aldehyde dehydrogenase activity in 50 mM sodium pyrophosphate buffer was 8.8. By contrast, enzyme activity in samples with a pH of either 8.4 or 9.2 was about 80%; for those with a pH of 7.2, 7.6, 8.0, 9.6 or 10.0, it was less than 50%. The optimal temperature for measurement was 24° at pH 8.8 and the reaction rate was linear for at least 10 min. When the reaction temperature was lower than 24° (20 or 16°), acetaldehyde dehydrogenase activity was also low; when the temperature was higher than 24° (28, 32 or 36°), the lineality of the enzyme activity disappeared within 10 min, though the enzyme activity itself was higher than that at 24°. Aldehyde dehydrogenase activity increased in proportion to the protein concentration up to 0.8 mg. Though the enzyme activity was somewhat higher in a solution containing a 1.0 mg protein than in that containing 0.8 mg protein, no significant difference was found. Consequently, 0.8 mg protein concentration was employed in these experiments.

Table 1 shows the effects of disulfiram and various cephem antibiotics on aldehyde dehydrogenase. Disulfiram induced a potent effect, while a significant effect was obtained even at a concentration of 10^{-8} M. Cefamandole, cefoperazone and cefmetazole caused a significant decrease in aldehyde dehydrogenase activity at respective concentrations of 10^{-4} M. Significant inhibition was also effected by 10^{-3} M concentrations of latamoxef, cefotetan or cefbuperazone. Cefotaxime, cefazolin, ceftezole and cefotiam did not inhibit the enzyme, even at concentrations of 10^{-3} M. However, higher concentrations (5×10^{-3} M) of cefazolin, ceftezole and cefotiam induced a significant decrease in the enzyme activity.

Table 1. Effects of disulfiram and various cephem antibiotics on aldehyde dehydrogenase activity

Reference drug	0	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4} (M)
Disulfiram	20.9 ± 0.3	20.2 ± 0.5	18.2 ± 0.4**	17.4 ± 0.5**	17.1 ± 0.5**	13.5 ± 1.0**	6.0 ± 0.4**
Test drugs	0	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	5×10^{-3} (M)
Cefotaxime	21.3 ± 0.2	21.2 ± 0.4	21.1 ± 0.7	21.3 ± 0.6	21.4 ± 0.6	21.1 ± 0.5	21.0 ± 0.4
Cefazolin	21.3 ± 0.4	21.1 ± 0.7	21.0 ± 0.5	21.0 ± 0.6	20.9 ± 0.6	19.7 ± 0.6	19.5 ± 0.5*
Ceftezole	21.0 ± 0.9	20.0 ± 0.6	19.6 ± 0.4	19.8 ± 0.9	19.4 ± 0.4	19.3 ± 0.5	18.1 ± 0.9*
Cefamandole	21.1 ± 0.5	20.3 ± 0.7	19.9 ± 0.2	19.7 ± 0.2	19.1 ± 0.3*	18.0 ± 0.6†	17.0 ± 0.8†
Cefoperazone	21.2 ± 0.5	19.9 ± 0.7	19.8 ± 0.9	19.2 ± 0.7	19.0 ± 0.6*	18.7 ± 0.5†	17.9 ± 0.9†
Cefotiam	21.2 ± 0.6	21.2 ± 0.6	20.7 ± 0.8	19.6 ± 0.4	19.6 ± 0.8	19.6 ± 0.6	18.8 ± 0.3†
Latamoxef	21.2 ± 0.5	20.4 ± 0.4	19.9 ± 0.6	20.1 ± 0.8	19.8 ± 0.8	19.0 ± 0.3*	15.7 ± 0.5†
Cefmetazole	21.0 ± 0.5	19.5 ± 0.9	19.1 ± 1.1	19.4 ± 1.1	18.1 ± 1.4*	17.2 ± 1.2†	17.2 ± 1.3†
Cefotetan	21.1 ± 0.6	20.9 ± 0.1	20.7 ± 0.2	19.4 ± 0.9	19.3 ± 0.4	18.7 ± 0.4*	18.4 ± 0.4*
Cefbuperazone	21.2 ± 0.4	20.6 ± 0.6	20.3 ± 0.8	20.0 ± 0.6	20.0 ± 0.9	19.5 ± 0.5*	18.3 ± 0.6†

Numbers indicate aldehyde dehydrogenase activity (nmol of NADH formed/min/mg of protein) and standard error of the mean of 6–8 experiments.

The assay mixture consisted of 50 mM sodium pyrophosphate buffer (pH 8.8), 0.5 mM NAD, 50 μM acetaldehyde, 0.1 mM pyrazole, 2 μM rotenone and 0.8 mg protein of rat liver mitochondria.

*, † Significantly different from the control values with $P < 0.05$ and $P < 0.01$, respectively.

Table 2 shows the effects of 1H-tetrazol derivatives on aldehyde dehydrogenase. TT and TZ caused significant inhibition of aldehyde dehydrogenase at respective concentrations of 10^{-3} M. The effect of MTT was more potent than those of TT and TZ, and significant inhibition was observed at a 10^{-4} M concentration. In addition, the effect of sodium MTT was almost equal to that of base MTT. DTT has a weak effect on aldehyde dehydrogenase; in fact, a concentration of 5×10^{-3} M was needed to obtain a significant effect. When absorption spectrum of NAD (0.5 mM) or NADH (0.5 mM) was measured at pH 8.8 by means of double beam spectrophotometer (Hitachi, Model 200–100) without enzyme protein, the addition of cephem antibiotics or side-chain related compounds at 10^{-3} M did not alter the spectrum; no enzyme activity was displayed without rat mitochondrial protein.

Figures 2–5 and Table 3 show the inhibition of aldehyde dehydrogenase by certain compounds. The apparent Michaelis constants K_m for NAD and acetaldehyde obtained from Lineweaver–Burk plots were 3.8×10^{-5} M and 4.0×10^{-5} M, respectively. As shown in Fig. 2, the enzyme inhibition caused by disulfiram, cefmetazole and MTT increased time-dependently. Disulfiram was a competitive inhibitor with respect to NAD but apparently uncompetitive with acetaldehyde; K_i values were 10^{-6}–10^{-7} M (Fig. 3 and Table 3). Cefamandole, cefmetazole, MTT and TT were also competitive inhibitors with respect to NAD and uncompetitive with acetaldehyde (Figs. 4 and 5). On the other hand, TZ displayed uncompetitive inhibition with either NAD or acetaldehyde (Fig. 6). Since the inhibitory effect of DTT was so weak, accurate determination of inhibition type with NAD or acetaldehyde was not possible.

When 10 mM of 2-mercaptoethanol was added to the assay medium together with a test drug such as disulfiram, cefmetazole or MTT, and each mixture was incubated with mitochondrial enzyme, the inhi-

Table 2. Effects of 1H-tetrazol analogues on aldehyde dehydrogenase activity

Drugs	0	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3} (M)
TZ	20.8 ± 0.4	20.3 ± 0.5	19.7 ± 0.8	19.8 ± 0.8	19.6 ± 0.4	18.6 ± 0.9*
TT	20.9 ± 0.5	20.3 ± 0.5	20.5 ± 0.9	20.1 ± 0.6	19.8 ± 0.3	18.5 ± 0.2*
MTT	20.6 ± 0.6	19.4 ± 0.3	19.1 ± 1.1	19.3 ± 0.5	18.4 ± 0.4†	17.4 ± 0.4†
DTT	20.7 ± 0.5	19.5 ± 0.3	19.6 ± 1.1	19.3 ± 0.6	19.3 ± 0.2	19.4 ± 0.5

Numbers indicate aldehyde dehydrogenase activity (nmol of NADH formed/min/mg of protein) and standard error of the mean of 6–8 experiments. The assay mixture consisted of 50 mM sodium pyrophosphate buffer (pH 8.8), 0.5 mM NAD, 50 μM acetaldehyde, 0.1 mM pyrazole, 2 μM rotenone and 0.8 mg protein of rat liver mitochondria.

*, † Significantly different from the control values with $P < 0.05$ and $P < 0.01$, respectively.

Abbreviations: TZ, 1H-tetrazol; TT, 1H-tetrazol-5-thiol; MTT, 1-methyl-1H-tetrazole-5-thiol; DTT, 1-(2-dimethylaminoethyl)-1H-tetrazol-5-thiol.

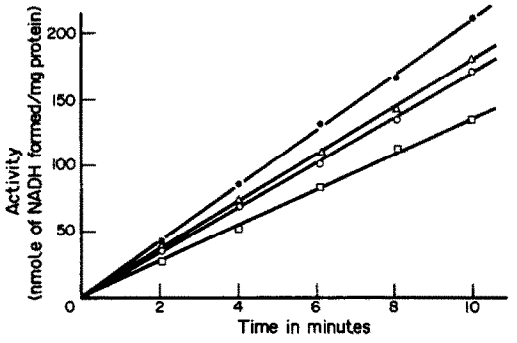


Fig. 2. The time-dependent increase of aldehyde dehydrogenase inhibition caused by disulfiram, cefmetazole and 1-methyl-tetrazol-5-thiol: ●—●, control; △—△, 5×10^{-3} M 1-methyl-1H-tetrazol-5-thiol; ○—○, 5×10^{-3} M cefmetazole; □—□, 10^{-5} M disulfiram.

bition of enzyme activity caused by these drugs was completely prevented (Table 4). The addition of 2-mercaptoethanol alone had no effect on enzyme activity. These results may indicate that this thiol compound protects the enzyme against inhibition. To investigate the persistence of the drug–enzyme interaction, reaction mixtures containing none or one of the test drugs were dialysed for 4 hr (Table 5). After a long period of dialysis, enzyme activity was lower than that determined before dialysis, even in control samples (approximately, 63% reduction). Enzyme activity was not restored completely under any of the test circumstances. The lowest removal

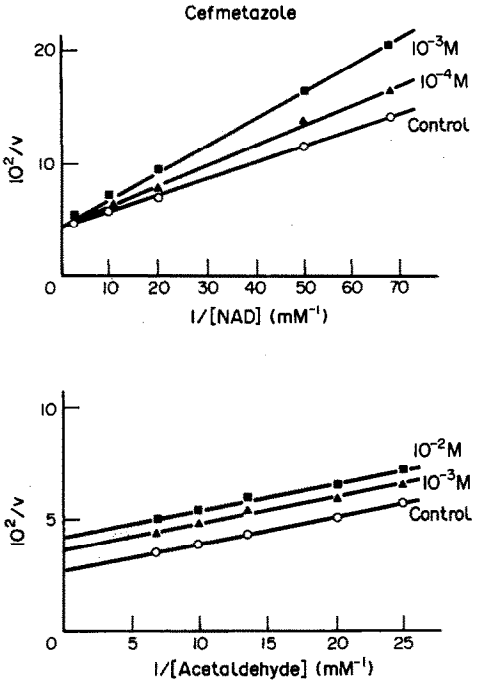


Fig. 4. The inhibition of aldehyde dehydrogenase by cefmetazole, "v" indicates the nmol of NADH formed/min/mg protein: upper, competitive inhibition with NAD; lower, uncompetitive inhibition with acetaldehyde.

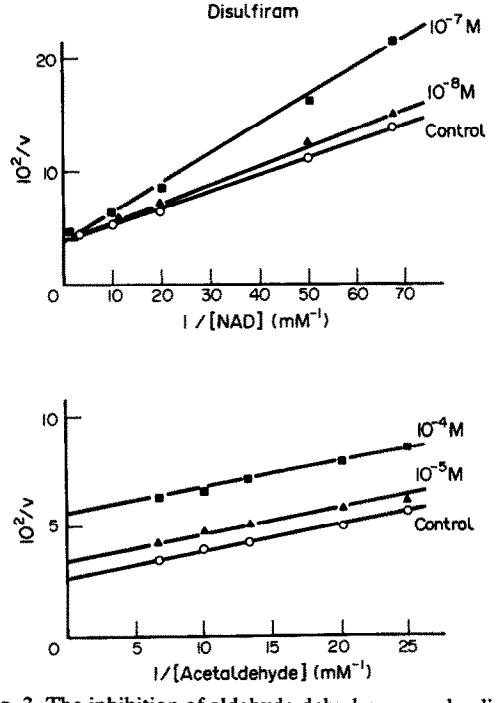


Fig. 3. The inhibition of aldehyde dehydrogenase by disulfiram; "v" indicates the nmol of NADH formed/min/mg protein: upper, competitive inhibition with NAD; lower, uncompetitive inhibition with acetaldehyde.

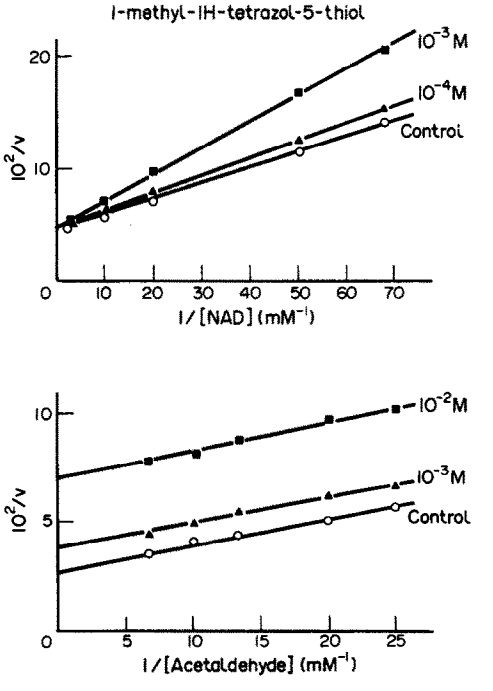


Fig. 5. The inhibition of aldehyde dehydrogenase by 1-methyl-1H-tetrazol-5-thiol; "v" indicates the nmol of NADH formed/min/mg protein: upper, competitive inhibition with NAD; lower, uncompetitive inhibition with acetaldehyde.

Table 3. Types of inhibition and K_i values for certain inhibitors of aldehyde dehydrogenase

Drugs	NAD		Acetaldehyde	
	Types of inhibition	K_i values (M)	Type of inhibition	K_i values (M)
Disulfiram	Competitive	1.0×10^{-7}	Uncompetitive	3.4×10^{-6}
Cefamandole	Competitive	3.8×10^{-3}	Uncompetitive	1.4×10^{-3}
Cefmetazole	Competitive	1.0×10^{-3}	Uncompetitive	1.6×10^{-3}
MTT	Competitive	9.5×10^{-4}	Uncompetitive	1.5×10^{-3}
TT	Competitive	1.0×10^{-3}	Uncompetitive	1.6×10^{-3}
TZ	Uncompetitive	2.8×10^{-3}	Uncompetitive	1.7×10^{-3}

was observed following disulfiram treatment but in the cases of cefmetazole and MTT, the enzyme activity regained over 80% of the control value.

The apparent Michaelis constant K_m for propionaldehyde was almost the same as that of acetaldehyde (K_m for propionaldehyde: 2.5×10^{-5} M; K_m for acetaldehyde: 4.0×10^{-4} M). In addition, no significant difference in inhibitory effects of cefmetazole and cefoperazone were noticed when either propionaldehyde or acetaldehyde was used as a substrate.

Changes in enzyme activity of aldehyde dehydrogenase obtained from Bakers yeast

The apparent Michaelis constants K_m of aldehyde dehydrogenase purified from Bakers yeast with respect to NAD, acetaldehyde and propionaldehyde were 3.10×10^{-5} M, 2.20×10^{-4} M and 8.30×10^{-4} M, respectively. Five micrograms of yeast aldehyde dehydrogenase dissolved in 2.5 ml of the assay mixture exhibit the enzyme activity of 22.3 nmol/NADH formed/min. Cefmetazole at concentrations of 10^{-3} M and 5×10^{-3} M caused a significant inhibition of yeast enzyme to the same extent as that of rat liver mitochondrial aldehyde dehydrogenase. At 10^{-3} M, the enzyme activity of 22.3 ± 0.5 (control value) was decreased to 19.3 ± 0.5 ($P < 0.05$, $N = 5$); at 5×10^{-3} M the control value was reduced to 18.2 ± 0.6 ($P < 0.01$, $N = 5$). Cefoperazone at concentrations of 10^{-3} M and 5×10^{-3} M also caused a significant inhibition of yeast aldehyde dehydrogenase similarly to that of rat liver aldehyde dehydrogenase (at 10^{-3} M, decreased to 20.4 ± 0.5, $P < 0.05$, $N = 5$; at 5×10^{-3} M, to 18.8 ± 0.4, $P < 0.01$, $N = 5$).

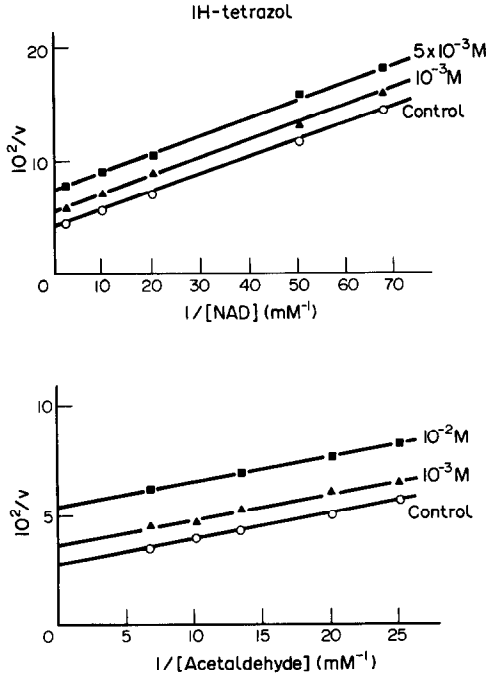


Fig. 6. The inhibition of aldehyde dehydrogenase by 1H-tetrazol; "v" indicates the nmol of NADH formed/min/mg protein: upper, uncompetitive inhibition with NAD; lower, uncompetitive inhibition with acetaldehyde.

DISCUSSION

As stated above, through our preliminary studies we determined that a pH of 8.8 and a reaction temperature of 24° were the optimal conditions for measuring the activity of aldehyde dehydrogenase prepared from rat liver mitochondria. This pH level is similar to those of rat liver samples described by Tottmar [7] and Shum and Blair [12]. Also, in almost all relevant previous experiments, temperatures of 20 to 25° were used [7, 11, 13]. In this experiment, it was found that pH of the assay mixture was not altered by addition of any test drug, and reduction of NAD or oxidation of NADH was not observed by test compounds. Therefore, it is conceivable to assume that the inhibitory effect elicited by cephems or related compounds on rat liver aldehyde dehydrogenase was not brought about in "non-enzymatic" manner.

In the present experiment, acetaldehyde was used as a substrate. Since the boiling point of acetaldehyde is 21°, some uncertainty regarding acetaldehyde concentration in the medium cannot be excluded completely. However, when propionaldehyde was used as substrate instead of acetaldehyde, K_m values of both propionaldehyde and acetaldehyde were almost the same.

K_m values of rat mitochondrial aldehyde dehydro-

Table 4. Effects of 2-mercaptoethanol on the activity of aldehyde dehydrogenase treated with certain inhibitors

Drugs	Concentration	2-mercapto-ethanol (10 mM)	Activity: nmol of NADH formed/min/mg of protein	Activity: relative activity (%)
Control		—	21.1 ± 0.5	100
		+	21.3 ± 0.4	101
Disulfiram	100 μM	—	6.1 ± 0.4	28.9
		+	21.4 ± 0.2	100
Cefmetazole	10 mM	—	17.0 ± 0.5	80.6
		+	21.1 ± 0.5	100
MTT	10 mM	—	16.4 ± 0.3	77.7
		+	21.4 ± 0.6	100

Each value was the mean ± SEM (N = 6). + (with) or − (without) 2-mercaptoethanol.

genase determined with respect to NAD, acetaldehyde, and propionaldehyde are comparable with those obtained by others, though in our case the K_m for acetaldehyde and propionaldehyde seem to be somewhat low. Tottmar *et al.* [7] described that the K_m for acetaldehyde was apparently less than 1×10^{-5} M, while that for NAD was $2\text{–}3 \times 10^{-5}$ M. According to the findings of Deitrich [13], the K_m for NAD was determined as 1.6×10^{-5} M, for propionaldehyde, 3.84×10^{-6} M.

In our experiments, it became apparent that cefamandole, cefoperazone and cefmetazole caused a relatively potent, and latamoxef, cefotetan and cefbuperazone a moderate inhibition of rat liver mitochondrial aldehyde dehydrogenase activity. We have reported earlier that these drugs cause a remarkable increase in the blood acetaldehyde level after ethanol administration, and that they have a 1-methyl-5-thiotetrazol side group at position 3 of the cephem nucleus [1]. Cefazolin and ceftezole also caused an increase in blood acetaldehyde level, although the extent was not so great as those of the drugs described above. Both cefazolin and ceftezole have a 1H-tetrazol group at position 7 of the cephem ring. In the present *in vitro* experiments, both drugs inhibited aldehyde dehydrogenase equally well, though a higher concentration was needed. Cefotaxime had no inhibitory effect on aldehyde dehydrogenase in accordance with the results obtained in *in vivo* experiments [1]. These findings suggest a good correlation between an increase of blood acetaldehyde level after ethanol administration and an inhibition of aldehyde dehydrogenase obtained from rat liver mitochondria.

Table 5. Recovery of the enzyme activity by dialysis

Drugs	Concentration	Activity (nmol of NADH formed/min/mg of protein): before dialysis	Activity (nmol of NADH formed/min/mg of protein): after dialysis
Control		21.3 ± 0.4 (100%)	13.5 ± 0.6 (100%)
Disulfiram	100 μM	6.2 ± 0.3 (29.1%)	4.9 ± 0.4 (36.3%)
Cefmetazole	10 mM	17.0 ± 0.8 (79.8%)	11.9 ± 0.8 (88.2%)
MTT	10 mM	16.3 ± 0.5 (75.5%)	11.2 ± 0.4 (83.0%)

Each value was the mean ± SEM (N = 6).
Relative activity was shown in the parentheses.

It is known that NAD is involved in many of the enzyme reactions which occur in rat mitochondria. In highly purified aldehyde dehydrogenase derived from Bakers yeast, K_m values determined for NAD, acetaldehyde and propionaldehyde are almost identical to those described by Tamaki *et al.* [15]. Cefmetazole and cefoperazone also inhibited the yeast aldehyde dehydrogenase to almost the same extent as observed in the mitochondrial aldehyde dehydrogenase. Therefore, it can be assumed that the effects of test compounds on rat mitochondrial enzyme properly reflects that elicited on the aldehyde dehydrogenase.

The structure–activity relationship of 1H-tetrazol related compounds showed that MTT provides more potent effect than TT, TZ and DTT. It was assumed that thiol group at position 5 of 1H-tetrazol ring is necessary to exert a competitive inhibition with NAD as shown in MTT and TT, and the substitution of methyl group at position 1 of tetrazol ring is indispensable to enhance the enzyme inhibition. It is well known that disulfiram causes a competitive inhibition with NAD, and uncompetitive inhibition with aldehyde compounds [5, 6]. The similar inhibitory pattern was also seen in cephem antibiotics and MTT. This finding seems to suggest that some common mechanism(s) may be exerted in inhibiting acetaldehyde dehydrogenase between these three groups of test compounds, that is disulfiram, MTT and cephems. Actually, the enzyme inhibition proceeded time-dependently in all cases and the simultaneous addition of 2-mercaptoethanol into the assay medium effectively protected the enzyme against the inhibition caused by these drugs. Furthermore, it was shown that removal of the test compounds by means of dialysis was not effective in restoring enzyme activity, especially in the case of disulfiram. This may indicate that drug–enzyme interaction takes place persistently.

REFERENCES

1. C. Kamei, Y. Sugimoto, N. Muroi and K. Tasaka, *J. Pharm. Pharmac.* **38**, 823 (1986).
2. J. Hald, E. Jacobsen and V. Larsen, *Acta Pharmac. Toxic.* **4**, 285 (1948).
3. E. B. Truitt and G. Duritz, in *Biochemical Factors in Alcoholism* (Ed. R. P. Maichel), pp. 61–69. Pergamon Press, London (1967).
4. O. Tottmar and E. Hellstrom, *Acta Pharmac. Toxic.* **45**, 272 (1979).
5. W. D. Graham, *J. Pharm. Pharmac.* **3**, 160 (1951).
6. R. A. Deitrich and L. Hellerman, *J. biol. Chem.* **238**, 1683 (1963).
7. S. O. C. Tottmar, H. Pettersson and K-H. Kiessling, *Biochem. J.* **135**, 577 (1973).
8. K. A. Bostian and G. F. Betta, *Biochem. J.* **173**, 773 (1978).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
10. R. C. Vallari and R. Pietruszko, *Science* **216**, 637 (1982).
11. T. M. Kitson, *Biochem. J.* **151**, 406 (1975).
12. G. T. Shum and A. H. Blair, *Can. J. Biochem.* **50**, 741 (1972).
13. R. A. Deitrich and V. G. Erwin, *Molec. Pharmac.* **7**, 301 (1971).
14. R. A. Deitrich, L. Hellerman and J. Wein, *J. biol. Chem.* **237**, 560 (1962).
15. N. Tamaki, M. Nakamura, K. Kimura and T. Hama, *J. Biochem.* **82**, 73 (1977).