

Acta Medica Okayama

Volume 52, Issue 1

1998

Article 7

FEBRUARY 1998

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Abstract

The main purpose of this study was to evaluate the inhibitory effects of 5-fluorouracil antineoplastics, cephem antibiotics containing the methyltetrazolylthiol (MTT) group and antidiabetics on aldehyde dehydrogenase (ALDH) activity in vivo and in vitro. In in vivo experiments, rats were given a 100 mg/kg dose of drugs (10 mg/kg for glibenclamide) orally or intraperitoneally. When each drug was administered singly immediately after an oral administration of 1.5 g/kg ethanol, only carmofur, an antineoplastic, produced marked increases in blood acetaldehyde concentrations. This action was also noted when ethanol was ingested 15 h after administration. The remaining drugs did not increase blood acetaldehyde concentrations. When rats were treated with carmofur at 12 h intervals for 3 consecutive days and were given 1.5 g/kg ethanol after the final treatment, blood acetaldehyde concentrations were elevated more significantly than with a single administration of carmofur. Furthermore, daily administration of cephem antibiotics containing the MTT group, latamoxef, cefamandole, cefoperazone and cefbuperazone, significantly increased blood acetaldehyde concentrations. Daily administration of sulfonylurea antidiabetics, chlorpropamide and acetohexamide, slightly increased blood acetaldehyde concentrations. Drugs causing increases in blood acetaldehyde concentrations when administration was combined with ethanol ingestion also inhibited ALDH activity in vitro. The results of the in vitro experiments roughly correlated with those of the in vivo experiments. The inhibitory effects of drugs on ALDH activity were in the following order: carmofur >> cephem antibiotics containing the MTT group > sulfonylurea antidiabetics.

KEYWORDS: toxicology, acetaldehyde, aldehyde dehydrogenase, disulfiram-like reaction, carmofur

*PMID: 9548989 [PubMed - indexed for MEDLINE]

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Effects of Antineoplastics, Antibiotics and Antidiabetics on Acetaldehyde Metabolism after Alcohol Ingestion

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The main purpose of this study was to evaluate the inhibitory effects of 5-fluorouracil antineoplastics, cephem antibiotics containing the methyltetrazolylthiol (MTT) group and antidiabetics on aldehyde dehydrogenase (ALDH) activity *in vivo* and *in vitro*. In *in vivo* experiments, rats were given a 100 mg/kg dose of drugs (10 mg/kg for glibenclamide) orally or intraperitoneally. When each drug was administered singly immediately after an oral administration of 1.5 g/kg ethanol, only carmofur, an antineoplastic, produced marked increases in blood acetaldehyde concentrations. This action was also noted when ethanol was ingested 15 h after administration. The remaining drugs did not increase blood acetaldehyde concentrations. When rats were treated with carmofur at 12 h intervals for 3 consecutive days and were given 1.5 g/kg ethanol after the final treatment, blood acetaldehyde concentrations were elevated more significantly than with a single administration of carmofur. Furthermore, daily administration of cephem antibiotics containing the MTT group, latamoxef, cefamandole, cefoperazone and cefbuperazone, significantly increased blood acetaldehyde concentrations. Daily administration of sulfonylurea antidiabetics, chlorpropamide and acetohexamide, slightly increased blood acetaldehyde concentrations. Drugs causing increases in blood acetaldehyde concentrations when administration was combined with ethanol ingestion also inhibited ALDH activity *in vitro*. The results of the *in vitro* experiments roughly correlated with those of the *in vivo* experiments. The inhibitory effects of drugs on ALDH activity were in the following order: carmofur \gg cephem antibiotics containing the MTT group $>$ sulfonylurea antidiabetics.

Key words: toxicology, acetaldehyde, aldehyde dehydrogenase, disulfiram-like reaction, carmofur

In social and legal medicine, the relationship of alcohol consumption to misconduct, accidents and crimes is of fundamental importance. When alcohol is mixed with other drugs, this relationship becomes increasingly complex. In estimating physical and mental conditions at the time of an accident, the following interactions between drugs and ethanol should be considered: a) depression of the central nervous system (CNS) due to the direct action of ethanol and drugs such as sedatives on the CNS (1); b) enhanced pharmacological effects due to ethanol-related promotion of drug absorption or inhibition of drug metabolism (2); c) enhanced inebriation due to drug-related promotion of ethanol absorption (1); and d) disulfiram-like reactions such as flushes, vertigo, nausea, sweating and tachycardia due to drug-related inhibition of the metabolism of the first metabolite of ethanol, acetaldehyde (3-7).

In a previous study, we examined the ethanol-enhanced pharmacological effects of drugs, especially antipsychotic agents, from the perspective of forensic toxicology (8-13), and reported that cerebral concentrations of triazolam, estazolam and meprobamate were markedly increased when combined with ethanol ingestion (8, 12, 13).

Concerning drug-related inhibition of acetaldehyde metabolism, Yanagihara *et al.* (14) demonstrated that cephem antibiotics containing the methyltetrazolylthiol (MTT) group release MTT in the intestinal tract, which inhibits aldehyde dehydrogenase (ALDH) activity, in-

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creasing blood acetaldehyde concentrations after combined usage with ethanol. Thereafter, this finding has been reported by many investigators (15–21). Furthermore, Öhlin *et al.* (22) and Jerntorp *et al.* (23) reported that a sulfonyleurea remedy for diabetes, chlorpropamide, inhibits ALDH activity. Several other drugs have also been reported to cause disulfiram-like reactions in clinical practice (24–26). However, the inhibitory effects of these drugs on ALDH activity have not yet been sufficiently investigated.

The main purpose of this study was to evaluate the inhibitory effects of 5-fluorouracil antineoplastics, cephem antibiotics containing the MTT group and antidiabetics on ALDH activity *in vivo* and *in vitro*.

Materials and Methods

Animals. Male Wistar rats weighing 250–350 g, purchased from Clea Japan Inc. (Tokyo), were used.

Chemicals. Drugs used in this experiment are as follows: MIFUROL® (Carmofur, Mitsui Pharmaceuticals, Tokyo, Japan), 5-FU (5-Fluorouracil, Kyowa Hakko Kogyo Co., Tokyo, Japan), FUTRAFUL® (Tegafur, Taiho Pharmaceutical Co., Tokyo, Japan), FURTULON® (Doxifluridine, Nippon Roche, Tokyo, Japan), SHIOMARIN® (Latamoxef, Shionogi & Co., Osaka, Japan), KEFDOL® (Cefamandole, Shionogi & Co., Osaka, Japan), CEFOBID® (Cefoperazone, Pfizer Pharmaceuticals, Tokyo, Japan), TOMIPORAN® (Cefbuperazone, Toyama Chemical Co., Tokyo, Japan), DIABINESE® (Chlorpropamide, Pfizer Pharmaceuticals, Tokyo, Japan), MELLITOS® (Tolbutamide, Ono Pharmaceutical Co., Osaka, Japan), DIMELIN® (Acetohexamide, Shionogi & Co., Osaka, Japan), EUGLUCON® (Glibenclamide, Yamanouchi Pharmaceutical Co., Tokyo, Japan), PANSPORIN® (Cefotiam, Takeda Chemical Industries, Osaka, Japan), NEUCEF® (Cefodizime, Hoechst Pharmaceuticals and Chemicals, Tokyo, Japan), CEFOTAX® (Cefotaxime, Chugai Pharmaceutical Co., Tokyo, Japan), EPOCELIN® (Ceftizoxime, Fujisawa Pharmaceutical Co., Osaka, Japan), MODACIN® (Ceftazidime, Tanabe Seiyaku Co., Osaka, Japan), CEFATREXYL® (Cefapirin, Bristol-Myers Squibb, Tokyo, Japan), ROCEPHIN® (Ceftriaxone, Nippon Roche, Tokyo, Japan), KEFLIN® (Cefalotin, Shionogi & Co., Osaka, Japan), CEFAMEZIN® (Cefazolin, Fujisawa Pharmaceutical Co., Osaka, Japan), KEITEN® (Cefpirome, Chugai

Pharmaceutical Co., Tokyo, Japan), TAKESULIN® (Cefsulodin, Takeda Chemical Industries, Osaka, Japan), FLUMARIN® (Flomoxef, Shionogi & Co., Osaka, Japan), PENTCILLIN® (Piperacillin, Toyama Chemical Co., Tokyo, Japan), LILACILLIN® (Sulbenicillin, Takeda Chemical Industries, Osaka, Japan), DOYLE® (Aspoxicillin, Tanabe Seiyaku Co., Osaka, Japan), PENTREX® (Ampicillin, Banyu Pharmaceutical Co., Tokyo, Japan), GEOPEN® (Carbenicillin, Pfizer Pharmaceuticals, Tokyo, Japan), AMASULIN® (Carumonam, Takeda Chemical Industries, Osaka, Japan), AZACTAM® (Aztreonam, Eisai Co., Tokyo, Japan), TIENAM® (Imipenem·cilastatin, Banyu Pharmaceutical Co., Tokyo, Japan), FOSMICIN® (Fosfomycin, Meiji Seika Kaisha, Tokyo, Japan), KANAMYCIN (Kanamycin, Banyu Pharmaceutical Co., Tokyo, Japan), BANAN® (Cefpodoxime proxetil, Sankyo Co., Tokyo, Japan), TOMIRON® (Cefteram pivoxil, Toyama Chemical Co., Tokyo, Japan), CEFSPAN® (Cefixime, Fujisawa Pharmaceutical Co., Osaka, Japan), KEFLEX® (Cefalexin, Shionogi & Co., Osaka, Japan), KEF-RAL® (Cefaclor, Shionogi & Co., Osaka, Japan), TARIVID® (Ofloxacin, Daiichi Pharmaceutical Co., Tokyo, Japan), CINOBACT® (Cinoxacin, Shionogi & Co., Osaka, Japan), BACCIDAL® (Norfloxacin, Kyorin Pharmaceutical Co., Tokyo, Japan), SUMIFON® (Isoniazid, Sumitomo Pharmaceuticals Co., Osaka, Japan), FLAGYL® (Metronidazole, Shionogi & Co., Osaka, Japan), FULCIN® (Griseofulvin, Zeneca Yakuhin, Osaka, Japan), TAGAMET® (Cimetidine, Smith Kline Beecham Seiyaku, Tokyo, Japan), CYANAMIDE (Cyanamide, Yoshitomi Pharmaceutical Industries, Osaka, Japan), and disulfiram (Wako Pure Chemical Industries, Osaka, Japan). Hexylamine and ALDH were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Boehringer Mannheim Co. (Mannheim, Germany), respectively. The other reagents used were of analytical grade.

The dose of all drugs except cyanamide (20 mg/kg) and glibenclamide (10 mg/kg) was 100 mg/kg. Antibiotics were administered intraperitoneally or through a stomach tube as 2% solutions. 5-Fluorouracil and hexylamine were orally administered as 2% solutions. Cyanamide was orally administered as a 0.4% solution. The remaining drugs were orally administered as 2% suspensions (0.2% suspension for glibenclamide) in 5% Arabic gum solution. Ten ml/kg of 15% (w/v) ethanol solution was orally administered.

Experiments with single administration of drugs. To establish whether ALDH activity is inhibited immediately after a single administration of 5-fluorouracil antineoplastics (carmofur, 5-fluorouracil, tegafur and doxifluridine), cephem antibiotics containing the MTT group (latamoxef, cefamandole, cefoperazone and cefbuperazone) and antidiabetics (chlorpropamide, tolbutamide, acetohexamide and glibenclamide), and the duration of the inhibition, ethanol was orally administered immediately before or 15 h after administration of these drugs to rats. Blood was collected from the tail vein 1 h after ethanol administration by slightly cutting the tail without anesthetization. Additionally, similar experiments were conducted on the other 36 drugs in common use to identify strong inhibitors of ALDH. As a negative control, ethanol alone was administered to the rats ($n = 53$).

Experiments with daily administration of drugs. The 5-fluorouracil antineoplastics, cephem antibiotics containing the MTT group and antidiabetics were administered to rats at 12 h intervals for 3 days. Ethanol was administered 15 h after the final administration. Blood was collected 1 h after ethanol administration. As a negative control, 5 ml/kg water was orally administered to the rats ($n = 14$) for 3 days instead of these drugs.

Apparatus. A Shimadzu gas chromatograph (GC-5A, Kyoto, Japan) equipped with a glass column (200 cm \times 0.3 cm intradiameter) containing 25 % polyethylene glycol 1000 on Shimalite 80-100 mesh and flame ionization detector was used for quantification of acetaldehyde. The column temperature was 60 °C. The temperature of the injection port and detector was 110 °C. The carrier gas was nitrogen at a flow rate of 50 ml/min.

A Shimadzu spectrophotometer (UV-160, Kyoto, Japan) was employed to measure ALDH activity.

Measurement of blood acetaldehyde concentrations. Head space gas chromatography described by Eriksson *et al.* (27) was modified as follows: One milliliter of 0.6 N perchloric acid (PCA)-physiological saline solution containing 0.012 % t-butanol as an internal standard was placed in a 15 ml glass vial with a silicon cap, weighed, and cooled on ice. Approximately 0.1 g of blood was collected and sealed in the glass vial. Immediately thereafter, the glass vial was agitated using a vortex mixer, then weighed. The vial was incubated in a water bath at 55 °C for 15 min, and 2 ml of air phase was injected into the gas chromatograph.

Measurement of in vitro activity of ALDH.

ALDH activity was measured by modifying the method described by Hasumura *et al.* (28). A 2.8 ml aliquot of 50 mM pyrophosphate buffer solution (pH 8.5) containing 2 μ M rotenone, 0.1 mM pyrazole, 0.5 mM NAD and 0.4 U ALDH was mixed with 0.1 ml of one of the above listed drugs in water, acetone or dimethylformamide then preincubated at 25 °C. Thereafter, 0.1 ml of 180 μ M acetaldehyde was added to the mixture. Absorbance at 340 nm was serially measured. As a control, the same solution, mixed with water, acetone or dimethylformamide without the addition of any of the above listed drugs, was measured for ALDH activity.

Statistical analysis. The Student's *t*-test was used to compare the experimental groups with the negative control groups. Differences at $P < 0.05$ were considered to be significant.

Results

Measurement of acetaldehyde. Acetaldehyde and t-butanol persisted on gas chromatography for 1.6 min and 5.0 min, respectively. When acetaldehyde was added to blood to adjust the level to 10 μ M or more and 5 μ M or less, the mean coefficients of variation ($n = 5$) of this method were 7.4 % and 11.8 %, respectively. The curve generated by plotting the ratios of peak heights between acetaldehyde and the internal standard to blood acetaldehyde concentrations showed good linearity at acetaldehyde concentrations ranging from 2.5 μ M to 50 μ M ($r = 0.996$).

Animal experiments. Blood acetaldehyde concentrations were determined 1 h after the administration of ethanol. The drugs possibly influencing the metabolism of alcohol were administered immediately after or 15 h before the treatment with ethanol. The results are summarized in Table 1. The mean blood acetaldehyde concentration in 53 rats given ethanol alone was $4.1 \pm 2.7 \mu$ M. Based on the mean value + 3 SD, we regarded 12.2 μ M as a tentative cut off point for significantly increased blood acetaldehyde concentration. When cyanamide used as a positive control for the inhibition of ALDH activity was administered immediately after or 15 h before ethanol treatment, the mean blood acetaldehyde concentrations were $43.4 \pm 13.1 \mu$ M ($n = 3$, $P < 0.05$) and $33.6 \pm 10.5 \mu$ M ($n = 3$, $P < 0.05$), respectively. When carmofur was administered immediately after or 15 h before ethanol treatment, the mean blood acetaldehyde concentrations were $24.6 \pm 4.3 \mu$ M ($n = 3$, $P < 0.05$) and $40.3 \pm 11.6 \mu$ M ($n = 3$,

Table 1 Blood acetaldehyde concentrations in rats given 1.5g/kg ethanol orally immediately before or 15h after a single administration of the following drugs

Drug ^a	Ethanol treatment		Drug ^a	Ethanol treatment	
	Immediately before	15h after		Immediately before	15h after
5-Fluorouracil antineoplastics			Others		
Carmofur	24.6 ± 4.3*	40.3 ± 11.6*	(continued from left column)		
5-Fluorouracil	1.9	2.8	Flomoxef	1.2	1.0
Tegafur	1.3	2.3	Piperacillin	1.8	2.9
Doxifluridine	1.9	2.3	Sulbenicillin	3.1	1.2
Cephem antibiotics containing the MTT group			Aspoxicillin	5.2	1.4
Latamoxef	7.1	4.9	Ampicillin	7.2	2.1
Cefamandole	3.3	3.7	Carbenicillin	2.1	1.6
Cefoperazone	7.7	4.0	Carumonam	2.7	3.1
Cefbuperazone	3.4	2.6	Aztreonam	4.3	2.9
Antidiabetics			Imipenem · cilastatin	5.1	2.5
Chlorpropamide	3.7	6.0	Fosfomycin	2.0	2.2
Tolbutamide	3.4	2.3	Kanamycin	3.7	2.2
Acetohexamide	8.9	5.5	Cefpodoxime proxetil	2.0	1.6
Glibenclamide (10mg/kg)	5.3	5.4	Cefteram pivoxil	0.8	1.5
Others			Cefixime	2.1	2.4
Cefotiam	8.4	4.9	Cefalexin	1.5	3.7
Cefodizime	4.4	1.9	Cefaclor	2.0	1.9
Cefotaxime	3.3	3.0	Ofloxacin	1.5	1.9
Ceftizoxime	6.2	3.7	Cinoxacin	6.2	2.4
Ceftazidime	5.6	2.6	Norfloxacin	1.3	2.2
Cefapirin	6.2	2.5	Isoniazid	2.9	2.1
Ceftriaxone	4.1	7.0	Metronidazole	2.6	2.3
Cefalotin	8.1	7.5	Griseofulvin	3.2	2.2
Cefazolin	1.9	1.6	Cimetidine	2.8	1.8
Cefpirome	1.6	2.2	Positive controls		
Cefsulodin	2.5	1.9	Cyanamide (20mg/kg)	43.4 ± 13.1*	33.6 ± 10.5*
			Disulfiram	2.3	26.9 ± 3.0*

A tentative cut off level for significantly increased blood acetaldehyde concentration was 12.2 μM.

Based on the cut off level, screening examinations were performed on all of the drugs.

In drugs having shown blood acetaldehyde concentrations above the cut off level, further investigations were conducted using 2 more animals. Blood samples were taken from the tail vein 1h after ethanol administration.

* Each value represents the mean of 3 animals ± SD and was statistically significant compared with the value of negative control group (n = 53). MTT: Methyltetrazolylthiol. ^a: The concentration of drugs is 100mg/kg except glibenclamide (10mg/kg) and cyanamide (20mg/kg).

$P < 0.05$), respectively. When disulfiram was administered immediately after ethanol treatment, there were no increases in blood acetaldehyde concentrations. However, ethanol treatment 15h after administration of this agent resulted in a mean blood acetaldehyde concentration of $26.9 \pm 3.0 \mu\text{M}$ ($n = 3$, $P < 0.05$). Even after a single administration of cephem antibiotics containing the MTT group and antidiabetics that were reported to show disulfiram-like actions, as well as other drugs, there were no increases in blood acetaldehyde concentrations.

Blood acetaldehyde concentrations when ethanol was

administered after daily administration of 5-fluorouracil antineoplastics, cephem antibiotics containing the MTT group and antidiabetics are shown in Figs. 1-3. The mean blood acetaldehyde concentration in 14 rats given water daily instead of the drugs was $3.0 \pm 1.6 \mu\text{M}$. Based on the mean value + 3SD, we regarded $7.8 \mu\text{M}$ as a tentative cut off point for significantly increased blood acetaldehyde concentration. Daily administration of carmofur, which significantly increased blood acetaldehyde concentrations after a single administration, increased blood acetaldehyde concentrations more significantly ($n =$

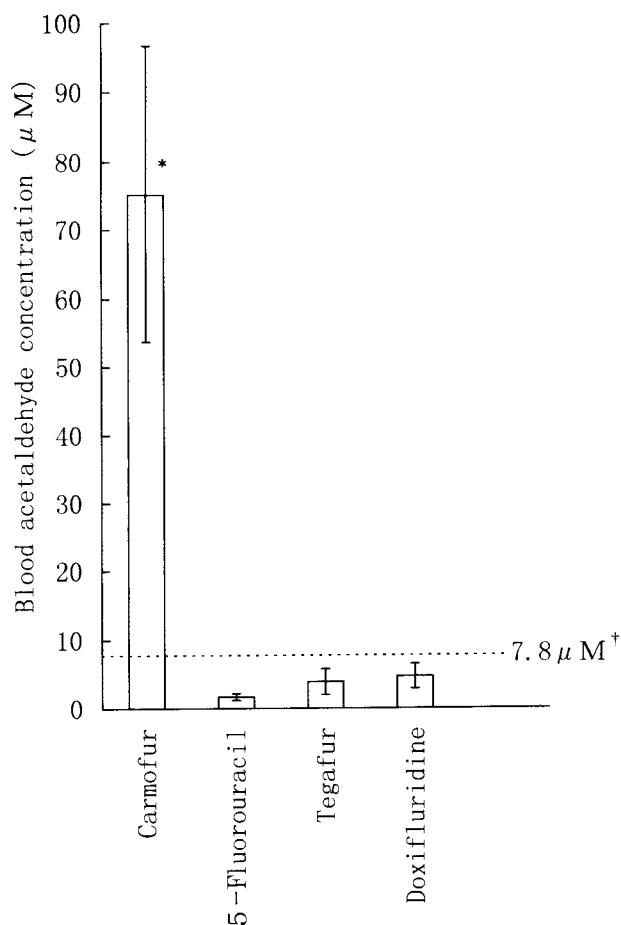


Fig. 1 Blood acetaldehyde concentrations in rats given 1.5g/kg ethanol orally 15h after treatment with 100mg/kg 5-fluorouracil antineoplastics at 12h intervals for 3 days. Blood samples were taken from the tail vein 1h after ethanol administration.

Each bar represents the mean of 3 animals ± SD.

* Statistically significant compared with the value of negative control group (n = 14).

† A tentative cut off level for significantly increased blood acetaldehyde concentration.

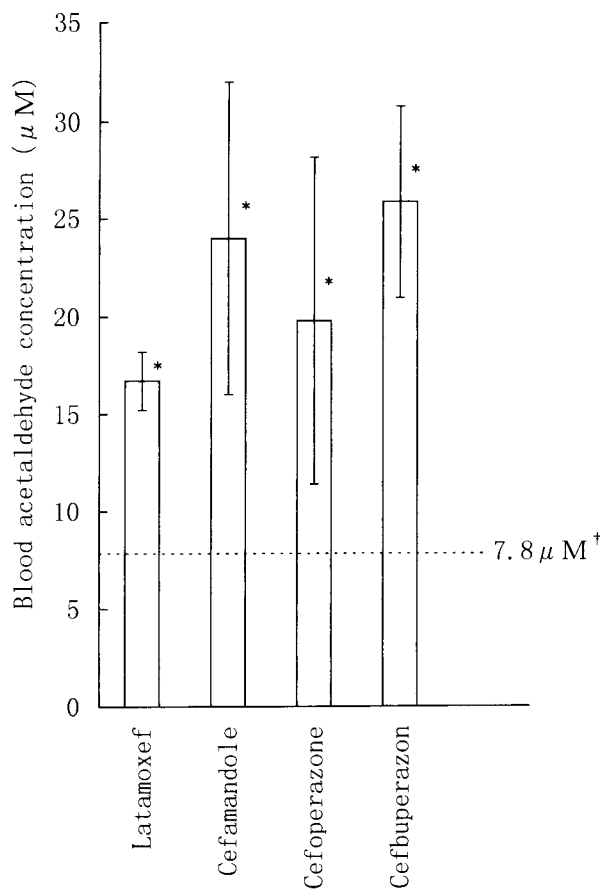


Fig. 2 Blood acetaldehyde concentrations in rats given 1.5g/kg ethanol orally 15h after treatment with 100mg/kg cephem antibiotics containing the methyltetrazolylthiol group at 12h intervals for 3 days. Blood samples were taken from the tail vein 1h after ethanol administration.

Each bar represents the mean of 3 animals ± SD.

* Statistically significant compared with the value of negative control group (n = 14).

† A tentative cut off level for significantly increased blood acetaldehyde concentration.

3, $P < 0.05$). However, when other 5-fluorouracil antineoplastics (5-fluorouracil, tegafur and doxifluridine) were administered daily, there were no increases in blood acetaldehyde concentrations (Fig. 1). Daily administration of cephem antibiotics containing the MTT group (latamoxef, cefamandole, cefoperazone and cefbuperazone) significantly increased blood acetaldehyde concentrations (n = 3, $P < 0.05$ for each drug), although there were no increases in blood acetaldehyde concentrations after a single

administration of these drugs (Fig. 2). As for the antidiabetics, daily administration of chlorpropamide and acetohexamide slightly increased blood acetaldehyde concentrations (n = 3, $P < 0.05$ for each drug). However, daily administration of tolbutamide or glibenclamide did not increase blood acetaldehyde concentrations (Fig. 3).

In vitro experiment. As shown in Table 2, carmofur completely inhibited ALDH activity at a low concentration of 1×10^{-6} M, although the inhibitory

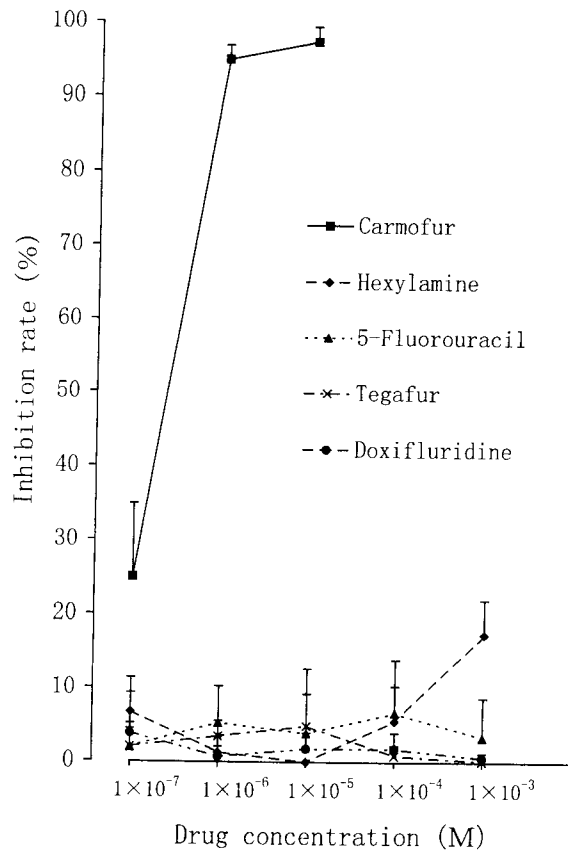
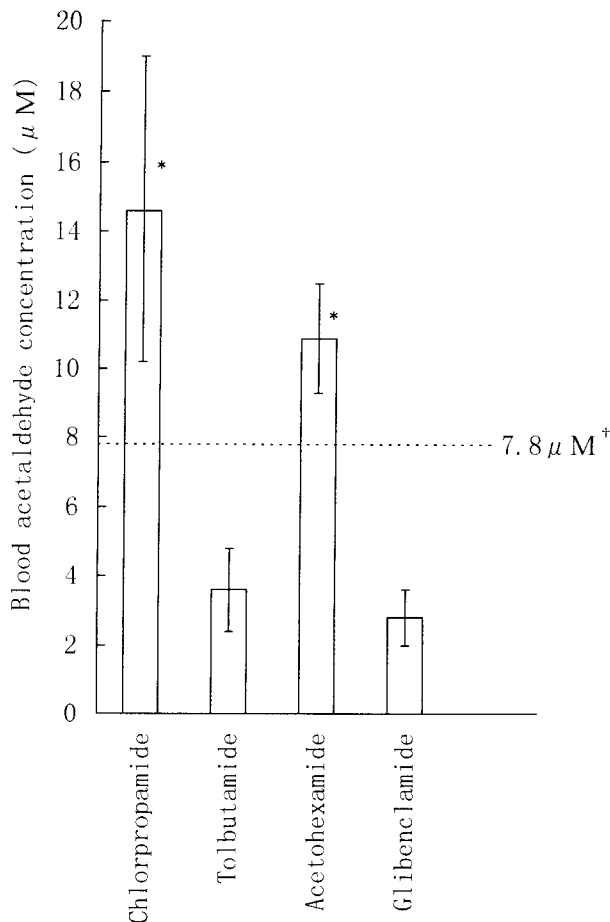


Fig. 3 (Left) Blood acetaldehyde concentrations in rats given 1.5g/kg ethanol orally 15h after treatment with 100mg/kg antidiabetics at 12h intervals for 3 days.

Blood samples were taken from the tail vein 1h after ethanol administration. Each bar represents the mean of 3 animals \pm SD. * Statistically significant compared with the value of negative control group ($n = 14$). † A tentative cut off level for significantly increased blood acetaldehyde concentration.

Fig. 4 (Right) Inhibition of aldehyde dehydrogenase (ALDH) activity by 5-fluorouracil antineoplastics and hexylamine. ALDH was added at the amount of 0.4 units. Each point represents the mean of 4 experiments \pm SD.

Table 2 Inhibitory effects of disulfiram and carmofur on aldehyde dehydrogenase (ALDH) activity

	Inhibition rate (%)				
	1 × 10 ⁻⁹	1 × 10 ⁻⁸	1 × 10 ⁻⁷	1 × 10 ⁻⁶	1 × 10 ⁻⁵ M*
Disulfiram	8.2 \pm 5.4	63.9 \pm 8.5	91.3 \pm 2.4	96.8 \pm 0.9	
Carmofur			25.0 \pm 10.0	95.0 \pm 1.9	97.4 \pm 2.0

* Drug concentration.

ALDH was added at the amount of 0.4 units.

Each value represents the mean of 4 experiments \pm SD.

effect was weaker than that of disulfiram. However, neither the side chain of carmofur, hexylamine, nor the

active metabolite of carmofur, 5-fluorouracil, inhibited ALDH activity (Fig. 4). Latamoxef, cefamandole, cefoperazone, cefbuperazone and MTT almost completely inhibited ALDH activity at 1 × 10⁻³M. A representative cephem antibiotic without the MTT group, cefotiam, inhibited ALDH activity by approximately 80% at 5 × 10⁻³M (Fig. 5). The inhibitory effects of acetohexamide and chlorpropamide on ALDH activity were similar to those of the cephem antibiotics containing the MTT group and cefotiam, respectively. Tolbutamide and glibenclamide inhibited ALDH activity by approximately 30% and 70%, respectively, at 5 × 10⁻³M (Fig. 6).

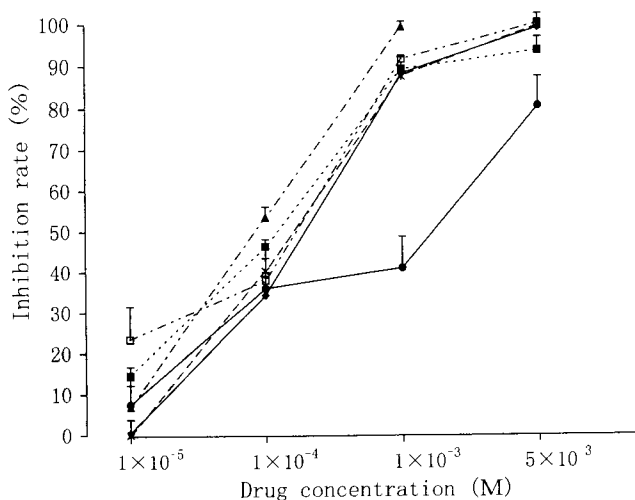


Fig. 5 Inhibition of ALDH activity by cephem antibiotics and methyltetrazolylthiol (MTT). ALDH was added at the amount of 0.4 units. ALDH: See Fig. 4. Each point represents the mean of 4 experiments + SD. —◆— Latamoxef, ...*... Cefamandole, ---■--- Cefoperazone, —▲— Cefbuperazone, —□— Methyltetrazolylthiol, —●— Cefotiam.

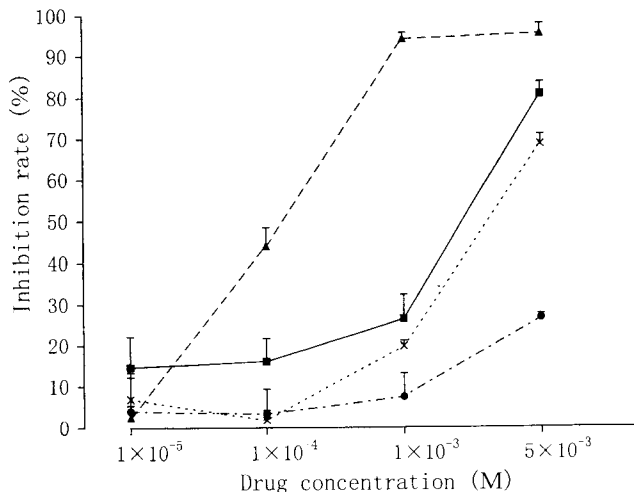


Fig. 6 Inhibition of ALDH activity by antidiabetics. ALDH was added at the amount of 0.4 units. ALDH: See Fig. 4. Each point represents the mean of 4 experiments + SD. —■— Chlorpropamide, ...●... Tolbutamide, —▲— Acetohexamide, ---x--- Glibenclamide.

Discussion

In measuring blood acetaldehyde concentrations by

head space gas chromatography, nonspecific oxidation of ethanol to acetaldehyde induced by the peroxidase-like actions of hemoglobin occurs during the procedure. Therefore, it has been reported that a method for analyzing the supernatant obtained by centrifugation following deproteinization with PCA is the best method for this kind of study (29). However, loss of acetaldehyde may occur during centrifugation. Therefore, blood samples were added to 0.6N PCA-physiological saline solution, rapidly agitated, and incubated without being centrifuged before head space gas chromatography. There was little nonspecific production of acetaldehyde. Both reproducibility and sensitivity were good. Therefore, this method was used to measure blood acetaldehyde concentrations because of its rapidity.

In this study, to identify drugs that markedly increase blood acetaldehyde concentrations when taken with alcohol among the many kinds of drugs tested in addition to 5-fluorouracil antineoplastics, cephem antibiotics containing the MTT group and antidiabetics, screening examinations were initially performed. After ethanol was administered to rats, blood acetaldehyde concentrations changed within a certain range until ethanol in blood completely disappeared (30). Therefore, the effect of each drug was evaluated based on blood acetaldehyde concentrations 1h after ethanol administration. After ethanol administration, blood acetaldehyde concentrations in rats were low, approximately 5 μM. However, there were day to day variations of 2 or 3 μM even in the same individuals (data not shown). In this study, blood acetaldehyde concentrations after administration of ethanol alone were measured in all rats treated with drugs. To determine whether or not the experimental drugs produced a significant increase in blood acetaldehyde concentration, a tentative cut off point of the mean value + 3SD was established.

Carmofur is metabolized to 5-fluorouracil in the liver. Four kinds of compounds with oxidized side chain have been reported as intermediate metabolites (31). Carmofur is rapidly absorbed after being orally administered to rats; the disappearance of carmofur and its metabolites from the blood and liver is slow (31). There were significant increases in blood acetaldehyde concentrations 1h after a single administration of carmofur to ethanol-treated rats. This may be because carmofur itself inhibited ALDH activity very strongly as demonstrated by our *in vitro* experiments. Furthermore, even when ethanol was administered 15h after a single administration of carmofur, blood acetaldehyde concentrations were significantly ele-

vated. This may have been caused primarily by the remaining carmofur itself, since neither the active metabolite, 5-fluorouracil, nor the side chain, hexylamine, inhibited ALDH activity. However, the possibility that the intermediate metabolites of carmofur inhibited ALDH activity cannot be excluded. Daily administration of carmofur increased blood acetaldehyde concentrations more significantly than a single administration of carmofur. This may be because liver concentrations of carmofur were increased, inhibiting ALDH activity more strongly. It has been noted that carmofur combined with alcoholic beverages may induce disulfiram-like reactions (25, 26). The results of this experiment suggest that the degree of this disulfiram-like reaction is very severe.

When an alcohol deterrent, cyanamide, used as a positive control for the inhibition of ALDH activity, was administered immediately after ethanol administration, blood acetaldehyde concentrations were significantly elevated at 1h. However, after a single administration of disulfiram, there were no rapid increases in blood acetaldehyde concentrations. When ethanol was administered 15h after disulfiram treatment, there were significant increases in blood acetaldehyde concentrations. Since disulfiram inhibited ALDH activity very strongly *in vitro*, delayed increases in blood acetaldehyde concentrations *in vivo* may be simply reflect the slow absorption of disulfiram in the digestive tract of rats.

The mechanism of increases in blood acetaldehyde concentrations related to cephem antibiotics with disulfiram-like actions is thought to involve inhibition of ALDH activity by the dimer of a metabolite, MTT (32). In this experiment, there were no increases in blood acetaldehyde concentrations even after a single administration of cephem antibiotics containing the MTT group to rats. This may be because the amount of accumulated MTT was small. MTT inhibited ALDH activity in an *in vitro* experiment. However, the inhibitory effect was much weaker than that of disulfiram or carmofur, which may be because MTT is in the form of monomer (16). The cephem antibiotics containing the MTT group and cefotiam without the MTT group slightly inhibited ALDH activity *in vitro*, suggesting that cephem antibiotics themselves inhibit ALDH activity regardless of the presence or absence of the MTT group, although the inhibitory effects are weak. Thus, increases in blood acetaldehyde concentrations after daily administration of the cephem antibiotics containing the MTT group may have been caused not only by accumulation of the dimer of MTT,

but also by accumulation of the parent drugs.

Sulfonylurea antidiabetics have also been reported to cause disulfiram-like actions when combined with alcohol (2). In this experiment, when alcohol was administered after daily administration of chlorpropamide and acetohexamide to rats, blood acetaldehyde concentrations were elevated. However, the degree of increase was much less than that after administration of cephem antibiotics containing the MTT group. Chlorpropamide increased blood acetaldehyde concentrations a little more than acetohexamide did, although the inhibitory effect of chlorpropamide on ALDH activity *in vitro* was weaker than that of acetohexamide. This may be because the plasma half-life (approximately 1.3h) of acetohexamide is much shorter than that (20–45h) of chlorpropamide (33). Accumulation of chlorpropamide in rat bodies may have been much greater than that of acetohexamide. One possible reason why the other two antidiabetics, tolbutamide and glibenclamide, caused no increases in blood acetaldehyde concentrations is that their inhibitory effects on ALDH activity *in vitro* were weaker than that of chlorpropamide, and that they may have accumulated in rat bodies to a much lesser degree than chlorpropamide did since their plasma half-lives are shorter than that of chlorpropamide (33).

In conclusion, this experiment demonstrated that an antineoplastic, carmofur, showed the strongest inhibitory effect on ALDH activity, followed by cephem antibiotics containing the MTT group and sulfonylurea agents used to treat diabetes. Our findings may be useful for evaluating interactions between ethanol and drugs in the practices of clinical and forensic toxicology.

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Received April 23, 1997; accepted September 24, 1997.