A Comparative Study on the Effects of Disulfiram and β-Lactam Antibiotics on the Acetaldehyde-Metabolizing System in Rats

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Abstract—Several β-lactam antibiotics, especially those containing N-methyl-tetrazolylthiomethyl groups at the 3-position of the cephalosporin nucleus, affect the alcohol-metabolizing system in rats. These effects were compared those with disulfiram, well-known as a potent inhibitor of aldehyde dehydrogenase (ALDH). Both disulfiram and antibiotics containing the N-methyltetrazolylthiomethyl group inhibited both mitochondrial low Km ALDH and acetaldehyde oxidation in rat livers. The high Km ALDH and alcohol dehydrogenase activities in livers were not affected by these treatments. When ethanol was given to rats pretreated with disulfiram or these antibiotics, the blood acetaldehyde concentration increased markedly concomitant with a decrease in activity of the low Km ALDH. Administration of N-methyltetrazolethiol alone suppressed the low Km enzyme activity and also increased the blood acetaldehyde level; both effects were pronounced and observed several hours after administration. β-Lactam antibiotics without N-methyltetrazolethiol in their molecule did not affect the liver mitochondrial enzyme activity or the blood acetaldehyde level.

Ethanol absorbed mainly from the digestive tract is first converted to acetaldehyde and then to acetic acid by liver enzymes. Enzyme systems that can catalyze the oxidation of ethanol to acetaldehyde in vitro are alcohol dehydrogenase (ADH), catalase and the microsomal ethanol-oxidizing system (MEOS); ADH is known to be the principal enzyme responsible for ethanol oxidation in the liver in vivo (1). Conversion of acetaldehyde to acetic acid is known to be catalyzed by aldehyde dehydrogenase (ALDH), which is present not only in the liver but also in other tissues (1). Many investigators have studied the factors affecting alcohol metabolism, and pyrazole and disulfiram have been shown to be potent inhibitors of ADH and ALDH, respectively (1–3).

Recent clinical work has shown that several β-lactam antibiotics cause a reaction similar to that of disulfiram when some, but not all, patients or volunteers treated with them ingest an alcohol-containing drink (4–11). Buening et al. and Yanagihara et al. have reported that some antibiotics, having an N-methyltetrazolylthiomethyl group at the 3-position of the cephalosporin nucleus, elevated blood acetaldehyde level during ethanol metabolism in rats (9, 12, 13). However, they did not check the alcohol-metabolizing enzyme activities. On the other hand, Yamanaka et al. (14) and Freundt et al. (15) reported on the inhibitory effects of several β-lactam antibiotics on ALDH and ADH, respectively. We thus chose to investigate the effects of several β-lactam antibiotics on the alcohol-metabolizing enzyme system and blood acetaldehyde level, and compared the findings with those for disulfiram. The findings presented here indicate a close correlation between the decrease in low Km ALDH activity in liver mitochondria and the increase in blood acetaldehyde level, when disulfiram and certain kinds of β-lactam antibiotics were administered.
Materials and Methods

Animals: Unless otherwise mentioned, Slc Wistar strain male rats (9 weeks old) were used. In some experiments, Jcl Sprague-Dawley (SD) strain male rats (9 weeks old) were also used. The animals were kept in an air-conditioned room (25±1 °C, 50-60% humidity) lighted 12 hr a day (8:00–20:00), and maintained on commercial rat chow (CA-1, Clea Japan, Inc., Tokyo) and water ad libitum. All animals were allowed at least 7 days to become acclimatized to the housing conditions prior to use in experiments.

Agents used: The structures of β-lactam antibiotics used in the experiments are depicted in Fig. 1. Cefamandole (CMD), moxalactam or latamoxef (LMOX), cephalothin (CET) and cefaclor (CCL) were obtained from Shionogi & Co. (Osaka), cefoperazone (CPZ) from Toyama Chemical Co. (Tokyo), cefmenoxime (CMX) and cefotiam (CTM) from Takeda Chemical Industries (Osaka), cefmetazole (CMZ) from Sankyo Co. (Tokyo), cefotetan (CTT) from Yamanouchi Pharmaceutical Co. (Tokyo) and cefazolin (CEZ) from Fujisawa Pharmaceutical Co. (Osaka). These antibiotics were dissolved in distilled water to the required potency concentrations just prior to administration, and the resulting solutions were administered subcutaneously at a dose of 2 ml/kg body weight. Only in the case of CCL was the antibiotics administered orally.

Disulfiram was suspended in 5% (w/v) arabic gum at 100 mg/ml and administered orally. Pyrazole was dissolved in distilled water at 34 mg/ml and administered intraperitoneally. N-Methyltetrazolethiol (NMTT) sodium salt (sodium 1-methyl-1H-tetrazole-...
5-thiolate) was dissolved in distilled water and the resulting solution was administered subcutaneously at a dose of 2 ml/kg body weight.

**Preparation of enzyme samples:** The animals were killed by decapitation and their livers quickly removed. Liver samples were homogenized in ice-cold 0.25 M sucrose containing 50 mM Tris-HCl buffer (pH 7.4), and the subcellular organelles of liver cells separated by differential centrifugation as described previously (16). The protein concentrations of the samples were determined by the method of Lowry et al. (17) using bovine serum albumin as a standard.

**Determination of enzyme activities:**

Aldehyde dehydrogenase activity was determined according to the method of Hasumura et al. (18) with slight modifications. The reaction mixture (3.0 ml) contained 50 mM pyrophosphate buffer (pH 8.5), 0.5 mM NAD, 2 μM rotenone (in methanol), 0.1 mM pyrazole, substrate (acetaldehyde) and the enzyme sample, and the absorbance change at 340 nm was recorded at 25 °C. To detect total and low Km enzyme activities, 5 mM and 180 μM acetaldehyde (at final concentrations), respectively, were added to the reaction mixture. High Km enzyme activity was obtained by calculating the difference between the total and low Km enzyme activities. The molar extinction coefficient of NADH employed to calculate the enzyme activity was 6.22×10³ M⁻¹ cm⁻¹ (19).

Alcohol dehydrogenase activity was measured as reported previously (20). Acetaldehyde oxidation activity in liver mitochondria was determined by detecting the decrease in acetaldehyde during the incubation. One milliliter of reaction mixture containing 68 mM pyrophosphate buffer (pH 8.5), 180 μM acetaldehyde, 0.75 mM NAD, 0.25 mg sodium deoxoycholate and liver mitochondria (about 1 mg protein) was incubated in a glass vial with an airtight puncture-type cap. The reaction was initiated by adding acetaldehyde through the rubber cap and the reaction mixture was incubated at 37°C for 3 min. Next, the reaction was terminated by adding 0.5 ml of 1 M perchloric acid (PCA) through the rubber cap. The amount of acetaldehyde in the vial was determined by the head-space gas chromatographic method described below.

**Determination of ethanol and acetaldehyde concentrations in blood:** Rats, fasted overnight, were given orally a 20% (w/v) solution of ethanol in water at 1.0 ml/100 g body weight. Blood samples from decapitated rats were collected in heparin-containing tubes 1 hr after ethanol treatment, and then the samples were treated essentially as described below. Blood (1.5 ml) was mixed with 4.5 ml of ice-cold 0.2% (w/v) deoxycholate (in physiological saline) followed by 1.5 ml of 1.5 N PCA. After centrifugation, a 2-ml aliquot of the deproteinized supernatant was transferred to a 15-ml glass vial fitted with an airtight puncture-type cap. The samples were tightly capped immediately after collection or transfer. In some experiments, 4-ml blood was mixed with 4 ml ice-cold distilled water followed by 4 ml of ice-cold 1.2 N PCA. After centrifugation, 1.5 ml of the resulting supernatant was transferred to the glass vial for analysis. The vials were then heated in a water bath at 64°C for 15 min, and 1 ml of the gas phase was collected with heated syringe and injected into a gas chromatograph. Analytical conditions for gas chromatography were as follows: instrument, Shimadzu GC-4CM-PF; column, chromosorb 101 (60/80 mesh, 3 mm i.d. ×100 cm); detector, FID; column temperature, 125°C; injector and detector temperature, 150°C; carrier gas, He; flow rate, 33 ml/min. Known amounts of ethanol and acetaldehyde were added to blood obtained from untreated rats and analyzed as described above. The concentrations of ethanol and acetaldehyde in the samples were calculated by comparing the peak areas with those of the standard.

**Chemicals:** Sodium salt of NMTT was prepared in this laboratory. NAD(H) was purchased from Oriental Yeast Co. (Tokyo). Disulfiram and pyrazole were obtained from Wako Chemical Industries (Osaka). Other chemicals, of the purest grade available, were obtained commercially and used for the experiments without further purification.

**Results**

Subcellular localization of alcohol-
metabolizing enzymes in rat liver cells: Subcellular distribution of enzymes participating in alcohol-metabolism in rat liver cells was studied to determine the suitable fractions to use for further studies. Low $K_m$ ALDH activity was distributed throughout the membranous fractions, with the highest being in the mitochondrial fraction. The cytosolic fraction also showed the low $K_m$ enzyme activity, although the activity was very low. On the other hand, high $K_m$ enzyme activity was detected mainly in microsomal and mitochondrial fractions. The activity of ADH was detected in cytosolic fraction. These results were almost the same as those reported previously (1, 21–24).

Hepatic alcohol-metabolizing enzyme activities in pyrazole- and disulfiram-treated rats were compared with those in control rats. Pyrazole administration resulted in a marked decrease of ADH activity in the cytosol, but no alteration was observed in the ALDH activities (Table 1). On the other hand, administration of disulfiram caused a decrease in low $K_m$ ALDH activity, but not in high $K_m$ enzyme activity, in both mitochondrial and cytosolic fractions (Table 1). These results are consistent with previous reports (1–3, 25). We thus concluded from the results shown in Table 1 and the data of subcellular enzyme distribution that mitochondria, microsomes and cytosol would be useful as enzyme sources to examine the effect of several drugs on the alcohol-metabolizing enzyme system.

Decrease of aldehyde dehydrogenase (ALDH) and acetaldehyde oxidation activities by disulfiram in vivo: The time course of the decrease of mitochondrial ALDH activity in rat liver was studied after oral administration of disulfiram, which caused a slow onset of decrease in mitochondrial low $K_m$ ALDH activity that reached a maximal effect 18 hr to 2 days after the treatment. The activity recovered gradually, reaching normal levels 7 days after treatment (Fig. 2, A). Acetaldehyde oxidation activity by mitochondrial enzyme(s) showed a pattern similar to that of low $K_m$ ALDH activity (Fig. 2, B). On the other hand, no alteration was observed in mitochondrial high $K_m$ ALDH and cytosolic ADH activities during the experimental period (data not shown). When ethanol was administered to disulfiram-pretreated animals, the blood acetaldehyde level increased markedly, concomitant with a decrease in the low $K_m$ ALDH activity. The highest concentration of acetaldehyde was observed when ethanol was administered 18 hr after the disulfiram treatment (Fig. 2, C). Ethanol administration 2 days after the disulfiram treatment did cause increase in the blood acetaldehyde level, but to a lesser extent. On the other hand, ethanol concentration in the blood was not affected

| Table 1. Effects of pyrazole and disulfiram administrations on alcohol-metabolizing enzyme activities in rat livers |
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| **Experiment I** | **Experiment II** |
| | | Control rat | Pyrazole-treated rat | Control rat | Disulfiram-treated rat |
| **Low $K_m$ ALDH activity** | | | | |
| Mitochondria | 16.66±1.75 | 14.03±0.60 | 16.01±0.72 | 3.76±0.43** |
| Cytosol | 2.47±0.22 | 2.03±0.26 | 2.93±0.38 | 1.04±0.12* |
| **High $K_m$ ALDH activity** | | | | |
| Mitochondria | 23.89±0.50 | 20.93±0.36 | 23.95±0.72 | 23.26±0.42 |
| Microsome | 58.13±0.11 | 54.80±1.50 | 66.58±1.30 | 59.39±2.41 |
| Cytosol | 2.25±0.25 | 3.10±0.46 | 1.81±0.30 | 2.89±0.52 |
| **ADH activity** | | | | |
| Cytosol | 118.9±1.8 | 66.3±5.9* | 102.8±7.4 | 117.6±4.5 |

Animals were given disulfiram (500 mg/kg, p.o.) or pyrazole (68 mg/kg, i.p.), and the liver samples were obtained 18 or 2 hr, respectively, after the administration. The activities are expressed as "nmoles/min/mg protein", and the values in the Table represent the mean±S.E. of 3 animals. *Statistically significant (P<0.05) against the control. **Statistically significant (P<0.01) against the control.
Fig. 2. Time course of the inhibitory effect of disulfiram on the alcohol metabolizing system. The animals were orally given disulfiram at a dose of 1,000 mg/kg and killed 3 hr to 7 days after the treatment. The activities of low \( K_m \) ALDH (A) and of in vitro acetaldehyde oxidation reaction (B) were determined using the isolated liver mitochondrial fraction as described in “Methods”. Disulfiram-pretreated animals were received ethanol (2,000 mg/kg, p.o.) 1 hr to 5 days after the disulfiram treatment as shown in the figure and their blood samples were obtained 1 hr after the ethanol administration. Blood acetaldehyde level (C) were determined as given in “Methods”. Each value represents the mean and standard error of 5 rats. *, **: significantly different from the corresponding control, \( P<0.05 \) and \( P<0.01 \), respectively.

Effect of cefamandole (CMD) on alcohol-metabolizing system in rats: Recently, some \( \beta \)-lactam antibiotics were found to exhibit a disulfiram-like activity in alcohol metabolism (4-11). Thus, the in vivo effects of \( \beta \)-lactam antibiotics on the alcohol-metabolizing system in rats were investigated using CMD as a model of \( \beta \)-lactam antibiotics. Rats were given a single subcutaneous administration of a high dose of CMD and the change of low \( K_m \) ALDH activity was investigated using isolated mitochondria. As shown in Fig. 3, A, CMD administration caused a slow onset of decrease in enzyme activity reaching maximal inhibition 18 hr to 48 hr after CMD treatment. The acetaldehyde oxidation activity of mitochondrial enzyme(s) displayed similar changes in activity, although a much more rapid fall in the activity was observed after treatment (Fig. 3, B). When ethanol was administered to CMD-pretreated rats 18-48 hr after the treatment, the blood acetaldehyde concentration increased remarkably (Fig. 3, C), but the blood concentration of ethanol was not altered (data not shown). The return to normal levels of the enzyme activities and the blood acetaldehyde level in CMD-treated rats after ethanol administration (Fig. 3) followed basically the same time schedule as that in the rats treated with disulfiram (Fig. 2). When LMOX, CMZ or CPZ was employed instead of CMD, the same pattern of effect on the alcohol-metabolizing system was observed (T. Matsubara et al., unpublished results).

As shown previously, multiple forms of ALDH with different subcellular localization and affinities for acetaldehyde have been found in rat liver by many investigators (21-24, 26, 27). Thus, the effect of CMD administration on ALDH activity was studied using various subcellular fractions of CMD-treated rat livers. A dose-dependent decrease in low \( K_m \) ALDH activity was observed in both mitochondrial and cytosolic fractions (Fig. 4A), while the high \( K_m \) enzyme activity in
Fig. 3. Time course of the effect of cefamandole on the alcohol metabolizing system in rats. Experimental conditions were almost the same as those in the legend of Fig. 2, except CMD (1,000 mg/kg) was administered subcutaneously instead of disulfiram. Figures (A), (B) and (C) indicate low Km ALDH activity, acetaldehyde oxidation activity and blood acetaldehyde concentration, respectively. Each value represents the mean and standard error of 4-5 rats. *, **: significantly different from the corresponding control, P<0.05 and P<0.01, respectively.

Fig. 4. Effect of various doses of cefamandole (CMD) on rat liver aldehyde dehydrogenase activity. Animals were subcutaneously administered CMD and their livers were obtained 18 hr later. The low Km and high Km ALDH activities in various subcellular fractions were determined and plotted as a function of CMD doses. Figure (A) indicates the low Km ALDH activity, and open and closed circles represent the mitochondrial and cytosolic enzyme activities, respectively. Figure (B) indicates the high Km ALDH activity, and open and closed circles represent the mitochondrial and microsomal enzyme activities, respectively. Each value represents the mean and standard error of 3 rats. *, **: significantly different from the corresponding control, P<0.05 and P<0.01, respectively.
both mitochondria and microsomes was not affected by CMD (Fig. 4B). Cytosolic ADH activity was also unchanged in the treated rats. The results shown in Figs. 3 and 4 indicate that CMD inhibits liver low K_m ALDH activity, which is then followed by the decrease in acetaldelyde oxidation activity. Administration of ethanol to the CMD-pretreated rats causes an increase in blood acetaldelyde concentration.

Effect of multiple administrations of CMD on low K_m ALDH and acetaldelyde oxidation activities in liver mitochondria: As described above, the effect of single administration of higher doses of CMD on the low K_m ALDH was long-lasting, with maximal effects at 1–2 days after the treatment (Fig. 3). We, therefore, thought that the inhibitory effect of CMD on the low K_m ALDH would be more pronounced if the animals were given multiple administrations of CMD, so the animals were given a higher dose of CMD (1,000 mg/kg/day) for 3 consecutive days. Liver mitochondrial low K_m ALDH and acetaldelyde oxidation activities were then estimated, for comparison with the data obtained after a single administration. A marked decrease in the enzyme activities was observed 1 day after the final dose of CMD: return of the activities followed approximately the same time schedule as in the case of a single administration (Fig. 5, A and B). When ethanol was given to rats given successive doses of CMD, the blood acetaldelyde level increased markedly 1 day after the final dose, and then the elevated acetaldelyde level fell gradually to the normal range (Fig. 5, C). These results indicate that the recovery process after suppression of low K_m ALDH by multiple administrations of CMD does not differ much from suppression by a single dose.

Comparative study on the effects of several β-lactam antibiotics on the alcohol-metabolizing system in rats: As mentioned above, treatment of animals with either disulfiram or CMD caused a marked decreases in the mitochondrial low K_m ALDH and acetaldelyde oxidation activities in livers. In addition, the concentration of acetaldelyde in the blood increased markedly when ethanol was given 18–24 hr after the rats had been treated with disulfiram or CMD (Figs. 2 and 3). The results indicate that the effect of various β-lactam antibiotics upon the alcohol-metabolizing system can be detected by measuring these parameters. As shown in Table 2, administrations of CPZ, CMX, CMZ, CTT and LMOX suppressed both liver mitochondrial low K_m ALDH and acetaldelyde oxidation activities, as was the case with disulfiram and CMD. All these β-lactam antibiotics possess NMTT as a common substituent of the 3-position of the cephalosporin nucleus (Fig. 1), and NMTT is thought to affect the acetaldelyde-metabolizing system. In fact, subcutaneous administration of NMTT to rats also suppressed enzyme
Table 2. Effects of administration of various \(\beta\)-lactam antibiotics and related compounds on mitochondrial acetaldehyde-metabolizing system and blood acetaldehyde level

| Expt group | Treatment of rat | Dose (mg/kg) | Activity (nmol/min/mg protein) | Blood acetaldehyde level (nmol/ml) |
|------------|-----------------|-------------|-------------------------------|----------------------------------|
|            |                 |             | Low K_m ALDH | Acetaldehyde oxidation |                                 |
| 1          | Control         | —           | 12.92±0.12 (4) | 29.16±0.78 (4) | 24.5±2.1 (5) |
|            | CMD             | 1,000       | 5.00±0.20** (4) | 9.04±0.59** (4) | 85.5±3.4** (5) |
| 2          | Control         | —           | 16.37±0.42 (4) | 36.15±1.99 (4) | 26.8±1.0 (5) |
|            | CPZ             | 1,000       | 5.16±0.19** (4) | 13.89±1.30** (4) | 96.3±8.9 (5) |
| 3          | Control         | —           | 16.17±0.75 (4) | 32.48±1.48 (4) | 18.4±1.9 (4) |
|            | CMX             | 1,000       | 6.18±0.23** (4) | 14.91±0.34** (4) | 148.6±25.4** (4) |
| 4          | Control         | —           | 23.04±1.05 (4) | 40.23±3.37 (4) | 17.3±1.3 (4) |
|            | CMZ             | 1,000       | 9.04±0.57** (4) | 18.21±1.23** (4) | 63.3±6.2** (5) |
| 5          | Control         | —           | 11.14±0.50 (4) | 27.25±0.89 (4) | 6.6±1.7 (4) |
|            | CTT             | 1,000       | 5.30±0.28** (4) | 14.26±1.27** (4) | 84.6±12.5** (4) |
| 6          | Control         | —           | 12.92±0.44 (4) | 37.07±0.72 (4) | 27.1±1.4 (5) |
|            | LMOX            | 1,000       | 5.66±0.17** (5) | 20.85±0.79** (5) | 136.1±3.8** (6) |
| 7          | Control         | —           | 10.80±0.22 (4) | n.d. | 17.3±1.3 (5) |
|            | CTM             | 1,000       | 12.11±0.38* (4) | n.d. | 21.0±1.0 (5) |
| 8          | Control         | —           | 11.73±0.25 (4) | 26.96±0.97 (4) | 17.3±1.3 (5) |
|            | CEZ             | 1,000       | 14.28±0.50** (4) | 31.55±2.03 (4) | 22.0±1.0 (5) |
| 9          | Control         | —           | 10.39±0.35 (4) | 32.03±0.95 (4) | 18.7±1.9 (4) |
|            | CET             | 1,000       | 11.35±0.67 (4) | 33.77±0.86 (4) | 20.3±2.8 (4) |
| 10         | Control         | —           | 11.73±0.25 (4) | 26.96±0.97 (4) | 9.4±0.3 (4) |
|            | CCL             | 1,000       | 15.50±0.66** (4) | 32.75±1.26 (4) | 14.9±1.8* (4) |
| 11         | Control         | —           | 12.44±0.70 (4) | 33.23±1.82 (4) | 17.3±1.3 (5) |
|            | NMTT            | 300         | 3.76±0.11** (4) | 11.04±1.26** (4) | 134.3±6.6** (5) |
| 12         | Control         | —           | 12.92±0.44 (5) | 37.07±0.72 (5) | 27.1±1.4 (5) |
|            | Disulfiram      | 500         | 3.53±0.22** (5) | 14.32±0.62** (5) | 213.4±25.6** (5) |

Most of the test compounds were given to rats subcutaneously, except for CCL and disulfiram which were given orally. Liver samples for determining the mitochondrial enzyme activities were obtained 18–24 hr after drug administration. To determine blood acetaldehyde concentration, ethanol was given orally at a dose of 2,000 mg/kg 18 hr after drug treatment, and blood samples were obtained 1 hr after the ethanol administration, except in the case of NMTT treatment, in which liver sampling and ethanol administration were done 5 hr after the treatment. The values in the Table represent the mean±S.E., and the numbers in parentheses indicate the number of animals used for the experiments. n.d.: not determined. *, **: statistically significant (P<0.05 and P<0.01, respectively) against the control.

activities, although several antibiotics (CTM, CEZ, CET and CCL) which do not contain NMTT as a substituent (Fig. 1) failed to affect the acetaldehyde-metabolizing enzyme activities (Table 2). Elevated blood acetaldehyde levels following administration of ethanol was observed when the animals were pretreated only with NMTT-containing antibiotics (Table 2).

The dose-response curve for single or multiple injections of several \(\beta\)-lactam antibiotics and disulfiram on acetaldehyde concentration in blood after administration of ethanol is shown in Fig. 6. When animals received either single or multiple injections of drugs, the dose-dependent increase in blood acetaldehyde concentration was observed with most of the drugs tested,
Fig. 6. Dose-response curve for the effects of β-lactam antibiotics and disulfiram on blood acetaldehyde concentrations in rats. The animals (SD strain rats) were given subcutaneous single administration of various amounts of antibiotics or disulfiram as shown in the figure (open circle), and ethanol was given 18 hr after the administration. Only in the case of disulfiram, animals were received the compound orally. In the multiple administration groups (closed circle), the animals were given antibiotics or disulfiram twice a day for 7 days, and then ethanol was administered 18 hr after the last administration. Dose of each injection was a half amount of daily dosage. Chemicals employed were (A) disulfiram, (B) CMD, (C) CMZ, (D) CPZ, (E) LMOX and (F) CEZ. Each value represents the mean and standard error of 5 animals. *, **: statistically significant (P<0.05 and P<0.01, respectively) against the control.

Discussion

Disulfiram-ethanol reactions, including flushing, headache and nausea, are considered to be due to increased acetaldehyde level in the body (28). Recently, disulfiram-like reactions were observed clinically in patients and in healthy volunteers who ingested ethanol after they had been given some newly developed β-lactam antibiotics (4–11, 29). Subsequently, an increase of the blood acetaldehyde level was demonstrated in rats pretreated with the NMTT-containing β-lactam antibiotics (9, 12, 13, 30). Yamanaka et al. reported the inhibition of mitochondrial low K_m ALDH activity in rat livers following the administration of these antibiotics (14). These reports indicate that some newly synthesized β-lactam antibiotics containing NMTT in their molecules affect the liver alcohol-metabolizing system and produce a “disulfiram-like reaction.” We thus compared the effect of disulfiram and CMD on liver alcohol-metabolizing enzyme system. Although ADH and high K_m ALDH activities in rat livers were not affected by the administration of CMD, low K_m ALDH activity in both mitochondria and cytosol showed a dose-dependent decrease (Fig. 4) similar to that

except CEZ. Interestingly, the dose-dependent increase of blood acetaldehyde concentration was almost the same in both treatment groups, i.e. those receiving either single or multiple injections of the drugs, although the increase of blood acetaldehyde level was more pronounced in those animals treated repeatedly with CMZ and CPZ (Fig. 7). Single or multiple administrations of various amounts of NMTT also caused dose-dependent elevation of blood acetaldehyde concentration; the changes in acetaldehyde concentration were similar for both the single and the multiple treatment groups (data not shown).
seen in the disulfiram-treated rats (Table 1). The time course of the suppression and recovery of the ALDH activity after the administration of NMTT-containing antibiotics was almost the same as that seen with disulfiram, with long action and the maximal effect 18–24 hr after administration (Figs. 2 and 3). Acetaldehyde oxidation activity in liver mitochondria showed a pattern similar to that of the low $K_m$ enzyme activity, and elevated blood acetaldehyde levels were observed when ethanol was administered to those rats showing lower enzyme activity (Figs. 2 and 3). These results clearly indicate that the principal effective site of disulfiram or CMD is the low $K_m$ ALDH, and the decreased enzyme activity causes the increase of acetaldehyde level in the body. The effect of single or multiple doses of CMD on the alcohol-metabolizing system in rats was dose-dependent; similarly, single and multiple doses of other antibiotics showed almost identical effects (Figs. 3–6). These results indicate that most likely a disulfiram-like reaction to $\beta$-lactam antibiotics will be detectable in rats with only a single dose of antibiotics.

Recovery following suppression of low $K_m$ enzyme activity after single and multiple doses of CMD (Figs. 3 and 4) was almost the same as that following disulfiram-treatment (Fig. 2). Since the suppression of low $K_m$ ALDH activity by disulfiram appears to be irreversible (31, 32), the slow recovery of enzyme activity might indicate the biosynthesis of new enzyme. It is interesting to note that the time required to normalize the mitochondrial low $K_m$ ALDH and acetaldehyde oxidation activities in rat livers was about 5–7 days following the administration of disulfiram or NMTT-containing $\beta$-lactam antibiotics, while the blood acetaldehyde level following ethanol treatment was normalized by 3–5 days after drug administration (Figs. 2, 3 and 5). We cannot presently explain the discrepancy between the low $K_m$ enzyme activity and the concentration of acetaldehyde in blood. The ALDH enzyme is localized not only in the liver but also in some other organs (1), and thus one possible explanation is that the enzyme(s) in other organs in addition to the liver may participate in the metabolism of acetaldehyde. This is a subject needing further study.

The relationship between the chemical structure of the antibiotics and their suppression of low $K_m$ ALDH activity is shown in Fig. 1 and Table 2. Antibiotics containing NMTT at the 3-position of the cephalosporin nucleus suppressed enzyme activity, as pointed out by Yamanaka et al. (14). These antibiotics have different substituents at 7-position of the nucleus and also have different nuclear structures (cephem and oxa-cephem), so these chemical structures are considered not to be participating in the development of the disulfiram-like reaction. As for the 3-position substituents, it is those antibiotics containing NMTT which seem to affect the alcohol-metabolizing system. Interestingly, CTM, whose 3-position is occupied by a substituent resembling NMTT (1-(2-dimethylamino)ethyl-1H-tetrazole-5-thiol) had no effect on the alcohol-metabolizing system (Table 2). Further studies on the structure-activity relationship of $\beta$-lactam antibiotics will be reported elsewhere.

The present investigation demonstrated that, like disulfiram, NMTT-containing $\beta$-lactam antibiotics inhibit liver mitochondrial low $K_m$ ALDH and acetaldehyde oxidation activities. The decreased enzyme activity causes a rise in blood acetaldehyde concentration if ethanol is administered 1–2 days after the antibiotic treatment. Recovery from the suppressed acetaldehyde-metabolizing activity after antibiotic injection is slow, requiring 5–7 days. Therefore, patients should be warned to avoid alcohol for several days after they are treated with NMTT-containing $\beta$-lactam antibiotics.

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