Effects of Alcohol-Metabolizing Enzyme Inhibitors and Beta-Lactam Antibiotics on Ethanol Elimination in Rats

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Abstract—The in vivo effects of alcohol-metabolizing enzyme inhibitors and beta-lactam antibiotics upon the ethanol elimination rate were examined in rats. Intravenous administration of ethanol caused a dose-dependent increase in blood ethanol level, and the ethanol elimination could be well described by a two compartment model. Pretreatment of rats with enzyme inhibitors caused a marked decrease in the ethanol elimination rate associated with the depression of the enzyme activities. Fasting of the animals caused a decrease in the ethanol elimination rate per animal associated with a decrease in the liver weight. However, no alteration was evident when the rate was expressed as the rate per g of liver. When animals were pretreated with a high dose of N-methyltetrazolethiol (NMTT)- containing beta-lactam antibiotics or NMTT itself, which causes a disulfiram-like reaction, the ethanol elimination rate per animal was depressed concomitant with an increase in the blood acetaldehyde level. The ethanol elimination rate in these animals showed lower values even when expressed as the rate per g liver. On the other hand, administration of cephems without NMTT, which cause no disulfiram-like reaction, led to a slight decline in the elimination rate per animal, although no alteration was detected when the rate was expressed as the rate per g liver. The findings indicated that the ethanol elimination in vivo per animal is regulated by the total capacity of the alcohol-metabolizing enzyme activities in the whole liver.

Disulfiram and calcium carbimide have been available for adjunctive use in the treatment of alcoholism (1). These compounds specifically inhibit hepatic aldehyde dehydrogenase (EC 1.2.1.3, ALDH), which is an enzyme primarily involved in the metabolism of ethanol-derived acetaldehyde to acetic acid (2). Recently, several beta-lactam antibiotics, having an N-methyltetrazolylthiomethyl group at the 3-position of the cephem nucleus, were reported to display a reaction similar to that of disulfiram (3–6). Subsequent studies showed that hepatic low Km ALDH activity was depressed markedly by the administration of these antibiotics (7–10). These results indicate that the administration of these antibiotics leads to a depression of liver low Km ALDH activity, which causes an increase in the blood acetaldehyde level during alcohol metabolism.

Freundt et al. (11) demonstrated that high concentrations of beta-lactam antibiotics, containing N-methyltetrazolethiol (1-methyl-1H-tetrazole-5-thiol, NMTT) as the 3'-position substituent of the cephem nucleus, noncompetitively inhibit alcohol dehydrogenase (EC 1.1.1.1, ADH) isolated from rat liver. Turcan et al. (12) reported the inhibition of 14CO2 production from [14C]-ethanol by several antibiotics. These findings suggest that the effect of beta-lactam anti-
biotics on the alcohol-metabolizing system should be checked by determining the blood ethanol level as well as liver ALDH activity and the blood acetaldehyde level. In fact, some investigators have been employing blood ethanol levels to check the disulfiram-like effects of antibiotics (5, 13-15). However, few reports have demonstrated the effect of beta-lactam antibiotics on blood ethanol elimination. Thus, we studied the effects of several factors, including antibiotics, on blood ethanol elimination. This report provides evidence that liver mass and alcohol-metabolizing enzyme activities are major factors in the regulation of blood ethanol elimination.

Materials and Methods

Animals: Unless otherwise stated, 9 weeks old Slc Wistar strain male rats were used for the experiments. 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 they were 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 aclimatized to the housing conditions prior to use in the experiments.

Antibiotics and chemicals: Cefamandole (CMD), latamoxef (LMOX) and flomoxef sodium (6315-S, FMOX) were obtained from Shionogi & Co. (Osaka) and cefotiam (CTM) from Takeda Chemical Industries (Osaka). Disulfiram and pyrazole were purchased from Wako Pure Chemical Industries (Osaka). Sodium salts of NMTT and hydroxyethyltetrazolethiol (1-[2-hydroxyethyl]-1H-tetrazole-5-thiol, HTT) were prepared in this laboratory. The antibiotics were dissolved in distilled water and administered intravenously or subcutaneously at a dose of 1,000 mg/kg. NMTT-Na or HTT-Na was dissolved in distilled water and administered subcutaneously at 200 mg/kg (as a free form). Disulfiram and pyrazole were purchased from Wako Pure Chemical Industries (Osaka). Sodium salts of NMTT and hydroxyethyltetrazolethiol (1-[2-hydroxyethyl]-1H-tetrazole-5-thiol, HTT) were prepared in this laboratory. The antibiotics were dissolved in distilled water and administered intravenously or subcutaneously at a dose of 1,000 mg/kg. NMTT-Na or HTT-Na was dissolved in distilled water and administered subcutaneously at 200 mg/kg (as a free form). Disulfiram was suspended in 5% (w/v) arabic gum and administered orally at 500 or 1,000 mg/kg. and pyrazole dissolved in distilled water was given intraperitoneally at a dose of 68 mg/kg.

Determination of enzyme activity: The animals were killed by decapitation, and their livers were 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 fractions were prepared by differential centrifugation as described previously (15, 16). Liver ALDH activity was determined at 25°C according to the method of Hasumura et al. (17) with slight modifications (10). ADH activity was measured at 25°C as reported previously (18). The protein concentration of the subcellular fractions was determined by the method of Lowry et al. (19) using bovine serum albumin as a standard.

Determination of ethanol and acetaldehyde concentrations in blood: Animals were given orally various doses of ethanol, and blood samples from decapitated rats were collected in heparin-containing tubes. Treatment of samples and gas chromatographic analyses of ethanol and acetaldehyde in blood samples were performed essentially as described previously (10). Isopropanol was added to the blood sample as the internal standard. In some experiments, animals were given intravenously 20% (w/v) ethanol in water at a dose of 1,000 mg/kg, and their blood samples (about 0.2 ml) were obtained periodically from the orbital vein. Blood (0.2 ml) was mixed with 2.5 ml of ice-cold 0.2% (w/v) deoxycholate (in physiological saline) followed by 1.0 ml of 2 M perchloric acid. Isopropanol was also added as the internal standard. After centrifugation at 1,000×g for 10 min, a 2-ml aliquot of the supernatant was transferred to a 15-ml glass vial fitted with an airtight puncture-type cap, and the concentrations of ethanol and acetaldehyde in these vials were determined by head-space gas chromatography (10).

Estimation of pharmacokinetic parameters: In the case of ethanol injection, the blood ethanol concentrations from individual rats were fitted to the following equation derived from a two compartment model with the Michaelis-Menten kinetic elimination process as shown in Chart 1 (20):

\[
-dC_1/dt = \left[ V_m/(K_m + C_i) + k_{12} \right] C_1 - k_{21} (V_2/V_1) C_2
\]

where \(-dC_1/dt\) is the rate of the decline of the blood ethanol concentration in com-
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Compartment 1 includes blood and rapidly equilibrating tissue and compartment 2, slowly equilibrating tissue. "k_{ij}" represents the apparent intercompartmental transfer rate constants.

Blood ethanol and acetaldehyde levels: Blood ethanol and acetaldehyde levels in normal and disulfiram-pretreated rats were determined after the oral administration of various amounts of ethanol. Increasing doses of ethanol resulted in dose-dependent increases in blood ethanol concentrations, with saturation of the increase occurring at the dose of 2,000 mg/kg. This suggested saturation of the absorption process at higher doses of ethanol. Blood ethanol level in disulfiram-pretreated rats showed the same pattern as that in normal rats (Fig. 1, solid line curve). The blood acetaldehyde level was negligibly low in normal rats with the administration of 3,000 mg/kg ethanol (Fig. 1, dashed line curve). Treatment of rats with disulfiram caused a marked decrease in the mitochondrial low Km ALDH activity (Table 1, Exptl group 1), and the blood acetaldehyde level increased dose-dependently when ethanol was given 18 hr after the disulfiram pretreatment. Interestingly, the blood acetaldehyde level in the disulfiram-pretreated rats showed saturation at the dose of 1,000 mg/kg of ethanol, indicating the probability of rate-limiting regulation in the conversion of ethanol to acetaldehyde. Administration of pyrazole resulted in a decrease in cytosolic ADH activity without alteration of the low Km ALDH activity (Table 1). When ADH and low Km ALDH activities were suppressed by the administration of both pyrazole and disulfiram, no increase in blood acetaldehyde level was observed even after the administration of a high dose (2,000 mg/kg) of ethanol (data not shown).

The time course of changes of blood ethanol and acetaldehyde levels after the oral administration of saturated amounts (2,000 mg/kg) of ethanol was studied in normal and disulfiram-pretreated rats. Blood ethanol levels in normal rats peaked at about 60 min after the oral administration of ethanol, and then the concentration decreased gradually with time. A similar pattern...
in the time course was detected in disulfiram-pretreated rats when ethanol was given 48 hr after the disulfiram treatment. The oral administration of ethanol 5 hr after disulfiram caused lower blood ethanol level even at the peak point (Fig. 2A). The results suggest that ethanol absorption was modified markedly by the contents in the gastrointestinal tract. When fed and fasted rats were given ethanol orally, a peak of blood ethanol level was observed on both fed and fasted rats at about 60 min after the administration, but a much higher ethanol level was detected in the fasted rats (data not shown). This supports the above assumption. On the other hand, the blood acetaldehyde level in the disulfiram-pretreated rats increased markedly depending on the decrease in the

Table 1. Alcohol dehydrogenase and aldehyde dehydrogenase activities in rats of various states

| Exptl group | Treatment of rat | Mitochondrial ALDH | Microsomal ALDH | Cytosolic ADH |
|-------------|-----------------|---------------------|-----------------|--------------|
|             |                 | low K_m ALDH | high K_m ALDH | high K_m ALDH |             |
| 1           | None (control)  | 15.27±0.84       | 20.48±0.54     | 44.29±0.75   | 52.63±3.78  |
|             | Disulfiram      | 3.06±0.13**      | 18.14±1.08     | 34.55±0.53** | 52.74±4.46  |
|             | Pyrazole        | 15.58±0.78       | 20.74±0.42     | 41.84±0.95   | 34.07±3.96* |
| 2           | None (fasted)   | 14.48±0.87       | 22.22±0.30     | 45.79±0.68   | 47.47±2.26  |
|             | Fasting         | 16.31±0.53       | 18.16±0.54**   | 36.65±1.42** | 62.14±2.50**|

Animals in Exptl group 1 were given disulfiram (1,000 mg/kg, p.o.) or pyrazole (68 mg/kg, i.p.), and their liver samples were obtained 18 hr or 30 min later, respectively. In Exptl group 2, liver samples were obtained from control rats (fed) and animals which had fasted for 2 days. Activities of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in subcellular fractions of liver cells were measured as described in "Methods". The values in the table represent the mean±S.E. of 4 animals. *, **: statistically significant (P<0.05 and P<0.01, respectively) against the control.

Fig. 2. Time course in the changes of blood ethanol and acetaldehyde levels after oral administration of ethanol. Animals were given orally disulfiram (1,000 mg/kg) and then ethanol (2,000 mg/kg) 5 or 48 hr later. Control rats were given ethanol alone. All animals were fasted overnight prior to the administration of ethanol. Blood samples were obtained 0.5 to 4 hr after the ethanol treatment, and ethanol (A) and acetaldehyde (B) concentrations were determined. Each value in the figure represents the mean and standard error of 4 animals. *, **: Statistically significant (P<0.05 and P<0.01, respectively) against the control.
low $K_m$ ALDH activity (Fig. 2B). This indicates that the oral administration of ethanol is not suitable for analyzing the effect of drugs on the blood ethanol concentration.

Pharmacokinetics of ethanol elimination after intravenous administration: The elimination of blood ethanol was studied after intravenous administration at 1,000 mg/kg. It could be well described by a two-compartment model with the Michaelis-Menten elimination process as shown in Fig. 3. The blood ethanol concentration-time curves in control rats showed that the distribution phase occupied the initial 10–15 min and then the ethanol concentration decreased gradually with time in the elimination phase. Treatment of animals with the alcohol-metabolizing enzyme inhibitors, pyrazole and disulfiram, caused decreases in ADH and low $K_m$ ALDH activities, respectively (Table 1, Exptl group 1). After intravenous administration of ethanol, the inhibitor-pretreated rats showed ethanol elimination profiles similar to those for the control rats. However, the pretreated animals showed a decrease in the ethanol disappearance rate and higher ethanol levels compared with the control value (Fig. 3). Although the fasted rats showed higher ADH activity (Table 1, Exptl group 2), the blood ethanol concentration-time curve for fed and fasted rats showed similar patterns when the animals were given ethanol by intravenous injection. The fasted animals displayed a lower disappearance rate (Fig. 4).

The pharmacokinetic parameters for ethanol clearance were estimated as described in "Methods", using the experimental data of Figs. 3 and 4. Interestingly, marked alterations in both $K_m$ and $V_m$ values were obtained in pyrazole-pretreated rats (Table 2, Exptl group 1), indicating that the inhibition

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**Fig. 3.** Mean blood concentration of ethanol after intravenous administration to normal and pyrazole- or disulfiram-pretreated rats. Animals were given pyrazole (68 mg/kg, i.p.) or disulfiram (1,000 mg/kg, p.o.) followed by ethanol (1,000 mg/kg) intravenously 30 min and 18 hr later, respectively. Blood samples were obtained periodically 2–150 min after the ethanol injection. Each value represents the mean and standard error of 4 rats. ****: Statistically significant ($P<0.01$) against the control.

**Fig. 4.** Mean blood concentration of ethanol after intravenous administration to fed and fasted rats. Ethanol (1,000 mg/kg) was injected intravenously to control (fed) and overnight-fasted rats, and their blood samples were obtained periodically. Each value represents the mean and standard error of 4 rats. *, **: Statistically significant ($P<0.05$ and $P<0.01$, respectively) against the control.
Table 2. Pharmacokinetic parameters for ethanol elimination

| Exptl group | Treatment of rat | $k_{12}$ (min$^{-1}$) | $k_{21}$ (min$^{-1}$) | $V_m$ (mg/ml/min) | $K_m$ (mg/ml) | $V_1$ (mg/g) |
|-------------|------------------|-----------------------|-----------------------|------------------|---------------|--------------|
| 1           | None (control)   | 0.315±0.080           | 0.339±0.040           | 0.018±0.002      | 0.118±0.038   | 0.407±0.021  |
|             | Disulfiram       | 0.056±0.019*          | 0.194±0.048           | 0.020±0.004      | 3.404±2.092   | 0.632±0.058* |
|             | Pyrazole         | 0.133±0.036           | 0.278±0.016           | 0.007±0.0004**   | 1.210±0.422   | 0.560±0.040* |
| 2           | None (fed)       | 0.234±0.030           | 0.356±0.050           | 0.014±0.001      | 0.188±0.056   | 0.471±0.019  |
|             | Fasting          | 0.157±0.029           | 0.293±0.025           | 0.013±0.001      | 0.562±0.132   | 0.538±0.021  |

Pharmacokinetic parameters were calculated from the data shown in Figs. 3 and 4. The values in the table represent the mean±S.E. of 4 animals. *: statistically significant (P<0.05 and P<0.01, respectively) against the control.

Table 3. Effects of enzyme inhibitor administration and fasting on the rate of ethanol elimination in vivo

| Exptl group | Body weight (g) | Liver weight (g) | Elimination rate | Elimination rate |
|-------------|-----------------|------------------|------------------|------------------|
|             |                 |                  | $\nu$ (mg/ml/min) | $\alpha$ (mg/hr/rat) | $\beta$ (mg/hr/g liver) |
| 1           | Control         | 240±6            | 9.59±0.46        | 0.016±0.002      | 92.1±4.6       | 9.71±0.84 |
|             | Disulfiram      | 246±4            | 9.79±0.34        | 0.006±0.001**    | 48.9±6.1**     | 5.05±0.71** |
|             | Pyrazole        | 243±5            | 9.83±0.34        | 0.003±0.001**    | 25.2±3.7**     | 2.55±0.35** |
| 2           | Control (fed)   | 266±6            | 11.25±0.39       | 0.011±0.001      | 85.3±3.2       | 7.58±0.13  |
|             | Fasted          | 237±4*           | 7.41±0.21**      | 0.008±0.001**    | 57.4±2.2**     | 7.75±0.37  |

Elimination rate of ethanol was calculated from the values shown in Table 2. Elimination rate from blood ($\nu$) and whole body ($\alpha$ and $\beta$) were estimated as follows:

$$\nu = \frac{V_mC_1}{K_m + C_1},$$

where the $C_1$ value used for the calculation is the blood ethanol level of 90 and 75 mg/dl in Exptl groups 1 and 2, respectively.

$$\alpha = \nu \cdot \text{(body weight)} \cdot V_1,$$

$$\beta = \frac{\alpha}{\text{liver weight}}.$$

The values represent the mean±S.E. of 4 animals. *: statistically significant (P<0.05 and P<0.01, respectively) against the control.
of hepatic ADH activity markedly affects ethanol elimination from the body. On the other hand, a higher \( K_m \) value without any alteration of the \( V_m \) value was obtained in disulfiram-pretreated rats, suggesting that the maximal rate of ethanol elimination is observable only in the presence of extremely high concentrations of ethanol. Fasting of the animals also caused a change in the \( K_m \) value, but not in the \( V_m \) value (Table 2, Exptl group 2).

**Ethanol clearance rate:** In the elimination phase of ethanol clearance, blood ethanol concentration decreased with time as shown in Figs. 3 and 4. Using the ethanol concentration in the elimination phase (40-120 min after the administration), the blood clearance rate of ethanol (\( v \)) was calculated with the equation derived from the Michaelis-Menten type elimination process (Table 3). The calculated values for ethanol elimination rates from blood (\( v \)) and the whole body (\( \alpha \)) were lower in disulfiram- and pyrazole-pretreated rats and also in fasted rats. When the data were expressed as rate per g of liver (\( \beta \)), the rate decreased significantly in the pyrazole- and disulfiram-pretreated rats, but no difference was obtained between fed and fasted animals (Table 3). Using the equation described previously (22, 23), the ethanol elimination rate was also estimated from the blood ethanol concentration only in the elimination phase, which showed almost the same results as those in Table 3 (data not shown). This indicates that the ethanol elimination rate can be estimated by analyzing the blood ethanol concentration-time curve in the elimination phase.

**Effect of beta-lactam antibiotics and their 3'-position substituents:** Animals were given various beta-lactam antibiotics and their 3'-position substituents subcutaneously followed by intravenous injection of ethanol. Blood samples in the ethanol elimination phase were obtained periodically 30-180 min after the injection, and the concentrations of ethanol and acetaldehyde in these samples were determined. Slower disappearance of blood ethanol was observed in rats pretreated with LMOX, CMD, NMTT or disulfiram, while CTM pretreatment showed little effect (Fig. 5). A marked increase in blood acetaldehyde concentration was observed after the intravenous administration.

![Fig. 5. Effects of antibiotics and related compounds on blood ethanol concentrations in rats.](image-url)

(A) Animals (7 weeks old) were given subcutaneously antibiotics (1,000 mg/kg) followed by ethanol (1,000 mg/kg, i.v.) 20 hr later. (B) Animals were given disulfiram (500 mg/kg, p.o.) or NMTT (200 mg/kg, s.c.) followed by ethanol intravenously 24 or 5 hr later, respectively. Blood samples were obtained 0.5-3.0 hr after the ethanol administration in both experiments (A) and (B). Each value in the figure represents the mean and standard error of 5 rats. *: Statistically different (\( P<0.05 \) and \( P<0.01 \), respectively) from the control.
of ethanol when animals were pretreated with either LMOX, CMD, NMTT or disulfiram, but not with CTM (Fig. 6). When animals were pretreated with FMOX or HTT, the blood ethanol concentration-time curve showed a pattern similar to that of CTM-pretreated rats, and no increase in the blood acetaldehyde level was observed (14). The ethanol elimination rates in these animals were calculated as described by Lumeng et al. (22). They were lower from the blood ($v'$) and from the whole body and liver ($\alpha'$ and $\beta'$) in rats pretreated with LMOX, CMD, NMTT or disulfiram. Administration of a high dose of CTM and FMOX caused a slight decrease in liver weight, and slightly lower values of ethanol elimination from the blood ($v'$) and the whole body ($\alpha'$) were obtained in these rats. However, no alteration was detected when the elimination rate was expressed as the rate per g liver, $\beta'$ (Table 4).

**Discussion**

Ethanol absorbed from the digestive tract is metabolized mainly in the liver, with a small fraction (normally less than 10%) being excreted into the urine and breath. Most fractions are metabolized in the liver (1, 2, 24), when ethanol is converted to acetaldehyde and then to acetic acid by ADH and low Km ALDH, respectively (1, 2). Although the participation of a microsomal ethanol oxidizing system and catalase has been well established in the conversion of ethanol to acetaldehyde, ADH seems to be the dominant rate-limiting enzyme, especially for low concentrations (<1 g/l) of ethanol (2, 25, 26). The lower blood concentration of acetaldehyde that we found compared with that of ethanol (Figs. 1 and 2), and the saturable pattern of acetaldehyde level in disulfiram-pretreated rats (Fig. 1) agreed well with the above assumption. In addition, no increase in blood acetaldehyde level was observed when ethanol was given to rats pretreated with both pyrazole and disulfiram (T. Matsubara et al., unpublished results). These results indicate the rate-limiting function of ADH in alcohol disappearance in vivo. On the other hand, fasted animals showed an increase in the specific activity of ADH (Table 1), although the rate of blood ethanol disappearance was slower in these rats (Fig. 4). The decrease in the ethanol disappearance rate in fasted rats agrees with generally reported findings (27, 28), but no correlation was thus obtained between the disappearance rate and the cytosolic ADH activity. On this point, Lumeng et al. (22) have demonstrated an increase in the specific activity of cytosolic ADH, but have observed a decrease in the total ADH activity in the whole liver due to a decrease in the liver mass of the fasted animals. All these results indicate that the hepatic content of ADH is a major rate-limiting factor of alcohol elimination (22, 29).

Liver ADH and low Km ALDH activities in rats were inhibited by the administration of pyrazole and disulfiram, respectively (Table 1). Interestingly, slower ethanol disappearance was detected not only in pyrazole-pretreated rats, but also in disulfiram-pretreated rats (Fig. 3). Close correlation between the depression of ADH activity and the decrease in the ethanol clearance rate in pyrazole-pretreated rats agreed well with the previous reports showing the physiological
Table 4. Effect of antibiotics and related compounds on the rate of ethanol elimination in vivo

| Exptl group | Treatment of animal | Body weight (g) | Liver weight (g) | $C_0$ (mg·ml$^{-1}$) | $r$ (mg·g$^{-1}$) | Ethanol elimination rate $v'$ (mg/ml/hr) | $\alpha'$ (mg/hr/rat) | $\beta'$ (mg/hr/g liver) |
|-------------|---------------------|-----------------|------------------|---------------------|------------------|----------------------------------------|----------------------|------------------------|
| 1           | Control             | 159±2           | 6.5±0.2          | 1.46±0.06           | 0.699±0.026      | 0.63±0.04                              | 68.1±2.8            | 10.71±0.48             |
|             | LMOX                | 154±2           | 5.6±0.1          | 1.42±0.04           | 0.707±0.024      | 0.48±0.01*                             | 51.9±1.3**          | 9.25±0.15              |
|             | CTM                 | 156±1           | 5.8±0.1          | 1.32±0.06           | 0.765±0.034      | 0.52±0.02                              | 62.3±2.8            | 10.51±0.39             |
|             | 6315-S              | 154±3           | 5.7±0.2          | 1.37±0.06           | 0.732±0.028      | 0.55±0.04                              | 61.6±2.3            | 10.83±0.50             |
| 2           | Control             | 162±3           | 6.5±0.2          | 1.30±0.06           | 0.776±0.041      | 0.50±0.04                              | 62.4±2.5            | 9.50±0.45              |
|             | NMTT                | 165±2           | 6.5±0.2          | 1.25±0.03           | 0.804±0.022      | 0.38±0.02*                             | 51.1±2.4*           | 7.87±0.32*             |
|             | HRT                 | 165±2           | 6.6±0.1          | 1.37±0.03           | 0.729±0.015      | 0.52±0.02                              | 62.8±3.0            | 9.58±0.45              |
|             | Disulfiram           | 161±2           | 6.9±0.2          | 1.31±0.04           | 0.766±0.022      | 0.42±0.02                              | 51.8±2.9*           | 7.54±0.31**            |
| 3           | Control             | 173±3           | 7.9±0.1          | 1.28±0.03           | 0.781±0.016      | 0.53±0.03                              | 72.0±2.0            | 9.10±0.33              |
|             | CMD                 | 172±2           | 6.9±0.2**        | 1.20±0.01*          | 0.834±0.012*     | 0.39±0.01**                            | 56.1±1.8**          | 8.12±0.26*             |

Animals (7 weeks old) were treated as described in Fig. 6, and the parameters were calculated from the data shown in Fig. 6. Ethanol concentration at zero time ($C_0$) was obtained by extrapolating the clearance curve to zero time, and the ratio of the volume of distribution for ethanol to body mass ($r$) was calculated as $A_0/(C_0 \times$ body weight), where $A_0$ is the amount of ethanol injected. Ethanol elimination rate from blood ($v'$) was obtained from the slope of the data in Fig. 6. The elimination rate from whole body was calculated as follows:

$\alpha' = v' \times r \times (\text{body weight})$

$\beta' = \alpha' / (\text{liver weight})$

The values in the table represent the mean±S.E. of 5 animals. *, **: Statistically different (P<0.05 and P<0.01, respectively) from the control.
function of ADH as the rate-limiting enzyme for alcohol oxidation (2, 25, 26). On the other hand, the blood ethanol concentration-time curve for the disulfiram-pretreated rats showed a slower ethanol elimination rate (Fig. 3), although the administration of disulfiram resulted in the depression of only low \(K_m\) ALDH activity, and not of ADH activity (Table 1). In vitro experiments also showed the inhibition of low \(K_m\) ALDH but no inhibition of ADH by disulfiram (30). Disulfiram treatment caused an elevation of the acetaldehyde level, as shown in Figs. 1, 2 and 6, due to depression of the acetaldehyde-oxidizing activity (8, 10, 31). Since the equilibrium in the reaction process of ADH is known to be toward the formation of alcohol rather than acetaldehyde (32), accumulation of or a high concentration of acetaldehyde may cause depression of ADH functioning, which would lead to decreased ethanol elimination. A marked increase in the \(K_m\) value without alteration of the \(V_m\) value in the disulfiram-pretreated rats (Table 2) agrees with the above assumption, suggesting that the presence of extremely high concentrations of ethanol compared with acetaldehyde sends the reaction in the direction of acetaldehyde formation even after disulfiram pretreatment.

Recent studies have demonstrated that the administration of beta-lactam antibiotics, having an N-methyltetrazolethiomethyl group at the 3-position of the cephem nucleus, causes a depression of liver low \(K_m\) ALDH activity, but not of ADH activity, associated with an increase in the blood acetaldehyde level following ethanol administration (7–10). Thus, these antibiotics seem to affect ethanol elimination like disulfiram-treatment. In fact, pretreatments of rats with NMTT-containing antibiotics (LMOX and CMD) and with NMTT itself caused a decrease in the ethanol elimination rate and an elevation of the blood acetaldehyde level during ethanol metabolism (Figs. 5 and 6). For the studies to examine the effects of antibiotics on blood ethanol level, we gave ethanol intravenously because oral administration would involve various processes in ethanol metabolism such as absorption, distribution, metabolism and elimination, and the ethanol absorption from the gastrointestinal tract is known to be variable even in the same individual (33). When ethanol was given orally to normal and disulfiram-pretreated rats, the peak level of blood ethanol was observed at about 1 hr after the ethanol administration (Fig. 2). A similar pattern in the blood ethanol concentration-time curve was obtained in antibiotic- and disulfiram-pretreated rats after oral ethanol administration, and the effects of these drugs on blood ethanol level were not clear (5). These results indicate that the intravenous injection of ethanol employed here is suitable for analyzing the effect of various drugs on blood ethanol elimination.

Since the blood ethanol concentration-time curves in rats after intravenous administration clearly showed the distribution and elimination phases in the process of ethanol disappearance (Figs. 3 and 4), elimination of ethanol was evaluated with the aid of a two-compartment model, as described previously (23, 34). The pharmacokinetic parameters estimated for pyrazole-pretreated rats showed a marked alteration of both \(K_m\) and \(V_m\) values, indicating also the important rate-limiting function of ADH for ethanol oxidation (Table 2). On the other hand, pretreatment of disulfiram caused a marked increase in the \(K_m\) value, but not in the \(V_m\) value. Fasting also caused a slight alteration of the \(K_m\) value (Table 2), which might correspond to the change in the total activity of ADH in the whole liver. Pharmacokinetic parameters were obtained in human subjects of various states. \(K_m\) and \(V_m\) values were 0.06–0.11 mg/ml and 0.16–0.22 mg/ml/hr (0.0027–0.0037 mg/ml/min), respectively (24, 35–40). They were almost comparable with those found for the normal rats described in this paper, although the \(V_m\) value in rats were slightly higher (Table 2). The rates of ethanol elimination from the blood and the whole body in normal rats were 10–14 nmol/l/hr (0.008–0.011 mg/ml/min) and 1.92 mmol/hr/rat (88.3 mg/hr/rat), respectively (22, 23). Wilkinson et al. (38) reported the apparent rate of ethanol elimination from blood in fasting volunteers to be 0.07–0.16 mg/ml/hr (0.001–0.003 mg/ml/min), although the rate varied dose-dependently. Using the equation shown in
Table 3, we also calculated the rate of ethanol elimination from the blood and the whole body (Table 3) and obtained values comparable to the reported ones. When the animals were pretreated with NMTT-containing antibiotics and NMTT itself, the calculated elimination rate clearly decreased together with an increase in the blood acetaldehyde level (Table 4, Figs. 5 and 6). On the other hand, the rate of ethanol elimination from the blood and the whole body were slightly lower in fasted and CTM- and FMOX-pretreated rats, although no alteration was evident when the rate was expressed per g liver (Tables 3 and 4). These results indicate the close correlation between the ethanol elimination rate and the activities of ADH and ALDH in the liver: Enzyme inhibitors, such as pyrazole and disulfiram, and beta-lactam antibiotics having a disulfiram-like reaction affect the alcohol-metabolizing enzyme activities, which is followed by the depression of the ethanol elimination rate. On the other hand, alteration of the liver weight or liver mass by fasting and the antibiotics having no disulfiram-like effect causes a change in the total activities of the alcohol-metabolizing enzymes in the liver, which may produce alterations in the ethanol elimination rate.

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