EFFECTS OF CEPHEM ANTIBIOTICS ON RAT LIVER ALDEHYDE DEHYDROGENASES

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Abstract—Effects of cepham antibiotics, which have a tetrazolethiol side chain, on rat liver mitochondrial aldehyde dehydrogenases (ALDH) were investigated in vitro and in vivo. The antibiotics tested were cefmetazole (CMZ), cefamandole (CMD), cefotiam (CTM), cefoperazone (CPZ) and latamoxef (LMOX). The antibiotics inhibited low-Vm ALDH activity by 17–30% at 5 mM in vitro. The degrees of inhibition were in the order: CMZ=CTM=CMD>LMOX>CPZ. Disulfiram inhibited the enzyme activity by 50% at approx. 40 ,WM. The antibiotics (except CTM) at a dose of 1,000 mg/kg i.v. inhibited the low-Vm ALDH activity by 36–52% of the control 24 hr after pretreatment, but did not alter the high-Vm ALDH activity. The degrees of inhibition were in the order: LMOX=CMD>CPZ>CMZ. Disulfiram at a dose of 300 mg/kg p.o. markedly inhibited the low-Vm ALDH activity, but did not alter the high-Vm ALDH activity. The blood acetaldehyde levels during ethanol metabolism were elevated 1.3–2.6 times in rats treated with the cepham antibiotics (except CTM) for 24 hr at a dose of 1,000 mg/kg i.v. The degrees of elevation at 1 hr after ethanol injection were in the order: LMOX>CMD>CPZ>CMZ. The present experiments demonstrated that the rise in blood acetaldehyde levels coincided with the inhibition rates of the low-Vm ALDH activity by the cepham antibiotics.

Many new semisynthetic, parenteral cepham antibiotics have been introduced as third generation antibiotics, which are active against Gram-positive and Gram-negative organisms, including Enterobacter, indole-positive Proteus and Pseudomonas aeruginosa. During clinical investigations of these antibiotics, disulfiram-like reactions have been observed in subjects taking cefoperazone (1-3), moxalactam (4, 5) and cefamandole (6). Buening et al. (5) have reported that moxalactam, cefamandole and cefoperazone increased blood acetaldehyde concentrations during ethanol metabolism in the rat. They also suggested that l-lactams with the methyltetrazolethiol side chain can alter the disposition of ethanol and increase acetaldehyde concentrations. Similar results have been reported by Yanagihara et al. (7). The purpose of the present paper is to elucidate the possible mechanism for the disulfiram-like reactions of these antibiotics by comparing the effects of these drugs with those of disulfiram on aldehyde dehydrogenases in rat liver.

Materials and Methods

Animals: Male Wistar strain rats weighing approx. 200 g were used in all experiments.

Materials: Antibiotics tested were: cefmetazole (CMZ) (Sankyo Co., Tokyo, Japan), cefamandole (CMD) (Shionogi, Osaka, Japan), cefotiam (CTM) (Takeda, Osaka, Japan), cefoperazone (CPZ) (Toyama, Tokyo, Japan) and latamoxef (LMOX) (Shionogi, Osaka, Japan). Tetraethylthiuram disulfide (disulfiram) was obtained from Wako Pure Chem. (Osaka, Japan). NAD+ and NADH
were supplied by Boehringer-Mannheim (W. Germany). Sodium desoxycholate and rotenone were purchased from Difco (Detroit, U.S.A.) and Aldrich (Milwaukee, U.S.A.), respectively. Pyrazole was obtained from Nakarai Chem. (Kyoto, Japan).

Determination of aldehyde dehydrogenase (ALDH) activity: The low- and high-Km ALDH activities were determined according to Tottmar et al. (8). Crude rat liver mitochondria was used as an enzyme source. Rat liver was homogenized in 10 vol. of 0.25 M sucrose, pH 7.0, and centrifuged at 700 x g for 15 min. The supernatant was filtered with 2 sheets of gauze and centrifuged at 10,000 x g for 20 min. The pellet was resuspended in 0.25 M sucrose, pH 7.0. Sodium desoxycholate at a concentration of 0.25 mg/mg protein was added to the samples to release latent activity and give clear solutions for spectrophotometric measurement. The incubation mixture consisted of 50 mM sodium pyrophosphate, pH 8.8, 0.5 mM NAD+, 0.8 mg protein of rat liver mitochondria, 0.1 mM pyrazole, 50 μM or 5 mM acetaldehyde for the low- or high-Km ALDH and 2 μM rotenone. The total volume was 3.01 ml. Incubations were carried out at room temperature for 2 min, and the reduction of NAD+ to NADH was measured spectrophotometrically at 340 nm (Hitachi 557 type). Rotenone was dissolved in methanol and added in a small volume (10 μl).

Effects of cephem antibiotics on ALDH in vitro: The enzyme preparation was preincubated for various times (1, 2, 5 and 10 min) at room temperature in 50 mM sodium pyrophosphate buffer (pH 8.8) containing 0.5 mM NAD and the various concentrations of the antibiotic (0.1–5.0 mM). Disulfiram was chosen as a standard inhibitor at the concentrations of 1–100 μM. Disulfiram was dissolved in ethanol, and the final incubation mixture contained 10% ethanol (v/v).

Determination of ethanol and acetaldehyde: The concentrations of ethanol and acetaldehyde in blood were determined principally according to Duritz and Truitt (9). The head space above a deproteinized blood sample was injected into a gas chromatograph, and the peaks from the samples were compared with those of suitable reference solutions. Blood was withdrawn via the inferior vena cava of the rats which were lightly anesthetized with pentobarbital at a dose of 50 mg/kg. One ml of blood was added into a 15-ml vial containing 0.5 ml of distilled water and 0.25 ml of 0.3 M zinc sulfate. The contents were mixed, and 0.25 ml of 0.3 M barium hydroxide was added 2 min later. Then, 1 ml of 0.5 ml/l n-propanol was immediately added as an internal standard. The vial was immediately stoppered and stored in a refrigerator. Duritz and Truitt claimed that samples could be stored at 5°C for 3–4 days without any change in the concentrations of acetaldehyde or ethanol.
Within 48 hr, the vial was placed for 15 min in an incubator at 55°C. One ml of the head space was withdrawn from the vial with a gas-tight syringe and injected into the gas chromatograph equipped with a flame ionization detector (GC-7A, Shimadzu) and a 1.1 m×3 mm glass column with 10% PEG 20 M on Uniport HP. The chromatographic conditions were as follows: column temperature, 60°C; injection port temperature, 100°C; helium flow rate, 30 ml/min; hydrogen flow rate, 50 ml/min, air flow rate, 0.4 l/min. Under the above conditions, retention times for acetaldehyde, ethanol and n-propanol were 0.6, 1.7 and 3.1 min, respectively.

Effects of the antibiotics on ethanol metabolism in vivo: Rats were pretreated 24 hr prior to ethanol administration with the antibiotics via the tail vein as 100 mg/ml solutions in distilled water at a dose of 1,000 mg/kg. One or 3 hr after ethanol administration at a dose of 1.5 g/kg i.p., rats were lightly anesthetized with 50 mg/kg pentobarbital i.p., and 1 ml of blood was taken via the inferior vena cava to determine blood acetaldehyde and ethanol levels.

Determination of protein contents: Protein contents were measured by the method of Lowrey et al. (10).

Statistical analysis: Statistical comparisons of inhibition rates of ALDH in vivo and blood acetaldehyde levels in the antibiotic-treated groups with those in the control group were conducted using the t-test for independent groups.

Results

Effects of cephem antibiotics on ALDH in vitro: The cephem antibiotics tested appeared to be weak inhibitors of low-\(K_m\) ALDH at high concentrations. As shown in Fig. 1A, a progressive decline in the enzyme activity was observed when the enzyme was preincubated with the cephem antibiotics (except CTM) at 5 mM in the absence of acetaldehyde, and similar results were observed in experiments with disulfiram (Fig. 2A). CTM, however, showed immediate apparent inhibition. The inhibition at different concentrations of the inhibitors

![Fig. 1](image-url)
after an incubation time of 10 min is shown in Fig. 1B. The enzyme activity was inhibited by 17–30% at 5 mM. The degrees of inhibition were in the order: CMZ=CTM=CMD>LMOX>CPZ. Disulfiram inhibited the enzyme activity by 50% at approx. 40 μM (Fig. 2B). The high-\( K_m \) ALDH activity was not inhibited by the cephem antibiotics and disulfiram at either of the concentrations used.

Effects of cephem antibiotics on ALDH in vivo: As shown in Table 1, preliminary experiments revealed that the cephem antibiotics (except CTM) inhibited the low-\( K_m \) ALDH activity, and the highest inhibition was observed 24 hr after drug treatment. These results were dose-related and similar to those of disulfiram. Effects of the cephem antibiotics on ALDH activity of rat liver mitochondria 24 hr after drug administration are shown in Table 2. The mean activities of low-\( K_m \) and high-\( K_m \) ALDH of rat liver mitochondria were 21.6±1.3 and 28.1±0.9 nmol/min/mg protein, respectively. The antibiotics
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Table 2. Effect of cephem antibiotics on rat liver ALDH in vivo

<table>
<thead>
<tr>
<th>Drug</th>
<th>Activity</th>
<th>N</th>
<th>low-(K_m)</th>
<th>high-(K_m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>% of control</td>
<td>11</td>
<td>21.6±1.3</td>
<td>28.1±0.9</td>
</tr>
<tr>
<td>CMZ</td>
<td>% of control</td>
<td>5</td>
<td>100±6</td>
<td>100±3</td>
</tr>
<tr>
<td>CMD</td>
<td>% of control</td>
<td>5</td>
<td>64±4*</td>
<td>105±6</td>
</tr>
<tr>
<td>CPZ</td>
<td>% of control</td>
<td>5</td>
<td>50±4*</td>
<td>113±4</td>
</tr>
<tr>
<td>CTM</td>
<td>% of control</td>
<td>5</td>
<td>58±4*</td>
<td>103±9</td>
</tr>
<tr>
<td>LMOX</td>
<td>% of control</td>
<td>5</td>
<td>112±6</td>
<td>98±5</td>
</tr>
<tr>
<td>Disulfiram</td>
<td>% of control</td>
<td>5</td>
<td>48±6*</td>
<td>105±3</td>
</tr>
</tbody>
</table>

*Significantly different from the control, \(P<0.01\). Each value represents the mean±S.E. Activity was measured 24 hr after treatment with the antibiotics at a dose of 1,000 mg/kg i.v. or disulfiram at a dose of 300 mg/kg p.o.

Table 3. Effect of cephem antibiotics on the blood acetaldehyde and ethanol levels in the rat

<table>
<thead>
<tr>
<th>Drug</th>
<th>Acetaldehyde ((\mu g/ml))</th>
<th>Ethanol (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hr</td>
<td>3 hr</td>
</tr>
<tr>
<td>Control</td>
<td>3.17±0.23</td>
<td>3.62±0.27</td>
</tr>
<tr>
<td>CMZ</td>
<td>4.65±0.25**</td>
<td>6.01±1.04**</td>
</tr>
<tr>
<td>CMD</td>
<td>5.46±0.48*</td>
<td>8.58±0.75*</td>
</tr>
<tr>
<td>CPZ</td>
<td>4.76±0.50*</td>
<td>4.72±0.63</td>
</tr>
<tr>
<td>CTM</td>
<td>3.30±0.22</td>
<td>3.94±0.61</td>
</tr>
<tr>
<td>LMOX</td>
<td>8.28±0.59*</td>
<td>8.59±0.92*</td>
</tr>
<tr>
<td>Disulfiram</td>
<td>14.74±2.56*</td>
<td>14.78±3.05*</td>
</tr>
</tbody>
</table>

*Significantly different from the control, \(P<0.01\). **Significantly different from the control, \(P<0.05\). Each value represents the mean±S.E. Rats were pretreated 24 hr prior to ethanol administration with the antibiotics at a dose of 1,000 mg/kg i.v. or disulfiram at a dose of 300 mg/kg p.o. Blood acetaldehyde and ethanol levels were determined 1 or 3 hr after ethanol administration at a dose of 1.5 g/kg i.p.

(\textit{except CTM}) at a dose of 1,000 mg/kg i.v. The blood acetaldehyde levels were 3.17 ±0.23 and 3.67±0.27 \(\mu g/ml\) at 1 and 3 hr after ethanol injection, respectively. When the cephem antibiotics (\textit{except CTM}) were injected, the blood acetaldehyde levels were elevated 1.3–2.6 times. The degrees of elevation at 1 hr after ethanol injection were in the order: LMOX>CMD>CPZ>CMZ. CTM did not influence either the low-\(K_m\) or the high-\(K_m\) ALDH activity. Disulfiram at a dose of 300 mg/kg p.o. markedly inhibited the low-\(K_m\) ALDH activity, but did not alter the high-\(K_m\) ALDH activity.

Effects of cephem antibiotics on the blood acetaldehyde and ethanol levels: As shown in Table 3, the blood acetaldehyde levels during ethanol metabolism were elevated in rats treated with the cephem antibiotics (\textit{except CTM}) for 24 hr at a dose of 1,000 mg/kg i.v. and 300 mg/kg p.o. The blood acetaldehyde levels were 3.17 ±0.23 and 3.67±0.27 \(\mu g/ml\) at 1 and 3 hr after ethanol injection, respectively. When the cephem antibiotics (\textit{except CTM}) were injected, the blood acetaldehyde levels did not
change. LMOX slightly increased the blood ethanol levels at 3 hr after ethanol injection, indicating the decreased rate of ethanol elimination. Disulfiram markedly elevated the blood acetaldehyde levels and decreased the rate of ethanol elimination.

Discussion

Although the mechanism of the disulfiram-alcohol reaction remains obscure, the appearance and disappearance of the reaction coincides with the rise and fall in blood acetaldehyde levels (11, 12). Disulfiram has been demonstrated to inhibit NAD-linked ALDH in vitro (13) and in vivo (14). Kinetic experiments suggested the possible existence of at least two different NAD-dependent ALDH's in rat liver mitochondria (8). Disulfiram has been reported to inhibit specifically low-Km ALDH and not to inhibit high-Km ALDH (15). Its inhibition appeared to be irreversible (14, 16). The present experiments demonstrated that CMZ, CMD, CPZ and LMOX also specifically inhibited low-Km ALDH. CTM, however, did not inhibit the enzyme in vivo. The in vitro and in vivo experiments revealed that these cephem antibiotics and disulfiram inhibited low-Km ALDH in a similar manner, and marked inhibition of the enzyme was observed even 48 hr after drug administration. These results suggest that the cephem antibiotics appeared to be irreversible inhibitors like disulfiram. The inhibition by the cephem antibiotics, however, was much weaker than that by disulfiram. Buening et al. (5) suggested that β-lactams with the 1-methyltetrazole-5-thiol side chain can alter the disposition of ethanol. They also reported that the effect of methyltetrazolethiols per se was much greater than the parent compound. CMZ, CMD, CPZ and LMOX have this moiety. CTM, however, has a 1-dimethylaminoethyltetrazole-5-thiol side chain. Therefore, slight modification of the structure of the side chain may abolish the inhibitory activity.

The present experiments demonstrated that the rise in blood acetaldehyde levels coincided with the inhibition rates of low-Km ALDH by the cephem antibiotics. Since an apparent Kₘ value below 10 nM for acetaldehyde is calculated for low-Km ALDH (8), the inhibition of this enzyme by the cephem antibiotics may cause the rise in blood acetaldehyde concentrations. Moreover, inhibition by these cephem antibiotics seemed to be irreversible. Therefore, patients should be cautioned to avoid alcohol for several days after treatment with CMZ, CMD, CPZ and LMOX to prevent disulfiram-like reactions.

References


