CEPHEM ANTIBIOTICS AND ALCOHOL METABOLISM

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(Received for publication December 17, 1984)

The cephem antibiotic is a general term for the cephalosporin antibiotics having an aminocephalosporanic acid nucleus in which a 6-membered ring is bonded to the β-lactam ring; the cephamycin antibiotics having a methoxy group introduced to the 7 position of the same nucleus, and the oxacephem antibiotics having the sulfur atom of the 6-membered ring of the nucleus substituted for with an oxygen atom.

A variety of such antibiotics have in recent years been synthesized as chemotherapeutic agents having a selective toxicity that they act on bacterial cells but not on the higher animal cells; and they are in the most widespread clinical use at the moment.

However, it has lately been found that when some of the patients treated with such cephem antibiotics ingested an alcohol-containing liquor, they showed a reaction similar to that under medication with disulfiram, an alcohol deterrent, and the reaction has been reported as disulfiram-like reaction due to the cephem antibiotics—the antibiotics are thus calling attention to their interaction with alcohol-containing liquor1−8).

It is said that disulfiram inhibits aldehyde dehydrogenase in the alcohol metabolic pathways, and the consequently elevated blood acetaldehyde leads to the development of such toxic manifestations as facial hot flushes, sweating, palpitation, dyspnea, tachycardia, hypotension, and nausea or vomiting.

Therefore, we investigated into the occurrence of the disulfiram-like reaction due to interaction between a variety of cephem antibiotics and alcohol, in terms of blood acetaldehyde level, and also into correlations of the structures to the activities of the cephem antibiotics causative of the disulfiram-like reaction, and further discussed the mechanism of development of the reaction.

Materials

1. Experimental animals

Wistar rats, weighing 250 to 270 g (reproduced by Kitayama Raves), were raised at room temperature in the range of 22±2°C and at 55±5% relative humidity for use in the experiments.

2. Agents used

Fig. 1 depicts the test agents: Cefazolin (CEZ; Fujisawa Pharmaceutical Co., Osaka), cefotiam (CTM; Takeda Chemical Industries, Osaka), cefsulodin (CFS; Takeda Chemical Industries, Osaka), cefotaxim (CFX; Merck-Banyu, Tokyo), cefotizoxime (CZX; Fujisawa Pharmaceutical Co., Osaka), cefmetazole (CMZ; Sankyo Co., Tokyo), ceftizoxime (CPZ; Toyama Chemical Co., Tokyo), cefamandole (CMD; Eli Lilly & Co., U.S.A.), latamoxef (LMOX; Shionogi and Co., Osaka), cefmenoxime (CMX; Takeda Chemical Industries, Osaka), and cefotetan (CTT; Yamanouchi Pharmaceutical Co., Tokyo). These test agents were dissolved in physiological saline to the required potency concentrations immediately before administration, and a 5 ml/kg volume of each solution was injected into the tail vein of the animals.

Disulfiram (DS; Wako Pure Chemical Industries, Osaka), as the standard drug, was suspended in 0.5% tragacanth, and administered orally. Also, sodium diethyldithiocarbamate (DDC; Wako Pure Chemical Industries, Osaka), a moiety of disulfiram, was dissolved in physiological saline, and injected
Fig. 1. Chemical structures of test compounds
into the tail vein. 1-Methyl-2-tetrazoline-5-thione (TZ; synthesized at the Central Research Laboratories, Takeda Chemical Industries, Osaka), having a chemical structure similar to the 3 position of the cephalosporin antibiotics, was first dissolved in 5 N NaOH, and then in physiological saline, and the solution was adjusted to a pH of 6.2 by the slow addition of NaHCO₃ (both 5 N NaOH and NaHCO₃ from Wako Pure Chemical Industries, Osaka). 1-(2-Dimethylaminoethyl)-2-tetrazoline-5-thione (MTZ; synthesized at the Central Research Laboratories, Takeda Chemical Industries, Osaka) was dissolved in physiological saline heated to 35°C. These solutions were also injected into the tail vein of the animals.

A gas chromatograph equipped with a hydrogen flame detector (GLC; the Shimadzu model GC-4CM gas chromatograph) was used in the assay of the blood for ethanol (EtOH) and acetaldehyde (AcH). EtOH (Wako Pure Chemical Industries, Osaka), AcH (Merck), 1-butanol (BuOH; Wako Pure Chemical Industries, Osaka) and 1-propanol (PrOH; Wako Pure Chemical Industries, Osaka) were used as the reagents in the assay.

Methods

1. Assay of the blood for EtOH and AcH by gas chromatography
   The assay as proposed by Hishida at al. was followed. To each of 10 ml clean test tubes (Rarubo; Terumo, Tokyo) was added 0.5 ml of physiological saline containing 0.8 mg/ml of PrOH and 20 mcg/ml of BuOH as internal standards; the tubes were immediately sealed with a plastic film (Sealon Film; Fuji Photo Film Co., Tokyo); and after the addition of 0.5 ml of physiological saline containing of various concentrations of EtOH and AcH and also blood, the tubes were again sealed. The tubes were then heated in a water bath at 55°C for 20 minutes, and 500 µl of the gas phase was injected into the gas chromatograph.

   The gas chromatography was performed at a temperature of 70°C, using a 3 × 2,000 mm glass chromatograph column packed with 20% PEG-1,000 (Chromosorb, AW) and He as the carrier gas (at a flow rate of 50 ml/min). For the assay for EtOH, the ratio of its peak to that of PrOH as the internal standard was used, and for the assay for AcH, the ratio of its peak to BuOH as the internal standard was used.

2. EtOH and AcH levels in the blood with EtOH loading following daily intravenous injection of various cephalosporin antibiotics and their side chains
   Five rats of each group, were injected intravenously with 100 mg/kg/time of the cephalosporin antibiotics, 5 mg/kg/time of TZ or DDC, or 100 mg/kg/time of MTZ twice a day (at 9:30 A.M. and 5:30 P.M.), or dosed orally with 200 mg/kg of DS once a day (at 5:30 P.M.). The drugs were administered for 3 consecutive days followed by a 17-hour fasting (during which period they were given free access to water), and they were give 2 g/kg of 20 w/v% EtOH orally.

   The blood sample was taken by cardiocentesis at 0.5, 1, 2, 4, 6 and 8 hours after the EtOH loading for assay of EtOH and AcH.

3. Duration of the effects of daily administered cephalosporin antibiotics on alcohol metabolism
   Out of the groups of 5 rats treated with the cephalosporin antibiotics, the group which had been injected intravenously with 100 mg/kg/time of CMX twice a day (at 9:30 A.M. and 5:30 P.M.) for 3 days, fasted for 17 hours, and then given 2 g/kg of 20 w/v% EtOH was designated as the group at 0 day of recovery. The groups which had been raised under normal condition for 1, 2, 3, 4 and 5 days, fasted for 17 hours, and then loaded with the EtOH, were designated as the groups at 1, 2, 3, 4 and 5 days of recovery. The blood was sampled from these groups during the first 1 hour after the EtOH loading, and the blood samples were assayed for EtOH and AcH by the aforementioned methods.

4. Effects of cephalosporin antibiotics and their side chains on the alcohol metabolism of bile duct-ligated rats
   The rats, divided into groups of 5 or 10, were laparatomized under ether anesthesia, and the common bile duct was ligated, to produce the bile duct-ligated rats. These rats were then given intravenously or orally 1,000 mg/kg of CMX or CMZ out of the cephalosporin antibiotics or 100 mg/kg of TZ.

   DDC was administered in an intravenous dose of 50 mg/kg. The rats were raised under normal condition for 17 hours after the administration of the test agents, and then given 2 g/kg of 20 w/v% EtOH orally. The blood was sampled from them during the first 4 hours after the EtOH loading for assay of EtOH and AcH.

   The results of these experiments were statistically analyzed by Student's t test.
Results

1. Gas-chromatographic assay of blood for EtOH and AcH

(1) Calibration curves for EtOH and AcH

The assay of the physiological saline or blood to which EtOH and AcH had been added at various concentrations gave such straight calibration curves which passed mostly through the base plots.

Regression analysis by the method of least squares of relations of the amounts of EtOH (Y mg) added to the blood to the ratio of its peak to that of the internal standard (X) gave a straight line of \[ Y = 0.924X - 0.110 \ (r=0.9995, n=25) \]; and that of relations of the amounts of AcH added to the blood (Y mcg) to the ratio of its peak to that of the internal standard (X) gave a straight line of \[ Y = 0.522X - 0.091 \ (r=0.9890, n=35) \].

Under these assay conditions, that portion of the calibration curve for 0.1 to 5.0 mg/ml of EtOH was mostly straight, and that for 0.01 to 20.00 mcg/ml of AcH, also mostly straight. Therefore, this method proved capable of assaying EtOH and AcH in these ranges of concentration, and was thought to be of very high precision. The chromatograms then obtained indicated that the retention times of the components were 1.0 minute for AcH, 2.3 minutes for EtOH, 3.9 minutes for PrOH, and 7.9 minutes for BuOH.

(2) Recovery rates of EtOH and AcH added to blood

The experiments by adding EtOH and AcH to the blood revealed that the recovery rate of EtOH was 103.0±1.24% when 0.1 mg/ml of EtOH was added, and 95.5±2.81% when 5.0 mg/ml was added (n=5, respectively), with almost the whole added amounts recovered, and that the recovery rate of AcH was 75.5±2.93% when 0.2 mcg/ml of AcH was added, and 85.6±2.08% when 10.0 mcg/ml was added (n=5, respectively). In other words, the recovery rate of AcH was slightly lower than that of EtOH, but was thought to offer no problems in particular.

2. EtOH and AcH levels in the blood with EtOH loading following daily intravenous injection of various cepham antibiotics and their side chains

(1) EtOH level in the blood

Changes in the EtOH level in the blood in all groups of rats treated with the cepham antibiotics, and also of those treated with DS, DDC, TZ and MTZ, remained the same as those in the control group.

(2) Changes in AcH level in the blood

Figs. 2–1 and 2–2 illustrate changes in the AcH level in the blood with EtOH loading after the daily administration of the test agents.

In the control group, the AcH level in the blood following the EtOH loading reached a peak in 1 hour, and then decreased slowly with the passage of time. In the groups dosed daily with CEZ, CTM, CFS, CFX and CZX out of the cepham antibiotics and the group dosed daily with MTZ, a compound having a structure similar to the 3 position of the structure of CTM, the AcH level in the blood did not differ significantly from the control value throughout the observation period.

In the groups dosed daily with CMZ, CPZ, CMD, LMOX, CMX and CTT, on the other hand, the AcH level in the blood was significantly high, compared with the level in the control group, and tended to be continuously high. In the group dosed daily with TZ, a compound having a structure similar
Fig. 2-1. Changes in blood AcH concentration with daily administration of cephem antibiotics not containing a methyl-tetrazole-thiol group, and other reference compounds

![Graph showing changes in blood AcH concentration with daily cephem administration.](image)

Fig. 2-2. Changes in blood AcH concentration with daily administration of cephem antibiotics containing a methyl-tetrazole-thiol group, and other reference compounds

![Graph showing changes in blood AcH concentration with daily cephem administration.](image)

to the 3 position substitute of the aminoccephalosporanic acid nucleus of the cephem antibiotics, the AcH level in the blood was elevated. Also in the group dosed daily with DS, the AcH level in the blood was elevated and remained very high. In the group treated daily with DDC, a moiety of DS, the AcH level in the blood was elevated.

3. Duration of the effects of daily administered cephem antibiotics on alcohol metabolism

(1) EtOH level in the blood

When the groups injected intravenously with 100 mg/kg/time of CMX, twice a day for 3 days were loaded with EtOH at 0 to 5 days of recovery, they showed such changes in the EtOH level in the blood which were similar to those in the control group.
Fig. 3. Recovery of AcH from the blood after daily intravenous injection of 200 mg/kg/day of CMX
Mean AcH concentration 1 hour after administration of EtOH

(2) AcH level in the blood
Fig. 3 shows the AcH levels in the blood of the rats 1 hour after loading with EtOH at 0 to 5 days of recovery following the intravenous injection of 100 mg/kg/time of CMX twice a day for 3 days.

The AcH levels in the blood of the rats at 0 to 5 days of recovery were lower in inverse proportion to the passage of the recovery period. In other words, in the group at 3 days of recovery, the AcH level in the blood reached a peak of 1.78 mcg/ml 1 hour after the EtOH loading, which was about 60% lower than the level at the same stage after the EtOH loading in the group at 0 day of recovery (4.43 mcg/ml). The AcH level in the blood of the group at 5 days of recovery did not differ significantly from the level in the control group at same stage after the EtOH loading.

4. Effects of cephem antibiotics and their side chains on the alcohol metabolism of bile duct-ligated rats

(1) EtOH level in the blood
There was no difference in the EtOH level in the blood between the test agent-intravenously or orally dosed groups of and the control group of the bile duct-ligated rats at any stage of observation.

(2) AcH level in the blood
Table 1 shows changes in the AcH level in the blood with EtOH loading after the administration of single doses of the test agents to the bile duct-ligated rats.

In the groups treated orally with CMZ, CMX and TZ and the group injected intravenously with DDC, the AcH level in the blood was elevated significantly at all stages of observation, as was noted the normal rats. In the groups injected intravenously with CMZ and CMX, however, such a significant elevation of AcH level in the blood as noted in the normal rats was not observed: in other words, the AcH levels in the blood of these groups of animals did not differ significantly from the level in the control group at any stage of observation. On the other hand, in the group injected intravenously with
Table 1. Changes in blood AcH concentration with administration of a single dose of CMX and other reference compounds to bile duct-ligated rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>n</th>
<th>AcH concentration in blood (mcg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Time after EtOH administration (hours)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>i.v.</td>
<td>10</td>
<td>0.20±0.009</td>
</tr>
<tr>
<td>CMZ</td>
<td>1,000</td>
<td>i.v.</td>
<td>5</td>
<td>0.43±0.358</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p.o.</td>
<td>5</td>
<td>6.04±2.176***</td>
</tr>
<tr>
<td>CMX</td>
<td>1,000</td>
<td>i.v.</td>
<td>10</td>
<td>0.41±0.216</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p.o.</td>
<td>10</td>
<td>5.33±1.707***</td>
</tr>
<tr>
<td>TZ</td>
<td>100</td>
<td>i.v.</td>
<td>10</td>
<td>0.78±0.393**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p.o.</td>
<td>10</td>
<td>3.31±1.831***</td>
</tr>
<tr>
<td>DDC</td>
<td>50</td>
<td>i.v.</td>
<td>5</td>
<td>1.06±0.262***</td>
</tr>
</tbody>
</table>

Significant difference from the control *: P<0.05, **: P<0.01, ***: P<0.001
EtOH was orally administered (2g/kg, 20 w/v%) to each rat.

TZ, the AcH level in the blood was significantly elevated, compared with the level in the control group.

Discussion

Reports have been published one after another on the development of disulfiram-like reaction following ingestion of alcohol-containing liquor as a drug interaction with some cephem antibiotics1-8). Disulfiram once used to be prescribed as an alcohol deterrent for the treatment of chronic alcoholism patients. Its action is derived from the mechanism that the agent inhibits aldehyde dehydrogenase of the alcohol metabolic pathways (EC1.2.1.3), and the consequently elevated AcH level in the blood leads to such toxic manifestations as facial hot flushes, sweating, palpitation, dyspnea, tachycardia, hypotension, and nausea or vomiting, to make the patient hate alcohol10-14).

For this reason, we investigated into when the disulfiram-like reaction would occur in rats as an interaction between many cephem antibiotics and alcohol, in terms of AcH level in the blood, and also discussed relations of the structures and activities of drugs to the development of this reaction, and the mechanism of development of this reaction.

Gas chromatograph is frequently used as the simplest and high precision assay of blood samples after EtOH loading for EtOH and AcH. Among various methods known for the preparation of EtOH and AcH assay samples, the head-space method of ERIKSSON15) and the method of HISHIDA9), both using gas chromatograph, are the most excellent. In a preliminary experiment, the calibration curves and the recovery rate of EtOH and AcH were studied by adding these agents to the blood, and the results were well consistent with the data reported by HISHIDA9). From this finding, we thought the assays used in this study were of sufficiently high precision and reproducibility. In the gas chromatograph assay for AcH, it is known that large amounts of AcH of unknown origin appear in processing human, monkey and bovine blood samples containing EtOH9,16). However, this AcH of unknown origin does not appear in the mouse and rat bloods9), and in this study using rats, this phenomenon was not observed.

Out of the cephem antibiotics used in this study, CEZ, CTM, CFS, CFX and CZX, on a dose schedule of 100 mg/kg/time by intravenous injection twice a day, for 3 consecutive days, caused no significant elevation of AcH level in the blood. On the other hand, CMZ, CPZ, CMD, LMOX, CMX and CTT, when administered daily by intravenous injection, elevated the AcH level in the blood significantly as did DS. It was thought therefore that these cephem antibiotics, like DS, were involved in alcohol
metabolism, to elevate the AcH level in the blood and consequently cause the disulfiram-like reaction. All the cephem antibiotics that elevated the AcH level in the blood significantly, i.e., CMZ, CPZ, CMD, LMOX, CMX and CTT, had the common substituting group in the 3 position of the aminoccephalosporanic acid nucleus with (1-methyl-1H-tetrazol-5-yl)thiomethyl. Therefore, TZ (1-methyl-2-tetrazoline-5-thione), having a similar common structure; MTZ, which has a dimethylaminoethyl group as the N-substituted group of TZ and a partial structure similar to the 3 position of CTM out of the cephem antibiotics; and DDC, a moiety of DS, were also examined for such an action. As a result, it was found that the daily intravenous injection of TZ and DDC resulted in a significant, persistent elevation of AcH level in the blood, but that of MTZ failed to elevated the level significantly.

The duration of the effects of the cephem antibiotics on alcohol metabolism was studied in the groups at 0 to 5 days of recovery, with the group after injected intravenously with 100 mg/kg/time of CMX twice a day for 3 consecutive days. The loading with EtOH by 4 days of recovery elevated the AcH level in the blood, while the loading at 5 days of recovery did not cause the AcH level in the blood to differ significantly from the level in the control group at same stage after the loading. Therefore, it was thought that the effects of the cephem antibiotics on alcohol metabolism were reversible, and that the patient on medication with or not more than 7 days after medication with such an antibiotic should refrain from the ingestion of alcohol-containing liquor.

The mechanism of development of disulfiram-like reaction by TZ and the cephem antibiotics causative of the reaction was studies in bile duct-ligated rats. The intravenous injection of CMX or CMZ failed to elevate the AcH level in the blood following EtOH loading, while the oral administration of either agent elevated the level significantly. The intravenous injection of TZ also elevated the AcH level in the blood significantly, though the elevation of the level was as low as about 1/5 that with the oral administration of the same agent. On the other hand, the intravenous injection of DDC increased the AcH level in the blood in the same manner as in the normal rats.

From these findings and with reference to literature data on the in vivo behavior of the cephem antibiotics17-20), the following was presumed to be the probable mechanism of development of disulfiram-like reaction with these antibiotics; the cephem antibiotics causative of disulfiram-like reaction, when administered intravenously, are not metabolized in the blood but in the intestinal tract after excretion into the bile, to release TZ, which in turn is absorbed, and inhibits alcohol metabolism.

**Summary**

Mechanisms of the disulfiram-like reaction of cephem antibiotics were studied. Changes in ethanol (EtOH) and acetaldehyde (AcH) levels in the blood with EtOH loading following daily intravenous administration of cephem antibiotics were determined in rats and the followings were found:

1. The daily intravenous injection of cefazolin, cefotiam (CTM), cefsulodin, cefoxitin or ceftizoxime in no way varied the changes in the EtOH and AcH levels in the blood with EtOH loading.

2. The daily intravenous injection of cefmetazole, cefoperazone, cefamandole, latamoxef, cefmenoxime or cefotetan caused the AcH level in the blood to be elevated significantly until at least 8 hours after the EtOH loading, but was inert on the EtOH level on the blood.

3. The daily administration of 1-methyl-2-tetrazoline-5-thione (TZ), a compound having a partial structure similar to those of the cephem antibiotics elevating the AcH level in the blood on EtOH loading, was inert on the EtOH level in the blood but elevated the AcH level in the blood. The daily administration of 1-(2-dimethylaminoethyl)-2-tetrazoline-5-thione (MTZ), a compound having a partial structure similar to that of CTM, was inert either on the EtOH or AcH level in the blood.

4. The cephem antibiotics elevating the AcH level in the blood all had a (1-methyl-1H-tetrazol-5-yl) thiomethyl group in the 3 position of the aminoccephalosporanic acid nucleus.

5. It was though that the disulfiram-like reaction caused by the cephem antibiotics was derived from the elevation of AcH level in the blood.
6. The disulfiram-like reaction was presumed to take place through the mechanism that the antibiotics, when injected intravenously, might be excreted into the bile to be metabolized in the intestinal tract, where they might release TZ, which in turn might inhibit aldehyde dehydrogenase.

7. The patient on treatment with or not more than 7 days after treatment with the cephem antibiotics causative of this phenomenon should refrain from the ingestion of alcohol-containing liquor.

References