Pharmacokinetics of Latamoxef and N-Methyltetrazolethiol in Rats Associated with the Development of Disulfiram-Like Effects

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Abstract—The disulfiram-like effect of beta-lactam antibiotics, having an N-methyltetrazolethiol (NMTT) as a 3'-position substituent of the cephalosporin nucleus, was determined in rats using latamoxef (LMOX) as a model. Intravenous and subcutaneous administrations of these antibiotics caused a decrease in the low Km aldehyde dehydrogenase (ALDH) activity in liver mitochondria and an increase in blood acetaldehyde level during ethanol metabolism, as in the case of disulfiram. When the antibiotic was administered intravenously to biliary fistula rats, the blood acetaldehyde level did not increase. On the other hand, oral administration of antibiotic to normal and biliary fistula rats caused pronounced development of disulfiram-like effects in both animals. When LMOX was injected to normal rats, the rapid and slow eliminations of LMOX and NMTT, respectively, were observed from blood and liver. After oral administration of LMOX, NMTT remained in the blood and liver for a long time with higher concentrations, although LMOX could not be detected in the body. With biliary fistula rats, intravenous injection of LMOX led to rapid urinary excretion of both LMOX and NMTT. These results indicate that the development of disulfiram-like effects of NMTT-containing antibiotics is closely related to the pharmacokinetic profile of NMTT released from its parent drugs.

Several beta-lactam antibiotics were found to show a reaction similar to that of disulfiram, when patients pretreated with some beta-lactam antibiotics ingested an alcohol-containing drink (1–9). Subsequently, several investigators demonstrated that pretreatment of rats with antibiotics having the N-methyltetrazoylthiomethyl group at the 3-position of the cephalosporin nucleus causes an increase in blood acetaldehyde levels when ethanol is administered 1 day after the pretreatment (6, 10–16). Administration of these antibiotics to rats resulted in a decrease in liver aldehyde dehydrogenase (ALDH) and acetaldehyde oxidation activities, as in disulfiram treatment (12, 15, 16). Treatment of animals with N-methyltetrazolethiol (1-methyl-1H-tetrazole-5-thiol; NMTT) also caused the rapid decrease in liver aldehyde-metabolizing enzyme activity (12, 15–17) and the elevation of blood acetaldehyde level during ethanol metabolism (6, 10–16). Thus, the disulfiram-like reaction of beta-lactam antibiotics is considered to be mediated by NMTT released from the parent drugs.

The pharmacokinetics and metabolic fate of cefmetazole (CMZ), one of the NMTT-containing beta-lactam antibiotics, were studied in detail and indicated the release of NMTT from CMZ in the body (18, 19). Nakamura et al. (20) and Uchida et al. (21) also demonstrated the release of NMTT from several beta-lactam antibiotics in various animals and humans. It should be interesting to clarify the pharmacokinetic profiles of antibiotics and released NMTT associated with the appearance of the disulfiram-like reaction. This report provides evidence showing that NMTT is released in the bile duct or gut from antibiotics excreted into the bile, which
causes NMTT to remain in the body for a long time. Thus, an NMTT-containing beta-lactam antibiotic can produce disulfiram-like effects when its administration is followed by ethanol.

**Materials and Methods**

**Animals and their treatments:** Jcl Sprague-Dawley strain male rats, 9-10 weeks old, were used for pharmacokinetic studies. For the detection of disulfiram-like effects of antibiotics, Slc Wistar strain male rats (9-10 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 some experiments, bile duct-ligated animals were used 1 hr after the ligation performed under pentobarbital anesthesia. Some animals were cannulated with a PE-10 polyethylene tube (Clay Adams, NJ, U.S.A.) inserted into their common bile ducts and a PE-66 tube into their urinary tracts under pentobarbital anesthesia. Cannulated animals were placed in a Bollman cage (KN-326, Natsume Co., Tokyo) and left for at least 1 hr to eliminate the effect of the anesthesia.

Antibiotics were dissolved in distilled water to the required potency concentrations just prior to their administration, and the resulting solution was administered intravenously or subcutaneously at 2 ml/kg body weight. In some experiments, antibiotics were administered orally.

**Determination of blood levels of ethanol and acetaldehyde:** Rats, fasted overnight, were given orally a 20% (w/v) solution of ethanol in water at a volume of 1.0 ml/100 g body weight (2.0 g ethanol/kg). Blood samples from decapitated rats were obtained 1 hr after ethanol treatment, and the concentrations of ethanol and acetaldehyde in blood were determined by the head-space gas chromatographic method as described previously (15, 16).

**Determination of aldehyde dehydrogenase (ALDH) activity:** Animals were treated with antibiotics and ethanol, and their livers were removed 1 hr after the ethanol treatment. Liver mitochondria were prepared as described previously (15), and the mitochondrial low K_m and high K_m ALDH activities were determined according to the method of Hasumura et al. (22) with slight modifications as described previously (15). Protein concentration of liver mitochondria was determined according to the method of Lowry et al. (23) using bovine serum albumin as a standard.

**Determination of latamoxef and NMTT:** Animals were given latamoxef or moxalactam (LMOX) intravenously or orally, and blood and liver samples were obtained 5 min to 4 hr after the treatment. Concentrations of LMOX in whole blood, plasma or liver samples were measured by high performance liquid chromatography (HPLC). First, 0.5 ml of blood or plasma sample was mixed with 0.5 ml of H_2O and 2.0 ml of ethanol-acetonitrile mixture (1:3, v/v), and then the mixture was centrifuged to remove the protein precipitate. The protein-free supernatant was used for HPLC analysis. When liver samples were used, liver tissue (0.5 g wet weight) in 0.5 ml of H_2O was mixed with 2.0 ml of ethanol-acetonitrile mixture (1:3, v/v) and then homogenized. The mixture was centrifuged, and the resulting supernatant was used for analysis. HPLC analysis was performed with an instrument equipped with a Waters Model 6000A pump, a Reodyne 7125 injector, a Shimadzu SPD-2A detector (set at 235 nm) and a Shimadzu C-R1B chromatopac. The column was Nucleosil 5C_18 (4.6 mm x 15 cm, Nagel) with a precolumn of RP-18 OD-GU (4.6 mm x 21 mm, Brownlee Lab.). The mobile phase was a mixture of 5 mM tetra-n-butyl-ammonium hydroxide (TBA) in 66 mM phosphate buffer (pH 6.0) and methanol (77:23, v/v) at the flow rate of 1.1 ml/min. To determine the NMTT concentration in biological samples, the samples (0.5 ml of blood or 0.5 g liver) were mixed with 50 µl H_2O and 1.0 ml acetonitrile, and the mixture was treated as in the case for LMOX. The following conditions were changed from those for LMOX in the HPLC analysis for NMTT: pump, Shimadzu LC-3A; mobile phase, mixture of 5 mM TBA in 66 mM phosphate buffer (pH 6.0) and tetrahydrofuran (98:2, v/v); flow rate, 1.5 ml/min;
detection, 254 nm.
For the determination of urinary and biliary excretion of LMOX and NMTT, urine and bile samples were periodically collected from the cannulated rats. Bile samples were neutralized by adding an equivalent volume of 0.2 M citrate-phosphate buffer (pH 6.25) soon after their collection to minimize the degradation of the antibiotics. Urine and bile samples were diluted with distilled water and then with an equivalent volume of "solution B." Next, the mixture was passed through a Millipore column guard (HV 0.45 μm, Nihon Millipore Kogyo), and the resulting filtrate was used for HPLC analysis. The gradient system using "solution A" (1 mM TBA in 13 mM phosphate buffer, pH 6.0) and "solution B" (mixture of solution A and acetonitrile, 60:40, v/v) as a mobile phase was employed to avoid interference from biological components. The pump and delivery system used were Toyo Soda SP-8700. The mobile phase (mixture of solutions A and B) was changed linearly from 90:10 (v/v) to 35:65 (v/v) over 24 min, and then the column was washed with solution B for 3 min. Before the next use, the column was equilibrated with a mixture of solutions A and B (90:10, v/v) for 13 min. LMOX and NMTT were detected at 254 nm in this experiment. Other HPLC conditions were as described above.

**Antibiotics and chemicals:** Cefamandole (CMD) and LMOX were obtained from Shionogi & Co. (Osaka). Disulfiram and TBA were purchased from Wako Chemical Industries (Osaka), and tetrahydrofuran was from Kanto Chemical Co. (Tokyo). Other chemicals of the purest grade available were obtained commercially and used for the experiments without further purification.

**Results**

Disulfiram-like effects of beta-lactam antibiotics: Effects of antibiotics on the liver mitochondrial acetaldehyde-metabolizing system and blood acetaldehyde level during ethanol metabolism were determined in rats using CMD and LMOX as models of the NMTT-containing antibiotics. As shown in Table 1, administration of disulfiram caused a marked decrease in acetaldehyde-metabolizing enzyme activities and an increase in the blood acetaldehyde level. Intravenous and subcutaneous injections of LMOX and CMD also led to a decrease in the enzyme activity, but to slight or no alteration in the blood acetaldehyde level under our experimental conditions (250 mg/kg dosage). On

Table 1. Effects of antibiotics and disulfiram administration on liver aldehyde-metabolizing enzyme activities and blood acetaldehyde level

| Expli group | Pretreatment of rat | Route of administration | No. of rat | Enzyme activity (nmol/min/mg protein) | Blood level of acetaldehyde (nmol/ml) |
|-------------|---------------------|-------------------------|-----------|--------------------------------------|--------------------------------------|
|             |                     |                         |           | Low K₉ ALDH | Acetaldehyde oxidation |                                      |
| 1           | None (control)      | —                       | 4         | 14.16±0.60 | 28.19±1.02 | 13.2±2.1 |
|             | CMD                 | i.v.                    | 4         | 8.75±0.19** | 16.86±1.11** | 17.8±6.7 |
|             | CMD                 | s.c.                    | 4         | 8.68±0.14** | 20.16±0.71** | 30.0±14.1 |
|             | CMD                 | p.o.                    | 4         | 7.46±0.26** | 17.73±1.18** | 90.5±9.7* |
| 2           | None (control)      | —                       | 3         | 16.85±1.24 | n.d. | 12.5±0.7 |
|             | LMOX                | s.c.                    | 3         | 10.12±0.43* | n.d. | 20.3±0.7 |
|             | LMOX                | p.o.                    | 3         | 5.73±0.16* | n.d. | 123.8±32.9 |
| 3           | None (control)      | —                       | 4         | 14.60±1.55 | 35.65±1.53 | 13.2±0.5 |
|             | Disulfiram          | p.o.                    | 4         | 5.45±0.86** | 14.39±1.89** | 177.9±2.8** |

Wistar strain male rats were given CMD (250 mg/kg), LMOX (250 mg/kg) or disulfiram (500 mg/kg), and then made to fast overnight. Next, the animals were given ethanol (2 g/kg) orally 18 hr after the pretreatment and blood and liver samples were obtained 1 hr later. The values in the table represent the mean±S.E. n.d.: not determined. *, **: statistically significant (P<0.05 and P<0.01, respectively) against the control.
the other hand, oral administration of antibiotics caused a marked increase in blood acetaldehyde level when ethanol was given 18 hr after the antibiotics treatment (Table 1). Large amounts of antibiotics (1,000 mg/kg) were given to the bile duct-ligated and biliary fistula rats, and their ALDH activity and blood acetaldehyde level were compared with those of control animals. Interestingly, the blood acetaldehyde level in the treated rats was not changed much by the intravenous and subcutaneous administrations, although the low \( K_m \) ALDH activity showed a significant decrease. On the other hand, oral administration of antibiotics caused a marked decrease in the mitochondrial low \( K_m \) enzyme activity and also an increase in the blood acetaldehyde level during ethanol metabolism (Table 2). The results indicated that the biliary excretion of antibiotics might participate in the development of the disulfiram-like effects when the antibiotics were administered intravenously or subcutaneously.

**Blood and liver concentrations of LMOX and NMTT in rats:** With LMOX as a model, the pharmacokinetic profiles of antibiotics were examined to obtain the correlation with the appearance of the disulfiram-like reaction.

The plasma concentration of LMOX showed a dose-dependent increase and then decreased rapidly (Fig. 1A). The blood concentration-time profiles were similar for whole blood and plasma. However, the LMOX level in whole blood was about 60% of that in plasma, suggesting that LMOX was hardly transported into blood cells (Fig. 1B). The LMOX level in liver also tended to show a dose-dependent increase, but no significant correlation was obtained between the dose and liver concentration of LMOX (Fig. 1C). The area under the plasma concentration-time curves (AUC) of LMOX, roughly calculated from the data shown in Fig. 1, demonstrated a dose-dependent increase in both plasma and whole blood, but the values in the liver were not correlated proportionally to the dose of LMOX (Table 2). In addition, LMOX concentrations in the liver were very low (concentration ratio of liver to plasma was 0.20–0.35) compared with those in plasma and whole blood (Fig. 1), and they decreased slowly from liver with longer half lives than those in plasma and whole blood at all dosages (Table 3).

HPLC analysis showed that NMTT was present in blood and liver samples obtained

### Table 2. Effect of LMOX and CMD administration on liver ALDH activity and blood acetaldehyde level in intact, bile duct-ligated and biliary fistula rats

| Exptl group | No. of rat | Enzyme activity (nmol/min/mg protein) | Blood acetaldehyde level (nmol/ml) |
|-------------|------------|--------------------------------------|-----------------------------------|
|             |            | Low \( K_m \) ALDH | High \( K_m \) ALDH |                                     |
| 1           | Control rat | 4 | 19.7±1.0 | n.d. | 4.0±0.7 |
|             | Bile duct-ligated rat | 4 | 20.7±0.7 | n.d. | 4.4±1.2 |
|             | +CMD (s.c.) | 4 | 8.9±0.3** | n.d. | 21.2±7.7 |
|             | +CMD (p.o.) | 4 | 3.3±0.3** | n.d. | 258.2±28.0** |
| 2           | Control rat | 3 | 20.4±2.3 | 22.9±1.5 | 8.0±2.9 |
|             | +LMOX (i.v.) | 3 | 7.7±1.0* | 25.7±1.3 | 52.7±16.6 |
|             | Biliary fistula rat | 3 | 20.3±0.5 | 21.6±1.9 | 11.9±3.5 |
|             | +LMOX (i.v.) | 3 | 11.5±0.7** | 22.7±0.4 | 10.3±1.9 |
| 3           | Control rat | 3 | 22.2±1.8 | 21.3±0.8 | 2.0±2.0 |
|             | +LMOX (p.o.) | 3 | 3.7±0.4** | 25.6±0.4* | 195.5±13.4** |
|             | Biliary fistula rat | 3 | 22.0±1.4 | 22.1±1.0 | 4.5±2.4 |
|             | +LMOX (p.o.) | 3 | 2.9±0.3** | 23.8±1.5 | 186.4±16.7** |

Wistar strain male rats were used for the experiments. Control and treated animals were given CMD or LMOX at a dose of 1,000 mg/kg, followed by ethanol (2 g/kg) 18 hr later. Blood and liver samples were obtained 1 hr after the ethanol treatment. n.d.: not determined. *, **: significantly different (P<0.05 and P<0.01, respectively) from the control.
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Fig. 1. LMOX concentrations in rat plasma, whole blood and liver after intravenous administration. Rats were given LMOX intravenously at the doses of 300 (●), 600 (●) and 1,000 (○) mg/kg. Each point represents the mean±S.E. of 4 animals.

Table 3. Pharmacokinetic parameters of LMOX and NMTT elimination in rats after intravenous administration of LMOX

| Compound determined | Dose of LMOX (mg/kg) | $T_{1/2}$(h) (min) | AUC ($\mu$g·hr/ml) |
|---------------------|----------------------|-------------------|------------------|
|                     | Plasma               | Whole blood       | Liver            | Plasma | Whole blood | Liver |
| LMOX                |                      |                   |                  |        |             |       |
| 300                 | 14.5                 | 14.8              | 20.8             | 485.0  | 266.0       | 131.1 |
| 600                 | 14.7                 | 15.5              | 18.2             | 860.4  | 515.5       | 180.4 |
| 1000                | 15.4                 | 15.3              | 20.9             | 1426.7 | 819.0       | 280.4 |
| NMTT                |                      |                   |                  |        |             |       |
| 300                 | 58.6                 | 61.9              | 39.7             | 11.84  | 9.99        | 2.49  |
| 600                 | 47.4                 | 37.3              | 39.0             | 18.46  | 14.53       | 7.59  |
| 1000                | 38.0                 | 43.8              | 32.9             | 21.79  | 21.83       | 10.15 |

Pharmacokinetic parameters were calculated with an automated pharmacokinetic analysis system using the mean values of LMOX and NMTT concentration shown in Figs. 1 and 2.

from LMOX-treated rats. NMTT concentrations in rat blood and liver after intravenous administration of LMOX showed a dose-dependent increase in NMTT concentrations (Fig. 2). NMTT levels decreased gradually with time, but were still detectable 60 min after the administration. Interestingly, NMTT concentrations in plasma and whole blood were almost the same, indicating that NMTT was incorporated easily into blood cells to the equilibrated states (Fig. 2, A and B). Liver concentrations of NMTT were about the half of those in plasma (Fig. 2C), and the concentration ratio of NMTT in liver to plasma (0.28–0.54) was high compared with that of LMOX (Fig. 1C). The results indicated that NMTT was released from the antibiotic in the body and was incorporated easily into intact cells. When rats were administered LMOX intravenously, the half life of the NMTT released was very long compared with that of LMOX (Table 3), indicating that NMTT remained in the body for a long time. The half lives of NMTT in plasma and whole blood were longer than that in liver.

Urinary and biliary excretion of LMOX and NMTT: Urinary excretion of LMOX and NMTT was determined after intravenous administration of various amounts of LMOX to intact rats. Figure 3A shows the cumulative
Fig. 2. NMTT concentrations in rat plasma, whole blood and liver after intravenous administration of LMOX. Biological samples were obtained from the animals used for the Fig. 1 experiment. Doses of LMOX were 300 (●), 600 (■) and 1,000 (○) mg/kg, and each point in the figure represents the mean ± S.E. of 4 animals.

Fig. 3. Urinary excretion of LMOX and NMTT after intravenous injection of LMOX. Rats were given LMOX intravenously at doses of 300, 600 and 1,000 mg/kg, and urine samples were collected periodically. Amounts of LMOX and NMTT in the urine were determined by HPLC, and cumulative amounts of LMOX (A) and NMTT (B) excreted are plotted as a function of time. Amounts of NMTT excreted were calculated as those equivalent to LMOX. Each point represents the mean ± S.E. of 4 rats.

Excretion of LMOX, which indicates that 75–85% of the LMOX administered was excreted rapidly into the urine by 2 hr after the treatment. On the other hand, NMTT was excreted slowly, being continuously detected even after 6 hr (Fig. 3B). Cumulative NMTT excretion for 6 hr was 7–10% of the dose (calculated as the amount of LMOX). Thus, almost 90% or more of the LMOX administered was excreted into the urine mostly as the unchanged form (LMOX) and partly as the decomposed form (NMTT). Slow elimination of NMTT into the urine, compared with the parent drug LMOX, indicated also
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that the NMTT released from LMOX remained for a long time in the body.

When LMOX was injected intravenously to biliary fistula rats, most of it was excreted into the urine and a small amount (less than 10% of the dose) into the bile (Fig. 4A). The urinary excretion profile of LMOX was similar to that in control rats (Fig. 3). On the other hand, the cumulative urinary excretion of NMTT was much less in biliary fistula rats compared with that in control rats, and the excretion was observed by 2–4 hr after the LMOX administration (Fig. 4B). Biliary excretion of NMTT was substantial. The results shown in Figs. 3 and 4 indicate that biliary excretion of LMOX causes a liberation of NMTT in the bile duct and gut followed by intestinal absorption, which causes a longer half life of NMTT in blood than that of LMOX and also slower urinary excretion compared with that of LMOX.

Pharmacokinetic profiles of LMOX and NMTT after oral administration of LMOX: Blood and liver levels of LMOX and NMTT in rats were examined after oral administration of LMOX. LMOX was not detected in the blood or liver, but HPLC chromatograms showed the existence of NMTT in both blood and liver samples. Then, blood and liver levels of NMTT after oral administration of LMOX were checked using overnight fasted animals. The blood concentrations showed rapid absorption of NMTT with a peak concentration at about 1 hr after the treatment. The concentrations of NMTT at 2 hr decreased markedly and was followed by a gradual increase showing a second peak at 4–6 hr after the treatment (Fig. 5A). A similar pattern was obtained for the NMTT concentrations in the liver, although the peak and trough concentrations did not differ much (Fig. 6A). It is interesting to note that the liver concentration of NMTT was about 4 \( \mu g/g \) wet weight of liver after 1,000 mg/kg oral dose of LMOX, and this level was continuously maintained during the 8 hr observation period (Fig. 5A). Similar pharmacokinetic profiles of NMTT were obtained when fed rats were used instead of fasted ones. However, the maximal blood level of NMTT was observed 2 hr after the administration supposedly due to the delay of intestinal absorption (Fig. 5B).

Figure 6 indicates the cumulative urinary and biliary excretion of LMOX and NMTT after oral administration of LMOX. Although LMOX was not detectable in blood and liver samples, small amounts of LMOX were excreted into the urine and bile; cumulative excretion was 2.6 and 1.4% of the dose for 24 hr urine and bile, respectively (Fig. 6A). On the other hand, about one-fourth of the LMOX administered was excreted in 24 hr-

![Fig. 4. Urinary and biliary excretion of LMOX and NMTT after intravenous administration of LMOX. Biliary fistula rats were given LMOX intravenously at the doses of 300, 600 and 1,000 mg/kg; and urine and bile samples were collected periodically. Cumulative amounts of LMOX and NMTT excreted are shown in the figure as a function of time. Each point represents the mean±S.E. of 4 animals.](image-url)
Fig. 5. Blood and liver levels of NMTT in rats after oral administration of LMOX. Control and overnight fasted rats received LMOX orally at 1,000 mg/kg, and their blood and liver samples were obtained 0.5–8 hr after the administration. NMTT concentrations in whole blood (○), plasma (●) and liver (●) were determined as described above. Each value represents the mean±S.E. of 3 rats.

Fig. 6. Urinary and biliary excretion of LMOX and NMTT after oral administration of LMOX. Biliary fistula rats were given LMOX orally at a dose of 1,000 mg/kg, and their urine and bile samples were collected periodically. Cumulative amounts of LMOX and NMTT excreted were calculated and are plotted as a function of time. Each point represents the mean±S.E. of 3 animals.

Discussion
Some beta-lactam antibiotics, having an N-methyltetrazolylthiomethyl group at the 3-position of the cephalosporin nucleus, have been found to show disulfiram-like effects on the acetaldehyde-metabolizing system in humans and rats. A time course study on the onset of the effects demonstrated clearly that NMTT released from these antibiotics, but not the parent drugs, is closely correlated with the development of the disulfiram-like effects (24). Yanagihara et al. (14) and Turcan et al. (25) reported that intravenous administration of NMTT-containing antibiotics to bile duct-ligated and biliary fistula rats did not increase the blood acetaldehyde level nor decrease the ethanol metabolizing activity. We obtained the same results as shown in Tables 1 and 2, which indicate the importance of biliary excretion of antibiotics.
in the release of NMTT in the body. Thus, we checked the pharmacokinetic profile of an NMTT-containing antibiotic, LMOX and the NMTT released from it. Although the profile of LMOX has been studied in detail in several experimental animals using therapeutic or lower doses of the drug (26, 27). When injected in higher or toxicological doses, LMOX was eliminated rapidly from the blood and liver with half lives of 15–20 min as shown in Fig. 1 and Table 3. On the other hand, NMTT released from LMOX in the body was eliminated slowly from the blood and liver with half lives of 40–60 and 30–40 min, respectively (Fig. 2 and Table 3). When NMTT itself was administered intravenously, its blood elimination was very fast with a half life of about 20 min (T. Matsubara, unpublished results). Similar results were obtained by using 14C-LMOX and 14C-NMTT (K. Mizoziri, personal communication). Therefore, the slow NMTT elimination after LMOX administration indicates that the NMTT liberation step is slow compared with the NMTT elimination step.

In conclusion, disulfiram-like effects of NMTT-containing antibiotics were marked when the antibiotics were administered orally. These effects were not observed after intravenous or subcutaneous administration of the antibiotics to bile duct-ligated and biliary fistula rats. Pharmacokinetic study demonstrated that the long life span of NMTT in the body led to the development of the disulfiram-like effects. Thus, the pharmacokinetic profile of NMTT is closely correlated with the development of disulfiram-like effects.

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