Potentiation of the Antitumor Activity of Tegafur in Sarcoma 180-Bearing Mice by Chlordiazepoxide, Diazepam and Oxazepam

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The effects of chlordiazepoxide, diazepam and oxazepam administration on the antitumor activity, acute toxicity and metabolism of tegafur were investigated in mice and compared with those in the case of 5-fluorouracil (5-FU). Tegafur was administered 24 h after the final injection of chlordiazepoxide, diazepam or oxazepam (100 mg kg^{-1} d^{-1} for 3 d, i.p.). The pretreatment with these drugs increased the antitumor activity of tegafur against the solid form of Sarcoma 180 and the acute toxicity. In chlordiazepoxide- and diazepam- or oxazepam-treated mice, after the administration of tegafur, the level of tegafur in the plasma was lower than that in untreated mice and a large amount of 5-FU was released. A low level of 5-FU in the plasma after the administration of 5-FU was also observed in chlordiazepoxide-, diazepam- or oxazepam-treated mice. In the liver and kidneys of chlordiazepoxide-, diazepam- or oxazepam-treated mice, the level of 5-FU after the administration of tegafur was higher. On the other hand, chlordiazepoxide, diazepam or oxazepam significantly enhanced the activities of hepatic drug-metabolizing enzymes. It can therefore be presumed that the antitumor activity of tegafur was enhanced by chlordiazepoxide, diazepam or oxazepam as a result of promotion of the conversion of tegafur to 5-FU, mainly via the induction of hepatic drug-metabolizing activities.

Keywords—chlordiazepoxide, diazepam, oxazepam, antitumor activity, tegafur, 5-FU, mouse

A masked form of 5-fluorouracil (5-FU), tegafur (1-(2-tetrahydrofuryl)-5-fluorouracil), has been shown to have a spectrum of antitumor activity similar to that of 5-FU, but with reduced side effects.\(^1\,2\) The conversion of tegafur to 5-FU occurs mainly in the microsomes of the liver and requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) and O\(_2\).\(^3\)

In recent years, Sasaki et al.\(^4\,5\) demonstrated that the antitumor activity of masked compounds such as cyclophosphamide and tegafur on Ehrlich and Sarcoma 180 solid tumor in mice was decreased by the intravenous injection of lipopolysaccharide (obtained from Escherichia coli, LPS). The results implied that LPS depresses the hepatic microsomal drug-metabolizing system.\(^6\) Inducers of microsomal cytochrome P-450 markedly enhance the rate of conversion of tegafur to 5-FU.\(^7\) Jori et al.\(^8\) and Jablonska et al.\(^9\) reported that chlordiazepoxide, diazepam and oxazepam induced hepatic microsomal drug-metabolizing enzymes in rats. These results suggested that these minor tranquilizers promote the conversion from tegafur to 5-FU and enhance the antitumor activity of tegafur.

In the present study, we examined the effects of chlordiazepoxide, diazepam and oxazepam on the antitumor activity, acute toxicity, and metabolism of tegafur or 5-FU in mice.

Materials and Methods

Animals—Male mice of ddY strain weighing 20—22 g were employed in all experiments. Food and water were
given freely.

Drugs—The drugs used were as follows: tegafur (1-(2-tetrahydrofuryl)-5-fluorouracil, Taiho Yakuhin Kogyo Co.), 5-fluorouracil (5-FU, Kyowa Hakko Kogyo Co.), diazepam (Maruko Seiyaku Co.), clordiazepoxide (Takeda Yakuhin Kogyo Co.) and oxazepam (extracted from commercial product, Banyu Seiyaku Co.). Drugs were suspended in 0.5% carboxymethylcellulose just before use.

Tumor Cells—-Sarcoma 180 ascites tumor cells that have been maintained in ddY mice by weekly passage in our laboratory were used.

Chemotherapy—Groups of ten mice were used. Solid-type Sarcoma 180 was obtained by the intramuscular injection of ascites cells (1 × 10⁶ cells) into the right thigh of mice. Tegafur (200 or 400 mg/kg) or 5-FU (25 or 50 mg/kg) was administered once intraperitoneally 24 h after tumor transplantation. Clordiazepoxide, diazepam or oxazepam (100 mg/kg) was administered intraperitoneally once a day for 3 d, and the antitumor drugs were administered 24 h after the final injection of these drugs. On the 20th day after Sarcoma 180 cell inoculation, mice were sacrificed, and the tumors were removed and weighed. The antitumor activity was evaluated in terms of the mean weight of tumors in treated mice as a percentage of the mean weight in control mice.

Acute Toxicity—Groups of ten mice were used. Tegafur or 5-FU was injected 24 h after the final injection of minor tranquilizers. The survival of mice was observed continuously for 15 d.

Assays of Tegafur and 5-FU in Plasma, Liver and Kidneys—Samples (0.3 ml of serum or 1 ml of 25% tissue homogenate) adjusted to pH 2.0 with 1 N HCl were extracted with 10 volumes of chloroform. Tegafur (extracted in the chloroform layer) and 5-FU (remaining in the aqueous layer) were assayed by the method of Fujiita et al. The ratio of 5-FU and fluorouridine (FUR) in tissue was determined by bioassay after separation of active metabolite by means of paper chromatography.

Preparation of Enzyme Source and Assays—The livers of mice were homogenized in 4 volumes of 1.15% KCl-10 mm sodium potassium phosphate buffer (pH 7.4) and the homogenate was centrifuged at 9000 × g for 20 min to remove cell debris, nuclei and mitochondria. The microsomal fraction of mouse liver was prepared by centrifugation at 105000 × g for 60 min. Microsomal cytochrome P-450 and cytochrome b₅ contents were measured according to the method of Omura and Sato with a spectrophotometer (UV 300 type, Shimadzu). Aminopyrine N-demethylase and aniline hydroxylase in hepatic 9000 × g supernatant fraction were assayed by the method of La Du et al. and Imai et al.

Protein Assay—Protein concentrations were determined by the method of Lowry et al., using bovine serum albumin as a standard.

Statistical Analysis—Student's t-test was used for statistical analysis. Values of p < 0.05 and p < 0.01 were used as criteria of significance.

Results

Effects of Clordiazepoxide, Diazepam and Oxazepam on the Antitumor Activities of Tegafur and 5-FU

The effects of clordiazepoxide, diazepam and oxazepam treatment on the antitumor activities of tegafur and 5-FU against the solid form of Sarcoma 180 in mice were examined, and the results are shown in Fig. 1. The solid tumor weight (mean ± S.E.) in control mice on the 20th day after the implantation of tumor cells was 6.44 ± 0.47 g. When tegafur 200 mg/kg or 400 mg/kg was injected once intraperitoneally 24 h after the cell inoculation, the solid tumor weight (mean ± S.E.) of tegafur-treated mice was 5.15 ± 0.51 g or 3.93 ± 0.43 g, the inhibition being about 20 or 40%. When tegafur was injected 24 h after the final injection of clordiazepoxide, diazepam or oxazepam (100 mg·kg⁻¹·d⁻¹ for 3 d, i.p.), the antitumor activity of tegafur was increased significantly by the pretreatment.

On the other hand, when 5-FU 25 mg/kg or 50 mg/kg was injected once i.p. 24 h after the cell inoculation, the solid tumor weight (mean ± S.E.) of 5-FU-treated mice was 5.03 ± 0.58 g or 3.81 ± 0.39 g, the inhibition being about 20 or 40%. The antitumor activity of 5-FU at a dose of 25 mg/kg was decreased most by the pretreatment with clordiazepoxide, while the activity of 5-FU at a dose of 50 mg/kg was decreased most by diazepam.

Effects of Clordiazepoxide, Diazepam and Oxazepam on the Toxicity due to Tegafur and 5-FU Administration

Toxic effects of tegafur and 5-FU, alone and in combination with clordiazepoxide, diazepam or oxazepam, were observed for 15 d in mice, and the results are shown in Fig. 2.
Fig. 1. Effects of Chlordiazepoxide, Diazepam and Oxazepam on the Antitumor Activities of Tegafur and 5-FU

Tegafur or 5-FU was injected once intraperitoneally 24 h after Sarcoma 180 cell inoculation. Chlordiazepoxide, diazepam or oxazepam (100 mg/kg) was injected intraperitoneally once a day for 3 d, and tegafur or 5-FU was injected 24 h after the final injection of these drugs. , saline (control); , chlordiazepoxide; , diazepam; , oxazepam. Significant differences from the control value are indicated as a) \( p < 0.05 \).

Fig. 2. Effects of Chlordiazepoxide, Diazepam and Oxazepam on the Lethality of Tegafur and 5-FU

Tegafur (800 mg/kg) or 5-FU (500 mg/kg) was injected once intraperitoneally 24 h after the final injection of chlordiazepoxide, diazepam or oxazepam (100 mg\cdot kg^{-1}\cdot d^{-1} for 3 d, i.p.). Survival was observed continuously for 15 d.

The pretreatment with chlordiazepoxide, diazepam or oxazepam (100 mg\cdot kg^{-1}\cdot d^{-1} for 3 d, i.p.) increased the lethality of tegafur (800 mg/kg, i.p.) and 5-FU (500 mg/kg, i.p.).

Effects of Chlordiazepoxide, Diazepam and Oxazepam on the Levels of Tegafur and 5-FU in Plasma, Liver and Kidneys

Figure 3 shows time courses of the levels of tegafur and 5-FU in the plasma of mice after
Fig. 3. Effects of Chlordiazepoxide, Diazepam and Oxazepam on the Levels of Tegafur and 5-FU in the Plasma of Mice after the Administration of Tegafur

Tegafur was given at a dose of 400 mg/kg (i.p.) 24 h after the final injection of chlordiazepoxide, diazepam or oxazepam (100 mg·kg⁻¹·d⁻¹ for 3 d, i.p.). Each point represents the mean of three values obtained from pooled plasma of three mice. ○, saline (control); ●, chlordiazepoxide; △, diazepam; ▲, oxazepam. Significant differences from the control value are indicated as a) $p < 0.05$ and b) $p < 0.01$.

Fig. 4. Effects of Chlordiazepoxide, Diazepam and Oxazepam on the Level of 5-FU in the Plasma of Mice after the Administration of 5-FU

5-FU was given at a dose of 50 mg/kg (i.p.) 24 h after the final injection of chlordiazepoxide, diazepam or oxazepam (100 mg·kg⁻¹·d⁻¹ for 3 d, i.p.). Each point represents the mean of three values obtained from pooled plasma of three mice. ○, saline (control); ●, chlordiazepoxide; △, diazepam; ▲, oxazepam. Significant differences from the control value are indicated as a) $p < 0.05$.

the administration of tegafur (400 mg/kg, i.p.). The level of tegafur in the plasma was lowered by the injection of chlordiazepoxide, diazepam or oxazepam (100 mg·kg⁻¹·d⁻¹ for 3 d, i.p.). The level of tegafur in the plasma 30 min after the injection in control mice was about
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<td>Saline (Control)</td>
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<td>Oxazepam</td>
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Tegafur (400 mg/kg) or 5-FU (50 mg/kg) was injected intraperitoneally 24h after the final injection of chlordiazepoxide, diazepam or oxazepam (100 mg·kg⁻¹·d⁻¹ for 3 d, i.p.). Mice were sacrificed 30 min after the administration of tegafur and 20 min after 5-FU. Each value represents the mean of six determinations on pooled tissue of three mice. Significant differences from the control value are indicated as a) p<0.05 and b) p<0.01.
550 μg/ml, and then gradually decreased, being undetectable at 8 h after the injection. The rate of decay of tegafur in the plasma of chlordiazepoxide-, diazepam- or oxazepam-treated mice may be more rapid than that of control mice. As regards 5-FU, the plasma level of 5-FU metabolized from tegafur reached a maximum in 30 min and then rapidly decreased. In contrast, the level of 5-FU liberated from tegafur in the plasma was higher in chlordiazepoxide-, diazepam- or oxazepam-treated mice.

On the other hand, after the administration of 5-FU (50 mg/kg, i.p.), the level of 5-FU in the plasma was determined at the times indicated in Fig. 4. The degradation of 5-FU was also influenced by chlordiazepoxide, diazepam or oxazepam (100 mg·kg⁻¹·d⁻¹ for 3 d, i.p.). The level of 5-FU in the plasma of chlordiazepoxide-, diazepam-, or oxazepam-treated mice was lower than that of control mice.

Further, the level of tegafur or 5-FU and the ratio of 5-FU to FUR in the liver and kidneys after the administration of tegafur or 5-FU were examined and the results are shown in Table I. In mice which were injected with chlordiazepoxide, diazepam or oxazepam (100 mg·kg⁻¹·d⁻¹ for 3 d, i.p.), the levels of 5-FU in the liver and kidneys 30 min after the administration of tegafur (400 mg/kg, i.p.) were higher than those in control mice, whereas in chlordiazepoxide-treated mice, the level of tegafur in the kidneys 30 min after the administration of tegafur was lower than that in control mice. However, no difference between control mice and chlordiazepoxide-, diazepam- or oxazepam-treated mice was observed as regards the level of 5-FU 20 min after the administration of 5-FU (50 mg/kg, i.p.) or the ratio of 5-FU to FUR at 30 min after the administration of tegafur or at 20 min after the administration of 5-FU in the liver and kidneys. FUR could not be detected in the plasma by the method employed in this paper.

Effects of Chlordiazepoxide, Diazepam and Oxazepam on the Hepatic Drug-metabolizing System

The effects of chlordiazepoxide, diazepam and oxazepam on the drug-metabolizing system are shown in Fig. 5. The activities of Aminopyrine N-Demethylase and Aniline Hydroxylase and Cytochrome P-450 and Cytochrome b₅ Contents in the Hepatic Microsomal Fraction of Mice

Mice were sacrificed 24 h after the final administration of chlordiazepoxide, diazepam or oxazepam (100 mg·kg⁻¹·d⁻¹ for 3 d, i.p.). Each column shows the mean activity, and horizontal bars represent the standard errors of the means of six mice. □, chlordiazepoxide; △, diazepam; ▽, oxazepam. Significant differences from the control value are indicated at a) p < 0.05 and b) p < 0.01.
enzymes and cytochrome P-450 and cytochrome b₅ contents were examined in the hepatic microsomal fraction of mice, and the results are shown in Fig. 5. Control values were as follow: aminopyrine N-demethylase: 1.82 ± 0.14 (nmol·30 min⁻¹·mg⁻¹ of protein); aniline hydroxylase: 2.04 ± 0.16 (nmol·30 min⁻¹·mg⁻¹ of protein); cytochrome P-450: 1.02 ± 0.08 (nmol/mg of protein); cytochrome b₅: 0.37 ± 0.03 (nmol/mg of protein). Activities of aminopyrine N-demethylase and aniline hydroxylase were significantly increased 24 h after the final injection of chlordiazepoxide, diazepam or oxazepam (100 mg·kg⁻¹·d⁻¹ for 3 d, i.p.). Oxazepam among the three drugs showed the greatest inducing effect on the drug-metabolizing enzyme activities in liver microsomes. Similarly, the level of cytochrome P-450 was also increased by pretreatment with these drugs, and the level of cytochrome b₅ was slightly increased by oxazepam.

Discussion

5-FU is converted to FUR and FUR is metabolized to 5-fluorouridine monophosphate, then into biologically active 5-fluorodeoxyuridine 5'-monophosphate (FdUMP), which is a potent inhibitor of thymidine synthetase. On the other hand, 5-FU is catabolized and excreted in the urine as 2-fluoro-5-ureidopropionic acid or 2-fluoro-β-alanine.

Previously, the authors reported that LPS, doxapram, aminophylline, propranolol and butocamidé affected the action of the antitumor drugs. These findings suggested that the hepatic drug-metabolizing system is inhibited or promoted by certain kinds of drugs, and that the action of the antitumor drugs is changed in combination with such drugs.

Stimulatory effects of chlordiazepoxide, diazepam and oxazepam on the hepatic microsomal drug-metabolizing enzymes in rats have been reported. Changes in hepatic microsomal metabolism provoked by the drugs are important from the viewpoint of the drug interaction. These drugs, benzodiazepines, are important as antianxietics. However, little work has been done on the drug interaction between minor tranquilizers and antitumor drugs.

In the present study, we investigated the effects of chlordiazepoxide, diazepam and oxazepam on the antitumor activities and plasma concentrations of tegafur and 5-FU, and on the hepatic drug-metabolizing system in mice.

Potentiation by chlordiazepoxide, diazepam and oxazepam of the antitumor activity of tegafur against mice bearing Sarcoma 180 was apparent. The antitumor activity of a single administration of tegafur 200 mg/kg or 400 mg/kg i.p. was increased by pretreatment with chlordiazepoxide, diazepam or oxazepam (100 mg·kg⁻¹·d⁻¹ for 3 d, i.p.) when tegafur was administered 24 h after the final injection of these drugs. On the other hand, the activity of a single administration of 5-FU 25 mg/kg or 50 mg/kg, i.p. was decreased by pretreatment with chlordiazepoxide or diazepam. These results indicate that chlordiazepoxide, diazepam and oxazepam potentiate the antitumor effect of tegafur, and that chlordiazepoxide and diazepam inhibit the effect of 5-FU, and thus suggest that these drugs promote the conversion from tegafur to 5-FU and the degradation of 5-FU.

It was reported that the antitumor activity on Sarcoma 180 solid tumor and the toxicity of tegafur and 5-FU are dose-dependent. Furthermore, the action of tegafur depends on its conversion to 5-FU and is weaker than that of 5-FU. In toxicity studies, it has become apparent that the prior administration of chlordiazepoxide, diazepam or oxazepam enhanced the toxicity of tegafur or 5-FU after 15 d. Consequently, it is considered that the increase in the toxicity of tegafur induced by chlordiazepoxide, diazepam or oxazepam is perhaps due to the increasing activation of tegafur. However, the reason for the increase in the toxicity of 5-FU induced by these drugs is not clear.

In the metabolic study of tegafur, we determined the time course of plasma level of
tegafur or 5-FU in mice after the administration of tegafur. Pretreatment with chlor diazepoxide, diazepam or oxazepam considerably affected the conversion of tegafur to 5-FU. The level of 5-FU released from tegafur in the plasma was maximum at 30 min after the administration of tegafur. In chlor diazepoxide-, diazepam- or oxazepam-treated mice, a lower concentration of tegafur and a higher concentration of 5-FU in the plasma after the administration of tegafur were observed as compared with those in mice treated with tegafur alone. On the other hand, the behavior of 5-FU level in the plasma after the administration of 5-FU was entirely opposite to that in the case of tegafur, that is to say, the level of 5-FU in the plasma after the administration of 5-FU was lower in chlor diazepoxide-, diazepam- or oxazepam-treated mice. In the liver and kidneys, the level of 5-FU after the administration of tegafur was higher in chlor diazepoxide-, diazepam- or oxazepam-treated mice than in control mice, but no effect of pretreatment with these drugs on the formation of FUR from 5-FU was observed. These findings indicated that chlor diazepoxide, diazepam and oxazepam promote the activation of tegafur and the degradation of 5-FU. Although it is known that 5-FU is converted to an active metabolite, FdUMP, through the intermediate FUR, the effect of chlor diazepoxide, diazepam or oxazepam upon the complex metabolic processes of 5-FU is still not well understood.

The influence of chlor diazepoxide, diazepam or oxazepam treatment on the activity of hepatic drug-metabolizing enzymes in mice was examined. Chlor diazepoxide, diazepam or oxazepam markedly enhanced the activities of aminopyrine N-demethylase and aniline hydroxylase in the 9000 x g supernatant of the liver homogenate and increased the cytochrome P-450 content in liver microsomes. Cytochrome b5 content in liver microsomes was slightly increased by oxazepam. The results indicated that chlor diazepoxide, diazepam or oxazepam stimulate the hepatic drug-metabolizing system, and this effect might explain the potentiation by chlor diazepoxide, diazepam and oxazepam of the antitumor activity of tegafur.

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References and Notes