STUDIES TO INCREASE THE ANTITUMOR ACTIVITY OF 1-(TETRAHYDRO-2-FURANYL)-5-FLUOROURACIL AND ITS METABOLIC ASPECTS BY COMBINED ADMINISTRATION WITH L-CYSTEINE

Nobuo KAWABATA, Seiyu SUGIYAMA, Tsukasa KUWAMURA, Tetsuo SATOH* and Haruo KITAGAWA*
Central Research Laboratory, SS Pharmaceutical Co., Ltd., 1143 Nanpeidai, Narita 286, Japan and *Laboratory of Biochemical Pharmacology and Biotoxicology, Department of Drug Evaluation and Toxicological Sciences, Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Chiba 260, Japan

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Abstract—The plasma and liver concentrations of both 1-(tetrahydro-2-furanyl)-5-fluorouracil (FT) and 5-fluorouracil (5-FU), an active metabolite of FT, increased very markedly after administration of FT (500 mg/kg, p.o.) combined with L-cysteine (L-CYS, 500 mg/kg, i.p. or p.o.) when compared to FT alone in rats. On the other hand, the oral acute toxicity of FT was also enhanced with the combined administration of FT and L-CYS. There was no difference in the in situ absorption rate of FT from the small intestine between rats treated with L-CYS (500 mg/kg, i.v.) and vehicle-treated controls. The inhibition of the disappearance of FT and the increase of the formation of 5-FU was observed in vitro after incubation of FT with liver microsomes from rats treated with L-CYS (500 mg/kg, p.o.) when compared to vehicle-treated controls. The presence of L-CYS significantly inhibited the in vitro degradation of 5-FU by non-treated rat liver homogenate. In the drug metabolizing enzyme activity of rat liver microsomes, aniline p-hydroxylase activity was inhibited, but aminopyrine N-demethylase activity was conversely activated by the combined administration of FT and L-CYS, but not by FT alone; furthermore, no change of cytochrome P-450 content was observed. In sarcoma 180 bearing mice, the oral antitumor activity of FT in combination with L-CYS (500 mg/kg, p.o. or i.p.) was about 1.1–2.0 times higher than that of FT alone. It was concluded from these findings that the drug metabolizing enzymes in liver involved in the conversion of FT into an active metabolite, 5-FU, are influenced by the combined administration of FT and L-CYS to give an increased organ level of 5-FU; and this resulted in the enhancement of the antitumor activity of FT.

1-(Tetrahydro-2-furanyl)-5-fluorouracil (FT) is now widely used in cancer chemotherapy and is considered as a prodrug of 5-fluorouracil (5-FU) (1–3). Several papers revealed that the cytochrome P-450-mediated drug metabolizing enzymes of liver microsomes may participate in the conversion of FT to 5-FU (1, 4). Many attempts have been made to keep the higher concentration of 5-FU in tissues as well as in blood by using either inhibitors of 5-FU degradation such as uracil (5–7) or drug metabolizing enzyme inducers such as phenobarbital (4). Uracil is known to inhibit 5-FU degradation and to have no influence on drug metabolizing enzyme activities (7, 8). When the drug
metabolizing enzyme activities are low in cancer patients (9), it seems difficult to obtain a high level of 5-FU in tissues by the combined administration with uracil. On the other hand, phenobarbital needs to be dosed for a few consecutive days before administration of FT to induce the drug metabolizing enzyme activities; furthermore, the pharmacological effects and development of tolerance of phenobarbital can not be disregarded either. The induction of the drug metabolizing enzymes by phenobarbital is enhanced by the combined administration with glutathione (GSH) because GSH increases the activities of drug metabolizing enzymes in the liver as a result of the inhibition of the lipid peroxidation which shares the electron transport system with the drug metabolizing enzymes (10, 11).

Recently, Yoshimura et al. (12) demonstrated that orally administered GSH is poorly absorbed from the digestive tract; but L-cysteine (L-CYS), a precursor of GSH, is well absorbed by the same route, resulting in the high liver level of GSH, which is synthesized from L-CYS in vivo, as reported previously (13, 14).

These findings prompted us to investigate the possibility for an increase in the concentrations of 5-FU in tissues by the treatment with FT combined with L-CYS, which might be followed by the enhancement in the antitumor activity of FT, and the effect of L-CYS on the metabolism of FT.

Materials and Methods

Animals: Male Wistar rats weighing 170–200 g and male ddY mice weighing 15–20 g were used in these experiments. The animals were maintained on a standard diet and water ad libitum in a room with controlled temperature (24°C) on a 12 hr light-dark cycle (7:00 a.m.–7:00 p.m.). Rats were fasted overnight before drug administration, but were given water ad libitum.

Chemicals: FT and 5-FU were purchased from Aldrich Chemical Co. (Milwaukee, WI) and Sigma Chemical Co. (St. Louis, MO), respectively. Orotic acid and L-CYS were obtained from Wako Pure Chemical Industries (Osaka) and Nippon Rikagakuyakuhin Co. (Tokyo), respectively. NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast Co. (Tokyo). Emulgen 913 was kindly supplied by Kao Atlas Co. (Tokyo). Ethereal diazomethane solution was prepared according to the method reported previously (15). All other reagents and solvents were analytical reagent grade. FT (150 mg/ml) and L-CYS (100 mg/ml) were administered in solutions of 1 M Na2CO3 and physiological saline, respectively. All solutions were prepared immediately before use, and all doses in this study referred to the free drug.

Preparation of plasma and liver microsomes: Rats were lightly anesthetized with ethyl ether at definite times after drug administration. Blood was drawn from the inferior vena cava into heparinized tubes after laparotomy. After centrifugation at 3,000 r.p.m. for 10 min, the resulting plasma was collected. The liver was perfused with 1.15% KCl and removed immediately after collecting blood which was omitted unless measuring the concentrations of FT and 5-FU in the plasma. The liver was minced and homogenized with 1.15% KCl or phosphate buffer with a Teflon-glass homogenizer. The homogenate was centrifuged at 10,000 g for 20 min, and the resultant supernatant was centrifuged at 105,000 g for 60 min. The microsomal pellet was washed with a small amount of 1.15% KCl and then suspended in 1.15% KCl to a protein concentration of about 10 mg/ml. All procedures were performed at 4°C. Plasma and liver microsomal suspensions were stored under nitrogen at −70°C for subsequent analyses.

Determination of FT and 5-FU in plasma
and liver: Rats were given FT alone at a dose of 500 mg/kg p.o. or combined with L-CYS at the same dose, i.p. or p.o., at 0, 3 and 6 hr after administration of FT. Plasma and livers were sampled at appropriate time intervals up to 12 hr after drug administration. Liver homogenate was prepared with 3 volumes of 0.25 M phosphate buffer (pH 6.0) in the manner described above.

The samples (1 ml) of plasma and 25% liver homogenate were deproteinized by adding 0.5 ml of ice-cold ethanol. After centrifugation at 3,000 r.p.m. for 10 min, 0.5 ml of the supernatant was removed and added to 0.5 ml of 0.25 M phosphate buffer (pH 6.0), then extracted with 8 ml of ethyl acetate. After centrifugation at 2,500 r.p.m. for 10 min, the organic layer was removed and evaporated to dryness under vacuum at room temperature. The residue was dissolved in 0.1 ml of orotic acid solution in methanol, corresponding to 30 and 150 µg/ml for plasma and liver, respectively, and 0.1 ml of ethereal diazomethane solution was added. The reaction mixture was evaporated to dryness under vacuum after standing for 30 min at room temperature, and the residue was dissolved in 100 µl of acetone. A 1–2 µl sample of this solution was injected into a Hewlett-Packard model 5710A GLC equipped with a nitrogen-phosphorus sensitive detector (NP-GLC). A 2 mm×3 mm I.D. glass column packed with 1% PEG-HT on 60–80 mesh Uniport HP (Gaschro Kogyo, Tokyo) was used for the chromatography. The temperatures of the injection port, column and detector were 300, 175 and 300°C, respectively. Helium, the carrier gas, was maintained at 37°C and was passed through the intestine at a flow rate of 3 ml/min. The rats were treated with L-CYS (500 mg/kg, i.v.) or the vehicle at 0, 0.5, 1 and 1.5 hr after the beginning of the perfusion. A 1 ml aliquot of the solution was removed at 0, 0.5, 1 and 2 hr after the perfusion was started. One half ml was used for the determination of FT by the spectrophotometric assay method (19), and the remaining 0.5 ml was used for the determination of phenol red (20).

In vitro metabolism of FT: The liver microsomal suspension was prepared from rats 2 hr after treatment with L-CYS (500 mg/kg, p.o.) in the manner described above. Control rats were treated with the identical volume of the vehicle. The incubation mixture contain-
ing 0.5 mM FT, 0.5 mM NADP, 5 mM glucose-6-phosphate, 12 units of glucose-6-phosphate dehydrogenase, 0.1 mM EDTA, 5 mM MgSO₄ and microsomal suspension (10 mg protein) in a total volume of 4 ml of 0.01 M phosphate buffer (pH 7.4) was incubated for 30 min at 37°C. The reaction was stopped by plunging it into an ice bath. A 1 ml aliquot of this solution was removed for subsequent determination of FT and 5-FU by NP-GLC in the manner described above.

In vitro degradation of 5-FU: A 25% (w/v) liver homogenate in 0.1 M phosphate buffer (pH 7.4) was prepared from non-treated rats in the manner described above. The incubation mixture with a total volume of 1 ml of the homogenate containing 7.7 μM 5-FU alone or combined with 1 or 10 mM L-CYS was incubated at 37°C for 1.5 hr. The reaction was stopped by plunging it into an ice bath at 0.5, 1 and 1.5 hr after the beginning of the incubation, and the concentration of remaining 5-FU was determined by NP-GLC in the manner described above.

Drug metabolizing enzyme activities: Rats were treated with FT (500 mg/kg, p.o.) alone or combined with L-CYS (500 mg/kg, i.p. or p.o.). Control rats were treated with the identical volume of the vehicle. Two hr later, microsomes were prepared in the manner described above. The content of cytochrome P-450 was measured by the method of Omura and Sato (21) using the extinction coefficient of 91 mM⁻¹ cm⁻¹. Aniline p-hydroxylase and aminopyrine N-demethylase activities were measured by determination of p-aminophenol formed according to the method of Schenkman (22) and formaldehyde formed by the method of Nash (23), respectively. Microsomal protein was determined by the method of Lowry et al. (24), using bovine serum albumin as the standard.

Chemotherapy experiments: Sarcoma 180 ascites tumor cells (5×10⁸) were transplanted subcutaneously into the right axilla of mice. These animals were divided into 3 groups. Ten animals of each group underwent each drug regimen starting 24 hr after tumor transplantation. One group of animals consisted of non-treated controls. A second group was given a single dose of FT (100 or 500 mg/kg, p.o.) alone or combined with L-CYS (500 mg/kg, i.p. or p.o.). A third group received FT (20, 60 or 100 mg/kg, p.o.) alone or combined with L-CYS (500 mg/kg, i.p. or p.o.) once a day for 7 consecutive days. Mice were sacrificed on day 15 after tumor transplantation. The tumors were excised and weighed. The antitumor activities of FT alone or combined with L-CYS were evaluated by the mean weight of the tumors in the treated mice as a percentage of their mean weight in the non-treated controls.

**Results**

**Plasma and liver concentrations of FT and 5-FU:** The concentrations of FT and 5-FU in plasma and liver obtained after administration of FT alone or combined with L-CYS (500 mg/kg, i.p. or p.o.) to rats are presented in Figs. 1 and 2. The concentrations of FT increased rapidly and reached the maximum value at 4–8 and 2–4 hr in plasma and liver, respectively. These concentrations were significantly different from those in the control group.

![Fig. 1. Concentrations of FT (---) and 5-FU (-----) in rat plasma after administration of FT (500 mg/kg, p.o.) alone or combined with L-CYS (500 mg/kg, i.p. or p.o.). Each point represents the mean±S.E. for 5 rats. ○: FT alone, ◊: FT+L-CYS, i.p., □: FT+L-CYS, p.o. Significantly different from FT alone. *P<0.05, **P<0.01, ***P<0.005.](image-url)
Table 1. Kinetic parameters obtained from the rats treated with FT alone or combined with L-CYS

<table>
<thead>
<tr>
<th>Kinetic parameter</th>
<th>FT</th>
<th>FT</th>
<th>FT</th>
<th>5-FU</th>
<th>5-FU</th>
<th>5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Liver</td>
<td>Plasma</td>
<td>Liver</td>
<td>Plasma</td>
<td>Liver</td>
</tr>
<tr>
<td>$\beta$ (hr$^{-1}$)</td>
<td>0.14</td>
<td>0.10</td>
<td>0.08</td>
<td>0.12</td>
<td>0.11</td>
<td>0.08</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>5.0</td>
<td>6.9</td>
<td>8.7</td>
<td>5.8</td>
<td>6.3</td>
<td>8.7</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/ml or g)</td>
<td>208.8 (1.2)</td>
<td>238.2 (2.0)</td>
<td>407.3 (2.0)</td>
<td>214.1 (1.4)</td>
<td>290.1 (1.7)</td>
<td>361.1 (1.7)</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hr)</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>$AUC_{0-12}$ (µg hr/ml or g)</td>
<td>2098 (1.2)</td>
<td>2415 (1.6)</td>
<td>3357 (1.6)</td>
<td>2159 (1.4)</td>
<td>3104 (1.5)</td>
<td>3327 (1.5)</td>
</tr>
</tbody>
</table>

$\beta$: 1st-order elimination rate constant. $t_{1/2}$: Biological half-life calculated by $0.693/\beta$. $C_{\text{max}}$: Maximum concentration. $T_{\text{max}}$: Time of $C_{\text{max}}$. $AUC_{0-12}$: Area under the plasma or liver concentration-time curve calculated by the trapezoidal rule.
liver, respectively, after administration of the drugs. 5-FU was released gradually from FT at concentrations approx. 1,000 times lower than those of FT for a long period. The time required to reach maximum concentrations of 5-FU in plasma and liver were around 8-12 hr after administration of the drugs.

The administration of FT combined with L-CYS resulted in significant increases of the concentrations of both FT and 5-FU in plasma and liver. Accordingly, the AUC0-12's of FT and 5-FU after administration of FT combined with L-CYS were approx. 1.2-1.6 and 1.7-2.0 times, respectively, greater than after FT alone in plasma and liver. The $t_1/2$'s of FT were prolonged in plasma as well as in liver, but as for 5-FU, such prolongation was not caused by the combined administration of L-CYS (Table 1).

**Acute toxicities:** As shown in Table 2, the LD50 in rats treated with FT alone (p.o.) was 1206 mg/kg, while after administration of FT (p.o.) combined with L-CYS (500 mg/kg, i.p. or p.o.), the toxicity was significantly enhanced with the potency ratios (LD50 of FT alone/LD50 of FT plus L-CYS, i.p. and p.o.) of 1.2. However, there was no significant difference in LD50 values between the two combined administration routes of L-CYS. After drug administration, death occurred primarily after 4 days in the FT alone group and between 2 and 4 days in the combined group.

**In situ absorption of FT:** FT was absorbed rapidly from the rat small intestine in situ as shown in Fig. 3. The percentages of the amounts of FT remaining in the perfusate of the vehicle-treated controls were 57.2, 30.2 and 16.1% at 0.5, 1.0 and 2.0 hr after the beginning of the perfusion, respectively.

**In vitro metabolism of FT:** The disappear-

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**Table 2. Effects of L-CYS on the 7 day LD50 of FT in rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LD50 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT alone</td>
<td>1206 (1076–1352)</td>
</tr>
<tr>
<td>FT+L-CYS (i.p.)</td>
<td>1002* (923–1087)</td>
</tr>
<tr>
<td>FT+L-CYS (p.o.)</td>
<td>1004* (924–1091)</td>
</tr>
</tbody>
</table>

Rats were given FT (p.o.) alone or combined with L-CYS (500 mg/kg, i.p. or p.o.). Seven rats were used for each treatment. Values in parentheses indicate the 95% confidence limits. Significantly different from FT alone, *P<0.05.
ance of FT and the formation of 5-FU in vitro with the liver microsomes of the rats treated with L-CYS at a dose of 500 mg/kg p.o. are shown in Table 3. When 0.5 mM FT was incubated with the microsomes from the vehicle-treated controls at 37°C for 30 min, 25.92 nmoles of FT per mg microsomal protein disappeared and 2.57 nmoles of 5-FU per mg microsomal protein was formed. With the microsomes obtained from the rats 2 hr after administration of L-CYS, the disappearance of FT was significantly inhibited by 44.8%, and conversely, the formation of 5-FU was stimulated by 14.8% when compared to the vehicle-treated controls.

In vitro degradation of 5-FU: The percent degradations of 5-FU after incubation of 5-FU alone or in the presence of L-CYS with the rat liver homogenate are shown as a function of time in Fig. 4. The concentration of 5-FU in the incubation mixture consisting of 25% rat liver homogenate with 7.7 μM 5-FU alone abruptly decreased to 34.0% of the initial concentration at 0.5 hr after the incubation, and thereafter, slowly diminished during the 1.5 hr incubation period. Whereas, such degradation of 5-FU was inhibited by L-CYS added in the incubation mixture in

![Fig. 3. Effects of L-CYS on the absorption of FT from rat small intestine in situ. Rats were treated with vehicle or L-CYS (500 mg/kg, i.v.) 0, 0.5, 1.0 and 1.5 hr after the beginning of the perfusion. Thirty ml of a solution of FT were recirculated through the small intestine for 2.0 hr. Each point represents the mean±S.E. for 3 rats. ●: vehicle-treated control. ○: treated with L-CYS.](image)

![Fig. 4. Effects of L-CYS on enzymatic degradation of 5-FU in rat liver homogenate. Homogenates were prepared from non-treated rats. Homogenates were incubated with 7.7 μM 5-FU alone or in the presence of 1 or 10 mM L-CYS at 37°C for 1.5 hr. The concentrations of 5-FU were measured as described under Materials and Methods. Each value represents the mean±S.E. for 3 experiments. ●: 5-FU alone, ○: 5-FU+1 mM L-CYS, □: 5-FU+10 mM L-CYS. Significantly different from 5-FU alone, *P<0.05, **P<0.005.](image)

**Table 3. Effects of L-CYS on metabolism of FT with rat liver microsomes**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FT disappeared (nmol/mg protein)</th>
<th>5-FU formed (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.92±1.94</td>
<td>2.57±0.24</td>
</tr>
<tr>
<td>L-CYS</td>
<td>14.30±3.29* (−44.8%)</td>
<td>2.95±0.15 (−14.8%)</td>
</tr>
</tbody>
</table>

Liver microsomes were prepared from vehicle-treated control rats or rats 2 hr after treated with L-CYS (500 mg/kg, p.o.). Incubation was carried out with 0.5 mM FT, 0.5 mM NADP, 5 mM glucose-6-phosphate, 12 units of glucose-6-phosphate dehydrogenase, 0.1 mM EDTA, 5 mM MgSO₄ and microsomes (10 mg protein) in a total volume of 4 ml of 0.01 M phosphate buffer (pH 7.4) for 30 min at 37°C. Each value represents the mean±S.E. for 3 rats with the percentage change from the vehicle-treated control in parentheses. Significantly different from the control, *P<0.05.
proportion to its concentration, and significant inhibitory effect was observed with 10 mM L-CYS in all incubation time examined.

Effects of drug treatment on the liver microsomal drug metabolizing enzymes: The drug metabolizing enzyme activities in liver microsomes of the rats 2 hr after receiving FT (500 mg/kg, p.o.) alone or combined with L-CYS (500 mg/kg, i.p. or p.o.) were measured, and the results are shown in Table 4. The concentrations of cytochrome P-450, obtained after administration of FT alone or combined with L-CYS, were almost similar, and they were not significantly different from those of the vehicle-treated controls. Aniline \( p \)-hydroxylase activity was not affected by the administration of FT alone, but lowered significantly to 79.7 and 93.2\% of the vehicle-treated controls by the administration of FT combined with L-CYS i.p. or p.o., respectively. Aminopyrine N-demethylase activity decreased only a little by the administration of FT alone, but increased to 115.9 and 163.5\% of the vehicle-treated controls by the administration of FT combined with L-CYS i.p. or p.o., respectively.

Chemotherapy experiments: The data obtained from 3 groups are summarized in Table 5.

1. Group I (control animals): The mean tumor weight in mice receiving no chemotherapy was 5.0±2.9 g (mean±S.E.) on day 15 after tumor transplantation. This number was used to calculate the T/C values after administration of FT alone or combined with L-CYS.

2. Group II (single administration of FT alone or combined with L-CYS): The antitumor activity after treatment of mice with FT alone at a dose of 100 mg/kg p.o. was not clearly recognizable, but treatment with a higher dose (500 mg/kg, p.o.) caused a definite reduction in tumor weight with a T/C value of 76\%. These activities were potentiated by the combined administration of L-CYS (500 mg/kg, i.p. or p.o.) as shown in Table 5. The reductions of tumor weight in mice treated with FT combined with L-CYS (500 mg/kg, i.p.) were in proportion to the doses of FT. After combined administration of L-CYS (500 mg/kg, p.o.), however, the tumor weight was not as reduced as after administration of L-CYS i.p.

3. Group III (repeated administration of FT alone or combined with L-CYS): No antitumor activity was obtained after adminis-

### Table 4. Effects of L-CYS on cytochrome P-450-dependent drug metabolism

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytochrome P-450 (nmol/mg protein)</th>
<th>Aniline ( p )-hydroxylase (nmol/min/mg protein)</th>
<th>Aminopyrine N-demethylase (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.99±0.10</td>
<td>0.59±0.01</td>
<td>2.08±0.06</td>
</tr>
<tr>
<td>FT alone</td>
<td>0.98±0.05 (-1.0%)</td>
<td>0.58±0.01 (-1.7%)</td>
<td>1.93±0.08 (-7.2%)</td>
</tr>
<tr>
<td>FT+L-CYS (i.p.)</td>
<td>0.89±0.01 (-10.1%)</td>
<td>0.47±0.01** (-20.3%)</td>
<td>2.41±0.16 (15.9%)</td>
</tr>
<tr>
<td>FT+L-CYS (p.o.)</td>
<td>1.06±0.03 (+7.1%)</td>
<td>0.55±0.01* (-6.8%)</td>
<td>3.40±0.39* (+63.5%)</td>
</tr>
</tbody>
</table>

Liver microsomes were prepared from vehicle-treated control rats or rats 2 hr after treatment with FT (500 mg/kg, p.o.) alone or combined with L-CYS (500 mg/kg, i.p. or p.o.). Concentrations of P-450, aniline (1 mM) \( p \)-hydroxylase and aminopyrine (1 mM) N-demethylase activities were measured as described under Materials and Methods. Each value represents the mean±S.E. for 4 rats with the percentage change from the vehicle-treated control in parentheses. Significantly different from the control, \(*P<0.025, **P<0.006.\)
L-CYS EFFECTS ON FT AND 5-FU LEVELS

Table 5. Effects of L-CYS on FT-induced inhibition of sarcoma 180 in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>FT (mg/kg, p.o.)</th>
<th>L-CYS (mg/kg)</th>
<th>Tumor weight(g) mean±S.E.</th>
<th>T/C (^a) (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>5.0±2.9</td>
<td></td>
</tr>
<tr>
<td>I(b)</td>
<td>100</td>
<td>0</td>
<td>4.7±2.0</td>
<td>94 (1.0)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>500 (i.p.)</td>
<td>4.1±2.8</td>
<td>82 (1.1)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>500 (p.o.)</td>
<td>2.7±1.2**</td>
<td>54 (1.7)</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0</td>
<td>3.8±2.3</td>
<td>76 (1.0)</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>500 (i.p.)</td>
<td>1.9±1.5</td>
<td>38 (2.0)</td>
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<tr>
<td></td>
<td>500</td>
<td>500 (p.o.)</td>
<td>2.8±1.8</td>
<td>56 (1.4)</td>
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<td>20</td>
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<td>5.1±2.0</td>
<td>102 (1.0)</td>
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<td>500 (i.p.)</td>
<td>3.7±3.2</td>
<td>74 (1.4)</td>
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<td>500 (p.o.)</td>
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<td>5.2±2.2</td>
<td>104 (1.0)</td>
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<td>500 (i.p.)</td>
<td>3.0±1.7</td>
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<td>500 (p.o.)</td>
<td>3.0±1.1</td>
<td>60 (1.7)</td>
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<td>0</td>
<td>3.8±1.7</td>
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<td>500 (i.p.)</td>
<td>2.7±1.9</td>
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<td></td>
<td>100</td>
<td>500 (p.o.)</td>
<td>1.9±1.2*</td>
<td>38 (2.0)</td>
</tr>
</tbody>
</table>

Mice were treated in a single dose or for 7 consecutive days with FT alone or combined with L-CYS starting on day 1. They were killed and tumor weights were measured on day 15 after tumor transplantation. Number of animals in each group was 10. \(^a\) Antitumor activity was evaluated by comparing the mean tumor weight (T) in the treated animals with that (C) in the non-treated controls. \(^b\): Non-treated control. \(^c\): II: Group of animals given a single dose of FT alone or combined with L-CYS. \(^d\): III: Group of animals given FT alone or combined with L-CYS, once a day for 7 days. Values in parentheses are inverse ratios of the mean tumor weight in mice treated with FT combined with L-CYS to that with FT alone. Significantly different from FT alone, \(*P<0.05, **P<0.01.\)

Discussion

When FT (500 mg/kg, p.o.) was administered with L-CYS (500 mg/kg, i.p. or p.o.) to rats, higher concentrations of FT and 5-FU in the plasma and liver were obtained as compared to the administration of FT alone (500 mg/kg, p.o.). The plasma and liver AUC\(_{0-12}\)'s of FT and 5-FU after administration of FT combined with L-CYS were approx. 1.2–1.6 and 1.7–2.0 times, respectively, greater than those after FT alone. The \(t_{1/2}\) of FT in plasma was estimated to be 5.0 hr after administration of FT (500 mg/kg, p.o.) in close agreement with a report by Cohen (1) who showed that the \(t_{1/2}\) of FT given i.v. at a dose of 100 mg/kg was approx. 5.0 hr in the rat. By the combined administration of L-CYS, however, the \(t_{1/2}\)'s of FT in plasma and liver were
prolonged, while those of 5-FU were not changed. To elucidate the mechanism of increment in the organ levels of FT and 5-FU after administration of FT combined with L-CYS, the present studies were approached with special reference to the effects of L-CYS on the metabolism of FT because little information from such a point of view is available.

Shimoyama et al. (25) have reported that FT was well absorbed from the small intestine of the rat, but scarcely from the stomach in an in situ recirculation system. Then, further investigation using the same system was aimed at examining whether L-CYS facilitates the intestinal absorption of FT. However, there was no difference in the absorption rate of FT from the small intestine between the rats treated with L-CYS (500 mg/kg, i.v.) and the vehicle-treated controls. Accordingly, the reason why the increased organ levels of FT and 5-FU were obtained by the combined administration of FT and L-CYS can not be explained by this absorption study.

It is well known that FT is biotransformed to an active metabolite, 5-FU, by microsomal drug metabolizing enzymes such as cytochrome P-450 in liver (1–3). Then it can be predicted that the increase of the formation of 5-FU should be accompanied by the decrease of the concentration of FT through the enzymatic conversion by cytochrome P-450 in the liver. On the contrary, in the present study, not only the concentrations of 5-FU but also those of FT increased together in plasma and liver after administration of FT combined with L-CYS when compared to FT alone. Furthermore, when FT was used as a substrate for the liver microsomal drug metabolizing enzymes of the rats 2 hr after receiving L-CYS (500 mg/kg, p.o.), the disappearance of FT was significantly inhibited by 44.8%, while the formation of 5-FU was enhanced by 14.8% after incubation for 30 min. Then the effect of L-CYS on the degradation of 5-FU formed in vivo after administration of FT was investigated in vitro with the liver homogenate of the nontreated rats, taking it into account that the degradation of 5-FU occurs predominantly in the liver cytosolic fraction (8). The degradation of 5-FU was inhibited by L-CYS in proportion to its concentration in the incubation mixture. These findings suggest that L-CYS may affect preferentially the drug metabolizing enzyme systems responsible for the conversion of FT into 5-FU and/or their degradation, rather than the absorption step described above.

It has been demonstrated that the decrease in the activities of aniline p-hydroxylase and aminopyrine N-demethylase is caused by the induction of lipid peroxidation which shares the electron transport system with the drug metabolizing enzymes (11), and the lipid peroxidation is inhibited by L-CYS and by GSH (10) which is formed from L-CYS in vivo (13, 14). In the present studies, the activity of aniline p-hydroxylase was inhibited, but the activity of aminopyrine N-demethylase was enhanced by the administration of FT (500 mg/kg, p.o.) combined with L-CYS (500 mg/kg, i.p. or p.o.). However, the result obtained in this study that the concentrations of both FT and 5-FU increased after administration of FT combined with L-CYS can not be elucidated only by the inhibition of lipid peroxidation, which, of course, can not be ruled out and may play a partial role.

Recently, it has been proved that there are multiple forms of cytochrome P-450 which catalyze drug metabolism with their own substrate specificities (26, 27), and the various forms of cytochrome P-450 also exhibit positional selectivity and stereoselectivity in the oxidation of drugs (27–29). Therefore, it can be supposed that the drug metabolizing enzyme activities in liver microsomes are influenced in the different manners
by the administration of FT combined with L-CYS, except by FT alone, without any change in the concentration of cytochrome P-450.

Based on these findings, we would like to present the speculative scheme (Fig. 5) in which two metabolizing routes, I and II, of FT catalyzed by cytochrome P-450 are proposed in order to explain the results obtained in this study, and the metabolites of FT reported and/or presumed up to the present are also depicted (30-33). It has been proposed that 5-FU is not formed directly from FT enzymatically by cytochrome P-450 in liver, but via labile metabolic intermediates which could be easily transformed enzymatically or nonenzymatically to 5-FU (30, 33, 34). Trans-3'-, cis-4'- and trans-4'-OH-FT are stable and not responsible either enzymatically or nonenzymatically for the formation of 5-FU as reported previously (33, 35). 4', 5'-Dehydro-FT is formed not only in vivo but also in vitro by rat liver microsomes (32). It is not apparent whether 4', 5'-dehydro-FT could be formed by the dehydroxylation of 4'- or 5'-OH-FT. Judging from the previous study that 4'-OH-FT could not be converted to 5-FU when incubated in plasma or the buffer (pH 9.0) at 37°C for 4 hr (35), 4', 5'-dehydro-FT might be formed via 5'-OH-FT from FT. In any case, 2'-, 5'-OH-FT and 4', 5'-dehydro-FT are chemically labile, so could undergo nonenzymatic transformation to 5-FU in vivo, and thus might serve as metabolic intermediates in the conversion of FT to 5-FU (30, 31, 33). The metabolic route I, through which 3'- and 4'-OH-FT could be formed, is perhaps inhibited by the administration of L-CYS and could result in the increase of the concentration of FT. In contrast with this, the other route II, responsible for the formation of 2'- and 5'-OH-FT, might be activated or at least unchanged by the administration of L-CYS. It is not clear whether this activation would be caused by the substantial activation of the cytochrome P-450's responsible for route II or attributable to the inhibition of those responsible for route I. The higher the concentration of FT that would be obtained as a result of the inhibition of route I, the more 5-FU could be formed via route II.

It was strongly suggested from these results that the high organ levels of FT and 5-FU after administration of FT combined with L-CYS might cause the enhancement in the antitumor activity of FT. Then, we conducted a preliminary experiment on the
antitumor effect of FT combined with L-CYS using mice. In the sarcoma 180 bearing mice, the oral antitumor activity of FT was enhanced about 1.1–2.0 times by the combined administration of L-CYS, i.p. or p.o., when compared to the effect chemotherapy with FT alone.

In summary, FT is converted to 5-FU via some metabolic intermediates formed through some of the different metabolizing routes in which cytochrome P-450’s might be activated or at least not changed by the administration of L-CYS. Other routes, which catalyze the metabolism of FT, not responsible for the formation of 5-FU are perhaps inhibited. Thus, the concentrations of FT and 5-FU increased in plasma and liver after administration of FT combined with L-CYS when compared to FT alone, resulting in the further increased anabolic metabolism of 5-FU, which subsequently exerts enhanced antitumor effects.

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