Effect of L-Cysteine on Plasma Concentration and Urinary Excretion of 1-(Tetrahydro-2-furanyl)-5-fluorouracil Metabolites¹⁾

Nobuo KAWABATA,* Kenichi YANO, Hiromitsu OHNO, and Toshiaki NAKASHIMA

Central Research Laboratory, SS Pharmaceutical Co., Ltd., 1143 Nanpeidai, Narita, 286, Japan. Received September 25, 1990

Effects of L-cysteine (CySH) on the plasma concentrations and the urinary excretion of 1-(tetrahydro-2-furanyl)-5-fluorouracil (FT) and its metabolites were studied by high performance liquid chromatography in rats.

Significantly higher plasma concentrations of FT, 5-fluorouracil (5-FU) and *cis*-4'-OH-FT were obtained after an oral administration of FT (500 mg/kg) combined orally with CySH (500 mg/kg) when compared to FT alone. The urinary excretions of 5-FU, *trans*-3'-OH-FT, *cis*-4'-OH-FT, *trans*-4'-OH-FT and 4',5'-dehydro-FT significantly decreased up to 12 h but that of α -fluoro- β -alanine significantly increased up to 24 h by the combined administration of CySH. Furthermore, the plasma concentration of 5-FU significantly increased at 0.5 h and its urinary excretion significantly decreased up to 4 h after an intraperitoneal administration of 5-FU (10 mg/kg) combined orally with CySH (500 mg/kg) when compared to 5-FU alone. The urinary pH significantly changed to acidic and the urinary volume significantly increased by the combined administration of CySH, so it was thought that the reabsorption of 5-FU through renal tubules from urine could increase and the increment of the urinary excretion of α -fluoro- β -alanine was caused by this. Then it was suggested that the increase of the plasma concentrations of 5-FU and *cis*-4'-OH-FT could be attributed to the decrease of their urinary excretions after an administration of FT combined with CySH when compared to FT alone.

Keywords 1-(tetrahydro-2-furanyl)-5-fluorouracil; cysteine; rat; metabolite; plasma concentration; urinary excretion

1-(Tetrahydro-2-furanyl)-5-fluorouracil (FT) is considered a prodrug of 5-fluorouracil (5-FU) and exerts antitumor activity through metabolic activation.^{2,3)} FT is metabolized to some metabolites by liver microsome and cytosol as shown in Fig. 1.⁴⁻⁹⁾ Among these metabolites, *trans*-3'-OH-FT, *cis*-4'-OH-FT, *trans*-4'-OH-FT and 4',5'-dehydro-FT exhibit antitumor activities as well as 5-FU.^{10,11)} However, the antitumor activities of these metabolites are lower than that of 5-FU.¹¹⁾

Many efforts have been made to increase the antitumor activity of FT by obtaining a high plasma concentration of 5-FU.^{12,13)} In the previous paper,¹⁾ we reported that the plasma and liver concentrations of FT and 5-FU increased after the administration of FT (500 mg/kg, *p.o.*) combined with L-cysteine (CySH, 500 mg/kg, *p.o.*) when compared to FT alone in rats.

In this study we tried to obtain some clue for elucidating

the mechanism of the CySH effect in the previous study,¹⁾ in terms of metabolic and excretory aspects, by examining the plasma concentrations and the urinary excretions of FT and its metabolites after the oral administration of FT alone or combined with CySH in rats.

Experimental

Materials FT and 5-FU were purchased from Aldrich Chemical Co. (Milwaukee, WI) and Sigma Chemical Co. (St. Louis, MO), respectively. α -Fluoro- β -alanine was obtained from Tokyo Kasei Kogyo Co. (Tokyo). CySH was purchased from Nippon Rikagakuyakuhin Co. (Tokyo). All other reagents and solvents were of reagent grade. FT (150 mg/ml) was dissolved in 1 M Na₂CO₃. 5-FU (5 mg/ml) and CySH (100 mg/ml) were dissolved in physiological saline. These solutions were administered immediately after preparation.

Animals Male Wistar rats weighing 135–170 g were used. Rats were fasted overnight before drug administration and during the experiment, and were given water *ad libitum*.

Preparation of Plasma and Urine Rats were orally given FT alone at

Fig. 1. Postulated Metabolic Pathways of FT

(a) Liver microsome (cytochrome P-450). (b) Liver cytosol (dehydrogenase, etc.). (c) Absolute configuration remains undetermined.

a dose of 500 mg/kg or combined with CySH at the same dose, 0, 3 and 6 h after the administration of FT. Rats were lightly anesthetized with diethyl ether at definite times after the administration of FT. Blood was drawn from the inferior vena cava into heparinized tubes after laparotomy. After centrifugation at 2500 rpm for 10 min the resulting plasma was collected. After the administration of FT alone or combined with CySH, urine was sampled up to 12 and 24 h by using metabolic cages and urine up to 4 h was used immediately after collection to determine the pH value.

In a separate experiment, other groups of rats were intraperitoneally given 5-FU at a dose of 10 mg/kg alone or combined orally with CySH at a dose of 500 mg/kg at 0 and 4 h before the administration of 5-FU. Plasma at 0.5 h and urine up to 4 h after the administration of 5-FU were prepared as described above.

The plasma and urine samples were frozen for subsequent analyses.

Determination of FT and Its Metabolites in Plasma and Urine FT and its metabolites except α -fluoro- β -alanine were extracted from plasma and urine according to the method of Wu *et al.*¹⁴⁾ The volume of samples (0.1–1 ml) of plasma or urine was adjusted to 1 ml with water, and mixed with 0.1 ml of 0.5 M NaH_2PO_4 . The mixture was extracted with 8 ml of ethyl acetate. After centrifugation at 2500 rpm for 10 min the organic layer was removed and evaporated to dryness under vacuum at room temperature. The residue was dissolved in 100 μl of methanol, and a 5–10 μl aliquot of this solution was injected into a Hitachi Model 635 high performance liquid chromatograph with a JASCO Model UVIDEC-100-V absorbance detector under the following conditions as previously reported^{15,16)}: analytical column, Nucleosil C_{18} (5 μm , octadecyl, 250 $\times$ 4.6 mm i.d., Gasukuro Kogyo Inc., Tokyo); mobile phases, 15% methanol in 0.01 M acetate buffer (pH 4.2) for analyzing FT and 5-FU, 0.01 M acetate buffer (pH 4.0): acetonitrile (95:5) for other metabolites; flow rate, 1 ml/min; column temperature, 50 $^\circ\text{C}$; detector, UV, 280 nm. *Trans*-3'-OH-FT, *cis*-4'-OH-FT, *trans*-4'-OH-FT and 4',5'-dehydro-FT were identified by comparing their retention times with the ones in the previous report.¹⁵⁾ They were quantitated using a standard curve of FT assuming identical chromatographic properties and extinction coefficients of 280 nm.⁷⁾

For the determination of α -fluoro- β -alanine in urine, urine samples (0.1 ml) were added to 0.4 ml water and 0.5 ml of 2% sulfosalicylic acid. After shaking for 15 min and subsequent centrifugation at 3000 rpm for 10 min, a 250 μl aliquot of the supernatant was injected into a Hitachi Model 835 amino acid analyzer under the following conditions: analytical

column, 2619 F (ion exchange resin, 150 $\times$ 2.6 mm i.d., Hitachi, Tokyo); mobile phase, citrate buffer (pH 3.0, 35 mM lithium citrate, 50 mM lithium chloride, 160 mM citric acid, 5% ethyl alcohol, 50 mM β -thiodiglycol, 0.8 mM polyoxyethylene (23) lauryl ether and 0.8 mM capric acid); flow rate, 0.37 ml/min.

Statistics Data were evaluated by the Student's *t*-test and the difference was considered significant at $p < 0.05$.

Results and Discussion

The plasma concentrations of FT and its metabolites could be measured by using 0.01 M acetate buffer (pH 4.0): acetonitrile (95:5) as a mobile phase (Fig. 2). Separation of 5-FU from an endogenous component was achieved by using 15% methanol in 0.01 M acetate buffer (pH 4.2) instead as a mobile phase. The concentrations of FT, 5-FU and *cis*-4'-OH-FT significantly increased after the administration of FT combined with CySH when compared to FT alone (Fig. 3) as reported in the previous study.¹⁾ In the case of the other metabolites, no significant difference was observed for *trans*-3'-OH-FT and *trans*-4'-OH-FT except at 8 and 24 h, respectively.

The urinary excretions of FT metabolites, except α -fluoro- β -alanine, were significantly suppressed up to 12 h and 5-FU was still significantly less excreted up to 24 h after the administration of FT combined with CySH when compared to FT alone (Fig. 4). The pH of urine obtained up to 4 h significantly changed to acidic and the urinary volume significantly increased (Table I).

On the other hand, CySH is metabolized to acidic compounds in rats.¹⁷⁾ It is thought that due to these formations the urinary pH might then change to acidic by the combined administration of CySH. 5-FU is a weakly acidic compound, and has a pK_a value of 8.0.¹⁸⁾ It is reported that 5-FU is reabsorbed from renal tubules in rats.¹⁹⁾ It is

Fig. 2. Chromatograms Obtained from Rat Plasma

(A) Blank plasma, (a) endogeneous component. (B) Plasma at 4 h after oral administration of FT (500 mg/kg); (b) 5-FU, 0.74 $\mu\text{g}/\text{ml}$; (c) *trans*-4'-OH-FT, 0.66 $\mu\text{g}/\text{ml}$; (d) *cis*-4'-OH-FT, 1.34 $\mu\text{g}/\text{ml}$; (e) *trans*-3'-OH-FT, 0.41 $\mu\text{g}/\text{ml}$; (f) FT, 127.6 $\mu\text{g}/\text{ml}$; (g) 4',5'-dehydro-FT, 6.62 $\mu\text{g}/\text{ml}$. Mobile phase; 0.01 M acetate buffer (pH 4.0): acetonitrile (95:5). The sensitivity of the instrument was lowered to 1/4 for 4',5'-dehydro-FT after other metabolites were detected. Other conditions are described in Experimental.

well known that the renal tubular reabsorption of weakly acidic compounds is greatly enhanced by the decline of urinary pH causing an increase in the concentration of their undissociated forms. Therefore, it is possible to consider that the tubular reabsorption of 5-FU increased due to the decline of the urinary pH caused by the combined administration of CySH. Furthermore, the plasma con-

centrations of 5-FU significantly increased from 1.53 to 1.88 $\mu\text{g/ml}$ at 0.5 h and concomitantly its urinary excretion significantly decreased from 2.1 to 1.6% in the percentage of the dose after the administration of 5-FU (10 mg/kg, i.p.) combined with CySH (500 mg/kg, p.o.) when compared to 5-FU alone. Accordingly, it was suggested that the increase of the plasma concentrations of 5-FU might be attributed, at least partially, to the decrease of its urinary excretion. And also, the increase of the plasma concentrations of *cis*-4'-OH-FT might be caused by the decrease of its urinary excretion in the same manner as 5-FU.

The distinct effect of CySH was not observed in FT metabolites other than 5-FU and *cis*-4'-OH-FT. In the case of these metabolites, the significant increase of their plasma concentrations may not have been distinctly observed in spite of the decrease of their cumulative urinary excretions (Fig. 4A).

The plasma concentrations of the FT metabolites did not decrease, but those of *cis*-4'-OH-FT as well as 5-FU (Fig. 3), significantly increased. Furthermore, the excreted amount of α -fluoro- β -alanine significantly increased (Fig. 4B), and was thought to be caused by the increase of the plasma concentrations of 5-FU. T_{max} of 5-FU was 8 h in plasma (Fig. 3). Some processes are needed for its metabolism⁹⁾ and then an appearance of significant increase in the excreted amount of α -fluoro- β -alanine might be observed not up to 12 h, but from 12 h after the ad-

Fig. 3. Plasma Concentrations of FT (○, ●), 5-FU (△, ▲), *trans*-3'-OH-FT (□, ■), *cis*-4'-OH-FT (◇, ◆), *trans*-4'-OH-FT (▽, ▼) and 4',5'-Dehydro-FT (☆, ★) after Oral Administration of FT (500 mg/kg) Alone (Open Symbol) or Combined with CySH (500 mg/kg, Closed Symbol)

Each point represents the mean $\pm$ S.E. for five rats. a) $p < 0.05$ and b) $p < 0.01$ when compared to FT alone.

TABLE I. Urinary pH and Urinary Volume Obtained after Oral Administration of FT (500 mg/kg) Alone or Combined with CySH (500 mg/kg)

	FT alone	FT + CySH
Urinary pH	7.87 ± 0.09	$6.76 \pm 0.22^a)$
Urinary volume (ml)	3.94 ± 0.43	$5.70 \pm 0.11^a)$

Each value represents the mean $\pm$ S.E. for five rats. a) $p < 0.01$ when compared to FT alone.

Fig. 4. Cumulative Urinary Excretions of FT (I), 5-FU (II), *trans*-3'-OH-FT (III), *cis*-4'-OH-FT (IV), *trans*-4'-OH-FT (V), 4',5'-Dehydro-FT (VI) and α -Fluoro- β -alanine (VII) after Oral Administration of FT (500 mg/kg) Alone (Open Symbol) or Combined with CySH (500 mg/kg Closed Symbol)

(A) and (B) show up to 12 and 24 h, respectively, after administration of FT alone or combined with CySH. Each column represents the mean $\pm$ S.E. for five rats. a) $p < 0.05$ and b) $p < 0.01$ when compared to FT alone.

ministration of FT combined with CySH. It was suggested that FT and 5-FU metabolism was not inhibited by the combined administration of CySH *in vivo*.

On the other hand, we obtained the result that CySH did not have any effect on FT absorption from the digestive tract *in situ* in the previous study.¹⁾ And there was no difference in the amount of FT remaining in the digestive tract after the administration of FT (500 mg/kg, *p.o.*) combined with CySH (500 mg/kg, *p.o.*) when compared to FT alone (data not shown).

Based on these results, it seems that other mechanisms (*e.g.*, an effect of CySH on FT and/or its metabolite distribution) for 5-FU and *cis*-4'-OH-FT, besides the one obtained in this study, might also be responsible for the increase of the plasma concentrations of FT (Fig. 3). In fact, the urinary volume significantly increased by the combined administration of CySH (Table I). And in relation to this, we obtained the preliminary result that a volume of distribution of FT significantly decreased when accompanied by the decrease of body fluid volumes, which is thought to be caused by an increase of urinary volume, after the administration of FT (100 mg/kg, *i.v.*) combined with CySH (500 mg/kg, *p.o.*) when compared to FT alone in rats (data not shown).

In conclusion, it was suggested that the decrease of urinary excretion of 5-FU and *cis*-4'-OH-FT might result, at least partially, in the increase of their plasma concentrations after the administration of FT combined with CySH when compared to FT alone. But, we could not successfully account for the mechanism of the increase in the plasma concentrations of FT. Further work is in progress on the aspects of FT and its metabolite distribution.

References

- 1) N. Kawabata, S. Sugiyama, T. Kuwamura, T. Satoh, and H. Kitagawa, *Jpn. J. Pharmacol.*, **33**, 735 (1983).
- 2) A. M. Cohen, *Drug Metab. Dispos.*, **3**, 303 (1975).
- 3) S. Fujimoto, T. Akao, B. Itoh, I. Koshizuka, K. Koyano, Y. Kitsukawa, M. Takahashi, T. Minami, H. Ishigami, Y. Nomura, and K. Itoh, *Cancer Res.*, **36**, 33 (1976).
- 4) Y. M. El Sayed and W. Sadee, *Biochem. Pharmacol.*, **31**, 3006 (1982).
- 5) Y. M. El Sayed and W. Sadee, *Cancer Res.*, **43**, 4039 (1983).
- 6) S. Kawata, Y. Minami, S. Tarui, T. Marunaka, M. Okamoto, and T. Yamano, *Jpn. J. Pharmacol.*, **36**, 43 (1984).
- 7) A. T. Wu, J. L. Au, and W. Sadee, *Cancer Res.*, **38**, 210 (1978).
- 8) T. Marunaka, Y. Minami, Y. Umeno, A. Yasuda, T. Sato, and S. Fujii, *Chem. Pharm. Bull.*, **28**, 1795 (1980).
- 9) K. L. Mukherjee and C. Heidelberger, *J. Biol. Chem.*, **235**, 433 (1960).
- 10) A. J. Lin, R. S. Benjamin, P. N. Rao, and T. L. Loo, *J. Med. Chem.*, **22**, 1096 (1979).
- 11) S. Fujii, *Eksp. Klin. Farmakoter.*, **12**, 84 (1983).
- 12) G. A. Belitsky, V. M. Bukhman, and I. A. Konopleva, *Cancer Chemother. Pharmacol.*, **6**, 183 (1981).
- 13) K. Tatsumi, M. Fukushima, T. Shirasaka, and S. Fujii, *Jpn. J. Cancer Res.*, **78**, 748 (1987).
- 14) A. T. Wu, H.-J. Schwandt, C. Finn, and W. Sadee, *Res. Commun. Chem. Pathol. Pharmacol.*, **14**, 89 (1976).
- 15) J. L. Au, A. T. Wu, M. A. Friedman, and W. Sadee, *Cancer Treat. Rep.*, **63**, 343 (1979).
- 16) J. L. Au and W. Sadee, *Cancer Res.*, **40**, 2814 (1980).
- 17) K. Yamaguchi, I. Ueda, T. Nakashima, Y. Kakimoto, M. Kizumi, E. Hasegawa, and H. Nakagawa, "Biochemical Experimental Lecture 11, Amino Acid Metabolism and Biogenic Amines," ed. by Y. Nishizuka, O. Hayaishi and H. Wada, Tokyokagakudojin, Tokyo, 1976, pp. 431-466.
- 18) I. Wempen, R. Duschinsky, L. Kaplan, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 4755 (1961).
- 19) J. L. Au, J. S. Walker, and Y. Rustum, *J. Pharmacol. Exp. Ther.*, **227**, 174 (1983).