Effect of L-Cysteine on Distribution Volume of 1-(Tetrahydro-2-Furanyl)-5-Fluorouracil

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Abstract—Higher plasma concentrations of 1-(tetrahydro-2-furanyl)-5-fluorouracil (FT) were obtained after administration of FT (100 mg/kg, i.v.) combined with L-cysteine (CySH, 500 mg/kg, p.o.). The volume of distribution (Vd) and body fluid volumes significantly decreased. These results suggest that the increase of the plasma concentrations of FT can be attributed to the decrease of the Vd of FT, which is considered to be based on the decrease of body fluid volumes by the combined administration of CySH.

1-(Tetrahydro-2-furanyl)-5-fluorouracil (FT) is considered a prodrug of 5-fluorouracil (5-FU) and exerts antitumor activity through metabolic activation (1–5). Many efforts have been made to increase the antitumor activity of FT by obtaining a high plasma concentration of 5-FU (6, 7). In the previous paper (8), we reported that the plasma concentrations of both FT and 5-FU increased after administration of FT (500 mg/kg, p.o.) combined with L-cysteine (CySH, 500 mg/kg, i.p. or p.o.) when compared to FT alone.

The purpose of this study was to elucidate the mechanism of the CySH effect in the previously reported results (8). We tried to examine the effect of CySH on FT distribution in terms of the pharmacokinetic aspects. Body fluid volumes were also measured to investigate its relationship with FT distribution after oral administration of FT alone or combined with CySH.

Male Wistar rats (150–178 g) were fasted overnight before drug administration and during the experiment, and they were given water ad libitum. Rats were given FT (100 mg/kg, i.v.; Aldrich) alone or combined with CySH (500 mg/kg, p.o.; Nippon Rikagaku-yakuhin, Co.) at 1 and 4 hr before and at 2 hr after administration of FT. Rats were lightly anesthetized with diethyl ether, and blood was drawn into heparinized tubes by the interorbital sinus bleeding technique at definite times after drug administration. After centrifugation at 2,500 r.p.m. for 10 min, the resulting plasma was collected.

In a separate experiment, other groups of rats were given FT (500 mg/kg, p.o.) alone or combined with CySH (500 mg/kg, p.o.). Urine was sampled up to 4 hr after drug administration, and the volume was measured.

FT was extracted from plasma according to the method of Wu et al. (9). After extraction, ethyl acetate was evaporated to dryness under vacuum at room temp. The residue was dissolved in 100 μl of methanol, and a 5-μl aliquot was injected into a Hitachi Model 635 high performance liquid chromatograph with a JASCO Model UVIDEC-100-V absorbance detector at 280 nm. The mobile phase was 15% methanol in 0.01 M acetate buffer (pH 4.2) at a flow rate of 1 ml/min. The precolumn (50×4.6 mm i.d.) and the analytical column (250×4.6 mm i.d.) were reverse phase columns (5 μm, octadecyl, Gasukuro Kogyo Inc., Tokyo) and were maintained at a temp. of 50°C.

Body fluid volumes in rats 4 hr after administration of FT (500 mg/kg, p.o.) alone or combined with CySH (500 mg/kg, p.o.) were determined according to the method of Bianchi et al. (10).

The hematocrit value was measured after
centrifugation of blood in a glass capillary tube at 10,000 r.p.m. for 5 min.

As shown in Fig. 1, significantly higher concentrations of FT were observed until 60 min after administration of FT combined with CySH than after FT alone. There was no significant difference in biological half-lives following the administration of FT combined with CySH (5.4 hr) and FT alone (6.5 hr). The plasma concentration at zero time (Cp⁰) and the volume of distribution (Vd) were 207.6 µg/ml and 0.486 liter/kg, respectively, after administration of FT alone. Significantly higher Cp⁰ of 229.6 µg/ml and smaller Vd of 0.437 liter/kg were observed after administration of FT combined with CySH than after FT alone.

The urinary volume and body fluid volumes observed 4 hr after administration of FT (500 mg/kg, p.o.) alone or combined with CySH (500 mg/kg, p.o.) are presented in Table 1. The urinary volume was 5.92 ml after administration of FT alone, whereas it significantly increased to 8.02 ml after administration of FT combined with CySH. Significant increase was also observed in the hematocrit value by the combined administration of CySH. The blood volume, plasma volume, extracellular fluid volume and interstitial fluid volume all significantly decreased, but no difference was observed in the plasma/interstitial fluid volume ratio after administration of FT combined with CySH when compared to FT alone.

In the previous paper (8), we reported that the plasma and liver concentrations of both FT and 5-FU increased after administration of FT (500 mg/kg, p.o.) combined with CySH (500 mg/kg, i.p. or p.o.) when compared to FT alone in rats, and also CySH

![Fig. 1. Plasma concentrations of FT after administration of FT alone or combined with CySH. ○: FT (100 mg/kg, i.v.) alone, ●: FT (100 mg/kg, i.v.) combined with CySH (500 mg/kg, p.o.). Each point represents the mean±S.E. for six rats. Significantly different from FT alone: *P<0.05.

| Parameter                              | FT alone       | FT+CySH        |
|----------------------------------------|----------------|----------------|
| Body weight                            | 169.8±2.5      | 164.0±1.7      |
| Urinary volume                         | 5.92±0.17      | 8.02±0.66*     |
| Hematocrit                             | 46.08±0.43     | 47.40±0.29*    |
| Blood volume                           | 16.66±0.33     | 15.46±0.35*    |
| Plasma volume                          | 9.37±0.22      | 8.50±0.15*     |
| Extracellular fluid volume             | 52.79±1.26     | 46.59±1.21**   |
| Interstitial fluid volume              | 43.42±1.16     | 38.09±1.29*    |
| Plasma/interstitial fluid volume ratio | 0.224±0.011    | 0.216±0.006    |

FT alone: FT 100 mg/kg, p.o.; FT+CySH: FT 100 mg/kg, p.o. combined with CySH 500 mg/kg, p.o. Each value represents the mean±S.E. for five rats. Significantly different from FT alone: *P<0.05, **P<0.01.
(500 mg/kg, i.v.) did not have any effect on FT absorption from the digestive tract in situ. Furthermore, there was no difference in the amount remaining in the digestive tract after administration of FT (500 mg/kg, p.o.) alone or combined with CySH (500 mg/kg, p.o.) (data not shown). On the other hand, we have also obtained the result that FT metabolism was not affected with liver homogenate obtained after administration of CySH (500 mg/kg, p.o.) and also by the existence of CySH with liver homogenate in vitro (data not shown). In this study, a significant decrease of Vd was observed by the combined administration of CySH; therefore, it was thought that the increase of the plasma concentrations of FT can be attributed, at least partially, to the decrease of the Vd of FT.

Benvenuto et al. (11) reported that the Vd of FT is 0.66 liter/kg, almost equal to the space of D2O in humans. Next we investigated the effect of CySH on the body fluid volumes to explain the decrease of the Vd of FT obtained by the combined administration of CySH. Significant decreases of the body fluid volumes including the blood volume, plasma volume, extracellular fluid volume and interstitial fluid volume were observed by the combined administration of CySH, and it could be supposed that the decrease of the body fluid volumes probably resulted in the increase of the urinary volume. Because there was no significant difference in the plasma/interstitial fluid volume ratio by the combined administration of CySH, it was thought that the decrease of the extracellular fluid volume occurred in such a way that the ratio of the plasma volume to the interstitial fluid volume was maintained. Additionally, the increase of the hematocrit value was considered to be naturally based on the decrease of the plasma volume.

In this study, the Vd of FT (78.21 ml/animal) was only 1.48 times as large as the extracellular fluid volume (52.79 ml/animal) after administration of FT alone. Accordingly, FT in the extracellular fluid is thought to form a considerable portion (67.5%) of the amount of FT distributed in the body. Consequently, it was speculated that the fluctuation of the body fluid volumes, especially the extracellular fluid volume, would be greatly reflected in the Vd of FT; because of this, the increase of the plasma concentrations of FT would be observed in the present study as well as in the previous one (8) after administration of FT combined with CySH when compared to FT alone. Furthermore, it can be thought that the increase of the plasma concentrations of 5-FU observed in the previous study (8) was also probably caused by the same mechanism as in the present study. Additionally, it can be supposed that the anabolism of 5-FU might increase because the concentrations of 5-FU increased as mentioned above; consequently, the antitumor activity of FT was enhanced by the combined administration of CySH as reported in the previous study (8).

In conclusion, the present results suggest that the increase of the plasma concentrations of FT can be attributed to the decrease of the Vd of FT, which is considered to be caused by the decrease of the body fluid volumes accompanying the increase of the urinary volume after administration of FT combined with CySH when compared to FT alone. Further study is necessary to elucidate the mechanism of the effect of CySH on the body fluid volumes.

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