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RAPID COMMUNICATION

MECHANISM OF POTENTIATION OF ANTITUMOR ACTIVITY OF 5-FLUOROURACIL AGAINST ADENOCARCINOMA 755 BY L-CYSTEINE

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5-Fluorouracil (FU), which has been used clinically for the treatment of gastrointestinal cancer, is known to degrade rapidly into inactive metabolites (1). To improve the chemotherapeutic effect of FU, its use in combination with various nucleosides and pyrimidine bases has been studied (2-9). Some compounds inhibit the degradation of FU, and therefore FU is retained for a longer time in the plasma and the tumor than after FU alone; its antitumor activity is enhanced but its toxicity for the host is also increased. Now we have found that the antitumor activity of FU is potentiated by the simultaneous i.v. injection of L-cysteine without increasing toxicity for the host. The FU level in the plasma and the tumor after administration of a combination of FU and L-cysteine was higher than after FU alone. Details of the antitumor activity of this combination will be reported elsewhere.

MATERIALS AND METHODS

Groups of six mice with body weights of 21-23 g were used. Tumors (adenocarcinoma 755, 5 x 10^5 cells/mouse) were implanted s.c. into BDF_1 mice (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan). Twenty-four hours after tumor implantation, FU alone or FU plus 100 mg of L-cysteine (Sigma Chemical Co., St. Louis, MO) per kg per day was injected i.v. once a day for 4 days.

Antitumor activity was evaluated by calculating the ratio of the average tumor weight in the treated groups to that in the control group on day 12.

For biochemical studies, a single dose of [6-^{3}H]FU (2.5 mCi/20 mg/kg) or [6-^{3}H]FU plus L-cysteine (100 mg/kg) was given i.v. to each of five mice bearing a 7-day-old tumor. In each experiment, mice were killed under ether anesthesia by exsanguination from the axillary artery and vein 30 min after drug administration. Methods of extraction and separation of FU from the plasma and the tumor were as reported previously, with the addition of fine mincing of solid tumors with scissors (10). Tumor-bearing mice were treated for 30 min with 50 μCi of [5-^{3}H]deoxycytidine (27.7 Ci/mmol) or [2,8-^{3}H]adenosine (32.2 Ci/mmol) concurrently with drug administration to measure DNA or RNA synthesis in the tumor respectively. To extract labeled DNA, the

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tumor was homogenized in 3 ml of 0.1 M Tris-HCl (pH 8.0) buffer containing 0.01 M EDTA and 1% sodium dodecyl sulfate in a Polytron homogenizer. Tissue extracts were mixed with 2 ml of water-saturated phenol and then 2 ml of chloroform. DNA (containing RNA) was precipitated from the aqueous phase at -20°C with 2% potassium acetate in 95% ethanol and incubated with RNase A (Type X-A, Sigma) and RNase T_1 (Sankyo Co. Ltd., Tokyo, Japan) at 37° C overnight to digest the RNA. Then DNA was precipitated with 2% potassium acetate in 95% ethanol. Labeled RNA was extracted as described previously (11).

Statistical significance of the difference between FU alone and its combination with L-cysteine was determined by Student's t-test.

RESULTS AND DISCUSSION

FU at 20 mg/kg/day was the maximum nontoxic dose in this schedule and inhibited tumor growth moderately (T/C value was 0.40). L-Cysteine alone had no effect. The combination of FU at 20 mg/kg/day and L-cysteine markedly inhibited the tumor growth as shown in Table 1 (T/C value was 0.13, $P<0.05$).

Table 1. Effect of L-cysteine on the antitumor activity of FU against solid tumor adenocarcinoma 755

FU dose (mg/kg/day)	Tumor weight (g)	
	FU alone	FU+L-Cysteine
0	2.93 ± 0.59	3.40 ± 1.28 (1.16)
10	1.84 ± 0.50 (0.63)	1.22 ± 0.31 (0.42)
20	1.17 ± 0.28 (0.40)	0.37 ± 0.17*(0.13)
30	Toxic, 5/6+	Toxic, 2/6

Drugs were given i.v. for 4 consecutive days starting on day 1 after tumor implantation. Each group contained six BDF_1 mice. L-Cysteine was given at a dose of 100 mg/kg/day. Values are expressed as mean ± S.E. (T/C).
* Significant difference from FU alone, $P<0.05$.
+ Toxic means mice died before day 12; figures show the number of mice that died before day 12/total number of treated mice.

To study the mechanisms of potentiation of the antitumor activity of FU by L-cysteine, we used ^{3}H-labeled FU and measured the incorporation of [^{3}H]FU into the plasma and tumor. Total radionuclide in the plasma was the same with and without L-cysteine. However, the plasma FU level after treatment with the combination of L-cysteine and [^{3}H]FU was significantly higher than after [^{3}H]FU alone (Table 2).

Table 2. Total radioactivity and levels of FU in plasma and tumor cells after administration of [^{3}H]FU alone or in combination with L-cysteine

	Total radioactivity dpm/μl or mg	Intact FU nmoles/ml or g	FU incorporation into RNA dpm/A_{260}
Plasma			
FU alone	2994 ± 233	2.5 ± 0.2 (3.0%)*	——
FU + L-Cysteine	3037 ± 86	3.8 ± 0.6+(4.5%)	——
Tumor‡			
FU alone	2627 ± 302	7.6 ± 1.0 (10.4%)	3108 ± 286
FU + L-Cysteine	2567 ± 204	14.4 ± 2.8+(20.2%)	2351 ± 498

Blood samples were collected from the axillary artery 30 min after administration of [^{3}H]FU (2.5 mCi/20 mg/kg) and L-cysteine (100 mg/kg). Values are expressed as mean ± S.E., N=5.
*Numbers in parentheses, ^{3}H-FU/total ^{3}H x 100 (%).
+Significantly different from FU alone, $P<0.05$.
‡About 400 mg of tumor was used.

In the tumor, total radioactivity 30 min after treatment with [^{3}H]FU alone or in combination with L-cysteine was almost the same. The level of intact FU in the tumor after the combination of [^{3}H]FU and L-cysteine was significantly higher than after [^{3}H]FU alone, but [^{3}H]FU incorporation into RNA was almost the same in the two cases. Moreover, DNA synthesis, as measured by incorporation of [^{3}H]deoxycytidine during 30 min after treatment, was markedly affected by the combination of FU and L-cysteine, showing 50% inhibition, but L-cysteine or FU alone had moderate effect (Table 3).

Table 3. DNA and RNA syntheses in tumor cells following treatment with FU and L-cysteine

	Incorporation of radioactivity	
	DNA dpm/A_{260}	RNA dpm/A_{260}
Control	4514 ± 518 (100)*	333 ± 58 (100)
L-Cysteine	3724 ± 199 (82)	284 ± 51 (85)
FU	3302 ± 389 (73)	353 ± 74 (106)
FU + L-Cysteine	2256 ± 409 (50)	222 ± 44 (67)

DNA and RNA syntheses were measured by the incorporation of [^{3}H]-deoxycytidine and [^{3}H]-adenosine. Values are the mean ± S.E. of five mice (DNA synthesis) and four mice (RNA synthesis).
*Numbers in parentheses are percent of control.

Moreover, there was slight difference between treatment with FU alone and in combination with L-cysteine in incorporation of [^{3}H]-adenosine into RNA during 30 min after treatment. These results suggest that potentiation by L-cysteine of the antitumor activity of FU may result in an increase in FU nucleotides, perhaps FdUMP, in the tumor. Increased FU levels in the plasma and the tumor also suggest that L-cysteine should be considered for use as a potentiator of FU.

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