Jpn. J. Cancer Res. 84, 783–786, July 1993

Effect of 5-Fluorouracil and UFT on Experimental Liver Metastasis Model of Colorectal Cancer Using Mouse Colon 26 Cells

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Effects of 5-fluorouracil (5-FU) and UFT on an experimental liver metastasis model were compared at equi-effective dosage levels against subcutaneous tumor of mouse colon 26. 5-FU at the dosage level of 40 mg/kg suppressed the subcutaneous tumor growth by 70.0% and 45.0% on day 13 and day 18, respectively, and UFT at 20 mg/kg provided almost equal suppression (63.0% and 48.0%). In the liver metastasis model, 5-FU at 40 mg/kg showed more potent prevention of the formation of metastatic foci (94.9%) than did UFT (60.4%) at 20 mg/kg. 5-FU at 40 mg/kg produced a much higher peak serum level of 5-FU than did UFT at 20 mg/kg and also showed a much higher AUC (area under the curve) level in the portal blood. These results suggest that oral administration of 5-FU might be useful in prevention of liver metastasis of colorectal cancer.

Key words: Colorectal cancer — Liver metastasis model — Colon 26 — 5-FU — UFT

The number of deaths due to colorectal cancer is increasing in Japan despite marked improvement in diagnostic techniques. Liver metastasis, which accounts for about half of all remote metastases, is considered to be a crucial factor in the prognosis of colorectal cancer. Thus, any therapy effective against liver metastases would be important for the prognosis of colorectal cancer patients. In clinical practice, postoperative chemotherapeutic drugs for colorectal cancer are used primarily to prevent liver metastases, and the usefulness of intraportal administration of mitomycin C (MMC) and 5-fluorouracil (5-FU) has been studied.

We previously reported that intraportal MMC showed excellent suppression of liver metastasis in an experimental liver metastasis model of colorectal cancer using mouse colon 26 cells.[1)]

In this study, we investigated the prevention of liver metastases with two oral fluoropyrimidine preparations, i.e., 5-FU and UFT (tegafur:uracil=1:4), which are frequently used in postoperative adjuvant chemotherapy of colorectal cancer.

MATERIALS AND METHODS

Animals Five- to six-week-old BALB/c and CDF_1 (BALB/c×DBA/2 F_1) mice obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, were used. All animal experiments were conducted with 5 mice per group.

Tumor A mouse transplantable experimental tumor cell line, colon 26, was kindly supplied by the Cancer Chemotherapy Center, the Japanese Foundation for Cancer Research, Tokyo. It was maintained by subcutaneous passage into the flank of BALB/c mice by implantation every three weeks.

Preparation of subcutaneous tumor models and liver metastasis models During the third week after subcutaneous implantation of colon 26, the tumor was isolated. After the addition of a small amount of physiological saline, the tumor tissue was minced and filtered through a #100 mesh filter to prepare a 20% tumor brei. A 0.1 ml aliquot of this brei was implanted subcutaneously into the flank of CDF_1 mice.

To create the liver metastasis model, colon 26 tumor cells were first treated with trypsin as previously reported,[1)] and suspended in RPMI-1640 at a density of 1×10^4 cells/ml. Then 0.1 ml of this cell suspension was implanted into the superior mesenteric vein of CDF_1 mice anesthetized with pentobarbital sodium.

Chemotherapy 5-FU was dissolved in distilled water and UFT in a 5% gum arabic solution. 5-FU at 20 to 40 mg/kg and UFT at 10 to 30 mg/kg (tegafur equivalent) were administered p.o. for 7 days beginning from the day after tumor implantation for the subcutaneous tumor model and for 15 consecutive days except Sundays for the liver metastasis model. In a preliminary study, there was no significant difference in the tumor size between the solvent control group given distilled water and that given the 5% gum arabic solution.

Antitumor activities In the subcutaneous tumor model, the long diameter (a) and the short diameter (b) of tumors were measured on days 13 and 18 after the implantation to calculate the tumor volume ($ab^2/2$). In the liver metastasis model, the liver was isolated and weighed on day 18 after the implantation, and the number of metastatic foci on the surface was counted.

Determination of 5-FU level in blood The mice were orally administered 5-FU at 40 mg/kg and UFT at 20 mg/kg. The peripheral blood was sampled by puncture of the jugular vein, and the portal blood sample was taken from the superior mesenteric vein after laparotomy under anesthesia. The 5-FU levels in the portal and peripheral blood were determined at various times. Quantitation of 5-FU followed the method of Masuike *et al.* (HPLC method).[2] The AUC (area under the concentration-time curve) was calculated according to a nonlinear least-squares method program, MULTI.

Statistical methods Differences between groups were evaluated using the *t* test, Mann-Whitney's U-test, and Scheffe's multiple range test.

RESULTS

Effects on subcutaneous tumor models The antitumor activities of 5-FU and UFT on the subcutaneous implantation model are shown in Table I. 5-FU at 40 mg/kg suppressed the tumor growth by 70.0% and 45.0% on days 13 and 18, respectively. UFT induced serious toxicities in the 30 mg/kg dose group, and some animals died. The UFT 20 mg/kg group exhibited 63.0% and 48.0% suppression on days 13 and 18, respectively.

Comparison of antitumor activities of 5-FU and UFT on liver metastasis model The preventive effects of 5-FU and UFT on metastases are compared in Table II. 5-FU at 40 mg/kg and 20 mg/kg showed 94.9% and 19.3% suppression of liver metastases, respectively. UFT showed 60.4% suppression of liver metastases at 20 mg/kg, but the metastatic foci were increased in the 10 mg/kg group.

The liver weight was significantly suppressed in the 5-FU 40 mg/kg and 20 mg/kg dosage groups and the UFT 20 mg/kg dosage group.

5-FU levels in portal and peripheral blood The 5-FU levels in the portal and peripheral blood at different times are shown in Table III, and the values are plotted in Fig. 1. The 5-FU levels in the portal and peripheral blood of the 5-FU group peaked at 5 min after administration. The peak level in the portal blood (9.90 μg/ml) was about three times higher than the peak in the peripheral blood (3.44 μg/ml). Meanwhile, in the UFT group, the 5-FU levels in both the portal and peripheral blood peaked at 15 min after the administration, with no difference between them. The peak 5-FU level in the 5-FU

Table I. Antitumor Activities of 5-FU and UFT on Murine Colon 26 Adenocarcinoma (s.c.-p.o.)

Drug	Dose (mg/kg/day)	Day 13		Day 18		ΔBW[a] (g)
		Tumor size (mm^3) (mean±SD)	T/C	Tumor size (mm^3) (mean±SD)	T/C	
Control	(water)	1557±185	–	2228±241	–	−0.8
5-FU	20	675±188[b]	0.43	1558±274[b]	0.70	+0.5
	40	466±50[b]	0.30	1222±95[b]	0.55	−1.0
UFT	10	843±113[b]	0.54	1390±336[b]	0.64	+0.6
	20	584±266[b]	0.37	1168±168[b]	0.52	−2.0
	30	toxic		toxic		

5-FU and UFT were administered p.o. daily from day 1 through day 7.
a) Average body weight change between day 1 and day 13.
b) $P<0.05$ by *t* test as compared with the control group.

Table II. Comparison of Antitumor Activities of 5-FU and UFT on Liver Metastasis Model of Colon 26

Drug	Dose (mg/kg)	Colony count (mean±SD)	Inhibition (%)	Liver weight (mean±SD)	ΔBW[a] (g)
Control	(water)	31.6±27.0	–	2.40±0.93	+2.7
5-FU	20	25.5±29.0	19.3	1.75±0.60[b]	+1.5
	40	1.6±2.8[b]	94.9	1.23±0.30[b]	+0.6
UFT	10	44.4±28.5	−40.5	2.43±1.10	+0.3
	20	12.5±12.5	60.4	1.50±0.10[b]	+1.5

Colon 26 cells (1×10^3/0.1 ml) were implanted into the superior mesenteric vein on day 0. 5-FU and UFT were administered p.o. on days 1–5, 7–12 and 14–17. Colonies of liver metastasis were counted on day 18.
a) Average body weight change between day 1 and day 18.
b) $P<0.05$ by Mann-Whitney's U-test as compared with the control group.

Table III. Comparison of Mean Serum 5-FU Levels after Oral Administration of 5-FU and UFT in Mice

Time (min)	Serum level of 5-FU (μg/ml) : mean±SD			
	5-FU: 40 mg/kg p.o.		UFT: 20 mg/kg p.o.	
	portal	peripheral	portal	peripheral
5	9.90±6.01	3.44±2.14	0.35±0.12	0.29±0.08
15	1.84±0.47	1.13±0.21	1.86±0.20	1.55±0.21
30	0.94±0.17	0.71±0.06	1.04±0.33	0.85±0.25
60	0.04±0.06	0.05±0.02	0.61±0.27	0.37±0.22
120	ND[a]	0.01±0.01	ND[a]	ND[a]
AUC[b]	119.4	58.6	77.2	57.8
Ratio	(1.0)	(1.0)	(0.65)	(0.99)

5-FU and UFT were administered p.o. to male CDF_1 mice. The 5-FU levels in the portal and peripheral vein were measured by HPLC.
a) Not detected (<0.01 μg/ml).
b) Area under the concentration-time curve (μg/ml·min).

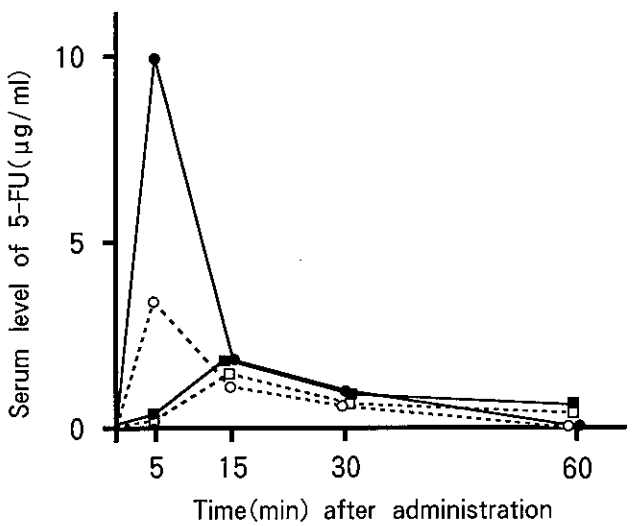

Fig. 1. Serum levels of 5-FU in the portal (——) and peripheral vein (– – –) after oral administration of 5-FU (●, ○) and UFT (■, □) to mice.

group was 5.3 times higher for the portal blood and 2.2 times higher for the peripheral blood when compared with the UFT group, and the time to reach the peak was different in the two groups.

Comparison of the AUC of 5-FU in the peripheral blood for the 5-FU and UFT groups showed no difference between the two drug treatments (AUC: 57.8 to 58.6 μg/ml·min). However, the AUC in the portal blood of the 5-FU group was 1.54 times higher than that in the UFT group (5-FU: 119.4 μg/ml·min, UFT: 77.2 μg/ml·min).

DISCUSSION

Colorectal cancer shows low sensitivity to chemotherapy, and many researchers doubt the clinical efficacy of currently available drugs. In postoperative adjuvant therapy of colorectal cancer, however, intraportal MMC, oral 5-FU preparations, etc., have been administered in attempts to prevent liver metastases and extrahepatic recurrence, which are negative prognostic factors.[3,4]

We previously reported the usefulness of intraportal administration of MMC in a liver metastasis model using mouse colon 26 cells.[1] In the present study, we investigated the usefulness of oral fluoropyrimidine preparations 5-FU and UFT in the expreimental model. UFT is a mixed compound of tegafur (a masked compound of 5-FU) and uracil, and is activated mainly in the liver, where it is converted into 5-FU. It is noteworthy that two fluoropyrimidine preparations with such different behavior were effective in the experimental model.

The efficacy on liver metastases of colorectal cancer can be considered from two aspects; one is the effect on existing metastatic foci, and the second is the preventive effect on metastases, which depends on the timing of the occurrence of metastasis and of the drug administration. Since the foci where metastases have been formed are supplied by arteries, anticancer drugs administered via the hepatic artery and transarterial embolization are mainly applied to them. Oral fluoropyrimidine preparations are frequently used as postoperative adjuvant therapy to prevent liver metastases and extrahepatic metastases.

In this study, the drug administration was initiated 24 h after the induction of metastases, and the effect in preventing the metastatic process and the proliferation of metastatic cells was investigated. Prior to investigating the prevention of liver metastases with oral fluoropyrimidine preparations, 5-FU and UFT, their activity against

mouse colon 26 in subcutaneously implanted tumors was tested. As a result, 5-FU at 40 mg/kg and UFT at 20 mg/kg were demonstrated to exhibit almost equivalent antitumor activities (T/C: 0.30 to 0.37) (Table I).

The effect in preventing liver metastases was then compared between 5-FU at 40 mg/kg and UFT at 20 mg/kg. The 5-FU group exhibited 94.9% suppression, which was more potent than the 60.9% suppression in the UFT group. These results revealed that 5-FU has higher activity against the liver metastasis model than UFT. Therefore, the behavior of 5-FU and UFT in the mice was compared to clarify the difference in their activity in the two models. In the experimental liver metastasis model, the treatment with the drugs was initiated 24 h after the intraportal implantation of the tumor cells and continued for 17 days. The metastatic foci in the liver were detected microscopically 3 to 5 days and macroscopically 14 days after the tumor implantation. Therefore, the expected antitumor activity might be attributable to a cytocidal effect on the tumor cells in the portal vein within 3 to 5 days after the tumor inoculation and thereafter, to growth inhibition of the tumor cells lodged in the liver.

Based on this concept, the 5-FU levels in the subcutaneous tumors and the metastatic foci in the liver after the administration of 5-FU and UFT were preliminarily investigated. Metastatic foci in the liver and subcutaneous tumors were established in the same mice, and the 5-FU level in each tissue was determined after oral administration of 5-FU at 40 mg/kg and UFT at 20 mg/kg. The 5-FU levels in the subcutaneous tumors and in the metastatic foci in the liver varied greatly. Thus, results explaining the difference in the activities of 5-FU and UFT in these two models were not obtained (unpublished data).

Then, considering the characteristic intraportal evolution of cancer cells in liver metastatic models, we studied the preventive effect on cancer cells metastasized in the early stage by comparing the intraportal 5-FU levels between 5-FU and UFT. The 5-FU levels in the portal and peripheral blood after oral administration of 5-FU at 40 mg/kg and UFT at 20 mg/kg were determined. In the UFT group, the 5-FU level showed exactly the same pattern in both the portal blood and the peripheral blood, and it reached a peak at 15 min after administration. In the 5-FU group, the peak 5-FU level was attained 5 min after administration, and it was significantly higher in the portal blood than in the peripheral blood. The intraportal peak level in the 5-FU group was 5.3 times higher than that in the UFT group. The AUC, which indicates the amount of drug absorbed, was almost the same for the two drugs in the peripheral blood, but the AUC in the portal blood was 1.54 times higher in the 5-FU group than in the UFT group.

Regarding the intraportal 5-FU level after oral administration, Douglass and Mittelman employed 5-FU-2-^{14}C in humans and reported in 1974 that a higher intraportal 5-FU level could be obtained by oral administration than by intravenous injection.[3] Inada *et al.* compared the intraportal 5-FU levels after oral administration of 5-FU and UFT and reported a significantly higher 5-FU level in the 5-FU group.[4] Since liver metastasis of colorectal cancer occurs by the transfer of cancer cells through the portal vein, a cytocidal effect on metastatic cancer cells in the portal vein seems to be an important factor affecting the prevention of liver metastases.

Though a conclusion can not yet be drawn regarding the difference in tissue distribution of the two drugs in the metastatic foci, our results are in good agreement with the clinical results reported by Inada *et al.*, indicating that the excellent preventive effect of 5-FU on liver metastases may be partly attributable to the high intraportal level of 5-FU. This suggests that immediate postoperative administration of 5-FU by continuous infusion into the portal vein might be effective in preventing postoperative liver metastases and also gives a theoretical basis to the argument that immediate initiation of postoperative chemotherapy with oral 5-FU is useful in the prevention of liver metastases.

(Received December 24, 1992/Accepted April 19, 1993)

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