STUDIES ON COMBINATION THERAPY WITH 1-(TETRAHYDRO-2-FURANYL)-5-FLUOROURACIL PLUS URACIL. I

Effect of Coadministration of Uracil on the Antitumor Activity of 1-(Terahydro-2-furanyl)-5-fluorouracil and the Level of 5-Fluorouracil in AH 130 bearing Rats

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Uracil has no antitumor activity, but it markedly potentiated that of 1-(tetrahydro-2-furanyl)-5-fluorouacil (FT) on AH 130, Sarcoma 180 and L 1210. It increased the 5-fluorouracil (5-FU) leves in the tumor and blood when administered orally with FT to AH 130 bearing rats.

The antitumor activity and 5-FU levels in the tumor and blood increased with increase in the molar ratio of uracil to FT: the 5-FU concentration in the blood increased more gradually than that in the tumor at low ratios of uracil to FT, but the 5-FU concentration in the blood increased greatly at high ratios. Consequently, the ratio of the 5-FU concentration in the tumor to that in the blood (T/B value) could be increased markedly by choosing appropriate ratios of FT and uracil. At a dose of less than 15 mg/kg of FT, a suitable ratio of uracil to FT was 1:2~1:5. With this ratio, we observed that the concentration of 5-FU in the tumor definitely increased more than that in the blood and was more than that with FT alone. UFT consists of FT and uracil in a molar ratio of 1:4. This is a reasonable ratio, judging from experimental findings. The antitumor activity of UFT on various tumor systems was about five times that of FT alone and the same as that of 5-FU. Moreover at low doses, UFT was more effective than FT or 5-FU on Lewis lung carcinoma and B 16 melanoma.

INTRODUCTION

UFT, a new antitumor agent, is composed of 1-(2-tetrahydrofuryl)-5-fluorouracil (FT) and uracil in a molar ratio of one to four.

FT was synthesized by HILLER *et al.*[1] as a masked form of 5-fluorouracil (5-FU). This compound is gradually converted to 5-FU *in vivo* without producing the marked side effects caused by administration of 5-FU. Thus in Japan FT is used as an oral antitumor agent. FT, however, seems to be somewhat less effective than 5-FU, and so must be adminsistered in a higher dose than the latter[2].

Recently, FUJII *et al.*[3~5] found that coadministration of uracil or its derivatives enhanced the concentration of 5-FU in tumors and the resulting antitumor activity of FT. In *in vitro* studies, uracil strongly inhibited the degradation of 5-FU to 2-fluoro-β-alanine (F-β-Ala). The cytotoxicity of 5-FU on *Staphylococcus aureus* 209 P was reversed by the presence of uracil, but that on mammalian cells, cultured FM 3 A/B and HeLa cells, was not reversed by even 1,000 times the concentration of 5-FU. Moreover in *in vivo*, the concentration of 5-FU in the tumor and blood of AH 130 bearing rats after oral administration of FT plus uracil was much greater than that after administration of FT alone. However, at suitable doses of FT and uracil, the concentration of 5-FU in the tumor increased more than that in the blood or other tissues. From these findings, they suggested that for clinical purpose, the optimum molar ratio

of uracil to FT should be 4.

These results were supported by the findings of TAGUCHI *et al.*[6-8] on the clinical pharmacology of UFT, and by the report of KIMURA *et al*[9] that administration of UFT to cancer patients sustained the 5-FU level in the tumor better than administration of FT alone, and that in tumors that did not respond to FT, but responded to UFT, a high concentration of 5-FU was observed on treatment with the latter drug.

This work was designed to confirm that UFT has higher antitumor activity than FT and to investigate the relation of the doses of FT and uracil on the 5-FU levels in the tumor and blood after administration of various doses of FT plus uracil to AH 130 bearing rats. The antitumor activity of UFT was also compared with those of FT and 5-FU.

MATERIALS AND METHODS

Chemicals: FT was synthesized by the method of HILLER *et al*[1]. in our laboratory. FT-6-^{3}H, specific activity 27.8 mCi/mmole, was obtained from the Japan Radioisotope Association, Tokyo, Japan. 5-FU was purchased from Sigma Chemical Co., U.S.A., and 20-methylcholanthrene, uracil and acasia were from Wako Pure Chemical Industries Ltd., Osaka, Japan.

Animals and tumors: The animals used in this study were Donryu rats (Nihon Rat Co., Urawa, Japan), ICR/JCL mice (Japan Clea Inc., Tokyo, Japan), ddY mice and Wistar rats (Tokushima Exp. Animal Lab., Tokushima, Japan), C 57 BL/6, DBA/2 and hybrid BDF_1 mice (Shizuoka Agr. Coop., Shizuoka, Japan). The tumors used were Walker 256, YOSHIDA sarcoma, AH 130, AH 44, AH 13, Sarcoma 180, EHRLICH carcinoma, L 1210, P 388, LEWIS lung carcinoma, B 16 melanoma and YM 12 (a fibrosarcoma induced in a C 57 BL/6 mouse with 20-methylcholanthrene). The tumors and host animals are listed in Table 1.

Antitumor activity: Male mice weighing 20~23 g and rats wieghing 120~150 g were used. The drugs were dissolved or suspended in 5% acasia solution and administered in a volume of 10 ml/kg. Each group consisted of 10 animals. For studies on tumor inhibition, tumors were inoculated into the right axilla of animals on day 0. The drugs were administered orally once daily for consecutive 7 days from day 1 to 7 after tumor implantation. The control group was given 5% acasia solution only in the same way. The percentage inhibition of tumor growth was calculated from the mean tumor weight of the treated group compared with that of the control group on day 10. With YM 12, LEWIS lung carcinoma and B 16 melanoma, treatment was started when the tumors grew to approximately 5 mm in diameter, and was continued for one or three weeks. The length and width of tumors were measured at intervals and the tumor weight were estimated from the following formula:

$$\text{weight (g)} = \frac{a \times b^2}{2} \quad a: \text{length(cm)}, b = \text{width(cm)}$$

For testing survival with L 1210 and P 388, BDF_1 mice were injected i.p. with these ascites cells on day 0. The drugs were given in the

Table 1 Test systems

Tumor	Host for propagation	Host for test	Inoculum
WALKER carcino-sarcoma 256	Wistar rats	Wistar rats	5×10^6
YOSHIDA sarcoma	Donryu rats	Donryu rats	$5\times10_9$
AH 130	Donryu rats	Donryu rats	5×10^6
AH 44	Donryu rats	Donryu rats	5×10^6
AH 13	Donryu rats	Donryu rats	5×10^6
Sarcoma 180	ICR/JCL mice	ICR/JCL mice	1×10^6
EHRLICH carcinoma	ddY mice	ddY mice	1×10^6
L 1210	DBA/2 mice	BDF_1 mice	5×10^5
P 388	DBA/2 mice	BDF_1 mice	5×10^5
LEWIS lung carcinoma	C57BL/6 mice	BDF_1 mice	1×10^5
B 16 melanoma	C57BL/6 mice	BDF_1 mice	1×10^5
YM 12, a 20-methylcholanthrene induced fibrosarcoma	C57BL/6 mice	BDF_1 mice	1×10^5

same way described above. The percentage increase in life span (ILS %) was calculated from the median survival period of the treated group compared with that of the control group.

Evaluation of drug toxicity: Groups of 10 male ICR/JCL mice, weighing 23±1 g, or male Donryu rats, weighing 130±10 g, were given various doses of UFT, FT or 5-FT orally once daily for 7 consecutive days. The LD_{10} value of each drug was calculated from the number of survivors on day 21 by the method of LITCHFIELD-WILCOXON[10].

Therapeutic index: The therapeutic indices (T. I.) of UFT, FT and 5-FU on the tumors used in this study were estimated from the following formula:

Inhibiotory systems

$$T.I. = \frac{LD_{10}(10\% \text{ lethal dose})}{ED_{50}(\text{dose for } 50\% \text{ inhibition of tumor growth})}$$

Survival systems

$$T.I. = \frac{ILS_{max} \text{ (optimum dose)}}{ILS_{30} \text{ (dose for } 30\% \text{ increase in life span)}}$$

Determination of 5-FU: AH 130 cells (1×10^7) were inoculated s. c. into the right axilla of Donryu rats. On day 8 after tumor inoculation, when tumors weighed about 3 g, the rats were starved

Table 2 Influence of uracil on growth of AH130, Sarcoma 180 and L 1210

AH 130 (s. c.-p. o.)

	Dose (mg/kg/day)	Body wt. change (g)	Tumor wt. Inhibition (g, mean ± S D)	(%)
Control	—	+46	2.11±0.62	—
Uracil	67.2	+39	1.96±1.35	7
	336	+41	2.07±1.20	2
	1,680	+31	2.02±0.59	4

Sarcoma 180 (s. c.-p. o.)

	Dose (mg/kg/day)	Body wt. change (g)	Tumor wt. Inhibition (g, mean ± S D)	(%)
Control	—	+3.0	1.07±0.10	—
Uracil	67.2	+2.9	1.08±0.08	−1
	336	+3.3	1.01±0.14	6
	1,680	+2.2	1.09±0.07	−2

L 1210 (i. p.-p. o.)

	Dose (mg/kg/day)	Survival time (day, mean ± SD)	ILS (%)
Control	—	8.29±0.49	—
Uracil	67.2	8.14±0.38	−2
	336	8.43±0.53	2
	1,680	8.43±0.53	2

Uracil was administered orally once daily for 7 consecutive days, starting 24 hr after implantation of AH 130 5×10^6 cells, Sarcoma 180 1×10^6 cells or L1210 5×10^5 cells. The control groups were treated similarly with 5% acacia solution alone. Growth of AH 130 and Sarcoma 180 was evaluated on day 10.

Table 3 Effect of uracil on antitumor activity of FT on AH 130 (s. c.-p. o.)

	Dose of FT (mg/kg/day)	Body wt. change (g)	Tumor wt. Inhibition (g, mean ± S D)	(%)
Control	—	+38	11.3±3.2	—
FT alone	200	+ 7	3.7±0.6	67
	133	+17	5.7±1.4	50
	89	+25	7.1±1.8	37
	59	+46	9.0±1.4	20
FT+Uracil	150	+11	3.3±0.7	71
(1 : 0.5)*	100	+15	5.3±1.4	51
	67	+29	7.2±2.0	36
	44	+48	9.8±2.2	13
FT+Uracil	100	− 1	2.8±0.8	75
(1 : 1)*	67	+11	4.7±1.2	58
	44	+45	6.7±1.2	41
	30	+50	10.0±2.4	11
FT+Uracil	80	−12	2.0±0.6	82
(1 : 2)*	53	+11	3.6±0.7	68
	36	+25	6.2±1.4	45
	24	+39	9.9±1.6	12
FT+Uracil	50	−22	1.5±0.4	87
(1 : 5)*	33	−21	2.4±0.3	79
	22	+21	4.9±1.1	57
	15	+22	8.2±1.4	27
FT+Uracil	30	−22	1.1±0.3	90
(1 : 10)*	20	+14	2.9±0.9	74
	13	+26	6.2±1.2	45
	9	+36	10.0±1.5	11
FT+Uracil	20	−19	2.1±0.6	81
(1 : 20)*	13	+10	3.1±0.8	73
	9	+14	5.6±1.1	50
	6	+31	10.9±1.7	4

( )* : molar ratio of uracil to FT

Donryu rats were inoculated s. c. with 5×10^6 cells of AH 130 on day 0. FT with or without uracil was adminstered once daily for 7 consecutive days from day 1. The percentage inhibition of tumor growth was evaluated on day 10.

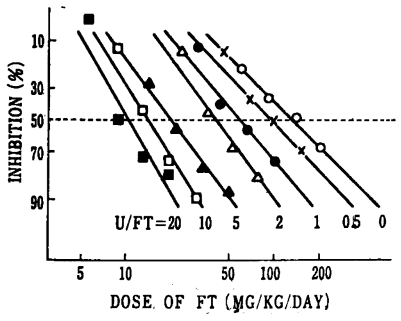
for 18 hr and then used for experiment. The rats were sacrificed at various periods after oral administration of various doses of FT-6-^{3}H alone or with uracil. The blood and tumors were rapidly removed, and homogenized with an equal volume of saline and samples of 1 ml were suspended in 7 volumes of cold methanol and centrifuged at 3,000 rpm for 10 min. The resulting precipitate was washed twice with 2 volumes of cold methanol and centrifuged. The supernatant was combined with the first supernatant and dried under a stream of nitrogen. The dried meterial was dissolved in 100 μl of 50% methanol and an aliquot (10 μl) was applied to a thin-layer chromatography plate (TLC plate, with Kiesel gel 60 F_{254} pre-coated, 2×20 cm, thickness 0.25 mm, Merck), to which carrier FT and 5-FU had been applied, and the plate was developed with chloroform: dioxane (1:1, v/v). In this way 5-FU (Rf 0.25) was separated from FT (Rf 0.75), and the spot of 5-FU was scraped off, placed in a vial and extracted with 0.2 ml of methanol for 2 hrs. Samples were mixed with 10 ml of scintillator containing 4 g of DPO, 0.4 g of dimethyl-POPOP and 100 g of naphthalene per liter of solvent (dioxane: toluene: ethyl cellosolve=15: 3:2, v/v/v), and the radioactivity was measured in an Aloka LSC 673 liquid scintillation spectrometer.

RESULTS

Influence of uracil on tumor growth: Table 2 shows the influence of uracil on the growth of AH 130, Sarcoma 180 and L 1210: it had no effect on these tumors at any dose tested.

Effect of uracil on the antitumor activity of FT: The combination ratios of uracil to FT mentioned below are all shown as molar ratios of uracil to FT.

Fig. 1 Dose response relations of effect of FT with or without uracil on AH 130 (s.c.-p.o.)
Values are from Table 3.
U/FT : molar ratio of uracil to FT



We compared the antitumor activities of FT with and without uracil on AH 130, Sarcoma 180 and L 1210. On AH 130 (Table 3 and Fig. 1) and Sarcoma 180 (Table 4 and Fig. 2), the inhibitory effect of FT on tumor growth was markedly enhanced by coadministration of uracil, namely, uracil shifted the dose-response lines for FT to the left hand and lowered the ED_{50} value. FT had only a slight inhibitory effect on AH 130 at

Table 4 Effect of uracil on antitumor activity of FT on Sarcoma 180 (s.c.-p.o.)

	Dose of FT (mg/kg/day)	Body wt. change (g)	Tumor wt. Inhibition (g, mean ± S D)	(%)
Control	—	+7.2	1.40±0.57	—
FT alone	150	+4.2	0.39±0.11	72
	100	+5.7	0.68±0.19	51
	67	+6.7	0.93±0.15	34
	44	+7.2	1.22±0.38	13
FT+Uracil	120	+2.5	0.40±0.13	71
(1 : 0.5)*	80	+5.2	0.59±0.20	58
	53	+7.0	0.82±0.28	41
	63	+6.7	1.35±0.44	4
FT+Uracil	80	+1.9	0.42±0.18	70
(1 : 1)*	53	+6.0	0.57±0.18	59
	36	+6.5	0.85±0.33	39
	24	+7.7	1.10±0.41	21
FT+Uracil	60	+0.1	0.35±0.05	75
(1 : 2)*	40	+5.6	0.49±0.22	65
	27	+5.5	0.71±0.14	49
	18	+6.5	1.26±0.35	10
FT+Uracil	40	−0.7	0.27±0.08	81
(1 : 5)*	27	+2.2	0.44±0.14	69
	18	+5.3	0.54±0.11	61
	12	+6.7	0.92±0.71	34
FT+Uracil	30	−7.3	0.14±0.01	90
(1 : 10)*	20	+3.5	0.45±0.18	68
	13	+5.3	0.59±0.15	58
	9	+6.8	1.07±0.60	24
FT+Uracil	20	−2.7	0.21±0.05	85
(1 : 20)*	13	+3.8	0.49±0.09	65
	9	+6.3	0.76±0.23	46
	6	+7.0	1.28±0.16	9

( )* : molar ratio of uracil to FT

ICR/JCL mice were inoculated s.c. with 1×10^6 cells of Sarcoma 180 on day 0. FT with or without uracil was administered once daily for 7 consecutive days from day 1. The percentage inhibition of tumor growth was evaluated on day 10.

Fig. 2 Dose response relations of effect of FT with or without uracil on Sarcoma 180 (s. c. -p. o.)
Values are from Table 4.
U/FT : molar ratio of uracil to FT

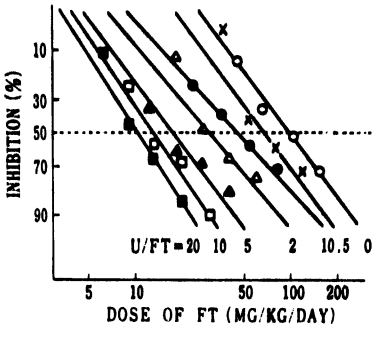
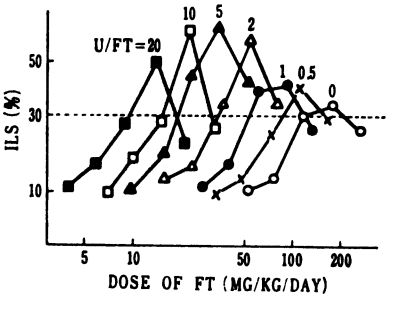


Fig. 3 Dose response curves of the effect of FT with or without uracil on L1210 (i. p.-p. o.)
Values are from Table 5.
U/FT : molar ratio of uracil to FT



less than 50 mg/kg and on Sarcoma 180 at less than 30 mg/kg, but on coadministration at these doses with uracil it had clear effects. At high doses or high combination ratios, FT plus uracil caused loss of body weight as well as high antitumor activity.

The effect of FT on survival of animals bearing L 1210 was also enhanced by coadministration of uracil. As shown in Table 5 and Fig. 3, the ILS_{max} % of FT alone was 34% (FT: 174 mg/kg), but those of FT plus uracil in ratios of 1:2, 1:5 and 1:10 were 59% (as FT: 54 mg/kg), 63% (as FT: 35 mg/kg) and 62% (as FT: 22 mg/kg), respectively. These findings suggest that the antitumor effect and toxicity of FT plus uracil depend on the dose of FT and the ratio of uracil to FT.

Effect of time of administration of FT and uracil: The effects on Sarcoma 180 and L 1210 administration of uracil at various intervals before or after the administration of FT were investigated. Uracil was most effective when administered simultaneously

Table 5 Effect of uracil on antitumor activity of FT on L 1210 (i. p.-p. o.)

	Dose of FT (mg/kg/day)	Survival time (day, mean±SD)	ILS (%)
Control	—	8.2±0.4	—
FT alone	261	10.3±0.9	26
	174	11.0±0.6	34
	116	10.7±2.0	30
	77	8.7±0.8	6
	52	8.3±0.5	1
FT+Uracil	165	10.6±1.1	29
(1 : 0.5)*	110	11.6±1.5	41
	78	10.0±0.8	22
	49	8.7±0.8	6
	33	8.3±0.5	1
FT+Uracil	137	10.2±0.6	24
(1 : 1)*	91	11.6±1.0	41
	61	11.4±1.8	39
	40	9.1±0.4	11
	27	8.4±0.8	2
FT+Uracil	81	10.9±0.9	33
(1 : 2)*	54	13.0±1.2	59
	36	11.0±1.8	34
	24	9.0±1.2	10
	16	8.6±0.5	5
FT+Uracil	53	11.8±1.5	44
(1 : 5)*	35	13.4±1.0	63
	23	11.9±1.1	45
	16	9.4±0.5	15
	10	8.3±0.5	1
FT+Uracil	33	10.3±0.6	26
(1 : 10)*	22	13.3±1.1	62
	15	10.4±0.5	27
	10	9.3±0.8	13
	7	8.2±0.4	0
FT+Uracil	21	9.8±1.1	20
(1 : 20)*	14	12.3±0.5	50
	9	10.3±0.5	26
	6	9.1±0.9	11
	4	8.4±0.5	2

( )* : molar ratio of uracil to FT

BDF_1 mice were inoculated i. p. with 5×10^5 cells of L 1210 on day 0. FT with or without uracil was administered once daily for 7 consecutive days from day 1. The percentage increase in the life span was evaluated from the mean survival days of the treated group compared with that of the control group.

Table 6 Influence of the time of uracil administered on the antitumor activity of FT

		Sarcoma 180 (s. c.-p. o.)			L 1210 (i. p.-p. o.)	
		Body wt. change	Tumor wt.	Inhibition	Survival time	ILS
		(g)	(g, mean±SD)	(%)	(day, mean±SD)	(%)
Control		+9.0	0.83±0.24	—	8.29±0.49	—
FT alone (30 mg/kg)		+9.1	0.74±0.24	11	8.71±0.49	5
Uracil alone (67.2 mg/kg)		+9.9	0.83±0.24	0	8.29±0.49	0
FT+Uracil	−4 hr	+8.3	0.78±0.21	6	8.57±0.53	3
(1 : 4)*	−2 hr	+8.4	0.78±0.24	6	8.86±0.38	7
	−1 hr	+7.8	0.47±0.13	43	9.14±0.69	10
	−0.5 hr	+8.3	0.43±0.09	48	11.71±0.49	41
	±0 hr	+4.2	0.21±0.06	75	13.57±1.62	64
	+0.5 hr	+7.7	0.43±0.08	48	12.86±1.86	55
	+1 hr	+8.1	0.44±0.09	47	11.43±0.53	38
	+2 hr	+8.0	0.59±0.11	29	9.43±1.27	14
	+4 hr	+8.7	0.60±0.07	28	8.86±0.69	7

( )* : molar ratio of uracil to FT

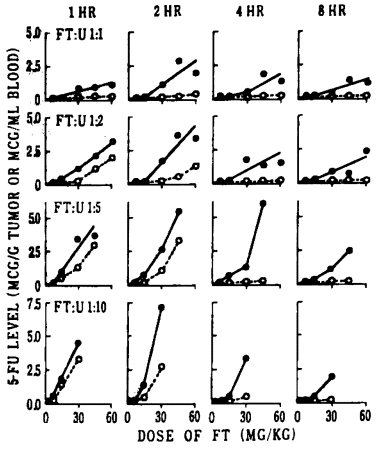
− : preadministration of uracil to FT + : postadministration of uracil to FT

Uracil was administered at vaious times relative to FT after implantation of tumor cells (Sarcoma 180 1×10^6 cells, L 1210 5×10^5 cells). Animals were given drugs orally from day 1 to 7 for 7 consecutive days.

Fig. 4 5-FU levels in tumor and blood after administration of FT plus uracil to AH 130 bearing rats

^{3}H-FT and uracil, at molar ratios of 1:1, 1:2, 1:5 and 1:10 were administered orally, and 1, 2, 4 and 8 hr later, rats were killed, and the 5-FU levels in the tumor and blood were determined. Results are means for 3 rats per group.

● in tumor ○ in blood



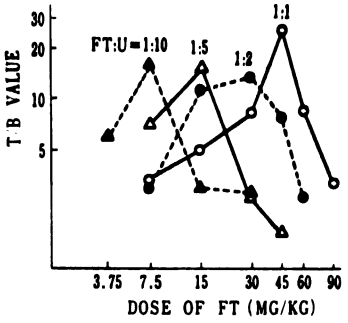
with FT in inhibiting Sarcoma 180 and prolonging the survival of animals bearing L 1210. It had little effect when administered more than 2 hrs before or after or after FT: the shorter the interval between the administrations of FT and uracil, more effect it had. This suggests that the reciprocal action of uracil and 5-FU, derived from FT, was influenced by the absorption and distribution of FT and uracil in the liver, which is the main site of metabolism of FT, 5-FU and uracil, as reported by FUJII *et al*[11].

Concentrations of 5-FU in the tumor and blood after coadministration of FT and uracil: For investigation of the relation between the doses of FT and uracil and the concentrations of 5-FU in the tumor and blood of AH 130 bearing rats, FT-6-^{3}H and uracil were administered simultaneously at various doses and various ratios. As shown in Fig. 4, coadministration of uracil increased the 5-FU levels in the tumor and blood. Furthermore these 5-FU levels increased with increase in the dose of FT at a fixed ratio, and in the ratio of uracil to FT at a fixed dose of FT. Moreover, the 5-FU level in the tumor tended to increase more rapidly and significantly than that in the blood. Though not shown in Fig. 4, the maximum concentration of 5-FU was 0.11~0.20 μg/ml in the blood and 0.22~0.35 μg/g in the tumor of AH 130 bearing rats treated with 90 mg/kg of FT, a dose

Fig. 5 Ratio of 5-FU levels in tumor and blood 2 hr after administration of FT plus uracil to AH 130 bearing rats

T/B value : ratio of the concentration of 5-FU in the tumor to that in the blood. T/B values 2 hr after adminstration of FT and uracil in various molar ratios were calculated from Fig. 4.

* : molar ratio of uracil to FT



shown to be effective[12] and scarcely toxic to rats[13]. Therefore, if the toxicity is proportional to the 5-FU level in the blood, 0.20 μg/ml of 5-FU in the blood should be the maximum non toxic level. At molar ratios of uracil to FT of 1:1, 1:2 and 1:5, the doses of FT which gave this level were less than 60 mg/kg, 30 mg/kg and 15 mg/kg, respectively, and these ratios increased the 5-FU level in the tumor significantly. The ratio of the 5-FU level in the tumor to that in the blood (T/B value) was 2 at most on administration of FT alone, but more than 2 on administration of FT plus uracil in the combination ratios described above; namely, doses of 45 mg/kg, 30 mg/kg and 15 mg/kg of FT at ratios of 1:1, 1:2 and 1:5 produced the maximum T/B value, which was much higher than that with FT alone. Thus, the 5-FU levels in the blood and tumor and the T/B value were affected by the dose of FT and the ratio of uracil to FT. That is to say, a suitable ratio of uracil to FT should have strong antitumor activity without severe toxicity.

The optimum ratio of uracil to FT in combination therapy: In combination therapy, it is important to decide the ratio of FT and uracil to use in clinical trials, when given orally, the clinical dose of FT used for therapy is about 800 mg/body/day[14]. Therefore, on the basis of the above findings, we examined the concentrations of 5-FU in the tumor and blood of AH 130 bearing rats after administration of 15 and 7.5 mg/kg of FT with uracil at ratios of 1:2～1:5. As shown in Table 7, high T/B

Table 7 5-FU levels in tumor and blood after oral administration of FT plus uracil in various molar ratio to AH 130-bearing rats

FT 15 mg/kg

Time (hr)	Tumor/Blood ratio of 5-FU			
	FT : U=1:2	1 : 3	1 : 4	1 : 5
1	0.163/0.050 (3.26)	0.382/0.112 (3.41)	0.458/0.294 (1.56)	0.979/0.640 (1.53)
2	0.146/0.013 (11.23)	0.482/0.022 (21.91)	0.353/0.032 (11.03)	0.613/0.041 (14.95)
4	0.137/0.011 (12.45)	0.195/0.009 (21.66)	0.230/0.014 (16.43)	0.401/0.019 (21.11)
8	0.073/0.006 (12.17)	0.082/0.005 (16.40)	0.119/0.007 (17.00)	0.316/0.011 (28.73)

FT 7.5 mg/kg

Time (hr)	Tumor/Blood ratio of 5-FU			
	FT : U=1:2	1 : 3	1 : 4	1 : 5
1	0.030/0.008 (3.75)	0.043/0.010 (4.30)	0.035/0.014 (6.07)	0.043/0.017 (2.53)
2	0.031/0.013 (2.38)	0.029/0.013 (2.23)	0.034/0.006 (5.67)	0.062/0.006 (10.33)
4	0.030/0.005 (6.00)	0.037/0.007 (5.29)	0.103/0.006 (17.17)	0.042/0.003 (14.00)
8	0.023/0.003 (7.67)	0.041/0.005 (8.20)	0.048/0.003 (16.00)	0.069/0.003 (23.00)

( ) : T/B values

^{3}H-FT (15 or 7.5 mg/kg) and uracil in molar ratios of 1 : 2, 1 : 3, 1 : 4 and 1 : 5 were administered orally and the 5-FU levels in the tumor (μg/g) and blood (μg/ml) were determined at the indicated times. Results are means for 3 rats/group.

Fig. 6 Inhibitory effects of UFT, FT and 5-FU on s. c. implanted tumors in rats
Each tumor (5×10^6 cells) was inoculated s. c. on day 0, UFT, FT and 5-FU were administered once daily for 7 consecutive days from day 1. The percentage inhibition of tumor growth was evaluated on day 10.
● UFT ○ FT △ 5-FU

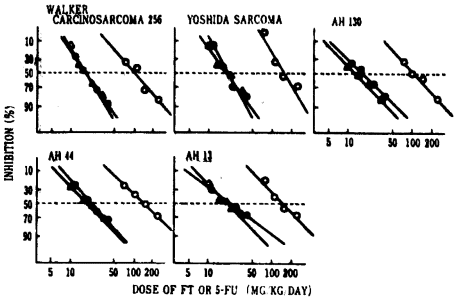
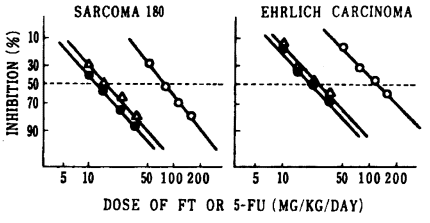


Fig. 7 Inhibitory effects of UFT, FT and 5-FU on s. c. implanted tumors in mice
Each tumor (1×10^6 cells) was inoculated s. c. on day 0, UFT, FT and 5-FU were administered orally once daily for 7 consecutive days from day 1. The percentage inhibition of tumor growth was evaluated on day 10.
● UFT ○ FT △ 5-FU



values were observed at ratios of 1:3~1:5 with 15 mg/kg of FT, and of 1:4 and 1:5 with 7.5 mg/kg of FT. These results confirm the reports of FUJII *et al*[4,5]. and TAGUCHI *et al*[7,8]. that a ratio of uracil to FT is suitable.

Antitumor activity of UFT: The antitumor activity of UFT, which is composed of FT and uracil in a molar ratio of 1:4, was compared with those of FT and 5-FU on various tumor systems. The doses of UFT, FT and 5-FU were established on the basis of LD_{10} values of these drugs when administered for 7 consecutive days to mice or rats.

Fig. 8 Effects of UFT, FT and 5-FU on survival of animals bearing L 1210 (i. p.-p. o.)
L 1210 cells (5×10^5) were inoculated i. p. on day 0. UFT, FT and 5-FU were administered orally once daily for 7 consecutive days from day 1. The antitumor activities of drugs were evaluated as the percentage increase in life span.
● UFT ○ FT △ 5-FU

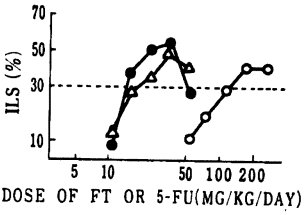


Table 8 Effects of UFT, FT and 5-FU on survival of mice hearing P 388(i. p.-p. o.)

	Dose (mg/kg/day)	Survival time (day, mean±SD)	ILS (%)
Control	—	10.86±0.53	—
UFT	180 (56)*	9.57±0.53	−12
	120 (37)*	9.86±0.69	− 9
	80 (25)*	13.14±2.04	21
	53 (16)*	12.29±0.76	13
	36 (11)*	10.86±0.38	0
FT	264	11.86±0.90	9
	176	10.86±0.69	0
	117	12.29±1.38	13
	78	12.00±1.29	10
	52	11.43±1.13	5
5-FU	53	10.00±0.00	− 8
	35	12.50±1.87	15
	23	12.86±0.38	18
	16	10.86±0.69	0
	10	11.29±0.76	4

( )* : dose of FT in UFT
P 388 cells (5×10^5) were inoculated i. p. on day 0. UFT, FT and 5-FU were administered orally once daily for 7 consecutive days from day 1. The antitumor activities were evaluated as percentage increases in the life span.

The inhibitory effect of UFT on s. c. transplanted tumors in rats and mice was about 5 times that of FT and the same as that of 5-FU. At higher doses these drugs suppressed growth or caused loss of body weight; the effect of 5-FU on the body weight of rats was somewhat more than those of the other drugs (data not shown).

Fig. 9 Inhibitory effects of UFT, FT and 5-FU on YM 12 (s.c.-p.o.)

YM 12 cells (1×10^6) were inoculated s.c. on day 0. Each drug was administered orally once daily for 1 week(a) or 3 weeks (b), starting when the tumors grew to about 5 mm in diameter. Tumor weights were calculated from the length and width of tumors.

( )* : dose of FT in UFT

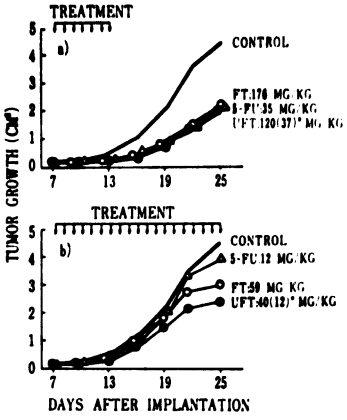


Fig. 10 Inhibitory effects of UFT, FT and 5-FU on Lewis lung carcinoma and B 16 melanoma (s.c.-p.o.)

Each tumor (1×10^5 cells) was inoculated s.c. on day 0. U. FT, FT and 5-UF were administered orally once daily for 3 weeks, starting when the tumors were about 5 mm in diameter. Tumor weights were calculated from the length and width of the tumors.

( )* : dose of FT in UFT

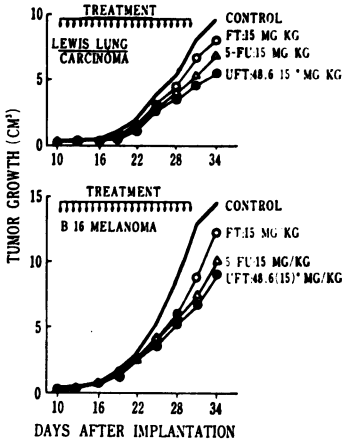


Table 9 Therapeutic indices of UFT, FT and 5-FU

		ED_{50}[a]	LD_{10}[b]	T.I.[e]
Walker 256	UFT	65(20)*	207(64)*	3.2
	FT	175	340	1.9
	5-FU	20	35	1.8
Yoshida s.	UFT	58(18)*	207(64)*	3.6
	FT	105	340	3.2
	5-FU	18	35	1.9
AH130	UFT	62(19)*	207(64)*	3.4
	FT	110	340	3.1
	5-FU	14	35	2.5
AH44	UFT	76(23)*	207(64)*	2.8
	FT	162	340	2.1
	5-FU	19	35	1.8
AH13	UFT	84(26)*	207(64)*	2.5
	FT	158	340	2.2
	5-FU	23	35	1.5
Sarcoma 180	UFT	42(13)*	120(37)*	2.8
	FT	75	176	2.3
	5-FU	15	35	2.3
Ehrlich ca.	UFT	71(22)*	120(37)*	1.7
	FT	129	176	1.4
	5-FU	26	35	1.3
		ILS_{50}[c]	ILS_{max}[d]	T.I.[f]
L 1210	UFT	49(15)*	120(37)*	2.5
	FT	122	264	2.2
	5-FU	19	35	1.8

( )* : dose of FT in UFT

Values of ED_{50}[a] were calculated from Figs. 6 and 7. Values of LD_{10}[b] were calculated by the method of Litchfield-Wilcoxon from results 21 days after administration of drugs for 7 consecutive days. Values of ILS_{50}[c] and ILS_{max}[d] were calculated from Fig. 8. T.I.[e]=LD_{10}/ED_{50} T.I.[f]=ILS_{max}/ILS_{50}

The effect of UFT on the survival of animals bearing L 1210 was greater than that of FT or 5-FU, the ILS_{max} % values being 53% for UFT (as FT : 37 mg/kg), 48% for 5-FU (35 mg/kg) and 43% for FT (264 mg/kg) as shown in Figs. 8. These drugs had a little effect on the life span of animals bearing P 388, the ILS_{max} % values for UFT, FT and 5-FU being 21%, 13% and 18%, respectively.

In animals bearing YM 12, the drugs were tested at their LD_{10} values for 1 week and at 1/3 of their LD_{10} values for 3 weeks. With the former regimen, their inhibitory effects were similar (Fig. 9 a). Even though the total dose was the same with the latter regimen, FT and 5-FU had a little effect, but UFT

was inhibitory (Fig. 9 b). These drugs had similar effects on LEWIS lung carcinoma and B 16 melanoma which grew slowly to those on YM 12 (Fig. 10). Thus at low doses, UFT is more effective than FT or 5-FU.

The therapeutic indices of UFT, FT and 5-FU on various tumor systems are shown in Table 9. The T.I. values of UFT are higher than those of FT or 5-FU. Since the LD_{10} is the lethal dose, the results show that at low doses UFT is more effective and less toxic than FT or 5-FU.

DISCUSSION

The selective toxicity of an antitumor agent, that is to say the concentration of the drug in tumor tissue compared with that in other tissues, is one factor governing its efficacy in cancer chemotherapy. The cytotoxicity of an antimetabolic agent is said to depend on the period of contact of its active form with tumor cells[15]. So, FT seems to have the merit that the concentration of its active form, 5-FU, in the tumor tissue and blood is maintained well. But, FT has less antitumor activity than 5-FU. ANADA *et al*[16]. reported that the antitumor activity of 5-FU on Sarcoma 180 and its concentration in the tumor tissue were similar to those of FT when it was administered at about 5 times the dose of 5-FU. This means that the concentration of 5-FU in the tumor tissue after administration of FT is too low to be effective. Thus for FT to be effective, conditions producing a higher 5-FU level in the tumor tissue must be found.

FUJII *et al*[11]. found that FT was mainly converted to 5-FU by the microsomal fraction of rat liver in the presence of NADPH, and suggested that the microsomal electron-transport system should be involved in this conversion. OHIRA *et al*[17]. demonstrated that the 5-FU level in the blood after administration of FT and the antitumor activity of FT could be increased by pre-treatment of animals with phenobarbital, which activates cytochrome P-450. On the other hand, FUJII *et al*[3-5]. finding that 5-FU was mainly degraded to F-β-Ala in the liver and that its degradation was inhibited by uracil, showed that the antitumor activity of FT could be enhanced by coadministration of uracil. JATO *et al*[18]. showed that coadministration of deoxyuridine enhanced the antitumor activities and toxicities of 5-FU and 5-fluorodeoxyuridine. According to HEIDELBERGER *et al*[19]., administration of thymine and azathymine to mice 10 min before 5-FU-6-C^{14} reduced the catabolism of the drug and also increased its toxicity, since it increased the amount of unchanged drug. Furthermore, WILSON *et al*[20]. demonstrated that uracil and thymine potentiated the teratogenic effect of 5-FU. Similarly in this study, we observed that treatment with high dose of FT or high ratio of uracil to FT had more antitumor activity and caused greater loss of body weight of animals (Tables 3 and 4). As described above, coadministration of a pyrimidine compound enhances the antitumor activity and toxicity of a fluorinated pyrimidine by increasing the 5-FU level in the body. In preliminary experiments, we confirmed that the ratio of the 5-FU levels in the tumor to that in the blood (T/B value) after administration of FT was 2 at the most, and that it was nearly the same at all doses of FT and at all times after FT adminstration. As shown in Fig. 4, the concentrations of 5-FU in the tumor and blood after coadministration of FT and uracil increased with increase in the dose of FT and the ratio of uracil to FT, but the increase of 5-FU in the tumor was more rapidly and greater than that in the blood. Therefore, the T/B values after coadministration of FT and uracil at various doses of FT and at various ratios of uracil to FT had the relations shown in Fig. 5. The increase of 5-FU in the tumor was greater than that in the blood at lower dose of FT than that giving the peak T/B value with the combination of FT and uracil. The antitumor activity and toxicity of 5-FU seem to be proportional to its concentrations in the tumor and blood, respectively. Thus in this combination therapy it is important that the 5-FU level in the blood should be low and that in the tumor should be high. At a dose of less than 15 mg/kg of FT, estimated to be effective from clinical use[14], we concluded that a suitable ratio of uracil to FT is 1:2~1:5. With these ratios, we observed that the concentration of 5-FU in the tumor was definitely increased more than that in the blood and was more than that with FT alone (Table 7). This observation is consistent with the results of FUJII *et al*[4,5].

UFT consists of FT and uracil in a molar ratio of 1:4. This seems to be a reasonable ratio from above findings. The antitumor activity of UFT was about 5 times that of FT, and the same as that of 5-FU (Figs 6, 7 and 8).

The therapeutic index of UFT was higher than

that of FT or 5-FU, but these values were calculated from the ED_{10} and LD_{10} values (Table 9). Namely, UFT had significant antitumor activity at a dose equivalent to a vary small dose of FT that was not effective alone. On long term administration at low dose, UFT was also more inhibitory than FT or 5-FU to YM 12, LEWIS lung carcinoma and B 16 melanoma (Figs. 9 and 10). These findings indicate that UFT is more effective than FT or 5-FU.

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