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Comparative Antitumor Activity of 5-Fluorouracil and 5′-Deoxy-5-fluorouridine in Combination with Radiation Therapy in Mice Bearing Colon 26 Adenocarcinoma

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The present study compared the antitumor activities of chemotherapy with 5-fluorouracil (5-FU) and with its prodrug 5′-deoxy-5-fluorouridine (5′-DFUR) in combination with radiotherapy on a solid colon 26 adenocarcinoma in the mouse. A single administration of 5′-DFUR immediately after local irradiation on day 10 after tumor inoculation produced more than additive antitumor effects, while only an additive effect was observed in the combined treatment with 5-FU and radiation. This over-additive effect of 5′-DFUR was more obvious in a fractionated-dose treatment schedule, where the same combined modality treatment was given three times on days 6, 10 and 14 after inoculation of the tumor cells. 5′-DFUR enhanced the radiation effects on the tumor in terms of the delay in tumor growth as well as the increase in the survival time. 5-FU produced only a marginal additive antitumor effect. Furthermore, radiation damage to normal tissues (skin damage by local irradiation and bone marrow and spleen damage by whole-body irradiation) was not enhanced by 5′-DFUR, though radiation damage to the thymus was additive. On the other hand, 5-FU produced toxic effects that were additive for all normal tissues tested. Thus, at doses that were the most effective against tumors, relative therapeutic gain factors (the ratio of the effect on tumors to that on the bone marrow) of 5′-DFUR and 5-FU were 1.24 and 0.49, respectively. These results suggest that 5′-DFUR will have a greater potential than 5-FU in combined modality treatment of cancer patients.

Key words: 5-Fluorouracil — 5′-Deoxy-5-fluorouridine — Radio-chemotherapy — Colon 26 adenocarcinoma

5-Fluorouracil (5-FU) is an established cytostatic for the treatment of a variety of neoplastic diseases, particularly for cancers of the breast and digestive organs, and is either used alone or in combination with other cytostatics. In combination therapy with radiation, 5-FU has also been used extensively for the treatment of squamous cell carcinomas.[1–3] However, 5-FU gave variable clinical results, casting doubt on the actual efficacy of 5-FU in combination with radiation. The use of 5-FU in preclinical *in vivo* studies has also resulted in variable effects,[4–8] although 5-FU clearly enhanced cell-killing by radiation in *in vitro* studies.[9–12]

5′-Deoxy-5-fluorouridine (doxifluridine, 5′-DFUR) is a prodrug from which 5-FU is generated by pyrimidine nucleoside phosphorylase,[13] mainly by uridine phosphorylase in mouse and by thymidine nucleoside phosphorylase in human tumors.[14, 15] 5′-DFUR has been shown to be a more effective agent with less toxicity, including immunosuppressive activity, than 5-FU, FUdR (2′-deoxy-5-fluorouridine) and tegafur,[16–19] and is now used clinically in the treatment of breast, stomach and colorectal cancers. The selective antitumor activity of 5′-DFUR has been shown to be mainly attributable to the unique tissue distribution patterns of the enzyme(s) responsible for its conversion to the active metabolite 5-FU.[13] Both in man and mouse, these enzymes are more abundant in tumors than in normal tissues, excepting the intestinal tract.[13] Consequently, 5′-DFUR was effectively converted to 5-FU in tumors after its administration.[20] Because of this characteristic of tumor-selective targeting, 5′-DFUR should be superior to 5-FU in combination therapy with radiation.

In the present study, we compared the antitumor activity of chemotherapy with 5-FU and with 5′-DFUR in combination with radiotherapy in mice bearing solid colon 26 adenocarcinoma, which contains a higher level of pyrimidine nucleoside phosphorylase than normal tissues.[21] In addition, since myelosuppression and skin damage are often observed in cancer patients who are receiving radiation therapy, we examined the influence of 5-FU and 5′-DFUR on such adverse effects of X-rays in this study and compared the relative therapeutic gain factors of X-rays in combination with each of these drugs. The study clearly indicated that the effect of 5′-DFUR on the tumor is greater than that on the normal tissues such as bone marrow, spleen and thymus. In addition, 5′-DFUR in combination with fractionated doses of radiation delayed the tumor growth and increased the survival time more than additively relative to the effects of the two modalities given independently. On the other hand, 5-FU in combination with X-rays given according to the same treatment schedule produced only marginal additive effects in antitumor activity and toxicity.

MATERIALS AND METHODS

Animals Male CDF$_1$ (BALB/c×DBA/2)F$_1$ mice (4 weeks old) and female BALB/c (11 weeks old) were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan. The mice were used after at least one week of observation.

Tumor cells Colon 26 adenocarcinoma cells were kindly supplied by Dr. T. Kataoka, Chemotherapy Center, Cancer Institute, Tokyo. The cells were maintained in monolayer culture in RPMI 1640 containing 10% fetal calf serum, penicillin G (50 U/ml) and streptomycin sulfate (100 μg/ml). A suspension of the tumor cells was prepared by the trypsinization of monolayers of the cells and 10^6 cells were inoculated intramuscularly in the right hind leg.

Cytostatics 5-Fluorouracil (5-FU) and 5′-DFUR (doxifluridine; 5′-deoxy-5-fluorouridine) synthesized by Hoffmann-La Roche, Basle, were dissolved in sterile water containing 0.5% carboxymethyl cellulose and administered by the po route.

Treatment schedules Treatment schedule 1 (single-dose treatment): Groups of 7 mice bearing the tumor were given either 5-FU, 5′-DFUR or the vehicle by the po route on day 10 after tumor inoculation. One hour thereafter, local irradiation of the tumor-bearing leg or whole-body irradiation was carried out by using an X-ray unit (Hitachi X-ray unit, model MBR-1505R, with 0.5 mm Al/0.1 mm Cu filter, 150 kVp, 0.45 Gy/min). The mice were injected intraperitoneally with pentobarbital (50 mg/kg) 30 min prior to the irradiation.

Treatment schedule 2 (fractionated-dose treatment): The same doses of X-rays and the drugs as in Treatment schedule 1 were given three times on days 6, 10 and 14 after the tumor inoculation.

Evaluation of antitumor effects Tumor size after treatment was determined every other day. The major (a cm) and minor (b cm) axes of the tumors were measured with a caliper and the tumor volume was calculated by using the formula $ab^2/2$ cm^3. The tumor volume was plotted as a function of time. From these lines, mean estimates were made of growth delay (T_{2000}). T_{2000} is the time taken for a tumor to grow to the size of 2,000 mm^3 starting from the day of treatment. Antitumor effects were also expressed in terms of the median survival days (MSD).

Evaluation of toxic effects Groups of 4 CDF$_1$ mice were treated with drugs and whole-body irradiation (0.5 Gy) either once or three times at days 0, 4 and 8. Procedures for X-ray irradiation were the same as described above. Four days after the last day of treatment, the weights of the spleen and thymus were measured, and the numbers of bone marrow cells and white blood cells were counted. In skin reaction tests, groups of 4 BALB/c mice were treated with drugs and local irradiation (30 Gy) to the right leg. Starting 4 days thereafter, the skin was examined three times per week for tissue damage and irritation ascribable to the irradiation, and the degree of the skin reactions (reddening, scray, crusting, breakdown with or without exudate, necrosis, etc.) was scored as described by Fowler *et al.*[22]

Calculations of DEF and TGF The radiation dose equivalent (RDE) of 5-FU and 5′-DFUR in combination with X-ray treatment is estimated by extrapolating from the radiation dose-effect relationship, the total X-ray dose that would have been required in the absence of drug to produce a given degree of effect.[23] RDEs were obtained for tumor growth delay (T_{2000}) and the increase of the MSD or for the degree of reduction of the cellularity of normal tissues. Using this RDE, the dose-effect factor (DEF) was calculated as follows:

DEF = RDE for the effect of combined therapy/actual radiation dose delivered.

A DEF value less than 1 indicates protection and a value greater than 1 indicates enhancement. For each combination, a therapeutic gain factor (TGF) was calculated as the ratio of the DEF for the tumor to that of normal tissues, as follows:

TGF = DEF(tumor)/DEF(normal tissue)

Statistical analysis Differences in the delay of tumor growth and the MSD of mice bearing tumors were compared by using the generalized Wilcoxon test. Differences in the cellularity of normal tissues were compared by using Student's t test. Dfferences were considered to be significant when the probability (P) value was <0.05.

RESULTS

Antitumor effects of single-dose treatments Tumor growth curves after a single-dose treatment with 5′-DFUR or 5-FU or a single local dose of X-rays with or without either drug are shown in Figs. 1 and 2. Mice bearing colon 26 (Exp. 1, 511 mm^3 ; Exp. 2, 1084 mm^3) at 10 days after the tumor inoculation were treated. A single dose of X-rays alone at 10 Gy delayed the tumor growth by 4 to 5 days, and its efficacy was significant at doses over 7.5 Gy. 5′-DFUR alone (4 mmol/kg) resulted in only a slight antitumor activity with T_{2000} of 2 to 3 days, while 5′-DFUR at doses of 2 and 4 mmol/kg in combination with X-rays (10 Gy) delayed tumor growth by about 10 days. On the other hand, 5-FU alone was ineffective, and the combined modality treatment produced a marginal additive effect only at a combination dose (1 mmol/kg of 5-FU and 10 Gy of X-rays) that was lethal to some animals.

Antitumor effects of fractionated-dose treatments The antitumor activity of 5′-DFUR administered either alone

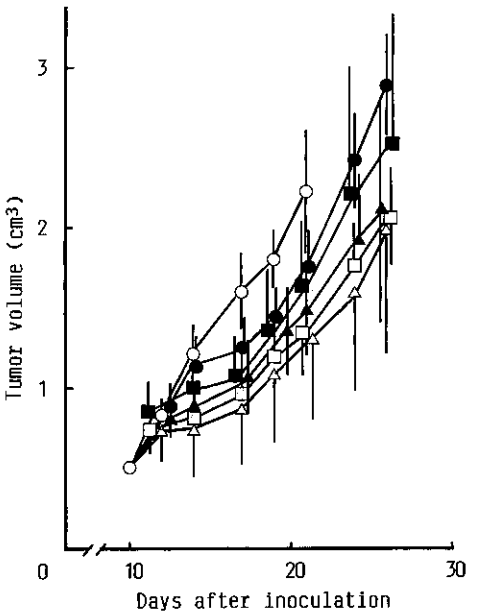

Fig. 1. Growth curves of colon 26 adenocarcinoma after a single X-ray treatment. Mice bearing colon 26 were locally irradiated with various doses (○, non-irradiated; ●, 5 Gy; ■, 7.5 Gy; ▲, 10 Gy; □, 12.5 Gy; △, 15 Gy) at day 10 after the tumor inoculation.

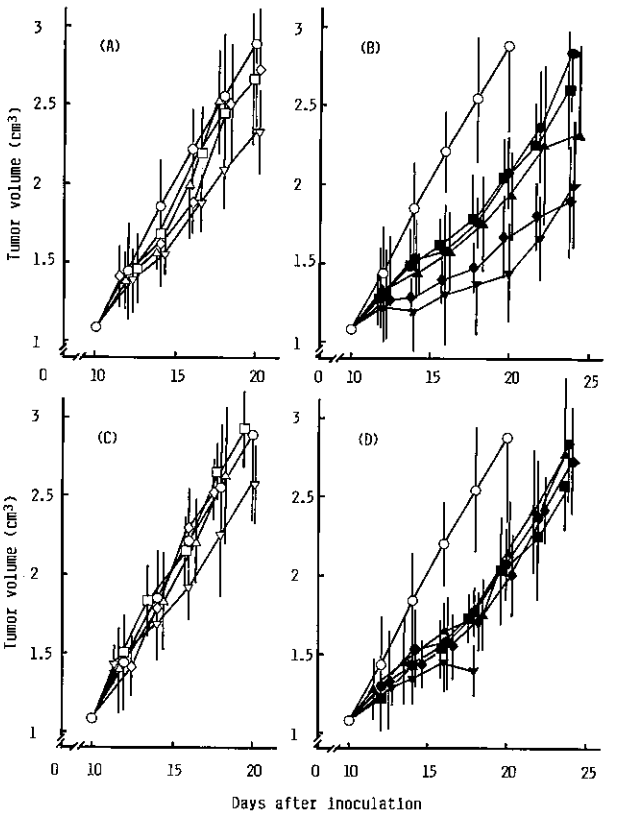

Fig. 2. Growth curves of colon 26 adenocarcinoma after a single treatment with X-rays and 5-FU or 5′-DFUR. Mice bearing colon 26 received 5-FU or 5′-DFUR in combination with (closed symbols) or without (open symbols) local radiation (10 Gy) at day 10 after the tumor inoculation. (A) and (B) 5′-DFUR (mmol/kg): ○ ●, vehicle; □ ■, 0.5; △ ▲, 1.0; ◇ ◆, 2.0; ▽ ▼, 4.0. (C) and (D) 5-FU (mmol/kg): ○ ●, vehicle; □ ■, 0.125; △ ▲, 0.25; ◇ ◆, 0.5; ▽ ▼, 1.0.

or in combination with X-rays was more obvious in a fractionated-dose treatment (Treatment schedule 2). In Fig. 3, tumor growth curves after three treatments with 5′-DFUR or 5-FU or local X-rays with or without either drug are shown. The treatment was given to mice bearing colon 26 (609 mm^3) at 6, 10 and 14 days after the tumor inoculation, and the growth in tumor size was measured every other day. The antitumor activity of these treatments in terms of delay in tumor growth (T_{2000}) is shown in Fig. 4. The X-rays alone (5 Gy) given three times delayed the tumor growth by 10 days. 5′-DFUR alone showed activity with T_{2000} of 5 to 15 days in a dose-dependent manner at doses ranging from 0.5 to 4 mmol/kg. 5′-DFUR in combination with 5 Gy of X-rays increased the T_{2000} by 16 to 35 days at the same dose range. On the other hand, 5-FU alone was effective only at 0.5 mmol/kg, with T_{2000} of 8.4 days, and was lethal at 1 mmol/kg. The combination of 5-FU at 0.5 mmol/kg and X rays additively increased T_{2000} as compared with either treatment separately.

In this experiment we also recorded the survival of mice that were treated with 5-FU and 5′-DFUR either as a single agent or in combination with X-rays. As Table I shows, X-rays alone increased the median survival days (MSD) by 15.5 to 44 days at doses in the range from 5 to 10 Gy, while 5-FU (0.5 mmol/kg) and 5′-DFUR (4 mmol/kg) alone showed only slight but significant increases of MSD of 7 days and 9 days, respectively. In combination with X-rays (5-Gy) 5-FU resulted in some additive effect only at a dose of 0.5 mmol/kg; the dose of 1 mmol/kg was lethal. On the other hand, combined treatments with 5′-DFUR and 5 Gy of X-rays increased the MSD much more than the sum of the effect of each treatment (20 days by 5′-DFUR at 4 mmol/kg).

Adverse effects of single-dose treatments In order to estimate the therapeutic potential of the combination of X-rays and 5-FU or 5′-DFUR, damage to normal tissues were observed at 4 days after a single treatment with the drugs and whole-body irradiation of 0.5 Gy in normal CDF_1 mice. In Table II, effects on body weight and

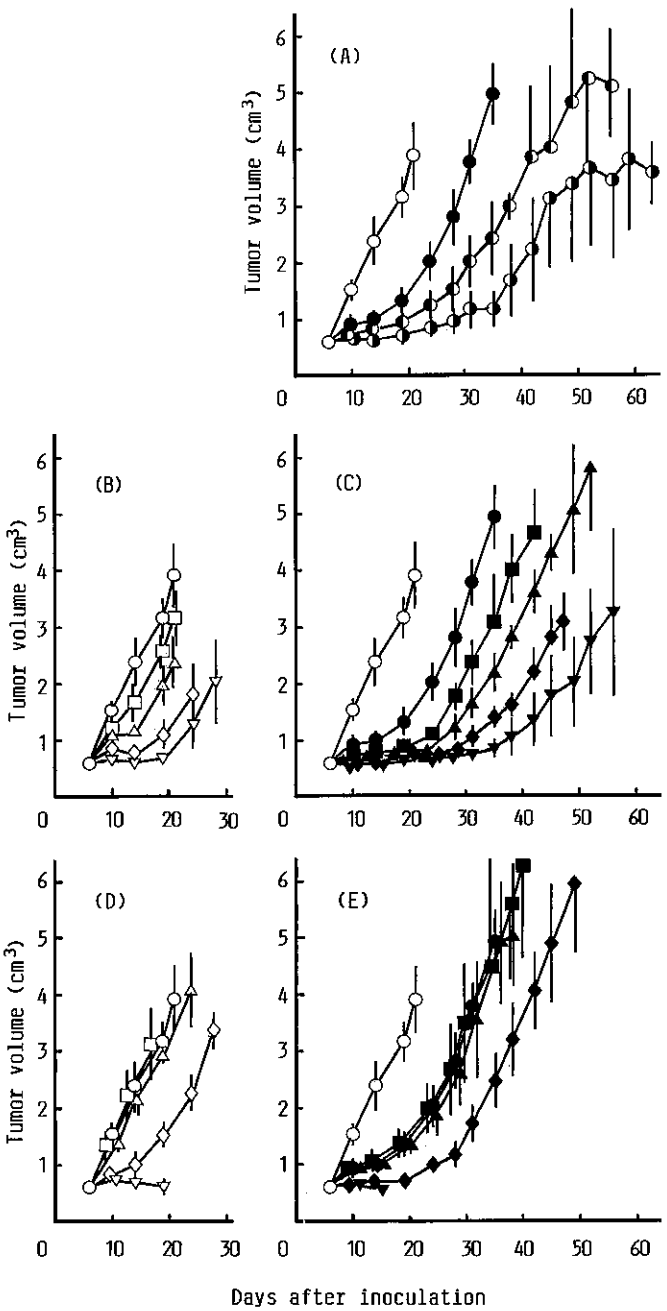

Fig. 3. Growth curves of colon 26 adenocarcinoma after fractionated-dose treatment with X-rays and 5-FU or 5′-DFUR. Mice bearing colon 26 received drugs in combination with (closed symbols, (C) and (D) 5 Gy/day) or without (open symbols) local radiation three times at days 6, 10 and 14 after the tumor inoculation. (A) X-rays (Gy/day) alone, ○, non-irradiated; ●, 5; ◐, 7.5; ◑, 10. (B) and (C) 5′-DFUR (mmol/kg/day): ○ ●, vehicle; □ ■, 0.5; △ ▲, 1.0; ◇ ◆, 2.0; ▽ ▼, 4.0. (D) and (E) 5-FU (mmol/kg/day): □ ■, 0.125; △ ▲, 0.25; ◇ ◆, 0.5; ▽ ▼, 1.0.

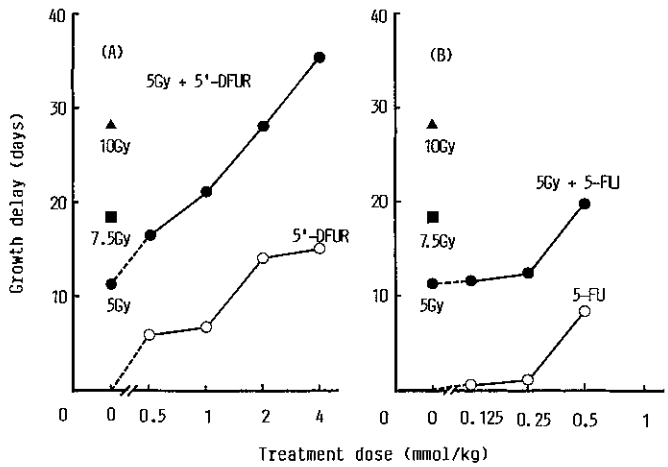

Fig. 4. Tumor growth delay caused by fractionated-dose treatment. Tumor growth delay (T_{2000}) was estimated from Fig. 3 and plotted against dosages of (A) 5′-DFUR and (B) 5-FU.

cellularity of normal tissues as a result of treatment with 5-FU, 5′-DFUR or X-rays and either drug in combination with X-rays are shown. 5-FU alone was toxic at doses of 0.5 and 1 mmol/kg, and the toxicities of 5-FU and X-rays administered in combination appeared to be additive. On the other hand, 5′-DFUR at the highest dose used in the antitumor testing, 4 mmol/kg (Fig. 2), was well tolerated by normal tissues with the exception of the thymus (toxic dose: 4 mmol/kg). Even in combination with X-rays at 0.5 Gy, 5′-DFUR at 4 mmol/kg was slightly toxic only to the thymus (53% reduction in the thymus cellularity). When the effective antitumor doses of 5-FU and 5′-DFUR administered as a single agent or in combination with X-rays (Fig. 2) are considered, 5′-DFUR is much less toxic to normal tissues and to the whole body than 5-FU.

Radiation therapy is also known to cause damage to the skin. After a single dose of local irradiation at 30 Gy to the right leg of normal mice, we observed various reactions of the skin (such as reddening, query, puffiness, breakdown with moist exudate, etc.) and scored their severity at 16 days after the irradiation. As Fig. 5 shows, X-rays caused skin damage, whereas 5′-DFUR did not, and it also did not enhance the damage caused by X-rays. 5-FU alone was also inactive. However, it delayed the skin reaction at a sublethal combination dose (1 mmol/kg) by about 7 days, possibly because of the immunosuppressive effect of 5-FU.

Adverse effects of fractionated-dose treatments Damage to normal tissues caused by the treatments was observed at 4 days after the last fractionated-dose treatment (Treatment schedule 2) with 5′-DFUR, 5-FU and/or the

Table I. The Median Survival Day of Mice Bearing Colon 26 and Given Fractionated-dose Treatment

Drug[a] (mmol/kg/day)		Median survival day					
		Without X-rays		With X-rays[b]			
Vehicle		21.5	(0)[c]	5	Gy/day	37[e]	(0)[d]
				7.5		57[e,f]	
				10		65.5[e,f]	
5′-DFUR	0.5	22	(0.5)	5	Gy/day	43.5[e]	(6.5)
	1	23	(1)	5		51[e]	(14)
	2	25.5[e]	(4)	5		47[e,f]	(10)
	4	30.5[e]	(9)	5		57.5[e,f]	(20.5)
5-FU	0.125	21	(−0.5)	5	Gy/day	40.5[e]	(3.5)
	0.25	22.5	(1)	5		39.5[e]	(2.5)
	0.5	28.5[e]	(7)	5		47[e]	(10)
	1	19.5	(−2)	5		16.5[e,f]	(−20.5)

a) Drugs were given three times at days 6, 10 and 14 after the tumor inoculation. The molecular weights of 5′-DFUR and 5-FU are 246 and 130, respectively.
b) Radiation was given three times at days 6, 10 and 14 after the tumor inoculation.
c) Difference in the median survival day between mice treated with vehicle (5% gum arabic) and drugs.
d) Difference in the median survival day between mice treated with X-rays (5 Gy) alone and combined modality treatment.
e) $P<0.05$ as compared with the group given the vehicle.
f) $P<0.05$ as compared with the group given X-rays alone.

Table II. Effects of Single-dose Treatment with X-rays and Drugs on Body Weight Gain and the Cellularity of Normal Tissues

X rays[a] (Gy)	Drug[a] (mmol/kg)		Body wt. gain(g)	Thymus wt.[b] (%)	Spleen wt.[b] (%)	PBL No.[b] (%)	BMC No.[b] (%)
—	—		0.92	100	100	100	100
0.5	—		1.03	69[c]	78[c]	73[c]	95
2	—		0.33	40[c,d]	55[c,d]	43[c,d]	32[c,d]
4	—		0.40	14[c,d]	30[c,d]	29[c,d]	7[c,d]
6	—		−0.19	11[c,d]	30[c,d]	26[c,d]	4[c,d]
—	5′-DFUR	0.5	1.24	80[c]	95	116	104
—		1	1.25	89	79[c]	110	110
—		2	0.91	64[c]	83[c]	100	97
—		4	0.55	40[c]	86[c]	94	73[c]
0.5	5′-DFUR	0.5	1.12	71[c]	74[c]	80[c]	81
0.5		1	1.10	70[c]	75[c]	75	91
0.5		2	1.17	60[c]	80[c]	66[c]	85
0.5		4	1.39	47[c,d]	61[c,d]	67[c]	60[c,d]
—	5-FU	0.125	1.04	96	96	115	127
—		0.25	1.19	64[c]	98	97	104
—		0.5	1.17	49[c]	76[c]	86[c]	63[c]
—		1	−0.77	24[c]	55[c]	52[c]	12[c]
0.5	5-FU	0.125	1.28	74[c]	81[c]	70	94
0.5		0.25	0.57	61[c]	68[c]	78[c]	108
0.5		0.5	0.37	30[c,d]	49[c,d]	59[c]	20[c,d]
0.5		1	−1.48	16[c,d]	38[c,d]	38[c,d]	4[c,d]

a) X-rays and drugs were given at day 0 to normal mice.
b) Cellularity of normal tissues were measured and expressed as a percentage of that of the non-treated mice.
c) $P<0.05$ for the control group (no treatment).
d) $P<0.05$ for the group that received only X-rays.

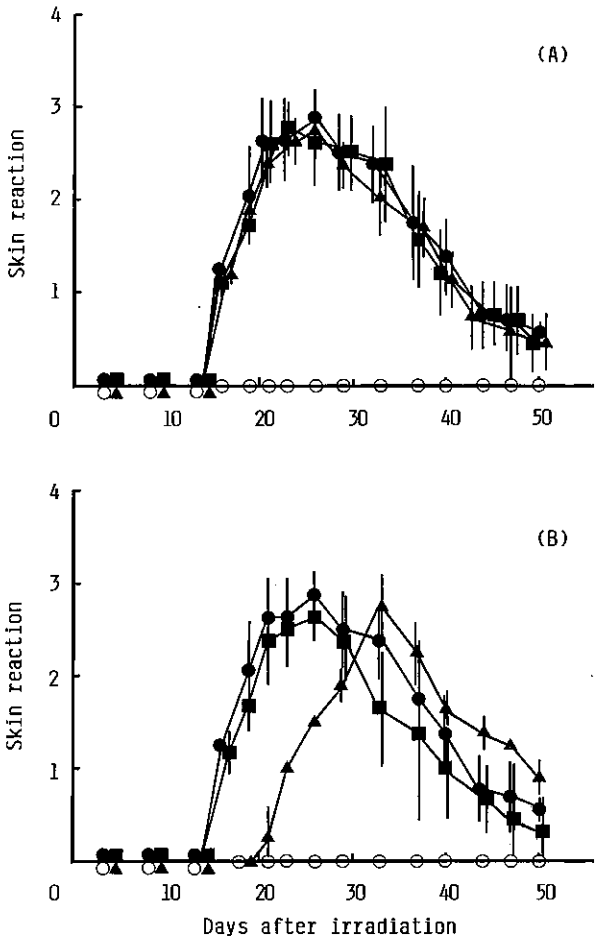

Fig. 5. Skin reaction of normal mice following fractionated-dose treatment. Normal BALB/c mice received drugs in combination with (closed symbols) or without (open symbols) local irradiation (30 Gy) to the skin. Then the skin reaction was observed, and the severity was scored as described in "Materials and Methods." (A) 5′-DFUR (mmol/kg): ○ ●, vehicle; ■, 2; ▲, 4. (B) 5-FU mmol/kg): ○ ●, vehicle; ■, 0.5; ▲ 1.0. 5-FU (1.0 mmol/kg) and 5′-DFUR (4.0 mmol/kg) alone did not cause any apparent skin reaction.

whole-body irradiation at 0.5 Gy in normal CDF_1 mice. In these treatments, either X-rays or 5-FU administered alone at doses over 1 to 2 Gy and 0.25 to 1 mmol/kg, respectively, reduced the cellularity of the spleen, thymus and bone marrow (Table III). On the other hand, mice were highly tolerant to 5′-DFUR at the doses used in this experiment. 5′-DFUR reduced the cellularity of only the thymus at the highest dose, 4 mmol/kg. In combination, 5′-DFUR did not enhance the toxicity of X-rays to normal tissues, and the toxicity of 5′-DFUR to the thymus was additive to that of X-rays at 0.5 Gy. In the fractionated-dose treatment, 5′-DFUR was shown to have a higher safety margin than 5-FU. Taking into consideration the higher antitumor efficacy of the combined modality treatment with 5′-DFUR than that with 5-FU, 5′-DFUR has a clear advantage over 5-FU.

Comparison of therapeutic gain factor of 5′-DFUR and 5-FU in combination therapy with X-rays In order to analyze the degree of modification to the radiation response caused by 5-FU and 5′-DFUR, we calculated the radiation dose effect factor (DEF) and therapeutic gain factor (TGF) from the results in the fractionated-dose treatment (Fig. 3 and Tables I and III). As Table IV shows, DEFs greater than 1 for tumor delay and survival were observed with 5′-DFUR at all dosages tested. 5′-DFUR also enhanced the damage caused by radiation to normal tissues (DEF$>$1), the spleen and bone marrow, to a slight extent. However, TGF, the ratio of the DEF for tumor growth to that for normal tissue damage, was greater than 1 for 5′-DFUR at 2 and 4 mmol/kg, indicating that 5′-DFUR at these dosages enhanced the effect of radiation, which was greater on the tumor than on the normal tissues.

On the other hand, 5-FU at only 0.5 mmol/kg enhanced the radiation effect on the tumor with a DEF greater than 1, whereas DEFs for the spleen and bone marrow were substantially greater as compared with that for the tumor. 5-FU appeared to affect the normal tissues somewhat selectively.

DISCUSSION

5′-DFUR has shown a better antitumor efficacy than 5-FU in various murine tumor models, particularly in terms of therapeutic indices.[17, 19, 24] The present study using mice bearing advanced colon 26 confirmed that 5′-DFUR is much more efficacious than 5-FU either in single-dose or fractionated-dose treatment but much less toxic to normal tissues than 5-FU. In combination with X-ray therapy the superiority of 5′-DFUR to 5-FU was also observed in the present animal tumor model. 5-FU is administered intravenously in combination with X-ray therapy of cancer patients. However, in the present study both drugs were orally administered and their efficacy was compared, because the antitumor efficacy of oral 5-FU was reported to be similar to that of intravenous 5-FU in experimental tumor models.[13, 25]

5′-DFUR was found to have antitumor activity with a high margin of safety. The fractionated-dose treatment with 5′-DFUR at doses of 1, 2 and 4 mmol/kg/day markedly delayed the tumor growth, whereas it caused damage only to the thymus at the highest dose (4 mmol/kg/day). This tumor-selective activity is attributed to its unique mode of conversion to the active metabolite 5-FU. 5′-DFUR is a prodrug which releases 5-FU when it is cleaved by pyrimidine nucleoside phosphorylase. The

Table III. Effects of Fractionated-dose Treatment with X-rays and Drugs on Body Weight Gain and the Cellularity of Normal Tissues

X rays[a] ×3 (Gy)	Drug[a] ×3 (mmol/kg)	Body wt. gain (g)	Thymus wt.[b] (%)	Spleen wt.[b] (%)	PBL No.[b] (%)	BMC No.[b] (%)
—	—	3.55	100	100	100	100
0.5	—	2.56	64[c]	75[c]	101	80
1	—	3.15	69[c]	60[c, d]	136	78
2	—	1.24	44[c, d]	29[c, d]	99	23[c]
—	5′-DFUR 0.5	2.41	104	99	86[c]	101
—	1	4.10	100	90	90	110
—	2	3.82	85	107	96	91
—	4	4.16	36[c]	104	94	85
0.5	5′-DFUR 0.5	2.01	85	69[c]	122	75
0.5	1	2.11	65[c]	69[c]	130	66[c]
0.5	2	2.24	67[c]	69[c]	113	68
0.5	4	2.52	11[c, d]	63[c, d]	122	67
—	5-FU 0.125	2.62	105	100	98	94
—	0.25	2.88	58[c]	93	108	81
—	0.5	3.16	36[c]	90	77[c]	46[c]
—	1	1.94	16[c]	61[c]	72[c]	10[c]
0.5	5-FU 0.125	3.30	76	78[c]	139	70[c]
0.5	0.25	3.59	71[c]	79	135	57[c]
0.5	0.5	2.16	11[c, d]	53[c, d]	79	42[c, d]
0.5[e]	1[e]	−3.08	0[c, d]	29[c, d]	55[c, d]	5[c, d]

a) X-rays and drugs were given three times at days 0, 4 and 8 to normal mice.
b) Cellularity of normal tissues were measured and expressed as a percentage of that of non-treated mice.
c) $P<0.05$ for the control group (no treatment).
d) $P<0.05$ for the group that received X-rays (0.5 Gy) three times.
e) Half the mice died of toxicity.

Table IV. Statistics for the Colon 26 and Normal Tissues after Fractionated-dose Treatment with X-ray Irradiation and 5-FU or 5′ -DFUR

Drug dose (mmol/kg)	Growth delay	Survival	Damage to spleen			Damage to bone marrow		
	DEF	DEF	DEF	TGF1	TGF2	DEF	TGF1	TGF2
5′ -DFUR								
0.5	1.37	1.16	1.54	0.89	0.75	1.50	0.91	0.77
1	1.64	1.42	1.54	1.06	0.92	1.96	0.84	0.72
2	1.99	1.28	1.54	1.29	0.83	1.82	1.09	0.70
4	2.36	1.65	1.94	1.22	0.85	1.90	1.24	0.87
5-FU								
0.125	1.04	1.06	1.06	0.98	1.00	1.74	0.60	0.61
0.25	1.08	1.02	0.98	1.10	1.04	2.4	0.44	0.42
0.5	1.58	1.28	2.5	0.63	0.51	3.2	0.49	0.40
1	—	0.21	3.9	—	0.05	5.2	—	0.04

TGF1: DEF (growth delay)/DEF (spleen or bone marrow).
TGF2: DEF (survival)/DEF (spleen or bone marrow).

enzymes with this activity are preferentially localized in animal tumor tissues,[13] and the levels in human cancers are higher than those in normal tissues adjacent to the cancer.[14, 15] Therefore, 5′-DFUR produces 5-FU more efficiently in tumor tissues[20] and shows potent antitumor activity.[13, 17, 24]

In combination with local irradiation, the antitumor efficacy of 5′-DFUR in terms of tumor growth delay (T_{2000}) appeared to be more than additive to that of X-rays in both single-dose and fractionated-dose treatments. This benefit of the combination was also observed in terms of increase of the survival days and again appeared to be more than additive. While 5′-DFUR alone at 4 mmol/kg increased the MSD by 9 days, and in combination with X-rays the MSD increased by 20 days as compared to that of X-ray alone. On the other hand, the toxicity of the compound to the thymus was only additive and there was no enhancement of other toxic effects of X-rays even at the highest dose used. The more than additive antitumor effects of 5′-DFUR and X-rays in combination may be attributed to the efficient formation of 5-FU from 5′-DFUR in the tumor tissues. 5-FU derived from 5′-DFUR at a concentration greater than its threshold concentration in tumor tissue may enhance tumor cell-killing by radiation. It is also possible that 5′-DFUR affects tumor cells selectively in such a way that the affected tumor cells become more susceptible to X-ray treatment. The mechanisms of the synergistic action remain to be elucidated.

5-FU was found to have a small safety margin. It was effective in terms of tumor growth delay only at doses causing damage to normal tissues, such as bone marrow, spleen and thymus. In combination with X-ray therapy, 5-FU enhanced the antitumor effects, although the effects were no more than additive to those of X-rays alone. In addition, 5-FU substantially enhanced the response of normal tissues to irradiation, to a greater extent than that of tumor tissue. TGF, the ratio of DEF for the tumor to that for the bone marrow was less than 0.5 (Table IV). Bone marrow cells appeared to be much more susceptible to 5-FU than the tumor tissues were. In the experiment on skin damage, neither drug enhanced the damage caused by X-rays, although 5-FU delayed the appearance of skin damage by X-rays, possibly through suppression of the inflammatory reaction. Thus, no advantage of the combined modality treatment with 5-FU and X-rays over either treatment alone was evident. Although radiosensitizing effects of 5-FU were suggested in *in vitro* studies,[9, 10, 12] the effects *in vivo* are controversial. A number of authors have reported an increase in tumor cell killing in a more than simply additive fashion in several animal tumor models,[7] whereas others reported only an additive effect.[4–6, 8] The efficacy of the combination may depend on the animal tumor models that were used.

Combined modality treatment with 5-FU and X-rays gave increased clinical responses with head and neck, esophageal, and cervical carcinomas in comparison to standard fractionated radiation alone.[2, 3] The present study with a mouse tumor model indicates that irradiation with 5′-DFUR produced higher TGF values than that with 5-FU in both single-dose and fractionated-dose treatments. 5′-DFUR therefore seems to have greater potential usefulness than 5-FU in combined modality treatment of cancer patients.

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