Comparative Studies on the Antitumor and Immunosuppressive Effects of the New Fluorouracil Derivative N^4-Trimethoxybenzoyl-5′-deoxy-5-fluorocytidine and Its Parent Drug 5′-Deoxy-5-fluorouridine

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N^4-Trimethoxybenzoyl-5′-deoxy-5-fluorocytidine (Ro 09-1390), a new prodrug of 5′-deoxy-5-fluorouridine (5′-dFUrd), was synthesized for the purpose of finding a drug with less intestinal toxicity than the parent compound. The present study compared the antitumor activity and immunotoxicity of Ro 09-1390 with those of 5′-dFUrd, 5-fluorouracil (5-FUra) and tegafur in various transplantable tumor models. The antitumor efficacy of Ro 09-1390 was comparable to 5′-dFUrd and these two agents were much more effective than the others. However, Ro 09-1390 was much less toxic to the intestinal tract and less immunosuppressive in both humoral and cellular immune reactions than 5′-dFUrd. Consequently, Ro 09-1390 showed higher therapeutic indices and higher efficacy than 5′-dFUrd at high dosages. The antitumor spectrum of Ro 09-1390 was somewhat different from that of 5′-dFUrd, though it shows the efficacy after it converts to 5′-dFUrd. The activity of Ro 09-1390 was partly associated with cytidine deaminase in the tumors treated. Ro 09-1390 appeared to be more effective against tumors with a high concentration of the enzyme by which the major metabolite 5′-deoxy-5-fluorocytidine (5′-dFCyd) is metabolized to 5′-dFUrd.

Keywords N^4-trimethoxybenzoyl-5′-deoxy-5-fluorocytidine (Ro 09-1390); 5′-deoxy-5-fluorocytidine (5′-dFCyd); 5′-deoxy-5-fluorouridine (5′-dFUrd); cytidine deaminase

Introduction

5′-Deoxy-5-fluorouridine (5′-dFUrd) is a prodrug from which 5-fluorouracil (5-FUra) is generated by pyrimidine nucleoside phosphorylases.[1] The enzyme activity in mice bearing transplantable tumors is localized preferentially in the tumors but not in normal tissues except within the intestinal tract.[1,2] Consequently, 5′-dFUrd was efficiently converted to 5-FUra in the tumors and to some extent in the intestine after its oral administration.[3] This tissue selective conversion of 5′-dFUrd to 5-FUra has explained its potent antitumor activity, and the dose limiting toxicity which induced diarrhea observed in clinical trials.[4,5] N^4-Trimethoxybenzoyl-5′-deoxy-5-fluorocytidine (Ro 09-1390) was synthesized with the aim of reducing intestinal toxicity. It is metabolized to 5′-dFUrd through 5′-deoxy-5-fluorocytidine (5′-dFCyd) after it enters the blood stream. It showed a similar antitumor activity to 5′-dFUrd but with much less intestinal toxicity in mice bearing Lewis lung carcinoma.[6]

In the present study we extensively compare the antitumor activity, and the intestinal and immunosuppressive toxicities of Ro 09-1390 with those of its parent compound 5′-dFUrd. The data clearly show that Ro 09-1390 is equally as effective as 5′-dFUrd but is less toxic. In addition, we discuss the role of cytidine deaminase, by which the major metabolite 5′-dFCyd of Ro 09-1390 is further converted to 5′-dFUrd, for the efficacy and toxicity of this compound.

Materials and Methods

Animals Male C57BL/6, BALB/c, BDF_1 (C57BL/6 × DBA/2)F_1, CDF_1 (BALB/c × DBA/2)F_1, B6C3F_1 (C57BL/6 × C3H/HeJ)F_1, ICR, and BALB/c nu/nu mice (4 weeks old) were obtained from Japan SLC Inc, Hamamatsu, Japan. The mice were used after at least one week of observation.

Tumor Cells Murine Lewis lung carcinoma, colon 26 adenocarcinoma, Meth A fibrosarcoma, and MX-1 human breast tumor cells were supplied by the Chemotherapy Center, Cancer Institute, Tokyo. UV2237 fibrosarcoma and CCM adenocarcinoma were kindly supplied by Dr. I.J. Fidler, MD Anderson Hospital and Tumor Institute, Houston, Texas and Dr. Iigo, of the National Cancer Center, Tokyo, respectively. All the tumors except for colon 26 and UV2237 were maintained by continuous passage in mice; C57BL/6 for Lewis lung carcinoma, BALB/c for Meth A, ICR for Ehlrich carcinoma and CCM, and BALB/c nu/nu for MX-1. A suspension of these tumor cells (2×10^5 to 5×10^6 cells) was inoculated subcutaneously into mice, except for MX-1. Small pieces of MX-1 (2 mm × 2 mm) were inoculated subcutaneously into mice by using a trochar. Colon 26 and UV2237 cells were maintained in *in vitro* cell cultures with RPMI1640 medium or Eagle's minimum essential medium (MEM) containing 10% fetal calf serum, penicillin G (50 U/ml) and streptomycin sulfate (100 μg/ml), respectively. A suspension of the tumor cells was prepared by the trypsinization of monolayers of the cells and 10^6 cells were inoculated subcutaneously to mice.

Antitumor Agents 5-FUra and UFT were purchased from Kyowa Hakko Co., Tokyo and Taiho Pharm. Co., Tokyo, respectively. Doxifluridine; 5′-dFUrd was synthesized at Hoffmann-La Roche, Basle, while we synthesized 5′-dFCyd and Ro 09-1390 as described elsewhere.[7] Ro 09-1390 is easily hydrolyzed to 5′-dFCyd in artificial gastric juice (pH 1.2) but not in a solution at a pH of over 4.0. Therefore, all the compounds were dissolved in or suspended with 40 mM citrate buffer (pH 6.0) containing 5% gum arabic as a vehicle and administrated by the *p.o.* route. This vehicle does not effect the antitumor activity and toxicity of the other cytostatics.

Evaluation of Toxicities For estimating the intestinal toxicity, the feces from 4 to 6 tumor-bearing mice were thoroughly examined. The toxicity was estimated from the degree of loose passage or diarrhea of the feces as follows: normal feces (N), loose passage (L), and diarrhea (D). In addition, the extent of occult blood in the feces was measured by using a test kit from Shionogi Pharma. Co., Osaka, Japan. For estimating other toxicities, weight gains of the thymus and spleen were measured and the number of the bone marrow cells of the femur was counted.

Measurement of Tumor Volume Tumor size and body weight were measured twice a week. The tumor volume was estimated by using the following equation, $ab^2/2$, where a and b are tumor length and width, respectively.

Preparation and Assay of Cytidine Deaminase Tumor tissues were homogenized with 4 volume of 0.1 M Tris–acetate buffer (pH 7.4) and then centrifuged at 100000 × g for 90 min at 4 °C. The pooled supernatant was used as a crude enzyme preparation. The enzyme activity was measured in terms of production of [2-^{14}C]uridine from [2-^{14}C]cytidine. Namely, the reaction mixture (0.05 ml) containing 5 μmol of Tris–acetate buffer (pH 7.4), 50 nmol of cytidine, 3.7 MBq of [2-^{14}C]cytidine and 10 μl of the crude enzyme was incubated at 37 °C for 15 min. The reaction was terminated by the addition of 50 μl of methanol. The resulting precipitate was removed by centrifugation (3000 rpm for 10 min), and 5 μl of the supernatant was spotted onto a silica gel thin-layer chromatography (TLC) plastic plate (Merck TLC plate with a silica gel $60F_{254}$). Cytidine and uridine were separated by the solvent ethyl acetate–methanol–ammonium water

(75:25:2) and the radioactivity of their spots was counted.

Allogenic Tumor Rejection The suppressive effect of drugs to cellular immunity was assessed in C3H/He mice ($H2^k$) inoculated with 10^6 cells of L1210 leukemic cells ($H2^d$) by observing the mortality for 21 d after inoculation of the tumor accompanying the decrease in the rate of rejection. Groups of six male C3H/He mice were used. Drugs were administered daily to the mice for 7 d from day 0, and a subline of L1210 resistant to 5-FUra was inoculated on day 3.

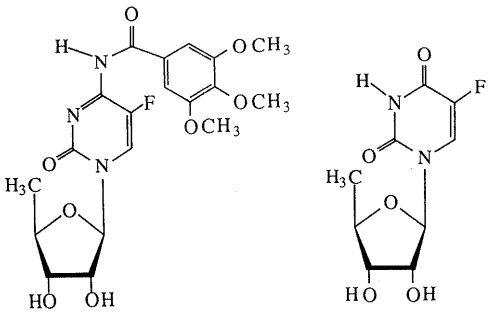
Assay for Serum Anti-SRBC Antibody BDF_1 mice were sensitized to SRBC (sheep red blood cell; 4×10^8 cells, i.v.) and then orally administered test drugs for each of 7 d beginning 1 d after the sensitization. The suppressive effect of drugs to humoral immunity was assessed in terms of the decrease in the titer of serum anti-SRBC, 8 d after the sensitization.

Statistical Analysis Statistical analyses of differences between the control group and the treatment groups were performed using Wilcoxon rank sum analysis. All p values reported were 2 tailed. The level of significance was set at $p < 0.05$.

Results

Antitumor Efficacy Ro 09-1390, 5′-dFUrd (Fig. 1) and other fluorinated pyrimidines was examined for their antitumor activity using various murine transplantable tumors and human tumor xenografts of MX-1 in nude mice. Tables I—VII indicate that Ro 09-1390 was as effective as 5′-dFUrd but much more effective than other fluorinated pyrimidines, 5-FUra and UFT tested. Accurately, the minimum effective doses of Ro 09-1390 and 5′-dFUrd were in similar ranges in mice bearing Lewis lung carcinoma, UV2237 fibrosarcoma, Ehlrich carcinoma and CCM adenocarcinoma. Those of Ro 09-1390 were about two times lower in mice bearing colon 26, Meth A and MX-1 tumors. However, Ro 09-1390 was less toxic than 5′-dFUrd, particularly to the intestinal tract. Consequently, Ro 09-1390 could be administered at higher doses and showed better efficacy at the maximum doses and higher therapeutic indices than 5′-dFUrd. When the doses causing a reduction in weight gain and intestinal toxicity (diarrhea and loose passage) were used as the toxicity parameters, overall therapeutic indices were about 2 and 4 times higher than those of 5′-dFUrd (Table VIII).

In order to understand the difference in the ratio of therapeutic indices of Ro 09-1390 to those of 5′-dFUrd among tumors, we compared the levels of cytidine deaminase activity in tumors. The enzyme is essential for conversion of the major metabolite of Ro 09-1390, 5′-dFCyd, to 5′-dFUrd. As Fig. 2 indicates, the activity of Ro 09-1390 was partly associated with the enzyme levels of the tumors being tested. Namely, the effective doses of Ro 09-1390 were relatively higher against tumors with low levels



Ro 09-1390 5′-dFUrd

Fig. 1. Chemical Structures of Ro 09-1390 and 5′-dFUrd

TABLE I. Antitumor Activity of Fluorinated Pyrimidines against Lewis Lung Carcinoma in BDF_1 Mice

Compound	Dose (mmol/kg/d)	Change of body weight on day 15 (g)	Tumor volume on day 21 (mm^3)	Inhibition $(1-T/C)\%$	Occult blood test on day 9	Fecal observation[a] on day 9
Ro 09-1390	0.25	+3.1	5121± 581	39[b]	−	N
	0.5	+2.9	3493± 311	53[b]	−	N
	1.0	+4.3	2203± 663	74[b]	±	N
	2.0	+2.1	513± 487	94[b]	+	N—L
5′-dFUrd	0.25	+1.3	5685±1345	32[b]	−	N
	0.5	+2.3	4006± 377	52[b]	−	N
	1.0	+2.9	2051± 676	75[b]	±	N
	2.0	—	(6/6 toxic)	—	++	D
5-FUra	0.25	−4.5	1501± 364 (4/6 toxic)	(82)	±	N
Control	—	+3.5	8362±2016	—	−	N

Male BDF_1 mice were subcutaneously implanted on day 0 with 10^6 cells/mouse of Lewis lung carcinoma cell. Compounds were administered daily from day 1 through 20. *a*) N, normal feces; L, loose passage; D, diarrhea. *b*) $p < 0.05$.

TABLE II. Antitumor Activity of Fluorinated Pyrimidines against UV2237 Fibrosarcoma in $B6C3F_1$ Mice

Compound	Dose (mmol/kg/d)	Change of body weight on day 15 (g)	Tumor weight on day 15 (g)	Inhibition $(1-T/C)\%$	Occult blood test on day 7	Fecal observation[a] on day 7
Ro 09-1390	0.25	+3.0	0.78±0.26	29[b]	−	N
	0.5	+2.9	0.84±0.27	23	±	N
	1.0	+3.6	0.31±0.18	71[b]	−	N
	2.0	+1.3	0.08±0.08	92[b]	+	N—L
5′-dFUrd	0.25	+1.3	0.71±0.38	34[b]	−	N
	0.5	+0.7	0.36±0.17	67[b]	+	N—L
	1.0	−0.7	0.09±0.04	92[b]	++	L
	2.0	−3.5	(6/6 toxic)	—	+++	D
Control	—	+1.1	1.09±0.27	—	−	N

Male $B6C3F_1$ mice were subcutaneously implanted on day 0 with 2×10^6 cells/mouse of UV2237 fibrosarcoma cell. Compounds were administered daily from day 1 through 14. *a*) N, normal feces; L, loose passage; D, diarrhea. *b*) $p < 0.05$.

Table III. Antitumor Activity of Fluorinated Pyrimidines against CCM Adenocarcinoma in ICR Mice

Compound	Dose (mmol/kg/d)	Change of body weight on day 15 (g)	Tumor weight on day 22 (g)	Inhibition $(1-T/C)\%$	Occult blood test on day 22	Fecal observation[a] on day 22
Ro 09-1390	0.25	+13.6	3.52±1.17	11	±	N
	0.50	+12.0	1.65±0.91	58[b]	±	N
	1.0	+13.4	1.54±1.46	61[b]	±	N
	2.0	+10.3	0.07±0.06	98[b]	+	L
5′-dFUrd	0.25	+12.2	2.75±1.15	30	±	N
	0.50	+14.5	2.43±1.15	38[b]	±	N
	1.0	+11.2	0.43±0.29	89[b]	+	L
	2.0	+8.9	0 ±0	100[b]	+	L
5-FUra	0.038	+14.0	4.58±2.26	N.E.[c]	±	N
	0.075	+12.6	4.42±1.05	N.E.[c]	±	N
	0.15	+13.2	2.81±2.23	29	±	N
	0.3	+13.0	2.58±1.00	35[b]	±	L
UFT	0.025	+15.4	5.24±2.53	N.E.[c]	±	N
	0.05	+15.1	4.71±2.17	N.E.[c]	±	N
	0.1	+14.2	3.62±0.91	8	±	N
	0.2	+11.2	1.98±1.65	50	+	L
Control	—	+14.3	3.95±1.47	—	±	N

Male ICR mice were subcutaneously implanted on day 0 with 2×10^6 cells/mouse of CCM adenocarcinoma cell. Compounds were administered daily from day 1 through 21. *a*) N, normal feces; L, loose passage; D, diarrhea. *b*) $p<0.05$. *c*) N.E. = not effective.

Table IV. Antitumor Activity of Fluorinated Pyrimidines against Ehrlich Carcinoma in ICR Mice

Compound	Dose (mmol/kg/d)	Change of body weight on day 21 (g)	Tumor weight on day 21 (g)	Inhibition $(1-T/C)\%$	Occult blood test on day 21	Fecal observation[a] on day 21
Ro 09-1390	0.25	+12.2	1.82±0.72	63[b]	±	N
	0.5	+10.5	1.41±0.87	71[b]	±	N
	1.0	+8.7	0.58±0.46	88[b]	±	N
	2.0	+7.3	0.15±0.07	97[b]	±	N
5′-dFUrd	0.25	+9.9	1.37±0.83	72[b]	±	N
	0.5	+9.6	1.01±0.52	79[b]	±	N
	1.0	+8.4	0.70±0.36	86[b]	±	N
	2.0	−5.5	0 ±0 (3/6 toxic)	(100)	+++	D
Control	—	+14.1	4.91±1.39	—	±	N

Male ICR mice were subcutaneously implanted on day 0 with 5×10^6 cells/mouse of Ehrlich carcinoma cell. Compounds were administered daily from day 1 through 20. *a*) N, normal feces; L, loose passage; D, diarrhea. *b*) $p<0.05$.

Table V. Antitumor Activity of Fluorinated Pyrimidines against Colon 26 Adenocarcinoma in CDF_1 Mice

Compound	Dose (mmol/kg/d)	Change of body weight on day 14 (g)	Tumor weight on day 14 (g)	Inhibition $(1-T/C)\%$	Occult blood test on day 14	Fecal observation[a] on day 14
Ro 09-1390	0.38	+1.2	0.36±0.08	40[b]	±	N
	0.75	+1.5	0.15±0.10	75[b]	±	N
	1.5	−0.5	0.07±0.02	88[b]	±	N
	3.0	−3.5	0.06±0.02	90[b]	±	N
5′-dFUrd	0.38	+1.0	0.14±0.03	77[b]	±	N
	0.75	+0.2	0.08±0.02	87[b]	±	N
	1.5	−3.5	0.07±0.03	88[b]	+	L
	3.0	−5.2	0.05±0.02	92[b]	++	L
Control	—	+0.8	0.60±0.08	—	±	N

Male CDF_1 mice were subcutaneously implanted on day 0 with 10^6 cells/mouse of Colon 26 adenocarcinoma cell. Compounds were administered daily from day 7 through 13. *a*) N, normal feces; L, loose passage; D, diarrhea. *b*) $p<0.05$.

of cytidine deaminase (colon 26, Meth A and MX-1) as compared with those of 5′-dFUrd.

Immunosuppressive Effects Ro 09-1390 was about two times less toxic to the organs responsible for immune reactions, the thymus, spleen and bone marrow, than 5′-dFUrd and much less toxic than 5-FUra (Fig. 3). In a functional assay for immunotoxicity, we examined the effects of Ro 09-1390, 5′-dFUrd and 5-FUra on the rejection of allogenic tumor cells in mice. A subline of L1210 leukemia resistant to 5-FUra was rejected in 100% of untreated C3H/He mice challenged with 10^6 cells, whereas the mice treated with high doses of each drug failed to reject the tumor (Fig. 4). The dose of Ro 09-1390 which lowered the rejecting rate by 50% was about two times higher than that of 5′-dFUrd, while 5-FUra was much more immunosuppressive than these two 5-FUra derivatives. In an assay

TABLE VI. Antitumor Activity of Fluorinated Pyrimidines against Meth A Fibrosarcoma in CDF_1 Mice

Compound	Dose (mmol/kg/d)	Change of body weight on day 8 (g)	Tumor weight on day 14 (g)	Inhibition $(1-T/C)\%$	Occult blood test on day 8	Fecal observation[a] on day 8
Ro 09-1390	0.38	+2.7	1.08±0.52	N.E.[b]	±	N
	0.75	+2.3	0.68±0.53	20	+	N
	1.5	+2.6	0.12±0.12	86[c]	+	N
	3.0	−0.5	0.18±0.12	79[c]	+	N
5′-dFUrd	0.38	+3.0	0.56±0.34	34	±	N
	0.75	+1.5	0.59±0.24	31	+	N
	1.5	−0.4	0.29±0.29	66[c]	+	L
	3.0	−5.5	(6/6 toxic)	—	+++	D
5-FUra	0.25	−1.2	0.34±0.22	60[c]	±	N
Control	—	+2.5	0.85±0.50	—	±—+	N

Male CDF_1 mice were subcutaneously implanted on day 0 with 2×10^5 cells/mouse of Meth A fibrosarcoma cell. Compounds were administered daily from day 1 through 7. *a*) N, normal; L, loose passage; D, diarrhea. *b*) N.E. = not effective. *c*) $p<0.05$.

TABLE VII. Antitumor Activity of Fluorinated Pyrimidines against MX-1 Human Mammary Adenocarcinoma Xenograft

Compound	Dose (mmol/kg/d)	Change of body weight on day 20 (g)	Relative tumor volume on day 30	Inhibition $(1-T/C)\%$	Fecal observation[a] on day 30
Ro 09-1390	0.25	+2.7	24.5	N.E.[b]	N
	0.5	+2.0	16.5	24	N
	1.0	+2.6	9.2	60[c]	N
5′-dFUrd	0.25	+2.9	17.7	18	N
	0.5	−0.6	10.1	55	L
	1.0	−2.7	(1.7)	(5/6 toxic)	D
5-FUra	0.075	+3.1	30.3	N.E.[b]	N
	0.15	+0.1	(6.7)	72 (3/6 toxic)	N
	0.3	—	—	(6/6 toxic)	—
UFT	0.038	+3.3	18.4	15	N
	0.075	+2.4	18.8	13	N
	0.15	—	(5.4)	(5/6 toxic)	D
Control	—	+2.4	21.4	—	N

Male Balb/c-nu/nu mice were subcutaneously implanted on day 0 with small pieces (2 mm × 2 mm) of MX-1 adenocarcinoma. Compounds were administered daily for 29 times from the day when the tumor volume reached to 150—200 mm^3. *a*) N, normal feces; L, loose passage; D, diarrhea. *b*) N.E. = not effective. *c*) $p<0.05$.

TABLE VIII. Therapeutic Indicies of 5′-dFUrd (a) and Ro 09-1390 (b)

Tumor	Schedule	Therapeutic indicies A		B		C	
		a	b	a	b	a	b
Meth A	×7[a]	1	>2	<1	>2	<1	1
Colon 26	×7[a]	8—16	>4	2—4	>4	2—4	2
UV2237	×14[b]	2	>2	1	>2	1	>2
LLC	×20[b]	2	>4	1	>4	2	>4
Ehrlich	×21[b]	4—8	>8	4	>8	4—8	>8
CCM	×21[b]	4—8	>4	<1	2	>2	>4
MX-1	×29[c]			0.5	>1.2		

A = ED_{max}/ED_{50}. B = maximum doses which maintain normal feces/ED_{50}. C = maximum doses which do not decrease body weight/ED_{50}. a = 5′-dFUrd. b = Ro 09-1390. Highest doses: *a*) 3, *b*) 2, *c*) 1 mmol/kg/d.

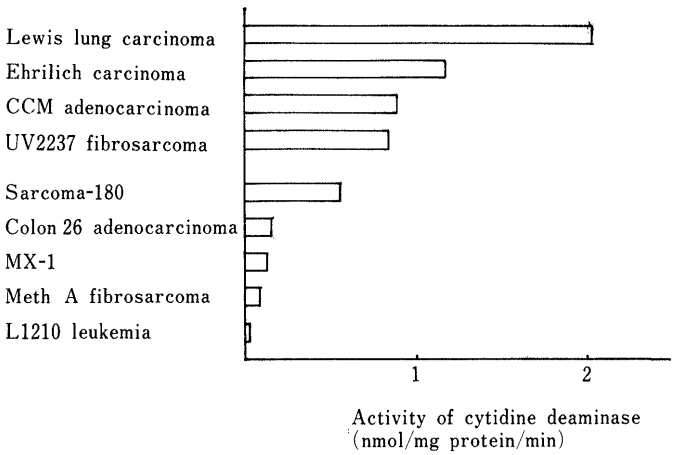


Fig. 2. Cytidine Deaminase Activity of Tumor Tissues in Mice

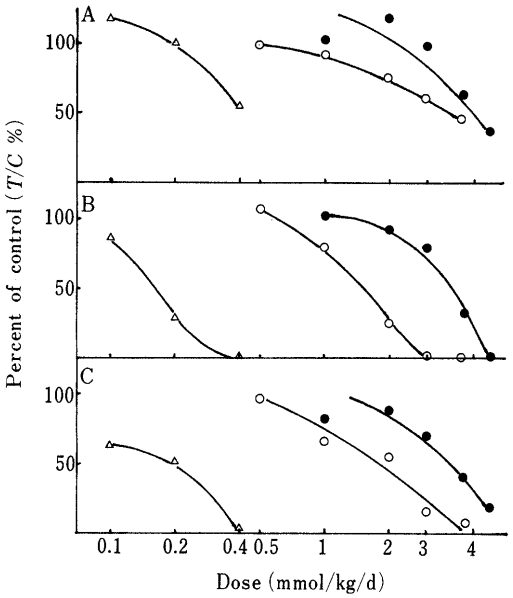


Fig. 3. Toxic Effects of Ro 09-1390, 5′-dFUrd and 5-FUra on the Spleen (A), Thymus (B) and Bone Marrow (C) in Mice

A, spleen weight; B, thymus weight; C, bone marrow cell number. ●, Ro 09-1390; ○, 5′-dFUrd; △, 5-FUra.

for suppressive effects on humoral antibody production, serum levels of anti-SRBC were measured at 8 d after the sensitization with the antigen. Both Ro 09-1390 and 5′-dFUrd inhibited the antibody production at their higher doses (Fig. 5). 5′-dFUrd was much less immunosuppressive than 5-FUra as described elsewhere,[8-10] but Ro 09-1390 was about two times less suppressive than 5′-dFUrd.

Discussion

5′-dFUrd is an orally administered cytostatic being clinically used for treatment of patients with stomach,

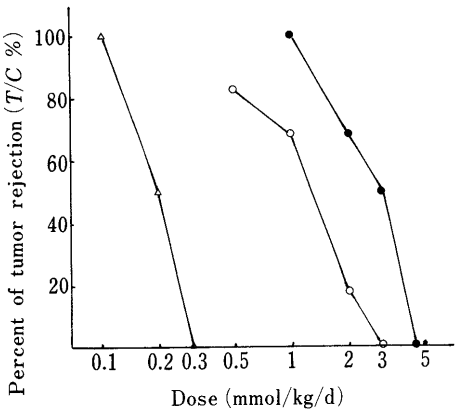


Fig. 4. Effects of Ro 09-1390, 5′-dFUrd and 5-FUra on Tumor-Allograft Rejection in Mice

●, Ro 09-1390; ○, 5′-dFUrd; △, 5-FUra.

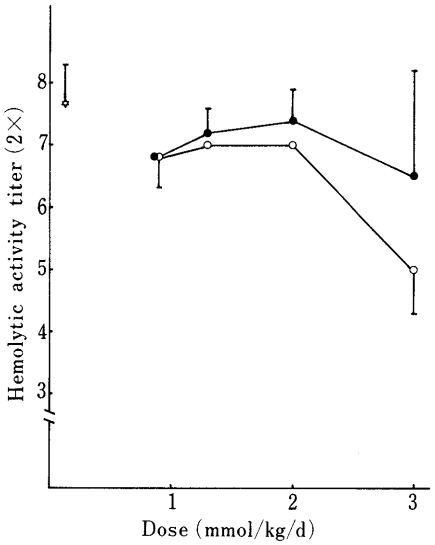


Fig. 5. Effects of Ro 09-1390 and 5′-dFUrd on the Production of Anti-SRBC Antibody in Mice

●, Ro 09-1390; ○, 5′-dFUrd; ✡, immune control.

colorectal and breast cancers.[11] It has been shown to be a more effective antitumor agent with less toxicity and immunosuppressive activity than other fluorinated pyrimidines in various tumor models.[12-14] The present study showed that a new prodrug of 5′-dFUrd, Ro 09-1390, is as effective as the parent 5′-dFUrd on molar basis but is less toxic, particularly much less toxic to the intestine. Consequently, Ro 09-1390 showed higher therapeutic indices than 5′-dFUrd and other fluorinated pyrimidines tested.

5′-dFUrd is converted to 5-FUra by pyrimidine nucleoside phosphorylase, which is preferentially located in the intestine as well as in tumor tissues.[1,2] The selective antitumor activity and its dose-limiting toxicity and diarrhea, has been shown to be mainly attributable to the unique tissue distribution pattern of the enzyme.[1,2,15-17] Ro 09-1390 is designed to generate 5′-dFUrd through 5′-dFCyd after it enters the blood stream. In fact, we have found that in mice bearing Lewis lung carcinoma 5′-dFUrd generated a substantial amount of the active metabolite 5-FUra in the intestine, whereas Ro 09-1390 produced only a small amount of 5-FUra here.[4] The difference in the extent of 5-FUra produced in the intestine explains why Ro 09-1390 was less toxic.

Ro 09-1390 and its metabolite 5′-dFCyd exhibit their antitumor effects after they were converted to 5′-dFUrd. Therefore, the antitumor activity and toxicity of Ro 09-1390 must be affected by the tissue concentrations of acylamidase and cytidine deaminase, which are the enzymes responsible for the conversion of Ro 09-1390 to 5′-dFCyd and then to 5′-dFUrd, respectively. In mice treated with Ro 09-1390, the major metabolites in the blood were 5′-dFCyd and 5′-dFUrd. Therefore, the antitumor activity must be affected not only by the concentration of 5′-dFUrd in the blood but also by the activity of cytidine deaminase in tumor tissues. The present study comparing the antitumor activity in various tumor models shows that Ro 09-1390 is more effective in tumors that have high cytidine deaminase activity. Namely, Ro 09-1390 on a molar basis was less effective than 5′-dFUrd against colon 26 and Meth A tumors, in which the enzyme activity was very low.

The activity and its distribution in tissue of cytidine deaminase are known to be quite different among species.[18] In humans, as well as mice, a sufficient amount of cytidine deaminase exists in the liver.[18] We found that acylamidase in the human liver converted Ro 09-1390 to 5′-dFCyd and in the intestinal tract, it converted the drug slightly. Furthermore, cytidine deaminase activity was higher in human cancerous tissues than in adjacent normal tissues.[19,20] We predict that Ro 09-1390 converts to 5′-dFUrd in humans as well as in mice. Studies on cytidine deaminase and acylamidase which are the responsible enzymes for conversion of Ro 09-1390 to 5′-dFUrd, such as studies on the distribution in tissue of the enzymes in mice and human, are necessary to predict the efficacy and toxicity of Ro 09-1390 in humans. If the conversion of Ro 09-1390 to 5′-dFUrd in mice is similar to that in humans, the drug will have greater potential than the prodrug 5′-dFUrd in the treatment of cancer patients particularly from a safety point of view.

Most cytostatics used in cancer therapy cause immunosuppression which, as one of the side effects, has limited the continuation of treatment with them. Host immune systems are needed for eradicating infections caused by microbials and for possibly destroying residual tumor cells after tumors have regressed due to cancer therapies. In mice, we have observed that 5′-dFUrd is less immunosuppressive in both humoral and cell-mediated immunities than 5-FUra, tegafur and UFT, while Bollag and Hartmann reported that 5′-dFUrd caused much less leukopenia than tegafur and 2′-dFUrd. The present study has proven that Ro 09-1390 is less toxic than 5′-dFUrd to the organs concerned with immune reactions, the thymus, spleen and bone marrow. It is also less immunosuppressive than 5′-dFUrd in SRBC antibody production and in allograft tumor rejection. Thus, Ro 09-1390 will have advantages over 5′-dFUrd and the other fluorinated pyrimidines in cancer therapy also from an immunotoxicological view point.

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