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A comparative study of the antitumor activities of 5′-deoxy-5-fluorouridine and its prodrug trimethoxy benzoyl-5′-deoxy-5-fluorocytidine (Ro09-1390) on human digestive organ cancer xenograft lines transplanted into nude mice

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5′-Deoxy-5-fluorouridine (5′-DFUR) is one of the oral fluoropyrimidines widely used in the treatment of gastric, colorectal and breast cancers in Japan. 5′-DFUR is converted to 5-fluorouracil by pyrimidine nucleoside phosphorylase in the tumor. 5′-DFUR has toxic effects on the intestine and may cause severe diarrhea. Trimethoxybenzoyl-5′deoxy-5-fluorocytidine (Ro09-1390) is a prodrug of 5′-DFUR, which was developed to reduce the intestinal toxicity of 5′-DFUR. The present study was designed to assess the antitumor activity and spectrum of Ro09-1390, and to compare its efficacy with that of 5′-DFUR. Six digestive organ cancer xenograft lines (two gastric, one esophageal, one colorectal, one gall bladder and one bile duct cancers) were s.c. transplanted into nude mice. The agents were orally administered daily for 14 days at doses of 0.08–0.64 mmol/kg (1–8 times the maximal clinical dose of 5′-DFUR). Both 5′-DFUR and Ro09-1390 significantly inhibited the growth of two gastric cancer lines, and the IC_{50}'s for Ro09-1390 in both lines were lower than the respective values for 5′-DFUR. The esophageal, colorectal, gall bladder and bile duct cancer lines, however, were resistant to both agents. 5′-DFUR at 0.64 mmol/kg significantly inhibited the growth of these cancers, but with high mortality, and most mice receiving this dose died within 14 days after the start of therapy, suffering from severe diarrhea and body weight loss. Ro09-1390 at the same dose resulted in low mortality, but evidenced similarly low antitumor activity. These results suggest that Ro09-1390 may be a more beneficial antitumor fluoropyrimidine, at least in the treatment of gastric cancer, than 5′-DFUR.

Key words: 5′-Deoxy-5-fluorouridine, human tumor xenograft, nude mouse, trimethoxybenzoly-5′-deoxy-5-fluorocytidine (Ro09-1390).

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Introduction

5′-Deoxy-5-fluorouridine (5′-DFUR) is one of the antitumor fluoropyrimidines used widely in Japan. 5′-DFUR is administered orally for the treatment of gastric, colorectal and breast cancers, singly or in combination with other cytotoxic agents.[1–4] 5′-DFUR is a prodrug, which is converted to 5-fluorouracil (5-FU) by pyrimidine nucleoside phosphorylase (PyNPase).[5–7] PyNPase activity is reportedly higher in neoplatic tissues and in the liver than in other normal tissues.[5,8] 5′-DFUR has toxic effects on the intestine and may cause severe diarrhea. This toxicity may be due to the metabolism of 5′-DFUR into 5-FU in the intestinal mucosa.[5,6] This intestinal toxicity is one of the dose-limiting factors in the clinical application of 5′-DFUR.[3,4] Recently, a prodrug of 5′-DFUR, trimethoxybenzoyl-5′-fluorocytidine (Ro09-1390), was developed to reduce the intestinal toxicity of 5′-DFUR. This prodrug, Ro09-1390, has been reported to have a similar or superior level of antitumor activity against murine tumors, with less intestinal toxicity than 5′-DFUR.[9] The effect of Ro09-1390 on human tumors, however, has yet to be reported. The present study was designed to evaluate the antitumor spectrum of Ro09-1390 for human digestive organ cancers and to compare this efficacy with that of 5′-DFUR.

Table 1. Human digestive organ cancer xenograft lines used in the present study

Line	Origin	Histology	Mean doubling time (days)
EC-YO	esophageal cancer	squamous cell carcinoma	6.6
GC-YN	gastric cancer	well differentiated adenocarcinoma	6.3
GC-SF	gastric cancer	poorly differentiated adenocarcinoma	14.1
CC-KK	colonic cancer	tubular adenocarcinoma	7.5
BDC-SN	bile duct cancer	undifferentiated carcinoma	4.9
GBG-GN	gall bladder cancer	well differentiated adenocarcinoma	7.0

Materials and methods

Animals

BALB/c nu^+/nu^+ male nude mice were purchased from CLEA, Tokyo, Japan. The mice were housed in a specific pathogen free environment at the Kyoto University Laboratory Animal Center.

Human tumor zenograft lines

Six human digestive organ cancer xenograft lines (two gastric, one esophageal, one colonic, one bile duct and one gall bladder cancers) were employed in the study (Table 1). All of the lines were established from specimens resected in our department and subsequently maintained by s.c. transplantation into the backs of nude mice.

5′-DFUR (frutulon) and Ro09-1390 (galocitabine)

5′-DFUR and Ro09-1390 were kindly supplied by Nippon Roche KK, Tokyo, Japan. Both agents were dissolved in a 40 mM citrate buffer (pH 6.0) containing 5% gum arabic as vehicle. Intragastric administration was carried out with a stainless steel catheter. The drugs were administered in the morning, once a day for 14 days, at doses of 0.08, 0.16, 0.32 and 0.64 mmol/kg/day (20, 40, 80 and 160 mg/kg for 5′-DFUR, and 35.6, 71.2, 142.4 and 284.8 mg/kg for Ro09-1390). The maximal clinical dose for 5′-DFUR, as approved by the Japanese Ministry of Welfare and Health, is 0.08 mmol/kg. The mice in the control group were given the vehicle alone.

Evaluation of antitumor activity

The tumors were cut into 2–3 mm fragments using scissors and these fragments were s.c. transplanted into the backs of nude mice. After transplantation, tumor sizes were serially measured with calipers. When the tumor had reached 100–300 mm^3 in size, the experiments were initiated. To estimate tumor volume (V) the following formula was used:[10]

$$V = \text{length} \times \text{width}^2 \times 1/2$$

The efficacy of each regimen was evaluated in terms of the tumor growth rate (TGR) and the inhibition rate (IR). The TGR and IR were calculated according to the following formulae:

$$\text{TGR} = V_n/V_0$$

$$\text{IR} = (1 - \text{TGR}_t/\text{TGR}_c) \times 100\ (\%)$$

Where V_n indicates the tumor volume estimated n days after the initial administration and V_0 is the tumor volume at the start of the experiment. TGR_t and TGR_c represent the TGRs of the test and control groups, respectively.

Drug toxicity was assessed in terms of percent mortality and body weight loss.

5-FU concentrations in the tumor

The mice were sacrificed by cervical dislocation and the tumors were subsequently removed. The tumors were homogenized with 4 volumes of ice cold saline and acetonitrile (1:1) using a Hiscotron homogenizer. An equal volume of acetonitrile was added to the supernatant of the homogenate, and the drugs and their metabolites were extracted. The content of the active metabolite 5-FU in the extract was measured by a bioassay method.[11]

Table 2. Comparative antitumor activities of 5′-DFUR and Ro09-1390 in sensitive human gastric cancer xenograft lines

Protocol (mmol/kg)		TGR (mean ± SD) (alive mice/tested mice)			
		GC-YN		GC-SF	
		day 7	day 14	day 7	day 14
Control (vehicle)		1.71 ± 0.39 (7/7)	3.48 ± 1.68 (7/7)	1.24 ± 0.33 (6/6)	1.38 ± 0.28 (6/6)
5′-DFUR	(0.08)[a]	1.62 ± 0.24 (7/7)	2.71 ± 0.61 (7/7)	1.50 ± 0.25 (5/5)	1.71 ± 1.36 (5/5)
	(0.16)	1.56 ± 0.26 (5/5)	2.15 ± 0.46 (5/5)	1.29 ± 0.51 (5/5)	1.48 ± 0.44 (5/5)
	(0.32)	1.55 ± 0.75 (6/6)	1.56 ± 0.69* (6/6)	1.17 ± 0.23 (4/4)	1.05 ± 0.37 (4/4)
	(0.64)	0.92 ± 0.18** (4/4)	0.75 ± 0.11* (4/4)	0.76 ± 0.33* (4/4)	0.68 ± 0.17** (4/4)
Ro09-1390	(0.08)	1.55 ± 0.37 (6/6)	2.33 ± 0.70 (6/6)	1.32 ± 0.58 (4/4)	1.54 ± 0.98 (4/4)
	(0.16)	1.45 ± 0.09 (6/6)	1.93 ± 0.31 (6/6)	0.90 ± 0.17 (5/5)	0.80 ± 0.16 (5/5)
	(0.32)	1.27 ± 0.17* (6/6)	1.11 ± 0.19** (5/6)	0.82 ± 0.20* (5/5)	0.65 ± 0.27** (5/5)
	(0.64)	1.19 ± 0.54# (4/4)	1.26 ± 0.96# (3/4)	0.89 ± 0.10# (4/4)	0.63 ± 0.20** (3/4)

* $p < 0.1$; * $p < 0.50$; ** $p < 0.01$ versus control.
The agents were intragastrically administered to the mice using a stainless steel catheter once daily from day 0 to 13.

Statistics

All results were expressed as the mean ± SD and Student's *t*-test was applied to determine *p* values. All data were analyzed by computer using Medical Plan II computer software (Sankyo Co. Ltd, Tokyo, Japan).

Results

The antitumor activities of the agents are summarized in Tables 2 and 3. The growth of two gastric lines out of six tested digestive organ cancer lines in the present study was significantly suppressed by both pharmaceutical agents (Table 2). The growth of GC-YN was significantly suppressed by 5′-DFUR at 0.32 and 0.64 mmol/kg or by Ro09-1390 at 0.16 and 0.32 mmol kg. The growth of GC-SF was significantly inhibited by 5′-DFUR at 0.64 mmol kg or by Ro09-1390 at 0.16, 0.32 and 0.64 mmol kg. These inhibitory effects were dose-dependent and Ro09-1390 demonstrated higher antitumor activity than 5′-DFUR at equivalent doses in mmol kg, although the antitumor effects of Ro09-1390 seemed to be maximal at 0.32 mmol/kg. The estimated doses to obtain 50% inhibition of tumor growth (IC_{50}) were obtained from dose–response curves (Figure 1). The IC_{50}'s for the GC-YN tumors were 0.23 mmol/kg (60 mg/kg) for 5′-DFUR and 0.17 mmol/kg (76 mg/kg) for Ro09-1390, and those for the GC-SF tumors were 0.65 mmol/kg (155 mg/kg) for Ro09-1390 and 0.46 mmol/kg (200 mg/kg) for Ro09-1390.

The other four cancer lines (esophageal, colonic, gall bladder and bile duct) were resistant to both pharmaceutical agents (Table 3). Although 5′-DFUR at 0.64 mmol/kg significantly inhibited the growth of these four lines by day 7 after the initiation of therapy, all of the mice died within 14 days. The mortality of mice following Ro09-1390 administration was lower than following 5′-DFUR administration at equivalent doses. However, the antitumor activity of Ro09-1390 was correspondingly low against these four lines. Ro09-1390 at 0.64 mmol kg caused only marginal inhibition ($p < 0.1$) of the growth of the gall bladder line GBC-GN.

Side effects were assessed by measuring the body weight and mortality of the mice, and overall results are summarized in Table 4. The percent mortalities (on day 14) were 5% for the control (vehicle)

Table 3. Comparative antitumor activities of 5'-DFUR and Ro09-1390 in resistant human digestive organ cancer xenograft lines

Protocol (mmol/kg)		TGR (mean ± SD) (alive mice/tested mice)							
		EC-YO		CC-KK		GBC-GN		BDC-SN	
		day 7	day 14	day 7	day 14	day 7	day 14	day 7	day 14
Control (vehicle)		5.46 ± 1.30 (16/16)	12.59 ± 3.03 (14/16)	1.89 ± 0.76 (8/8)	2.55 ± 0.76 (8/8)	2.44 ± 0.80 (16/16)	4.85 ± 2.46 (16/16)	4.71 ± 1.41 (25/27)	11.17 ± 4.28 (25/27)
5'-DFUR	(0.16)[a]	4.66 ± 1.68 (7/7)	10.17 ± 3.56 (7/7)	1.35 ± 0.38 (8/8)	2.05 ± 1.05 (8/8)	2.98 ± 0.66 (11/11)	5.58 ± 1.59 (11/11)	4.16 ± 1.62 (8/10)	11.45 ± 5.17 (8/10)
	(0.32)	5.72 ± 1.58 (6/6)	13.26 ± 4.39 (6/6)	1.30 ± 0.29 (8/8)	2.18 ± 1.10 (8/8)	2.73 ± 0.83 (11/11)	3.92 ± 1.33 (11/11)	5.22 ± 1.95 (11/11)	13.83 ± 4.83 (11/11)
	(0.64)	1.29 ± 0.40*** (6/8)	(0/8)	NT[a]	NT	0.98 ± 0.34*** (5/6)	(0/6)	2.54 ± 0.68*** (12/12)	(0/12)
Ro09-1390	(0.16)	4.65 ± 0.97 (6/6)	10.68 ± 2.94 (6/6)	1.28 ± 0.28 (9/10)	2.08 ± 0.60 (9/10)	2.81 ± 0.68 (11/13)	5.62 ± 0.68 (11/13)	3.99 ± 1.12 (13/13)	9.91 ± 3.59 (13/13)
	(0.32)	5.87 ± 2.90 (4/4)	12.88 ± 5.50 (4/4)	1.70 ± 0.75 (7/7)	2.48 ± 1.15 (7/7)	3.39 ± 1.25 (11/13)	6.44 ± 2.15 (11/13)	4.23 ± 1.87 (14/14)	9.66 ± 4.30 (14/14)
	(0.64)	5.21 ± 1.72 (8/8)	10.70 ± 3.47 (6/8)	NT	NT	1.67 ± 0.69 (7/7)	2.66 ± 1.69 (5/7)	5.74 ± 1.71 (12/12)	11.73 ± 4.14 (8/12)

[a] Not tested.
*** $p < 0.001$ versus control.
The agents were intragastrically administered to the mice using a stainless steel catheter once daily from day 0 to 13.

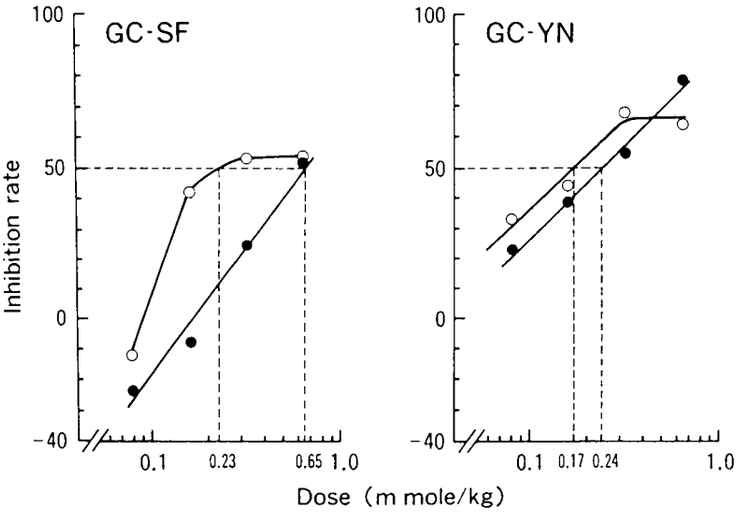

Figure 1. Dose–response curves for 5'-DFUR and Ro09-1390 in sensitive human gastric cancer xenograft lines. The relationship between dose and mean IR on day 14 is shown. The IRs were calculated as described in Materials and methods. ●, 5'-DFUR. ○, Ro09-1390.

Table 4. Body weight loss and mortality (overall)

Protocol (mmol/kg)		Body weight (g ± SD)			Percent mortality (died mice/total mice)	
		day 0	day 7	day 14	day 7	day 14
Control	(vehicle, $n = 80$)	24.6 ± 3.5	25.8 ± 3.8	26.4 ± 3.7	3% (2/80)	5% (4/80)
5'-DFUR	(0.08, $n = 12$)	21.8 ± 1.9	21.1 ± 2.1	21.7 ± 2.5	0% (0/12)	0% (0/12)
	(0.16, $n = 47$)	26.4 ± 4.1	26.7 ± 4.1	26.7 ± 4.1	9% (4/47)	9% (4/47)
	(0.32, $n = 46$)	25.1 ± 3.5	25.3 ± 4.1	25.6 ± 5.3	0% (0/46)	0% (0/46)
	(0.64, $n = 34$)	23.8 ± 3.0	19.5 ± 2.3*	19.4 ± 2.2*	9% (3/34)	77% (26/34)
Ro09-1390	(0.08, $n = 10$)	21.8 ± 1.5	20.9 ± 2.1	20.1 ± 3.0	0% (0/10)	0% (0/10)
	(0.16, $n = 51$)	24.8 ± 3.4	25.4 ± 4.1	25.7 ± 4.1	6% (3/51)	6% (3/51)
	(0.32, $n = 49$)	24.0 ± 3.2	24.4 ± 3.1	24.9 ± 3.6	4% (2/49)	6% (3/49)
	(0.64, $n = 35$)	23.5 ± 3.0	23.4 ± 3.5	24.8 ± 5.3	0% (0/35)	29% (10/35)

* $p < 0.001$ versus control.
Data are shown as cumulative results.

Table 5. 5-FU concentrations in human digestive organ cancer xenograft lines

Protocol (mmol/kg)		5-FU concentration[a] (ng/g tissue ± SD)			
		GC-YN ($n = 2$–4)	GC-SF ($n = 2$–4)	CC-KK ($n = 4$)	EC-YO ($n = 3$–4)
Control	(vehicle)	ND[b]	ND	ND	ND
5'-DFUR	(0.16)	ND	ND	8.4 ± 3.6	51.9 ± 16.1
	(0.32)	203.4 ± 64.1	ND	24.7 ± 22.0	75.3 ± 24.6
Ro09	(0.16)	ND	ND	11.2 ± 6.0	63.7 ± 19.1
	(0.32)	ND	ND	17.3 ± 10.1	70.9 ± 23.2

[a] 5-FU concentration was measured 24 h after the last administration of the agents, as described in Materials and methods.
[b] Not detected.

groups, 9% for the groups given 5′-DFUR at 0.16 mmol/kg and 6% for the groups given Ro09-1390 at either 0.16 or 0.32 mmol/kg. However, these results were caused by technical errors during intragastric administration. 5′-DFUR at 0.64 mmol/kg was associated with a notably high mortality rate (77%) on day 14 and with significant body weight loss caused by severe diarrhea. Body weight loss was not, however, noted in mice administered Ro09-1390 at 0.64 mmol/kg and the percentage mortality of these mice was significantly lower than that of mice administered 5′-DFUR at an equivalent dose.

The 5-FU concentrations in the tumors were measured 24 h after the last administration of the agents at 0.32 and 0.64 mmol/kg in four out of the six cancer lines tested, and these results are summarized in Table 5. In the two gastric lines sensitive to 5′-DFUR, 5-FU was undetectable after the administration of Ro09-1390. After the administration of 5′-DFUR, 5-FU was similarly undetectable in the 5′-DFUR-sensitive GC-SF tumors. 5-FU became detectable in the tumors of 5′-DFUR-sensitive GC-YN only after the administration of 0.32 mmol/kg of the drug. In the 5′-DFUR-resistant tumors, however, high concentrations of 5-FU were detected.

Discussion

5′-DFUR is metabolized to 5-FU by PyNPase in both neoplastic and liver tissue, as well as in the intestine.[5,6] This metabolism, causes diarrhea, which is one of the primary reasons for dose restrictions in clinical application of this drug. Ro09-1390 was developed to reduce the intestinal toxicity of 5′-DFUR. Ro09-1390 is a 5′-deoxy-5-fluorocytidine (5′-DFCR)-related compound,[9] which is converted to 5′-DFUR by cytidine deaminase, an enzyme found mainly in the liver,[12,13] then metabolized to 5-FU. 5′-DFCR is not, however, easily absorbed by the gut and Ro09-1390 has subsequently been synthesized to improve the absorption capabilities of 5′-DFCR. This prodrug has reportedly demonstrated higher antitumor activity against Lewis lung carcinomas transplanted into BDF_1 mice.[9]

In the present study, Ro09-1390 displayed almost the same antitumor spectrum as 5′-DFUR and was an effective inhibitor of the growth of human gastric cancer lines. Colorectal, esophageal, gall bladder and bile duct cancer lines, however, were resistant to both pharmaceutical agents. The IC_{50}'s for Ro09-1390, in the inhibition of 5′-DFUR sensitive lines, were lower than those for 5′-DFUR and the toxicity was similarly lower at equivalent doses.

The major cause of death in mice following 5′-DFUR administration at 0.64 mmol/kg appeared to be diarrhea followed by dehydration. The body weight loss of these mice was severe by day 7 following 5′-DFUR administration. In contrast, Ro09-1390 at 0.64 mmol/kg did not cause a significant body weight loss and the diarrhea was less severe than that caused by 5′-DFUR administered an equivalent dose. These results suggest that Ro09-1390 may work as expected.

Morphological examination has revealed that the administration of 5′-DFUR results in damage to the intestinal mucosal membrane, and causes diarrhea in normal mice, whereas Ro09-1390 is much less toxic to the intestine.[9] Furthermore, 5-FU concentrations in the intestine are reported to be much higher in mice administered 5′-DFUR than in those administered Ro09-1390 at equivalent doses, while 5-FU concentrations in tumor tissue are virtually the same.[9] These observations suggest that Ro09-1390 can be administered at higher doses than 5′-DFUR, which may result in more effectual chemotherapy.

5′-DFUR and Ro09-1390 are the prodrugs of 5-FU, and their antitumor effects are dependent upon the actions of this metabolite. Accordingly, the 5-FU concentration in tumor tissue is thought to correlate with the efficacy of these prodrugs. As such, 5-FU concentrations in various tumors were evaluated in the present study. Curiously, 5-FU concentrations in 5′-DFUR-sensitive tumors were lower than in resistant tumors following the administration of 5′-DFUR or Ro09-1390. The reasons for this controversial observation remain unclear. One possible explanation is the rapid metabolism of 5-FU into its active form, 5-fluorodeoxyuridine monophosphate (FdUMP) in the 5′-DFUR sensitive cells. The rate of metabolism to FdUMP may, for example, be lower in the resistant tumor lines than in the sensitive tumor lines.

Regardless, the present study indicates that Ro09-1390 may be more efficacious in the treatment of human gastric cancer than 5′-DFUR and may be clinically applied.

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