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Antitumor effect and tumor level of 5-fluoro-2′-deoxyuridylate following oral administration of tetradecyl 2′-deoxy-5-fluoro-5′-uridylate

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The antitumor effect and tumor levels of 5-fluoro-2′-deoxyuridylate (FdUMP) following oral administration of tetradecyl 2′-deoxy-5-fluoro-5′-uridylate (TT-62) were compared with those attained following intravenous (i.v.) or intraperitoneal (i.p.) administration of 5-fluorouracil (5-FU) or 5-fluoro-2′-deoxyuridine (FUdR) in BDF_1 mice bearing murine mammary adenocarcinoma 755 and athymic mice bearing the transplantable human colon adenocarcinoma LS174T. Oral administration of TT-62 showed a stronger antitumor effect against adenocarcinoma 755 than FUdR. The maximum effect of TT-62 was similar to that of 5-FU. However, TT-62 and FUdR treatments were more effective than i.v. administration of 5-FU against LS174T. Thus, oral administration of TT-62 showed marked antitumor activity in both tumor systems. The maximum tolerated dose of FUdR resulted in a much higher level of free FdUMP in the LS174T tumor than that obtained with 5-FU. After oral administration of TT-62 the levels of FdUMP in the tumor were about 10 times those attained with 5-FU, but significantly lower than the levels obtained following i.v. administration of FUdR. With TT-62 the levels of FdUMP in the tumor reached their peak at 60 min following the administration and gradually decreased thereafter. However, FdUMP levels after administration of FUdR decreased rapidly. Three hours after the administration of TT-62 and for up to 24 h the FdUMP levels in the LS174T tumor were almost the same as after administration of FUdR, i.e. effective levels of FdUMP were maintained for a long time with TT-62.

Key words: Colon adenocarcinoma LS174T, FdUMP derivative, TT-62.

Introduction

5-Fluorouracil (5-FU) is still the drug of choice for chemotherapy of gastrointestinal cancers in spite of the agent's limited effectiveness: the reported response rates are between 10 and 20%.[1] In recent years, 5-FU used in combination with leucovorin has proven more effective than 5-FU alone.[2–4] Since 5-FU is transformed by cells into 5-fluoro-2′-deoxyuridylate (FdUMP), a powerful irreversible inhibitor of thymidylate synthase,[5,6] the higher the level of FdUMP in the tumor, the stronger the antitumor effect that can be attained. The FdUMP levels in the tumor following intravenous (i.v.) administration of 5-fluoro-2′-deoxyuridine (FUdR) is markedly high compared with i.v. administration of 5-FU,[7] but FUdR is too toxic to the host.[8] FUdR derivatives with strong antitumor activity and reduced toxicity should be developed; therefore, we investigated new FUdR derivatives using murine mammary adenocarcinoma 755 and human colon adenocarcinoma LS174T. We found that tetradecyl 2′-deoxy-5-fluoro-5′-uridylate (TT-62) attained a high level of FdUMP in the tumor, and showed marked antitumor activity by oral administration compared with 5-FU and FUdR by i.v. administration.

Materials and methods

Drugs

5-FU and FUdR were obtained from Sigma (St Louis, MO, USA). TT-62 was kindly provided by Teijin Ltd (Tokyo, Japan) (Figure 1).

Animals

Groups of six male athymic (*nu/nu*) mice (BALB/c) weighing 18–20 g (CLEA Japan, Tokyo) and six male BDF_1 mice weighing 20–23 g (Japan SLC, Hamamatsu, Japan) were housed in plastic cages with sterilized woodchip bedding. Athymic

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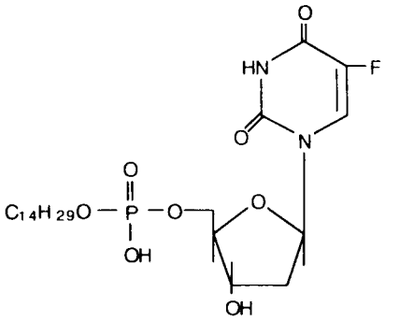

Figure 1. Structure of TT-62.

mice received CMF pellets (Oriental Yeast Co. Ltd, Tokyo, Japan) and BDF_1 mice received CA-1 pellets (CLEA Japan, Inc., Tokyo, Japan). The mice were allowed to drink sterilized water *ad libitum*. All the experiments were performed in an animal laboratory at a controlled temperature (25°C).

Treatments and antitumor activity

Murine mammary adenocarcinoma 755 (5×10^5 cells/BDF_1 mouse) or human colon adenocarcinoma LS174T tumor (20 mg/athymic mouse) were implanted subcutaneously (s.c.) on day 0 into the right thigh. Twenty-four hours after implantation of adenocarcinoma 755, the mice were treated i.p. or p.o. with 5-FU, FUdR or TT-62. Treatment was continued for five consecutive days. Tumors from treated and untreated mice were weighed on day 11 and the ratio of average tumor weight in the treated group to that of the control group (T/C %) was determined. For the LS174T tumors, as soon as the tumors were palpable (about 3–5 mm at day 10–14), the mice were randomized before being allocated to different groups of six animals each. 5-FU, FUdR and TT-62 were administered three times a week at doses of 20 (i.v.), 100 (i.v.) and 20 (p.o.) mg/kg, respectively. The growth of the implanted tumor LS174T was monitored by measuring the perpendicular diameters with calipers, and the tumor volume (mm^3) was calculated by the formula: (1/2) × (major diameter in mm) × (minor diameter in $mm)^2$.

Measurement of intratumor FdUMP pools following 5-FU, FUdR and TT-62 treatment

A single dose of 5-FU (20 mg/kg, i.v.), FUdR (100 mg/kg, i.v.) or TT-62 (20 and 40 mg/kg, p.o.) was administered to mice bearing a 2-week-old LS174T tumor. The mice were sacrificed 30 min, 1, 3, 6, 15 and 24 h later (more than three mice at each determination point); then the tumors were removed and frozen as quickly as possible in dry-ice acetone. Free FdUMP in the tumor was determined as reported by Moran *et al.*[9]

Results and discussion

Oral administration of TT-62 showed a marked antitumor effect on adenocarcinoma 755 (Table 1). Maximum effects of TT-62 were comparable to those of 5-FU. FUdR showed weak antitumor effect on this tumor. However, the antitumor activity of FUdR against tumor LS174T was significantly high as compared with the control, whereas 5-FU showed almost no antitumor activity. TT-62 at a dose of 20 mg/kg p.o. was as active as FUdR (100 mg/kg, i.v.) (Figure 2). There were no significant differences between the antitumor activities at 20 and 40 mg/kg of TT-62.

In the LS174T tumor, the peak levels of free FdUMP following a single oral administration of TT-62 at doses of 20 and 40 mg/kg were 3300 ± 640 pmol/g (mean ± SE) ($n = 3$) and 5380 ± 1480 pmol/g ($n = 9$), respectively. These peak levels were observed 60 min after administration and they gradually decreased thereafter (Figure 3). These peak levels were higher than those

Table 1. Inhibition of growth of adenocarcinoma 755 by 5-FU derivatives

	Dose (mg/kg/day)	Route	T/C % (mean ± SE)	No. of experiments
5-FU	10	i.p.	82 ± 9	5
	20	i.p.	43 ± 11	5
	30	i.p.	11 ± 2	4
	50	i.p.	toxic (7/12)	2
	25	p.o.	57 ± 6	2
	50	p.o.	toxic (6/18)	3
FUdR	100	i.p.	34 ± 1	2
	200	i.p.	toxic (7/12)	2
	50	p.o.	83 ± 11	2
	100	p.o.	32 ± 3	2
	200	p.o.	toxic (2/6)	1
TT-62	12.5	p.o.	54 ± 7	2
	25	p.o.	48 ± 9	4
	50	p.o.	15 ± 3	4
	100	p.o.	toxic (4/6)	1

The number in parentheses is the ratio of dead mice to total of mice.

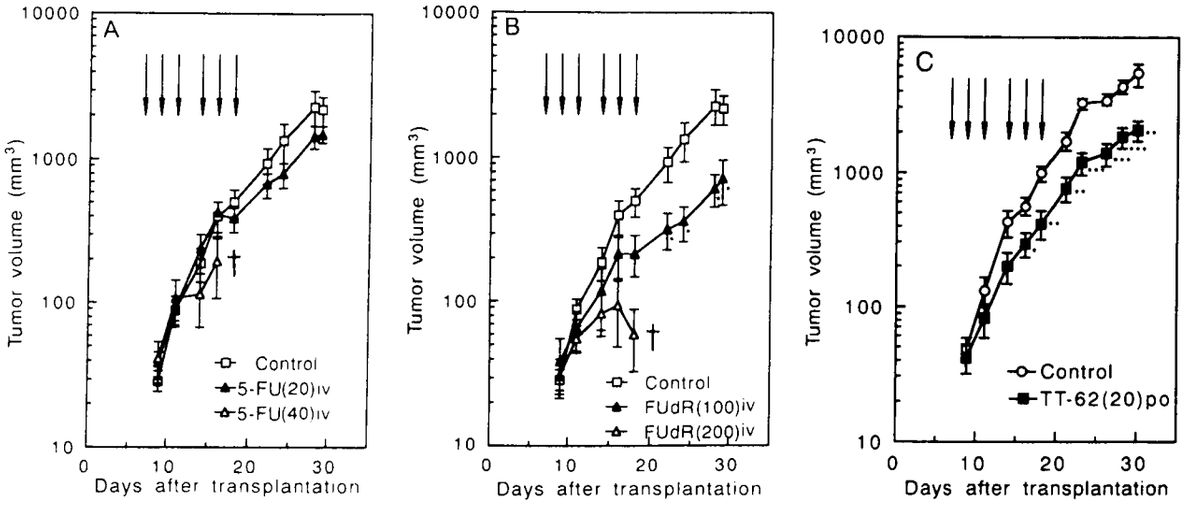

Figure 2. Effects of 5-FU (A), FUdR (B) and TT-62 (C) on the growth of LS174T tumor. Each point represents the mean ± SE ($n = 6$). The arrows mean administration of drugs. $^{*}p \leq 0.05$ compared with control; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$. † Toxic death. The number in parentheses is the dose in mg/kg.

attained with 5-FU, where the peak level was 350 ± 40 pmol/g ($n = 6$), 30 min after a single i.v. administration. The maximum level of free FdUMP in the tumor of animals administered FUdR (i.v.) was much higher (31 600 ± 19 200 pmol/g) than that attained with 5-FU or TT-62. In this case the level of FdUMP decreased rapidly up to 3 h after the administration and gradually thereafter. The levels of FdUMP between 3 and 24 h after administration of TT-62 (20 or 40 mg/kg, p.o.) did not differ from those observed with FUdR (100 mg/kg, i.v.).

FUdR is reported to induce periportal fibrosis of bile ducts and biliary sclerosis[8,10] and chemical hepatitis is frequently observed among patients undergoing treatment with FUdR.[10] It may be caused by marked high levels of FUdR and its metabolites distributed in the liver. However, TT-62 (20 mg/kg, p.o.) showed high levels of FdUMP in the tumor and an antitumor effect similar to that attained with FUdR (100 mg/kg, i.v.). These results suggest that oral administration of TT-62 may present therapeutic advantages over 5-FU and FUdR. This should undergo further evaluation regarding any treatment schedule as well as when used in combination with leucovorin.

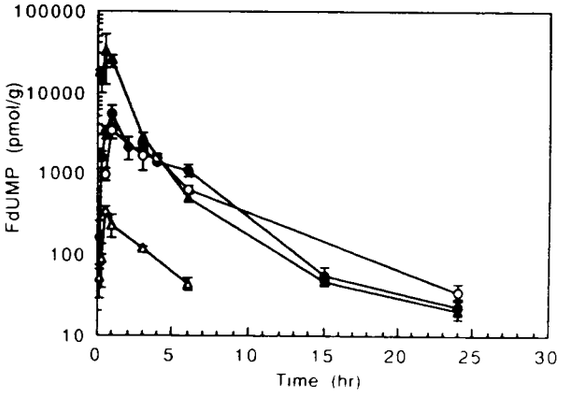

Figure 3. Free FdUMP levels in LS174T tumor following administration of 5-FU (20 mg/kg, i.v., △), FUdR (100 mg/kg, i.v., ▲), and TT-62 at 20 (○) and 40 mg/kg, p.o. (●). Each point represents mean ± SE ($n = 3$–9).

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