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ORIGINAL ARTICLE

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Comparative study between daily and 5-days-a-week administration of oral 5-fluorouracil chemotherapy in mice: determining the superior regimen

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Abstract *Background*: Oral administration of derivatives of 5-fluorouracil (5-FU) is currently used to treat colorectal cancer in the United States. Oral chemotherapy possesses certain advantages: it is simple, easy to administer, and has few side effects. We compared conventional daily oral administration of 5-FU (daily schedule) with administration on 5 consecutive days followed by 2 drug-free days (5-days-a-week schedule) in a mouse tumor model. *Methods*: The maximal tolerated dose (MTD) in the 5-days-a-week schedule and in the daily schedule were determined in 6-week-old non-tumor-bearing CDF1 male mice. In antitumor experiments, CDF1 mice were inoculated subcutaneously with Colon26 cells (1×10^6 per mouse). Antitumor efficacy was evaluated in terms of the ratio of tumor size in treated to control mice (T/C ratio). *Results*: The MTD of 5-FU in the 5-days-a-week schedule was 42 mg/kg, and in the daily schedule was 29 mg/kg. In the 5-days-a-week schedule dose escalation nearly 1.4 times that in the daily schedule was possible, although the total dose over 7 days was similar between the two schedules (203 mg/kg and 210 mg/kg, respectively). When the doses of 5-FU were compared under the condition of no body weight loss, the 5-days-a-week schedule produced a comparative dose escalation of 2.1 times per day (from 20 to 42 mg/kg), and 1.5 times per total weekly amount (from 140 to 210 mg/kg) compared to the daily schedule. With regard to the antitumor effect as indicated by the T/C ratio, the 5-days-a-week schedule produced over 70% tumor suppression, whereas the daily schedule produced only 50% suppression at the MTD. Therapeutic efficacy was calculated in terms of the ratio of body weight change to antitumor effect (T/C ratio), and revealed that the MTD of 42 mg/kg 5-FU in the 5-days-a-week schedule produced a therapeutic efficacy almost three times that of the MTD of 29 mg/kg 5-FU in the daily schedule ($P < 0.001$). *Conclusions*: Using oral administration of 5-FU, we confirmed that the 5-days-a-week schedule allowed dose intensity escalation and was superior to the daily schedule in both enhancement of antitumor effect and protection against adverse effects.

Keywords 5-Days-a-week administration · Oral 5-fluorouracil chemotherapy · Mouse

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Introduction

Oral administration of 5-fluorouracil (5-FU) and its derivatives as adjuvant chemotherapy after curative surgery for gastrointestinal cancer is generally accepted in Japan and is usually administered daily [1, 2, 3]. On the other hand, in Western countries, oral administration of 5-FU has not yet been approved [4], and the regimen of 5 days i.v. administration of 5-FU followed by 2 days free is the standard, perhaps because of the difficulty of injection over the weekend [5]. Oral administration of the drug has several benefits: it is simple to administer, it is less toxic, and it shortens the hospital stay or reduces the time of a hospital visit. In the present study, in addition to i.v. administration of 5-FU, the significance of the free 2 days with oral administration of 5-FU was examined in experimental mice, and the superiority of the 5-days-a-week schedule over daily administration was shown in terms of the balance of antitumor effect and adverse effects such as body weight loss.

Animals and methods

Experiment 1

The maximal tolerated doses (MTD) of oral 5-FU (Kyowa Hakko Co. Tokyo, Japan) in the 5-days-a-week schedule and in the daily

schedule were determined. A total of 70 6-week-old non-tumor-bearing CDF1 male mice (average weight 25 ± 1 g) purchased from Japan SLC Company (Shizuoka, Japan) were maintained at five animals per cage under conventional conditions in an air-conditioned room at a constant 21ºC and under a 12-h light cycle. Standard laboratory chow and tap-water were available ad libitum. Animal handling procedures were approved by the Animal Care and Use Committee of Tottori University.

The mice were divided into 5-days-a-week groups (60, 50, 42, 35, 29, and 24 mg/kg per day, and control; five mice in each group) and daily groups (35, 29, 24, 20, 17, and 14 mg/kg per day, and control; five mice in each group). The initial dose was set at 20 mg/kg per day for daily administration based on the previous findings that the a LD_{10} of mice receiving daily injection of 5-FU over 16 consecutive days is 20 mg/kg [6]. The dose of 5-FU was changed by 20%, using 20 mg/kg as the standard. For the 5-days-a-week groups, the standard dose was calculated as 35 mg/kg per day (1.75 times the 20 mg/kg daily dose LD_{10}), and the dose was increased or decreased by 20%. Administration of 5-FU was continued for 4 weeks and the mice were followed-up for another 4 weeks. Observation was performed daily and body weights were recorded on days 0, 2, and 4 of every week.

Experiment 2

A group of 80 CDF1 mice inoculated subcutaneously with Colon26 cells [7] (1×10^6 per mouse) were used for the antitumor experiments. Doses of 5-FU were as follows: 17, 20, 24 and 29 mg/kg, and control, for the daily schedule and 24, 29, 35 and 42 mg/kg, and control, for the 5-days-a-week schedule, according to the results of experiment 1 (five mice in each group). 5-FU dissolved in 0.2 ml distilled water was administered orally 9 days after inoculation for 4 weeks using a metallic cannula daily until the end of the experiment in the daily groups. Only water was administered to the control animals. Tumor size was measured twice a week and tumor volume was calculated according to the following formula: tumor volume (cm^3) = $ab^2/2$ (where a and b are perpendicular diameters measured in centimeters). The ratio of treated to control tumor sizes (T/C ratio, %) was used for the evaluation of antitumor efficacy.

Statistical analysis

Analysis of variance was used for statistical analysis to compare the results from the different groups. *P*-values less than 0.05 were considered statistically significant.

Results

Experiment 1

In the daily schedule, four of five mice treated at 35 mg/kg died, but no deaths were observed in mice treated at 29 mg/kg or less. On the other hand, all five mice treated at 60 mg/kg died, and four of the five mice treated at 50 mg/kg died, but there were no deaths among those treated at 42 mg/kg in the 5-days-a-week schedule. Therefore, the MTD of 5-FU in the 5-days-a-week schedule was 42 mg/kg, and in the daily schedule was 29 mg/kg. These results are summarized in Table 1. In the 5-days-a-week schedule, including 2 days free, dose escalation nearly 1.4 times that in the daily schedule was possible, although the total dose per 7 days was similar between the two schedules (203 mg/kg and 210 mg/kg, respectively).

Table 1 MTD of each schedule of oral 5-FU administration and mortality in mice

Schedule	Dose		Mortality
	mg/kg	mg/kg/week	
Daily	35	245	4/5[a]
	29	203	0/5
	24	168	0/5
	20	140	0/5
	17	119	0/5
	14	98	0/5
5-days-a-week	60	300	5/5[b]
	50	250	3/5[c]
	42	210	0/5
	35	175	0/5
	29	145	0/5
	24	120	0/5

[a]Died on days 20, 24, 24, and 28
[b]Died on days 13, 14, 14, 16, and 18
[c]Died on days 17, 23, and 24

Body weight changes

The daily schedule groups showed a dose-dependent body weight loss at doses from 24 to 35 mg/kg. Considering the persistent body weight loss during the administration period as an adverse effect, the dose of 20 mg/kg oral 5-FU may be the MTD for daily administration when administration is prolonged for more than 4 weeks (Fig. 1). However, there was no body weight loss in mice treated in the 5-days-a-week schedule at 42 mg/kg or less (Fig. 2). When the doses of 5-FU were compared under the condition of no body weight loss, the 5-days-a-week schedule allowed increments in single doses of about twice (from 20 to 42 mg/kg) and in weekly doses of 1.5 times (from 140 to 210 mg/kg) compared to the daily schedule.

Tumor study

In the daily schedule groups, a greater antitumor effect was observed to accompany dose escalation. The T/C ratios revealed that regrowth occurred at 2 weeks in the 17 mg/kg group and at 3 weeks in the 20 and 24 mg/kg groups, although the 29 mg/kg (MTD) group showed over 50% tumor suppression during the experimental period (Fig. 3).

In the 5-days-a-week groups, a dose-dependent antitumor effect was also seen. The T/C ratio revealed over 70% tumor suppression in the 42 mg/kg (MTD) group and regrowth was not seen at any dose (Fig. 4). To clearly compare the T/C ratio between the two administration schedules, the ratios of the groups at the MTD and the next dose below the MTD are shown in Fig. 5. The 5-days-a-week schedule produced 15–20% more tumor suppression than did the daily schedule comparing the MTD groups. Nevertheless, daily administration of 24 mg/kg (168 mg/week) and 5-days-a-week administration of 35 mg/kg (175 mg/week) were similar in total

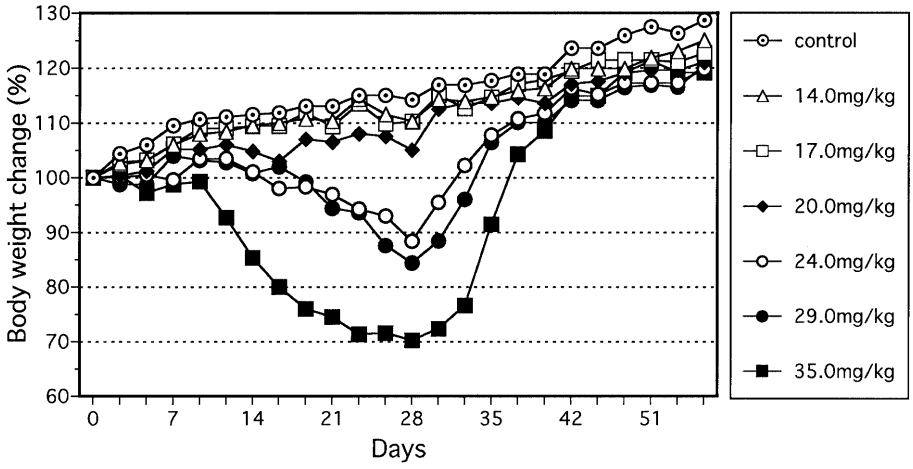

Fig. 1 Body weight changes in the daily oral 5-FU administration groups at various dosages. Dosages over 24 mg/kg had resulted in persistent weight loss by the end of administration (day 28). Four of five mice died on days 20, 24, 24, and 28 at the 35 mg/kg dose

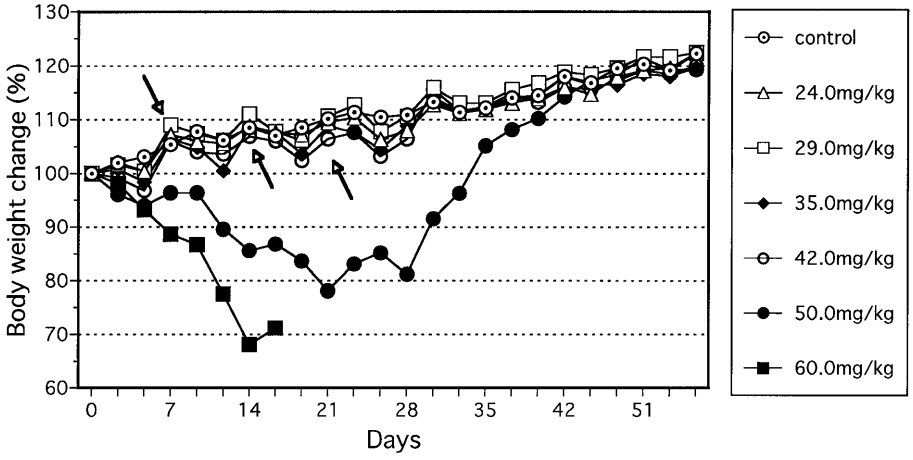

Fig. 2 Body weight changes in the 5-days-a-week oral 5-FU administration groups at various dosages. Dosages over 50 mg/kg 5-FU resulted in persistent weight loss. All mice died in the 60 mg/kg dose group, and four of five mice died in the 50 mg/kg dose group. Weight gain was observed during the two drug-free days following 5-FU (*arrows*)

weekly dose, and the antitumor effect of the former, as judged by the T/C ratio, was equal to daily administration of 29 mg/kg (203 mg/week). Although the total weekly doses were equal, the 5-days-a-week schedule was superior to the daily schedule in antitumor effect.

Therapeutic efficacy

To estimate the therapeutic benefit, we used the ratio of body weight change to antitumor effect (T/C ratio) as an indicator of therapeutic efficacy (Fig. 6). For example, for a 20% weight loss and a 10% tumor reduction (T/C ratio 90%), the therapeutic efficacy was calculated as $0.8/0.9 = 1.08$. A dose of 42 mg/kg 5-FU (MTD) in the 5-days-a-week schedule produced significantly higher therapeutic efficacy than 35 mg/kg in the 5-days-a-week schedule and higher than 29 mg/kg and 24 mg/kg in the daily schedule ($P < 0.001$, respectively).

Discussion

In Japan, oral 5-FU or 5-FU derivatives are frequently used for postoperative adjuvant chemotherapy, and the dose of 5-FU is typically 150 to 200 mg/body two or three times daily. However, it is as yet unclear whether daily oral administration of 5-FU is optimal in terms of antitumor effect, toxicity and adverse effects. In the present study, 5 consecutive days' administration followed by 2 free days (5-days-a-week schedule) was compared with a daily schedule. In the dose escalation study, the 5-days-a-week schedule produced a comparative dose escalation of 2.1 times per day, and 1.5 times per total weekly amount. This dose escalation might have been achieved as a result of recovery during the two drug-free days and an increase in dietary intake as judged from the curve of body weight change.

Watanabe [8] investigated the adverse effects on the gastrointestinal mucosa of rats comparing daily administration of 5-FU 40 mg/kg (total 280 mg/kg) and intermittent administration of 5-FU (35 mg/kg, given for 4 days, then not given for 3 days; total 280 mg/kg). Gastric mucosal erosion and intestinal mucosal damage, including shortening of villi, destruction of the glands, and diarrhea occurred in 30% of the rats receiving daily 5-FU, whereas these results were hardly found in the rats receiving intermittent 5-FU. Sadahiro et al. [9] also noted recovery of body weight during drug-free days using oral UFT (tegafur and uracil) as an anticancer drug.

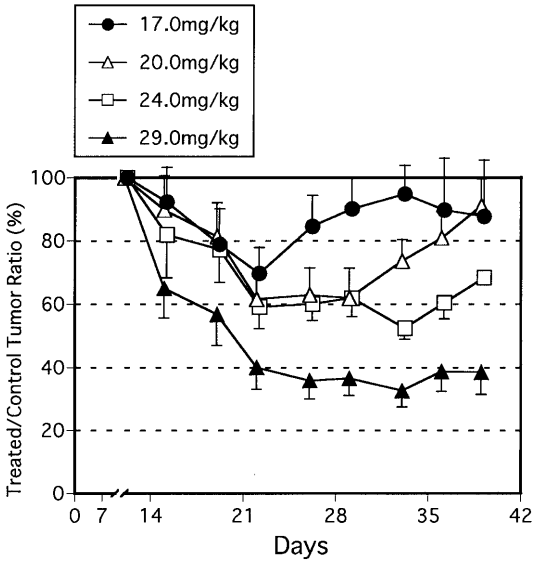

Fig. 3 Antitumor effects in terms of T/C ratios in the daily oral 5-FU administration groups. 5-FU was administered for 4 weeks from day 9 after tumor inoculation

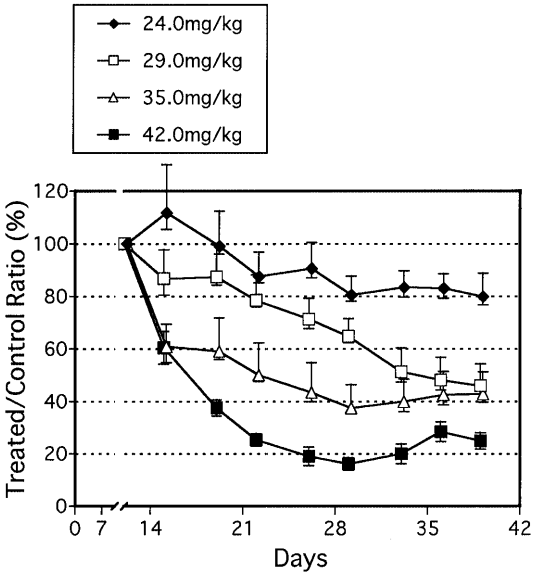

Fig. 4 Antitumor effect in terms of T/C ratios in the 5-days-a-week oral 5-FU administration groups. 5-FU was administered for 4 weeks from day 9 after tumor inoculation

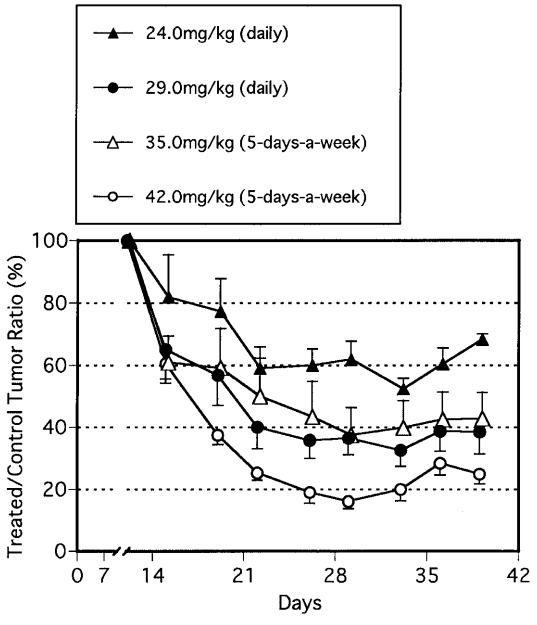

Fig. 5 Comparison of antitumor effects in terms of T/C ratios among the daily and the 5-days-a-week oral 5-FU administration groups. 5-FU was administered for 4 weeks from day 9 after tumor inoculation

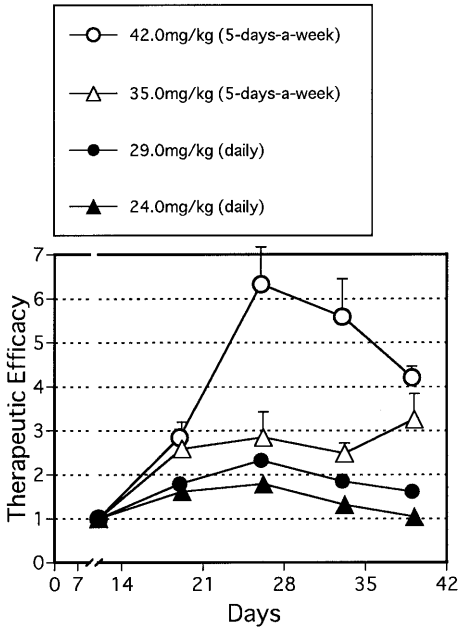

Fig. 6 Comparison of therapeutic efficacy (ratio of body weight change to antitumor effect) among the daily and the 5-days-a-week oral 5-FU administration groups

With regard to the influence of the schedule of 5-FU on its antitumor effect, Bateman et al. [10] have demonstrated that once weekly oral administration of 5-FU is as effective in the treatment of disseminated gastrointestinal cancer as weekly intravenous administration. In the intravenous administration of 5-FU, protracted administration has been recognized to be valuable since Lokich et al. [11] reported the superiority of 10 weeks continuous infusion compared to administration once a day for 5 consecutive days in the treatment of colorectal cancer, based on the findings of a similar antitumor effect and fewer adverse effects of the former. With oral administration of 5-FU in this study, the 5-days-a-week schedule mimicking the intermittent schedule in the study of Lokich et al. was found to be superior to the daily schedule. This may be explained by the fact that

the escalated dose of 5-FU in the 5-days-a-week schedule resulted in a higher 5-FU concentration in the tumor compared with daily administration, although it has been reported that 5-FU administered orally dose not reach an effective concentration in the blood and tumor so readily as 5-FU administered i.v. [12].

Considering the effective 5-FU concentration in the blood, it is understandable that the 5-days-a-week schedule, the total weekly dosage of which was over twice that following daily administration, achieved a higher drug concentration and resulted in a better antitumor effect. In this experiment, 35 mg/kg 5-FU in the 5-days-a-week schedule produced an antitumor effect equivalent to 29 mg/kg 5-FU in the daily schedule, even though the total amount of 5-FU was less in the former schedule (35 mg/kg 5-FU 5 days a week, 175 mg/kg per week; 29 mg/kg 5-FU daily, 203 mg/kg per week). Another possible explanation for the superior antitumor effect of the 5-days-a-week schedule is that the lower weight loss compared to that found with the daily schedule could have resulted in a less-damaging effect on host immunity, although we have no data in support of this. We used the ratio of body weight change to antitumor effect as an indicator of therapeutic efficacy. An extremely high therapeutic efficacy ratio (2.8–6.3, i.e. overall benefit) was obtained at 42 mg/kg 5-FU in the 5-days-a-week schedule from 2 weeks after the start of treatment, and these values are more than twice those obtained at the MTD of 5-FU in the daily schedule (29 mg/kg).

It is difficult to determine the best schedule of oral administration of 5-FU, Although our findings confirmed that in the 5-days-a-week schedule the dose intensity was escalated and this schedule was superior to the daily schedule in both higher antitumor effect and lower adverse effects. In the clinical setting, we have already introduced a 5-days-a-week schedule of oral 5-FU without any trouble and with better compliance (data not shown), but there is no definite evidence of superiority in antitumor effect. Furthermore, 2 days rest from oral 5-FU may be valuable and sometimes inevitable for combinations with another anticancer drug that induces nausea and vomiting such as cisplatin or irinotecan.

New oral anticancer drugs, such as 1 *M* Tegafur/0.4 *M* 5-chloro-2,4-dihydroxypyrimidine/1 *M* potassium oxonate (S-1) [13] and capecitabin (N4-pentyloxycarbonyl-5′-deoxy-5-fluorcytidine) [14], are now available. Our results could provide information useful for further investigation of the optimal schedule of administration of these and similar drugs.

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