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# Antitumor activity and low intestinal toxicity of S-1, a new formulation of oral tegafur, in experimental tumor models in rats

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**Abstract** S-1, a new oral antitumor agent, is composed of 1-(2-tetrahydrofuryl)-5-fluorouracil (Tegafur, FT), 5-chloro-2,4-dihydroxypyridine (CDHP) and potassium oxonate (Oxo) in a molar ratio of 1 : 0.4 : 1. FT which is a masked compound of 5-fluorouracil (5-FU) acts as an effector, while both CDHP and Oxo which do not have antitumor activity themselves act as modulators. In this study, the antitumor activity and intestinal toxicity of S-1 were investigated using experimental tumor models in rats, and compared with those of other oral fluoropyrimidines, namely 5-FU, FT, FCD (1 *M* FT/0.4 *M* CDHP) and UFT (combination of FT and uracil). In rats bearing subcutaneous Yoshida sarcoma, S-1 inhibited tumor growth at the lowest dose ($ED_{50}$ value: S-1 5, UFT 22, FT 82, FCD 5, and 5-FU 19 mg/kg per day), and induced the least host body weight suppression, leading to the highest therapeutic index (TI) (S-1 4.5, UFT 1.4, FT 1.8, FCD 2.0, and 5-FU 1.4). S-1 also showed a higher therapeutic effect than UFT against AH-130 and Sato lung carcinoma. After administration of S-1 and UFT at equitoxic doses, S-1 showed a higher and more prolonged concentration of 5-FU than UFT both in plasma ($AUC_{0-\infty}$: S-1 28 nmol h/ml, UFT 15 nmol · h/ml) and in tumor tissue ($AUC_{0-\infty}$: S-1 95 nmol h/g tissue, UFT 52 nmol h/g tissue), leading to a higher 5-FU level incorporated into the RNA fraction (F-RNA level) in tumor tissue ($AUC_{0-24}$: S-1 7.0 nmol h/mg RNA, UFT 4.3 nmol h/mg RNA) and 5–8% higher thymidylate synthase (TS) inhibition in tumor tissue at every time-point through 24 h. Compared with other oral fluoropyrimidines after administration of the maximal tolerable dose (MTD), S-1 caused the lowest rates of intestinal toxicities, such as diarrhea and occult blood in feces. S-1 also showed a higher antitumor effect on Yoshida sarcoma implanted intracolonically than UFT at an equitoxic dose (tumor weight: S-1 $64 \pm 30$ mg, UFT $133 \pm 52$ mg; $P < 0.05$). These results suggest that CDHP, which is a potent inhibitor of 5-FU degradation, increases the antitumor activity of FT, and that Oxo, which is an inhibitor of 5-FU phosphorylation, locally protects the gastrointestinal tract from 5-FU-induced toxicity without decreasing the antitumor activity.

**Key words** S-1 · Biochemical modulation · Rat · Metabolism · Intestinal toxicity

**Abbreviations** *5-FU* 5-fluorouracil · *BWC* body weight change · $BWC_{50}$ dose for 50% BWC · *CDHP* 5-chloro-2,4-dihydroxypyridine · *CR* complete response · *CVI* continuous venous infusion · *DLT* dose-limiting toxicity · *DPD* dihydropyrimidine dehydrogenase · $ED_{50}$ dose for 50% tumor growth inhibition · *F-RNA* 5-FU incorporated into RNA fraction · *FCD* 1 *M* tegafur/0.4 *M* CDHP · *FdUMP* 5-fluorodeoxyuridine 5′-monophosphate · *FT* tegafur · *FUMP* 5-fluorouridine 5′-monophosphate · *HPMC* hydroxypropylmethylcellulose · *GI* gastrointestinal · *i.v.* intravenous · *MTD* maximal tolerable dose · *Oxo* potassium oxonate · *S-1* 1 *M* tegafur/0.4 *M* 5-chloro-2,4-dihydroxypyridine/1 *M* potassium oxonate · *s.c.* subcutaneous · *SLC* Sato lung carcinoma · *TGI* tumor growth inhibition · *TI* therapeutic index · *TS* thymidylate synthase · *TSIR* thymidylate synthase inhibition rate

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## Introduction

5-Fluorouracil (5-FU), first synthesized in 1957 [6], is one of the most important antineoplastic agents and is widely used in clinical practice for the treatment of solid tumors [11, 12, 26]. In an effort to improve therapeutic efficacy, many methods of administration have been studied clinically. Recently, continuous venous infusion (CVI) has been used for the effective administration of 5-FU to patients with gastric, colorectal and breast cancers [1, 3, 4, 10, 13, 24, 28, 37]. Lokich et al. [18] have also reported that long-term CVI of 5-FU results in a higher response rate than intravenous (i.v.) bolus administration as adjuvant chemotherapy of metastatic colorectal cancers. In this method of treatment, the dose-limiting toxicity (DLT) is not myelosuppression but gastrointestinal (GI) toxicity.

A combination of tegafur (FT), a masked form of 5-FU [33], and uracil at a molar ratio of 1:4 (UFT) is used as an oral antitumor agent. Since FT is gradually converted to 5-FU in the liver and uracil competitively inhibits 5-FU degradation by dihydropyrimidine dehydrogenase (DPD; EC1.3.1.2) [14], leading to the prolonged retention of 5-FU in the blood [16], UFT is experimentally more effective than FT and 5-FU [7, 8]. In Japan, UFT has been widely used for adjuvant chemotherapy after surgery [2, 17, 20, 22, 36].

More recently, we have developed S-1 as a new and superior antitumor drug compared to UFT in terms of biochemical modulation. S-1 consists of FT, 5-chloro-2,4-dihydroxypyridine (CDHP) and potassium oxonate (Oxo) (Fig. 1). As shown in Fig. 2, FT acts as an effector, while both CDHP and Oxo, which do not have antitumor activity themselves, act as modulators. CDHP inhibits 5-FU degradation approximately 180 times more effectively than uracil in vitro [32]. Oxo competitively inhibits the phosphorylation of 5-FU to 5-fluorouridine-5′-monophosphate (FUMP) by pyrimidine phosphoribosyltransferase (EC2.4.2.10) both in vitro and in vivo [30]. Oxo is mainly distributed in the GI tract after oral administration to rats, leading to a decrease in GI toxicity due to 5-FU [30]. Using Yoshida sarcoma-bearing rats, Uchida et al. [35] showed that combined treatment with FT, CDHP and Oxo at a molar ratio of 1:0.4:1 produces optical therapeutic effects based on a comparison of the balance between antitumor effect and toxicity induced by various combination ratios of these three compounds.

In the present study, the antitumor effect and the intestinal toxicity of S-1 were compared with those of other oral fluoropyrimidines namely 5-FU, FT, 1 *M* FT/0.4 *M* CDHP (FCD) and UFT using experimental tumor models in rats. In addition, the 5-FU levels in plasma and tumor tissue, the 5-FU levels incorporated into the RNA fraction (F-RNA levels) in the tumors and the thymidylate synthase (TS) inhibition rate (TSIR) in the tumors were investigated to clarify the mode of action of CDHP and Oxo.

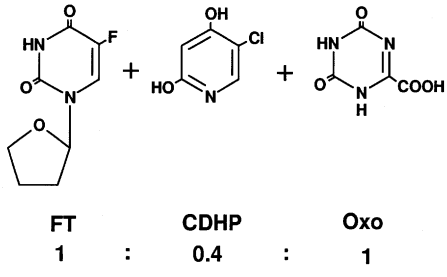


**Fig. 1** Chemical structure of the components of S-1

## Materials and methods

### Preparation of drugs

FT, CDHP and Oxo were synthesized in our laboratory. Uracil was purchased from Yamasa Co., Chiba, Japan; 5-FU was purchased from Wako Pure Chemical Industries, Osaka, Japan; [6-$^{14}$C]FT (9.9 MBq/mmol) was obtained from Amersham International, Buckinghamshire, England; and [6-$^{3}$H]5-fluorodeoxyuridine 5′-monophosphate (FdUMP) (551 GBq/mmol) was synthesized enzymatically from [6-$^{3}$H]5-fluorodeoxyuridine (New England Nuclear, Boston, MA). All other chemicals used were the highest standard grade commercially available. S-1 was prepared by mixing FT, CDHP and Oxo at a molar ratio of 1:0.4:1. FCD was prepared by mixing FT and CDHP at a molar ratio of 1:0.4. UFT was prepared by mixing FT and uracil at a molar ratio of 1:4. S-1, FT and 5-FU were dissolved in 0.5% (w/v) hydroxypropylmethylcellulose (HPMC) solution. UFT was suspended in 0.5% HPMC

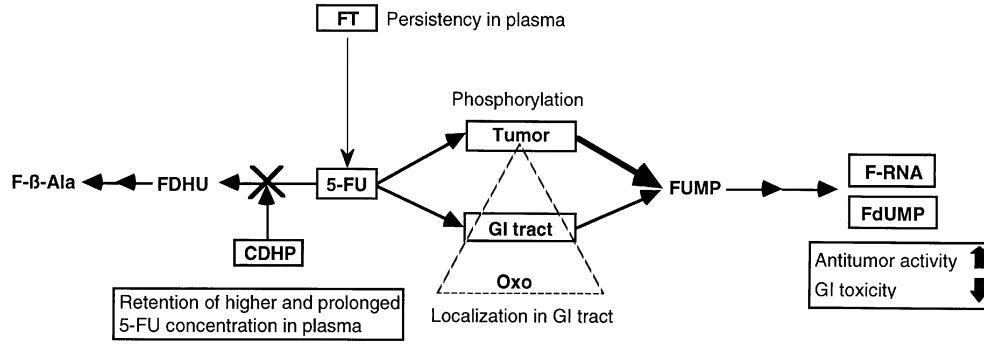


**Fig. 2** Summary of the mode of action of S-1

solution because of the insolubility or uracil. Since the active component in both S-1 and UFT is FT, only the amount of FT is used to describe the dosages of these two drugs.

### Animals and tumors

Male Donryu rats were purchased from Japan SLC, Hamamatsu. Ascites tumor cells of Yoshida sarcoma, AH-130 (rat ascitic hepatoma) and Sato lung carcinoma (SLC) were maintained by intraperitoneal transfer into Donryu rats, and collected for experiments 4 to 7 days after inoculation.

### Subcutaneous implantation of tumor cells

Tumor cells suspended in 0.1 ml saline were transplanted subcutaneously (s.c.) into the right axilla of 5-week-old Donryu rats. The numbers of cells injected were as follows: Yoshida sarcoma $2 \times 10^4$ cells/rat, AH-130 $5 \times 10^6$ cells/rat, and SLC $2 \times 10^5$ cells/rat. Rats were randomized into treatment and control groups (seven rats/group) based on body weight just after implantation (day 0).

### Intracolonic implantation of tumor cells

The technique performed was based on the method described by Morikawa et al. [23]. In brief, rats were anesthetized with diethylether, and the colon was exteriorized under sterile conditions Yoshida sarcoma cells ($1 \times 10^5$ cells/0.025 ml saline) were injected into the colonic wall approximately 5 cm above the anus from the serosal side with a 30-gauge needle. The wound was closed with metal clips. All rats survived surgery. The following day (day 1), rats were randomized into treatment and control groups (five rats/group) based on body weight.

### Antitumor activity

The drugs for the treatment groups and the vehicle solution for the control group were orally administered in a volume of 10 ml/kg by gavage once daily for 7 consecutive days from day 1 to day 7. On day 8, rats were weighed before being sacrificed with diethylether, and the tumors were removed and weighed. The values for criteria of antitumor activity and toxicity were calculated as follows: tumor growth inhibition (TGI, %) = [1 − (mean tumor weight of treated group)/(mean tumor weight of control group)] × 100; $ED_{50}$ = dose for 50% TGI; body weight gain (g) = (body weight on day 8) − (body weight on day 0) − (tumor weight); body weight change (BWC, %) = (mean body weight gain of treated group)/(mean body weight gain of control group) × 100; $BWC_{50}$ = dose for 50% BWC; and therapeutic index (TI) = $BWC_{50}/ED_{50}$. Based on the median effect equation according to Chou and Talalay [5], $ED_{50}$ and $BWC_{50}$ values were estimated from the regression line of log-logit plots of dose vs TGI or BWC, respectively. Logit values were calculated from the following equation: $\text{logit}\, p = \ln p/(100 - p)$, where $p$ is TGI or BWC.

### Intestinal toxicity

The intestinal toxicity of rats undergoing the Yoshida sarcoma (s.c.) experimental protocol described above was investigated on day 8. The condition of the feces of each group was observed and scored [25] as: − normal ± loose and + diarrhea. Occult blood in approximately 1 g of feces collected at random from each group was measured using the rat hemoglobin enzyme assay method (Panapharm Laboratories, Kumamoto, Japan).

### Equitoxic doses

Based on the regression lines of dose vs BWC (Fig. 3B), the doses of S-1 and UFT inducing equivalent host toxicity were established as 15 mg/kg per day and 30 mg/kg per day, respectively.

### Collection of specimens

Eight days after s.c. implantation of Yoshida sarcoma cells in the manner described above, tumor-bearing rats were divided into two groups (five rate per group), and then S-1 and UFT including $^{14}$C-FT for the determination of the 5-FU levels) were administered orally. At the scheduled time after administration, rats were sacrificed and blood and tumors were collected. Blood samples were collected into tubes containing heparin and centrifuged immediately to separate the plasma.

### Determination of 5-FU levels

The tumors were homogenized in three volumes of saline using a polytron homogenizer (Biotron, Kimura, Japan). Samples were mixed with methanol to remove coagulated protein. The supernatant was dried under nitrogen gas. The residues were dissolved in 60% methanol and subjected to thin layer chromatography [15]. In brief, samples were applied to silica gel plates (Kieselgel 60 F254, 3 × 20 cm, Merk, Germany) and first developed to approximately 5 cm in ethanol/1 *M* ammonium acetate (4 : 1, v/v), pH 9.5, and then twice to approximately 15 cm in diethylether/acetone/chloroform/water (50 : 50 : 40 : 1, v/v/v/v). 5-FU fractions were scrapped off and eluted in 0.5 ml 60% methanol. Following the addition of 10 ml scintillator (ACS-II, Amersham), the radioactivity of each extract was measured with a liquid scintillation counter (Beckman, Palo Alto, CA).

### Determination of F-RNA levels

Isolation and quantification of 5-FU incorporated into the RNA fractions were performed using the methods described by Uchida et al. [34]. In brief, the RNA fractions in the tumors were extracted and separated by the method described by Schneider [29]. For isolation of 5-FU, the RNA fractions were heated to 100 °C in 6 *N* HCl and hydrolyzed for 24 h. Finally, 5-FU was determined using gas chromatography-mass spectrometry (Model JGS-20kp, Model JMS-D 300, Jeol, Tokyo, Japan) [21].

### Determination of TS inhibition rate

TS activity was determined by estimating the [6-$^3$H]FdUMP binding sites in 105 000 *g* supernatants (cytosol) of tumor tissue homogenates, based on the method described by Spears et al. [31]. The samples for the determination of total TS were prepared by causing the ternary complex present in the cytosol to be fully dissociated to unbound TS at pH 8.0 during the preincubation period. In the case of the samples for the determination of free TS, preincubation for the dissociation process was omitted. Total TS and free TS samples were incubated with [6-$^3$H]FdUMP in the presence of 5,10-methylenetetrahydrofolate for 20 min at 30 °C and the radioactivity in the acid-insoluble fractions was measured using a liquid scintillation counter. The TSIR was calculated from the following equation: $\text{TSIR}\ (\%) = (1 - \text{TS}_{\text{free}}/\text{TS}_{\text{total}}) \times 100$.

### Statistics

The significance of differences between means was assessed by Student's *t*-test.

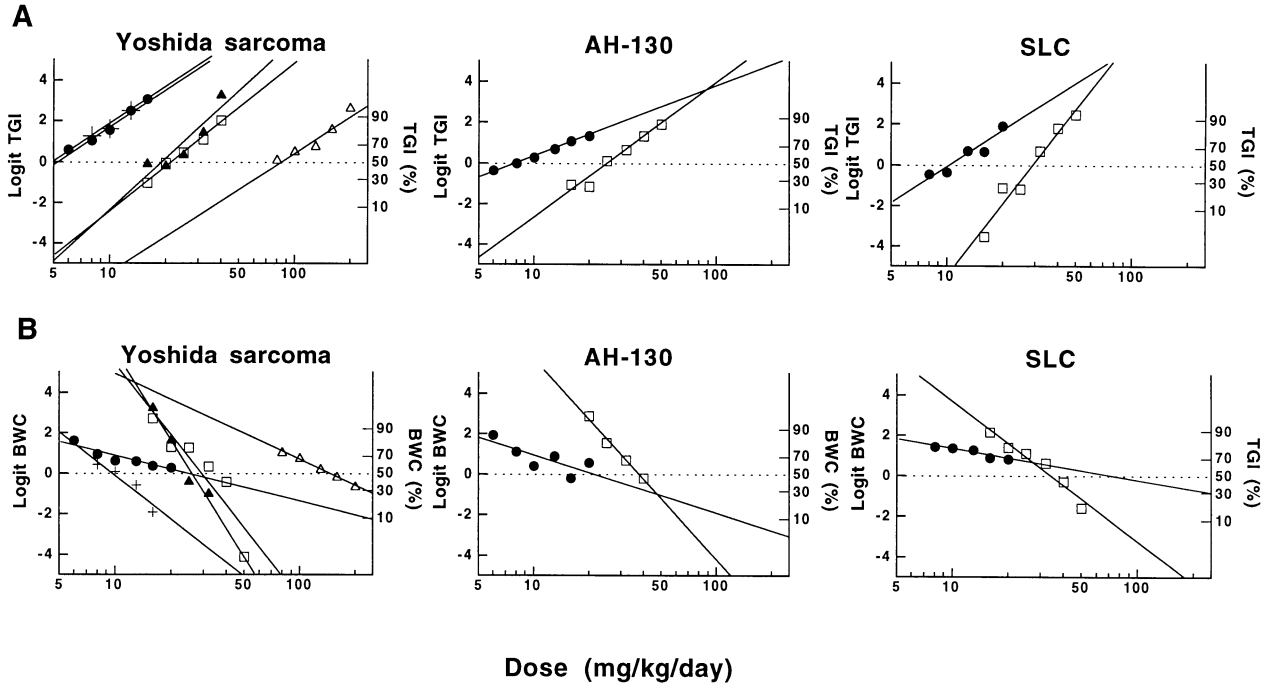


**Fig. 3A, B** Inhibitory effects of oral fluoropyrimidines on tumor growth and body weight in experimental tumor-bearing rats. **A** Tumor growth inhibition (TGI); **B** body weight change (BWC). Each tumor was implanted s.c. on day 0. Drugs were orally administered once daily for 7 consecutive days from day 1. On day 8, tumor weight and body weight were measured. TGI and BWC induced by S-1 (●), UFT (□), FT (△), FCD (+) and 5-FU (▲) were calculated as described in Materials and methods. Values are the means of seven rats, converted as follows: F(X) = log(X) and F(Y) = logit(Y). The regression lines were fitted according to the method of least squares

## Results

### Antitumor effect of S-1 on experimental rat tumors

Using s.c. tumor models in rats, the correlation between the dose of fluoropyrimidines and TGI was determined as shown in Fig. 3A. Based on these graphs, the $ED_{50}$ value of each drug was estimated (Table 1). The $ED_{50}$ values of S-1 and FCD were lower than those of 5-FU, FT and UFT. The ratio of each $ED_{50}$ value was as follows: Yoshida sarcoma, S-1/UFT/FT/FCD/5-FU = 1.0/4.4/16.4/1.0/3.8; AH-130, S-1/UFT = 1.0/3.3; and SLC, S-1/UFT = 1.0/2.9.

The maximal TGIs of each drug against Yoshida sarcoma after administration of the maximal tolerable doses (MTD) (S-1 20 mg/kg per day, UFT 50 mg/kg per day, FT 200 mg/kg per day, FCD 20 mg/kg per day, and 5-FU 40 mg/kg per day) were as follows: S-1 100% (complete response 7/7), UFT 100% (CR 7/7), FT 94%, FCD 100% (CR 7/7), and 5-FU 97% (CR 3/7). The maximal TGIs against AH-130 were S-1 79% and UFT = 87%, and against SLC were S-1 87% and UFT 92%. There was no significant difference between the maximal TGI of S-1 and that of UFT.

**Table 1** Antitumor activity of S-1 and other oral fluoropyrimidines against rat tumors. Tumor cells were implanted s.c. on day 0. Drugs were administered once daily consecutively from day 1 to day 7. Tumor weight was measured on day 8. Values were derived from the regression lines shown in Fig. 3A (*NT* not tested)

| | $ED_{50}$ (mg/kg/day) | | | | |
|---|---|---|---|---|---|
| Tumor | S-1 | UFT | FT | FCD | 5-FU |
| Yoshida sarcoma | 5 | 22 | 82 | 5 | 19 |
| AH-130 | 8 | 25 | NT | NT | NT |
| SLC | 10 | 29 | NT | NT | NT |

### Therapeutic efficacy of S-1 in tumor-bearing rats

In general, it is considered that body weight loss is typical of the toxicity caused by fluoropyrimidines in rats. To collectively estimate the antitumor effect and the toxicity of S-1, the TI, i.e. the index representing the balance between TGI and BWC, was calculated (Table 2). The TI of S-1 was the highest of all oral fluoropyrimidines. The TI ratios of each drug were S-1/UFT/FT/FCD/5-FU 1.0/0.3/0.4/0.4/0.3 for Yoshida sarcoma, S-1/UFT 1.0/0.6 for AH-130, and S-1/UFT 1.0/0.2 for SLC.

**Table 2** Therapeutic efficacy of S-1 and other oral fluoropyrimidines in tumor-bearing rats. Tumor cells were implanted s.c. on day 0. Drugs were administered once daily consecutively from day 1 to day 7. Tumor weight and body weight was measured on day 8. Values were calculated as described in Materials and methods (*NT* not tested)

| | Therapeutic index ($BWC_{50}/ED_{50}$) | | | | |
|---|---|---|---|---|---|
| Tumor | S-1 | UFT | FT | FCD | 5-FU |
| Yoshida sarcoma | 4.5 | 1.4 | 1.8 | 2.0 | 1.4 |
| AH-130 | 2.7 | 1.5 | NT | NT | NT |
| SLC | 6.0 | 1.2 | NT | NT | NT |

## 5-FU levels in plasma and tumors, F-RNA levels in tumors and TSIR in tumors

To demonstrate the high therapeutic efficacy of S-1 from a pharmacokinetic angle, the levels of 5-FU in plasma and tumors were determined after oral administration of equitoxic doses of S-1 and UFT to Yoshida sarcoma-bearing rats. As shown in Fig. 4A, B, the $AUC_{0-\infty}$ in the S-1 group compared with the UFT group was 1.9 times higher in plasma (S-1 28 nmol h/ml, UFT 15 nmol h/ml) and 1.8 times higher in tumor tissue (S-1 95 nmol h/g tissue, UFT 52 nmol h/g tissue). The plasma 5-FU level in the S-1 and UFT groups decreased to 0.5 nmol/ml at 11.7 h and 6.9 h, respectively. The 5-FU level in tumors of the S-1 group also tended to remain elevated for longer periods than that in the UFT group.

F-RNA levels and TSIR in tumor tissue are shown in Fig. 4C, D. The $AUC_{0-24}$ of the F-RNA level in tumors of the S-1 group was 1.6 times higher than in the UFT group (S-1 7.0 nmol h/mg RNA, UFT 4.3 nmol h/mg RNA). The TSIR of the S-1 group was 70% at 4 h and 56% at 24 h, and was 5–8% higher than in the UFT group at every time-point. These differences were significant ($P < 0.01$).

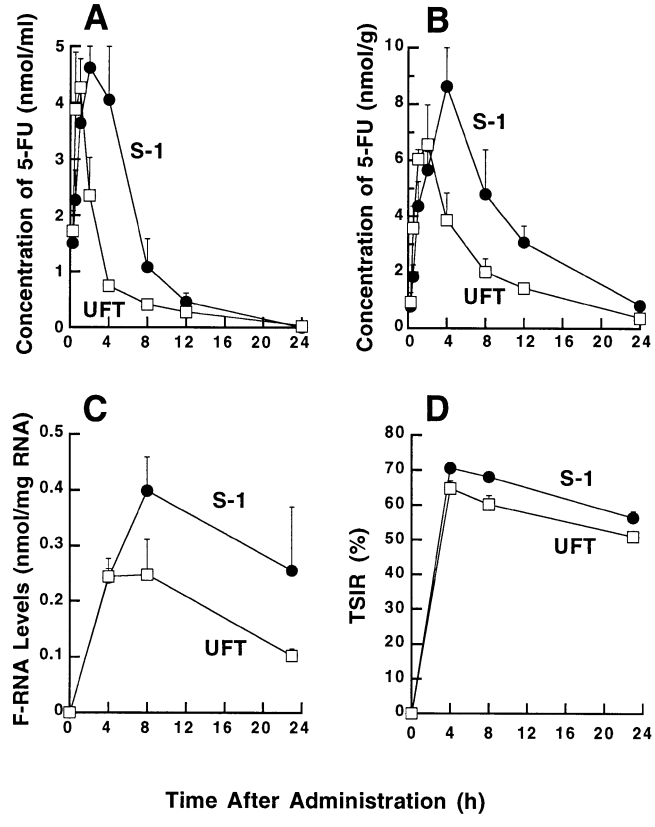


**Fig. 4A–D** Levels of 5-FU and F-RNA, and TS inhibition rate (TSIR) in plasma and/or tumor tissue after administration of S-1 or UFT. **A** 5-FU levels in plasma; **B** 5-FU levels in tumor tissue; **C** F-RNA levels in tumor tissue; **D** TSIR in tumor tissue. The equitoxic dose of each drug (S-1 15 mg/kg, UFT 30 mg/kg) was administered to Yoshida sarcoma-bearing rats. After administration, the blood and tumor tissues were removed at 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h for **A** and **B**, and at 4, 8 and 24 h for **C** and **D**. The concentration of 5-FU and F-RNA, and the TSIR were determined as described in Materials and methods. Values are the means ± SD (*bars*) of individual samples from five rats

## Intestinal toxicity of S-1 in tumor-bearing rats

One of the causes of the high therapeutic efficacy of S-1 is considered to be the presence of Oxo which decreases the intestinal toxicity induced by 5-FU [30]. We investigated the intestinal disorders which appeared on day 8 after administration of fluoropyrimidines at the MTD. Table 3 shows that the faces of the S-1 group were normal, and the amount of occult blood in feces in the S-1 group was 0.58 μg/g, which was less than in the FCD, UFT and 5-FU groups.

**Table 3** Intestinal toxicity of S-1 and other oral fluoropyrimidines on Yoshida sarcoma-bearing rats. Yoshida sarcoma cells were implanted s.c. on day 0. Drugs were administered once daily consecutively from day 0 to day 7. On day 8, feces of each MTD group were collected. Values and scores were determined as described in Materials and methods. Occult blood values are the means of each group. Fecal scoring: − normal, ± loose, + diarrhea

| Criterion | S-1 | UFT | FT | FCD | 5-FU |
|---|---|---|---|---|---|
| Occults blood (μg/g feces) | 0.58 | 1.03 | 0.29 | 0.62 | 0.74 |
| Fecal score | − | + | ± | + | + |

## Antitumor effect of S-1 on the tumor located in the colonic wall

Eight days after the injection of Yoshida sarcoma into the colonic wall from the serosal side, gross and microscopic anatomy revealed that the tumor had grown to approximately 7 mm in diameter and had invaded both the mucosal and the serosal sides of the tract.

S-1 and UFT at equitoxic doses were administered for 7 consecutive days. Table 4 shows that each drug induced equivalent BWC. However, the mean tumor

**Table 4** Effect of S-1 and UFT on Yoshida sarcoma located in the colonic tract. Yoshida sarcoma cells ($10^5$ cells) were implanted intracolonicaly on day 0. Drugs were administered once daily from day 1 to day 7 consecutively. On day 8, whole body weight and tumor weight were measured

| Criterion | Control | S-1 | UFT |
|---|---|---|---|
| Body weight gain (g, mean ± SD) | 60 ± 6 | 33 ± 9 | 33 ± 8 |
| Body weight change (% of control) | 100 | 55 | 56 |
| Tumor weight (mg, mean ± SD) | 446 ± 196 | 64 ± 30* | 133 ± 52 |
| Tumor growth inhibition (%) | 0 | 86 | 70 |

*$P < 0.05$ vs UFT group

weight in the S-1 group was half that in the UFT group, and this difference was significant ($P < 0.05$). S-1 practically inhibited tumor growth in the intestinal tract, while S-1 did not seem to decrease tumor invasion.

## Discussion

This study investigated the experimental efficacy of S-1 in comparison with other oral fluoropyrimidines from the perspectives of antitumor activity, intestinal toxicity and FT metabolism. In a series of experiments, we used a rat-tumor system which was highly advantageous for estimating antitumor effects on intracolonic tumors and intestinal toxicity as well as antitumor effects on s.c. tumors.

The $ED_{50}$ values for S-1 and FCD were the same, and were about one-sixteenth of the value for FT and about one-fourth of the value for UFT in Yoshida sarcoma-bearing rats. Additionally, S-1 was pharmacokinetically compared with UFT, which results in longer retention time for 5-FU in the blood than FT or 5-FU [15, 16] after the administration of equitoxic doses. The $AUC_{0-\infty}$ of 5-FU in the S-1 group was approximately twice as high both in plasma and in tumor tissue as that for the UFT group. Fujii et al. [9] reported that CVI of 5-FU at 20 mg/kg per day into Yoshida sarcoma-bearing rats for 6 days induces a plasma 5-FU level of 30 to 60 ng/ml (0.23 to 0.46 nmol/ml) and a 43% TGI. When the plasma 5-FU level inducing a TGI of approximately 50% was estimated at 0.5 nmol/ml, the plasma 5-FU level in the S-1 group was maintained for 11.7 h above that level, and lasted 1.7 times longer than that in the UFT group (6.9 h). Moreover, the F-RNA level in the tumors of the S-1 group was 1.6 times higher than that in the UFT group, and a TSIR above 56% was maintained for 24 h in the S-1 group. These results suggest that CDHP, which is a more effective inhibitor of DPD than uracil in vitro [32], strongly inhibited the degradation of 5-FU to increase the $AUC_{0-\infty}$ and prolong the retention time in both plasma and tumor tissue leading to a higher antitumor activity of S-1 than UFT. In other words, S-1 might more closely approach the CVI of 5-FU which is the most effective current dosing schedule for 5-FU [1, 3, 4, 10, 13, 18, 24, 28, 37] in terms of pharmacokinetics.

S-1 did not markedly suppress the body weight gain in rats even at high doses. Therefore, the TI of the S-1 group was the highest. Since Oxo has been shown to distribute mainly in the GI tract after oral administration [30], the intestinal toxicity was further investigated. Intestinal disorders such as diarrhea and occult blood in feces in the S-1 group were milder than in the FCD group and the other groups. These results suggest that Oxo, which is an inhibitor of 5-FU phosphorylation to FUMP [30], decreased the cytotoxicity of 5-FU in the intestinal tract resulting in a reduction in intestinal disorders. As well as CVI, administration of FT with a DPD inhibitor such as CDHP may also cause intestinal DLT clinically because of the long retention of 5-FU in the plasma. Because of the effect of Oxo, the dose intensity of FT could be elevated, allowing S-1 to have a potent antitumor activity in comparison with FCD. In rats, however, the MTD of S-1 was equivalent to that of FCD, when the DLT seemed to be myelosuppression.

Fluoropyrimidines are often used clinically in the treatment of GI neoplastic tumors [1, 3, 4, 18, 19, 24, 26–28, 37–39]. The clinical investigation of S-1 is currently in progress mainly against GI tumors. To demonstrate the antitumor activity of S-1 against tumors in the GI tract, we used experimental intracolonic implants of Yoshida sarcoma. In these experiments, S-1 was no less effective against intracolonic tumors than against s.c. tumors. The biochemical features of Oxo that protect GI tract tissue from the cytotoxicity of 5-FU are now being investigated further.

In conclusion, our results in an experimental rat-tumor system suggest that CDHP, a strong inhibitor of 5-FU degradation, seemed to increase the $AUC_{0-\infty}$ and retention time of 5-FU in the plasma and tumor tissue, while Oxo protected the GI tract from the cytotoxicity of 5-FU without loss of antitumor effect even with the tumor located in the colon. Therefore, S-1 enables a single oral drug to show the highest antitumor activity among oral fluoropyrimidines, while avoiding intestinal disorders that are a particular problem in patients treated with fluoropyrimidines [1, 18]. S-1 could prove a highly efficacious in the clinical treatment of solid tumors, including GI tumors.

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