Persistent Induction of Apoptosis and Suppression of Mitosis as the Basis for Curative Therapy with S-1, an Oral 5-Fluorouracil Prodrug in a Colorectal Tumor Model

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Abstract

In an effort to improve the therapeutic selectivity of 5-fluorouracil (FUra) against colorectal cancer, S-1, a combination agent including a prodrug of FUra with two modulators, was recently developed by Taiho Pharmaceuticals Co. S-1 is a combination of tegafur (FT), 5-chloro-2,4-dihydroxypyridine, and potassium oxonate in the molar ratio of 1.0:0.4:1.0, with the latter two components as inhibitors of dihydropyrimidine dehydrogenase and phosphoribosylpyrophosphate transferase, respectively. In this study, the therapeutic selectivity and efficacy of S-1 (oral) was compared with FT (oral) and FUra (i.v. infusion) in rats bearing advanced colorectal cancer by using clinically relevant schedules. The maximum tolerated doses (MTDs) of S-1, FT, and FUra were 31.5, 200, and 25 mg/kg/d for 7 days and 22.5, 150, and 12.5 mg/kg/d for 28 days, respectively. The therapeutic index of S-1 was 4- to 5-fold higher than that of either FT or FUra. S-1 achieved 100% complete tumor regression (CR) at its MTD in both 7-day and 28-day schedules. Furthermore, the high incidences of stomatitis, alopecia, and diarrhea observed with FUra and FT, were not observed with S-1. In an attempt to understand the basis for the observed superior therapeutic selectivity with S-1, we studied pharmacokinetic analysis of FUra, drug-induced apoptosis, suppression of mitosis, and inhibition of thymidylate synthase (TS) after S-1, FUra, or FT administration. The peak plasma FUra concentrations derived from FUra or S-1 (FT) at comparable MTDs were similar, but the plasma level of FUra was higher with S-1 than with FUra. Induction of high and sustained apoptosis was achieved with S-1. Although the initial level of apoptosis induced by FUra was comparable to S-1, it was not sustained. The sustained level of apoptosis appears to correlate with tumor growth inhibition. Mitotic figures were more greatly suppressed with S-1 treatment than with FUra. Studies on TS inhibition indicated that, although both S-1 and FUra caused a 4- to 6-fold induction of total TS protein, single oral administration of S-1 was superior to 24-h infusion of FUra in suppressing free TS. The data are consistent with the observation that the therapeutic efficacy of S-1 (100% cure) over FUra is associated with high and sustained levels of drug-induced apoptosis, greater suppression of mitosis, and inhibition of free TS in tumor tissues.

Introduction

Improving therapeutic selectivity is a major goal of anticancer drug development and biochemical modulation. After the success of FUra/LV3 therapy in patients with advanced colorectal cancer, TS has remained an important target for the design of more selective and efficacious drugs. Although FUra/LV therapy increased tumor response rate in patients with colorectal cancer compared with FUra alone, no significant improvement on patient survival was observed (1–3). Severe side effects such as diarrhea, mucositis, and neutropenia were observed in FUra/LV therapy and, thus, limited the therapeutic selectivity (4–6). Similarly, hand and foot syndrome is the dose-limiting toxicity in protracted infusion of FUra, although the latter can be successfully used in colorectal cancer to achieve response rates similar to those achieved with FUra/LV modulation (7, 8). To improve the therapeutic selectivity of FUra/LV modulation, new and more specific TS inhibitors such as Tomudex (ZD1694) and AG-337, are under extensive preclinical and clinical evaluation. However, the initial response rates in colorectal cancer and the toxicity profile from these new drugs are similar to those that have been observed for FUra/LV therapy (9, 10).

In clinical and preclinical model systems, FUra is eliminated rapidly from the plasma with a t1/2 of less than 10 min (11, 12). Furthermore, more than 90% of the injected dose of FUra is metab...
FUra is inactivated by DPD in normal and tumor tissues (13). The remaining 10% of FUra is activated via the anabolic pathway with a major fraction incorporated into cellular RNA (14–16). In addition, GI toxicity is caused by the phosphorylation of FUra in the digestive tract, and the level of thymidine/uridine phosphorylase is higher in normal tissues than in tumor tissue (17, 18). This suggests that the therapeutic selectivity of FUra may be improved by selective inhibition of phosphorylation of FUra in normal tissue. Thus, new treatment modalities are being developed using the combination of FUra or its prodrug with an inhibitor of DPD to prevent FUra degradation and/or with an inhibitor of PRPPT to prevent FUra phosphorylation into fUDP in normal tissue (19). Examples of this approach are UFT/LV (20, 21), FUra/EU (22–24), FT/EU (25), and S-1 (19, 26), which are presently under preclinical and clinical evaluation.

S-1 is a new oral pyrimidine fluoride-based anticancer agent in which FT is combined with two classes of modulators, CDHP and Oxo, at a molar ratio of 1:0.0:4:1.0 for FT:CDHP: Oxo, respectively (19, 26). FT is inactive until it is metabolized to FUra by thymidine/uridine phosphorylase. CDHP is a potent inhibitor of DPD, the enzyme responsible for degradation of FUra into therapeutically inactive but toxic 5-fluoro-dihydouracil (27); CDHP is 180 times more effective than uracil in the inhibition of DPD in vitro (27). Oxo is a potent inhibitor of PRPPT, an enzyme responsible for the metabolic activation of FUra to fUDP. It has been demonstrated that PRPPT inhibition occurs mainly in the normal GI tract because of the selective distribution of Oxo in GI tissues; this results in the significant reduction of FUra incorporation into cellular RNA and in decreased toxicity (18, 26, 27). S-1 is in phase I and II clinical trials in patients with advanced colorectal cancer in Europe and Japan (28, 29) and will be in clinical trials in the United States soon.

In this study, we report on the antitumor activity and toxicity of S-1 in comparison with FUra and FT using clinically relevant schedules in rats bearing advanced colorectal cancer. Reduced host toxicity, persistent induction of apoptosis, greater suppression of mitosis, and prolonged TS inhibition by S-1 seem to be the basis for the therapeutic superiority of S-1 over FUra and FT in this model system.

Materials and Methods

Drugs. S-1 was obtained from Taiho Pharmaceutical Co., Ltd. (Hanno-City, Japan). FT was purchased from Sigma Chemical Co. (St. Louis, MO). FUra was purchased from Hoffmann-La Roche, Inc. (Nutley, NJ). All of the drugs were dissolved in sterile 0.9% NaCl solution.

Rats. Female Fisher 344/HSD rats (ages, 8–12 weeks; body weight, 150–180 g) were obtained from Harlan Sprague Dawley Inc. (Indianapolis, IN) and kept at four rats to a cage with water and food ad libitum according to an institutionally approved animal protocol.

Tumor. The chemically induced Ward colorectal carcinoma, which has been extensively investigated in this laboratory, was used (20, 22). Nonnecrotic tumor pieces (100 mg) were transplanted s.c. via trocar under light ether anesthesia. Treatment was initiated 12–14 days after transplantation when tumors weighed ~3 g.

Chemotherapy. S-1 and FT were given p.o., once a day for 7 or 28 days. FUra was given for 7 or 28 days by continuous i.v. infusion (31). The control group was given 0.9% saline via respective routes and schedules. Each treatment group had four rats per experiment, and each experiment was repeated three to seven times.

Tumor Response. CR was defined as the disappearance of tumor for more than 90 days posttherapy. Partial tumor regression was defined as >50% reduction in tumor size with subsequent regrowth.

Determination of Therapeutic Index. Therapeutic indices were calculated as the ratio of MTD:MED. As defined previously (22), the MTD was the maximum dose that did not cause drug-related lethality, and the MED was the minimum dose with which 10–25% CRs were achieved in tumor-bearing animals.

Antitumor Activity and Host Toxicity Assessments. Measurements of tumor size and animal body weight were carried out as described previously (22). Toxicities, consisting of body weight loss, diarrhea, stomatitis (mouth ulceration), alopecia (hair loss), and lethality, were assessed daily for a minimum of 4 weeks after drug treatment.

Pharmacokinetics. Repetitive blood sampling from individual rats, plasma extraction, and high-performance liquid chromatography quantitation of uracil, FUra, and FT were carried out as described previously (32). For high-performance liquid chromatography analysis, typical column retention times for uracil, FUra, FT, and bromouracil (internal standard) were 3.4, 3.6, 47.7, and 9.4 min, respectively.

Spontaneous and Drug-induced Apoptosis. Rats were treated on day 8 after tumor transplantation (tumor weight ~1 g) with a single dose of S-1 (22.5 mg/kg p.o.) or FUra (25 mg/kg, 24-h infusion). Tumors were removed at pretreatment (0 h), and 24, 48, and 72 h after the initiation of drug treatment; they were then weighed, fixed in 10% neutral buffered formalin, dehydrated, and embedded in paraffin. Apoptosis was evaluated by morphology after H&E staining; distinct morphological features of apoptotic cells included nuclear condensation and fragmentation. Apoptosis indices were calculated as the percentage of apoptotic cells among three hundred tumor cells in a randomly selected nonnecrotic portion of tumor. Apoptotic cells at the edges of necrotic foci commonly seen in untreated tumors were considered as spontaneous apoptosis, and they were not scored. The presence of apoptotic cells was confirmed by terminal deoxynucleotidyl transferase (TdT)-mediated nick and labeling (TUNEL) immunohistochemical assay on paraffin section (Apop Tag® Plus in situ detection kit, Oncor, Gaithersburg, MD) according to the manufacturer’s instruction.

Mitotic Figures in Tumor Tissues. H&E-stained slides of tumor tissue for the study of apoptosis were also evaluated for mitotic figures. Mitotic figures included cells staging from prophase to telophase of the M phase of cell cycle. For each slide, mitotic figures were counted on 10 randomly selected fields at ×400. For each group, three slides from independent tumor samples (different animals) were evaluated. All of the morphological study was done by a pathologist (K. T.).
TS Enzyme Assays. Tumors were removed and frozen immediately in liquid nitrogen and kept at −70°C until TS assays were performed. Frozen tumor pieces (0.2–0.5 g) were broken into powder and then extracted with a Polytron homogenizer for 3 × 10 s at 4°C in 1–2 ml extraction buffer [20 mM Tris/HCl (pH 7.5), 250 mM sucrose, 2 mM DTT, 1.5 mM MgCl₂, and 1 mM EDTA]. Tissue homogenates were then centrifuged at 77,000 × g for 30 min at 4°C, and the supernatants were used for TS assays. Assays of free and total TS levels (dUMP-bound plus dUMP-free) were based on quantitating the stoichiometric formation of covalent ternary complexes of TS, [6-3H]dUMP, and 5,10-CH₂H₄PteGlu, as described previously (33). For the determination of total TS level in FUra- or S-1-treated tumor samples, 30 μl of tumor extract were mixed with 30 μl of dissociation buffer [0.6 M NH₄HCO₃ (pH 8.0), 15 mM cytosine monophosphate, 100 mM NaF, and 100 mM β-mercaptoethanol] and preincubated for 3 h at 30°C. At the end of preincubation, 20 μl of [6-3H]dUMP (830 nM) and 20 μl of CH₂H₄PteGlu (500 μM) were added to the above mixture and incubated further for 20 min at 30°C. Free TS was measured as described above without the preincubation step. At end of the 20-min incubation, samples (50 μl each) were mixed with 10 μl of 6% SDS and boiled for 3 min. Ternary complexes were separated from unreacted [6-3H]dUMP by centrifuging 25 μl of boiled sample through a Sephadex G-25 mini-column (400 μl). The radioactivity in the filtrate was quantitated. Free and total TS levels ([6-3H]dUMP binding sites) were expressed as pmol/g.

Results

Kinetics of Tumor Response to S-1. The data in Fig. 1 are a summary of the antitumor activity of S-1 with different doses administered p.o. to rats bearing advanced colorectal cancer, using daily × 7 and × 28 day schedules. The MTDs of S-1 were 31.5 mg/kg and 22.5 mg/kg for 7-day and 28-day schedules, respectively. The kinetics of achieving CR seems to be similar with the two schedules: 100% of treated animals achieved complete and sustained CR (cure) within 14–16 days. S-1 at lower doses than MTD, i.e., 18–27 mg/kg for 7-day schedule and 13.5–18 mg/kg for 28-day schedule, were also able to produce 100% CR.

Comparative Antitumor Activity of S-1 with FUra and FT. The antitumor activity (CR rates) of S-1 (p.o.) was compared with FT (p.o.) and FUra (i.v. infusion) at the MTD and ½ MTD (Fig. 2 and Table 1). The therapeutic efficacy of S-1 was superior to FUra and FT in both schedules. Whereas S-1 achieved 100% of CR at its MTDs of both schedules, FUra and FT achieved 50% and 19% of CR, respectively, only in the 7-day schedule. Neither CR nor partial tumor regression was achieved by FUra and FT even at their MTDs with 28-day schedule (Fig. 2B). The data in Table 1 indicate that S-1 produced CR at lower doses than the MTD. However, optimal therapeutic efficacy of FUra and FT was achieved only at the MTD, which indicates the potential for less toxicity and a superior margin of safety with S-1 over FUra and FT. Although FT achieved minimal tumor response with either schedule, continuous i.v. infusion of FUra was highly schedule-dependent. Seven-day schedule was more active than the 28-day schedule.

Fig. 1 Antitumor activity of S-1 in rats bearing advanced colorectal cancer. ○, control; ●, 9 mg/kg; □, 13.5 mg/kg; ■, 18 mg/kg; ▼, 22.5 mg/kg (MTD for 28-day schedule); ▲, 27 mg/kg; ●, 31.5 mg/kg (MTD for 7-day schedule). S-1 was administered p.o. The doses indicated were the daily doses. Data point, 16–32 rats of 4–8 independent experiments.

Toxicity Profiles. The toxicity profiles of S-1, FT, and FUra were evaluated, and the results are summarized in Table 2. FUra induced diarrhea in 25% (7-day schedule), stomatitis in 25–38%, and alopecia in 25% of treated animals (both schedules). FT induced stomatitis in 25–75% and alopecia in 100% of treated animals with both schedules. No significant toxicities were observed after S-1 treatment at the MTD or lower doses. S-1 also induced less weight loss in treated animals than did FT or FUra treatment, although all of the animals were fully recovered in weight loss within 1–2 weeks after treatment with S-1, FT, and FUra at their MTDs.

Therapeutic Indices of S-1, FT and FUra. The therapeutic indices of S-1, FT, and FUra are summarized in Table 3. The data show that the therapeutic index of S-1 is 4- to 5-fold higher than that of FT or FUra.

Pharmacokinetics. Plasma concentrations of FUra derived from S-1 (22.5 mg/kg, p.o. × 1), FT (150 mg/kg, p.o. × 1), and FUra (25 mg/kg, 24-h i.v. infusion) were determined (Table 4). Although the peak plasma concentration of FUra derived from S-1, FT, and FUra was similar immediately after drug administration, the plasma level of FUra derived from S-1 was sustained at higher concentrations than from FT and FUra. No FUra in plasma was detected at 4 h after cessation of FUra infusion.
Apoptosis and Mitotic Figures. In this tumor, spontaneous apoptotic cells were clearly observed. Spontaneous apoptotic cells were sparsely scattered in the viable portions of tumor tissue, or they were formed characteristically on the border between necrotic and nonnecrotic tumor tissues (Fig. 3A). Higher levels of apoptosis were detected after S-1 treatment (Fig. 3B).

In control tumors (treated with saline), the apoptotic indices averaged between 6.5 and 10%, mitotic figures averaged from 4 to 10 per high-power field in a section, and the tumor doubled in weight from 1 g to 2 g within 3 days (Fig. 4). The apoptotic index induced by FUra (25 mg/kg, 24-h infusion) in tumor cells was higher than in the control group at the end of infusion (24 h); however, the level of apoptosis was similar to that in the control group after 48 h. Mitotic figures were decreased to a level lower than in the control group at the end of FUra infusion but then recovered to a level comparable to the control group (Fig. 4B). Tumor growth was slower in the FUra-treated group than in the control group (Fig. 4C).

S-1 treatment were suppressed to a level lower than that in the control and FUra-treated groups (Fig. 4B). The tumor sizes decreased from 1 g to 0.8 g within 3 days after a single oral dose of S-1 (Fig. 4C). Inhibition of TS. In parallel with apoptosis studies, the effects of S-1 and of FUra on the degree and duration of TS inhibition in tumor tissue were also evaluated. A lower level of free TS was observed with S-1 than with FUra at various times after drug administration (Fig. 5). There was no significant difference between S-1 and FUra in inhibitory rates and induction of TS protein (total TS) level, which was elevated 4- to 6-fold above control values.

Discussion

Although drug development has produced significant advances in the treatment of patients with colorectal cancer in recent years, the therapeutic selectivity remains a major obstacle to curative therapy. Recent clinical trials have demonstrated that UFT is effective, with reduced side effects, in patients with advanced colorectal cancer (21). Preclinical studies with FUra and FT modulated by EU, an inhibitor of DPD, showed improved therapeutic efficacy and selectivity (22, 23, 25). Thus, the selective activation of FUra prodrug in tumor tissues with the concomitant inhibition of the FUra degradative enzyme DPD seems to be a useful approach.

In general, the inhibition of TS has been associated with the antitumor activity of FUra-based therapy and drug incorporation into normal tissue cellular RNA associated with side effects (14–17). Attempts have been made to reduce drug incorporation into normal GI tissues and simultaneously to inhibit the degradation of FUra, thus forcing FUra to be metabolized to fUMP and/or into fUTP (17, 18). S-1 has been developed as a formulation consisting of FT (a prodrug of FUra), CDHP (an inhibitor of DPD), and Oxo (an inhibitor of PRPP) in the molar ratio of 1.0:0.4:1.0.

Recent studies by Shirasaka et al. (19) have demonstrated high therapeutic activity of S-1 in nude rats implanted orthotopically with human colon carcinoma. This agent is also under clinical evaluation in Europe and Japan with encouraging results (28, 29). In the present study, the antitumor activity and toxicity of S-1 were evaluated and compared with those of FUra and FT with clinically relevant schedules in rats bearing advanced colorectal cancer. The results can be summarized as follows: (a) S-1 is p.o. bioavailable with superior antitumor activity (100% cure) in this model without the significant toxicity generally associ-
ated with FUra and FT such as diarrhea, mucositis, and alopecia (Tables 1 and 2); (b) because FT itself was not highly active in this model, the improvement in the therapeutic efficacy with S-1 may be related to the inhibition of DPD by CDHP, with decreasing FUra elimination via the anabolic pathway, which results in high levels of the drug in tumor tissues and increasing antitumor activity through TS inhibition (a potent and sustained inhibition of free TS was observed; Fig. 5); (c) evidence for decreased FUra incorporation into normal tissue cellular RNA through the inhibition of PRPPT by Oxo was deduced by the absence of diarrhea and mucositis after S-1 treatment (Table 2) and on the basis of previous findings of Shirasaka et al. (18); and (d) although a sustained blood level of FUra was observed after S-1 treatment and resembled the blood level obtained with continuous i.v. infusion of FUra, the observed differences in therapeutic response between oral S-1 and continuous infusion of FUra can hardly be explained by the pharmacokinetic data. Our data does not support the hypothesis that the oral S-1 is acting solely as a continuous infusion of FUra. It is clear that—in addition to the prolonged exposure of tumor tissues to the active metabolite of FUra derived from S-1, persistent induction of apoptosis and the inhibition of TS in tumor are also contributors to the effectiveness of S-1.

The therapeutic efficacy of S-1 was superior to that of FUra alone as well as when it was modulated by LV (32). Complete (100%) CR was achieved in rats bearing advanced colorectal cancer treated with S-1 but not in rats that received FUra/LV treatment with various schedules of drug administration (32).

Because catabolism into 5-fluoro-dihydrouracil and α-fluoro-β-alanine represent a major route of FUra clearance, inactivation of the catabolic enzyme DPD should reduce host toxicity including neurological toxicity. Results reported by Davis et al. (34) documented that neurological toxicity associated with FUra in dogs can indeed be elevated by EU via inhibition of DPD.

To provide the basis for high tumor response to S-1 in this model system, the induction of apoptosis, the decrease in mitotic index (inhibition of cell proliferation), and the degree and duration of TS inhibition in tumor tissues were investigated at various times after drug treatment. Higher levels of apoptosis were observed after S-1 or FUra treatment (Fig. 4A). These drug-induced apoptotic cells were located differently compared

Table 2  Toxicity profiles of S-1, FT, and FUra at their MTDs

Each treatment group had 16–32 rats from 4–8 independent experiments.

<table>
<thead>
<tr>
<th>MTD (mg/kg/d)</th>
<th>Incidence (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>7-day schedule</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-1 (31.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11.8 ± 2.4b</td>
</tr>
<tr>
<td>FT (200)</td>
<td>25</td>
<td>0</td>
<td>100</td>
<td>13.2 ± 2.5</td>
</tr>
<tr>
<td>FUra (25)</td>
<td>38</td>
<td>25</td>
<td>25</td>
<td>15.4 ± 3.0</td>
</tr>
<tr>
<td>28-day schedule</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-1 (22.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13.4 ± 3.1</td>
</tr>
<tr>
<td>FT (150)</td>
<td>75</td>
<td>0</td>
<td>100</td>
<td>15.6 ± 2.2</td>
</tr>
<tr>
<td>FUra (12.5)</td>
<td>25</td>
<td>0</td>
<td>25</td>
<td>15.8 ± 2.6</td>
</tr>
</tbody>
</table>

a MWL, maximum weight loss of percentage of pretreatment body weight.
b Mean ± SD.

Table 3  Therapeutic index of S-1, FT, and FUra in rats bearing advanced colorectal cancer

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Schedule</th>
<th>MTD (mg/kg/d)</th>
<th>MED (mg/kg/d)</th>
<th>Therapeutic index (MTD/MED)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-1</td>
<td>7-day</td>
<td>31.5</td>
<td>6</td>
<td>5.3</td>
</tr>
<tr>
<td>FT</td>
<td>7-day</td>
<td>200</td>
<td>200</td>
<td>1.0</td>
</tr>
<tr>
<td>FUra</td>
<td>7-day</td>
<td>25</td>
<td>20</td>
<td>1.25</td>
</tr>
<tr>
<td>S-1</td>
<td>28-day</td>
<td>22.5</td>
<td>&gt;150</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>FT</td>
<td>28-day</td>
<td>150</td>
<td>&gt;150</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>FUra</td>
<td>28-day</td>
<td>12.5</td>
<td>&gt;12.5</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

Table 4  Plasma concentration of FUra in rats after administration of S-1, FT, and FUra

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>Plasma FUra (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 h*</td>
<td>8 h</td>
</tr>
<tr>
<td>S-1</td>
<td>p.o.</td>
<td>22.5</td>
<td>2.3 ± 0.2b</td>
</tr>
<tr>
<td>FT</td>
<td>p.o.</td>
<td>150</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>FUra</td>
<td>24-h infusion</td>
<td>25</td>
<td>2.0 ± 0.3</td>
</tr>
</tbody>
</table>

a S-1 and FT after the initiation of a single oral dose, and FUra during infusion.
b Mean ± SD. The data represent 3–6 independent samples (different animals) for each treatment group.
with spontaneous apoptotic cells, i.e., in nonnecrotic regions of tumor tissue (Fig. 3B). Spontaneous and drug-induced apoptotic cells morphologically are the same, but they may be distinguished based on their localization. There was a correlation between drug-induced apoptosis and tumor response. When tumors were retreated with a second dose of S-1 (22.5 mg/kg) after 72 h (when the level of apoptosis was similar to that in the control group), a high level of apoptosis was induced (data not shown). No apoptosis was detected when a noneffective dose of S-1 (4.5 mg/kg, data not shown) was given. Collectively, these data indicate that apoptosis is an important determinant of response to S-1 in this model system.

In preclinical and clinical studies, it has been demonstrated that enhanced apoptosis by FUra in tumor cells was closely associated with its antitumor activity in human gastric and colon tumor xenografts (35) and in patients with gastric carcinoma (36). In our studies, although significant apoptotic responses were induced in tumor tissues after both S-1 and FUra treatment, the high and sustained apoptosis was only observed with S-1 treatment in parallel tumor growth inhibition (Fig. 4). Despite the fact that infusion of FUra induced an apoptotic response comparable to that induced by S-1 at 24 h after initiation of drug treatment, the level of apoptosis rapidly decreased to that of control at 24 h after infusion (Fig. 4A). The apoptosis after S-1

Fig. 3 Characteristic distribution of apoptotic cells and bodies (arrows) in tumor sections after H&E staining. A, untreated tumor with spontaneous apoptotic cells gathered at the edge of necrotic area. B, drug-induced apoptotic cells in nonnecrotic portion of the tumor (at 24 h after S-1, 22.5 mg/kg, p.o. × 1). The presence of apoptotic cells has been confirmed by terminal deoxynucleotidyl transferase (Tdt)-mediated nick end labeling (TUNEL) immunohistochemistry.

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treatment persisted and was associated with the cessation of proliferation among tumor cells, as evidenced by the decrease in mitotic index (Fig. 4B). The transient induction of apoptosis after FUra treatment may not be sufficient to produce tumor regression in some tumors. TS inhibition induced by S-1 or FUra also differ in that S-1 (single oral dose) caused a greater suppression of free TS than FUra (24-h infusion; Fig. 5). Thus, induction of high and sustained apoptosis, suppression of cell proliferation, and greater inhibition of free TS by S-1 may be the basis for its superior antitumor activity (100% cure) in this model system.

In brief, the data reported herein demonstrated that the inactivation of a FUra degradative enzyme, DPD, with concurrent inhibition of FUra incorporation into normal tissues RNA and with selective tumor tissue activation of FT to FUra can lead to a highly active and selective treatment for colon cancer in this model system. These data suggest that this approach could offer a therapeutic advantage in the treatment of colorectal cancer and other malignancies amenable to fluoropyrimidine therapy. Unlike other fluoropyrimidines, S-1 is highly effective at lower doses than the MTD and offers the possibility of greater therapeutic selectivity in combination with other drugs with different mechanisms of action and toxicity profiles. For example, the combination chemotherapy using drugs directed against targets other than TS, e.g., CPT-11 (a topoisomerase I inhibitor) or cisplatin (a DNA cross-link agent) with limited overlapping toxicity, could offer the hope to maximize antitumor activity with manageable toxicity. However, the validity of this concept must be verified clinically.

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References


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