Therapeutic Effect of 1 M Tegafur-0.4 M 5-Chloro-2,4-dihydroxypyridine-1 M Potassium Oxonate (S-1) on Liver Metastasis of Xenotransplanted Human Colon Carcinoma

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S-1 [1 M tegafur (FT)-0.4 M 5-chloro-2,4-dihydroxypyridine (CDHP)-1 M potassium oxonate (Oxo)], was developed as a new oral antineoplastic agent based on biochemical modulation of fluorouracil (5-FU) by CDHP and Oxo. The therapeutic effect of S-1 on human colon cancer xenografts (TK-13) with high metastatic potential to the liver was evaluated. Small pieces of TK-13 were sutured into the cecal wall of 52 nude mice, and the animals were randomly divided into 3 groups [control ($n=17$), UFT (combination of 1 M FT and 4 M uracil) ($n=18$) and S-1 ($n=17$)]. S-1 or UFT was administered orally at an equitoxic dose (S-1, 7.5 mg/kg; UFT, 17.5 mg/kg as FT) for 37 consecutive days beginning 10 days after the transplantation. S-1 showed higher tumor growth inhibition than UFT ($P<0.05$) and also showed a significant anti-metastatic effect on liver metastasis, while UFT did not. Liver metastasis developed in only 2 of the 17 mice (12%) in the S-1 group, whereas it developed in 9 of the 17 (53%) and 7 of the 18 (39%) in the control and UFT group, respectively. Analysis of AUC (area under the curve) revealed that S-1 yielded higher 5-FU levels in both tumor tissue (1.6 times) and plasma (2.5 times) than UFT. These results suggest that S-1 will show a higher clinical therapeutic effect against human colorectal cancer than UFT.

Key words: S-1 — Colon cancer — Liver metastasis — UFT

Fluorouracil (5-FU) has been widely used clinically in the treatment of solid tumors.^{1,2} Many attempts have been made to develop new superior 5-FU derivatives, but sufficient therapeutic efficacy has not yet been obtained. Enhancement of the therapeutic effect without any increase in adverse effects would be beneficial for patients with digestive organ malignancies, including colorectal carcinoma, because 5-FU derivatives are commonly chosen as treatment for them.

S-1 is a new oral antineoplastic agent based on biochemical modulation of 5-FU. It is a combination of tegafur (FT), 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate (Oxo) in a molar ratio of 1:0.4:1.³ Neither CDHP nor Oxo has antitumor activity itself, and they play a role as modulators. CDHP competitively inhibits dihydroxypyridine dehydrogenase, an enzyme that degrades 5-FU,⁴ resulting in prolonged maintenance of 5-FU concentration in the circulation. Oxo is mainly distributed in the gastrointestinal tract and acts to reduce the toxicity of 5-FU.⁵ S-1 showed a better therapeutic effect in various rat tumors and human xenografts than other oral fluoropyrimidines.^{6,7}

Orthotopic transplantation of human tumor xenografts has been widely used to produce “natural” metastasis in

nude mice as a model analogous to cancer patients.^{8–10} We have produced liver metastasis of human colon cancer in several xenografts¹¹ and found that one of them, TK-13, has high metastatic potential to the liver.

We therefore applied this xenograft to evaluate the anti-tumor and antimetastatic effects of S-1 and UFT. Furthermore, to clarify the pharmacological characteristics of S-1, 5-FU levels in the tumor tissue and plasma after administration of S-1 were compared with those after administration of UFT.

UFT is composed of FT (prodrug of 5-FU) and uracil in a molar ratio of 1:4. FT is a form of 5-FU, and is gradually converted to 5-FU. UFT has been shown to be more effective experimentally than FT and 5-FU¹² and is widely used as an oral antineoplastic agent in Japan.^{13–16}

MATERIALS AND METHODS

Preparation of agents FT, CDHP, and Oxo were synthesized by Taiho Pharmaceutical Co. (Tokyo). Uracil was purchased from Yamasa Co. (Chiba). All other chemicals used were standard commercial products of the highest grade. S-1 was prepared by mixing FT, CDHP, and Oxo in a molar ratio of 1:0.4:1. S-1 was dissolved in a 0.5% (w/v) hydroxypropylmethylcellulose (HPMC) solution. UFT was suspended in a 0.5% HPMC solution, because of its insolubility. Since the active component in both S-1 and

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UFT is FT, only the amount of FT in both agents was considered in calculating the dosage. The dose of each agent for *in vivo* treatment was set at the equitoxic level, i.e., S-1, 7.5 mg/kg/day, and UFT, 17.5 mg/kg/day, in a volume of 0.1 ml/10 g, and was administered once daily. The equitoxic dose of each agent was determined in a preliminary study.

Animals Male BALB/c *nu/nu* mice, obtained from Clea Japan (Tokyo) at 4 weeks of age were used for this study at 5 weeks of age.

Human colon cancer Human colon cancer xenograft TK-13 was established in our department from a metastatic liver lesion of a 44-year-old Japanese female with sigmoid colon cancer, and was maintained by passage in nude mice (BALB/c *nu/nu* males) for about 3 years. The tumor was a well-differentiated adenocarcinoma that maintained its original features after inoculation.

Experimental protocol The tumor transplantation and evaluation methods have already been reported.¹⁷⁾ Briefly, small pieces of TK-13 tumor tissue (5 mm in diameter and about 100 mg in weight) were resected from subcutaneous tumors in the exponential growth phase, and the pieces were sutured to the wall of the cecum of nude mice with 6-0 Dexon (Davis-Geck, Manati, PR) after removal of the serosa. The mice were divided into 3 groups: a control group ($n=17$), a UFT group ($n=18$) and an S-1 group ($n=17$). Animals in the UFT group received intravenously 17.5 mg/kg of UFT solution and those in the S-1 group received 7.5 mg/kg of S-1 solution daily from day 10

after transplantation. Animals in the control group received saline solution. On day 47 after transplantation, all mice were weighed and killed to evaluate transplanted tumor growth and liver metastasis. The cecal tumors were removed and weighed and the metastatic foci of the liver were counted by careful macroscopic examination. The specimens were examined histologically in the usual manner.

Determination of plasma 5-FU levels Three weeks after orthotopic transplantation of TK-13, pharmacokinetic analysis of S-1 was performed. Mice were killed and tumor tissues and plasma were collected 30 min, 1 h, 2 h, 4 h, 8 h and 24 h ($n=5$, each) after oral administration of S-1 (7.5 mg/kg) or UFT (17.5 mg/kg). Plasma and tumor samples were mixed with methanol, and the coagulated protein was removed by centrifugation. 5-FU determination was based on the method of Marunaka *et al.*¹⁸⁾ and was performed with a gas chromatograph-mass spectrometer (Models JGS-20-kp and JMS-D 300 (JEOL, Tokyo)). The pharmacokinetic parameter AUC (area under the curve) was calculated by the trapezoidal method.

Statistics Dunnett's *t* test and the χ^2 test were used for statistical analysis. A *P* value of less than 0.05 was considered significant.

RESULTS

Inhibitory effect of S-1 on tumor growth The microscopic appearance of the transplanted tumor and the macroscopic appearance of the liver metastatic foci are shown

Fig. 1. Histological appearance of the TK-13 tumor transplanted in the cecum. Well differentiated adenocarcinoma, which invaded the muscle layer of the cecum, was demonstrated. Scale bar = 100 μ m.

Fig. 2. Macroscopic appearance of liver metastasis in the control group at the experimental termination. Arrows indicate the metastatic foci.

Table I. Inhibitory Effect of S-1 on Tumor Growth

Group	Dose (mg/kg)	<i>n</i>	Tumor weight (Mean±SD)	Tumor growth inhibition (%)
Control		17	2.11±0.778	—
UFT	17.5	18	2.28±0.614	-8.0
S-1	7.5	17	1.46±0.668*	30.6

* Significantly different from the control and UFT groups at $P<0.05$.

in Fig. 1 and Fig. 2, respectively. Table I shows the therapeutic effects of S-1 and UFT. Administration of 17.5 mg of UFT had no effect on tumor growth, whereas 7.5 mg of S-1 showed a significant inhibitory effect. Fig. 3 shows body weight in the three groups at the end of the experiment; no significant difference was observed among the three groups. The equitoxicity of the two agents was well demonstrated.

Inhibitory effect of S-1 on liver metastasis Table II shows the numbers of mice with liver metastasis and metastatic foci in the three groups. A significant inhibitory effect of S-1 on liver metastasis was demonstrated compared with the control group.

5-FU levels in plasma after administration of S-1 Fig. 4 shows the plasma FT and 5-FU levels at various times after administration of the equitoxic dose of both agents,

Fig. 3. Body weight in the control group ($n=17$), the UFT group ($n=18$) and the S-1 group ($n=17$) at the experimental termination. There was no significant difference among the three groups.

and Table III showed the AUC of FT and the 5-FU concentration. Although both tumor and plasma FT levels after S-1 administration were significantly lower, both

Table II. Inhibitory Effect of S-1 on Liver Metastasis

Group	<i>n</i>	Liver metastasis (No. of mice with metastasis)	Metastatic foci (Mean±SD)
Control	17	9	7.9±11.5
UFT	18	7	5.3±2.9
S-1	17	2*	2.5

* Significantly different from the control group at $P<0.05$.

Table III. AUC of FT and 5-FU Concentration

Group	Plasma (ng×h/ml)		Tumor tissue (ng×h/g)	
	AUC of FT	5-FU conc.	AUC of FT	5-FU conc.
UFT	6509.2	383.4	4657.6	2968.2
S-1	2334.7	940.3	1731.6	4783.7

Fig. 4. FT and 5-FU levels in plasma and tumor tissue following oral administration of S-1 and UFT to TK-13-bearing mice. TK-13 tumor was orthotopically transplanted into the cecum of mice, and 7.5 mg/kg of S-1 or 17.5 mg/kg of UFT was orally given to the mice. At the indicated times, mice were killed and blood (A and C) and tumor (B and D) were removed to determine FT (A and B) and 5-FU (C and D) concentrations. Values are means±SD for 5 mice. ● S-1, 7.5 mg/kg; ○ UFT, 17.5 mg/kg.

tumor and plasma 5-FU levels were significantly higher than those after UFT administration.

DISCUSSION

The clinical response rate to 5-FU alone has been only 10–30%. One of the crucial factors in this clinical inade-

quacy of 5-FU is its rapid catabolism. Addition of uracil to FT (prodrug of 5-FU) increases the efficacy of 5-FU, because uracil inhibits the hepatic degradation of 5-FU.¹²⁾ CDHP, a new pyridine-based inhibitor of dihydropyridine dehydrogenase, is a 180 times more potent inhibitor of 5-FU degradation than uracil. Gastrointestinal toxicity is also a crucial factor in the clinical inadequacy of 5-FU.

It has been reported that Oxo is mainly distributed in the gastrointestinal tract after oral administration to Yoshida sarcoma-bearing rats, and that it inhibits the formation of 5-fluorouridine 5'-monophosphate and F-RNA from 5-FU in the small intestine and markedly reduces injury of the gastrointestinal tract and severe diarrhea without affecting the antitumor effect of UFT.⁵⁾ These findings suggest that Oxo could selectively decrease the anabolism and cytotoxicity of 5-FU in normal intestinal tissue. S-1 is composed of a suitable combination of an effector compound, FT, and CDHP and Oxo as biological modulators, and possesses a potent therapeutic effect and low toxicity, as demonstrated in the present study.

In a previous paper, the therapeutic effect of S-1 on tumor growth and lung metastasis of human colorectal adenocarcinoma cell line KM12C in nude rats was reported.⁶⁾ The therapeutic effect on liver metastasis was not examined, because liver metastasis does not develop in that model. However, the most frequent cause of death of patients with colorectal cancer is liver metastasis. In the present study, the therapeutic effects of S-1 on tumor growth and liver metastasis of human colon cancer xenograft, TK-13, were clearly demonstrated. The results of the analysis of the AUC for 5-FU demonstrated that S-1 administration maintained a high concentration of 5-FU in the tumor tissue, and a prolonged high concentration of 5-FU is expected to provide a superior inhibitory effect on tumor growth. The advantage of maintaining a high 5-FU

concentration has already been demonstrated clinically, because continuous infusion of 5-FU was superior to bolus injection of 5-FU.^{19, 20)}

Regarding the inhibitory effect on liver metastasis, it is possible that the decrease in the number of cancer cells in the transplanted tumor by S-1 may contribute to decreasing the likelihood that cancer cells will enter the circulation. In addition, the high concentration of 5-FU in the plasma may eliminate cancer cells from the circulation, so that few cancer cells reach the target organ. To our knowledge, this is the first report to demonstrate the inhibitory effect of oral antineoplastic agents on liver metastasis of a human colon cancer xenograft.

The excellent inhibitory effect of S-1 on liver metastasis shown in this study may contribute to the prolonged survival of patients with colorectal cancer. Although adverse effects, including hematotoxicity, have been observed in a clinical trial, the new oral antineoplastic agent S-1 can be expected not only to shrink the primary tumor, but also to inhibit liver metastasis of colorectal cancer.

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