SPANDIDOS PUBLICATIONS

Preclinical analysis of the antitumor efficacy of TS-1 using human uterine cervical cancer tumor xenografts

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Abstract. We investigated the antitumor activity of TS-1 in comparison with that of UFT and cisplatin (CDDP) against cervical cancer using xenografts of a human uterine cervical squamous cell cancer cell line, CaSki, transplanted into female Balb/cA JcL-nu mice. CaSki cell xenografts were prepared by subcutaneous (s.c.) implantation of $3x10^6$ cells/animal into the right dorsal region of the mice. The tumor volume was measured twice a week and the relative tumor volume (RTV) was calculated. We divided the animals into four groups according to the treatment administered; TS-1 (10 mg/kg orally, once daily for 14 consecutive days), UFT (24 mg/kg orally, once daily for 14 consecutive days), CDDP (7.6 mg/kg injected intravenously once on the 1st day) and control (no treatment) groups. The antitumor effects of the drugs were measured. On the 35th day after the completion of treatment, the mean tumor volume in the mice treated with TS-1 or CDDP changed from 132.873±11.783 mm^3 to 706.401±613.122 mm^3 and 133.809±19.366 mm^3 to 722.630±855.509 mm^3, respectively. The mean tumor volume in the groups treated with TS-1 or CDDP was significantly lower compared to that in the control group ($p<0.001$; one-tailed Student's t-test). The relative inhibition of the tumor growth was 65.31 in the TS-1 group, 48.31 in the UFT group and 64.51 in the CDDP group. We conclude that TS-1 administered orally for 14 consecutive days showed the highest antitumor activity.

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Abbreviations: CDDP, cisplatin; RTV, relative tumor volume; RI, relative inhibition of tumor growth; NAC, neoadjuvant chemotherapy; 5-FU, 5-fluorouracil; FT, tegafur; TP, thymidine phosphorylase; DPD, dihydropyrimidine dehydrogenase; FBAL, F-ß-alanine; CDHP, 5-chloro-2,4-dihydroxy-pyridine

Key words: TS-1, uterine cervical cancer, antitumor efficacy, preclinical study

Introduction

Treatment of advanced uterine cervical carcinoma has been mainly dependent on surgery and radiotherapy, however, recently improved efficacy of chemotherapy with multiple agents has been demonstrated (1-3) and its usefulness pointed out. As a result, chemotherapy has been administered not only as adjuvant therapy to surgery and radiotherapy, but also as neoadjuvant chemotherapy (NAC) before surgical treatment. 5-Fluorouracil (5-FU), an antitumor pyrimidine, has been frequently used clinically to treat patients with gastrointestinal, head and neck and uterine cancers. UFT is an oral fluoropyrimidine agent containing a masked form of 5-FU, prepared as a mixture of tegafur (FT) and uracil at a molar ratio of 1:4 (4). Conversion of tegafur to 5-FU is catalyzed by cytochrome P-450 in the hepatic microsomes (CYP2A6) and thymidine phosphorylase (TP) in other tissues. Thereafter, UFT is converted to FdUMP or FUMP in the tumor cells and inhibits the synthesis of DNA and RNA. On the other hand, the half-life of 5-FU in the blood is short (6-20 min), because the compound is rapidly inactivated by hepatic or intracellular dihydropyrimidine dehydrogenase (DPD) and excreted in the urine as F-ß-alanine (FBAL) (5). Thus, the activity of DPD is a major impediment to the maintenance of an effective concentration of 5-FU; however, the uracil moiety in UFT is a DPD-inhibitory fluoropyrimidine (6) that competitively inhibits DPD activity and suppresses the degradation of 5-FU, with a resultant increase in the plasma 5-FU level. Our previous study indicated that the tumor DPD activity in advanced cervical carcinoma is a determinant of the tumor sensitivity to UFT, suggesting an association between UFT therapy and the induction of apoptosis, and measurement of the tumor DPD activity before UFT therapy may possibly be used as an indicator of the tumor sensitivity to oral fluoropyrimidines (7).

TS-1 is a newly developed novel form of an FT-based antitumor agent (Fig. 1) consisting of 1 M FT (8), a pro-drug of 5-FU, 0.4 M 5-chloro-2,4-dihydroxy-pyridine (CDHP) (9), a potent inhibitor of DPD and 1 M potassium oxonate (Oxo) (10), an inhibitor of orotate phosphoribosyltransferase, which thereby acts as a protector against the gastrointestinal toxicity of 5-FU.

In this study, we investigated the antitumor activity of TS-1 in comparison with that of UFT and cisplatin (CDDP) against

cervical cancer using xenografts of a human uterine cervical squamous cell cancer cell line, CaSki, transplanted into female Balb/cA JcL-nu mice.

Materials and methods

TS-1 and UFT were products of Taiho Phamaceutical Co., Ltd (Tokyo, Japan). CDDP was provided by Bristol-Myers Squibb Co., Ltd (Tokyo, Japan).

Animals and cancer cells. Five-week-old Balb/cA JcL-nu female mice were purchased from CLEA Japan Inc. (Tokyo, Japan). The mice were kept in laminar air-flow units throughout the period of the therapeutic experiments and fed a sterilized pellet diet and autoclaved water. The human cervical squamous cancer cell line, CaSki, was provided by the Health Science Research Resource Bank (Osaka, Japan).

The handling of animals were performed appropriately in accordance with 'Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Notification No. 88, April 28, 2006 of the Prime Minister's Office)' and 'Guidelines on Methods of Sacrificing Laboratory Animals (Notification No.59, December 1, 2000 of the Ministry of Environment)'.

Antitumor experiments. In a preliminary experiment, we investigated the change in the tumor volume and implantation rate of CaSki cells in the mice by using $3x10^5$, $1x10^6$, $3x10^6$ and $1x10^7$ cells/animal, and based on the results, selected the cell density of $3x10^6$ cell/animal, which showed the highest rate of tumor growth, for the implantation (Fig. 2). Table I shows the human cancer cell xenograft, the drugs and the treatment schedules used in this study. Human cancer cell xenografts were prepared by subcutaneous (s.c.) implantation of $3x10^6$ cells/animal of the tumor cell line into the right dorsal region of the mice, which weighed 22.22±0.85-22.58-0.79 g at the time of the experiment.

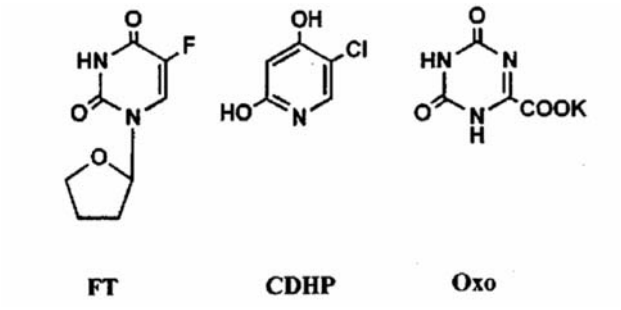

Figure 1. Chemical structure of TS-1. TS-1 consists of FT (Tegafur) as an effector of 5-FU and two modulators, CDHP (5-chloro-2,4-dihydroxy-pyridine) and Oxo (potassium oxonate).

The tumor volume (mm^3) [(major axis)x(minor axis)x1/2] was measured twice a week throughout the experiment and the relative tumor volume (RTV) was calculated as RTV = (mean tumor volume during or after treatment)/(mean tumor volume at the start of the treatment).

We divided the mice into four treatment groups: the TS-1, UFT, CDDP and control groups. When the tumor volume in the tumor-bearing mice reached 130 mm^3, we selected mice so that the average tumor volume in each treatment group was the same. Rationale for dose selection: to the tumor-bearing mice subcutaneously implanted with Sarcoma-180, Leis lung carcinoma, and colon 26 adenocarcinoma, TS-1 and UFT were administered once daily for 9 consecutive days, and the doses of 50% tumor growth inhibition (ED50) was determined in the control groups. As the result, ED50 was 9.3 to 19.3 mg/kg/day for TS-1, and 24.1 to 34.8 mg/kg/day for UFT. Consequently, for the present study, 14-days oral administration was selected for TS-1 and for UFT, at the doses of 10 mg/kg/day for TS-1, and 24 mg/kg/day for UFT. For the nude mice implanted with UCC-8-JCK, uterine cervical cancer, a single dose of CDDP was intravenously injected at the dose of 7.6 mg/kg, 1/2 quantity of LD50 value of CDDP. As a result, relative

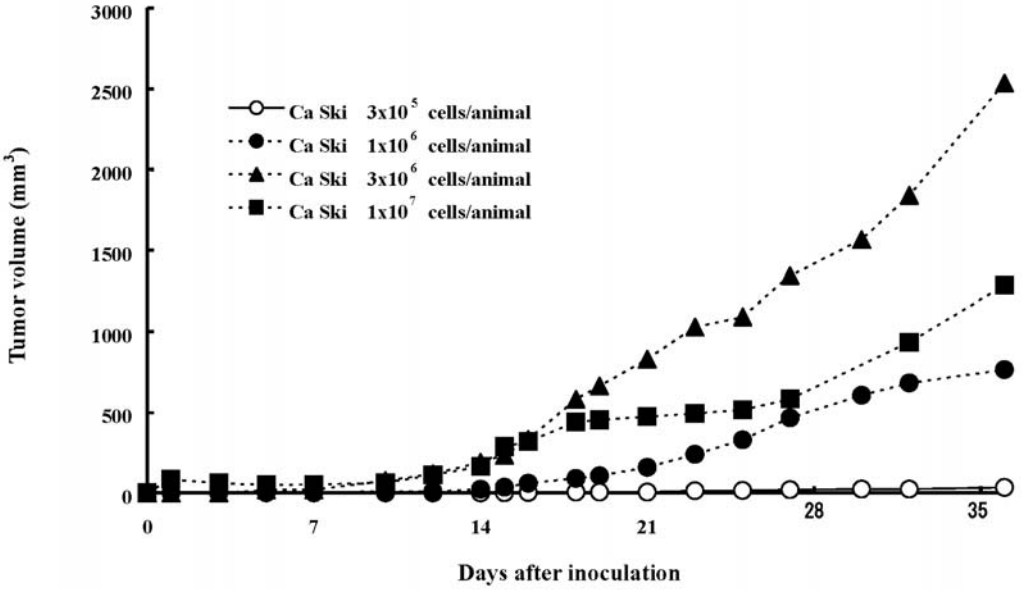

Figure 2. Changes in the tumor volume in the CaSki-tumor-bearing mice. Each point represents the mean value of the tumor volume. The experiment was replicated five or six times.

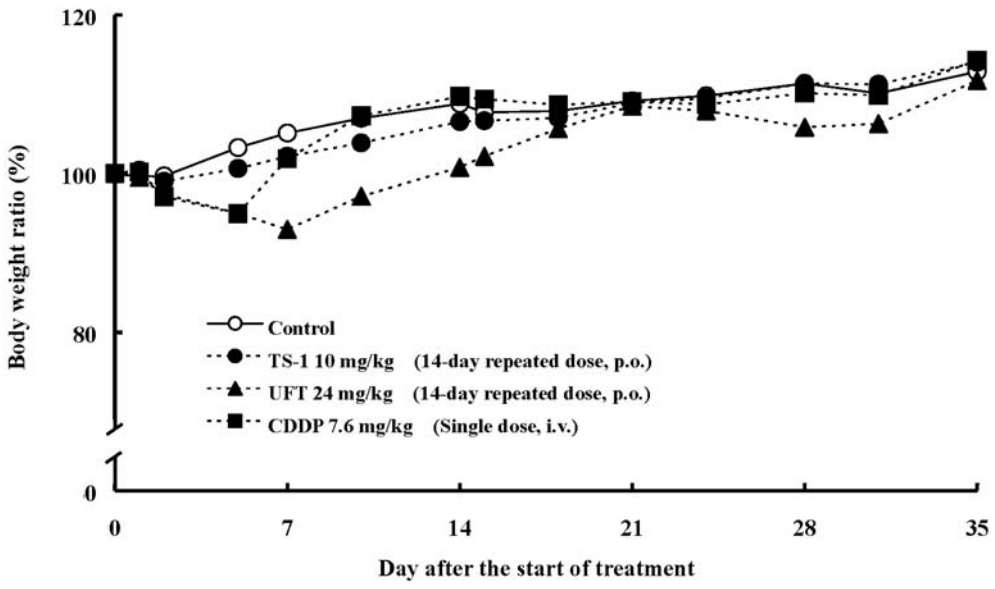

Figure 3. Body weight ratio of the CaSki-tumor-bearing mice treated with TS-1, UFT and CDDP. Each point represents the mean value of the body weight ratio. The experiment was replicated ten times. CDDP, cisplatin.

Table I. Animal, tumor and treatment schedules.

Animal	Cell line	Tumor type	Drug	Dose (mg/kg)	Schedule
Nude mice	CaSki	Uterine Cervix	TS-1	10	Daily x 14 (oral)
			UFT	24	Daily x 14 (oral)
			CDDP	7.6	Day 1st (i.v.)

CDDP, Cisplatin and i.v., intravenous.

inhibition of tumor growth was 61%. Consequently, for the present study, a single dose intravenous administration at the dose of 7.6 mg/kg was selected for CDDP.

The day the drug administration commenced was designated as the 1st day. TS-1 10 mg/kg and UFT 24 mg/kg were administered orally once daily for 14 consecutive days via the feeding tube. CDDP was injected intravenously at the dose of 7.6 mg/kg once on the 1st day (Table I). The drugs were administered to the mice for 14 days starting at 14-20 days after the tumor implantation. The antitumor effect of the drugs was measured using the following equation: relative inhibition of tumor growth (RI, %) = [1-(mean RTV in the drug-treated group/mean RTV in the control group)]x100.

We observed the general appearance and activity and checked on the general condition of the CaSki-tumor-bearing mice more than once a day before, during and after the drug administration. The body weight of the CaSki-tumor-bearing mice was measured before the start of treatment, on the 1st, 2nd, 5th, 10th and 14th day (during the treatment), and on the 15th, 18th, 21st, 24th, 31st and 35th day (after the completion of treatment). Host weight was calculated as the body weight minus the tumor volume. The body weight ratio (%) was calculated as follows: Body weight ratio = (body weight during or after completion of treatment/body weight before the start of treatment)x100.

Statistical analysis. The significance of differences between the animal groups and/or drugs was assessed using one-tailed Student's t-test or the one-tailed Aspin-Welch t-test.

Results

In this experiment, no significant differences in the body weight change or host weight change in the CaSki-tumor-bearing mice were noted among the TS-1, UFT, CDDP and control groups.

Tables IIA and IIB show the general appearance of the CaSki-tumor-bearing mice treated with TS-1, UFT or CDDP and the control group. As compared with the mice in the control group and the TS-1-treated group, the animals treated with UFT or CDDP suffered from pasty or soft stools from the 2nd to the 18th day. In the group treated with UFT as compared with the other groups, the body weight ratio was significantly decreased from the 8th to the 14th day ($P<0.01$), to increase gradually thereafter (Fig. 3). Only one mouse, which had received CDDP, died on the 34th day (after completion of treatment).

Fig. 4 shows the tumor volumes (mm^3) in the CaSki-tumor-bearing mice treated with TS-1, UFT or CDDP and the control group. Each point represents the mean value of the tumor volume. On the 35th day (after completion of treatment),

Table IIA. General appearance of the CaSki-tumor-bearing mice treated with TS-1, UFT and CDDP.

Group	Findings	Grouping	Days after the start of treatment																			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Control	Number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	No abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mass in the right dosal region	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Pasty stool or soft stool	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Jaundice	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Dead	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TS-1 10 mg/kg																						
(14-day consecutive dosing, p.o.)	Number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	No abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mass in the right dosal region	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Pasty stool or soft stool	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Jaundice	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Dead	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UFT 24 mg/kg																						
(14-day consecutive dosing, p.o.)	Number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	No abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mass in the right dosal region	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Pasty stool or soft stool	0	0	5	10	10	9	9	9	7	7	7	6	6	6	4	3	1	1	1	0	0
	Jaundice	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Dead	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CDDP 7.6 mg/kg																						
(single dose, i.v.)	Number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	No abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mass in the right dosal region	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Pasty stool or soft stool	0	0	7	10	10	7	7	3	3	1	0	0	0	0	0	0	0	0	0	0	0
	Jaundice	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Dead	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

CDDP, Cisplatin; p.o., oral and i.v., intravenous.

Table IIB. General appearance of the CaSki-tumor-bearing mice treated with TS-1, UFT and CDDP.

Group	Findings	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Control	Number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	No abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mass in the right dosal region	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Pasty stool or soft stool	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Jaundice	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Dead	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TS-1 10 mg/kg																
(14-day consecutive dosing, p.o.)	Number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	No abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mass in the right dosal region	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Pasty stool or soft stool	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Jaundice	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Dead	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UFT 24 mg/kg																
(14-day consecutive dosing, p.o.)	Number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	No abnormal findings	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2
	Mass in the right dosal region	10	10	10	10	10	10	10	10	10	8	8	8	8	8	8
	Pasty stool or soft stool	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Jaundice	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Dead	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CDDP 7.6 mg/kg																
(single dose, i.v.)	Number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	No abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mass in the right dosal region	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Pasty stool or soft stool	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Jaundice	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
	Dead	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

CDDP, Cisplatin; p.o., oral and i.v., intravenous.

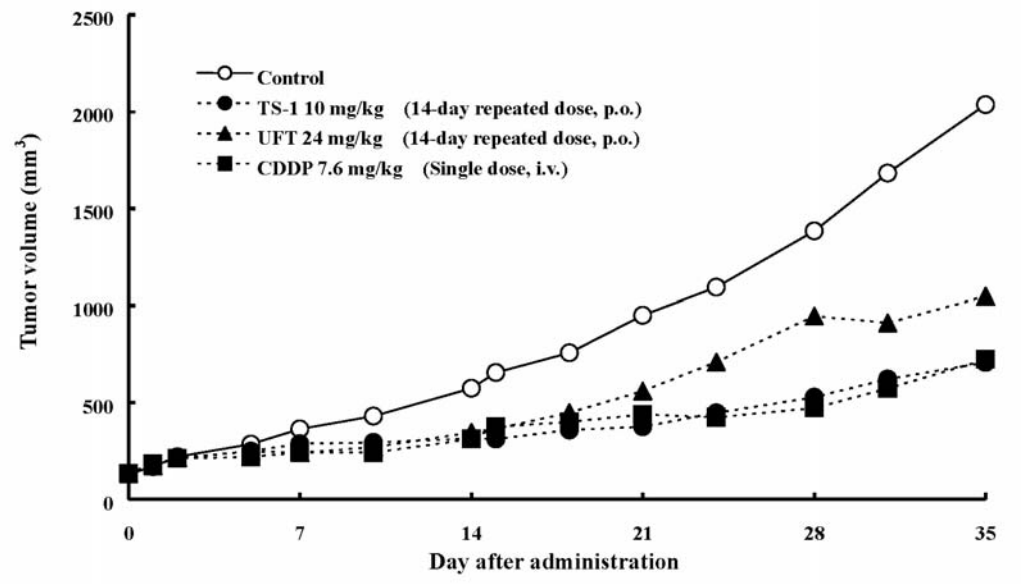

Figure 4. Tumor volume in the CaSki-tumor-bearing mice treated with TS-1, UFT and CDDP. Each point represents the mean value of the tumor volume. The experiment was replicated ten times. CDDP, cisplatin.

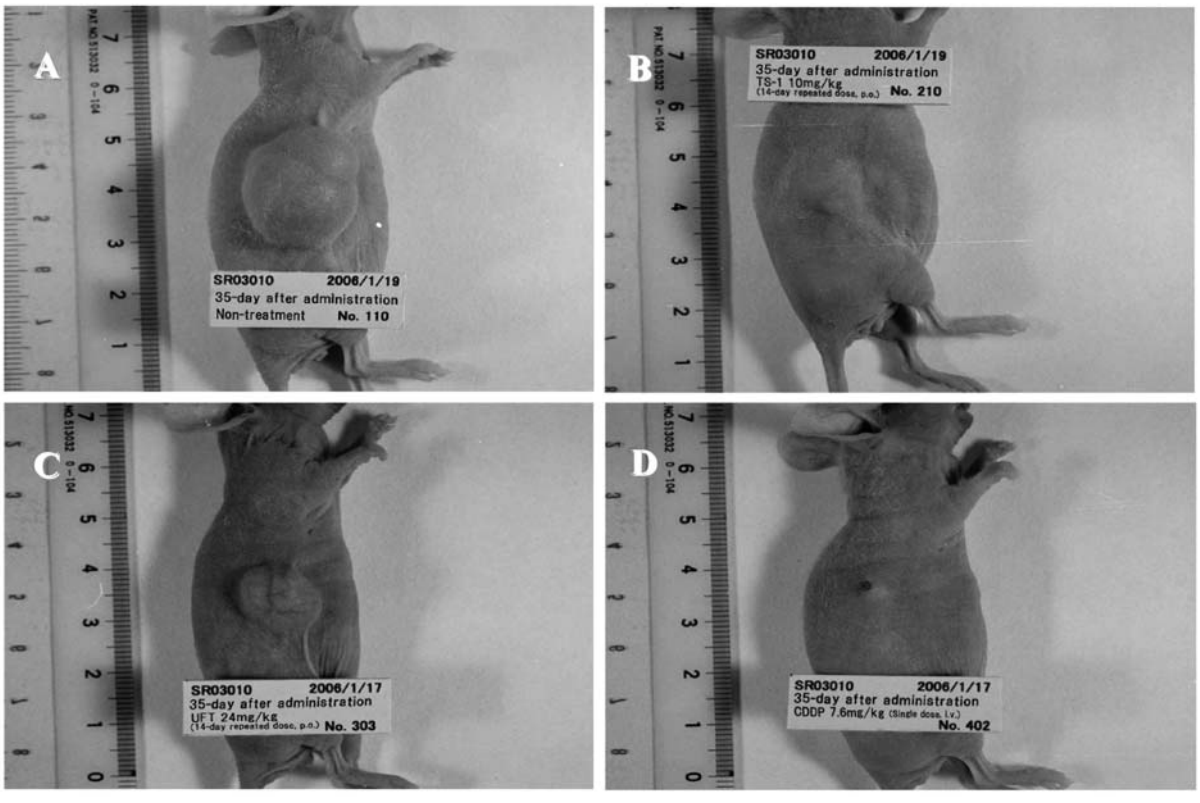

Figure 5. The external appearance of mice with tumor. (A) Thirty-fifth day (after completion of treatment). Control. Animal no. 110; (B) 35th day (after completion of treatment). TS-1 10 mg/kg (14-day consecutive dosing, p.o.). Animal no. 210; (C) 35th day (after completion of treatment). UFT 24 mg/kg (14-day consecutive dosing, p.o.). Animal no. 303; (D) 35th day (after completion of treatment). CDDP 7.6 mg/kg (single dose, i.v.). Animal no. 402.

the mean tumor volume in the mice treated with TS-1 or CDDP had increased from 132.873±11.783 mm^3 to 706.401±613.122 mm^3 and 133.809±19.366 mm^3 to 722.630±855.509 mm^3, respectively. Thus, on the 35th day (after treatment completion), the mean tumor volume in the groups treated with TS-1 or CDDP was significantly lower as compared with that in the control group ($P<0.001$; one-tailed Student's t-test). The mean tumor volume in the mice treated with UFT was also lower than that in the control group ($P<0.005$; one-tailed Student's t-test) (Fig. 5).

Table III shows that relative inhibition of the tumor growth among the groups treated with TS-1, UFT and CDDP. On the 35th day (after treatment completion), the relative inhibition of tumor growth was 65.31 in the TS-1 group, 48.31 in the UFT

Table III. Relative inhibition of the tumor growth in the CaSki-tumor-bearing mice treated with TS-1, UFT and CDDP.

Group	No. of animals	Day after start of treatment												
		1	2	5	7	10	14	15	18	21	24	28	31	35
TS-1 10 mg/kg (14-day consecutive dosing, p.o.)	10	6.10	3.43	12.57	20.80	31.68	45.16	52.36	52.73	60.59	59.11	61.85	63.07	65.31
UFT 24 mg/kg (14-day consecutive dosing, p.o.)	10	2.20	1.82	13.16	33.78	37.29	39.77	44.27	40.84	41.16	35.14	31.60	45.86	48.57
CDDP 7.6 mg/kg (single dose, i.v.)	10	7.86	3.48	23.14	33.04	43.74	45.73	42.66	47.06	53.92	61.27	65.87	65.98	64.51

group and 64.51, in the CDDP group. From the above results, it was concluded that TS-1 administered orally for 14 consecutive days yielded the best antitumor activity.

Discussion

One of the important disadvantages of 5-FU is its rapid degradation *in vitro* catalyzed by the liver DPD. Our previous study indicated that the tumor DPD activity is a determinant of the tumor sensitivity to 5-FU in uterine cervical cancer, and the tumor DPD activity measured prior to the start of treatment may be used as an indicator of the sensitivity to oral fluoropyrimidines (7).

The uracil moiety in UFT is a DPD-inhibitory fluoropyrimidine (6), which competitively inhibits DPD activity and suppresses the degradation of 5-FU, with a resultant increase of the plasma 5-FU level. The response rate of advanced cervical carcinoma to UFT alone has been reported to be 16.0% (4/25). In regard to the relationship of the tumor histology on the tumor sensitivity to chemotherapeutic agents, it has been reported that the response rate is 16.7% in patients with large-cell non-keratinized squamous cell carcinoma (12).

UFT has long been used for adjuvant chemotherapy in patients undergoing surgery and radiotherapy. Studies including a multicenter trial in Japan have shown that the addition of UFT can significantly improve the disease-free survival in patients receiving radiotherapy (13), indicating that it may also have a recurrence-preventing effect.

The novel oral fluoropyrimidine TS-1 was developed as a mixture of FT, CDHP, a potent and reversible inhibitor of DPD, and Oxo, which characteristically protects against the gastrointestinal toxicity of 5-FU (11). As a result, high plasma 5-FU levels are maintained for a prolonged period of time after oral administration of TS-1 to yield potent antitumor activity with low gastrointestinal toxicity in experimental murine tumor models (14,15).

In this study, we investigated the antitumor activity of TS-1 against the human cervical squamous cell cancer cell line, Caski, xenografts transplanted into female Balb/cA JcL-nu mice to predict its clinical efficacy in comparison with that of UFT and CDDP. TS-1 showed excellent antitumor activity against the human cervical cancer cells as compared with that of UFT and nearly equivalent anti-tumor activity to that of CDDP. Although we did not investigate the pharmacokinetic behavior of TS-1, UFT or CDDP in this experiment, we speculate that the prolonged retention of 5-FU in the blood is related to the potent antitumor activity of the drug (16,17) as compared with that of UFT (Table III).

In one clinical investigation, TS-1 showed excellent antitumor activity against stomach (17,18), colorectal (19), breast (20), lung (21) and head and neck cancers (22), with response rates of 44-49, 35.5, 42.0, 22.0 and 28.8%, respectively. Thus, the addition of chemotherapy in the treatment of advanced cervical cancer has improved the survival by controlling both the primary lesion and the distant metastases. Previously, neoadjuvant chemotherapy with a platinum-based regimen for operable cervical cancer yielded high response rates, ranging from 40 to 82% (23-25). In this experiment, the antitumor activity of TS-1 was excellent, and

the relative inhibition of tumor growth in the CaSki-tumor-bearing mice was equal to or greater than that obtained with CDDP.

We, therefore, conclude that TS-1 may be an effective agent both for adjuvant chemotherapy and neoadjuvant chemotherapy in cases of advanced uterine cervical cancer.

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