Effect of combination of TS-1 and low-dose cisplatin on sarcoma-180 mouse sarcoma

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TS-1 contains tegaful (FT), 5-chloro-2,4-dihydroxypyridine (CDHP; an inhibitor of 5-fluorouracil (5-FU) degradation) and potassium oxonate (Oxo; an inhibitor of 5-FU assimilation mainly in the digestive tract) in a molar ratio of 1:0.4:1. We evaluated the combination of TS-1 and low-dose cisplatin on mouse sarcoma. Male ddy strain mice at 6 weeks of age were s.c. transplanted with 5×10^6 sarcoma-180 (S-1800) cells and divided into groups of seven animals each: Group A, no treatment; Group B, 5-FU alone by continuous i.p. infusion of 10 mg/kg with a minipump (Alzet); Group C, TS-1 10 mg/kg p.o. alone; Group D, cisplatin 0.2 mg/kg i.p. alone; Group E, B + D; Group F, C + D. Treatment was given for 5 days. Antitumor activity was evaluated on the basis of the tumor weight on day 8, and white blood cell count, red blood cell count, platelet count, BUN, GOT and GPT were determined to detect adverse effects. Tumor weights (g, mean ± SD) were 0.54 ± 0.15 in Group A, 0.52 ± 0.17 in Group B, 0.34 ± 0.05 in Group C, 0.46 ± 0.12 in Group D, 0.34 ± 0.07 in Group E and 0.16 ± 0.03 in Group F. There were no noticeable adverse effects. The combined TS-1 + cisplatin regimen showed considerably enhanced antitumor activities since sarcomas were significantly ($p < 0.05$) decreased as compared with tissue. Mean AUC_{0-12} (ng/ml·h) estimated in the groups receiving 5-FU + cisplatin or TS-1 alone was measured to calculate AUC_{0-12} by the trapezoidal rule. 5-FU concentrations in blood and tumor from blood concentration data were 435 in Group B, 2651 in Group C, 343 in Group E and 1538 in Group F, while mean AUC_{0-12} (ng/g·h) estimated from tumor tissue concentration data were 345 in Group B, 3548 in Group C, 324 in Group E and 2020 in Group F. Cisplatin acted as a modulator of 5-FU, suggesting clinical benefits of the combination of TS-1 and low-dose daily cisplatin. *Anti-Cancer Drugs* 14:475–479

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Introduction

The combination of protracted continuous i.v. infusion of 5-FU and low-dose cisplatin (low-dose FP therapy) has been widely used with good clinical results in advanced or recurrent solid cancers of the stomach, esophagus, rectum and colon, head and neck, lung, breast, liver, and uterine cervix [1–10]. The mechanism of action of this combination therapy has been investigated [11–13]. It has been suggested that the combination is as effective as or more effective than conventional chemotherapy regimens, and that it improves patient quality of life (QoL) by reducing adverse reactions such as diarrhea, stomatitis, anorexia and hematopoietic disorders. A protracted chemotherapy and good compliance possibly lead to improved survival. At present, however, the chemotherapy is not always satisfactory in view of the response rate. Continuous i.v. infusion has been recommended as an optimal mode of administration for 5-FU [14,15], but places burdens on patients since it requires them to be hospitalized and to carry infusion pumps with them. Efforts were directed toward preparing an oral formulation with increased antitumor activity and reduced gastrointestinal toxicity so as to improve patient QoL; the novel oral anticancer drug TS-1 was thus developed. This is an oral fluoropyrimidine with enhanced efficacy and decreased toxicity that follows a new concept for cancer chemotherapy—the 5-FU self-rescuing concept (SRC). TS-1 is an oral anticancer drug containing tegaful (FT), a prodrug of 5-FU, and two modulators: 5-chloro-2,4-dihydroxypyridine (CDHP), which is an inhibitor of 5-FU degradation and keeps drug levels in blood and tumor tissue high for a protracted time, and potassium oxonate (Oxo), which specifically inhibits gastrointestinal toxicity of 5-FU. The molar ratio of the three components is 1:0.4:1 [16–18]. TS-1 is a drug that satisfies the SRC [19].

This study was undertaken to evaluate antitumor activity and adverse effects of TS-1 plus low-dose cisplatin in

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comparison with continuous i.p. infusion of 5-FU plus low-dose cisplatin, and to demonstrate the effectiveness of this oral preparation in tumor-bearing mice.

Materials and methods

Animals

Male mice of the ddy strain, 6 weeks of age and weighing about 30 g, were used. Sarcoma-180 (S-180) grown in mice was selected because this cell line has confirmed sensitivity to both 5-FU and cisplatin, as we reported before [8]. To induce experimental tumors, 5×10^6 S-180 cells were s.c. transplanted into the animals. The mice were divided into six groups of seven animals each: A, no treatment (serving as a control); B, continuous i.p. infusion of 5-FU 10 mg/kg/day through a minipump (model 1007D; Alzet, Cupertino, CA); C, oral TS-1 10 mg/kg/day; D, i.p. cisplatin 0.2 mg/kg/day; E, continuous i.p. infusion of 5-FU 10 mg/kg/day concomitantly with i.p. cisplatin 0.2 mg/kg/day; F, oral TS-1 10 mg/kg/day concomitantly with i.p. cisplatin 0.2 mg/kg/day. Treatment started 24 h after tumor transplantation and continued for 5 consecutive days in every group.

Antitumor activity

Following 5-day treatment that started 24 h after tumor transplantation, tumors were isolated and weighed on day 8 to evaluate antitumor activity.

5-FU concentrations in blood and tumor

On day 8 after tumor transplantation, blood was collected, and the tumor isolated and weighed; all samples were promptly stored at −80°C. To 0.5 ml of serum was added 0.5 ml of saturated ammonium sulfate and 0.1 ml of the internal standard solution (IS for 5-FU: 5-bromouracil at 4 μl/ml). After agitation, 5 ml of chloroform was added, shaken vigorously at room temperature for 10 min and centrifuged. The resulting aqueous phase to which was added 4 ml of ethyl acetate was evaporated to dryness under a stream of N_2 and dissolved in 200 μl of pure water. This was used for quantification of 5-FU. An aliquot of 0.5 g from tissue sample to which was added 2 ml of cold acetonitrile was homogenized and centrifuged; 1 ml of the supernatant was evaporated to dryness. To the residue was added 0.5 ml of pure water and 0.5 ml of saturated ammonium sulfate, and the procedure described for serum was followed. 5-FU concentrations in blood and tumor were determined by HPLC under the following operative conditions to calculate AUC_{0-12} using the trapezoidal rule:

- Column: Chemcosorb 300-5C_{18} (4.6 × 250mm)
- Mobile phase: 10 mM monopotassium phosphate solution containing 2 mM tetrabutyl-ammonium (pH 5.0)
- Flow rate: 1.0 ml/min
- UV wavelength: 270 nm

Adverse effects

White blood cell count, red blood cell count, platelet count, BUN, GOT and GPT were measured 8 days after tumor transplantation to detect adverse effects. Animals were weighed daily.

Statistical analysis

Data were analyzed by the *t*-test with $p < 0.05$ considered statistically significant.

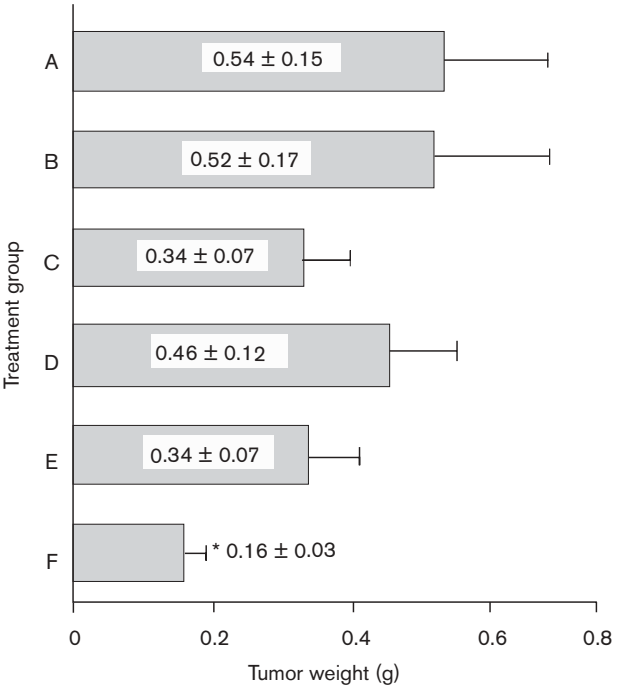
Results

Antitumor activity

Figure 1 shows that tumor weights (g, mean ± SD) were 0.54 ± 0.15 in Group A, 0.52 ± 0.17 in Group B, 0.34 ± 0.07 in Group C, 0.46 ± 0.12 in Group D, 0.34 ± 0.07 in Group E and 0.16 ± 0.03 in Group F.

TS-1 combined with cisplatin (Group F) significantly decreased tumor weight as compared with continuous 5-FU (Group B) or TS-1 alone (Group C) ($p < 0.05$). Comparison of the combination regimens revealed that tumor weight was significantly lower after TS-l plus cisplatin (Group F) than after 5-FU plus cisplatin (Group E) ($p < 0.05$). Thus, the combination of TS-1 and low-dose daily cisplatin showed more potent antitumor activity.

Fig. 1



Antitumor activity evaluated by tumor weight after treatment in S-180-bearing mice (mean ± SD of seven mice). Group A, untreated; Group B, 5-FU alone; Group C, TS-1 alone; Group D, cisplatin alone; Group E, 5-FU + cisplatin; Group F, TS-1 + cisplatin. *$p < 0.05$ (versus Group B, C or E).

AUC_{0-12} calculated from 5-FU concentrations in blood and tumor tissue

Figure 2 shows that mean AUC_{0-12} (ng/ml·h) of 5-FU in blood and tumor tissue was 435 and 345 in Group B, 2651 and 3548 in Group C, 343 and 324 in Group E, and 1538 and 2020 in Group F, respectively. TS-1 alone and TS-1 plus low-dose daily cisplatin yielded remarkably higher 5-FU concentrations in both samples than other regimens did, with a trend of blood containing more 5-FU than tumor tissue. There was a trend toward decreased blood and intratumoral 5-FU with TS-1 plus cisplatin as compared with TS-1 alone, though the difference was not significant.

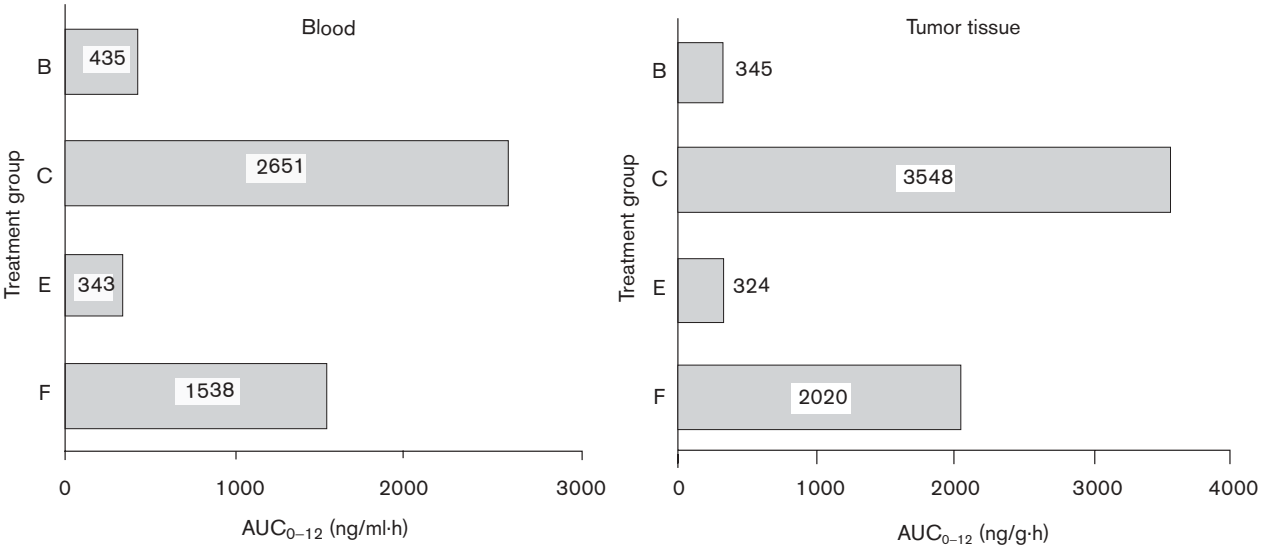
Adverse effects observed during evaluation of anti-tumor activity

Table 1 shows that white blood cells were significantly decreased after TS-1 alone (Group C) and TS-1 plus cisplatin (Group F), as compared with the control count ($p < 0.05$). Though the antitumor activity was enhanced in Group F over Group C, there was no difference in white blood cell count between these groups. In Group F, however, the platelet count was significantly decreased ($p < 0.05$). BUN, GOT and GPT values did not differ among groups. White blood cell count and platelet count were significantly lower in the TS-1 plus cisplatin group than in the control (Group A) ($p < 0.05$), while there was no significant difference between Groups C and F in any of parameters indicative of adverse effects. Figure 3 shows that there was little difference in body weight change during the experimental chemotherapy study among groups.

Discussion

5-FU is a highly time-dependent drug and *in vitro* assays demonstrate that in medium it is hardly decomposed, but is efficiently assimilated and metabolized into FdUMP, resulting in inhibition of DNA synthesis and exerting cytocidal effects. This contrasts with the *in vivo* series where most of the dose administered is found to be

Fig. 2



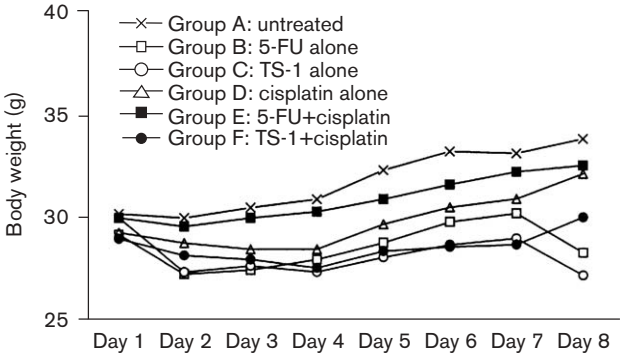
AUC_{0-12} calculated from 5-FU concentrations in blood and tumor tissue following administration of 5-FU or TS-1 for 5 consecutive days in S-180-bearing mice. Each data presents the mean of three mice. Group B, 5-FU alone; Group C, TS-1 alone; Group E, 5-FU + cisplatin; Group F, TS-1 + cisplatin.

Table 1 Adverse effects observed during the antitumor activity study—adverse effect (1)

	Group A (untreated)	Group B (5-FU alone)	Group C (TS-1 alone)	Group E (5-FU + cisplatin)	Group F (TS-1 + cisplatin)
White blood cells ($\times 10^3$/mm^3)	8.28 ± 2.63	9.50 ± 3.82	4.60 ± 1.42[a]	6.53 ± 2.41	4.43 ± 1.05[a]
Red blood cells ($\times 10^4$/mm^3)	870 ± 24	771 ± 165	839 ± 113	811 ± 199	750 ± 181
Platelets ($\times 10^4$/mm^3)	29.2 ± 4.7	41.5 ± 15.5	33.1 ± 12.2	33.3 ± 7.7	18.7 ± 6.1[a]
BUN (mg/dl)	25.8 ± 1.9	21.4 ± 4.9	25.6 ± 4.5	22.8 ± 2.7	18.7 ± 1.5
GOT (IU/l)	301 ± 56	330 ± 36	288 ± 90	363 ± 67	246 ± 24
GPT (IU/l)	44.0 ± 7.7	44.0 ± 7.7	38.8 ± 9.5	45.8 ± 8.5	35.6 ± 2.9

Mean ± SD of seven mice.
[a] $p < 0.05$ (versus Group A).

Fig. 3



Body weight changes during the antitumor activity study—adverse effect (2) (mean of seven mice).

rapidly decomposed by dihydropyrimidine dehydrogenase, an enzyme decomposing 5-FU in the liver, and to be excreted in the urine in the form of F-β-alanine. The large discrepancy between *in vitro* and *in vivo* metabolic data makes it difficult to estimate the *in vivo* antitumor activity of 5-FU from its blood concentration and duration a sustained concentration, or the exact relationship between blood concentration and antitumor activity. Fujii *et al.* [20] determined blood concentrations, antitumor activity and toxicity of 5-FU as precisely as possible under conditions similar to the *in vitro* environment, by combining a masked form of 5-FU with an inhibitor of the 5-FU degradation enzyme DPD to obtain constant blood drug concentrations. The optimal mode of administration they recommended was to maintain blood 5-FU at approximately 200 ng/ml for a protracted time (6 days). Clinical studies in advanced or recurrent colorectal cancer reported response rates ranging from 23.4 to about 30.0% with a protracted (4 weeks or longer) continuous i.v. infusion of 5-FU [14,15]. The dose-limiting factors with this continuous i.v. infusion therapy were hand and foot syndrome, and gastrointestinal disorders. Under these circumstances, an oral preparation was desired that would achieve high blood 5-FU concentrations and keep them there for a long time, and that would reduce gastrointestinal disorders, possible adverse reactions to 5-FU, with improved tumor selectivity or tolerance. The combination of the oral derivative of 5-FU and its two modulators was then evaluated.

TS-1 is an oral anticancer drug combining three agents in a molar ratio of 1:0.4:1: FT (a prodrug of 5-FU), CDHP (an inhibitor of 5-FU degradation) and Oxo (an inhibitor of gastrointestinal toxicity of 5-FU).

Effective blood 5-FU concentrations are prolonged with TS-1 despite its oral route of administration, preventing or reducing the dose-limiting gastrointestinal disorders expected of 5-FU. Good tolerance and clinical benefits of TS-1 have been reported [21,22]. Future research will be focused on combination regimens containing TS-1.

Various aspects of biochemical modulation of 5-FU resulting in enhanced antitumor activity have been investigated by many authors. The advent of cisplatin promotes this and the combination of 5-FU plus cisplatin proves effective against solid cancers including advanced or recurrent gastrointestinal cancer. It is being demonstrated that in addition to its known direct action, i.e. direct binding to DNA and inhibition of DNA synthesis, cisplatin has an indirect action, i.e. it plays a role as a modulator of 5-FU. (i) Cisplatin does not bind directly to DNA, but acts on the cell membrane and inhibits incorporation of methionine into cells; the portion of methionine binding to protein or albumin can also inhibit cellular incorporation of methionine. (ii) Intracellular methionine decreases while intracellular reduced-type folate (5,10-methylene-tetrahydrofolate) increases. (iii) As a result, the covalently bound ternary complex is increased to potentiate the antitumor activity of 5-FU. Combination therapy with 5-FU and cisplatin has been established on the basis of biochemical modulation theory and proves to be of clinical usefulness. Experimental studies demonstrated that single high-dose treatment was not necessary since the modulating action showed on multiple low doses [11–13].

A high antitumor activity was observed in our animal study with the combination of TS-1 and low-dose daily cisplatin without any increase in adverse effects. Despite the trend toward increased 5-FU concentrations in blood and tumor tissue after TS-1 alone as compared with TS-1 plus cisplatin, the former regimen was markedly inferior to the latter in antitumor activity. It has been suggested that once 5-FU had reached a certain concentration in the organism, its efficacy might depend on the presence of a modulator. Therefore, to estimate the antitumor activity of 5-FU from blood and tumor drug concentrations alone did not seem reasonable. The modulation effect of cisplatin does not come from elevation of 5-FU concentrations in blood and tumor, but from an increase in reduced-type folate in tumor cells and from enhanced inhibition of thymidylate synthase by FdUMP. The considerably improved efficacy and the low incidence of adverse effects of this therapy will facilitate ambulatory treatment with promising clinical usefulness for the QoL of cancer patients.

Conclusion

A high antitumor activity was observed in this animal study with the combination of TS-1 and low-dose daily cisplatin without any increase in adverse effects. Despite the trend toward increased 5-FU concentrations in blood

and tumor tissue after TS-1 alone, as compared with TS-1 plus cisplatin, the former regimen was significantly inferior to the latter in antitumor activity. Cisplatin acted as a modulator of 5-FU, suggesting clinical benefits of the combination of TS-1 and low-dose daily cisplatin.

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