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Research paper

Anticancer activity and toxicity of S-1, an oral combination of tegafur and two biochemical modulators, compared with continuous i.v. infusion of 5-fluorouracil

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S-1 is an oral combined form of 1 M tegafur [a prodrug of 5-fluorouracil (5-FU)], 0.4 M 5-chloro-2,4-dihydroxypyridine (a reversible inhibitor of dihydropyrimidine dehydrogenase) and 1 M potassium oxonate (an inhibitor of orotate phosphoribosyltransferase). S-1 has been shown to exert a potent antitumor effect with low gastrointestinal toxicity in experimental tumor models. We have therefore compared the antitumor effect of oral S-1 with that of continuous infusion of 5-FU in rats bearing transplants of human and murine tumors. Almost complete inhibition of the tumor growth was obtained on 7 day schedules in Yoshida sarcoma-bearing rats by consecutive administration of 30 mg/kg/day of oral S-1 and 40 mg/kg/day infusion of 5-FU. However, a significant difference between the incidence of toxicities of S-1 and 5-FU, including body weight loss and diarrhea, was noted. The rats given the 5-FU infusion had marked weight loss and severe diarrhea, while those given oral S-1 had neither. Although about 50% inhibition of the tumor growth was attained with 15 mg/kg/day of oral S-1 and 30 mg/kg/day infusion of 5-FU in nude rats with xenografted human colon cancer (KM12C), the rate of body weight loss in the 5-FU-treated group was distinctly higher than in the S-1-treated group. The ratio of the 5-fluoronucleotide concentrations in gastrointestinal tissue to that in the tumor was lower in the S-1-treated rats than in the 5-FU-treated rats. In conclusion, the results suggest that oral S-1 might be more effective in the treatment of cancer patients than continuous infusion of 5-FU, from the standpoint of antitumor potency and toxicity.

Key words: 5-Fluoronucleotide, 5-fluorouracil, antitumor activity, CDHP, continuous infusion, potassium oxonate, S-1, tegafur.

Introduction

5-Fluorouracil (5-FU) is still the most prescribed anticancer drug for the treatment of stomach, colorectal, and head and neck cancer, either alone or in combination with other drugs or biochemical modulators. In general, the clinical response to bolus 5-FU has been found to be low (about 10–20%),[1] and this low response to 5-FU has been mainly attributed to side effects such as myelotoxicity and gastrointestinal (GI) toxicity, which prevent its administration for long periods and/or at high doses, and to its extremely rapid catabolism in the body before it can exert a notable antitumor action. During the past 10 years, continuous infusion (CI) of 5-FU has been suggested to be a useful method of administration to obtain a relatively higher response rate (about 30%).[2–9] Nevertheless, dose-limiting toxicities such as diarrhea, stomatitis and hand-foot syndrome during CI of 5-FU remain unsettled as reported by Caballero *et al.*[10] and Lokich *et al.*,[7] and circadian variations and patient-to-patient variations in plasma 5-FU concentrations caused by dihydropyrimidine dehydrogenase (DPD) (EC 1.3.1.2) in the liver and peripheral mononuclear cells of patients are crucial factors limiting the clinical efficacy of 5-FU.[11–13]

Although biochemical modulators of 5-FU such as leucovorin (LV) or folinic acid have been reported to improve the clinical response rate to CI of 5-FU,[1,14,15] the incidence and degree of dose-limiting toxicity seem to increase in parallel with the response rate to 5-FU.[14,15] Consequently, it is very important to inhibit the rapid degradation of 5-FU and to prevent 5-FU-induced toxicities such as diarrhea and stomatitis.

In 1987, we found a new reversible inhibitor of DPD,[16] 5-chloro-2,4-dihydroxypyridine (CDHP), and demonstrated that high and constant 5-FU concentrations were maintained in rats during CI of 5-FU in combination with CDHP in rats.[17] We also showed that potassium oxonate (Oxo), an inhibitor of orotate

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phosphoribosyltransferase (OPRT) (EC 2.4.2.10), prevented 5-FU-induced GI toxicity without loss of antitumor activity of 5-FU.[18]

To develop a new conventional anticancer drug to replace CI of 5-FU, we used clinically available tegafur (FT),[19] a prodrug of 5-FU that remains in the blood for a long time after oral administration and is gradually hydrolyzed to 5-FU by liver microsomes,[20] as an effector. By simultaneously administering a combination of FT with two biochemical modulators, CDHP and Oxo, in a molar ratio of 1:0.4:1, we demonstrated that this new oral formulation of 5-FU, called S-1, provides persistently high plasma 5-FU concentrations with less 5-FU-induced GI toxicity and body weight loss.[21] The result showed that S-1 exerted a potent antitumor activity with low side effects in various murine and human tumor models.[22,23] In this paper, we report on the antitumor activity, toxicity and biochemical characteristics of S-1, compared to CI of 5-FU alone, in rats with murine and human tumors.

Materials and methods

Chemicals

FT, CDHP and Oxo were products of Taiho Pharmaceutical (Tokyo, Japan). 5-FU was purchased from Sigma (St Louis, MO.). [6-^{3}H]5-FU (655 GBq/mmol) and [2-^{14}C]FT (2.0 GBq/mmol) were obtained from Amersham (Amersham, UK) and DuPont (Boston, MA), respectively. All other chemicals used were commercially available products. S-1 was prepared by simultaneous mixing of FT, CDHP and Oxo in a molar ratio of 1:0.4:1. The doses of S-1 are expressed as doses of FT.

Animals and tumors

Four-week-old Donryu rats were purchased from Charles River Japan (Tokyo, Japan), and supplied *ad libitum* with a commercial diet and autoclaved water until used. F344/N Jcl-rnu nude rats (5 weeks old) were obtained from CLEA Japan (Tokyo, Japan) and maintained in clean cabinets until the end of the therapeutic experiment. Ascitic Yoshida sarcoma cells were supplied by Sasaki Research Institute (Tokyo, Japan) and passaged in Donryu rats weighing about 150 g by i.p. inoculation of 1×10^{-4} cells at weekly intervals. KM12C human colorectal cancer cells were kindly provided by Dr K Morikawa (Iwamizawa Workers' Compensation Hospital, Hokkaido) and passaged in nude rats weighing 150–200 g by s.c. implantation of approximate 2 mm^3 cubed fragments into the right axilla of the rats.

Chemotherapy

Solid-type Yoshida sarcoma was prepared by implantation of 1×10^5 cells into the s.c. tissue of the back of Donryu rats on day 0. The rats were divided in two groups: one group was used for oral therapy and the other for infusion therapy. The rats in the infusion group were then operated on as described below within 8 h. A 10 or 30 mg/kg dose of S-1 was given to rats orally daily for seven consecutive days, starting 24 h after tumor implantation. Control rats received 0.5% hydroxypropylmethylcellulose (HPMC) solution alone. A 20 or 40 mg/kg/day dose of 5-FU was continuously infused for 7 days by the same schedule in the other group of rats. The control rats for this group received saline alone. On day 8, the rats were sacrificed, and their body weight and tumor weight were measured. In the human tumor model, about 8 mm^3 cubed fragments of KM12C colon cancer was implanted s.c. into the back of the nude rats and about 14 days later, rats were subjected to the same procedures as described above. The antitumor activity of the drugs was evaluated as described previously.[21–23]

Continuous venous infusion

There are several techniques of catheterization to infuse animals with the drug.[24,25] We used the method described previously.[17] Briefly, a 16 gauge, 60 cm, silicon catheter (Nipro, Osaka, Japan) was inserted into the right cardiac vein via the artery of the tumor-bearing rats under diethylether anesthesia. The catheter was then tunneled through the s.c. tissue to the back of the neck and passed through a harness having a 30 cm spring. The rats were placed in metabolic cages and the other end of the catheter was attached to an infusion pump (Termo, Tokyo, Japan). The rats were free to move in this system, and the were supplied with food and water *ad libitum* for at least 10 days without any damage to the catheter. Saline alone was infused for about 16 h before the start of drug infusion.

Extraction and determination of 5-fluoronucleotides formed from 5-FU

S-1 containing 5 mg/kg of [2-^{14}C]FT (9.25 MBq/kg), 1.46 mg/kg of CDHP and 4.88 mg/kg of Oxo was given to the tumor-bearing rats orally. Rats were sacrificed at the times indicated, and their tumors and normal tissue were removed and immediately frozen

at −80°C. In a separate experiment, [6-^{3}H]5-FU (20 mg/kg, 18.5 MBq/kg) was continuously infused for 2 days, and at the end of the infusion the rats were rapidly sacrificed and their tissues were removed and frozen. The frozen tissues were homogenized with 4 volumes of ice-cold 5% TCA and centrifuged at 3000 r.p.m. for 10 min. The supernatant fluid was neutralized with 2 M KOH and centrifuged. An aliquot of the neutralized sample was then loaded onto a small column (1 ml of bed volume) of packed Dowex 1 × 4 (HCl form) resin. Radiolabeled FT and 5-FU were eluted unbound through the column by washing with 10 ml of water (2 ml × 5). Radiolabeled 5-fluoronucleotides as total phosphorylated products from 5-FU bound to the column were then eluted with 4 ml of 0.1 HCl. An aliquot of the HCl-eluate was added to a vial containing 10 ml of scintillation fluid (ACS-II) to count the radioactivity.

Statistical analysis

The significance of differences between means in the antitumor experiments were assessed using Dunnett's *t*-test (Yoshida sarcoma model) or Welch's test (KM12C xenograft model).

Results

Antitumor activity and toxicity of oral S-1 and infusion 5-FU in Yoshida sarcoma-bearing rats

Murine Yoshida sarcoma-bearing rats received 7 day chemotherapy consisting of oral S-1 or infusion of 5-FU. Antitumor activity and changes in body weights are shown in Table 1. Tumor growth was significantly inhibited by oral S-1 and CI of 5-FU at the doses used in the study, compared to tumor growth in the control rats. At 10 and 30 mg/kg of S-1 there was 35 and 99% inhibition, respectively, of the growth of Yoshida sarcoma, and at 20 and 40 mg/kg/day of 5-FU infusion there was 41 and 99% inhibition, respectively. Thus, 30 mg/kg of oral S-1 and 40 mg/kg of 5-FU infusion resulted in almost completely tumor-free rats. However, a significant difference in side effects, such as diarrhea and body weight loss, was seen between oral S-1 and 5-FU infusion. The body weight of rats given 40 mg/kg of 5-FU decreased greatly (−140% of non-treated rats) compared to the rats given 30 mg/kg of oral S-1 (about −10% of control rats). Moreover, three of the eight rats in the 5-FU infusion group died of severe toxicity during treatment and the remaining rats had watery diarrhea. The presumably equitoxic doses of S-1 and 5-FU infusion, 30 and 20 mg/kg /day, respectively, resulted similar body weight loss, −10 and −28%, respectively of the control values. The antitumor effect (tumor growth inhibition rate) of oral S-1, however, was clearly higher than that of 5-FU infusion (99 versus 41% inhibition). Figure 1 shows the relationship between antitumor activity and body weight loss as a result of oral S-1 and 5-FU infusion. Oral S-1 was shown to have a potent antitumor effect without extreme body weight loss, whereas weight loss with 5-FU infusion was extreme.

Efficacy of S-1 and 5-FU on the growth of human colorectal cancer xenografts in nude rats

We previously evaluated the antitumor effect of S-1 on various human cancer xenografts in rats administering it for 14 days because of the slow growth of the

Table 1. Antitumor and toxic effect of oral S-1 and 5-FU infusion in rats with Yoshida sarcoma

Drugs	Dose (mg/kg/day)	*n*	Tumor weight (g, mean ± SD)	TGI[a] (%)	Incidence of diarrhea (*n*)	Body weight gain	
						(g, mean ± SD)	% control
S-1 (oral)	—	10	2.31 ± 0.72		0	57.2 ± 6.7	—
	10	10	1.50 ± 0.74*	35.1	0	18.5 ± 10.1*	32.4
	30	10	0.02 ± 0.05*	99.0	0	−5.7 ± 13.3*	−10.0
5-FU (CI)	—	7	3.07 ± 0.89		0	22.9 ± 16.0	—
	20	8	1.82 ± 0.80*	40.8	0	−6.4 ± 11.2*	−27.9
	40	5[b]	0.03 ± 0.03*	99.0	5	−32.1 ± 8.7*	−140.2

Drugs were administered or infused for 7 days, starting 24 h after the tumor implantation. The antitumor effect and toxicities of oral S-1 and 5-FU infusion were evaluated on day 8.
[a]Tumor growth inhibition.
[b]Three of the eight animals died of toxicity.
Significantly different from each control group at *$p < 0.01$ by Dunnett's *t*-test.

tumors.[21] In the present study, administration of 5-FU for more than 10 days was abandoned because of damage to the catheter, and the antitumor effect of oral S-1 and 5-FU infusion was subsequently evaluated by 7 day administration. As shown in Table 2, both oral S-1 (15 mg/kg/day) and 5-FU infusion (30 mg/kg/day) were significantly effective against KM12C colorectal cancer, RI values being about 53 and 48%, respectively. The body weight loss in the 5-FU group, however, was significantly greater than in the S-1 group.

5-Fluoronucleotide levels in the tumor and GI tissue

To clarify the reason for the marked difference in degree of side effects, such as diarrhea and/or the body weight loss, between oral S-1 and 5-FU infusion, 5-fluoronucleotides, active metabolites produced from 5-FU, were determined in the tumor and normal GI tissue after oral administration of radiolabeled S-1 including [6-^{14}C]FT or during CI of [6-^{3}H]5-FU to Yoshida sarcoma-bearing rats. As shown in Figure 2, the 5-fluoronucleotide concentration of the tumor increased markedly, reaching a maximum of 0.75 nmol/g tumor at 6 h after oral administration of 5 mg/kg (25 μmol/kg) of S-1, while the levels in GI

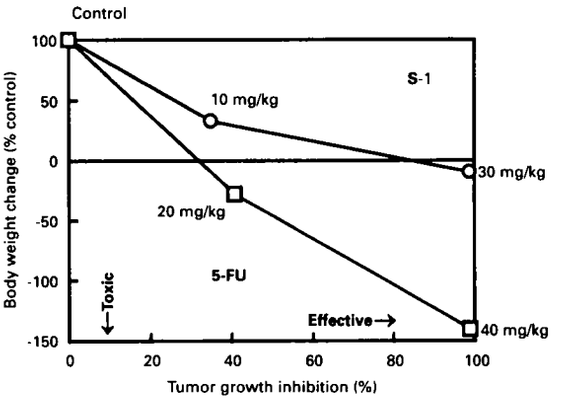

Figure 1. Relationship between antitumor activity and body weight loss after oral S-1 and 5-FU infusion in Yoshida sarcoma-bearing rats. Experimental conditions are summarized in Table 1. Tumor growth inhibition (%) and body weight change (% control) in each treatment group have been plotted.

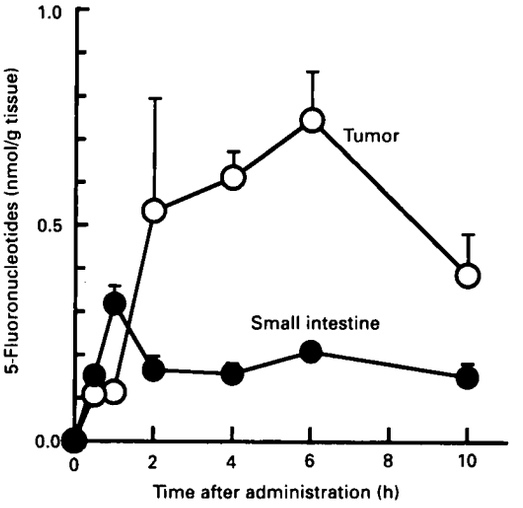

Figure 2. 5-Fluoronucleotide levels in the tumor and GI tissue of Yoshida sarcoma-bearing rats following oral administration of radiolabeled S-1 and UFT. A 5 mg/kg dose of S-1 containing 9.25 MBq/kg of [2-^{14}C]FT was administered orally to the tumor-bearing rats and at the times indicated, rats were sacrificed and their tumor (○) and GI tissue (●) was rapidly removed and frozen at −80°C. The 5-fluoronucleotides in the tissue were extracted and determined by the method described in Materials and methods. Values are means ± SD for three rats.

Table 2. Antitumor and toxic effect of oral S-1 and 5-FU infusion in nude rats with xenografted human colon cancer KM12C

Drugs	Dose (mg/kg/day)	*n*	Relative tumor volume (mean ± SD)	RI[a] (%)	Body weight gain		
					(g, mean ± SD)	($BWG_{treat} - BWG_{count}$)[b]	
S-1	—	10	3.05 ± 0.28	—	10.5 ± 4.2		
	15	10	1.44 ± 0.36**	52.8	−7.9 ± 4.5	−18.4	#
5-FU	—	10	1.80 ± 0.66	—	−22.4 ± 14.8		#
	30	6	0.93 ± 0.51*	48.4	−53.9 ± 11.2	−31.5	#

Human colon cancer KM12C was implanted into the right axilla of nude rats. About 14 days later, S-1 and 5-FU were administered for 7 days, and the antitumor effect and gain or loss of body weight were evaluated on day 8.
[a]Relative inhibition of tumor growth.
[b]Difference in body weight change between the treated and control group.
Significantly different from each control group at $*p<0.05$ and $**p<0.01$, respectively, by Welch's test.
Significantly different at $\#p<0.05$ by Welch's test.

tissue rapidly declined and remained at low levels under 0.2 nmol/g, suggesting that Oxo, one component of S-1, strongly inhibited the phosphorylation of 5-FU in the GI tract but not in tumor tissue, as described previously.[18,21]

The tissue 5-fluoronucleotide levels in tissues during CI of 5-FU were also determined (Figure 3). When 20 mg/kg/day (154 μmol/kg/day) of 5-FU was infused constantly, about 1.7, 1.0, 0.9 and 1.7 nmol of 5-fluoronucleotides were formed from 5-FU in 1 g of the tumor, small intestine, large intestine and bone marrow, respectively. However the ratio of 5-fluoronucleotide concentration in the tumor to that in GI tissue was less than 2.0. This suggested that the selectivity of the antitumor effect of S-1 was higher than that of 5-FU infusion.

Discussion

CI of 5-FU has been shown to provide relatively higher response rates in cancer patients compared to conventional bolus administration of 5-FU[7,9] and an even greater response to CI of 5-FU has been achieved by combination with leucovorin (LV), the most available biochemical modulator.[1] However, the metabolic disadvantage of 5-FU and the 5-FU-induced toxicities, such as diarrhea, mucositis and myelosuppression, are unresolved problems. Circadian and patient-to-patient variations in plasma 5-FU concentration caused by DPD is one of the response-limiting factors during CI of 5-FU, and 5-FU-induced diarrhea and stomatitis are dose-limiting toxicities. Consequently, if these factors can be eliminated by using a new 5-FU derivative or combination with a biochemical modulator, it would be possible to achieve higher tumor-selective efficacy of 5-FU than by CI of 5-FU alone.

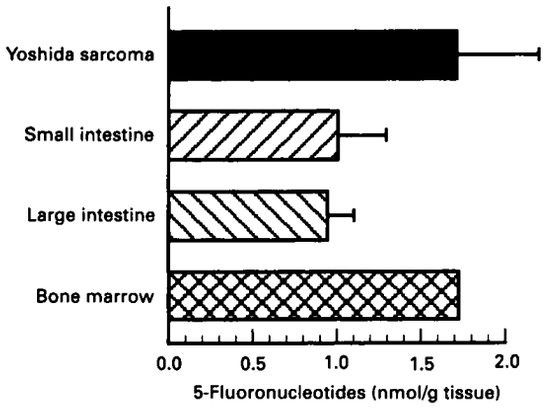

Figure 3. 5-Fluoronucleotide levels in the tumor and normal tissues of Yoshida sarcoma-bearing rats during CI of 5-FU. A 20 mg/kg/day dose of [6-^{3}H]5-FU (18.5 MBq/kg) was continuously infused into Yoshida sarcoma-bearing rats for 2 days. At the end of the infusion, rats were immediately sacrificed, and tumor, GI tissue and bone marrow tissue was removed and frozen. The 5-fluoronucleotide levels in the tissue was determined by the methods described in Materials and methods. Bone marrow tissue from all rats tested were pooled. Values are means ± SD for four rats.

For this purpose, we used CDHP, a new reversible inhibitor of 5-FU degradation catalyzed by DPD,[16] and Oxo, an inhibitor of OPRTase and a strong preventer of 5-FU-induced GI toxicity, as biochemical modulators. We used them in an attempt to develop a conventional oral form of FT,[19] well known as a prodrug of 5-FU, that would be maintained in the blood for a long time after administration. S-1, formulated with 1 M FT, 0.4 M CDHP and 1 M Oxo, is an oral FT-based antitumor drug characterized by high and long-lasting 5-FU levels in the blood and tumor tissue. S-1 provides a potent antitumor effect with lower 5-fluoronucleotide concentrations in the GI tract, thereby preventing 5-FU-induced GI toxicity.[21] Thus, it became important to know whether oral S-1 is superior to 5-FU infusion in terms of antitumor activity and GI toxicity. In this paper, the antitumor effects and side effects (toxicity) of S-1 were compared with those of 5-FU infusion in rats with solid-type Yoshida sarcoma and in nude rats with xenografted human colon cancer (KM12C). As shown in Table 1, 30 mg/kg/day of oral S-1 and 40 mg/kg/day of 5-FU infusion almost completely inhibited the growth of Yoshida sarcomas. The toxicities, such as diarrhea and the marked body weight loss, were observed in the 5-FU-treated group but not in the S-1-treated group.

At the minimum toxic dose, which is under 30 mg/kg for S-1 and about 20 mg/kg for 5-FU infusion, the antitumor effect of oral S-1 was clearly higher than that of 5-FU infusion (Figure 1). As described previously,[18] Oxo, a component of S-1, has been demonstrated to inhibit the formation of 5-fluoronucleotides from 5-FU in the GI tract but not in tumor tissue. When 5 mg/kg of S-1 containing [2-^{14}C]FT (9.25 MBq/kg) was given to Yoshida sarcoma-bearing rats orally, the 5-fluoronucleotide concentration in the tumor and GI tissue was about 0.75 and 0.21 nmol/g, respectively, at 6 h, i.e. there was about 3-fold distinction between the tumor and GI tissue (Figure 2).

In a previous paper,[22] we reported on the antitumor effect of S-1 compared with another FT-based antitumor drug, UFT formulated with 1 M FT and 4 M uracil,[26] which is used clinically in Japan.[27] When UFT was orally administered, the 5-fluoronucleotide concentration in the tumor and GI tissue was almost the same, suggesting that GI toxicity in UFT-

treated rats is expressed in parallel with the antitumor effect of UFT (data not shown). In a separate experiment, the 5-fluoronucleotide concentration in the tumor, small intestine and bone marrow during CI of rats with [6-^{3}H]5-FU (20 mg/kg, 18.5 MBq/kg) was about 1.7, 1.0 and 1.7 nmol/g, respectively (Figure 3). Thus, the difference in 5-fluoronucleotide concentration between the tumor and GI tissue was small, suggesting that a high dose of infusion 5-FU has both potent antitumor activity and severe toxicity, including diarrhea and marked body weight loss.

The antitumor effect of S-1 had also been evaluated in 2 week therapeutic periods in nude rats implanted with human tumors. However, the antitumor and toxic effects of oral S-1 and 5-FU infusion were only evaluated in 7-day therapeutic periods in this study because CI of 5-FU to rats could not be continued for 14 days by our method. Both oral S-1 and 5-FU infusion were significantly effective, with RI values of 53 and 48%, respectively, for KM12C tumor, but the weight loss in the 5-FU-treated group tended to be larger than in the S-1 group. In addition, CI itself caused slight body weight loss in the control group.

It has been reported that weekly 24 h infusion of high-dose 5-FU (2600 mg/m^2/week) plus folinic acid (500 mg/m^2/week), a regimen that has been used often recently, caused neurotoxicity (7.5% incidence) to cancer patients.[28] Since later catabolites of 2-fluoro-β-alanine (2-fluoroacetic acid or 2-fluorocitric acid) are probably related to the development of the neurotoxicity,[29] combination of 5-FU or its derivative with an inhibitor of 5-FU degradation should decrease the incidence of such toxicity. Suitable combination of two modulators, CDHP and Oxo, with FT might potentiate the antitumor activity of 5-FU and decrease 5-FU-induced GI toxicity and/or other toxicities in cancer patients. Moreover, our results suggest that the combination of oral S-1 and other modulators such as LV might produce much higher response in cancer patients than infusion of 5-FU plus LV.

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