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# Pharmacokinetic Modulation of Plasma 5-Fluorouracil Concentrations to Potentiate the Antitumor Activity of Continuous Venous Infusion of 5-Fluorouracil

Setsuro Fujii, Masakazu Fukushima, Yuji Shimamoto and Tetsuhiko Shirasaka

*Biwako Research Institute, Otsuka Pharmaceutical Co., Ltd., 11-1, 1-chome, Karasaki, Ohtsu, Shiga 520-01*

**Methods for pharmacokinetic modulation of the plasma 5-fluorouracil (5-FU) level to increase antitumor activity during continuous venous infusion (CVI) of low doses of 5-FU were examined in Yoshida sarcoma-bearing rats. These methods were additional infusion of 5-FU for a short period (4 h) or oral administration of UFT or Tegafur during long-term CVI of 5-FU that alone gave a plasma 5-FU level of about 50 ng/ml. The antitumor effect on Yoshida sarcoma was markedly potentiated when an additive dose of 5-FU combined with 3-cyano-2,6-dihydroxypyridine (CNDP), a potent inhibitor of 5-FU degradation, giving a plasma level of about 500 ng/ml, was infused for 4 h. A similar increase in the antitumor effect was observed with oral administration of a conventional dose of UFT during CVI of 5-FU without CNDP, giving a plasma level of 30 to 60 ng/ml. These results suggest that the antitumor effect of CVI of 5-FU can be potentiated by pharmacokinetic modulation of the 5-FU concentration in the blood.**

Key words: 5-Fluorouracil — UFT — Antitumor activity — Continuous infusion — Pharmacokinetic modulation

The antimetabolite 5-fluorouracil (5-FU), seems potentially useful clinically as an antitumor agent. But, although it is effective in some cancer patents when given alone or in combination with other agents, most clinicians report that the response rate to 5-FU is only about 10 to 30% and remission, if achieved, is generally of short duration. On the basis of the time-dose relationship for 5-FU cytotoxicity against human malignant tumor cells and murine tumor cells *in vitro*,[1-4] the cytotoxic effects of 5-FU on tumors in patients have been evaluated by using two schedules of continuous venous infusion (CVI) of 5-FU. One is short-term CVI of a high dose (1–2 g/m$^2$) of 5-FU,[5-8] and the other is long-term CVI of low doses (300–500 mg/m$^2$).[9-14] However, possibly because of variations in 5-FU catabolism in patients from day to day and during the day and night,[15-17] the response rates and toxic effects observed have been variable. Recently, in an attempt to improve the antitumor activity of 5-FU, we examined the 5-FU level in the blood and the persistence of this level during CVI of 5-FU plus 3-cyano-2,6-dihydroxypyridine (CNDP),[18] a potent inhibitor of 5-FU degradation, in Yoshida sarcoma-bearing rats. Results showed that the circadian variation in the plasma concentration of 5-FU of rats during its continuous infusion was completely blocked by simultaneous infusion of CNDP and that long-term infusion of 5-FU plus CNDP at a dose that gave a plasma 5-FU level of about 200 ng/ml resulted in almost complete inhibition of tumor growth.[19] We also investigated schedules of administration of 5-FU that resulted in high antitumor activity but low toxicity during CVI of 5-FU into rats. The present paper describes methods for pharmacokinetic modulation of the plasma 5-FU concentration to potentiate the antitumor activity of 5-FU during its CVI into rats at low doses.

Inocula of $2\times10^5$ Yoshida sarcoma cells were implanted into subepidermal tissues in the back of Donryu strain rats (6–7 weeks old; Laboric Service Co., Shiga). Then a silicone catheter was inserted into the right cardiac vein from the right jugular under anesthesia and the outer end of the catheter was attached to an infusion pump (Terumo Co., Tokyo). In the first experiment, 5-FU combined with a 10-fold molar excess of CNDP was infused continuously for 6 days from 24 h after tumor implantation. During this infusion, a further dose of 5-FU plus CNDP was infused additively for 4 h in a day. In the second experiment, derivatives of 5-FU, Tegafur and UFT, were orally administered during a 6-day continuous infusion of 5-FU without CNDP. These administrations were repeated once a day for 6 consecutive days. On day 7, the rats were killed and their tumors were removed and weighed to evaluate the antitumor effects (T/C, %) of these schedules. Blood samples were taken from the left cardiac vein of rats during the infusion for measurement of plasma 5-FU levels by a reported method.[19]

Recently, we observed high antitumor activity against Yoshida sarcomas in rats when 5-FU was given by infusion at 5 mg/kg with 52.3 mg/kg of CNDP over 24 h for

6 consecutive days (total dose of 5-FU, 30 mg/kg), which resulted in a plasma 5-FU level of about 200 ng/ml.[19] In the present study we used conditions giving high levels of plasma 5-FU for a short time and low levels for a long time each day during continuous infusion of 5-FU for 6 days. For 6 consecutive days, every day 5-FU was infused at 1 to 2 mg/kg in combination with a 10-fold molar excess of CNDP over 4 h and then 1.7 mg/kg of 5-FU with CNDP was infused over 20 h, which gave a plasma 5-FU level of about 50 ng/ml. These schedules and the resultant 5-FU levels in the blood of the rats are shown in Fig. 1, and the antitumor effects in Table I. The greatest antitumor activity, with a slight decrease in the white blood cell (WBC) count, was achieved by 4-h infusion of high doses of 5-FU plus CNDP and then 20-h infusion of a low dose of 5-FU plus CNDP (schedules C and D). Antitumor activity was less with the other schedules. These results suggested that the optimum schedules were combinations of a high level of 5-FU for 4 h and a low level for 20 h every day for 6 consecutive days, giving plasma 5-FU levels of 300 to 500 ng/ml at 4 h and of about 50 ng/ml for 20 h. By these schedules (especially schedule D), almost complete tumor suppression without a severe decrease in WBC count was achieved with total doses of 5-FU of up to 22 mg/kg, whereas the previous experiment of constant long-term infusion of 5-FU plus CNDP had necessitated a total dose of 30 mg/kg for a similar antitumor effect and resulted in an appreciable decrease in the WBC count (schedule E, not done simultaneously with schedules A to D).

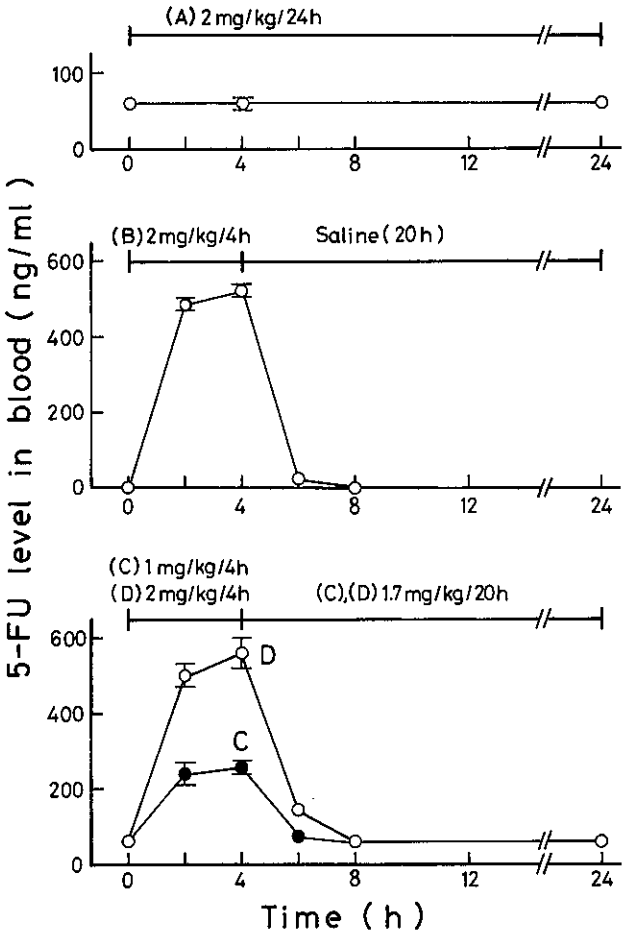


Fig. 1. 5-FU levels in the blood of rats during CVI of various doses of 5-FU combined with CNDP. Yoshida sarcoma-bearing rats received continuous venous infusion (CVI) of 5-FU with CNDP by the schedules shown in Table I for 6 consecutive days. On days 3 to 4, blood samples were removed from the left cardiac vein of rats at the times indicated after the start of the daily schedule, and their 5-FU levels were determined as described.[19] Values are means±SE for 4 rats.

Table I. Antitumor and Toxic Effects of Various Schedules of Infusion of 5-FU plus CNDP in Yoshida Sarcoma-bearing Rats

| Schedule | Dose of 5-FU (mg/kg) | | | Total dose of 5-FU (mg/kg/6 days) | n | T/C (%) | WBC[a] count ($\times 10^2$/mm$^3$) | Body wt. change (g) |
|---|---|---|---|---|---|---|---|---|
| | Short time (4 h) | | Long time (20–24 h) | | | | | |
| A | — | | 2.0 per 24 h | 12.0 | 8 | 61±9 | 112±11 | +4±3 |
| B | 2.0 per 4 h | | — (Saline) | 12.0 | 4 | 81±5 | 100±13 | −14±6 |
| C | 1.0 per 4 h | plus | 1.7 per 20 h | 16.0 | 4 | 12±7 | 82±10 | −5±6 |
| D | 2.0 per 4 h | plus | 1.7 per 20 h | 22.0 | 13 | 7±3 | 49±6 | −30±4 |
| E[b] | — | | 5.0 per 24 h | 30.0 | 20 | 2±1 | 28±2 | −25±3 |

Yoshida sarcoma cells ($2\times10^5$) were implanted into rats and from 24 h later, rats received various schedules of infusion of 5-FU combined with a 10-fold molar excess of CNDP (schedules A, B, C, D and E) for 6 consecutive days. On day 7, rats were killed and their tumors were removed and weighed. Values are means±SE for 4 to 20 rats.

*a*) WBC, white blood cell.

*b*) Data reported previously.[19]

Based on these preliminary findings on the method for achieving pharmacokinetic modulation of the plasma 5-FU level of rats to increase the antitumor effect of 5-FU, we next examined the combined effect of long-term CVI of 5-FU with oral UFT or Tegafur. In this experiment, 5-FU without CNDP was continuously infused. In the absence of CNDP, a 10-fold higher dose of 5-FU was required to give a plasma 5-FU level of about 50 ng/ml as compared to the case of the presence of CNDP.[19] Tumor-bearing rats were given UFT or Tegafur orally (20 mg/kg) once a day for 6 consecutive days during CVI of 5-FU alone (20 mg/kg/day for 6 days). As shown in Fig. 2 and Table II, the most effective combination in this experiment was oral administration of UFT plus CVI of 5-FU, which gave a maximum plasma 5-FU level of 253 ng/ml at 0.5 h and then maintained a level of 30 to 60 ng/ml for a long time. Oral co-administration of Tegafur, which gave a maximal plasma 5-FU level of about 100 ng/ml at 1 h, was less effective. These results suggest that on administration of UFT, a higher 5-FU concentration in the blood of rats was attained through inhibition of degradation of 5-FU derived from Tegafur and from CVI of 5-FU by uracil.[20] In separate trials, we observed that CVI of 5-FU without CNDP at 50 mg/kg over 24 h for 6 consecutive days brought about both an almost complete antitumor effect and a severe decrease in WBC count (about 1700 cells/mm$^3$)(data not shown).

Many clinical data suggest that long-term infusion of 5-FU at a low dose is more effective than its bolus injection or short-term infusion at a high dose. Our previous study,[19] in which CNDP, a potent inhibitor of 5-FU degradation, was simultaneously infused with 5-FU to block the circadian variation of the activity of dihydrouracil dehydrogenase in the liver, suggested that long-term infusion of a clinical dose of 5-FU (300–500 mg/m$^2$), which gave a plasma 5-FU level of about 50 ng/ml, had little antitumor activity or toxic effect but that a high dose of 5-FU, giving a plasma 5-FU level of over

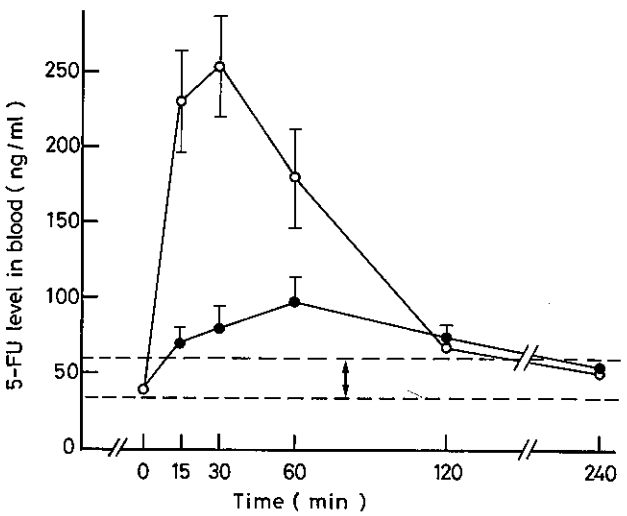


Fig. 2. 5-FU levels in the blood of rats after oral administration of UFT or Tegafur during CVI of 5-FU without CNDP. Yoshida sarcoma-bearing rats received continuous infusion of 5-FU alone at 20 mg/kg over 24 h and oral UFT or Tegafur (each 20 mg/kg) once daily for 6 consecutive days. After oral administration of UFT (○) or Tegafur (●) on day 3, blood samples were removed from the left cardiac vein and the 5-FU levels were determined. The arrow indicates the range of 5-FU levels in the blood of rats (30 to 60 ng/ml) during CVI of 5-FU alone. Values are means±SE for 3 to 4 rats.

Table II. Antitumor Effects and Toxicities in Yoshida Sarcoma-bearing Rats Treated with Combinations of Oral UFT or Tegafur plus Continuous Venous Infusion of 5-FU without CNDP

| Schedule | Dose (mg/kg/day) | Total dose (mg/kg) | n | T/C (%) | WBC[a] count (×10$^2$/mm$^3$) | Body wt. change (g) |
|---|---|---|---|---|---|---|
| 5-FU by CVI[b] | 20 | 120 | 11 | 57±5 | 98±8 | −1±6 |
| Oral Tegafur | 20 | 120 | 6 | 76±15 | 170±14 | +50±7 |
| Oral UFT | 20 (as Tegafur) | 120 | 8 | 59±6 | 123±10 | +56±1 |
| 5-FU by CVI plus oral Tegafur | 20 +20 | 120 +120 | 11 | 32±4 | 71±4 | −12±6 |
| 5-FU by CVI plus oral UFT | 20 +20 | 120 +120 | 9 | 10±1 | 44±8 | −11±4 |

Yoshida sarcoma cells (2×10$^5$) were implanted into rats and from 24 h later, the rats received oral UFT or Tegafur once daily for 6 consecutive days during CVI of 5-FU alone. Values are means±SE for 6 to 11 rats.
*a*) WBC, white blood cell.
*b*) CVI, continuous venous infusion.

200 ng/ml, had higher antitumor activity and greater toxic effects. These findings indicated the necessity of establishing an administration schedule of 5-FU that gave high antitumor activity but without severe toxic effects. The present study showed that this could be achieved by pharmacokinetic modulation of the plasma 5-FU concentration by a combination of infusion of a high dose of 5-FU plus CNDP for a short period (4 h), giving a plasma 5-FU level of about 500 ng/ml, with long-term CVI of a low dose of 5-FU plus CNDP, which gave a plasma 5-FU concentration of 50 ng/ml. Moreover, similar pharmacokinetic modulation of the plasma 5-FU concentration to increase the antitumor effect of 5-FU was attained by a combination of oral administration of a 5-FU derivative such as UFT with long-term CVI of a low dose of 5-FU without CNDP, as commonly used in clinical therapy, giving a plasma 5-FU level of about 30 to 60 ng/ml. This pharmacokinetic modulation of the plasma 5-FU concentration by a combination of oral treatment with 5-FU derivatives and CVI of 5-FU at a low dose should be clinically feasible and useful.

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