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Clinical significance of monitoring serum levels of 5-fluorouracil by continuous infusion in patients with advanced colonic cancer*

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Summary. Serum concentrations of 5-fluorouracil (5-FU) given by continuous infusion to 19 patients with advanced colonic cancer were measured by an HPLC method, and steady-state concentration (SSc), area under the curve (AUC_{72}) and total body clearance (Cl) were calculated as pharmacokinetic parameters. The serum level of 5-FU rapidly increased, reaching a plateau within 2 h after the start of administration. There were positive correlations between the dose and both SSc ($r = 0.578$, $P < 0.01$) and AUC_{72} ($r = 0.558$, $P < 0.05$). When the patients were divided into toxic and non-toxic groups according to the degree of toxicity, the values for SSc and AUC_{72} in the toxic group were significantly higher than those in non-toxic patients. The Cl value in the toxic group was also significantly different from that in the non-toxic group when data were calculated on a log scale. Furthermore, no differences in these parameters between effective and non-effective groups were detected when the patients were divided into two groups according to anti-neoplastic responses. These results indicate that increased serum concentration does not always provide therapeutic benefits to patients receiving continuous infusions of 5-FU.

Introduction

5-Fluorouracil (5-FU) is commonly used against gastrointestinal cancers [9]. Many studies on the antitumor activity of this drug by various regimens, including combination and biochemical modulation, have been reported. Of these regimens, continuous intravenous infusion gives relatively good clinical responses [1, 3, 8], but a certain degree of toxicity may occur according to the doses given. Although the pharmacokinetics of continuous administration of this drug have been investigated [11, 13], the possible therapeutic benefit in terms of clinical response and toxicity obtained by prolonging the drug delivery period and/or increasing the blood concentration are not yet known. The aim of the present study was to clarify whether the dose of 5-FU is related to tumor response and/or toxicity when the drug is given by continuously infusion.

Patients and methods

Patient selection and treatment schedule. A total of 19 patients with advanced colonic cancer, treated by continuous intravenous infusion of 5-FU with or without hyperalimentation, were included in this study. Informed consent was obtained from all patients. Infusion was performed either by gravity flow or with an electric pump on a non-random basis for 1 week. The 5-FU dose was varied and treatment was continued for >28 days in seven patients, although the dose level was diminished after the monitoring period.

Evaluation of toxicity and tumor response. Patients were divided into toxic and non-toxic groups according to the degree of toxicity, defined by World Health Organization (WHO) criteria [15]; patients exhibiting toxicity greater than grade 2 were placed in the toxic group. Patients were also divided into effective and non-effective groups; those who achieved a complete or partial response as defined by WHO criteria or showed a reduction of >50% in the serum levels of some tumor markers were included in the effective group.

Determination of 5-FU in serum. Blood samples (5 ml) were taken from the patients before and at 24, 48 and 72 h after the start of treatment and were then centrifuged at 3,000 rpm for 10 min at 4° C. The collected serum was kept at a temperature of –80° C until analysis. Aliquots of the serum (0.5 ml) were adjusted with distilled water to a total volume of 1.0 ml, followed by the addition of 0.2 ml 0.5 *M* KH_2PO_4 buffer and 8 ml ethyl acetate. Samples were shaken on a mechanical shaker for 10 min, centrifuged at 3,000 rpm for 10 min and evaporated to dryness at 40° C under a stream of nitrogen. Extracted serum samples were reconstituted in 0.5 ml high-performance liquid chromatography (HPLC)-grade water by mixing and were then applied on a Bond Elut SAX column (Analychem International, Harbor City, Calif., USA). Then, 20 µl eluted material was chromatographed on a C_{18} µ-Bondapak column (3.9 × 300 mm) (Waters Associates, Milford, Mass., USA) with a mobile phase of 2%

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Table 1. 5-FU dose, pharmacokinetic parameters, patient response and drug toxicity

Patient number	Dose (mg/m^2)	SSc ($\mu g/ml$)	AUC_{72} ($\mu g\ h^{-1}/ml^{-1}$)	Cl (dose/AUC)	Toxicity	Response
1	190	0.050	3.00	63.3	–	+
2	191	0.080	5.56	34.4	+	+
3	288	0.010	0.65	444.4	–	+
4	390	0.130	7.94	49.1	+	–
5	400	0.077	4.58	87.3	–	–
6	400	0.105	5.89	67.9	–	–
7	407	0.129	5.94	68.5	–	–
8	426	0.317	19.80	21.5	+	+
9	449	0.077	4.44	101.1	–	+
10	460	0.119	7.64	60.2	+	–
11	472	0.138	8.80	53.6	+	+
12	477	0.095	6.08	78.5	–	–
13	489	0.289	19.28	25.4	+	–
14	495	0.162	9.86	50.2	–	+
15	510	0.302	18.66	27.3	+	+
16	543	0.215	14.45	37.6	–	–
17	564	0.217	13.72	41.0	+	+
18	570	0.144	8.34	68.5	–	+
19	600	0.187	11.40	52.6	+	+

SSc, steady-state concentration; AUC_{72}, area under the curve from zero to 72 h after the start of chemotherapy; Cl, total body clearance

methanol at a flow rate of 0.7 ml/min. the HPLC system was equipped with an automated sample injector (Waters 712 WISP), a programmable pump (Waters 600), a variable-wavelength UV detector (Waters 490), and a printer/integrater (Waters 741). Quantitation was carried out at 260 nm.

Pharmacokinetic evaluation. Three parameters were evaluated for determination of the pharmacokinetics of the continuous venous infusion. Steady-state serum concentrations (SSc) were calculated from the means of the serum concentration values obtained at 24, 48 and 72 h after the start of treatment. Areas under the curves from time zero to 72 h (AUC_{72}) were calculated by the trapezoidal rule. Total body clearances (Cl) were calculated according to the formula 5-FU dose/AUC. For statistical analysis of the data, Bonferroni's corrected P value was used [14].

Results

In all, 6 women and 13 men with a median age of 59 years (range, 32–76 years) were studied and each received 1 course of chemotherapy. Daily 5-FU doses ranged from 190 to 600 mg/m^2. Nine patients were included in the toxic group and the rest, in the non-toxic group. Stomatitis, anorexia, nausea and vomiting, disorientation and toxic dermatitis of greater than grade 2 were reported. Also, there were ten patients in the effective group, including three who achieved a partial response, and nine in the non-effective group. Serum levels of 5-FU rapidly increased, reaching a plateau within 2 h after the start of treatment. After the plateau had been reached, steady-state concentrations were maintained during the monitoring period, although wide variation was seen within this period in a minority of cases.

The doses and pharmacokinetic parameters calculated are listed in Table 1. There were positive correlations between the dose and both SSc ($r = 0.578$, $P < 0.01$) and AUC_{72} ($r = 0.558$, $P < 0.05$), as well as between SSc and AUC_{72} ($r = 0.993$, $P < 0.01$). The dose and the values for SSc and AUC_{72} in the toxic group were higher than those in the non-toxic group, and the Cl value was lower in the latter group when Cl was calculated on a log scale. The differences in these parameters were statistically significant according to comparative analyses of values obtained in the toxic vs non-toxic groups. In addition, all parameters of the effective group were higher than those of non-effective patients, however, no significant differences in any of the parameters were detected between these two groups (Table 2).

Discussion

Anti-cancer drugs have essential effects on cancer cells and adverse effects on normal cells. Many clinical trials of

Table 2. Relationship of statistical differences between pharmacokinetic parameters and toxicity or response

Group	Dose	SSc	AUC_{72}	Cl
Toxic	456±118	0.198±0.088*	12.53±5.55*	40.6± 14.1**
Non-toxic	422±115	0.106±0.059*	6.32±3.84*	106.7±120**
Effective	457±129	0.163±0.097	10.12±6.05	89.5±126.8
Non-effective	417±100	0.134±0.074	8.31±5.21	59.7± 19.5

* Significant difference ($P < 0.05$) between toxic and non-toxic groups

** Significant difference ($P < 0.01$) between toxic and non-toxic groups as calculated on a log scale

5-FU given by continuous infusion have reported relatively good response rates [1, 3, 8], but various degrees of toxicity have also been reported [2, 5]. Determination of an ideal 5-FU regimen requires that the optimal dose for obtaining maximal anti-cancer effects and minimal adverse effects be established. We found that the degree of adverse effect is dependent on the AUC or concentration-time product, whereas that of anti-tumor effect is not. Our data show that the dose intensity for the minimal anti-tumor effect obtained with a continuous infusion of 5-FU was 0.01 μg/ml (SSc) or 0.65 μg h^{-1} ml^{-1} (AUC) and that a certain degree of toxicity occurred when SSc and AUC_{72} values were >0.08 μg/ml and 5.56 μg h^{-1}/ml^{1}, respectively. Our results also suggest that the administration of 407 mg/m^2 daily is enough to obtain such dose intensities (Table 1); this finding is almost in agreement with previously reported data [13]. These results indicate that high-dose 5-FU therapy does not always provide therapeutic benefits under the conditions of the present study.

In contrast, Hillcoat et al. [4] reported that responders had significantly higher concentration-time product values than did non-responders, although there were no differences in this parameter between toxic and non-toxic patients [4]. However, these authors also stated that 5-FU concentrations, determined by a mass-spectrometric method, varied widely in many patients and that the definition of responder was different from the WHO criteria used in the present study. Recent findings [7] seem to support our data because the effects of anti-tumor agents are not simply proportional to the drug concentration, but rather are due to the sensitivity of tumor cells to the drug. Although it would be very interesting to determine the reason for the discrepancy between anti-tumor effects and dose intensity, it remains unclear in clinical studies. Analyses of the active metabolite of 5-FU and of drug resistance in tumor cells are necessary.

The kinds of parameter selected for pharmacokinetic analysis are important because the cell-killing action of an agent is commonly related with the cell-cycle phase specificity by which the parameters vary. Anti-cancer agents have been classified as follows: non-cell-cycle phase-specific agents, as "concentration-dependent" drugs; and cell-cycle phase-specific agents, as "time-dependent" drugs [12]. 5-FU is considered to be an anti-metabolite and belongs to the group of S-phase-specific agents. It is not necessarily appropriate to evaluate the cell-killing action of cell-cycle phase-specific agents by AUC and/or time-concentration products, although the cytotoxicity of drugs lacking cell-cycle phase specificity can be evaluated by such parameters [10]. However, these parameters have actually been selected in several studies analyzing the pharmacokinetics of 5-FU [4, 13] and the antitumor activity of 5-FU has been reported to be correlated with AUC within the drug concentration range between 0.02 and 0.4 μg/ml [6], which is almost the same concentration range usually shown in clinical studies. Accordingly, the use of these parameters is considered to be suitable within the range of doses used in the present study.

Continuous venous infusion of 5-FU seems to be an effective regimen against advanced colonic cancer. We did not clarify whether prolongation of the infusion period or administration of an initially high dose followed by a continuous low dose provides benefits to patients with gastrointestinal cancer. Further pharmacokinetic analyses of this agent are necessary in the near future.

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