Fluctuation in Plasma 5-Fluorouracil Concentration During Continuous 5-Fluorouracil Infusion for Colorectal Cancer

HIDEO MATSUMOTO[1], HIDEO OKUMURA[1], HARUAKI MURAKAMI[1], HISAKO KUBOTA[1], MASAHARU HIGASHIDA[1], ATSUSHI TSURUTA[1], KAORU TOHYAMA[2] and TOSHIHIRO HIRAI[1]

Departments of [1]Digestive Surgery and [2]Laboratory Medicine, Kawasaki Medical School, Kurashiki City, Okayama, Japan

Abstract. *Background/Aim: It is generally believed that the plasma concentration of 5-fluorouracil (5-FU) is constant when 5-FU is continually administered for chemotherapy. The aim of the present study was to verify whether this is true. Patients and Methods: Nine patients with colorectal cancer were enrolled in this study. All patients received chemotherapy; four patients received FOLFIRI (leucovorin, 5-fluorouracil, irinotecan) and five received FOLFOX (leucovorin, 5-fluorouracil, oxaliplatin). 5-FU was administered continuously (2400 mg/m^2) for 46 h. Serum was collected at 12 points after the start of administration. The concentration of 5-FU was evaluated using a new immunoassay method and gas chromatography–mass spectrometric (GC/MS) method. Results: The concentrations of 5-FU fluctuated dramatically over time, with greater than 3-fold changes in each individual, and the pattern was not constant. Conclusion: Because the serum concentration of 5-FU fluctuates and displays various patterns, the dosage should not be based on body surface area. A new individualized method for determining the 5-FU dosage should be developed.*

5-Fluorouracil (5-FU)-based chemotherapy regimens combined with L-leucovorin, irinotecan, or oxaliplatin with/without targeted monoclonal antibodies have dramatically prolonged the life expectancy of patients with colorectal cancer. Recently, it was advocated that the response to 5-FU treatment is dependent on the value of the area under the plasma 5-FU concentration–time curve (AUC), not body surface area (BSA) (1-4). Furthermore, toxicity has been shown to be correlated with 5-FU concentration and the AUC (2, 4). Pharmacokinetically-guided dose adjustments for 5-FU were found to be ideal for improving the therapeutic outcome (5).

The concentration of 5-FU is typically measured by using gas chromatography–mass spectrometry (GC/MS) (6). Although this method provides accurate results, it is complex and time-consuming. Recently, the My5-FU™ immunoassay (Saladax Biomedical, Inc., Bethlehem, PA, USA) method for measuring the concentration of 5-FU was developed. It is a competitive homogeneous two-reagent nanoparticle agglutination immunoassay method based on changes that occur in light scattering or absorbance when nanoparticles aggregate (6-8), and its results can be obtained quickly and with ease. Therefore, this assay might be useful for therapeutic drug monitoring of 5-FU in clinical settings.

Generally, the AUC of 5-FU is calculated based on the assumption that the plasma concentration of 5-FU is constant during continuous infusion. In the present study, our aim was to verify the constancy, or lack thereof, of the 5-FU concentration during treatment with FOLFOX and FOLFIRI using the My5-FU™ immunoassay, and to compare the results of the My5-FU assay and GC/MS methods.

Patients and Methods

Patients who had received chemotherapy of 5-FU regimen were enrolled in this prospective study, between March 2012 and February 2014, only after informed consent was obtained.

The 5-FU dosage was 2,400 mg/m^2 continuously for 46 h in FOLFIRI and FOLFOX regimens. 5-FU was administered using a balloon-type infuser (Baxter infuser SV 2.5; Baxter™, Deerfield, IL, USA) in the first six cases and an electromechanical-type infusion pump (Infusion pump TE-331S; TERUMO™, Tokyo, Japan) in the remaining three cases.

Plasma was collected into EDTA tubes containing a stabilizing agent [a derivative of uracil with properties that irreversibly inhibit dihydropyrimidine dehydrogenase (DPD)] at 0.25, 1.5, 3, 6, 9, 12, 18, 24, 32, and 46 h after the start of administration, and at 1 and 1.5 h after completion of administration. The serum concentration of 5-FU was measured using the My5-FU™ assay in our Hospital

Correspondence to: Hideo Matsumoto, MD, Ph.D., Department of Digestive Surgery, Kawasaki Medical School, 577 Matsushiama, Kurashiki City, Okayama, 701-0192, Japan. Tel: +81 864621111, Fax: +81 864621199, e-mail: h-matsu@med.kawasaki-m.ac.jp

Key Words: Concentration of 5-FU, 5-FU-based chemotherapy, My5-FU™ immunoassay, FOLFIRI, FOLFOX.

Table I. *Patient's characteristics.*

Case	Age, years	Gender	Primary lesion	Chemotherapy	Target	Treatment
1	56	M	Rectum	P-mab+FOLFOX	Primary lesion	Primary
2	64	M	Sigmoid colon	FOLFOX6	Adjyvant chemotherapy	Primary
3	36	M	Rectum	P-mab+FOLFIRI	Recurrence (peritonium)	Primary
4	49	M	Rectum	FOLFOX6	Recurrence (liver)	Primary
5	74	F	Sigmoid colon	FOLFIRI	Recurrence (peritonium)	Secondary
6	50	M	Rectum	FOLFIRI	Recurrence (local)	Primary
7	53	F	Rectum	BV+FOLFOX	Metastasis (liver)	Secondary
8	60	M	Rectum	FOLFOX	Metastasis (liver)	Secondary
9	62	F	Rectum	FOLFIRI	Primary lesion	Primary

M: Male, F: female, P-mab: panitumumab, BV: bevacizumab, FOLFIRI: leucovorin, 5-fluorouracil, irinotecan. FOLFOX: leucovorin, 5-fluorouracil, oxaliplatin.

Table II. *Serum level (ng/ml) measured by My5-FU™ assay. Values in parentheses (ng/ml) were those as measured by gas chromatographic–mass spectrometric method.*

	Time after the start of administration (h)										Time after the completion (h)	
Case	0.25	1.5	3	6	9	12	18	24	32	46	1	1.5
1	171.4	380.3	164.1	173.5 (79.5)	216.6	167.0	541.3	171.8	310.4	204.0	242.4	200.9
2	86.7 (10.3)	384.5 (268.5)	85.5 (20.8)	173.5 (110.1)	294.4 (185.4)	572.3 (459.8)	549.3 (441.1)	436.0 (347.4)	305.4 (213.3)	699.5 (543.7)	74.1 (17.2)	84.5 (4.7)
3	138.0	224.3	207.6	223.9	173.9	173.9	225.1	132.6	289.6	353.5	104.6	67.3
4	65.8	307.8	164.3	348.2	388.4	377.1	250.1	460.6	671.2	399.8	84.5	90.9
5	235.2	252.1	238.8	382.9	361.1	651.8	656.9	617.9	586.3	39.0	44.8	33.4
6	707.0	212.9	186.8	143.4 (43.6)	478.9 (352.1)	190 (120.7)	315.8	320.5	371.1	82.3	69.0 (N.D.)	89.0 (N.D.)
7	284.0	104.8	258.0	147.3	254.5	236.9	469.3	354.2	292.8 (181.5)	333.3 (271.5)	19.5	12.0
8	1837.8 (1657.3)	43.9 (5.0)	339.9 (250.6)	499.8 (445.5)	295.3	395.4	527.6 (481.8)	272.3 (13.5)	518.6 (388.5)	369.6 (316.6)	24.6 (4.3)	45.7 (1.6)
9	42.2	247.0	206.2	330.9	472.4	317.9 (242.7)	145.7 (104.4)	508.3 (430.3)	392.6 (298.1)	473.2 (399.9)	0	25.7

laboratory unit. For cases 6-9, the 5-FU concentration was measured by using GC/MS at some time points (Table II). The points measured by using GC/MS were chosen for the following reasons: i) to include low and high values measured using My5-FU™, ii) to avoid bias for a particular regimen, iii) to include consecutive points without large variations, iv) to include all time points from start to finish, v) and to ensure constraints on cost. The samples were centrifuged immediately and plasma was stored at under 80˚C until analysis. The plasma levels of 5-FU were assessed by gas chromatography-mass spectrometry (GC-MS) (14). GC-MS was carried-out using the Trace GC and Trace MS with an Xcalibur (Ver. 1.2) control system (Thermo Electron K.K., Yokohama, Japan). The methodology of GC-MS has been previously described in detail (9).

The concentration curves were drawn using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) software to draw scatter plots. The concentration of 5-FU was displayed on both a logarithmic scale and a normal scale.

This study protocol was approved by the Institutional Review Board of Kawasaki Medical School (Approved No. 908, 908-1, 908-2). Informed consent was obtained from all patients.

Results

Nine patients, six men, and three women, were enrolled in the study between March 2012 and February 2014 (Table I). The mean age was 56 years. Seven patients had rectal cancer, and two had sigmoid colon cancer. The reasons for administration of chemotherapy were as follows: recurrence in four cases, unresectability in two, simultaneous metastasis to the liver after resection of the primary lesion in two, and adjuvant therapy in one case. Five patients received a FOLFOX regimen, and four patients received a FOLFIRI

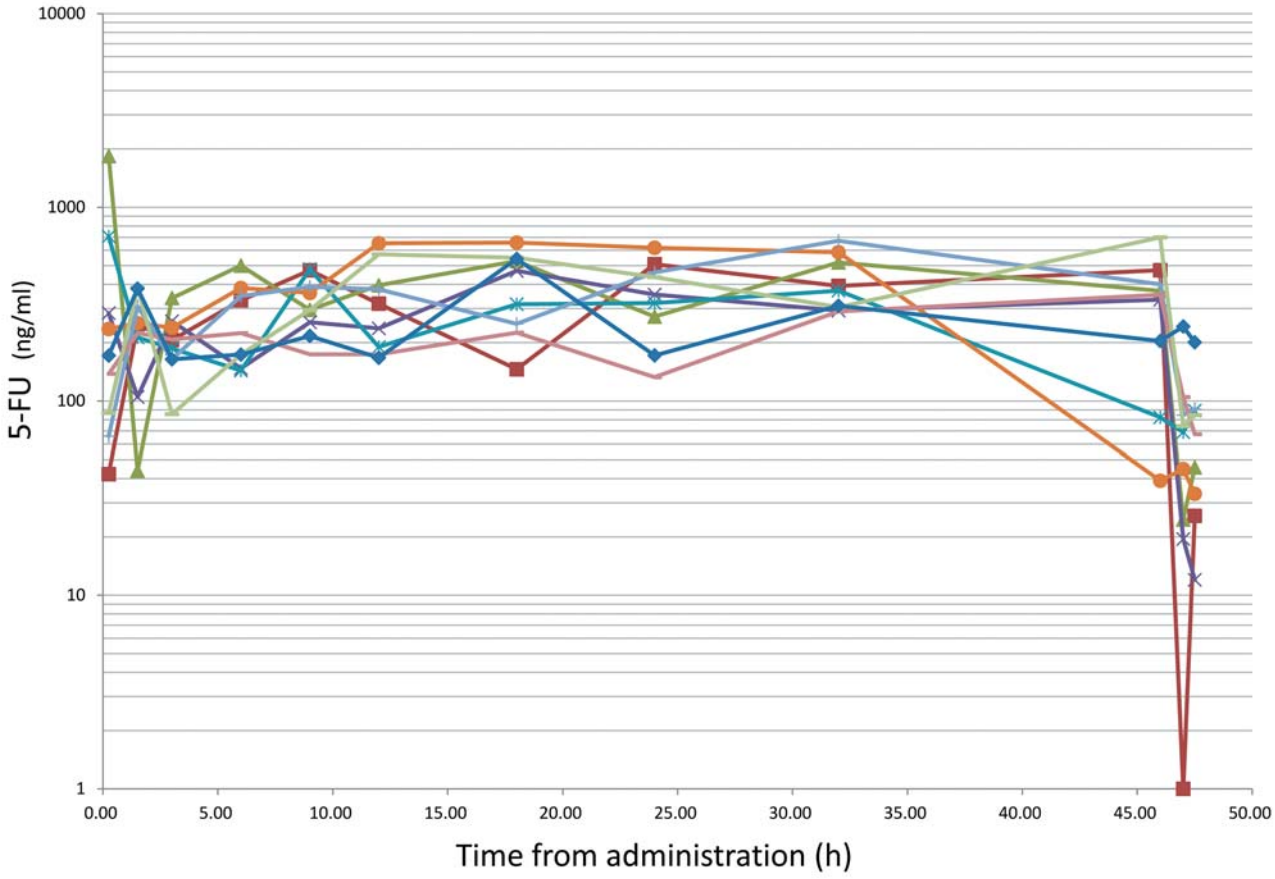

Figure 1. *Concentration curve with vertical axis on log.*

regimen. Three patients also received molecularly-targeted therapy, including two who received panitumumab and one who received bevacizumab (Table I).

Concentration curves for all patients are shown in Figure 1. The concentration curves of each case are individually shown in Figure 2. As seen in Figures 1 and 2, the concentration of 5-FU was not constant but changed dynamically over time. In fact, for all patients, the concentration of 5-FU varied more than 3-fold over time. Furthermore, the change in the concentration curve was quite different for each individual.

In general, the concentration measured by using My5-FU™ was higher than that measured by using GC/MS (Table II). For case 8, the concentration of 5-FU at 0.25 h after the start of administration was extremely high, and therefore, 5-FU was re-measured by using the GC/MS method at 10 of the 12 time points, each of which revealed the same tendency. The values of 5-FU measured by using My5-FU™ were not completely consistent with the values measured by using GC/MS. However, the form of the concentration curves generated by using the two methods was similar (Figure 3). This applied not only to case 8 but also to case 2. Although the 5-FU concentration measured using My-5 FU™ was not equal to that measured by using GC/MS, the concentrations were important in reflecting the trend of change. For those samples measured using GC/MS, 5-FU concentration was re-measured by FALCO Biosystems, Ltd. (Kyoto, Japan) using the My5-FU™ assay. No differences were detected between the values measured in our laboratory by using the My5-FU™ method and those obtained by using FALCO Biosystems.

Discussion

It has been generally believed that the plasma concentration of 5-FU is constant during continuous infusion. However, our data reveal that fluctuations in the concentration of 5-FU were dynamic, individual, and without a regular pattern. We were unable to identify any point that could represent an individual's 5-FU concentration during continuous infusion of this drug.

Recently, it was proposed that the AUC-based dosing of 5-FU was superior to BSA-based dosing of 5-FU in terms of

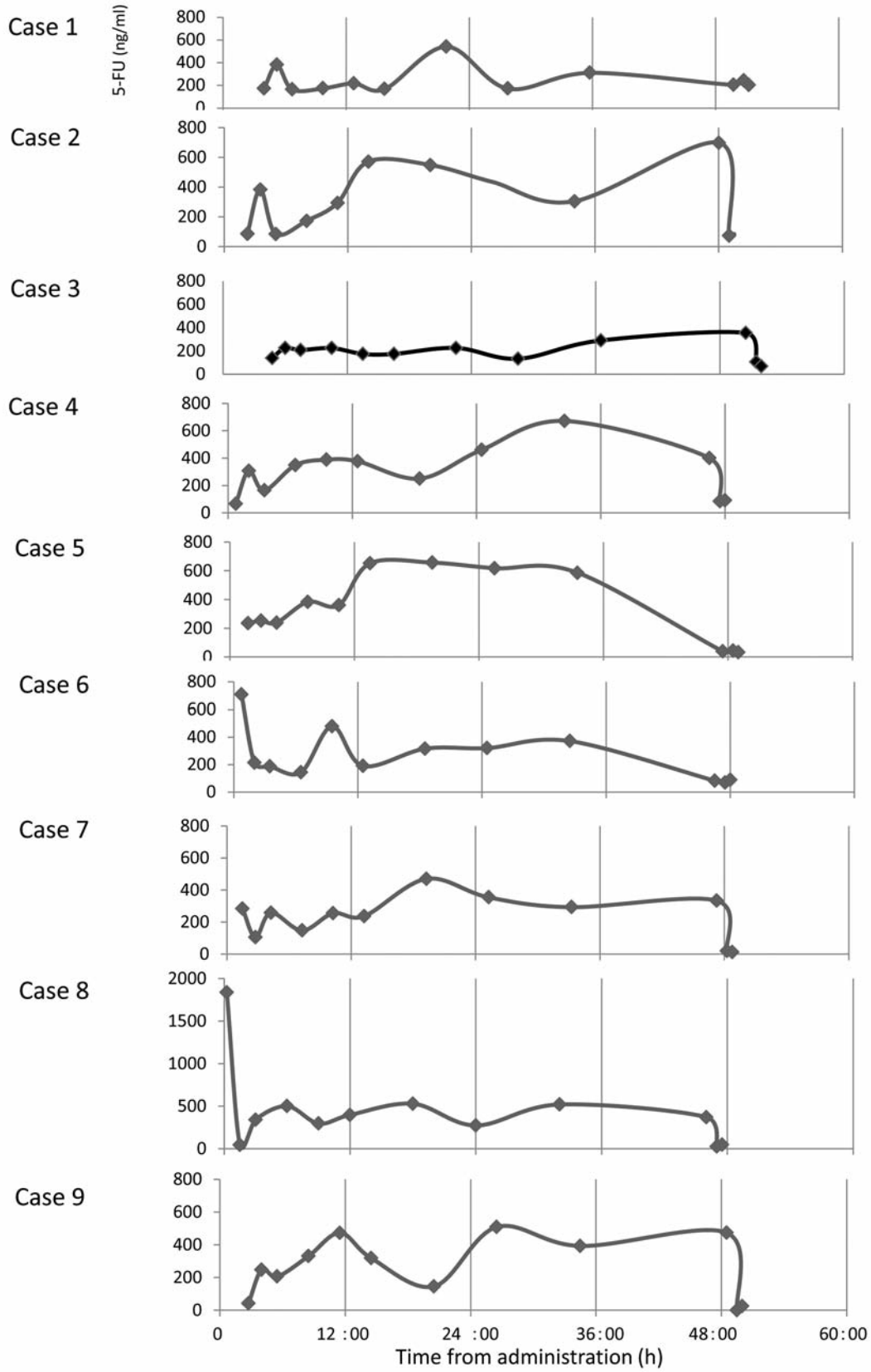

Figure 2. *Concentration curves of all cases with a scatter plot.*

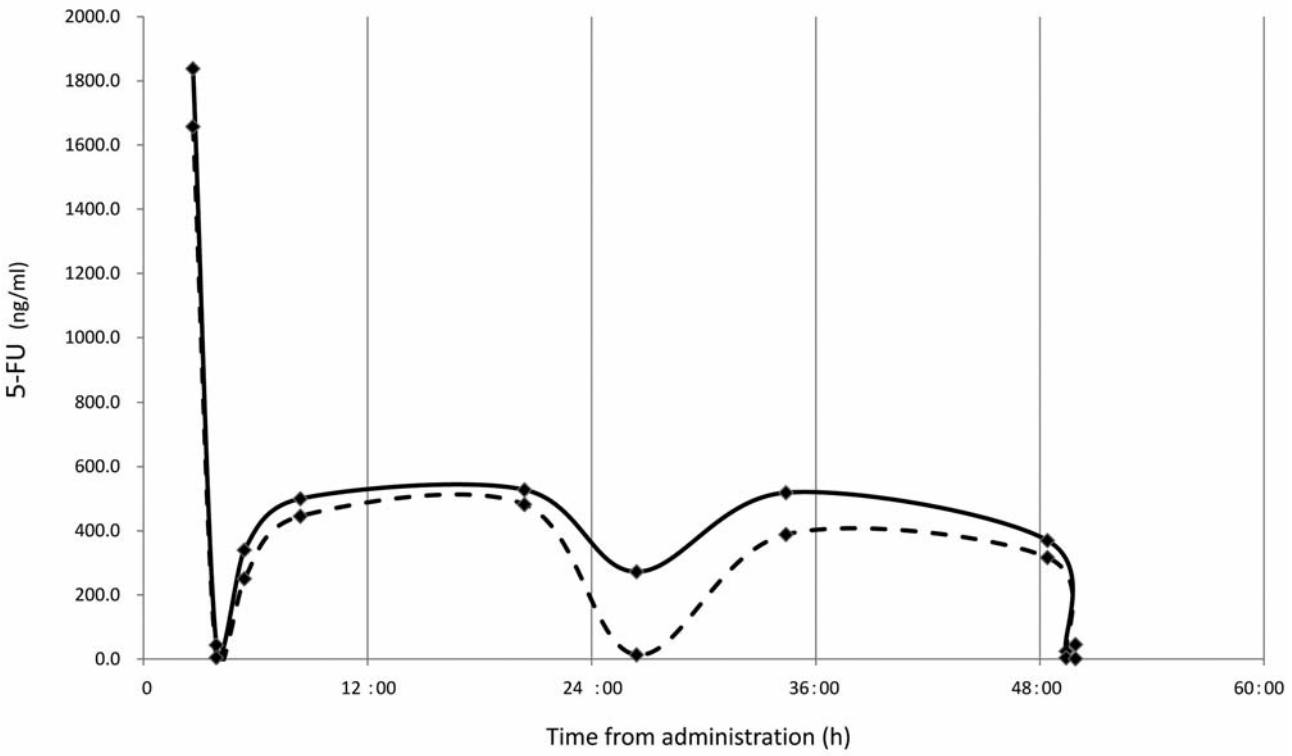

Figure 3. *Concentration curve of case 8: Solid line connects the values measured by My5-FU™ immunoassay method and dashed line connects the values measured by the GC-MS method.*

efficacy and toxicity (1, 2, 5, 10, 11). Gamelin *et al*. reported on the priority of 5-FU dose adjustments for patients with colorectal cancer (1, 2, 12). 5-FU concentrations were measured during the first cycle of the FOLFOX regimen, and the 5-FU dose of the second and subsequent cycles was decided according to a dose-adjusted chart, which is the outcome of their research (2, 4). Their data on the AUC were based on the 5-FU concentration at two time points: 3 and 7 h after beginning the infusion.

However, Adjei *et al*. reported that the 5-FU concentration for individual patients fluctuated over a 2- to 34-fold range (median=14-fold) during the 24-h observation period for continuous venous injection of 5-FU. They measured the 5-FU concentration 16 times during 24 h (13). Similarly, Au *et al*. reported that the 5-FU concentration fluctuated 6- to 130-fold during five days of continuous venous infusion of 5-FU, measured at 2 h and every 24 h (14).

A circadian variation was reported to affect the pharmacokinetics of 5-FU-based chemotherapy (16, 17). In consideration of the circadian variation, chronotherapy was proposed in order to enhance the effect and reduce the toxicity of 5-FU (18, 19). It was reported that circadian variation affected the pharmacokinetics of 5-FU *via* a change in DPD activity. In that report, maximum DPD activity exceeded minimum activity by approximately 2-fold, and the maximum 5-FU concentration exceeded the minimum concentration by almost 5-fold. However, the times of peak DPD activity and 5-FU concentration did not coincide (15).

Recent reports have shown that pharmacokinetically-guided dose adjustments, using the nanoparticle-based immunoassay used in this study, were successfully employed for dose optimization (20, 21). It was reported that this immunoassay method might be suitable for monitoring 5-FU in patients with cancer because the assay results correlated with liquid chromatography–MS results (8). In our findings, however, the values of 5-FU measured by using My5-FU™ were not completely consistent with the values measured by the GC/MS method, but the trend in the values over time was nearly identical (22, 23).

Compared to GC-MS, the immunoassay gave higher values for all measured parameters. This could be because of assay cross-reactivity with uracil, which is present in plasma. Normally, the cross-reactivity rate for uracil is thought to be 9.9%, so it should not affect the performance of the assay (24).

In previous studies, few measurements of 5-FU concentration were used to evaluate dose adjustment. Kaldate *et al*. measured the 5-FU concentration at only three time points, the beginning of infusion (2 h), the middle of infusion

(22 h), and toward the end of infusion (44 h) (19), and Patel *et al*. measured the 5-FU concentration at only one point during the 2-44 hours after the start of continuous infusion (20).

The system used to deliver continuous infusion might be thought to have an effect on the 5-FU concentration. In this study, the balloon-type infuser was used routinely in the first six cases, but for the latter three cases, an electromechanical-type infusion pump was used to take into account possible variation. Our results indicate that the concentration was not dependent on the delivery system but rather, fluctuated individually, as described.

Soh *et al*. concluded that pharmacokinetically guided 5-FU dose adjustments did not result in a greater number of Asian patients achieving target AUC. Furthermore, the AUC levels of 5-FU did not correlate with toxicity. At the 2015 American Society of Clinical Oncology Gastrointestinal Cancer Symposium, it was proposed that larger numbers of patients are needed to confirm the clinical utility and benefit of pharmacokinetically guided 5-FU dosing in Asian patients (25).

However, the theory of the AUC-based dosing of 5-FU is reasonable and would be ideal. It might be necessary to determine the time points of sample collection that could lead to dose intensification or to determine how to best predict the fluctuation in concentration.

One limitation of this study was its small sample size. Therefore, future studies incorporating more cases are necessary as it might then be possible to determine a specific rule for predicting the fluctuation of 5-FU.

In conclusion, we were unable to determine a rule for predicting the AUC of 5-FU because the fluctuation over time was dramatic, and the concentration varied by more than 3-fold.

Conflicts of Interest

There are no conflicts of interest to declare.

Acknowledgements

The Authors would like to express their gratitude to Mr. K. Kohguchi and Ms. S. Furukawa, who are members of our hospital laboratory unit and who cooperated with us during this study, and to Mr. H. Yamane of FALCO Biosystems, who gave advice on writing this manuscript. They would also like to thank Editage (www.editage.jp) for English language editing.

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Received July 14, 2015
Revised September 1, 2015
Accepted September 17, 2015