Evaluation of the 5-fluorouracil plasma level in patients with colorectal cancer undergoing continuous infusion chemotherapy

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Abstract. 5-Fluorouracil (5-FU) dosing has traditionally been based on the body surface area (BSA) in colorectal cancer treatment. However, there is accumulating evidence that dosing based on BSA may be of limited use. The purpose of the present study was to evaluate the changes in 5-FU plasma levels and tumor response as well as the severity of adverse events in patients with cancer treated with 5-FU combined chemotherapy. The dosing amount of 5-FU was determined based on the BSA. Blood samples were collected, and 5-FU plasma levels in 15 patients with colorectal cancer were measured three times (0, 22 and 40 h before and after the start of infusion) during constant-infusion of 5-FU for 46 h by an immunoassay. 5-FU plasma levels were significantly higher at 22 and 40 h compared with at 0 h (P<0.001), when all 15 patients were analyzed. Notably, the tumor response of the partial response/stable disease group showed significant increases in 5-FU plasma levels at 40 h compared with at 22 h (P<0.01), while the progressive disease group showed no significant increase. In addition, the 5-FU plasma level in the adverse event level of grade ≥2 was higher than that of grade <2 at 40 h after the start of infusion. Collectively, these observations indicated that during continuous infusion of 5-FU, the 5-FU plasma level increased significantly, and the tumor response (such as partial response, stable or progressive disease) may be influenced by the increase of 5-FU plasma level from the start of infusion. Therefore, the 5-FU plasma level may be a predictive factor for maximizing the tumor response and minimizing the risk of severe adverse events.

Introduction

Colorectal cancer is one of the leading causes of cancer mortality worldwide (1,2). Since the introduction of 5-fluorouracil (5-FU) in the 1960s, 5-FU has been widely used in colorectal cancer treatment, as well as for other malignancies, including gastric cancer, breast cancer, and head and neck cancer (3). Subsequently, 5-FU-based combination therapies, such as 5-FU plus folinic acid-oxaliplatin (FOLFOX) and 5-FU plus folinic acid-irinotecan (FOLFIRI) regimens, have been developed (4-6). Previously, the addition of anti-epidermal growth factor receptor monoclonal antibodies, such as cetuximab and panitumumab, and humanized anti-vascular growth factor receptor monoclonal antibodies, such as bevacizumab, have also proven to enhance the anti-tumor effect (7-12). Despite the development of combined therapy, 5-FU has continued to be the main drug for the treatment of colorectal cancer. However, the calculation of the dosing amount of 5-FU is still simply based only on the body surface area (BSA) (13).

Dosing based on BSA is considered convenient and easy to use, yet previous studies have indicated that many patients treated with 5-FU are not given the appropriate dose to achieve optimal plasma concentration (13,14). According to a previous study, only 20-30% of patients are treated with the appropriate therapeutic range, while 40-60% are underdosed and 10-20% are overdosed (15). Previous results have suggested the existence of interpatient and intrapatient pharmacokinetic (PK) variation in 5-FU clearance (13,16-19). Collectively, these previous results indicated that dosing based on BSA is of limited use (13,19,20). Therefore, the concept of direct monitoring of 5-FU plasma concentrations with appropriate dose adjustments has been introduced. A number of previous studies, including a large multicenter randomized trial, have shown that PK-guided dose adjustment of 5-FU in metastatic colorectal cancer has resulted in an improvement in therapeutic outcomes and reduced the severity of adverse events (14,20). The aim of the present study was to determine an optimal method of determining the 5-FU dosage for patients with colorectal cancer undergoing chemotherapy. In the present prospective study, the transition of 5-FU plasma levels in patients with colorectal cancer during treatment with...
5-FU combined chemotherapy was monitored. Furthermore, the association between the tumor response and adverse events [classified by the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0] (21) based on the 5-FU plasma levels was evaluated.

**Patients and methods**

*Patient treatment and eligibility.* The present prospective study was performed using 15 patients with colorectal cancer treated with 5-FU combined chemotherapy at The Tobu Chiiki Hospital. All patients were subjected to standardized clinical practice, according to the Japanese Society for Cancer of the Colon and Rectum Guidelines 2016 for the Treatment of Colorectal Cancer (22). 5-FU combined chemotherapy included modified FOLFOX6 (mFOLFOX6) (n=6): Oxaliplatin 85 mg/m² for 2 h, leucovorin (folinic acid) 200 mg/m² for 2 h, 5-FU 400 mg/m² bolus and 5-FU 2,400 mg/m² continuous intravenous infusion for 46 h; or FOLFIRI (n=9): Irinotecan 150 mg/m² for 2 h, leucovorin 150 mg/m² for 2 h, 5-FU 400 mg/m² bolus and 5-FU 2,400 mg/m² continuous intravenous infusion for 46 h. All patients received the dose of 5-FU based on their BSA (5-FU 400 mg/m² bolus and 5-FU 2,400 mg/m² continuous intravenous infusion for both mFOLFOX6 and FOLFIRI regimens). The Eastern Cooperative Oncology Group Performance Status (ECOG-PS) classification (23) was used to evaluate the performance status (PS) of each patient. Patients were required to have a PS ≤2. The criteria for the classification of PS were as follows: i) PS 0, fully active, able to carry on pre-disease performances without restriction; ii) PS 1, restricted in physically strenuous activity but ambulatory and is able to carry out work of a light or sedentary nature; and iii) PS 2, ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours. All the patients included in the present study were treated under the guidance of professional medical staff members. Patients were required for 4 days in admission for treatment and were readmitted every 2 weeks for 3 months (a total of six admissions). The present study was approved by the Ethics Committee of Tobu Chiiki Hospital (approved December 21, 2015; IRB nos. 15 and 4), and performed between January 1, 2017 and March 31, 2017. The present study was conducted in accordance with the principles of the amended ‘Declaration of Helsinki and Ethical Guidelines for Epidemiological Research’ (established by the Japanese Government in 2008) (24). Written informed consent was obtained from all patients before they were enrolled in the present study. Patients with PS >2 or requiring radiation or dialysis, or those unable to provide informed consent were excluded.

*Measurement of 5-FU plasma levels.* Venous blood samples (10 ml) were collected three times during admission (prior to the start of infusion, and 22 and 40 h after the start of continuous 5-FU infusion). 5-FU plasma levels were measured using the My-5FU® assay, a competitive homogeneous nanoparticle agglutination immunoassay (FALCO Biosystems Ltd.). This assay is used for patients receiving 5-FU by continuous infusion to facilitate PK dose adjustment at the next cycle and drug monitoring to achieve an optimal plasma level of the drug. The assay uses two reagents and when they are mixed, the nanoparticles aggregate together. Therefore, the amount of aggregation of nanoparticles determines the 5-FU concentration in plasma samples. 5-FU plasma levels at 0 (prior to the start of infusion), 22 and 40 h after the start of continuous 5-FU infusion in each patient were calculated as median values of six admissions and statistically analyzed.

*Analysis of tumor response and adverse events.* Tumor response to treatment was classified according to Response Evaluation Criteria in Solid Tumors Group (RECIST 1.1) criteria (25) and was evaluated after the 3 month monitoring period. A complete response required the disappearance of every lesion. A partial response required a ≥30% reduction in the cross-sectional area of all lesions. Stable disease required a lesion size decrease <30%. Progressive disease categorization encompassed any situation in which any one lesion increased in cross-sectional size by >25% or a new lesion appeared.

Adverse events during chemotherapy were evaluated just after the infusion of 5-FU (for 46 h) on the 4th day of admission, according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 (21), based on physical examination and laboratory tests. The severity of adverse events was graded between 1 and 5. Grade 1 consists of the following criteria: i) Mild; ii) asymptomatic or mild symptoms; iii) clinical or diagnostic observations only; and iv) intervention not indicated. Grade 5 is associated with death. Adverse events were assessed on the last day before discharge for each patient.

*Statistical analysis.* The plasma levels of 5-FU are presented as box-and-whisker plots (containing the minimum, first quartile, median, third quartile and maximum values), unless otherwise stated. The 5-FU plasma levels were measured three times (0 h prior to the start of infusion, 22 and 40 h after the start of continuous 5-FU infusion) in each patient, calculated as median values of six admissions, and statistically analyzed. Statistical significance was determined by one-way ANOVA with Bonferroni’s post hoc test using GraphPad Prism 7 (GraphPad Software, Inc.). Statistical significance for the different tumor responses (complete response, partial response, stable disease and progressive disease) as well as the response rate and disease control rate between mFOLFOX6 and FOLFIRI was determined by a bivariate analysis using JMP 13 software (SAS Institute, Inc.). P<0.05 was considered to indicate a statistically significant difference.

*Results.*

*Patients.* A total of 15 patients (5 female and 10 male) were enrolled in the present study. The patient characteristics are summarized in Table I. The mean age was 69.0±7.3 (mean ± SD, range of 52-79) years and the mean BSA was 1.57±0.18 m². Based on the ECOG-PS classification (23), 1 patient was categorized as PS 0, 11 patients were categorized as PS 1, and 3 patients were categorized as PS 2. Only 1 patient had a tumor located in the descending colon, 10 patients had a tumor located in the sigmoid colon and 4 patients had a tumor located in the rectum. In total, 4 patients had a pathological diagnosis of well differentiated adenocarcinoma, 10 patients were diagnosed with moderately differentiated adenocarcinoma and 1 patient was diagnosed with papillary adenocarcinoma, according
to the Japanese Classification of Colorectal Carcinoma (8th Edition) (26). A total of 9 patients enrolled were treated for a primary tumor, while 6 patients were treated for a recurrent tumor. Among the 9 patients, 8 patients were diagnosed as Stage IV and 1 patient was diagnosed as Stage IIIb, based on the Japanese Classification of Colorectal Carcinoma (8th Edition) (26) (data not shown). The 9 patients were diagnosed with resectable tumors, and thus they were not treated for a neoadjuvant setting. A total of 14 patients had metastasis. The most frequent metastatic site (6 patients) was the liver; other metastasis sites included the lung (1 patient) and peritoneum (2 patients). A total of 5 patients had multiple metastatic sites. A total of 11 out of 15 patients underwent operations.

**Changes of the plasma level of 5-FU during infusion.** Before the start of infusion, the plasma level of 5-FU was 0 ng/ml in all patients (n=15). During infusion of 5-FU for 46 h, the level at 22 h was 460.5 ng/ml (median value), while the level at 40 h reached 682.0 ng/ml. The levels at 22 h and 40 h were significantly higher than that at 0 h (P<0.001; Fig. 1). Notably, the level at 40 h was significantly higher than at 22 h (P<0.01).

**Adverse events in different tumor response groups.** Table III shows the adverse events of grade ≥2 in the different tumor response groups; grade 2, according to CTCAE v5.0, which is defined according to the following criteria: i) Moderate; ii) minimal, local or noninvasive intervention indicated; and iii) limiting age-appropriate instrumental activities of daily living. In the partial response group, numbness was the most common adverse event (75%). In the stable disease group, numbness and diarrhea were observed (both 50%). In the progressive disease group, numbness (80%), appetite loss (60%) and hand foot syndrome (60%) were observed. Adverse events were evaluated after the infusion of 5-FU for 46 h on the 4th day of admission. Therefore, this suggests that the 5-FU plasma level at 40 h after the start of infusion may reflect the results of the adverse events.

**Plasma levels of 5-FU in the patients with different severities of adverse events.** Among the 15 patients, 13 patients were included in the adverse event level of grade ≥2. In the partial response/stable disease group, 8 out of 9 patients were included in the adverse event level of grade ≥2, whereas in the progressive disease group, the 5-FU plasma level at 22 h after the start of infusion was 460.5 ng/ml (median value), while the level at 40 h reached 690.9 ng/ml (Fig. 2A). The levels at 22 and 40 h were significantly higher than at 0 h (P<0.001). Notably, the level at 40 h was significantly higher than at 22 h (P<0.01). In the progressive disease group, the 5-FU plasma level at 22 h after the start of infusion was 435.1 ng/ml, while the level at 40 h reached 525.9 ng/ml (Fig. 2B). The levels at 22 h and 40 h were significantly higher than at 0 h (P<0.05). However, the level at 40 h was not significantly higher than at 22 h.

Furthermore, the mean 5-FU plasma level at 40 h after the start of infusion between the partial response/stable disease group and the progressive disease group was compared (Fig. 2). The 5-FU plasma level of the partial response/stable disease group (776.2±286.9 ng/ml; mean ± SD) was higher than that of the progressive disease group (681.9±369.4 ng/ml; data not shown), although there was no significant difference between the two groups, as assessed by one-way ANOVA analysis with Bonferroni’s post hoc test.
Table II. Tumor response and 5-fluorouracil regimens.

<table>
<thead>
<tr>
<th>Tumor response</th>
<th>mFOLFOX6 (n=6)</th>
<th>FOLFIRI (n=9)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>Partial response</td>
<td>2</td>
<td>3</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Stable disease</td>
<td>1</td>
<td>3</td>
<td>0.60</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>3</td>
<td>3</td>
<td>0.52</td>
</tr>
<tr>
<td>Response rate</td>
<td>33%</td>
<td>33%</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Disease control rate</td>
<td>50%</td>
<td>67%</td>
<td>0.52</td>
</tr>
</tbody>
</table>

In total, 6 patients were treated with the mFOLFOX6 regimen and 9 patients were treated with the FOLFIRI regimen. The number of patients with different tumor responses (complete response, partial response, stable disease and progressive disease) in each regimen are listed. The response rate is defined as the proportion of patients with complete or partial response among each regimen. The disease control rate is defined as the proportion of patients with complete, partial response or stable disease among each regimen. Statistical significance was determined by a bivariate analysis. There was no significant difference in each tumor response, response rate or disease control rate between the two regimens. mFOLFOX, modified 5-fluorouracil plus folinic acid-oxaliplatin; FOLFIRI, 5-fluorouracil plus folinic acid-irinotecan; N/A, not applicable.

Discussion

5-FU dosing has traditionally been based on BSA in colorectal cancer treatment. However, there is accumulating evidence that the dosing based on BSA may be of limited use (13). The purpose of the present study was to evaluate the changes in 5-FU plasma levels and the tumor response as well as the severity of adverse events in patients with cancer treated with 5-FU combined chemotherapy (mFOLFOX6 and FOLFIRI). The dosing amount of 5-FU was determined based on the BSA, and the transition of 5-FU plasma levels in 15 patients with colorectal cancer was monitored three times (0,22 and 40 h) before and after the start of infusion during constant-infusion of 5-FU for 46 h. The present study demonstrated that 5-FU plasma levels were significantly higher at 22 and 40 h compared with at 0 h, when all 15 patients were analyzed. Notably, the partial response/stable disease group showed significant increases in 5-FU plasma levels at 40 h compared with at 22 h, although the progressive disease group showed no significant increase. In addition, the 5-FU plasma level in the adverse event level of grade ≥2 was higher than that of grade <2 at 40 h after the start of infusion.

A significant increase in the 5-FU plasma level at 40 h compared with 22 h after the start of infusion was observed. In order to explain the wide range of increases in 5-FU plasma levels, a number of previous studies suggested that dihydropyrimidine dehydrogenase (DPD), an initial rate-limiting enzyme related to 5-FU metabolism, plays a role in determining the plasma levels of 5-FU (27-30). Therefore, the significant increase in 5-FU plasma levels after continuous intravenous infusion (particularly between 22 and 40 h) observed in the partial response/stable disease group may be due to the decreased 5-FU-metabolizing activity of DPD. However, the non-significant increase of 5-FU plasma level in the progressive disease group (between 22 and 40 h) may suggest that the 5-FU-metabolizing activity of DPD is higher than that of the partial response/stable disease group. Furthermore, the present results suggested that the tumor response may be influenced by the increase of 5-FU plasma level (possibly based on the low DPD activity) after the start of continuous infusion, as observed in the higher plasma 5-FU level in the adverse event level of grade ≥2. 5‑FU plasma levels at 40 h after the start of infusion in the adverse event level of grade ≥2 were significantly higher than that of grade <2 with the partial response/stable disease group compared with at 0 h, when all 15 patients were analyzed. Notably, the partial response/stable disease group showed significant increases in 5-FU plasma levels at 40 h compared with at 22 h, although the progressive disease group showed no significant increase. In addition, the 5-FU plasma level in the adverse event level of grade ≥2 was higher than that of grade <2 at 40 h after the start of infusion.

In contrast to a previous study (18), in which the 5-FU plasma level reached a plateau at 22 h after the continuous infusion with an electronegative pump, the present study showed that the 5-FU
plasma level continued to significantly increase between 22 and 40 h after infusion. In the previous study (18), 5-FU continuous infusion relied on an elastomeric infusion pump that is suitable for ambulatory patients treated in an out-patient clinic. In the present study, professional staff members constantly monitored the electronical infusion pump to prevent the discontinuity of 5-FU during admission. Therefore, the infusion methods used in the present study may explain a continuous increase of 5-FU plasma levels during 40 h from the start of admission. Furthermore, the increase in the 5-FU plasma level may contribute to the significant increase of the plasma level of 5-FU at 40 h in the present study. Furthermore, it has also been suggested in a previous study utilizing an electronic infusion pump that acute toxicity was correlated with a high 5-FU plasma level (35). Taking these observations into consideration, it was hypothesized that the high plasma level of 5-FU observed in the partial response/stable disease group may reflect the severity in adverse events shown in the present study.

However, the present study has several limitations. The number of patients enrolled in the present study was limited and the tumor response was evaluated for only a short term of 3 months. Therefore, future studies should monitor patients for a longer period to evaluate the prognosis and overall survival. However, despite these limitations, the present study supports the accumulating evidence that measuring 5-FU plasma levels may contribute to the improvement of a positive tumor response as well as to minimize the risk of severe adverse events (14,20).

In conclusion, during continuous infusion of 5-FU, the 5-FU plasma level increased significantly. The tumor response may be influenced by the increase of 5-FU plasma level from the start of infusion. The 5-FU plasma level may be a predictive factor for maximizing the tumor response and minimizing the risk of severe adverse events.

Table III. Tumor response and severity of adverse events.

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Partial response, %</th>
<th>Stable disease, %</th>
<th>Progressive disease, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand foot syndrome</td>
<td>50</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Edema</td>
<td>0</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>50</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Appetite loss</td>
<td>25</td>
<td>25</td>
<td>60</td>
</tr>
<tr>
<td>Numbness</td>
<td>75</td>
<td>50</td>
<td>80</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Nausea/Vomit</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Facial flushing</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0</td>
<td>50</td>
<td>20</td>
</tr>
</tbody>
</table>

The proportion of adverse events among patients of Grade ≥2 (n=13) treated with modified 5-fluorouracil plus folinic acid-oxaliplatin or 5-fluorouracil plus folinic acid-irinotecan is shown for each tumor response (partial response, n=4; stable disease, n=4; progressive disease, n=5). Grade 2 is defined according to the following criteria of the Common Terminology Criteria for Adverse Events v5.0: i) Moderate; ii) minimal, local or noninvasive intervention indicated; and iii) limiting age-appropriate instrumental activities of daily living.
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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors’ contributions

YA and TO designed the research. YA, NS, TS, KK, KNa, AN, MK, TW, KNi and TO performed the clinical study. YA, TO and IN analyzed the data. YA and IN prepared the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Tobu Chikhi Hospital (approved December 21, 2015; IRB nos. 15 and 4), and performed between January 1, 2017 and March 31, 2017. The present study was conducted in accordance with the principles of the amended ‘Declaration of Helsinki and Ethical Guidelines for Epidemiological Research’ (established by the Japanese Government in 2008). Written informed consent was obtained from all patients before they were enrolled in the present study.

Patient consent for publication

All the participants enrolled in the present study provided written informed consent for the publication of any associated data.

Competing interests

The authors declare that they have no competing interests.

References


