Correlation between the Urinary Dihydrouracil-uracil Ratio and the 5-FU Plasma Concentration in Patients Treated with Oral 5-FU Analogs

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Abstract. Background: The determination of dihydropyrimidine dehydrogenase (DPD) deficiency is important in avoiding severe 5-fluorouracil (FU) toxicity. The dihydrouracil (UH2)-uracil (Ura) ratio (UH2/Ura) in plasma might be an important indicator of the risk of 5-FU catabolic deficiency. In order to clarify this possibility, the pyrimidine metabolites and the UH2/Ura were measured in urine and the plasma level of 5-FU was evaluated in patients with gastric and colorectal cancer. Patients and Methods: Patients with primary gastric (n=14) and colorectal (n=8) cancer who had undergone surgery were recruited in this study. These patients were divided into the S-1 treatment group, which drug is a novel oral formulation of tegafur, oxonic acid and 5-chloro-2, 4-dihydroxypyridine (CDHP) (n=14) and a group receiving other drugs which include UFT (Uracil/Tegafur) or oral doxifluridine (n=8). The urinary levels of UH2 and Ura were measured by high-performance liquid chromatography (HPLC) using column switching. The plasma level of 5-FU was assessed by gas chromatography-mass spectrometry (GC-MS). Results: The UH2/Ura or UH2/Ura (treated/no treated) in the S-1 group significantly decreased in comparison to that in the other-drug group and the plasma 5-FU concentration levels significantly increased compared to that in the group treated with other drugs. The plasma 5-FU concentration levels significantly indicated a positive correlation with urinary Ura. Moreover, UH2/Ura treated with 5-FU analogs or UH2/Ura (treated/no treated) significantly showed a negative correlation with the plasma 5-FU concentration levels. Conclusion: Our findings indicate that either urinary Ura, the UH2/Ura or UH2/Ura (treated/no treated) can predict the plasma 5-FU concentration levels or DPD deficiencies.

5-fluorouracil (5-FU) and its analogs remain widely used in the treatment of gastrointestinal carcinomas and other carcinomas. Recently, S-1, a novel oral formulation of tegafur, oxonic acid and 5-chloro-2, 4-dihydroxypyridine (CDHP) at a molar ratio of 1:0.4:1, is frequently used for patients with gastric or colorectal cancer in Japan. This drug displayed powerful effects with a reported response rate of 53.6% for recurrent gastric cancer in an early phase II study (1). However, as CDHP is a dihydropyrimidine dehydrogenase (DPD) inhibitor, the rate of the adverse bone marrow suppression after S-1 administration increased in comparison with that after the administration of other oral 5-FU analogs (1). Moreover, some powerful combination regimens of chemotherapy for colorectal cancer, such as oxaliplatin/5-FU/leucovolin (LV) combination and irinotecan/bolus 5-FU/LV combination, are now used in Japan. Several pharmacological population studies indicated a higher 5-FU concentration with these new drugs or regimens in comparison to those under previous regimens (2-4). Therefore, accurately predicting the adverse side-effects of 5-FU is considered clinically important.

DPD is the key enzyme of pyrimidine and fluoropyrimidine catabolism. In pyrimidine metabolism, uracil (Ura) and thymidine are first converted to dihydrouracil (UH2) and dihydrothymidine by DPD and are then metabolized to β-ureidopropionic acid and β-ureidoisobutyric acid, respectively, by dihydropyrimidinase. These metabolites are converted subsequently into β-alanine and β-aminoisobutyric acid, respectively (5). Approximately 80% of an administered dose of 5-FU is degraded by this pathway. The remnant of 5-FU is then metabolized and exerts its cytotoxic effect. DPD is mainly expressed in the liver, but it is also present in such tissues as peripheral blood mononuclear cells (PBMCs), bone marrow, intestinal mucosa and the spleen (6). The
pharmacokinetic data on 5-FU clearance showed that 60-90% of the administered 5-FU is metabolized by DPD in the liver (7, 8), thus, suggesting that DPD in the liver is critically important in determining 5-FU toxicity. However, collecting hepatic samples in order to measure the DPD levels remains difficult to perform.

Patients with genetic fluorouracil catabolic deficiencies are at high risk for severe toxicity of pyrimidine chemotherapy agents, such as 5-FU or 5-FU analogs. Previous population studies indicated that a complete DPD deficiency is estimated to occur in about 0.1% of patients and the relative DPD deficiency is projected to have an incidence of 2.7% to 5.8% in cancer patients (8-10). Therefore, a prediction of such DPD deficiency is important in avoiding severe 5-FU toxicity.

To predict 5-FU catabolic deficiencies and toxic side-effects, there are several methods available for measuring DPD levels. In general, the DPD activity is measured in PBMCs by ELISA. However, this method is both time-consuming and expensive. Moreover, the DPD levels in PBMCs are not significantly correlated to either the 5-FU systemic clearance or liver DPD levels (11). On the other hand, Gamelin et al. indicated the importance of the UH2/Ura ratio in plasma level as an indicator of the risk of 5-FU catabolic deficiency (12). They measured the plasma pyrimidine and dehydrogenated metabolite levels using high performance liquid chromatography (HPLC). The urinary pyrimidine metabolites were measured and the importance of the UH2/Ura ratio level as an indicator of either a 5-FU catabolic or a DPD deficiency was evaluated in this study.

Patients and Methods

Patients. Between December 2001 and April 2003, patients with primary gastric (n=14) and colorectal (n=8) cancer who had undergone surgery at the Department of Surgery 1, University Hospital of Occupational and Environmental Health, Japan, were recruited into this study. The clinical data for these patients are summarized in Table I. The patients were divided into the S-1 treatment group (n=14), and the group treated with other drugs which include UFT (Uracil/Tegafur) or oral doxifluridine (n=8). Informed consent was obtained from all patients prior to starting the study.

Urine and blood samples. To evaluate the 5-FU plasma levels, venous blood samples were collected using a tube containing EDTA-Na. The samples were centrifuged for 10 min at approximately 1000 xg. Plasma was then collected 4 h after administration of 5-FU analogs and samples were stored at <–20°C until analysis. To evaluate the urinary levels of Ura and UH2, urine samples were also collected 4 h after administration of 5-FU analogs and were stored at <–20°C until analysis.

Measurement of urinary levels of Ura and DH2. The urinary levels of Ura and UH2 were measured by high-performance liquid chromatography (HPLC) with column switching (13), while urinary Cre was measured using an autoanalyzer (13-15). The HPLC system consists of a reversed-phase column as the first column, a cation-exchange column as the second column, four sets of ultraviolet absorbance detectors, a microcomputer and other conventional equipments. The detailed procedures of HPLC with column switching have been previously described (13).

Measurement of 5-FU plasma levels. The plasma level of 5-FU was assessed by gas chromatography-mass spectrometry (GC-MS) (16). GC-MS was carried out using Trace GC and Trace MS (Thermo Electron K.K., Yokohama, Japan) with Xcalibur (Ver. 1.2) (Thermo Electron K.K., Yokohama, Japan) as a control system. The detailed procedures of GC-MS were previously described (16).

Evaluation of the parameters. UH2 and Ura levels and the UH2/Ura ratio were compared between the S-1 group and the other-drug group. The UH2/Ura (treated/no treated) ratio indicates the UH2/Ura ratio in treated state with drugs-the UH2/Ura ratio in no treated state with 5-FU analogs were not different in the two groups (Table II). On the other hand, in the S-1 group, the concentration of Ura was increased and the concentration of UH2 significantly decreased compared to that in the other-drug group which is treated with UFT or oral doxifluridine. The UH2/Ura ratio treated with drugs in the S-1 group was significantly decreased in comparison to that in the group treated with other drugs and the plasma 5-FU concentration treated with drugs in S-1 group significantly increased compared to that in other-drug group. Moreover, the UH2/Ura (treated/no treated)

Table I. Characteristics of the patients.

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of patients</th>
<th>Site of carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-1</td>
<td>14</td>
<td>stomach</td>
</tr>
<tr>
<td></td>
<td></td>
<td>colorectum</td>
</tr>
<tr>
<td>others</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Results

The urinary concentration of Ura, UH2 and the UH2/Ura ratio in no treated state with 5-FU analogs were not different in the two groups (Table II). On the other hand, in the S-1 group, the concentration of Ura was increased and the concentration of UH2 significantly decreased compared to that in the other-drug group which is treated with UFT or oral doxifluridine. The UH2/Ura ratio treated with drugs in the S-1 group was significantly decreased in comparison to that in the group treated with other drugs and the plasma 5-FU concentration treated with drugs in S-1 group significantly increased compared to that in other-drug group. Moreover, the UH2/Ura (treated/no treated)
ratio was significantly decreased in the S-1 group relative to that in the group treated with other drugs (Table II).

S-1 significantly increased the urinary Ura levels and decreased the UH2/Ura ratio compared to values in the other group (Table III). On the other hand, the other drugs significantly increased the UH2 levels compared to levels in the other group (Table III).

The plasma 5-FU concentration levels significantly indicated a positive correlation with urinary Ura under medication with 5-FU analogs \((p=0.0452, r=0.430)\) (Figure 1). However, plasma 5-FU concentration levels did not correlate with urinary UH2 concentration levels under medication with 5-FU analogs (data not shown). Though the urinary UH2/Ura ratio under no medication with 5-FU analogs did not correlate with plasma 5-FU concentration levels (data not shown), the urinary UH2/Ura ratio treated with 5-FU analogs were significantly correlated with plasma 5-FU concentration levels \((p=0.0067, r=-0.552)\) (Figure 2). Moreover, the urinary UH2/Ura (treated/no treated) ratio significantly correlated with the plasma 5-FU concentration levels \((p=0.0006, r=-0.657)\) (Figure 3).

**Discussion**

Measuring the plasma 5-FU level is considered important for the efficacy and safety of this treatment modality. The response to the treatment has been correlated with the 5-FU dose, more precisely with the 5-FU area under the curve (AUC) or the 5-FU plasma concentration at steady-state (17,18). There is, however, a high individual variability of 5-FU pharmacokinetics in the plasma and a close link between the toxic side-effects and individual pharmacokinetic parameters was demonstrated (12, 19). There is also a correlation between the 5-FU plasma levels, toxicity and efficacy (12). These relationships point out a problem of the polymorphism of 5-FU metabolism, the utility of the therapeutic range determination and of the individual 5-FU dose adaptation (12). It is therefore important to identify patients with a DPD deficiency who show unexpectedly high concentrations of 5-FU.

In the present study, no patients showed any adverse drug reactions related to 5-FU. In a population of 150 Japanese, Ogura et al. reported one healthy volunteer (0.7% of the population) who showed peripheral blood mononuclear cell (PBMC) DPD activity due to heterozygosity for a mutant allele of the DPYD gene (20). However, the frequency of heterozygotes in the normal population was...
estimated to be as high as 3% (21). In addition, the frequency of the relative DPD deficiency detected by PBMC DPD activity was projected to have an incidence of 2.7% of cancer patients in France (10), 2.7% of patients underwent laparotomy in France (8) and 5.8% of the breast cancer patients in USA (9). On the other hand, several studies reported the findings of genetic analysis of the DYPD gene. There were several mutant DPYD alleles in patients suffering from severe toxicity after the administration of 5-FU (22-26). The G to A mutation in the invariant GT splice donor site [IVS14+1G>A] is the most frequent of these mutations (27). The presence of IVS14+1G>A mutation was examined for screening individuals among various nationalities (23, 24, 28-30). This mutation was identified in Dutch (23), German (24), Turkish (28), Finnish (29) and Taiwanese (29), but not in Japanese (29, 30) or African-Americans (29). Therefore, we speculate that little difference exists in the DPD activity or DYPD mutation between the Japanese and people from other countries.

Several strategies exist for predicting DPD deficiencies prior to and during 5-FU treatment, including measurement of the PBMC DPD activity, a mutation analysis of the DYPD gene and the measurement and the assessment of pyrimidines in plasma or urine. According to previous experience, the DPD activity in PBMCs instead of the liver was determined (10, 31), as PBMCs are much more accessible than is a liver biopsy. However, the method for detection of PBMC DPD activity was found to be time-consuming and expensive. In addition, PBMC DPD levels were not significantly correlated to 5-FU systemic clearance (11). It has become possible to predict the metabolism of FU-derived anticancer agents by DPD activity through the determination of plasma or urinary pyrimidine levels. The measurement of Ura and UH2 levels may provide an estimate of 5-FU degradation as well as of DPD levels because 5-FU and Ura are catabolized by DPD. Therefore, several studies measured plasma pyrimidine and their dehydrogenated metabolite levels with HPLC (32-34). It was speculated that the plasma UH2/Ura ratios might be helpful in identifying patients with a partial or complete DPD deficiency, thereby allowing us to identify the risk of 5-FU toxicity. Ohba et al. previously screened Japanese infants for abnormalities in pyrimidine metabolism and detected a case of infantile dihydropyrimidinuria using a method that permits the analysis of UH2 and Ura in small volumes of urine (14). Moreover, Hayashi et al. analyzed urinary UH2 and Ura levels in 1133 adults by HPLC with column switching and detected a dihydropyrimidinuria (35). In addition, significant linear correlations with liver DPD levels were demonstrated for the plasma and urinary UH2/Ura ratio in a rat experimental model (36). Therefore, we measured the urinary pyrimidine levels and evaluated the importance of the UH2/Ura ratio in urinary levels as an indicator of DPD deficiency or 5-FU concentration.

In this study, some parameters were evaluated to predict the 5-FU concentration. Pretreatment urinary Ura, UH2 and UH2/Ura did not significantly correlate with plasma 5-FU concentration (data not shown). Though the number of patients recruited in this study is small, pretreatment urinary pyrimidine and UH2/Ura might not be suitable for the prediction of the plasma 5-FU concentration. The urinary Ura concentration 4 h after administration of the 5-FU analogs positively correlated with plasma 5-FU concentration (p=0.0452, r=0.430). Moreover, urinary UH2/Ura treated with 5-FU analogs negatively correlated with the plasma 5-FU concentration (p=0.0067, r=-0.552). Based on our results, a strong relationship was identified between the plasma 5-FU concentration levels and the UH2/Ura
treated/no treated) ratio (p=0.0006, r=−0.657). The reason for this may be that this ratio may reflect the true individual value of the systemic DPD level. The most suitable predictive parameter for the plasma 5-FU concentration may thus be the urinary UH2/Ura (treated/no treated) ratio.

The results of the present study suggest that urinary pyrimidine metabolites or their parameters may help us to effectively predict the plasma 5-FU concentration levels and the urinary UH2/Ura or UH2/Ura (treated/no treated) ratios might possibly predict DPD deficiencies. Since a correlation between 5-FU plasma levels and toxicity is thus considered to be important for predicting DPD deficiency. The measurement of urinary pyrimidine metabolites is non-invasive, safe and easy to perform. Additional studies are required to clarify the utility of the measurement of urinary pyrimidine metabolite levels and their parameters.

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References


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