The Relationship between Treatment Time of Gemcitabine and Development of Hematologic Toxicity in Cancer Patients

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Although gemcitabine is frequently used in the treatment of cancer, it is associated with myelosuppression. An animal study showed that the tolerability of gemcitabine varied with changes in treatment time; however, no clinical data have verified this finding. The purpose of this study was to determine the relationship between treatment time and development of hematologic toxicity in patients treated with gemcitabine. Gemcitabine-induced hematologic toxicity was retrospectively investigated in 77 patients. Patients were divided into two treatment-time groups: 9:00 and 15:00. Hematologic toxicity was evaluated on day 8 and 15 after treatment. On day 8 and 15, the changing count of white blood cells was significantly reduced in patients treated at 15:00 compared with those treated at 9:00 (p<0.01 and p<0.05, respectively). On days 8 and 15, the changing count of platelet was significantly reduced in patients treated at 15:00 compared with those treated at 9:00 (p<0.05). The incident of over 2.92. In conclusion, this cohort study demonstrated that gemcitabine-induced hematologic toxicity could be alleviated by treating patients at 9:00.

Key words chronotherapy; circadian rhythm; gemcitabine; hematological toxicity

The sleep-wake cycle, hormonal secretion, and drug metabolism are all affected by a circadian rhythm. For example, dihydropyrimidine dehydrogenase, the rate-limiting enzyme in the metabolism of 5-fluorouracil, displays a circadian variation of enzyme activity in human peripheral blood mononuclear cells. This variation results from circadian changes in cellular mechanisms involving metabolism, repair, protection, and proliferation. These findings support the hypothesis that drug tolerability may be affected by the sleep-wake cycle in mice or humans. Improved tolerability has been seen with chronotherapy for fluoropyrimidine, anthracyclines, and platinum complexes in humans.

Gemcitabine (2′, 2′-difluoro-2′-deoxycytidine; GEM) is a deoxycytidine analog with a broad spectrum of anticancer activity against several solid tumors. GEM is available for non-small-cell lung cancer and pancreatic cancer. At standard doses, the weekly treatment of GEM is associated with several side effects, including myelosuppression, hepatopathy, nausea, vomiting, and alopecia. Myelosuppression is a dose-limiting toxicity of GEM. A previous study identified an association between the circadian rhythm and the tolerability and hematologic toxicity of GEM in mice. The tolerability of GEM was enhanced by drug treatment in the early dark phase, when mice were active. However, there has been no clinical evidence to support this finding. The purpose of this retrospective observational study was to reveal whether the treatment time influences hematologic toxicity in patients treated with GEM.

MATERIALS AND METHODS

Experimental Design We retrospectively evaluated seventy-seven patients with advanced and/or metastatic gastrointestinal or pulmonary stage III or IV carcinomas who had never received granulocyte colony-stimulating factor. Patients were undergoing outpatient chemotherapy at Kumamoto Red Cross Hospital. The mean±S.D. age of patients was 69.3±9.0 years. Inclusion criteria were adequate bone marrow function (white blood cell count ≥3000/μl, platelet count ≥7.5×10^4/μl); adequate liver function (serum aspartate transaminase and serum alanine transaminase <2.5 times normal value); and adequate renal function (serum creatinine <1.5 times normal value). These criteria were set referring to a previous Phase II study. GEM at a dose of 1000 mg/m² was given by intravenous administration over 30 min. Dose modifications were based on hematologic or some severe non-hematologic toxicities (hepatopathy, nausea, vomiting or malaise) before injections. The dose was reduced by 80% for grade 2 hematologic or liver toxicity and omitted for grade 3 toxicity or greater. The toxicity grade was determined by common terminology criteria for adverse events (CTCAE) version 4.0 criteria. Since this retrospective study was an epidemiological observation analysis using patient’s clinical record, the approval for the use of patient data was obtained from the clinical research ethics committee of Kumamoto Red Cross Hospital. The data from patient’s record were completely encrypted and data analysis was performed after processing to remove all personally identifiable information.

From April 2007 to March 2011, a total of ninety outpatients underwent gemcitabine monotherapy at Kumamoto Red Cross Hospital. Chemotherapy for outpatients in Kumamoto Red Cross Hospital are routinely begun at 9:00, 10:30, 13:00, and 15:00. Time of administration was decided at random. Thirteen patients excluded from this study because GEM had not been administrated at 9:00 and 15:00. Seventy-seven patients were divided into two different groups at time of administration: 9:00 and 15:00. GEM was administrated
to 9:00 group between 9:00 to 9:59, 15:00 group between 15:00 to 15:59. We evaluated the difference of hematologic toxicity between the first time administration of GEM (on day 1) and on day 8 or 15 after treatment. In addition, red blood cell, hematocrit, and hemoglobin, serum aspartate transaminase, and serum alanine transaminase levels were assessed on day 8 and 15. Among patients treated at 9:00, a total of 72 and 49 administration of GEM were treated on day 8 and day 15, respectively. Among patients treated at 15:00 group, a total of 42 and 12 administration of GEM were treated on day 8 and day 15, respectively.

**Statistical Analysis** All data are expressed as mean±S.D. Statistical analysis of patient backgrounds and hematologic toxicity were performed using the Student’s *t*-test or Welch *t*-test for parametric data, and Mann-Whitney *U* test for nonparametric data. Toxicity grade of data were analyzed statistically by Fisher’s exact test. A *p*-value less than 0.05 were considered statistically significant.

**RESULTS**

As shown in Table 1, there were no significant differences in patient backgrounds between those treated at different times. On day 8 and 15, a significant difference in the changing count of white blood cell (WBC) was seen between patients treated at 9:00 and patients treated at 15:00 (*p*<0.01 and *p*<0.05, respectively) (Figs. 1B, C). As shown in Fig. 1D and E, a significant difference in changing count of platelet was seen between patients treated at 9:00 and patients treated at 15:00 on day 8 and 15 (*p*<0.05). Furthermore, the WBC count had completely recovered on day 15 among patients treated at 9:00, whereas it was not completely recovered in patients treated at 15:00. As shown in Table 2, the incident of over grade 2 WBC decreased was significantly reduced in patients treated at 15:00 compared with those treated at 9:00 (*p*=0.048, odds ratio=2.92). In turn, there were no significant differences in the incident of over grade 2 platelet decreased between those treated at different times. However, grade 2 platelet decreased was experienced in 2 patients treated at 15:00, there was no patient treated at 9:00. Red blood cell, hematocrit, and hemoglobin levels did not differ significantly between groups on day 8 or 15 (Fig. 2). In addition, treatment time did not affect liver function. Data for two liver function markers, that is, serum aspartate transaminase

<table>
<thead>
<tr>
<th>Table 1. Patient Background</th>
<th>Treatment-time at 9:00</th>
<th>Treatment-time at 15:00</th>
<th><em>p</em>-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient number</td>
<td>41</td>
<td>36</td>
<td>0.53</td>
</tr>
<tr>
<td>Age (range)</td>
<td>70.1±8.9</td>
<td>68.3±9.0</td>
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</tr>
<tr>
<td>(43—85)</td>
<td>(46—84)</td>
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</tr>
<tr>
<td>Gender (male/female)</td>
<td>24/17</td>
<td>20/16</td>
<td></td>
</tr>
<tr>
<td>Dose of GEM (mg)</td>
<td>1417.6±239.8</td>
<td>1401.1±195.2</td>
<td>0.37</td>
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<tr>
<td>Count of WBC before treatment (/μl)</td>
<td>5632.2±2209.7</td>
<td>6213.1±2217.3</td>
<td>0.87</td>
</tr>
<tr>
<td>Count of platelet before treatment (×10^9/μl)</td>
<td>23.6±9.9</td>
<td>26.3±12.0</td>
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<td>Cancer location</td>
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<tr>
<td></td>
<td>Bile duct: 10</td>
<td>Bile duct: 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung: 6</td>
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<tr>
<td>Disease state (I/II/III/IV)</td>
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<tr>
<td>Performance status (0/1/2/3/4)</td>
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<td>6/2/5/5/0</td>
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</table>

All data were expressed as mean±S.D. Data were analyzed statistically by Student’s *t*-test or Welch *t*-test for parametric data, and Mann-Whitney *U* test for nonparametric data. Differences with a *p*-value of less than 0.05 were considered statistically significant.
It resulted in a 5-fold reduction in the risk of diarrhea compared with a constant rate infusion of the same total dose.5) It was suggested that the circadian variation in human peripheral blood mononuclear cell and intestinal dihydropyrimidine dehydrogenase expression, which is the main enzyme involved in 5-fluorouracil catabolism, was closely related to the 5-fluorouracil-induced gastrointestinal toxicity.2,13) On the other hand, GEM is extensively and rapidly metabolized by cytidine deaminase in the liver, kidneys, and plasma.14) A previous report showed that serum cytidine deaminase expression has a circadian rhythm and reaches a peak in the early active phase in humans.15) It is possible that the hematologic toxicity associated with GEM can be alleviated by choosing a treatment time that reflects the early active phase in humans, which would ultimately decrease GEM exposure. It is thought that the anticancer efficacy might decrease. These results indicate that the serum concentrations of GEM and serum cytidine deaminase expression might be closely related. Future studies are needed to confirm this observation.

The proportion of S-phase cells in the bone marrow of mice is highest in the late active phase.6,16) GEM is an S-phase-specific agent and induces apoptosis.17) This process can be prevented by B-cell lymphoma-2 (BCL-2) expression and accelerated by Bcl-2 associated X protein (BAX) expression.18) BAX expression level was shown to be highest and BCL-2 expression level was shown to be lowest in the late active phase of mice.19) Thus, the lower the proportion of S-phase cells and the lower the level of BAX expression, the higher the tolerability of GEM. It is possible that rhythms in S-phase distribution and BCL-2/BAX expression are consistent with the better tolerability of GEM in the early active phase in humans. Meanwhile, in this study, drug treatment time had no influence on changes in red blood cells, hematocrit, or hemoglobin level. In general, the proliferation of WBC and platelets occurs more rapidly than that of red blood cells, hematocrit, and hemoglobin; therefore, tissue with high proliferation rates may be more easily affected by drug treatment time.

In conclusion, this retrospective finding revealed a relationship between treatment time and the development of hematologic toxicity in cancer patients treated with GEM. GEM-induced hematologic toxicity could be alleviated using a treatment time of 9:00. A larger sample size and prospective measurements of plasma concentrations of GEM are needed to confirm these results.

REFERENCES

6) Tampellini M., Filipski E., Liu X. H., Lemaigre G., Li X. M., Vrig-