Severe Drug Toxicity Associated with a Single-Nucleotide Polymorphism of the Cytidine Deaminase Gene in a Japanese Cancer Patient Treated with Gemcitabine plus Cisplatin

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Abstract Purpose: We investigated single-nucleotide polymorphisms of the cytidine deaminase gene (CDA), which encodes an enzyme that metabolizes gemcitabine, to clarify the relationship between the single-nucleotide polymorphism 208G>A and the pharmacokinetics and toxicity of gemcitabine in cancer patients treated with gemcitabine plus cisplatin.

Experimental Design: Six Japanese cancer patients treated with gemcitabine plus cisplatin were examined. Plasma gemcitabine and its metabolite 2',2'-difluorodeoxyuridine were measured using a high-performance liquid chromatography method, and the CDA genotypes were determined with DNA sequencing.

Results: One patient, a 45-year-old man with pancreatic carcinoma, showed severe hematologic and nonhematologic toxicities during the first course of chemotherapy with gemcitabine and cisplatin. The area under the concentration-time curve of gemcitabine in this patient (54.54 µg hour/mL) was five times higher than the average value for five other patients (10.88 µg hour/mL) treated with gemcitabine plus cisplatin. The area under the concentration-time curve of 2',2'-difluorodeoxyuridine in this patient (41.58 µg hour/mL) was less than the half of the average value of the five patients (106.13 µg hour/mL). This patient was found to be homozygous for 208A (Thr70) in the CDA gene, whereas the other patients were homozygous for 208G (Ala70).

Conclusion: Homozygous 208G>A alteration in CDA might have caused the severe drug toxicity experienced by a Japanese cancer patient treated with gemcitabine plus cisplatin.

Gemcitabine (2',2'-difluorodeoxycytidine) is a deoxycytidine analogue that is efficacious against non–small cell lung cancer and pancreatic carcinoma, as a single agent or in platinum combination therapy (1, 2). Its major adverse effects are hematologic toxicity, weakness, and emesis, and its dose-limiting toxicity is hematologic toxicity, including leukocytopenia, anemia, and thrombocytopenia (1). Single-agent and platinum combination gemcitabine therapy is relatively well tolerated, but hospitalization is occasionally required due to significant hematologic toxicity (1, 2), and it has been difficult to predict the toxicity.

Gemcitabine is activated by intracellular phosphorylation to gemcitabine monophosphate by deoxycytidine kinase, which is subsequently phosphorylated to the higher-order phosphates, gemcitabine diphosphate followed by gemcitabine triphosphate. Gemcitabine triphosphate can be incorporated into DNA followed by one more deoxynucleotide, after which DNA polymerization stops. This process is referred to as “masked chain termination” (3, 4).

Gemcitabine and gemcitabine monophosphate are deaminated to the inactive metabolite 2',2'-difluorodeoxyuridine (dfdU) and 2',2'-difluorodeoxyuridine monophosphate by cytidine deaminase (CDA) and dCMP deaminase, respectively. Multiple mechanisms potentiate the activity of gemcitabine both by increased formation of active gemcitabine diphosphate and gemcitabine triphosphate and decreased elimination of gemcitabine, as follows: (a) gemcitabine diphosphate, through its inhibition of ribonucleotide reductase, depletes the deoxycytidine pool available for DNA synthesis and repair; (b) the decreased concentration of dCTP activates deoxycytidine kinase, which accelerates phosphorylation of gemcitabine;...
and (c) an inactivating enzyme, dCMP deaminase, is inhibited by the decreased concentration of intracellular dCTP and increased concentration of gemcitabine triphosphate (5–7). Polymorphisms of the DNAs encoding the above enzymes may influence the pharmacokinetics and pharmacodynamics of gemcitabine.

To establish the medical guidelines for treatment based on individual genetic polymorphisms, we have launched multicenter, prospective, pharmacogenomic trials (as the Millenium Genome Project) of antineoplastic agents, such as gemcitabine, paclitaxel, irinotecan, and other commonly used drugs.

At the time point when 97 gemcitabine-treated patients had been recruited, we experienced extremely severe toxicities in one patient. Because this patient was coadministered cisplatin in addition to gemcitabine, we compared the clinical data, pharmacokinetics and CDA genotype between this patient and the other five control patients, who were also coadministered the two drugs.

**Patients and Methods**

**Selection of patients and treatment schedule.** Patients being treated with gemcitabine plus cisplatin were eligible for the trial if they met all of the following inclusion criteria: histologically or cytologically proven carcinoma, no prior treatment with gemcitabine, age above 20 years, Eastern Cooperative Oncology Group performance status between 0 and 2, absence of severe infectious or neurologic disease, and no evidence of heart or interstitial lung disease. Other requirements included adequate bone marrow function (WBC ≥3,000/μL, neutrophils ≥1,500/μL, and platelets ≥75,000/μL), hepatic function (serum total bilirubin ≤3 mg/d, aspartate aminotransferase and alanine aminotransferase less than five times the upper limit of normal), and renal function (serum creatinine within the upper limit of normal). The trial was approved by the Ethics Review Committees of the National Cancer Center Hospital and NIH Sciences, and oral and written informed consent was obtained from all patients before entering.

Gemcitabine was given to all patients at a dose of 1,000 mg/m² (30-minute infusion) on days 1, 8, and 15 and followed by 1 week of rest. If adequate bone marrow function (WBC ≥2,000/μL, neutrophils ≥1,000/μL, and platelets ≥70,000/μL) was confirmed, gemcitabine was given on days 8 and 15.

Cisplatin was given at a dose of 80 mg/m² (150-minute infusion) on day 1, immediately after gemcitabine. All patients received antiemetic prophylaxis with granisetron plus dexamethasone. Granulocyte-colony stimulating factor was not given routinely. The treatment schedule was repeated every 28 days until disease progression or unacceptable side effects occurred.

Toxicity was scored according to the National Cancer Institute Common Toxicity Criteria ver 2.0. A complete blood cell count and serum chemistry were repeated weekly. At the start of every new course, the dose was reevaluated according to toxicity. If the WBC count was <2,000/μL and the platelet count was <70,000/μL, then treatment was delayed until the recovery of bone marrow function. If grade 4 leukocytopenia, neutrocytopenia, or thrombocytopoenia was observed in the previous course, the gemcitabine dose was reduced to 800 mg/m² in subsequent courses.

**Blood sampling.** Before the start of the treatment, a 5-mL heparinized blood sample was collected to measure CDA activity, and a 14-mL blood sample, to which EDTA was added, was collected to extract leukocyte DNA for genetic analysis. On day 1 of the first course, a 5-mL heparinized blood sample for gemcitabine and metabolite analysis in plasma was collected from the opposite arm before the infusion, at 3 minutes before the end of the infusion, and 15, 30, 60, 90, 120, and 240 minutes after the end of the infusion, and 50 μL of 10 mg/mL tetrahydouridine (Wako Junyaku, Co., Ltd., Osaka, Japan) was immediately added to each of the samples. The samples were centrifuged at 3,000 x g for 5 minutes at 4°C, and the plasma was collected and stored at −70°C until analyzed.

**Analysis of gemcitabine and its metabolite, 2’-defluorodeoxycytidine.** The concentrations of gemcitabine and dFdU in the plasma were determined by the method of Venook et al. with slight modifications (8). A 25 μL volume of 25 mg/mL 3’-deoxy-3-fluoro-thymidine (Aldrich Chem. Co., St. Louis, MO) was added to an 0.25-mL aliquot of plasma sample containing 0.1 mg/mL tetrahydouridine as an internal standard. After adding 1 mL of acetonitrile, the mixtures were centrifuged at 12,000 x g for 5 minutes, and the supernatant was evaporated to dryness under a nitrogen stream. The residue was dissolved in 0.25 mL of 15 mmol/L ammonium acetate buffer (pH 5.0), and the solution was filtered twice through Ultrafree-MC (0.45 μm; Millipore Corp., Billerica, MA) and Microcon YM-10 (10,000 MW; Amicon). Twenty microliters of sample were loaded into a high-performance liquid chromatography system (HP 1100 model) with diode array detection and electro spray-mass spectrometry detection. The chromatographic conditions were as follows: column, CAPCELL PACK C18 MG column (5 μm, 2.0 × 150 mm; Shiseido Co., Ltd., Tokyo, Japan) with a CAPCELL C18 MG S-5 guard cartridge (4.6 mm i.d. × 10 mm; Shiseido); column temperature, 40°C; mobile phase, 15 mmol/L ammonium acetate (pH 5.0)/methanol; running program of the mobile phase: 95:5 (0 minute), 75:25 (10-15 minutes), 60:40 (20-25 minutes), 55:45 (30-40 minutes); flow rate: 0.3 mL/min; diode array detection: 268 nm for gemcitabine, 258 nm for dFdU, and 266 nm for 3’-deoxy-3-fluoro-thymidine; electrospray-mass spectrometry: m/z 264 for gemcitabine, m/z 265 for dFdU, and m/z 245 for 3’-deoxy-3-fluoro-thymidine. Detection and integration of chromatographic peaks were done by the HP Chemstation data analysis system (Hewlett-Packard, Les Ulis, France).

**Pharmacokinetic analysis.** Compartment model independent pharmacokinetic variables were calculated using WinNonlin software, ver. 4.1 (Pharsight Co., Mountain View, CA). The values are expressed as means ± SD, for those of the patient with severe toxicity.

**DNA sequencing.** DNA used for sequencing was extracted from peripheral blood. All of the four exons of CDA were amplified from 100 ng of genomic DNA using multiplex primers listed in Table 1 (PCR). The PCR conditions have been described previously (9). After the second amplification for each exon, the PCR products were purified and directly sequenced on both strands with the sequencing primers listed in Table 1 (sequencing), as described previously (9). All variations were confirmed by repeating the sequence analysis from the first-round PCR with DNA. National Center for Biotechnology Information accession no. NT_004610.16 was used for the reference sequence.
Results

We encountered a patient treated with gemcitabine and cisplatin who developed extremely severe toxicities (grade 4 neutropenia, thrombocytopenia, and stomatitis and grade 3 rash, fatigue, and febrile neutropenia). To clarify the cause of these life-threatening toxicities, we determined the plasma levels of gemcitabine and its metabolite, dFdU, and the genotypes of CDA encoding a major gemcitabine-metabolizing enzyme, cytidine deaminase, of this patient (patient 1) and the other five gemcitabine/cisplatin-administered patients (patients 2-6).

Pharmacokinetics. Plasma concentration-time profiles of gemcitabine and dFdU are shown in Figs. 1 and 2, and pharmacokinetic variables are summarized in Table 2. The maximum plasma gemcitabine concentration ($C_{\text{max}}$) and area under the concentration-time curve of patient 1 were about twice and five times higher, respectively, than the average values of patients 2 to 6. The area under the concentration-time curve ratio (dFdU/gemcitabine) of patient 1 was about one tenth of the average value in patients 2 to 6.

Genotypes. The results of CDA genotyping analysis are shown in Table 3. We only found three known single nucleotide polymorphisms (SNP) in the coding regions in these patients. Patient 1 was homozygous for 208G>A (Ala70 Thr) in exon 2 (10), but had homozygous wild-type alleles for the other SNPs in exons 1 and 4. All of the other patients carried the homozygous wild-type alleles in exon 2. Thus, it was assumed that the increased plasma gemcitabine levels in patient 1 might have been caused by the Ala70 Thr substitution in cytidine deaminase.

Discussion

There was no nephrotoxicity or neurotoxicity in patient 1, which is specifically associated with cisplatin (11). In addition,
the skin rash observed in patient 1 is common (with a reported 30.1% occurrence) in patients treated with gemcitabine in single-agent therapy (1). Therefore, we considered that the severe toxicity profile observed in patient 1 was mainly caused by the administration of gemcitabine.

Because the average pharmacokinetic profiles of gemcitabine and dFdU in patients 2 to 6 were almost the same as the population pharmacokinetic profiles in phase I and late phase II trials in Japan (12–14), the pharmacokinetic profiles of patients 2 to 6 can be regarded as standard for a Japanese population. Therefore, the plasma gemcitabine levels of patient 1 were remarkably high. Because the DLST of gemcitabine and cisplatin were negative in patient 1, the toxicities, especially a severe systemic rash including stomatitis and purpura, were unlikely to have been caused by drug allergies, such as Stevens-Johnson syndrome. Thus, the exposure to increased levels of gemcitabine is most likely responsible for the severe toxicities experienced in patient 1.

The patient backgrounds showed no major difference in age, body surface area, and performance status among patients with and without severe toxicities; age ranged from 45 to 69 years, the bovine serum albumin ranged from 1.42 to 1.78 m², and Eastern Cooperative Oncology Group performance status ranged from 0 to 1 (patient 1: 45 years, 1.78 m², performance status 0). None of the patients had received any prior chemotherapy or radiotherapy. It was unlikely that the patient backgrounds other than the CDA genotype caused the abnormal pharmacokinetics observed in patient 1.

Patient 1 was homozygous for the SNP 208G>A (Ala⁷⁰Thr), and all of the other patients carried the homozygous wild-type allele. Patient 1 carried no other known nonsynonymous and synonymous CDA polymorphisms (79A>C and 435C>T, respectively). The variant CDA enzyme with Thr⁷₀ was reported to show 40% and 32% of the activity of the wild-type for cytidine and 1-β-D-arabinofuranosylcytosine substrates in an in vitro experiment, respectively (10). Thus, the

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Table 2. Compartment-independent pharmacokinetic variables of gemcitabine and its metabolite, dFdU

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patients 2-6 (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gemcitabine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>46.42</td>
<td>22.28 ± 5.08</td>
</tr>
<tr>
<td>AUC$_{\infty}$ (µg hour/mL)</td>
<td>54.54</td>
<td>10.88 ± 1.64</td>
</tr>
<tr>
<td>Cl (L per h per m²)</td>
<td>18.34</td>
<td>93.17 ± 15.61</td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>0.97</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>$V_z$ (L/m²)</td>
<td>25.62</td>
<td>35.2 ± 7.47</td>
</tr>
<tr>
<td><strong>dFdU</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>8.19</td>
<td>28.75 ± 4.09</td>
</tr>
<tr>
<td>AUC$_{\infty}$ (µg hour/mL)</td>
<td>41.58</td>
<td>106.13 ± 31.44</td>
</tr>
<tr>
<td>Cl/F (L per h per m²)</td>
<td>24.05</td>
<td>10.04 ± 2.98</td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>2.17</td>
<td>2.46 ± 0.52</td>
</tr>
<tr>
<td>$V_z$ (L/m²)</td>
<td>75.4</td>
<td>34.29 ± 6.6</td>
</tr>
<tr>
<td>AUC ratio (dFdU/gemcitabine)</td>
<td>0.76</td>
<td>9.68 ± 2.05</td>
</tr>
</tbody>
</table>

Abbreviations: $C_{\text{max}}$, maximum plasma concentration; AUC$_{\infty}$, area under the concentration-time curve; Cl, clearance; $T_{1/2}$, terminal-phase half-life; $V_z$, = Dose / ($\lambda_e$ × AUC); $\lambda_e$, elimination rate constant at terminal phase; F, metabolite fraction (F can be assumed to lie between 0.90 and 0.95).

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Table 3. Genotypes of the three known polymorphic loci in exons of the CDA gene

<table>
<thead>
<tr>
<th>Patient</th>
<th>Exon 1, 79A&gt;C</th>
<th>Exon 2, 208G&gt;A</th>
<th>Exon 4, 435C&gt;T,</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K27Q</td>
<td>A70T</td>
<td>T145T (silent)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>A/A</td>
<td>A/A</td>
<td>C/C</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A/C</td>
<td>G/G</td>
<td>C/T</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>A/A</td>
<td>G/G</td>
<td>C/C</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>A/A</td>
<td>G/G</td>
<td>C/T</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>A/A</td>
<td>G/G</td>
<td>C/C</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>C/C</td>
<td>G/G</td>
<td>C/T</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: K27Q, A70T, T145T (silent)
reduced activity of the variant enzyme with Thr70 might have resulted in the abnormal pharmacokinetics in patient 1.

The allelic frequency of the 208G>A polymorphism of the CDA gene in the Japanese population is 4.3% (10). Recently, genetic polymorphisms in the gemcitabine metabolic pathway, including CDA SNPs in Europeans and Africans, were reported by Fukunaga et al. (15). The SNP 208G>A was not detected in Europeans, whereas the allelic frequency of 208A was 0.125 in Africans (15). According to the two previous studies (10, 15), frequencies of homozygous 208G>A individuals in the Japanese and African populations were estimated to be about 0.18% and 1.56%, respectively. Therefore, severe toxicity caused by 208G>A could occur more frequently in Africans than in Japanese.

Based on the results of the analyses of the pharmacokinetic profiles and the 208G>A SNP, we can conclude that decreased CDA activity might have been responsible for the severe drug toxicity observed in this Japanese cancer patient.

Acknowledgments

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References

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