The agent 5-fluorouracil (5-Fu) and its derivatives are widely used in the treatment of gastrointestinal, breast, and head and neck cancers. Dihydropyrimidine dehydrogenase (DPD) is the initial and rate-limiting enzyme in the catabolism of the pyrimidine bases uracil and thymine [1], and is also known to be the key enzyme catalyzing the metabolic degradation of 5-Fu [2]. Patients with DPD deficiency are prone to develop severe 5-Fu-associated toxicity, and the use of 5-Fu in such patients may even result in their death [3, 4]. We report the case of an adult male with gastric cancer who was treated with oral 5-Fu and developed severe toxicity but recovered after intensive conservative treatment.

Case Report

A 63-year-old Japanese male with stomach adenocarcinoma received oral 5-fluorouracil derivative, cisplatin and trastuzumab chemotherapy. On day 8, severe diarrhea and mucositis developed; chemotherapy was stopped. On day 14, the patient developed renal dysfunction and febrile neutropenia. He also suffered from pneumonia due to Candida albicans. Systemic symptoms improved after intensive conservative treatment. Best supportive care was continued until the patient died from gastric cancer. The dihydropyrimidine dehydrogenase protein level was low at 3.18 U/mg protein. The result of DPYD genotyping revealed three variants at positions 1615 (G > A), 1627 (A > G), and 1896 (T > C) in exons 13, 13, and 14, respectively.

Key words: 5-fluorouracil, dihydropyrimidine dehydrogenase deficiency, DPYD variant, gastric cancer

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to deteriorate rapidly (PS 3), preventing him from taking anything orally. Chemotherapy was stopped on day 9, and total parental nutrition was introduced.

On day 14, the patient developed grade 2 renal dysfunction and grade 3 febrile neutropenia (neutrophil 247/mm³). Granulocyte-colony stimulating factor, meropenem, and micafungin were administered. On day 22, he developed grade 4 oral mucositis, grade 4 febrile neutropenia, and grade 3 renal dysfunction. He suffered from pneumonia (Fig. 1), and a bronchoalveolar lavage revealed infection by Candida albicans. The anti-fungal agent was changed to amphotericin B, and the patient’s systemic symptoms improved. He was discharged on day 55. He declined to continue chemotherapy and received best supportive care for 3 months until he died from gastric cancer (Fig. 2).

Based on the patient’s clinical course, DPD deficiency was suspected. A blood sample was obtained, and DPD protein was measured using an enzyme-linked immunosorbent assay (ELISA) [5].

Fig. 1 Pneumonia was shown in the right upper lobe on chest X-ray and CT.

Fig. 2 The patient’s clinical course. Symptoms were assessed using CTCAE ver. 4.0. Abbreviations were as follows: P + Tmab: capecitabine + cisplatin + trastuzumab, G-CSF, granulocyte-stimulating factor; MEPM, meropenem; MCFG, micafungin; L-AMB, liposomal amphotericin B; VRCZ, voriconazole.
protein level in peripheral blood mononuclear cell (PBMCs) was markedly low at 3.18 U/mg protein, and a diagnosis of DPD deficiency was established. A gene analysis was performed as described [6]. The sequencing of all 23 exons from the patient’s sample-derived DNA revealed three variants in the nucleotide substitutions at positions 1615 (G > A) in exon 13, 1627 (A > G) in exon 13, and 1896 (T > C) in exon 14. In addition, five single nucleotide polymorphisms in intron 13 (IVS13 +39C > T and IVS13 +40G > A), intron 15 (IVS15 +75A > G), intron 22 (IVS22 +55C > T), and intron 23 (IVS23 − 69A > G) were detected. Our institution’s ethical committee approved this clinical study (approval no. 288). Written informed consent for his case to be published was obtained from the patient.

Discussion

DPD is the initial and rate-limiting enzyme in the catabolic pathway. A partial or complete DPD deficiency is associated with different degrees of 5-Fu toxicity, the most frequent manifestations of which include neutropenia, mucositis, and diarrhea [7]. Although DPD is widely expressed in human tissues, the liver is the major source of this enzyme, harboring 80% of the total body supply, with the kidneys, spleen, lungs, and marrow being minor sources [8]. DPD activities are high in PBMCs, with the pattern of DPD activities expressed in PBMCs very similar to that found in the liver. Therefore, PBMCs are used to measure DPD activities [9].

The human DPD gene has been mapped to chromosome 1p22 and is shown to consist of 23 exons [10], and a variety of mutant alleles of the DPD gene has been reported [11]. These variants are mainly point variants and proteins that have missing amino acid sequences due to the deletion of several bases. However, it is impossible to analyze all known genes, and DPD genotypes and phenotypes do not coincide [12]. The DPD gene is transmitted to offspring by autosomal recessive inheritance [13-15].

Although numerous variants within the gene coding for DPD have been described, only a few have been demonstrated to result in reduced DPD enzyme activity [16]. In our patient, three point variants in exons of the DPD gene were detected. The 1627A > G and 1615G > A variants in exon 13 are missense variants, while the 1896 (T > C) in exon 14 is a silent variant. The first variant, 1627A > G (I543V), is known as rs1801159 [16]. The frequency of I543V variants is relatively high at 18.5%, especially in East Asians and Americans (26.6% and 27%, respectively). In 1,070 Japanese individuals, 21 allelic variants of DPD were identified and the frequency of 1627A > G was 25.68% [17]. The clinical behavior of the I543V variant was favorable and the clinical significance was reported to be benign [18, 19]. However, Teh et al. indicated that the co-existence of 1627A > G and 1896 T > C (rs17376848) could potentially be used as a predictive marker for neutropenia during 5-Fu treatment [20]. The second variant, 1615G > A in exon 13 (G539R), is known in the genome database as rs142619373. The frequency of this mutant allele is very low at 0.7%, and it is detected mainly in Africans. Although the in vitro DPD enzyme activity of the G539R variant was reported to be similar to that of the wild-type DPD, the clinical significance is unknown [21]. Further research is needed to determine whether the G539R variant can influence the clinical course in patients taking 5-Fu.

In Japan, a total of 19 patients with DPD deficiency, including the present patient, have been reported [22-38]. Their characteristics are summarized in Table 1. The patients were 12 men and seven women (median age, 64 years; range, 39-78 years). There were 11 patients with colorectal cancer, six with gastric cancer, one with esophagogastric junction cancer, and one with breast cancer. Three patients received 5-Fu intravenously, and the remaining 16 received oral 5-Fu derivatives such as S-1, uracil/tegafur, or capecitabine. The median period from the first administration of 5-Fu to the onset of adverse events was 7.5 days (range 2-17 days). DPD gene variant was detected in only 3 of the patients, including our patient. The mortality rate from adverse events was approx. 21% and was not significantly associated with age, gender, cancer type, administration route, or the treatment administration period.

Several methods exist to identify DPD deficiency; as mentioned above, DPD activities in PBMCs may be used as a marker for DPD activity in general [39, 40]. It was revealed that DPD levels measured by ELISA correlated well with DPD activities in various cancer types [5]. Another method, i.e., the measurement of dihydrouracil and uracil in urine, is simple and useful but it presents problems with respect to screening for carriers and partial deficiency [41]. The 2-13C-uracil breath test
rapidly discriminates between normal, partially, and profoundly DPD-deficient individuals and offers a useful screening method that could be applied in most clinical settings [42, 43].

In a multicenter prospective cohort study, the determination of DPD deficiency in cancer patients prior to or during 5-Fu treatment was shown to be beneficial in improving the clinical effectiveness of 5-Fu [44]. Hernicks et al. reported that in a cohort of 40 patients with heterozygous DPD deficiency treated with a 5-Fu dose reduced by approx. 50%, the dose reduction did not reduce the effectiveness of 5-Fu-based chemotherapy compared with patients without a DPD deficiency, and that the reduced dose provided significantly improved patient safety [45]. The cost of hospital admission for severe chemotherapy-related toxicity is significantly higher than the cost of prospective DPYD testing of each patient commencing fluoropyrimidine chemotherapy [46]. Therefore, screening for DPD deficiency would enable us to continue 5-Fu-based treatments while maintaining adequate drug exposure in DPD-deficient patients.

As mentioned above, although pharmacokinetic and pharmacogenomic tests in general have the potential to improve clinical outcomes by increasing efficacy and avoiding toxicity, their use in routine clinical practice is still limited. This also holds true for the use of DPYD genotyping prior to the start of treatment with 5-Fu. In general, pharmacogenomics has the potential to result in safer uses of drugs; unfortunately however, these tests have not resulted in the clinical implementation of DPYD screening in the oncology field.

### Table 1 Clinical characteristics of the 19 reported cases of Japanese patients with DPD deficiency in the literature

<table>
<thead>
<tr>
<th>Reported year</th>
<th>First author</th>
<th>Age</th>
<th>Gender</th>
<th>Cancer site</th>
<th>Administration route of 5-Fu</th>
<th>Onset day of adverse events</th>
<th>Diagnostic methods</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>Kouwaki M</td>
<td>57</td>
<td>Female</td>
<td>Breast</td>
<td>Intravenous</td>
<td>7</td>
<td>DPD activity recovery in urine</td>
<td>Recovered</td>
</tr>
<tr>
<td>1998</td>
<td>Kobayashi K</td>
<td>65</td>
<td>Female</td>
<td>Stomach</td>
<td>Intravenous</td>
<td>17</td>
<td>DPD activity Uracil in urine</td>
<td>Recovered</td>
</tr>
<tr>
<td>1999</td>
<td>Inada T</td>
<td>44</td>
<td>Female</td>
<td>Stomach</td>
<td>Intravenous</td>
<td>2</td>
<td>DPD activity Uracil in plasma</td>
<td>Recovered</td>
</tr>
<tr>
<td>2006</td>
<td>Hashimoto T</td>
<td>39</td>
<td>Male</td>
<td>Stomach</td>
<td>Orally</td>
<td>5</td>
<td>DPD activity</td>
<td>Died</td>
</tr>
<tr>
<td>2008</td>
<td>Takaba T</td>
<td>72</td>
<td>Male</td>
<td>Colorectal</td>
<td>Orally</td>
<td>6</td>
<td>DPD activity Uracil in urine</td>
<td>Died</td>
</tr>
<tr>
<td>2008</td>
<td>Kai K</td>
<td>75</td>
<td>Male</td>
<td>Colorectal</td>
<td>Orally</td>
<td>2</td>
<td>Uracil in urine</td>
<td>Recovered</td>
</tr>
<tr>
<td>2010</td>
<td>Aragane H</td>
<td>70</td>
<td>Female</td>
<td>Stomach</td>
<td>Orally</td>
<td>14</td>
<td>DPD mRNA</td>
<td>Died</td>
</tr>
<tr>
<td>2010</td>
<td>Iwamoto A</td>
<td>75</td>
<td>Male</td>
<td>Colorectal</td>
<td>Orally</td>
<td>3</td>
<td>DPD activity recovery in plasma</td>
<td>Recovered</td>
</tr>
<tr>
<td>2013</td>
<td>Tsukiyama G</td>
<td>64</td>
<td>Male</td>
<td>Colorectal</td>
<td>Orally</td>
<td>8</td>
<td>DPD activity recovery in plasma</td>
<td>Recovered</td>
</tr>
<tr>
<td>2014</td>
<td>Sakaguchi H</td>
<td>70</td>
<td>Male</td>
<td>Colorectal</td>
<td>Orally</td>
<td>12</td>
<td>DPD protein recovery in plasma</td>
<td>Recovered</td>
</tr>
<tr>
<td>2014</td>
<td>Matsumoto A</td>
<td>64</td>
<td>Female</td>
<td>Colorectal</td>
<td>Orally</td>
<td>9</td>
<td>DPD activity recovery in plasma</td>
<td>Recovered</td>
</tr>
<tr>
<td>2015</td>
<td>Nagai K</td>
<td>75</td>
<td>Male</td>
<td>Colorectal</td>
<td>Orally</td>
<td>5</td>
<td>DPD protein Uracil in urine</td>
<td>Recovered</td>
</tr>
<tr>
<td>2015</td>
<td>Kinoshita H</td>
<td>51</td>
<td>Male</td>
<td>Stomach</td>
<td>Orally</td>
<td>ND†</td>
<td>DPD protein recovery in plasma</td>
<td>Recovered</td>
</tr>
<tr>
<td>2015</td>
<td>Mita Y</td>
<td>78</td>
<td>Female</td>
<td>Colorectal</td>
<td>Orally</td>
<td>14</td>
<td>DPD protein recovery in plasma</td>
<td>Recovered</td>
</tr>
<tr>
<td>2015</td>
<td>Yoshida Y</td>
<td>73</td>
<td>Male</td>
<td>Colorectal</td>
<td>Orally</td>
<td>11</td>
<td>DPD protein recovery in plasma</td>
<td>Recovered</td>
</tr>
<tr>
<td>2017</td>
<td>Sakata H</td>
<td>58</td>
<td>Male</td>
<td>Colorectal</td>
<td>Orally</td>
<td>7</td>
<td>DPD protein recovery in plasma</td>
<td>Recovered</td>
</tr>
<tr>
<td>2018</td>
<td>Watanabe H</td>
<td>57</td>
<td>Female</td>
<td>Colorectal</td>
<td>Orally</td>
<td>9</td>
<td>DPD protein recovery in plasma</td>
<td>Recovered</td>
</tr>
<tr>
<td>2018</td>
<td>Inoue H</td>
<td>61</td>
<td>Male</td>
<td>EGJ§</td>
<td>Orally</td>
<td>14</td>
<td>DPD protein recovery in plasma</td>
<td>Recovered</td>
</tr>
<tr>
<td>2019</td>
<td>Our case</td>
<td>63</td>
<td>Male</td>
<td>Stomach</td>
<td>Orally</td>
<td>5</td>
<td>DPD protein recovery in plasma</td>
<td>Recovered</td>
</tr>
</tbody>
</table>

5-FU*, 5-fluorouracil; DPD†, dihydoropyrimidine dehydrogenase; ND‡, not described; EGJ§, esophagogastric junction
When DPD deficiency has been demonstrated after chemotherapy that included 5-Fu, a re-administration of 5-Fu must be re-evaluated. The following should be considered: whether to reduce the amount of 5-Fu and continue chemotherapy; whether to continue chemotherapy using another regimen that does not include 5-Fu; and whether to simply provide supportive care. Although chemotherapy without 5-Fu or best supportive care has been selected in most cases, Yoshida et al. reported that capecitabine was administered to their patient with low DPD activity in incrementally increased doses every 14 days, beginning with a single pill (300 mg) and then a gradual increase of the capecitabine dose to 1800 mg [11]. Henricks et al. showed that a patient with a complete DPD deficiency could be safely treated with a very low dose (0.8% of original dose) of a 5-Fu derivative [47].

In conclusion, we described the case of a Japanese patient with gastric cancer who developed severe toxicity associated with three mutant alleles of the DPYD gene. If severe toxicity occurs after the administration of 5-Fu, it is important to consider the possibility of DPD deficiency and to stop chemotherapy immediately, and then to provide appropriate intensive supportive care. In clinical practice, DPD deficiency screening prior to the administration of 5-Fu should be considered to avoid severe 5-Fu toxicity.

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