Lethal Outcome of a Patient with a Complete Dihydropyrimidine Dehydrogenase (DPD) Deficiency after Administration of 5-Fluorouracil: Frequency of the Common IVS14+1G>A Mutation Causing DPD Deficiency

André B. P. van Kuilenburg,2 Erik W. Muller, Janet Haasjes, Rutger Meinsma, Lida Zoetekouw, Hans R. Waterham, Frank Baas, Dick J. Richel, and Albert H. van Gennip

Emma Children’s Hospital and Department of Clinical Chemistry, Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam [A. B. P. v. K., J. H., R. M., L. Z., H. R. W., F. B., D. J. R., A. H. v. G.], and Slingeland ziekenhuis, 7009 BL Doetinchem [E. W. M.], the Netherlands

ABSTRACT

Dihydropyrimidine dehydrogenase (DPD) is the initial and rate-limiting enzyme in the catabolism of 5-fluorouracil (5FU), and it is suggested that patients with a partial deficiency of this enzyme are at risk from developing a severe 5FU-associated toxicity. In this study, we demonstrated that a lethal toxicity after a treatment with 5FU was attributable to a complete deficiency of DPD. Analysis of the DPD gene for the presence of mutations showed that the patient was homozygous for a G→A mutation in the invariant GT splice donor site flanking exon 14 (IVS14+1G>A). As a consequence, no significant residual activity of DPD was detected in peripheral blood mononuclear cells. To determine the frequency of the IVS14+1G>A mutation in the Dutch population, we developed a novel PCR-based method allowing the rapid analysis of the IVS14+1G>A mutation by RFLP. Screening for the presence of this mutation in 1357 Caucasians showed an allele frequency of 0.91%. In our view, the apparently high prevalence of the IVS14+1G>A mutation in the normal population, with 1.8% heterozygotes, warrants genetic screening for the presence of this mutation in cancer patients before the administration of 5FU.

INTRODUCTION

5FU3 remains one of the most widely used chemotherapeutic agents for the systemic treatment of cancers of the gastrointestinal tract, breast, and head and neck. As a single drug, 5FU has only limited efficacy. To improve the clinical response of 5FU, optimal administration schedules as well as the combination of 5FU with other drugs, which should increase its antitumor activity or decrease the host toxicity, have been investigated. It has been shown that long-term continuous i.v. infusion of 5FU is superior to bolus injections of 5FU in terms of response rate, however, only a small increase in median survival was observed (1), and high inter- and intrapatient variations in the plasma concentrations of 5FU have been observed during prolonged infusion of the drug (2). In addition, a relationship between the 5FU dose intensity and the therapeutic response, as well as toxicity, has been noted (3, 4).

An important determinant in predicting the toxicity as well as the efficacy of 5FU might be the activity of DPD. DPD is the initial and rate-limiting enzyme in the catabolism of the pyrimidine bases uracil and thymine, but also of the pyrimidine analogue 5FU. It has been reported that >80% of the administered 5FU is catabolized by DPD (5). Furthermore, a correlation has been observed between the pretreatment activity of DPD in PBM cells and the systemic clearance of 5FU in cancer patients (6). The pivotal role of DPD in chemotherapy using 5FU has been shown in cancer patients with a complete or near-complete deficiency of this enzyme. These patients suffered from severe toxicity, including death, after the administration of 5FU (7–11). It was shown that a number of these patients were genotypically heterozygous for a mutant DPD allele (9–12). To date, 17 variant DPDY alleles have been identified in pediatric patients suffering from a complete or near-complete DPD deficiency, or in tumor patients with decreased DPD activity (12–15). Analysis of the frequency of the various mutations among DPD patients has shown that the splice-site mutation, IVS14+1G>A, was by far the most common one (52%; Ref. 13).

To date, the frequency of the splice site mutation IVS14+1G>A in the normal population is not known. On the basis of population analysis of the DPD activity, the frequency of heterozygotes has been estimated to be as high as 3% (6). Such individuals might be at risk of developing severe toxicity after the administration of 5FU. Furthermore, only a small number of cases have been reported regarding lethal toxicity after the administration of 5FU. In none of these cases have the
molecular mechanisms underlying the 5FU-induced death been resolved. In this paper, we describe a simple genotyping procedure to test for the presence of the IVS14+1G>A mutation. Furthermore, we describe the first patient with lethal toxicity, after the administration of 5FU, who proved to be homozygous for the IVS14+1G>A mutation.

MATERIALS AND METHODS

**Chemicals.** [4,14C]-thymine (1.85–2.22 GBq/mmol) was obtained from Moravek Biochemicals (Brea, CA). Lymphoprep (specific gravity, 1.077 g/ml; 280 mOsm) was obtained from Nycomed Pharma AS (Oslo, Norway). LeucoSep tubes were obtained from Moravek Biochemicals (Brea, CA). Lymphoprep (specific gravity, 1.077 g/ml; 280 mOsm) was obtained from Nycomed Pharma AS (Oslo, Norway). LeucoSep tubes were supplied by Greiner (Frickenhausen, Germany). HAM-F10 medium with 20 mM HEPES was obtained from Life Technologies, Inc. (Breda, the Netherlands). AmpliTaq Taq polymerase Big-Dye-Terminator-Cycle- Sequencing-Ready Reaction kits were supplied by Perkin-Elmer Corp. (Foster City, CA). Restriction endonuclease Ndel was obtained from Roche Diagnostics Nederland B.V. (Almere, the Netherlands). A Qiaquick Gel Extraction kit was obtained from Qiagen (Hilden, Germany). All other chemicals used were of analytical grade.

**Analysis of Pyrimidine Bases.** The concentrations of the pyrimidine bases, uracil and thymine in plasma, were determined using reversed-phase HPLC combined with diode-array detection, as described before (16).

**Culture Conditions of Human Fibroblasts.** Fibroblasts were cultured from skin biopsies obtained from controls and the index patient. Biopsies were incubated at 37°C for 10 min. To lyse the erythrocytes, the pellet was resuspended in 7 ml of ice-cold ammonium chloride solution (155 mM NH₄Cl, 10 mM KHCO₃, and 0.1 mM EDTA) and kept on ice for 5 min. After the addition of 10 ml of ice-cold supplemented PBS, the solution was centrifuged at 250 × g at 4°C for 10 min. The pellet was collected and subjected to another lysis step as described above. The pellet containing the granulocytes was washed once more with supplemented PBS, and the final cell pellet was frozen in liquid nitrogen and stored at −80°C until further analysis.

**PCR Amplification of Exon 14.** DNA was isolated from purified granulocytes by standard procedures. PCR amplification of exon 14 and its flanking intronic regions was carried out using the primer sets DPD14f and DPD14r, as specified in Table 1. Amplification of exon 14 was carried out in a 50-μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl₂, 10 pmol each primer, 200 μM each deoxynucleotide triphosphate, and 2 units of Taq polymerase. After initial denaturation for 5 min at 95°C, amplification was carried out for 35 cycles (1 min 95°C, 1 min 55°C, 1 min 72°C). The PCR product was separated on 1% agarose gels, visualized with ethidium bromide, and purified using a Qiaquick Gel Extraction kit or used for direct sequencing.

**RFLP of Exon 14.** PCR amplification of exon 14 and its flanking 5’ donor intronic region was carried out using the primer sets NDEr and NDe, as specified in Table 1. Amplification of exon 14 was carried out in a 25-μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM MgCl₂, 5 pmol each primer, 200 μM each deoxynucleotide triphosphate and 2 units of Taq polymerase. After initial denaturation for 5 min at 96°C, amplification was carried out for 35 cycles (0.5

### Table 1 Oligonucleotides used for genomic PCR of exon 14 of the *DPYD* gene

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Direction</th>
<th>Positiona</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPD14f</td>
<td>5’–TCCTCTGCAAAATGAGAGGACC–3’</td>
<td>Sense</td>
<td>451–477</td>
</tr>
<tr>
<td>DPD14r</td>
<td>5’–TCACCAAGTTGCACATTCCT–3’</td>
<td>Antisense</td>
<td>762–783</td>
</tr>
<tr>
<td>NDe</td>
<td>5’–ATCAGAGACTGTGACATATGTTC–3’</td>
<td>Sense</td>
<td>565–589</td>
</tr>
<tr>
<td>NDe</td>
<td>5’–CTGTTTTAGATGTTAATCCACACATA–3’</td>
<td>Antisense</td>
<td>736–762</td>
</tr>
</tbody>
</table>

* Numbering according to the intron sequences flanking exon 14 of *DPYD* as published by Vrek et al. (19).
* The NdeI restriction sites introduced by site-directed mutagenesis (primers NDef and NDer) are underlined. The single-base mismatches introducing the NdeI restriction site are depicted in boldface.
RESULTS

Clinical Evaluation. The patient was a female 44 years of age. At the age of 40, she underwent a curative resection of a Dukes B2 moderately differentiated rectum carcinoma and subsequent adjuvant radiotherapy. Three years later, the patient presented with an ileus caused by an intra-abdominal recurrence of the tumor at the right hypochondrium. Debulking of the tumor was performed as well as a partial ileal resection and extirpation of the uterus including the adnexae. No measurable lesions were present after the operation. After a period of 11 months, a palpable mass was present in the right lower abdomen and palliative chemotherapy was started with 5FU (900 mg) and leucovorin (200 mg), which was administered i.v. on a weekly basis. Five days after the first treatment, the patient developed thrombocytopenia (420 × 10^9/l) and thrombocytopenia (10.0 × 10^9/l), which was administered i.v. on a weekly basis. Five days after the second injection with 5FU, the patient developed a severe pancytopenia for which she received a transfusion with erythrocytes and thrombocytes.
A splice site mutation in only a very limited number of alleles of the gene encoding DPD in a tumor patient experiencing DPD deficiency is scarce (7, 22). Of DPD (9–12), the data regarding tumor patients with complete deficiency of DPD, are, therefore, at risk. Although a number of tumor patients before the administration of 5FU. In this way, the genetic screening for the presence of this mutation in cancer patients heterozygous or homozygous for the IVS14 A mutation in adjuvant therapy, the apparently high prevalence of the expected prevalence of a DPD deficiency is high. Considering the common use of 5FU in the treatment of cancer patients and the increasing percentage of patients receiving high doses of 5FU in adjuvant therapy, the apparently high prevalence of the IVS14+1G>A mutation in the normal population warrants genetic screening for the presence of this mutation in cancer patients before the administration of 5FU. In this way, the serious and sometimes lethal 5FU-related toxicities encountered in patients heterozygous or homozygous for the IVS14+1G>A mutation might be prevented.

ACKNOWLEDGMENTS

We thank Fiona Ward for critical reading of the manuscript.

REFERENCES


Lethal Outcome of a Patient with a Complete Dihydropyrimidine Dehydrogenase (DPD) Deficiency after Administration of 5-Fluorouracil: Frequency of the Common IVS14+1G>A Mutation Causing DPD Deficiency

André B. P. van Kuilenburg, Erik W. Muller, Janet Haasjes, et al.