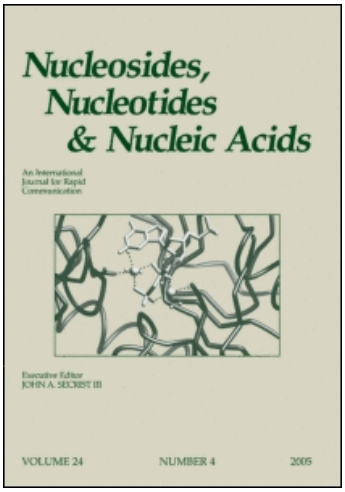

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Pharmacokinetics of 5-Fluorouracil in Patients Heterozygous for the IVS14+1G > A Mutation in the Dihydropyrimidine Dehydrogenase Gene

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PHARMACOKINETICS OF 5-FLUOROURACIL IN PATIENTS HETEROZYGOUS FOR THE IVS14+1G>A MUTATION IN THE DIHYDROPYRIMIDINE DEHYDROGENASE GENE

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□ *5-Fluorouracil (5FU) and capecitabine are two of the most frequently prescribed chemotherapeutic drugs for the treatment of patients with cancer. Administration of test doses of 5FU to eight patients heterozygous for the IVS14+1G>A mutation and five control patients showed that the AUC and clearance were weak parameters with respect to the identification of patients with a DPD deficiency. However, highly significant differences were observed for the terminal half life of 5FU between DPD patients and controls. Thus, a DPD deficiency could be predicted from 5FU blood concentrations measured after the administration of a test dose of 5FU.*

Keywords Dihydropyrimidine dehydrogenase; 5-fluorouracil; *DPYD*; pharmacokinetics; toxicity

INTRODUCTION

5-Fluorouracil (5FU) and the oral prodrug capecitabine (Xeloda) are two of the most frequently prescribed chemotherapeutic drugs for the curative and palliative treatment of patients with cancers of the gastrointestinal tract, breast and head and neck.[1,2] Nevertheless, approximately 30% of the patients with stage III colorectal cancer will still die from metastatic

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disease, despite surgery and adjuvant chemotherapy while the toxicity can be profound.[1]

In order to exert its cytotoxicity 5FU must first be metabolized to the nucleotide level. Although the cytotoxic effects of 5FU are probably directly mediated via the anabolic pathways, the catabolic route plays a significant role since more than 80% of the administered 5FU is catabolized by dihydropyrimidine dehydrogenase (DPD). The pivotal role of DPD in chemotherapy using 5FU has been shown in cancer patients with a complete or partial deficiency of this enzyme. These patients suffered from severe toxicity, including death, following the administration of 5FU.[3–6] It was shown that a number of these patients were genotypically heterozygous or homozygous for a mutant *DPYD* allele.[5,7,8] Analysis of the prevalence of the various mutations reported in cancer patients suffering from severe 5FU-associated toxicity showed that the splice-site mutation, IVS14+1G>A, was by far the most common mutation.[4,9,10]

Various strategies have been proposed to screen for patients with a DPD deficiency.[11] Controlling the AUC of 5FU seems an attractive approach as a clear relationship has been demonstrated between the plasma levels of 5FU and toxicity in addition to response.[12–15] To date, very limited information is available regarding the pharmacokinetics of 5FU in patients with a partial DPD deficiency.[14,16] In this study, we investigated the pharmacokinetics of 5FU in patients heterozygous for the IVS14+1G>A mutation in the *DPYD* gene to establish whether a DPD deficiency could be predicted from 5FU blood concentrations measured after the administration of a test dose of 5FU.

MATERIALS AND METHODS

Patients and Pharmacokinetic Analysis

All tumor patients participated in a protocol that had been designed to study 5FU pharmacokinetics. The protocol was approved by the Medical Ethics Review Committee of the Ludwig-Maximilians Universität München in Germany. Patients received 5-FU, via a 2 min intravenous bolus administration, at 300 mg/m^2 (first test dose) and 450 mg/m^2 (second test dose, 1–20 days after the first test dose), respectively. Blood (plasma) was taken prior to injection at 5, 10, 15, 30, 45, 60, 90, and 120 minutes after 5-FU injection from the vein of the other arm. Plasma samples were analyzed for 5FU concentrations by high-performance liquid chromatography, as described before.[17] The area under the curve (AUC), terminal half-life, and the average systemic clearance were determined by noncompartmental analysis in MW\Pharm (v3.5, Mediware, Groningen, The Netherlands).

Screening for IVS14+1G>A Mutation in DPYD

Carriership for the IVS14+1G>A mutation was investigated in all subjects by RT-PCR analysis of exon 14, as described before.[9] In short, leucocytes were isolated from EDTA blood by hypotonic lysis using Erythrocyte Lysis Buffer (Qiagen, Germany) and subsequent centrifugation. RNA from leucocytes was isolated using TRIZOL (Invitrogen, Germany) according to the manufacturer's recommendations. RNA was reverse transcribed with hexamers (GE Healthcare, Germany) into cDNA using Moloney murine leukemia virus reverse transcriptase (Invitrogen, Germany) according to the manufacturer's instructions. PCR was performed with Platinum Supermix (Invitrogen, Germany) and 12 μM of forward and reverse primer, respectively. Amplified regions and PCR conditions were described previously.[9]

RESULTS

Carrier analysis for the IVS14+1G>A mutation in *DPYD* of all patients showed that eight patients were heterozygous for the IVS14+1G>A mutation whereas this mutation could not be detected in the remaining five patients. For that reason, the five patients without the IVS14+1G>A mutation served as controls with respect to the pharmacokinetic analysis of 5FU.

Analysis of the plasma levels of 5FU in the control patients and patients heterozygous for the IVS14+1G>A mutation showed that the clearance of 5FU was considerably slower in the patient group when compared to that observed in the controls (Figures 1 and 2). Furthermore, the AUC was

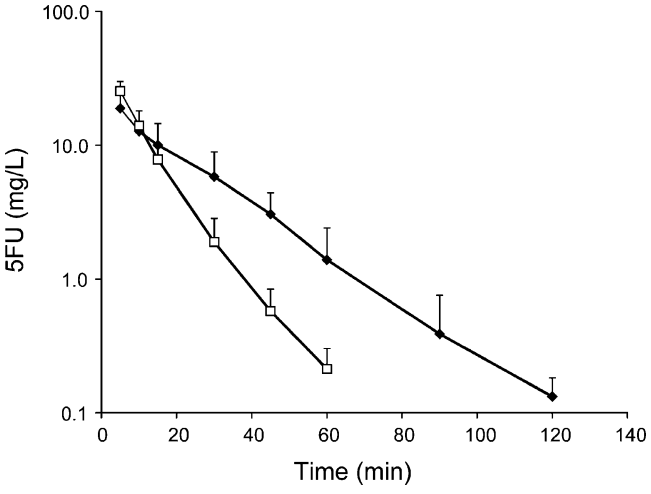

FIGURE 1 Plasma levels of 5FU after the bolus administration of 5FU (300 mg/m^2). The plasma levels of 5FU (mean + SD) are shown for five controls (□) and eight patients heterozygous for the IVS14+1G>A mutation (♦).

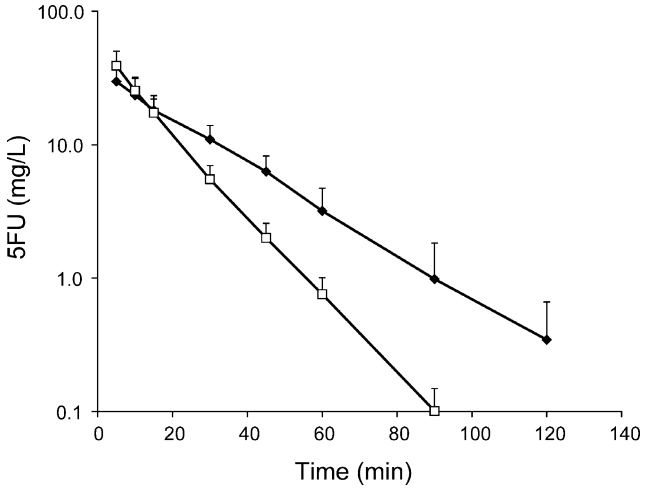

FIGURE 2 Plasma levels of 5FU after the bolus administration of 5FU (450 mg/m^2). The plasma levels of 5FU (mean + SD) are shown for five controls (□) and eight patients heterozygous for the IVS14+1G>A mutation (♦).

1.3-fold and 1.4-fold higher in DPD patients treated with 300 mg/m^2 and 450 mg/m^2, respectively, when compared to controls (Table 1). However, only when a test dose of 450 mg/m^2 was administered, was a significant difference observed for the clearance and AUC between carriers for the IVS14+1G>A mutation and controls (Table 1). In contrast, a highly significant difference in the terminal half life of 5FU was observed at both test doses between DPD patients and controls. The mean average terminal half

TABLE 1 Pharmacokinetic parameters

Model parameter	DPD patients[a] (n = 8)	Controls[b] (n = 5)	P
5FU 300 mg/m^2			
AUC (mg.h/l)			
Mean ± SD	8.2 ± 2.7	6.2 ± 1.6	P = 0.17
Clearance (l/h)			
Mean ± SD	78 ± 36	102 ± 25	P = 0.23
$T^1/_2$ (h)			
Mean ± SD	0.21 ± 0.04	0.13 ± 0.03	P = 0.001
5FU 450 mg/m^2			
AUC (mg.h/l)			
Mean ± SD	16.7 ± 4.9	11.6 ± 2.3	P = 0.028
Clearance (l/h)			
Mean ± SD	54 ± 19	79 ± 19	P = 0.041
$T^1/_2$ (h)			
Mean ± SD	0.28 ± 0.08	0.17 ± 0.02	P = 0.005

[a]Cancer patients heterozygous for the IVS14+1G>A mutation.
[b]Cancer patients without the IVS14+1G>A mutation.

life of 5FU was 60% longer at both dose levels in DPD patients when compared to that observed in controls.

DISCUSSION

5-Fluorouracil (5FU) and the oral prodrug capecitabine (Xeloda) are frequently being used for the curative and palliative treatment of patients with cancers of the gastrointestinal tract and breast.[1,2] Several 5-FU containing chemotherapy regimens nowadays exist, including 5-FU dose levels ranging from 500 mg/m^2 per 21-day cycle in breast cancer to 2000 mg/m^2 per 14-day period in colorectal cancer.[18,19] Despite extensive safety data available from dose finding studies, the therapy-induced toxicity can be profound and the identification of patients with an increased risk of development of severe 5FU-associated toxicity is, therefore, a challenging field. The identification of these patients, prior to the start of the therapy, would allow either dose-adaptation or the use of other non-fluoropyrimidine-based chemotherapeutic drugs. There is now overwhelming evidence that those patients with a deficiency of dihydropyrimidine dehydrogenase (DPD) are at risk of developing severe and sometimes lethal 5FU-associated toxicity.[3–7] More than 80% of the administered 5FU is catabolized by DPD and patients with a complete or partial DPD deficiency have a strongly reduced capacity to degrade 5FU and therefore, an increased likelihood of suffering from severe toxicity.[14,16,20] The importance of a DPD deficiency in the aetiology of unexpected severe 5FU toxicity has been demonstrated by the fact that in 39–61% of the cases, a decreased DPD activity could be detected in peripheral blood mononuclear (PBM) cells.[5,6,21] There are currently a number of procedures to test for the presence of a DPD deficiency, including genotyping, measurement of the uracil/dihydrouracil ratio, the assessment of the DPD activity in peripheral blood mononuclear cells and an oral loading test using stable-isotope labelled uracil.[11,22]

In this study, we have investigated the pharmacokinetics of 5FU in patients heterozygous for the IVS14+1G>A mutation in the DPD gene. This mutation leads to skipping of exon 14 immediately upstream of the mutated splice donor site in the process of DPD pre-mRNA splicing. As a result the mature DPD mRNA lacks a 165 nt segment encoding the amino acids 581-635.[23,24] Analysis of the prevalence of the various mutations among DPD patients has shown that the IVS14+1G>A mutation is by far the most common one.[4,9,10] In addition, there is a relatively high frequency of this mutation in the populations from Northern Europe.[4]

Noncompartimental pharmacokinetic modeling revealed that AUC and clearance were weak parameters with respect to the identification of patients with a DPD deficiency. These findings can be explained by the fact that the AUC of bolus injected 5-fluorouracil is largely determined by the

peak concentration reached immediately after injection. This peak value is hardly influenced by the DPD status. Our results are in line with the recent pharmacokinetic profiling of patients treated with 5FU adjuvant therapy, administered according to the Mayo Clinic regimen.[15] Although a fairly linear trend was observed between the AUC and toxicity grade, the AUC proved not to be a very sensitive marker for predicting severe to life-threatening toxicity.[15] Furthermore, the administration of a test dose of 5FU (250 mg/m^2) to cancer patients did not yield any valuable pharmacokinetic parameters associated with toxicity experienced by these patients upon subsequent treatment with regular doses of 5FU.[25] Indeed, our results showed that only when a higher test dose of 450 mg/m^2 was administered, the mean AUC and clearance of 5FU was significantly different in DPD patients compared to that observed in control patients.

However, the terminal half life of 5FU proved to be a valuable pharmacokinetic parameter to identify patients with a DPD deficiency since highly significant differences were observed at both test doses between DPD patients and controls. The increased AUC and terminal half-life and the decreased clearance of 5FU in the DPD patients is in line with the fact that the mean DPD activity in patients heterozygous for a mutation in the DPD gene is 48% of that observed in controls.[26]

Considering the common use of 5FU-based chemotherapy in the treatment of cancer patients and the severe 5FU-related toxicities in patients with a partial DPD deficiency and the relatively high frequency of the IVS14+1G>A mutation in the normal population, screening of patients for a DPD deficiency is warranted. In this study, we showed that pharmacokinetic analysis of 5FU in plasma after the administration of a test dose can clearly identify patients with a DPD deficiency.

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