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SHORT COMMUNICATION

**Joseph Ciccolini · Cedric Mercier · Laetitia Dahan
Alexandre Evrard · Jean-Christophe Boyer
Karine Richard · Jean-Philippe Dales · Alain Durand
Gerard Milano · Jean-François Seitz · Bruno Lacarelle**

Toxic death-case after capecitabine + oxaliplatin (XELOX) administration: probable implication of dihydropyrimidine deshydrogenase deficiency

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Abstract This report here is the case of a 52-year-old male patient who suffered from extremely severe haematological toxicities (G4 neutropenia, G4 thrombocytopenia) while undergoing Xelox (Xeloda + Oxaliplatin) treatment for his multifocal hepatocarcinoma. Despite appropriate supportive treatment, his condition quickly deteriorated and led to death. It was hypothesized that dihydropyrimidine deshydrogenase (DPD) gene polymorphism could be, at least in part, responsible for this fatal outcome. To test this hypothesis, both phenotypic and genotypic studies were undertaken, and fully confirmed the DPD-deficient status of this patient. Uracil to dihydrouracil ratio in plasma was evaluated as a surrogate marker for DPD deficiency, and showed values out of the range previously recorded from a reference, non-toxic population. Interestingly, the canonical IVS14 + 1G > A single nucleotide polymorphism, usually associated with the most severe toxicities reported with 5-fluorouracil (5-FU), was not found in this patient, but further investigations showed instead a heterozygosity for the 1896C > T mutation located in the exon 14 of the DPYD gene. Taken together, the data strongly suggest for the first time that a toxic-death case after capecitabine-containing protocol could be, at least in part, linked with a DPD-deficiency syndrome. The case reported here warrants therefore systematic detection of patients at risk, including when oral capecitabine is scheduled.

Keywords Capecitabine · Oxaliplatin · Gene polymorphism · Dihydropyrimidine deshydrogenase

J. Ciccolini · A. Durand · G. Milano · B. Lacarelle
Clinical Pharmacokinetics Department, La Timone University Hospital, Marseille, France

C. Mercier (✉)
Medical Oncology Unit, La Timone University Hospital,
235 rue St Pierre, 13385 Marseille cedex 05, France
E-mail: cedric.mercier@mail.ap-hm.fr
Tel.: +33-491-835509
Fax: +33-491-835667

L. Dahan · K. Richard · J.-F. Seitz
Gastroenterology Unit, La Timone University Hospital, Marseille, France

A. Evrard · J.-C. Boyer
Clinical Biochemistry and Toxicology Department, Caremeau General Hospital, Nîmes, France

J.-P. Dales
Anatomopathology Department, Northern School of Medicine, Marseille, France

Introduction

Dihydropyrimidine deshydrogenase (DPD) deficiency is a pharmacogenetic syndrome usually associated with the occurrence of severe, when not lethal, toxicities in cancer patients undergoing 5-fluorouracil (5-FU) chemotherapies [1, 2]. Since DPD is the first and exclusive step of fluoropyrimidines catabolism, partial or total deficiency leads to dramatic plasma overexposure in patients treated with standard dosage, with subsequent exacerbation of drug-induced toxicities. Till date, sparse and contradictory data have been published on the relationships between DPD impairment and toxicities with capecitabine (Xeloda®), a 5-FU pro-drug recently launched as a convenient and specific oral alternative in the setting of ambulatory chemotherapies requiring fluoropyrimidine drugs [3–5].

Case report

The case presented here is that of a 52-year-old male patient, who was hospitalized for a multifocal hepatocarcinoma. CT-scan imaging (Fig. 1a, b) detected several nodules, confirmed by histologic diagnosis on percutaneous biopsies. Essentially, this patient was non-cirrhotic, and major parts of his liver remained

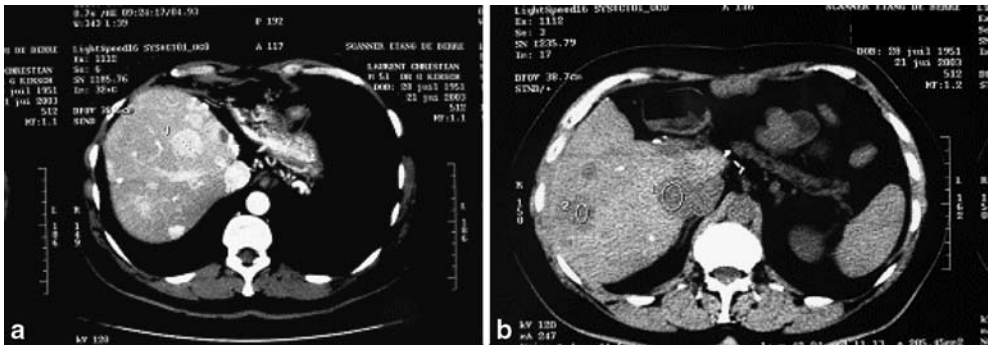

Fig. 1 CT scans showing lesions in high density (**a**) of hepatocarcinoma, as seen with iodine injection (liver segment IV–VII–VIII). **b** lesions in low density of hepatocarcinoma seen in CT scan without iodine injection (liver segment I–VI)

healthy, an observation fully consistent with the fact that functional exploration of this organ was found to be normal.

This patient was treated with a standard Xelox (a.k.a. Capox) protocol, scheduled as following: oxaliplatin: 130 mg/m^2 (total dose: 215 mg) on D1 and D21 and capecitabine: 1,000 mg/m^2 (total dose: 1,650 mg) twice daily from D1 to D14. Patient had to be re-hospitalized on emergency on D9 due to the outbreak of extremely severe toxicities (rectal haemorrhage, G4 oesophagitis, G4 thrombocytopenia, G4 neutropenia, standard WHO grading) starting home on D7. Capecitabine intake was stopped right then. Despite the appropriate symptomatic treatments (Ciflox, Claventin, Neupogen, and daily infusions of platelet concentrates) undertaken as soon as the patient presented back in the unit, his condition quickly deteriorated, and fatal outcome was eventually observed on D21.

Since no signs of peripheral neurotoxicity usually associated with oxaliplatin were observed, it was hypothesized that the capecitabine intake could be, at least in part, at the origin of this treatment-related death. As a triple pro-drug, capecitabine is rationally designed to be preferentially activated to 5-FU in tumour cells by cytidine deaminase and thymidine phosphorylase [6]. Since both the enzymes can also be expressed in the liver, unscheduled 5-FU synthesis may occur there, thus putting DPD-deficient patients at risk. To test this hypothesis, both phenotypic and genotypic investigations were carried out to establish the DPD status of this patient.

Evaluation of his U/UH2 ratio in plasma was first performed, as a surrogate marker for DPD activity [7, 8]. Patient's U/UH2 ratio in plasma was measured and compared with values previously recorded from a reference population. Regarding the Gaussian distribution of the reference ratios, and after an inter-institute, cross-validation study [9], the cut-off indicative of a DPD deficiency suspicion was set at 2 (the higher the ratio, the stronger the deficiency). The U/UH2 score recorded for this patient (ratio = 5) was found to be more than three times higher than median value observed in a reference, non-toxic population ($n = 60$, mean 1.5), and 2.5 times greater than the cut-off above which DPD deficiency is suspected in this institute [8, 9]. This strongly suggests that the patient displayed markedly impaired DPD activity indeed (Fig. 2).

Genetic studies were next undertaken from liver biopsies previously obtained for diagnostic, to check whether this deficiency was related to a genetic mutation on the DPYD gene.

The exon 14-skipping mutation IVS14 + 1G > A of the DYPD gene was first checked after DNA extraction from formalin-fixed paraffin embedded tissue by means of standard procedure. The assay was based on different melting temperatures (T_m) of fluorescent-labelled oligonucleotide hybridization probes using single-step assay that combine fluorescence PCR and melting curve analysis (Light-Cycler methodology) using a method adapted from Nauck et al. [10]. A 198 bp amplicon was amplified by using sens (5′-GTTTTCAGCTGCTTGATGG-3′) and antisens (5′-AAGGCATGTATGTTGGCCTC-3′) primers. Melting curve analysis was performed from 45 to 80°C with continuous fluorescence detection by using detection (5′-ATCACACTTACGTTGTCTGG-3′-fluorescein) and anchor (LCred640-5′-AAGTCA

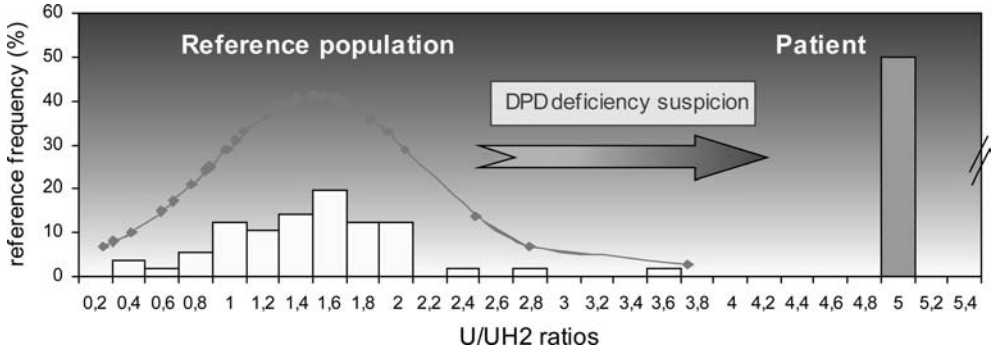

Fig. 2 Comparison between U/UH2 ratio distribution recorded from a reference population ($n = 60$, median ratio = 1.4) and the patient with lethal toxicity (ratio = 5). Above 2 ratios are considered as indicative of DPD deficiency (the higher the ratio, the greater the deficiency)

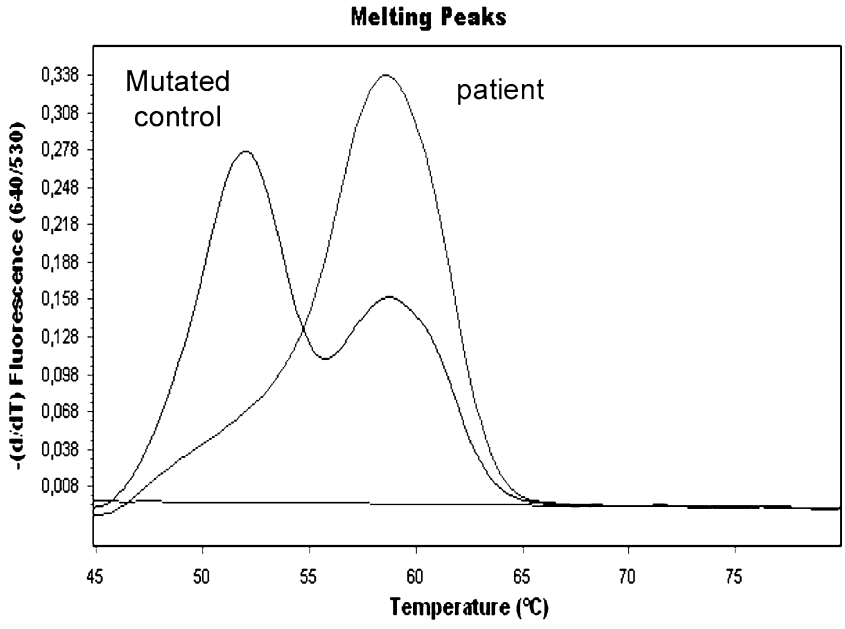

Fig. 3 Melting curve analysis of hybridization probes for IVS14 + 1G > A single nucleotide permutation. No such mutation was found in this patient

GCCTTTAGTTCAGTGACA-3′) probes. Fluorescence genotyping of exon 14 polymorphism was also confirmed by a RFLP assay.

Surprisingly, the canonical IVS14 + 1G > A single nucleotide polymorphism, usually associated with the most severe toxicities reported with fluoropyrimidine drugs [11], was not found in this patient (Fig. 3), a negative finding confirmed next by RFLP analysis (data not shown).

Finally, exons 2, 4, 10, 12, 13, 14, 22, and 23 were chromatographed at the optimized melting temperatures following the method adapted from Ezzeldin et al. [11]. Patient sample was analysed both mixed and unmixed with wild-type standards at temperatures used for mutation detection, to separate the homozygote from the heterozygote genotypes. Changes in chromatogram, as compared with wild-type patterns, were purified and subsequently sequenced following standard procedures. The interpretation of the variation was performed using the Locus Specific Data Base dedicated to DPYD gene (UMD–DPYD at www.umd.be). Interestingly, this analysis fully confirmed the absence of the IVS14 + 1-G > A single nucleotide permutation, but revealed instead a heterozygosity for the 1896C > T mutation located in the exon 14 of the DPYD gene. This mutation creates a potential enhancer splice site (ESE) for the SRp40 and SC35 proteins, with possible impact on DYPD mRNA and, subsequently, enzyme expression.

Discussion

Taken together, the data strongly suggest, that this toxic-death case in a patient undergoing capecitabine-containing protocol could be, at least in part, linked with a DPD-deficiency syndrome. Interestingly, it was found that it is not the usual IVS14 + 1G > A mutation [12], but instead a 1896C > T single nucleotide polymorphism located on the exon 14 of the DPYD gene, that seems driving this marked deficiency, and the subsequent lethal toxicities upon Xeloda. To the best of our knowledge, this is the first time ever that a treatment-related death involving capecitabine can be linked with DPD gene polymorphism, pharmacogenetic syndrome usually associated with lethal toxicities with 5-FU only [1, 2].

The data presented here, raise therefore the question of systematic detection of patients at risk prior to the administration of widely prescribed fluoropyrimidine-drugs, including when oral Xeloda is scheduled. Strong pharmacokinetics support (e.g. pharmacokinetically guided dosing methods) should help to reduce the risk, by tailoring 5-FU or capecitabine dosage according to the DPD status of cancer patients, thus ensuring an optimal therapeutic efficacy with a maximum safety.

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