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Letter to the Editors

A well-tolerated 5-FU-based treatment subsequent to severe capecitabine-induced toxicity in a DPD-deficient patient

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5-Fluorouracil (5-FU) is widely administered as a continuous infusion to treat gastrointestinal tract or head and neck cancers, and as bolus injections in breast cancer chemotherapy regimens. Grade 3–4 toxicity occurs in about 30% of patients receiving 5-FU as a continuous infusion, and proves lethal in 0.5% of these patients [1]. 5-FU-related toxicity has mostly been reported with intravenous administration [1–3]. However, some cases of severe toxicity, including deaths, have been described after oral administration of 5-FU derivatives, such as capecitabine (Xeloda®) [4, 5]. A polymorphism of the dihydropyrimidine dehydrogenase (DPD) gene has been identified as a frequent cause of such toxicity [6, 7]. DPD catalyses the rate-limiting step of fluoropyrimidine catabolism. Partial or total DPD deficiency therefore leads to substantial overexposure in patients treated with the standard dose, exacerbating drug toxicity. This raises questions about possible screening for DPD deficiency before the administration of fluoropyrimidine drugs, including their oral forms. Patients found to have a deficiency on screening before treatment or following signs of toxicity during a previous course are usually given alternative treatments based on nonfluoropyrimidine compounds. However, 5-FU is highly active and its use may be essential in patients who fail to respond to other treatments. We report the case of a patient with DPD deficiency detected due to severe toxicity during capecitabine treatment, who has since received a continuous infusion of 5-FU at almost the standard dose with no significant signs of toxicity.

A 34-year-old woman was treated with capecitabine monotherapy at a dose of 1200 mg m^{-2} twice daily for 14 days as second-line chemotherapy for metastatic breast cancer, with the liver the main site involved. She took no other drugs during this treatment. She had received six cycles of 5-FU, epirubicin and cyclophosphamide (FEC) 100 [500 mg m^{-2} 5-FU (i.v. bolus), 100 mg m^{-2} epirubicin and 500 mg m^{-2} cyclophosphamide] as an adjuvant chemotherapy 2 years previously for her breast cancer. Two days after the beginning of the first cycle of capecitabine treatment, the patient complained of diarrhoea, which progressively worsened, leading to hospitalization on day 16 for severe toxicity including grade 4 mucositis, grade 3 diarrhoea, fever and dehydration. She exhibited grade 4 febrile neutropenia (neutrophil count = 20 mm^{-3}) and intestinal occlusion at day 19. The patient eventually recovered with symptomatic treatment.

We carried out phenotypic and genotypic analyses of the *DYPD* gene in this patient. We tested for relevant *DYPD* single nucleotide polymorphisms (SNPs) by pyrosequencing, as previously described [8]. Plasma uracil (U) and dihydrouracil (UH_2) concentrations were then determined by liquid chromatography [9] and the UH_2/U ratio was characterized as a surrogate marker for DPD activity, rather than measuring DPD activity directly in mononuclear cells [10]. The patient was found to be heterozygous for the splice site mutation IVS14 + 1G→A (*DYPD*2A*). The plasma uracil concentration was normal (9.5 ng ml^{-1}, see Table 1 for reference values), but the UH_2/U ratio (patient's ratio = 3.6) was below the 10th percentile of the reference distribution (Table 1) [8]. We therefore concluded that the patient had probably been overexposed to 5-FU due to DPD deficiency.

Treatment was limited to a single 14-day cycle of capecitabine, but nonetheless resulted in a significant decrease in hepatic lesions, as shown by computed tomography (CT) scans. However, such severe toxicity was observed that capecitabine was considered definitively contraindicated in this patient. Two subsequent lines of chemotherapy were administered over a 6-month period, but both failed, leaving the patient with progressive disease. Finally, given the high level of activity of fluoropyrimidine derivatives, as observed during capecitabine treatment, it was decided to reintroduce 5-FU, but at a lower dose. A continuous infusion schedule was chosen so that plasma 5-FU concentration could be measured daily, making it possible to adapt the dose, as appropriate, during the course of treatment.

The patient received a combination of vinorelbine (Navelbine®) and 5-FU, scheduled every 21 days, as follows: Navelbine® 25 mg m^{-2} (days 1–8) and 5-FU 225 mg m^{-2} per day as a continuous infusion over 5 days (days 1–5). This reduced the 5-FU dose administered to 30%, the theoretically recommended dose (750 mg m^{-2} day^{-1}). During each course of treatment, plasma concentrations of 5-FU were determined on days 1 and 2, for calculation of the partial area under the curve ($AUC_{0–48h}$), and again on days 3, 4 and 5, for calculation of $AUC_{0–120h}$. AUCs were obtained using the formula $AUC = \Sigma\ C_i \times 24$, where C_i is the steady-state concentration measured on the i^{th} day of treatment. The AUCs were compared with reference values obtained during systematic 5-FU therapeutic drug monitoring carried out at our institution for patients treated with doses of 600 and 750 mg m^{-2} day^{-1} (Figure 1 and Table 1) [11]. During the first course, $AUC_{0–48h}$ was low (5520 μg l^{-1} h^{-1}). It was therefore decided to increase the dose for the remaining 3 days of treatment (Table 2). The increase in daily dose to 450 mg m^{-2} day^{-1} provided an $AUC_{0–120h}$ of 27 240 μg l^{-1} h^{-1}. No toxicity was observed during this first course, so the initial dose was set at 450 mg m^{-2} day^{-1} for the next course. Plasma concentrations on days 1 and 2 of the second course ($AUC_{0–48h}$ = 8160 μg l^{-1} h^{-1}) remained below reference values. Given the good immediate tolerance observed, the treatment was intensified by increasing the dose to 600 mg m^{-2} day^{-1} for the last 2 days. The $AUC_{0–120h}$ for this second course was 46 080 μg l^{-1} h^{-1}, with a mean daily dose of 510 mg m^{-2}. The patient had fever and mouth ulcers for 3 days, accompanied by grade 2 neutropenia (neutrophil count = 929/mm^3), but this toxicity was

Table 1

Reference values used for interpretation of the patient's data: plasma uracil distribution, dihydrouracil/uracil (UH_2/U) distribution and 5-FU area under the curve

Reference values for uracil (U) and dihydrouracil/uracil ratio (UH_2/U)*		
	Plasma uracil (μg l^{-1})	UH_2/U ratio
Number of subjects	252	252
Median	13	7.3
Minimum	5.7	1.3
Maximum	70.6	17.1
Threshold value for toxicity	15	6

Reference values for 5-FU AUC using our calculation method				
5-FU dose (continuous infusion)	600 mg m^{-2} day^{-1}†		750 mg m^{-2} day^{-1}‡	
Number of courses	96		91	
	$AUC_{0–48h}$	$AUC_{0–120h}$	$AUC_{0–48h}$	$AUC_{0–120h}$
Median (mg.l^{-1}.h)	8 880	20 724	16 080	51 120
Minimum (mg.l^{-1}.h)	2 640	7 800	5 040	15 360
Maximum (mg.l^{-1}.h)	38 520	107 520	97 920	121 760
25th percentile	6 750	17 400	11 280	39 900
75th percentile	14 160	29 682	21 600	67 980
90th percentile	20 400	39 360	30 060	81 672

*See Boisdron-Celle et al. [8]. †See Beneton et al. [11]. ‡Unpublished.

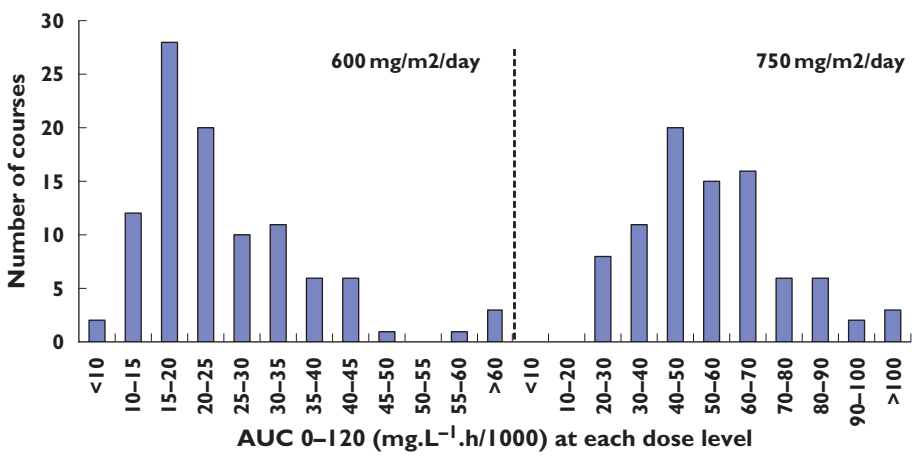

Figure 1

Reference area under the curve ($AUC_{0–120h}$) for 5-fluorouracil, as obtained in our institution and classified according to the dose administered

Table 2

Doses of 5-FU administered on three separate occasions (courses 1, 2 and 3), with corresponding 5-FU plasma concentrations and cumulative AUCs

Course and day numbers		5-FU dose (mg m^{-2} day^{-1})	5-FU concentration (mg l^{-1})	Cumulative 5-FU AUC (mg.l^{-1}.h)
Course 1	day 1	225	100	
	day 2	225	130	5 520
	day 3	450	250	
	day 4	450	340	
	day 5	450	315	27 240
Course 2	day 1	450	310	
	day 2	450	300	8 160
	day 3	450	350	
	day 4	600	400	
	day 5	600	460	46 080
Course 3	day 1	600	340	
	day 2	600	270	
	day 3	600	380	
	day 4	600	265	
	day 5	600	310	37 560

not considered limiting and the dose of 600 mg m^{-2} day^{-1} was maintained for the third course. The third course was associated with acceptable levels of tolerance and an AUC_{0-120h} of 37 560 mg.l^{-1}.h. This result is consistent with moderate DPD deficiency, the AUC level being close to the 90th percentile of the reference distribution for this dose. A further course of the same chemotherapy regimen was administered on an outpatient basis [no therapeutic drug monitoring (TDM) performed] at the same dose (600 mg m^{-2} day^{-1} for 5 days) without toxicity. However, after four courses of treatment, progression of the liver metastasis was observed on CT scan. The patient died of terminal liver failure a few weeks later.

We describe here the administration of 5-FU, without major toxicity, to a DPD-deficient patient carrying the IVS14 + 1G→A splice-site mutation, who displayed severe signs of toxicity during her first cycle of treatment with capecitabine.

Population analyses have shown that the mutated allele of the *DYPD* gene is prevalent, the frequency of heterozygotes being 0.9–3.06% [12, 13]. This mutation is one of the most common found in patients suffering from severe toxicity and, despite conflicting results concerning prevalence, which has been reported to be between 2% and 28% in such patients [7, 10, 14], treatments based on 5-FU are generally considered contraindicated in patients with complete deficiency carrying this mutation [8, 10].

In her initial phase of treatment, the patient received six courses of FEC 100 as adjuvant chemotherapy with radiotherapy. She reported nausea, but no immediate or delayed toxicity was observed after these courses. Capecitabine treatment was thus initiated at the standard dose when required due to tumour progression. This treatment was immediately stopped on the observation of signs of toxicity and 5-FU was eliminated from subsequent treatments due to the severity of the toxicity observed with capecitabine. Two subsequent lines of chemotherapy proved ineffective, and the decision was eventually taken to administer intravenous 5-FU. As the patient had been shown to have DPD deficiency, the dose of the first course was initially set at 30% the theoretical recommended dose, and was subsequently adapted, using pharmacokinetic monitoring [10]. The treatment was well tolerated. The dose was therefore gradually increased to 600 mg m^{-2} day^{-1}, not far below the standard dose of 750 mg m^{-2} day^{-1}.

It remains unclear why this patient was able to tolerate this dose, despite her DPD deficiency. The positive predictive value of detection of a variant of the *DYPD* gene for the development of severe 5-FU toxicity has recently been evaluated in a prospective study [8]. The presence of one relevant SNP was associated with a positive predictive value of 0.75, based on a prevalence of 10%. The specific risk associated with the IVS14 + 1G→A mutation could not be estimated from this study, due to the low prevalence of this mutation in the population studied (three of 252 patients, 1.19%). However, one of the two patients with this mutation only, and the third patient with both this and another mutation, displayed grade 3–4 toxicity after the first course of 5-FU. Similar results have been obtained by Magné *et al.*, who observed grade 3–4 toxicity in two patients carrying this mutation [14]. Thus, patients having the IVS14 + 1G→A mutation are clearly predisposed to severe 5-FU toxicity. Our patient presented severe side-effects after capecitabine intake, but not after continuous infusion of 5-FU, albeit at a dose below the standard dose. We have previously shown that there is indeed an exposure–toxicity relationship for 5-FU during 5-day continuous infusions at a dose of 1000 mg m^{-2} day^{-1}, but not at a dose of 600 mg m^{-2} day^{-1}, for which 5-FU exposure rarely

exceeds 40 000 $\mu g\ l^{-1}\ h^{-1}$. When treated at this dose, the AUCs measured in our patient approximated this value, consistent with good tolerance. However, 5-FU exposure might have been expected to increase to much higher levels, due to both DPD deficiency in this patient and the nonlinear pharmacokinetics of 5-FU [15]. In fact, 5-FU concentrations approximated theoretical values when the drug was administered by continuous infusion, whereas oral administration of capecitabine was associated with severe toxicity. The reasons for this remain unclear. Could the effects of the nonlinear pharmacokinetics on intracellular 5-FU levels be more marked with the administration of capecitabine as a single daily dose rather than as a continuous infusion? Could the characteristics of the tumour account for this phenomenon? Presant *et al.* have showed that 5-FU trapping by the tumour is highly variable [16]. We can hypothesize that an increase in 5-FU levels in the tumour would lead to lower concentrations of 5-FU in the plasma. In our experience of TDM for 5-FU, we have often found it necessary to increase the dose, to ensure that the target concentration is reached during tumour progression [17, 18]. CT scans for our patient at the time of 5-FU administration showed much greater hepatic involvement than during the period in which capecitabine was administered. If these findings reflect reality, then 5-FU administration in DPD-deficient patients should always be considered with caution, taking the stage of the cancer into account.

Differences in the metabolic routes followed by 5-FU and capecitabine may also account for severe toxicity developing within a few days of the initiation of capecitabine treatment, whereas 5-FU infusions were well tolerated. Capecitabine is a prodrug that is activated through three steps leading to the generation of 5-FU in target cells [19]. The first of these steps is catalysed by hepatic and plasma carboxylesterase and yields 5′deoxyfluorocytidine (5′DFCR). Cytidine deaminase (CDA) then generates 5′deoxyfluorouridine (5′DFUR) from 5′DFCR, and in the final step, 5′DFUR is specifically activated by conversion to 5-FU in tumour cells, mediated by thymidine phosphorylase. CDA activity levels are high in about 10% of the population [20]. This has led to the suggestion that a possible CDA extensive-metabolizer phenotype might have favoured the overactivation of capecitabine to generate 5-FU in this patient. The patient's serum CDA activity was assessed, using a published assay based on the release of ammonium from cytidine [20]. Serum CDA activity was found to be no higher than normal (patient, 2.45 U mg^{-1} protein; reference population, 3.6 $\pm$ 1.6 U mg^{-1} protein). Intracellular overexposure to locally produced 5-FU might also be observed, due to the role of DPD in detoxification [21].

Intravenous 5-FU and oral fluoropyrimidine derivatives are widely prescribed. Specific analyses of DPD activity and screening for selected mutations, including the IVS14 + 1G→A mutation, should be routinely carried out before fluoropyrimidine administration, given the severity of treatment-related toxicity and the prevalence of patients with low levels of DPD activity. However, the case reported here illustrates a new difficulty in the management of DPD-deficient patients, as the selection of alternative treatments may not be the only possible choice. Indeed, our observation indicates that, once this deficiency has been identified on the basis of both DPD genotype and phenotype, it is possible to tailor 5-FU dose in DPD-deficient patients, using TDM.

Competing interests

None declared.

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