Dihydropyrimidine dehydrogenase activity and the IVS14+1G>A mutation in patients developing 5FU-related toxicity

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What is already known about this subject

- Dihydropyrimidine dehydrogenase (DPD) deficiency is known to be a major cause of severe 5FU-related toxicity.
- A link has been previously shown between 5FU toxicity and either low enzyme activity measured in peripheral blood mononuclear cells (PMNC) or germinal DPD mutations.
- The link between the most common DPD mutation (IVS14+1G>A) and PMNC enzyme activity is poorly documented.

What this study adds

- This paper provides the largest series of case-reports ($n = 131$) with 5FU-related toxicity, analyzed for both PMNC-DPD activity and the IVS14+1G>A mutation.
- A very low incidence of the IVS14+1G>A mutation (2.2%) was observed in this selected Caucasian population.
- Present data suggest that IVS14+1 mutation screening has limited effectiveness in identifying patients at risk for severe 5FU toxicity. Also, patients with normal PMNC-DPD activity may develop life-threatening toxicity.

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Keywords

dihydropyrimidine dehydrogenase, fluoropyrimidine, gene polymorphism, pharmacogenetics, toxicity

Aims

To examine retrospectively the relationship between DPD phenotype/genotype and the intensity of 5FU toxicity.

Methods

One hundred and thirty-one case-reports (81 women, 50 men) with 5FU-related toxicity were analyzed.

Results

The lower the DPD activity ($10\text{--}504 \text{ pmol min}^{-1} \text{ mg}^{-1}$), the higher the toxicity grade was scored ($P < 0.01$). Toxicity-related deaths occurred in nine patients (eight women) who significantly expressed lower DPD activity than other patients. Two of the deceased patients had normal DPD activity. The IVS14+1G>A mutation, analyzed in 93 patients, was detected in two patients (nonlethal toxicity).

Conclusions

The IVS14+1G>A mutation may not help prevent toxicity and patients with normal DPD activity may develop life-threatening 5FU toxicity.

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Introduction

Fluoropyrimidine-based treatment remains the most used chemotherapy worldwide. A meta-analysis has shown that grade 3–4 toxicity occurs in 30% of 5-fluorouracil (5FU)-treated patients, with 0.5% mortality [1]. Deficiency in dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme of pyrimidine catabolism, is a major cause of severe 5FU-related toxicity [2, 3], which has been linked to low enzyme activity measured in peripheral blood mononuclear cells (PBMC) or to germinal *DPYD* mutations [2, 3]. The relationship between the most commonly studied functional IVS14+1G>A mutation and DPD-PBMC enzyme activity is poorly documented. We now describe the largest series of case-reports ($n = 131$) with severe 5FU-related toxicity, analyzed for PBMC-DPD activity and the IVS14+1G>A mutation. This work is an extension of a previous case-report analysis from our group [3].

Methods

Patients

Between January 1993 and July 2004, blood samples from 131 Caucasian cancer patients with well-documented severe 5FU-related toxicity (at least one episode = grade 3 or neurotoxicity = grade 2) were referred to our institute, including the 53 patients previously reported [3]. There were 81 women and 50 men (mean age 57 years, range 31–94 years). Cancer localizations were colorectal ($n = 88$), breast ($n = 30$), pancreatic ($n = 9$) or oesophagus ($n = 4$). Chemotherapy was either FOLFOX4 ($n = 38$), FEC100 ($n = 30$), FOLFIRI ($n = 24$), FUFOL Mayo ($n = 26$) or cisplatin/continuous FU ($n = 13$). The median interval between blood sampling and toxicity was 3 weeks. Toxicity was evaluated according to standard WHO criteria (one observation per patient). The majority of cases were seen at the first 5FU-chemotherapy cycle (106/131).

DPD analyses

Blood was collected in the morning and PBMC separation was immediately performed according to a standard protocol [4]. DPD activity was then measured using a radioenzymatic assay (sensitivity limit 10 pmol min⁻¹ mg⁻¹ protein), as previously described [4]. The *DPYD* IVS14+1G>A mutation was screened using a PCR-RFLP method [5].

Data analysis

A global toxicity score (ranging from 3 to 18, median 10) was computed by adding up all the observed toxicity grades (i.e. neutropenia + thrombopenia + mucositis + diarrhoea + neurotoxicity). The nonparametric Spear-

Figure 1

Frequency distribution of PBMC-DPD activity in 131 cases of 5FU-related toxicity (1B (■), mean 200, median 188, range 10–504 pmol min⁻¹ mg⁻¹) and in our previous population study conducted on 185 unselected cancer patients (1A (■), mean 222, median 211, range 65–559 pmol min⁻¹ mg⁻¹) [4]

man rank correlation and Mann–Whitney tests were used. For the latter, two-sided exact *P* values were calculated based on the distribution of the test statistics. Two-tailed tests were used. Statistical analysis was performed using SPSS software version 13.0 (Chicago, USA).

Results and Discussion

In this series of case-reports of patients developing severe 5FU-related toxicity, PBMC-DPD activity ranged between 10 and 504 pmol min⁻¹ mg⁻¹ (mean 200, median 188, $n = 131$). Comparison of the present DPD distribution with that of a previous population study from our group conducted on 185 unselected consecutive cancer patients [4] is shown in Figure 1. The majority of the present cases had DPD values overlapping those reported in our previous population study on cancer patients. Even though DPD activity distributions were similar, DPD activity in patients with 5FU toxicity was significantly lower than that of the general cancer population ($P = 0.02$). Thirteen percent of the present cases ($n = 17$) exhibited a marked DPD deficiency (between 50 and 100 pmol min⁻¹ mg⁻¹) compared with only 2.7% in the general cancer population. Six percent ($n = 8$) exhibited a severe DPD deficiency (below 50 pmol min⁻¹ mg⁻¹) compared with 0% in the population study.

We observed 70% grade 3–4 mucositis, 31% grade 3–4 diarrhoea, 63% grade 3–4 neutropenia, 47% grade 3–4 thrombocytopenia and 15% grade 2 central neurotoxicity. Even though these adverse effects were 5FU-specific, we cannot exclude a possible influence

Table 1

DPD activity and 5FU toxicity in this case series of 131 patients

		DPD activity (pmol min ⁻¹ mg ⁻¹)			Statistics Mann–Whitney test
		<i>n</i>	median	Q1–Q3*	
Lethal toxicity	No	122	193	134–271	<i>P</i> = 0.002
	Yes	9	61	22–169	
Central neurotoxicity	Grade 0–1	112	207	146–273	<i>P</i> < 0.001
	Grade 2	19	66	44–130	
Mucositis	Grade 0-1-2-3	82	214	155–274	<i>P</i> < 0.001
	Grade 4	49	135	64–251	
Diarrhoea	Grade 0-1-2-3	109	209	143–273	<i>P</i> < 0.001
	Grade 4	22	120	68–176	
Neutropenia	Grade 0-1-2-3	94	210	152–272	<i>P</i> < 0.001
	Grade 4	37	122	72–235	
Thrombopenia	Grade 0-1-2-3	98	207	144–274	<i>P</i> = 0.005
	Grade 4	33	131	85–239	

*Q1 and Q3 represent the first and third quartile.

of the co-administered drugs. Nine patients died from toxicity, including seven patients with DPD activity below 100 pmol min⁻¹ mg⁻¹ (10, 10, 34, 49, 61, 70, 85 pmol min⁻¹ mg⁻¹) and two with normal DPD activity (252, 277 pmol min⁻¹ mg⁻¹). Even though gender and age were not statistically related to toxicity scores, it was striking that among the nine patients who died from toxicity, eight were women (Exact test, *P* = 0.15). Correlation analysis between the global toxicity score and the DPD activity revealed a weak but significant negative correlation (*r* = −0.334, *P* < 0.001). In addition, separate analyses of each toxicity pattern showed that the lower the DPD activity, the greater the mucositis grade (*P* < 0.001), the diarrhoea grade (*P* = 0.008) and the neutropenia grade (*P* = 0.002), even though the correlation coefficients were somewhat low (<0.31). Table 1 shows that median DPD activities were 1.6–3.2-fold lower in patients developing grade 4 toxicity (or grade 2 for neurotoxicity) than in patients with grade 0-1-2-3 toxicity (or grade 0–1 for neurotoxicity).

The prevalence of the IVS14+1G>A mutation is as low as 0.9% in the Caucasian population [6, 7]. In the present study, the IVS14+1G>A mutation was screened in 93 of the 131 patients included in this series. Only two patients (i.e. 2.2%) carried the mutation (heterozygous patients). Such a low frequency in a series of patients with severe 5FU toxicity is a novel finding contrasting with data from Raida [7] and Van Kuilenburg [8] who reported 24% and 28% of patients with the IVS14+1 mutation, in selected populations of patients with 5FU toxicity. This discrepancy may be due to the different ethnic origins of the patients, even though the above

studies were conducted on Caucasians from Northern Europe [7, 8]. Similarly, the absence of the IVS14+1 mutation was reported in a previous study of 23 French DPD-deficient patients [9].

In the present case-report analysis, the two patients carrying the mutation were women with low DPD activities (44 and 142 pmol min⁻¹ mg⁻¹). Both exhibited a high global toxicity score at first 5FU cycle (16 and 18, respectively), but recovered. Among the 93 patients screened for the IVS14+1G>A mutation, four developed lethal 5FU-related toxicities but did not carry the mutation (i.e. 5/9 patients with lethal toxicity were not tested for the IVS14+1G>A mutation). The present data suggest that screening for the IVS14+1G>A mutation alone in the French population may have limited effectiveness in identifying patients at risk of lethal 5FU toxicity. Prospective analysis of the IVS14+1G>A mutation would be needed to determine the cost-effectiveness of a genetic strategy for DPD screening.

The present findings show that DPD deficiency only partially explains 5FU-related toxicity, since some patients with normal DPD activity may also develop life-threatening 5FU toxicity. In addition, the use of a single value of PBMC-DPD activity as a means of identifying patients at risk is weakened by the existence of a circadian rhythm [10]. The recent report by Ezzeldin *et al.* [11] showing that methylation of the *DPYD* promoter region is associated with down-regulation of DPD activity in patients, suggests that epigenetic mechanisms may also contribute to the regulation of DPD activity.

To minimize the occurrence of 5FU toxicity, large scale screening of DPD may be beneficial, provided that the test has good sensitivity and specificity, is suitable for routine use and has a low cost. Determination of PBMCDPD activity using current biochemical techniques, or IVS14+1G>A mutation screening, does not represent a feasible large scale approach. An alternative marker of DPD activity may be the plasma or urinary dihydrouracil : uracil ratio [12]. Mattison *et al.* [13] recently reported a ¹³C-uracil breath test for the rapid identification of DPD-deficient individuals. Further prospective studies aimed at comparing sensitivity and specificity, along with positive and negative predictive values of these different methods, are needed for the development of an effective DPD screening policy.

Competing interests: None declared.

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