Cancer Chemother Pharmacol (2010) 65:403–406
DOI 10.1007/s00280-009-1147-x

SHORT COMMUNICATION

The contribution of deleterious DPYD gene sequence variants to fluoropyrimidine toxicity in British cancer patients

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Received: 14 August 2009 / Accepted: 16 September 2009 / Published online: 1 October 2009

Abstract
Purpose The fluoropyrimidines have been extensively used for almost five decade worldwide for the treatment of solid cancers. However, severe toxicity is a major clinical problem and has been reported in association with deleterious sequence variants in dihydropyrimidine dehydrogenase (DPD) coding-gene (*DPYD*), causing DPD deficiency. Genetic DPD deficiency has previously been considered to be insignificant in the British population. The study aim was to assess the contribution of deleterious *DPYD* sequence variants to fluoropyrimidine toxicity amongst British cancer patients.
Methods Sequencing of the coding region of *DPYD* was undertaken in 47 patients (27 female, mean age 61 years), mainly with GI malignancy, experiencing grade 3 or 4 toxicity on fluoropyrimidines according to CTCAE criteria.
Results Myelotoxicity (37.5%) and diarrhoea (37.5%) were the most frequent toxicities followed by mucositis (19.6%), hand–foot syndrome (3.6%) and neurotoxicity (1.8%). 4 of 47 (8.5%) patients carried the 1905+1G>A splice site variant. All 4 cases were female and 3 of 4 suffered severe diarrhoea. A further five cases carried other sequence variants (2846A>T $n = 4$, 1679T>G $n = 1$). In total, 9 (19%) patients carried deficiency associated *DPYD* sequence variants.
Conclusions Contrary to previous estimates for a UK population, genetic DPD deficiency accounts for around 19% of cases of severe fluoropyrimidine toxicity. The influence of DPD deficiency is such that toxicity can be avoided by prior testing and appropriate 5-FU dose/regimen alteration.

Keywords Dihydropyrimidine dehydrogenase (DPD) · Dihydropyrimidine dehydrogenase gene (*DPYD*) · Fluoropyrimidines · Toxicity · 5-Fluorouracil (5-FU) · Capecitabine

Introduction

The fluoropyrimidines, 5-fluorouracil (5-FU) and its prodrug capecitabine have been extensively used for almost five decade worldwide [1].These drugs are given either as monotherapy or combination therapy for a variety of solid cancers including gastrointestinal tract and breast. However, even though the benefits of fluoropyrimidine based chemotherapy are well established, adverse drug reactions are a major clinical problem often necessitating treatment discontinuation. Patients can experience severe myelosuppression, cardiac toxicity, mucositis and hand–foot syndrome [2]. In a meta-analysis of 1,219 colorectal cancer (CRC) patients receiving 5-FU, grade 3–4 toxicity was encountered in 31–34% of patients, with 0.5% mortality [3].

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The cytotoxic effect of 5-FU depends on the generation of 5-fluoro(deoxy)nucleotides, which then produce cytotoxicity through inhibition of thymidylate synthase activity and/or incorporation into RNA and DNA as false base [4].

Dihydropyrimidine dehydrogenase (DPD), converts 80% of administered 5-FU to dihydrofluorouracil (DHFU) in the liver [5]. However, genetic polymorphisms in *DPYD* results in wide inter-individual variation in DPD activity [6]. A patient with partial or complete DPD deficiency will accumulate active metabolites and could suffer serious toxicity or even rare fatality, when exposed to fluoropyrimidines. Several studies have highlighted the role of DPD deficiency in the development of severe 5-FU related toxicity [4]. In the Northern European population, 3–5% are heterozygous with partial deficiency and 0.2% are homozygous with complete deficiency [7]. A total of 39 sequence variants in the coding region of *DPYD* have been reported [4]. Although some of the variants are polymorphisms with no obvious deleterious effect, more than 13 sequence variants, including the most common 1905+1G>A splice site variant, result in DPD deficiency [8]. To date there is little data for British patients. Indeed, Ridge and colleagues reported that genetic DPD deficiency was not a significant cause of 5-FU toxicity in the British population, with the 1905+1G>A splice site variant being a very rare event [9]. Furthermore, the respective frequencies of the different deficiency associated sequence variants and their effect on fluoropyrimidine toxicity have not been adequately investigated [10].

In this study, we investigated the incidence of deleterious *DPYD* sequence variants, in a retrospective cohort of 47 British patients who developed severe early grade 3–4 toxicity following the administration of fluoropyrimidine based chemotherapy.

Materials and methods

Participants and laboratory investigations

The study cohort consisted of 47 Caucasian British cancer patients with well documented severe fluoropyrimidine related early toxicity (at least one episode = grade ≥ 3), who had been referred to our laboratory for the determination of DPD deficiency between 2005 and 2007 (Table 1). Toxicity was recorded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 (CTCAE). In all the cases, toxicity was seen in the first four chemotherapy cycles. DPD enzyme assay on peripheral mononuclear cells was performed on 13 patients using a radiochemical method [11] (control range 4.3–9.9 nmol/mg prot/h). Normal levels of urinary uracil and thymine [12] was used to exclude complete DPD deficiency in 33 patients.

Table 1 Patient and treatment characteristics

Patients with grade 3–4 toxicity ($N = 47$)	
Demographic details	
Females *N* (%)	27 (57.5)
Mean age (years)	61
Age range (years)	32–74
Cancer type *N* (%)	
Colorectal	31 (66.0)
Breast	12 (25.5)
Oesophagus	3 (6.4)
Gastric	1 (2.1)
Chemotherapy regimens *N* (%)	
5-FU + folinic acid or leucovorin	29 (61.7)
5-FU + Oxaliplatin	6 (12.8)
5-FU + Irinotecan	1 (2.1)
Capecitabine monotherapy	10 (21.3)
Capercitabine + Oxaliplatin	1 (2.1)
Grade 3–4 toxicity according to CTCAE *N* (%)	
Mucositis	11 (19.6)
Diarrhoea	21 (37.5)
Hand–foot syndrome	2 (3.6)
Neutropenia	21 (37.5)
Neurological	1 (1.8)

The study had been approved by the local Ethics Committee and approved signed written informed consent was obtained from all study patients.

*g*DNA sequencing analysis

DNA was extracted from whole blood using a QIAamp DNA Mini Kit (Qiagen Ltd., Crawley, West Sussex, UK). Intron located primers for each *DPYD* exon were designed using the web-based tool primer3 (http://frodo.wi.mit.edu/) and synthesized by MWG Biotech, (Ebersberg, Germany). PCR products were amplified using Hot Start DNA Polymerase (Rovalab, Teltow, Germany). PCR conditions were 1 min denaturation at 94°C then 35 cycles of (30 s denaturation at 94°C, 30 s annealing at 48°C and 30 s extension at 72°C) and 1 last cycle consisting of 5 min extension at 72°C. PCR products were purified using QIAquick PCR purification kit (Qiagen Ltd., Crawley, West Sussex, UK). Dye-terminator cycle sequencing was performed using the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Warrington, UK). Excess dye terminators were removed using Agencourt CleanSeq (Beckman Coulter (UK) Limited, Biomedical Research, High Wycombe, UK). Samples were run on an ABI PRISM 3130xl Genetic Analyser (Applied Biosystems). Sequences were analysed by Mutation Surveyor Local v3.20 (Softgenetics LLC, State College, PA, USA).

Results

All 23 exons of *DPYD* were sequenced in 47 patients with severe early fluoropyrimidine related toxicity. Several patients experienced multiple side effects. Myelotoxicity (38%) and diarrhoea (38%) were the most frequent toxicities followed by mucositis (18%), hand–foot syndrome (4%) and neurotoxicity (2%).

4 of 47 (8.5%) patients carried the 1905+1G>A (*DPYD* 2A) splice site variant. All 4 cases were female and 3 of 4 suffered severe diarrhoea. 4 of 47 (8.5%) carried the 2846A > T (*DPYD* 9B) sequence variant. 1 of 47 (2%) carried the 1679T>G (*DPYD* 7) sequence variant. In total, 9 (19%) out 47 patients who developed fluoropyrimidine related early toxicity carried *DPYD* sequence variants (Table 2).

In the 33 patients where urine was available for analysis, urinary uracil and thymine levels were not elevated, excluding complete DPD deficiency. DPD activity in peripheral mononuclear cells was measured in 13 of 47 patients. Two out of seven patients with normal DPD activity carried either the 1905+1G>A or 2846 A>T *DPYD* sequence variant. Two out of six patients with reduced DPD activity carried either the 1905+1G>A or the 2846 A>T *DPYD* sequence variant.

Discussion

There is growing awareness of the pivotal role that genetic DPD deficiency plays in life-threatening toxicities from 5-FU and capecitabine [2]. According to the literature, the prevalence of *DPYD* deleterious variant heterozygous genotypes at risk of severe fluoropyrimidine toxicity is about 3–5%, with the splice site variant 1905+1G>A being considered the most common [10]. However, genetic DPD deficiency, has not been considered to be a significant cause of fluoropyrimidine toxicity in British cancer patients [9].

As toxicity remains the major limitation to adequate dosing, the ability to predict toxicity before chemotherapy and to provide individualised treatment should result directly in reduced toxicity and improved clinical response. This would not only greatly improve the quality of life of cancer patients, but will also be cost-effective by reducing hospital admissions. Fluoropyrimidine toxicity, is a major healthcare cost and cause for prolonged length of stay [13].

This is the first UK study in which all 23 exons of *DPYD* have been sequenced for mutations in a cohort of British Caucasian cancer patients with severe fluoropyrimidine related early toxicity. Three sequence variants 1905+1G>A ($n = 4$), 2846A>T ($n = 4$) and 1679T>G ($n = 1$) accounted for 19% cases of early grade 3–4 toxicity in our cohort of patients. These three *DPYD* sequence variants detected have been previously described in the literature as deleterious variants, causing DPD deficiency [10, 14].

There was a poor correlation between the presence of a deleterious sequence variant and decreased DPD enzyme activity. Both the 1905+1G>A and 2846A>T variants were detected in patients with low and normal activity. The DPD enzyme assay has a number of pitfalls which may explain the poor concordance between genotype and enzyme assay phenotype [15].

The finding from our study are in accordance with a recent study where deleterious *DPYD* variants were detected in 29% of patients with severe early toxicity [10], with 1905+1G>A and 2846A>T being predominant types. However, 30% of patients with either 1905+1G>A or the 2846A>T variant had no severe toxicity, although a significant proportion of this group had pre-treatment dose reduction. In another recent study only 8% of patients with severe toxicity possessed deleterious *DPYD* sequence variants [16]. In that study the toxicity related to 1905+1G>A variant was strongly related to male sex and likely to produce mucositis and neutropenia, whereas in our study the findings were contradictory with all four cases being female and 3 out of 4 suffering severe diarrhoea.

Finally, in 38 out of 47 patients with severe early toxicity, representing 81% of the cohort, no deficiency associated *DPYD* coding variants were detected. The majority of this unexplained toxicity may be associated with deleterious sequence variants in other enzymes such as thymidylate synthase, 5,10-methylenetetrahydrofolate reductase and dihydropyrimidinase, involved in fluoropyrimidine anabolism and catabolism [17].

Conclusion

Screening for three deleterious *DPYD* sequence variants will identify 19% of patients at risk for severe early

Table 2 Clinical characteristics of patients with *DPYD* sequence variants

DPYD sequence variant	Amino acid change	*N* (%)	Diarrhoea	Neutropenia	Mucositis	Male (M)/female(F)
1905+1G>A (Exon 14 skipping)	Exon 14 skipping	4 (8.5)	3	1	0	0 = M/4 = F
2846A>T (D949V)	D949V	4 (8.5)	2	1	1	3 = M/1 = F
1679T>G (I560S)	I560S	1 (2.0)	0	1	0	0 = M/1 = F
Total		9 (19.2)	5	3	1	3 = M/6 = F

fluoropyrimidine toxicity. Considering the morbidity and economic costs associated with grade 3–4 toxicity and the frequent use of 5-FU and capecitabine in Great Britain and worldwide, pre-treatment detection of genetic DPD deficiency should be considered. Although these patients are likely to tolerate reduced therapy, further studies are needed to determine whether efficacy is maintained. Patients may, however, tolerate and respond to treatment with an alternative thymidylate synthase inhibitor such as Raltitrexed, which is not catabolised through DPD. In addition, further studies are required to confirm the predictive value of *DPYD* mutations in the context of fluoropyrimidine toxicity, due to the contradictory nature of recent studies, but also to determine the role of genetic deficiency of other enzymes involved in fluoropyrimidine anabolism and catabolism.

Acknowledgments We acknowledge funding from Purine Metabolic Patient Association (PUMPA) and CANHELP.

Conflict of interest statement None.

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