Cancer Chemother Pharmacol (2009) 64:615–618
DOI 10.1007/s00280-009-0970-4

SHORT COMMUNICATION

Lack of large intragenic rearrangements in dihydropyrimidine dehydrogenase (*DPYD*) gene in fluoropyrimidine-treated patients with high-grade toxicity

Ivana Ticha · Petra Kleiblova · Julie Fidlerova · Jan Novotny · Petr Pohlreich · Zdenek Kleibl

Received: 4 February 2009 / Accepted: 20 February 2009 / Published online: 14 March 2009

Abstract
Purpose Deficiency of dihydropyrimidine dehydrogenase (DPD) has been associated with severe fluoropyrimidines (FP) toxicity. Mutations in DPD-coding gene (*DPYD*) were shown to increase the risk of severe toxicity in FP-treated cancer patients. However, the majority of *DPYD* alterations characterized in these patients has been considered as polymorphisms and known deleterious mutations are rare and present in only limited subgroup of patients with high toxicity. Recently, the common fragile site FRA1E was mapped within *DPYD* locus but intragenic rearrangements in *DPYD* gene were not studied so far.
Methods We performed the analysis of intragenic rearrangements of *DPYD* using multiplex ligation-dependent probe amplification in 68 patients with high-grade gastrointestinal and/or hematological toxicity developed at the beginning of FP treatment.
Results We did not detect any deletion/duplication of one or more *DPYD* exons in analyzed patients.
Conclusions We assume that rearrangements in *DPYD* gene play insignificant role in the development of serious FP-related toxicity.

Keywords Dihydropyrimidine dehydrogenase (DPD) · Dihydropyrimidine dehydrogenase gene (DPYD) · 5-Fluorouracil · Fluoropyrimidines · Toxicity · Multiplex ligation-dependent probe amplification (MLPA)

Introduction

5-Fluorouracil (5-FU) and its prodrugs (e.g. capecitabine) belong to the most often used chemotherapeutics for the systemic treatment of a broad spectrum of solid tumors. 5-FU exerts cytotoxic effects on rapidly proliferating cells because of its inhibition of thymidylate synthase and interference with RNA and DNA metabolism [1]. Over 80% of administered 5-FU is rapidly inactivated in fluoropyrimidines catabolic pathway involving dihydropyrimidine dehydrogenase (DPD), the introductory and rate limiting enzyme of fluoropyrimidine degradation. It was shown that the inhibition of DPD activity causes life-threatening toxicity and mutations in this enzyme are now accepted to predispose to development of fluoropyrimidine toxicity in their carriers [2]. During past decade over 50 alterations in the *DPYD* gene were characterized; however, majority of them represent missense or intronic variants with unknown biological importance and clinical significance [3, 4]. Only a limited number of patients carry nonsense or frameshift mutations (including the most prevalent IVS14 + 1G > A mutation) significantly affecting DPD catalytic activity [5, 6]. The *DPYD* gene spans nearly 900 kilobases (kb) region on chromosome 1p22 [7]. Recently, Hormozian et al. [8] characterized the common fragile site FRA1E mapped to the 370 kb region within the *DPYD* gene and raised the question whether presence of FRA1E could contribute to *DPYD* genomic rearrangements in patients suffering from 5-FU toxicity. During the last few years, multiplex

I. Ticha, P. Kleiblova contributed equally to this work.

I. Ticha · P. Kleiblova · J. Fidlerova · P. Pohlreich · Z. Kleibl (✉)
Institute of Biochemistry and Experimental Oncology,
1st Faculty of Medicine, Charles University in Prague,
U Nemocnice 5, 128 53 Prague 2, Czech Republic
e-mail: zdekleje@lf1.cuni.cz

J. Fidlerova · J. Novotny
Department of Oncology,
1st Faculty of Medicine and General Teaching Hospital,
Charles University in Prague, U Nemocnice 2,
128 53 Prague, Czech Republic

ligation-dependent probe amplification (MLPA) analysis has become widely used as a simple and reliable method for the detection of intragenic rearrangements [9].

In this report, we present the results of MLPA analysis of the *DPYD* gene in the set of 68 cancer patients with serious or life-threatening fluoropyrimidine-related toxicity.

Materials and methods

Patients and samples

The test group included 68 patients with serious (grade 3–4) hematological (leukopenia, neutropenia or thrombocytopenia) and/or gastrointestinal (mucositis, emesis, diarrhea) toxicity classified according to the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) scale 3.0 (Table 1). All patients were recruited between January 2002 and May 2008 from regional oncology centers in the Czech Republic under the supervision of Comprehensive Oncology Center of the General Teaching Hospital in Prague. Inclusion criteria for enrollment of patients into the study involved development of serious gastrointestinal and/or hematological toxicity during the first or the second cycle of fluoropyrimidine-based chemotherapy. Four patients, three with combined and one with isolated gastrointestinal toxicity, died of fatal complications attributable to the fluoropyrimidine-based chemotherapy. All samples analyzed in this study were previously genotyped for the presence of *DPYD* coding sequence variants and IVS14+1G > A (Table 1) [10]. None *DPYD* sequence variant was found in 13 out of 68 patients (19.1%) suffering from high-grade toxicity. Three negative controls were prepared by pooling of 20 DNA samples of anonymized non-cancer individuals. All patients and controls were Caucasians of the Czech origin. Signed written informed consent approved by the ethics committee of the General Teaching Hospital in Prague was obtained from all study participants.

Multiplex ligation-dependent probe amplification (MLPA) analysis

Genomic DNA was isolated from peripheral blood samples of patients and controls using the Wizard Genomic DNA Purification Kit (Promega). One hundred nanograms of DNA were used for MLPA analysis performed with SALSA MLPA P103 DPYD kit (MRC-Holland) under conditions specified by manufacturer [11]. Amplified PCR products were separated in the 36 cm capillary filled with POP-7 polymer on an ABI PRISM 3130 analyzer (Applied Biosystems). The collected data were analyzed using Gene Mapper 4.0 software (Applied Biosystems). The MLPA

Table 1 Basic clinical characteristics of analyzed patients treated by 5-fluorouracil (5-FU) or capecitabine-based chemotherapeutic regimes

Patients with toxicity (toxicity grade 3–4) *N* = 68	
Demographic parameters	
Females *N* (%)	36 (52.9)
Mean age (years ± SD)	60.9 ± 10.5
Age range (years)	30–75
Males, *N* (%)	32 (47.1)
Mean age (years ± SD)	63.2 ± 7.2
Age range (years)	47–73
Cancer diagnose *N* (%)	
Esophageal cancer	2 (2.9)
Gastric cancer	4 (5.9)
Colorectal cancer	48 (70.6)
Biliary cancer	2 (2.9)
Pancreatic cancer	1 (1.5)
Laryngeal cancer	1 (1.5)
Breast cancer	10 (14.7)
Chemotherapy regimens *N* (%)	
Bolus 5-FU	17 (25.0)
Continuous 5-FU	29 (42.6)
Capecitabine	7 (10.3)
FOLFIRI	1 (1.5)
FOLFOX	12 (17.7)
Other	2 (2.9)
Toxicity grade 3–4 according to NCIC CTC *N* (%)	
Overall gastrointestinal only	35 (51.5)
Overall hematological only	9 (13.2)
Overall gastrointestinal and hematological	24 (35.3)
Mucositis	23 (33.4)
Emesis	13 (19.1)
Diarrhea	37 (54.4)
Leucopenia	10 (14.7)
Neutropenia	32 (47.1)
Thrombocytopenia	12 (17.7)
DPYD alterations[a]	
c.85T > C (C29R)	24 (35.3)
c.496A > G (M166 V)	19 (27.9)
c.775 A > G (K259E)	1 (1.5)
c.1050 G > A (R357H)	1 (1.5)
c.1236 G > A (E412E)	2 (2.9)
c.1601G > A (S534 N)	4 (5.9)
c.1627A > G (I543 V)	18 (26.5)
c.1896T > C (F632F)	3 (4.4)
IVS14 + 1G > A (e14 del)	5 (7.4)
c.2194G > A (V732I)	10 (14.7)

[a] Results of mutation analysis of *DPYD* gene performed in all patients analyzed in this study were published previously [10]

chromatograms were evaluated by both visual examination of peak profiles and quantitative comparison of peak areas. Visual examination of peak profiles with representative control samples was performed by three independent researchers in a blinded manner. Samples with discordant results (i.e. result reported as "uncertain" at least by one reviewer) were reanalyzed by the same procedure. The quantitative comparison of peak areas was performed from data exported from Gene Mapper to spread sheet calculator. The individual peak area was normalized to sum of that in particular sample MLPA analysis. As a significant for gain or loss of genetic material in heterozygotes, a 35–50% decrease or increase of relative peak area of the amplification product in comparison with averaged normalized signals of control samples was considered.

Results

To evaluate the prevalence of *DPYD* genomic rearrangements in fluoropyrimidine-treated patients, we screened a series of 68 unrelated cancer patients by MLPA analysis. We did not find a deletion/duplication of one or more *DPYD* exons in any of these high-grade toxicity patients. Particularly, no differences were found in signal intensities of 15 probes covering the whole FRA1E site (exon 9–18) and seven probes located in maximum breakage region (exons 13–16). The MLPA analysis clearly recognized all five previously characterized heterozygotes carrying the c.1905 + 1G > A (IVS14 + 1G > A) mutation resulting in exon 14 skipping (Fig. 1). We did not notice any influence of previously characterized *DPYD* coding sequence alterations in these patients on results of MLPA analysis (Table 1).

Discussion

Similar to other cytotoxic drugs, fluoropyrimidines have a narrow therapeutic index combined with high interpatient pharmacokinetic variability resulting in severe toxicity development that negatively affects the therapeutic outcome [12]. Pharmacological interconversion of fluoropyrimidines is dependent on DPD activity introducing their catabolism. Genetic variants of the *DPYD* gene resulting in the synthesis of protein with reduced DPD activity thereby influence 5-FU pharmacokinetics and toxicity profile [13]. Numerous studies proved that DPD deficiency leads to severe toxicity with 5-FU and capecitabine exposure. Identification of patients at risk of serious and life-threatening toxicity development become an important challenge for therapy individualization in clinical oncology. Different laboratory approaches used for prediction of fluoropyrimidine-related toxicity based on *DPYD* genotyping, quantification of its expression, measurement of DPD enzymatic activity or determination of overall (fluoro)pyrimidine catabolic pathway capacity were introduced and have been applied in clinical settings [14, 15].

Previously reported mutation analyses performed in individual cases and small groups of patients considered that IVS14 + 1G > A (*DPYD**2A allele) has been the most significant *DPYD* mutation [16, 17]; however, recent larger studies have shown that early reports overestimated frequency of IVS14 + 1G > A and that this alteration is responsible for about 6% of high-grade toxicity cases [5, 18]. Contradictory results were published about the significance of frequent missense variants in association with fluoropyrimidine-related toxicity [3, 19]. The presence of large intragenic alterations in *DPYD* gene was not analyzed

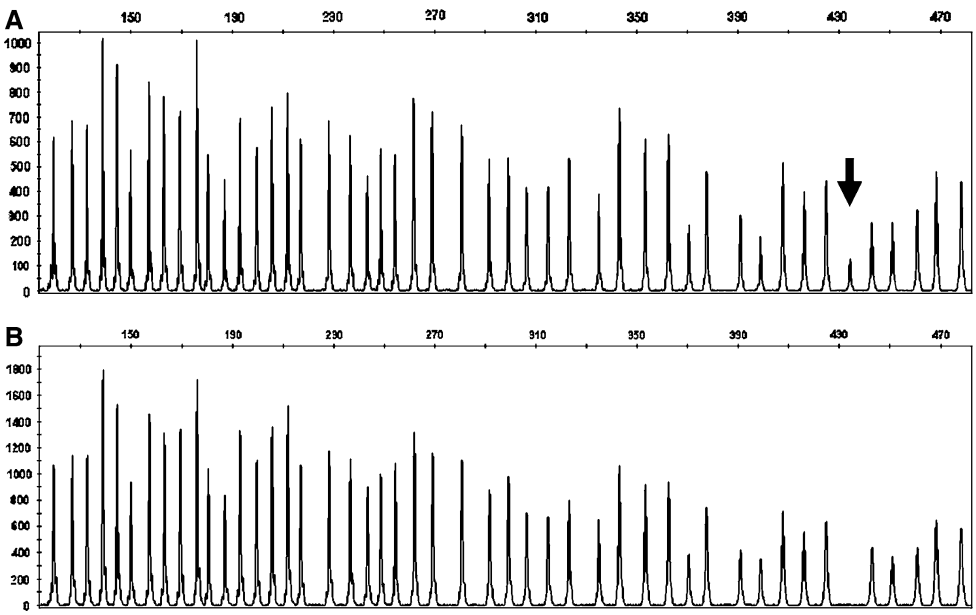

Fig. 1 Typical results of *DPYD* MLPA analysis. The MLPA probes cover all of the 23 *DPYD* exons, 12 exons were covered by two probes and three probes were present for exon 1. Single probe served for recognition of the IVS14 + 1G > A mutation (**a** peak signal in heterozygotic carrier marked by *black arrow*) absent in sample without mutation (**b**)

so far, moreover, recent characterization of common fragile site FRA1E mapped to the 370 kb region of the *DPYD* gene between introns 8 and 18 with the region of maximum breakage spanning 185 kb of genomic sequence between introns 12 and 16 had further emerged its possibility [8]. Lack of rearrangements in *DPYD* gene tested from DNA samples isolated from peripheral blood in our set of patients suffering from high fluoropyrimidine-related toxicity indicates that these inherited intragenic changes unlikely contribute to this pharmacogenomic syndrome. On the other hand, presence of FRA1E within *DPYD* sequence may raise the question of whether increased frequency of the *DPYD* gene disruption could be found in cancer cells acquiring impaired DNA repair capacity? As low DPD level is believed to be advantageous for the tumor response, patients with somatic *DPYD* intragenic rearrangements in tumor tissue could benefit from fluoropyrimidine-based therapy in terms of its efficacy [20].

Conclusion

Steadily increasing number of patients with cancer worldwide including those treated by fluoropyrimidine-based chemotherapy call for methods enabling reliable individual prediction of treatment-related toxicity. Numerous methods have been used for this prediction including combinatorial strategies implementing both genotypic and phenotypic approaches. Our findings show that large *DPYD* intragenic rearrangements were absent in 68 high toxicity patients who represent the subpopulation with a highest probability of their occurrence. This negative result indicates that the large genomic rearrangements in *DPYD* gene play insignificant role in the development of serious toxicity. Although the result of our study need to be further validated in other populations, we assume that the contribution of intragenic *DPYD* rearrangements to fluoropyrimidines toxicity development is infrequent and, therefore, the MLPA analysis of *DPYD* gene has little clinical relevance and could be omitted in *DPYD* genotyping.

Acknowledgments This study was supported by the grant of Internal Grant Agency of the Ministry of Health of the Czech Republic No. 1A8708-4/2005 and the Research Project of The Ministry of Education, Youth and Sports of the Czech Republic No. MSM0021620808.

References

1. van Kuilenburg AB (2004) Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. Eur J Cancer 40:939–950
2. Diasio RB (1998) Sorivudine and 5-fluorouracil; a clinically significant drug-drug interaction due to inhibition of dihydropyrimidine dehydrogenase. Br J Clin Pharmacol 46:1–4
3. Collie-Duguid ES, Etienne MC, Milano G et al (2000) Known variant DPYD alleles do not explain DPD deficiency in cancer patients. Pharmacogenetics 10:217–223
4. Maekawa K, Saeki M, Saito Y et al (2007) Genetic variations and haplotype structures of the DPYD gene encoding dihydropyrimidine dehydrogenase in Japanese and their ethnic differences. J Hum Genet 52:804–819
5. Schwab M, Zanger UM, Marx C et al (2008) Role of genetic and nongenetic factors for fluorouracil treatment-related severe toxicity: a prospective clinical trial by the German 5-FU toxicity study group. J Clin Oncol 26:2131–2138
6. Bosch TM, Bakker R, Schellens JH et al (2007) Rapid detection of the DPYD IVS14 + 1G > A mutation for screening patients to prevent fluorouracil-related toxicity. Mol Diagn Ther 11:105–108
7. Johnson MR, Wang K, Tillmanns S et al (1997) Structural organization of the human dihydropyrimidine dehydrogenase gene. Cancer Res 57:1660–1663
8. Hormozian F, Schmitt JG, Sagulenko E et al (2007) FRA1E common fragile site breaks map within a 370kilobase pair region and disrupt the dihydropyrimidine dehydrogenase gene (DPYD). Cancer Lett 246:82–91
9. den Dunnen JT, White SJ (2006) MLPA and MAPH: sensitive detection of deletions and duplications. In: Ujhazy V (ed) Current protocols in human genetics, Wiley, New York, pp 7.14.1–7.14.20
10. Kleibl Z, Fidlerova J, Kleiblova P et al (2009) Influence of dihydropyrimidine dehydrogenase gene (DPYD) coding sequence variants on the development of fluoropyrimidine-related toxicity in patients with high-grade toxicity and patients with excellent tolerance of fluoropyrimidine-based chemotherapy. Neoplasma 56 (accepted)
11. MRC-Holland. http://www.mlpa.com/, SALSA MLPA kit P103 DPYD
12. Ploylearmsaeng SA, Fuhr U, Jetter A (2006) How may anticancer chemotherapy with fluorouracil be individualised? Clin Pharmacokinet 45:567–592
13. Maring JG, van Kuilenburg AB, Haasjes J et al (2002) Reduced 5-FU clearance in a patient with low DPD activity due to heterozygosity for a mutant allele of the DPYD gene. Br J Cancer 86:1028–1033
14. Ezzeldin HH, Diasio RB (2008) Predicting fluorouracil toxicity: can we finally do it? J Clin Oncol 26:2080–2082
15. Mercier C, Ciccolini J (2006) Profiling dihydropyrimidine dehydrogenase deficiency in patients with cancer undergoing 5-fluorouracil/capecitabine therapy. Clin Colorectal Cancer 6:288–296
16. Saif MW, Ezzeldin H, Vance K et al (2007) DPYD*2A mutation: the most common mutation associated with DPD deficiency. Cancer Chemother Pharmacol 60:503–507
17. van Kuilenburg AB, Meinsma R, Zoetekouw L et al (2002) High prevalence of the IVS14 + 1G > A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. Pharmacogenetics 12:555–558
18. Morel A, Boisdron-Celle M, Fey L et al (2006) Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. Mol Cancer Ther 5:2895–2904
19. Gross E, Busse B, Riemenschneider M et al (2008) Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. PLoS ONE 3:e4003
20. Imyanitov EN, Moiseyenko VM (2007) Molecular-based choice of cancer therapy: realities and expectations. Clin Chim Acta 379:1–13