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Original Research Article

The use of 5-FU pharmacogenomics from paraffin blocks in patients with breast cancer receiving fluorouracil therapy

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Abstract

The pyrimidine analog 5-fuorouracil (5-FU) is a cytostatic drug that is widely used for the treatment of many solid carcinomas including breast cancer. More than 80 – 85% of administered 5-FU is quickly metabolized in the liver through a series of metabolic steps involving an enzyme dihydropirimidine dehydrogenase (DPD). Patients with low DPD activity (approximately 2-4% of population) cannot effectively inactivate 5-FU which leads to high and sometimes even lethal toxicity. DPD is encoded by the DPYD gene. The mutant allele DPYD*2a is caused by a congenital mutation in the splicing sequence of intron 14 (IVS14+1G>A) of the DPYD gene. Patients with the IVS14+1G>A mutation produce a non-functional enzyme DPD and, when treated with 5-FU, toxic metabolites are accumulated in their bodies and it may lead to severe toxic reactions. Detection of the IVS14+1G>A mutation should be considered the important factor of toxicity prediction prior to the beginnig of 5-FU therapy. In our study the IVS14+1G>A mutation was detected using the certified kit PGX-5FU Strip Assay (ViennaLab Diagnostics), which combines in vitro PCR and reversible hybridization. DNA was isolated from paraffin blocks, not from blood, which accelerated the whole process of diagnostic analysis. 40 female patients with histologically verified breast cancer (Grade I.-III.), who were treated in the Department of Oncology, (First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague), participated in this pilot study. DPYD genotyping was performed in all patients. One patient was a carrier of the IVS14+1G>A mutation in a heterozygous state and no patient was a carrier of homozygous mutation. On the basis of molecular analysis and symptom-atology the heterozygous patient received only a minimum effective dose of 5-FU.

Keywords: 5-Fluorouracil, Breast carcinoma, DPYD, FFPET, Pharmacogenomics, Toxicity

INTRODUCTION

The pyrimidine analog 5-fluorouracil (5-FU) is widely used for chemotherapy of many solid tumors including breast carcinomas. The mechanism of its effect on target cells lies in inhibition of DNA synthesis and in interruption of RNA synthesis and translation due to the incorporation of 5-fluorouridine triphosphate. More than 80 – 85% of

administered 5-FU is rapidly detoxified in the liver through a multistep metabolic pathway involving dihydropirimidine dehydrogenase (DPD) as an initial and rate-limiting enzyme converting 5-FU into 5.6-dihydrofluorouracil (5.6-DHFU) that is subsequently degraded by dihydro-pyrimidinase to fluoro-beta-ureidopropionate and is finally excreted in the form of fluoro-beta-alanine (Lévy et al., 1998; Matsusaka and Lenz, 2015). Remaining 15 – 20% of 5-FU is transported to a tumor. Possible side effects of 5-FU therapy are regarded as a serious clinical problem in the treatment of solid tumors. 5-FU toxicity is mainly caused by malfunctions of enzymes participating in the catabolic pathways of 5-FU metabolism.

Patients with low DPD activity (2 – 4% of population) cannot effectively inactivate 5-FU and may develop severe or even lethal toxicity. Almost 1% of those patients dies of 5-FU toxicity. The effective function of DPD enzyme is the key factor in 5-FU metabolism. DPD enzyme is encoded by the DPYD gene. This gene is located in the 1q22 area occupying the fragment of 950 kpb. The entire coding sequence is spread out over the whole gene in 23 exons. More than 40 existing gene alterations have been described. Seven alterations (M166V, M182K, V335L, I560S, A777S, D949V and IVS14+1G>A) are believed to be the cause of toxicity related to 5-FU therapy (Omura, 2003; Saif et al, 2007). The IVS 14+1G>A mutation is considered to be the most important and concurrently the most common one. This mutation is associated with the mutant alele DPYD*2A which is caused by the congenital mutation in the splicing sequence of intron 14 (IVS14+1G>A) of the GPYD gene. As a consequence the splice location is incorectly recognized and it results in 165 bp deletion in exon 14.

Patients with the mutant allele DPYD*2A produce a non-functional enzyme DPD. Then, during 5-FU therapy, toxic metabolites are accumulated in their bodies which can lead to severe or even lethal toxic reactions. Determination of DPD activity and the IVS14+1G>A mutation detection would be the important toxicity predictors prior to the beginning of 5-FU therapy. Heterozygous patients should be given only a minimal effective dose of 5-FU, homozygous patients (where there is a risk of severe complications) should receive another medical treatment (Ursula et al., 2011; VanKuilenburg, 2004; VanKuilenburg et al., 2002). Preventively, it is possible to recommend genetic examination of the DPYD*2A allele in blood relatives of positively diagnosed patients. With regard to the fact that the DPYD*2A allele is not so commonly detected it is possible to assume the occurence of other mutations in the DPYD gene, including mutations of other genes influencing 5-FU metabolism (e.g.*TYMS, MTHFR*). For the IVS14+1G>A mutation detection we can use the whole range of methods, for ex-ample the certified kit PGX-5FU StripAssay (ViennaLab Diagnostics) which combines *in vitro* PCR and reversible hybridization.

MATERIALS AND METHODS

Patients

40 female patients with histologically verified breast cancer treated in the Department of Oncology (First Faculty of Medicine, CharlesUniversity in Prague and GeneralUniversityHospital in Prague) participated in out pilot study. 30 patients were with a median age of 49.4 years (14 postmenopausal [46.7%] and 16 premenopausal [53.3%]) with detected HER-2 FISH amplification (histologically 18 patients with HER-2 subtype which means without hormone receptor positivity and 12 patients with luminal B subtype and thus hormone receptor positive). All patients were without evidence of distal metastases (6 in stage I, 13 in stage II and 11 in stage III). A control group was formed of 10 patients with a median age of 49.2 years (4 postmenopausal [40.0%] and 6 premenopausal [60.0%]) and without HER-2 amplification (histologically 7 patients with luminal A subtype, 2 patients with luminal B subtype and 1 patient with basal-like subtype). All patients of the control group were without evidence of distal metastases (2 in stage I, 6 in stage II and 2 in stage III). All patients signed the written informed consent.

Processing of the tissue

All specimens were fixed in 10% formalin and embedded in paraffin.

DNA isolation

Deparaffinized sections of formalin-fixed, paraffin-embedded tissue and isolation of DNA were performed using standard procedures. First, the sections were deparaffinized in xylene and the DNA was then extracted using a QIAamp DNA mini kit (Qiagen, Hamburg, Germany).

Concentration of DNA

Concentration was measured in a spectrophotometer NanoDrop 2000; ThermoScientific and tune measured in duplicates.

Analysis of DPYD genes

Genomic DNA of concentration 10 ng/µl was amplified

Table 1. The results indicate the analysis of hormone receptors: estrogen receptors (ER), progesterone receptors (PR), proliferation index (Ki 67), the status of the Her2 / neugene(0 negativ/1 positiv) and DPYD gene.

Sample	Grade	ER	PR	Ki67	HER2/neu	DPYD
1	3	90	90	40	0	wt
2	2	80	80	8	0	wt
3	3	0	0	40	0	wt
4	3	75	0	10	0	wt
5	1	65	90	8	0	wt
6	3	40	0	5	0	wt
7	1	70	90	10	0	wt
8	2	100	100	25	0	wt
9	2	30	0	40	0	invalid
10	2	100	90	4	0	wt
11	3	30	0	40	1	wt
12	2	80	10	40	1	wt
13	2	0	95	20	1	wt
14	3	0	0	50	1	wt
15	2	0	0	25	1	wt
16	3	0	0	40	1	wt
17	3	0	0	20	1	wt
18	3	0	0	40	1	wt
19	2	30	30	5	1	wt
20	3	90	65	15	1	wt
21	3	60	NA	NA	1	wt
22	2	0	0	5	1	wt
23	2	0	0	40	1	heterozigot
24	3	0	0	30	1	wt
25	3	30	0	50	1	wt
26	1	90	90	40	1	wt
27	3	0	0	NA	1	wt
28	2	0	0	70	1	wt
29	3	20	0	20	1	wt
30	3	0	0	60	1	invalid
31	2	0	75	25	1	wt
32	2	0	0	35	1	wt
33	2	0	0	NA	1	wt
34	2	60	0	35	1	wt
35	2	0	0	40	1	wt
36	3	15	15	40	1	invalid
37	3	0	0	30	1	wt
38	2	80	5	20	1	wt
39	3	0	0	NA	1	wt
40	2	0	0	55	1	wt

and hybridized using strips kit PGF-5-FU StripAssay Kit CE IVD (Pentagen, VienaLab) and robotic hybridisation Bee20 (BeeRobotics). Results were evaluated with Software (VienaLab).

RESULTS

DPYD genotyping was perfomed. One patient (2.5%) was a carrier of the IVS14+1G>A mutation in a heterozygous state and no patient was a carrier of this mutation in a homozygous state. 36 patients (90%) showed normal DPYD function (WT). It was not possible to carry out genetic analysis of the DPYD gene in 3 patients (7.5%). (table 1)

DISCUSSION

StripAssay method helped us to detect the DPYD*2A allele in a heterozygous state in one out of 40 patients (2.5%), and none in a homozygous state. Detected DPYD*2A allele frequency of 1.25% does not correspond to European population data as it is 1.37x higher compared to the stated frequency of 0.91%. However, our result cannot be regarded as a significant one since the only small group of patients was examined. We showed that the analysis can be modified for DNA

isolated from paraffin blocks instead of blood, which can make the whole diagnostic process easier and quicker prior to the choice of the most appropriate therapy. Due to the modification from paraffin blocks it is possible to carry out histological verification, to determine the proliferation index and the state of hormone receptors including the Her2/neu gene amplification and other tests. Possible disadvantage of this certified method is the fact that it is restricted only to the IVS14+1G>A mutation detection. The IVS14+1G>A mutation is only one out of seven mutations responsible for 5-FU toxicity, nevertheless, it accounts for 99% of the DPYD gene mutations. Our captured heterozygous variant in a patient after the relapse in supraclavicular lymph nodes in August 2008 was treated with a combination of oral navelbine, capecitabine and due to HER-2 receptors positivity also with Herceptin. During the therapy diarrhea G1-2appeared, but it is necessary to mention that the patient received a slightly reduced capecitabine dose (75% dose). In January 2009, complete remission was achieved on CT and the patient continued with Herceptin maintenance therapy. Progression occurred in January 2010 when it was deployed trastuzumab chemotherapy with paclitaxel, however, at the first restaging progression in bone and lymph nodes was demonstrated. He was again deployed capecitabine (again in 75% reduction, for IVS14 + 1G> A mutation in a heterozygous state) in combination with lapatinib. Treatment was complicated by the occurrence of hand foot syndrome and diarrhea G2 G1-2 and capecitabine had to be reduced again. The patient was treated in this way until August 2012, when it was discontinued due to progression.

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CONCLUSION

Determination of a mutational state of the DPYD gene using the StripAssay method is really uncomplicated.DNA isolation from paraffin blocks, from which it is practicable to carry out histological verification, determine the state of receptors and amplification of the Her2/neu gene, makes the whole diagnostic process faster. Our results do not allow us to draw a conclusion how to select patients suitable for the 5-FU therapy. Nevertheless, in our small patient group we managed to detect one patient with the IVS14+1G>A mutation and thus to prevent 5-FU toxicity.

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