Personalised medicine of fluoropyrimidines using DPYD pharmacogenetics

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CHAPTER 1

General introduction

Fluoropyrimidines

5-Fluorouracil (5-FU) and capecitabine belong to the group of fluoropyrimidines, which represent the backbone of anti-cancer treatment for various types of cancer, such as colorectal, breast and gastric cancer. Fluoropyrimidines are used by millions of patients worldwide each year[1-3] and are often combined with other chemotherapeutic drugs (e.g. irinotecan or oxaliplatin), immunotherapeutic drugs or act as a radio-sensitizer in chemo-radiotherapy.[4,5]

5-FU was developed by Heidelberger *et al.* in the 1950's.[6] The anti-cancer effect of 5-FU is caused by three active metabolites, as shown in Figure 1. The first is 5-fluoro-2'-deoxyuridine-5'-monophosphate (5-FdUMP), which inhibits the enzyme thymidylate synthase (TS). The inhibition of TS leads to a reduced production of deoxythymidine monophosphate (dTMP), resulting in the inhibition of DNA synthesis and repair. Two other metabolites, fluorouridine triphosphate (FUTP) and fluorodeoxyuridine triphosphate (FdUTP), are incorporated into RNA and DNA, respectively. This results in RNA and DNA damage and ultimately cell death.[7]

In February 2001, European approval and market authorization for Xeloda® (capecitabine) was given, the first oral pro-drug of 5-FU used in the treatment of metastatic colorectal cancer. Besides the advantage of oral administration, capecitabine is also a tumour-specific therapy for colorectal and breast cancer. Thymidine phosphorylase (TP), the third enzyme converting capecitabine into 5-FU, was found to be more expressed in breast and colorectal tumour cells compared to normal tissue. This leads to higher 5-FU levels in tumour cells compared to plasma, and thus a higher anti-cancer effect of capecitabine with less toxicity.[8-10]

5-FU has a relatively narrow therapeutic index and, depending on the type of treatment regimen, up to 30% of patients suffer from severe toxicity such as diarrhoea, nausea, (oral) mucositis, myelosuppression and hand-foot syndrome (HFS). These side-effects can lead to mortality in approximately 1% of patients.[11,12] Toxicity is classified using the common terminology criteria for adverse events (CTC-AE) and grades 3 and higher are considered severe toxicity (range 0–5).

Dihydropyrimidine dehydrogenase

The enzyme dihydropyrimidine dehydrogenase (DPD) plays a key role in the metabolism of 5-FU. It is the rate limiting enzyme degrading over 80% of the drug into the inactive metabolite 5-fluoro-5,6-dihydrouracil (DHFU). Because of this, DPD plays an important role in the development of toxicity.[13-16] DPD is mainly expressed in the liver, but also in other tissues.[17,18] DPD shows great interpatient and intrapatient variability, is influenced by circadian rhythm[19,20] and possibly gender.[21-24] Some patients are partially DPD deficient (incidence 3–8%) or completely DPD deficient (incidence 0.2%).[23-25] DPD deficient patients have higher levels of active 5-FU metabolites and therefore an increased risk to develop severe or even fatal fluoropyrimidine-induced toxicity.[26-28] In addition, the onset of toxicity occurs faster in DPD deficient patients compared to patients with a normal DPD enzyme activity.[28] Up to 60% of the patients who experienced severe fluoropyrimidine-induced toxicity were DPD deficient.[21,22,27,28]

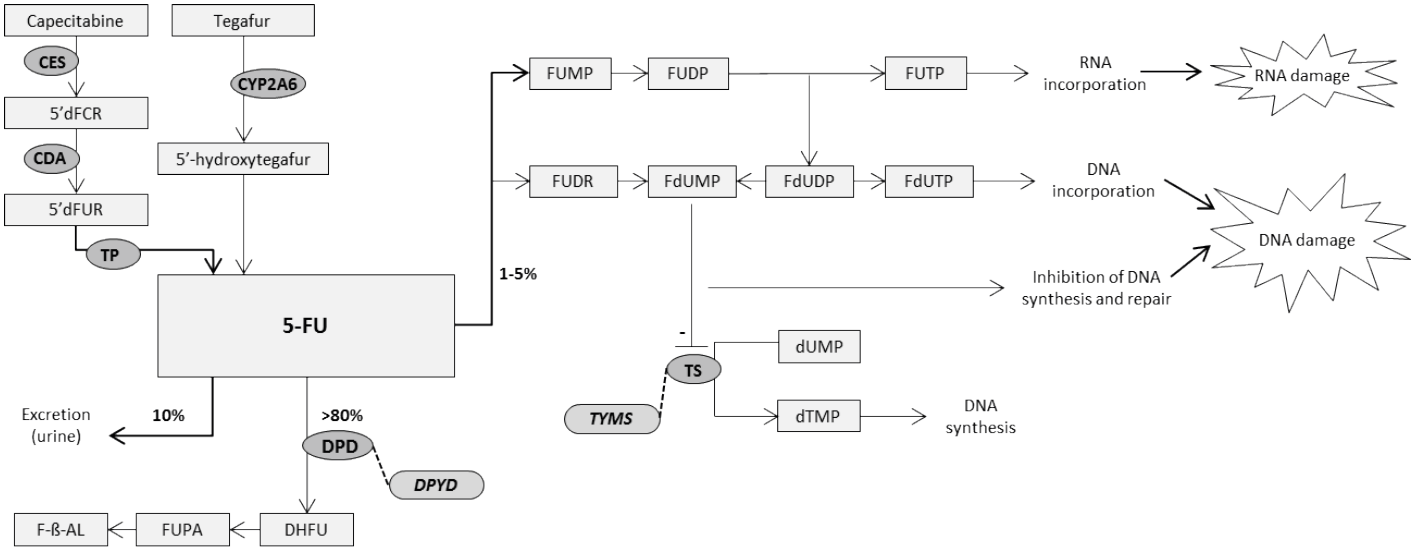

Figure 1. Metabolic pathway of fluoropyrimidines

Abbreviations: CES: carboxylesterase; 5'dFCR: 5'-deoxy-5-fluorocytidine; CDA: cytidine deaminase; 5'dFUR: 5'-deoxy-5-fluorouridine; TP: thymidine phosphorylase; 5-FU: 5-fluorouracil; FUMP: fluorouridine monophosphate; FUDP: fluorouridine diphosphate; FUTP: fluorouridine triphosphate; RNA: ribonucleic acid; FUDR: fluorodeoxyuridine; FdUMP: fluorodeoxyuridine monophosphate; FdUDP: fluorodeoxyuridine diphosphate; FdUTP: fluorodeoxyuridine triphosphate; DNA: deoxyribonucleic acid; TS: thymidylate synthase; *TYMS*: gene encoding TS; dUMP: deoxyuridine monophosphate; dTMP: deoxythymidine monophosphate; DPD: dihydropyrimidine dehydrogenase; *DPYD*: gene encoding DPD; DHFU: 5,6-dihydrofluorouracil; FUPA: fluoro-ß-ureidopropionate; F-ß-AL: fluoro-ß-alanine.

Personalised medicine

In order to prevent severe fluoropyrimidine-induced toxicity, interpatient differences must be overcome and treatments must be individualized (personalised medicine). As DPD is an important factor for the onset of severe fluoropyrimidine-induced toxicity, DPD deficient patients are an interesting target for personalised medicine. Yet, DPD deficient patients generally do not show specific phenotypic features and must be identified otherwise. One way to use personalised medicine, is through pharmacogenetics or pharmacogenomics (PGx). In PGx, the influence of human genetic variation in drug metabolic pathways or molecular drug targets on drug therapy response (both efficacy as toxicity) is studied.

DPD is encoded by the *DPYD* gene, which consists of 26 exons and is located on chromosome 1p21.3.[29,30] Over 1,000 variants or single nucleotide polymorphisms (SNPs) are known in *DPYD*, some leading to altered DPD enzyme activity.[31-33] A well-known example is the variant *DPYD**2A, which is located at the intron downstream of exon 14. This point mutation at a splice donor site leads to skipping of exon 14 and results in a catalytically inactive enzyme.[34]

Heterozygous carriers of *DPYD**2A are partially DPD deficient. Of four variants (*DPYD**2A, rs3918290, c.1905+1G>A, IVS14+1G>A; *DPYD**13, rs55886062, c.1679T>G, I560S; c.2846A>T, rs67376798, D949V; c.1236G>A/HapB3, rs56038477, E412E) sufficient evidence has been provided showing the association with severe fluoropyrimidine-induced toxicity.[13,35-41] Other *DPYD* variants have been described, however evidence on the association with toxicity is limited or missing.

Previously, Deenen *et al.* have shown that prospective genotyping of *DPYD**2A, followed by initial dose reductions in heterozygous carriers, resulted in a reduction of severe fluoropyrimidine-induced toxicity in these patients.[42] In this study, 28% of the *DPYD**2A variant allele carriers treated with reduced dosages experienced severe fluoropyrimidine-induced toxicity compared to 73% of *DPYD**2A variant allele carriers treated with regular dosages in a historic cohort. The risk of toxicity for *DPYD**2A variant allele carriers was reduced to the wild-type level of 23%. Efficacy of the treatment was not expected to be reduced, as exposure to active metabolites of 5-FU were similar in *DPYD**2A variant allele carriers treated with a reduced dose and wild-types. In addition, the study showed that prospective screening was feasible and did not increase costs.

Over time, genotyping in general has become very attractive for routine diagnostics, with decreasing costs of the assays and better interpretation of the data. Yet, implementation of prospective *DPYD* genotyping remained limited for a substantial period, as evidence of its effectivity from a randomized clinical trial (RCT) was lacking.

Aim and outline of this thesis

The general aim of this thesis is to study how to further reduce severe fluoropyrimidine-induced toxicity, in addition to genotyping of *DPYD**2A, while keeping aspects of implementation of any method in clinical practice in mind.

The first part of the thesis is entitled "*DPYD* genotyping: proof of principle and implementation in clinical practice". In chapter 2 we present a review, in which we summarize the evidence on the association with severe fluoropyrimidine-induced toxicity for four *DPYD*

variants. In addition, we discuss the advantages and disadvantages of *DPYD* genotyping.[43] In chapter 3, literature is extensively checked to discuss the effect of four *DPYD* variants on DPD enzyme activity. This is converted into a gene activity score for each *DPYD* variant, which will be used in PGx guidelines to translate the *DPYD* genotype into a DPD phenotype.[44] Chapter 4 contains the Dutch Pharmacogenetics Working Group (DPWG) PGx guideline for *DPYD* and fluoropyrimidines. The guideline provides a dose reduction advice for heterozygous *DPYD* variant allele carriers of *DPYD**2A, *DPYD**13, c.2846A>T and c.1236G>A. In addition, a statement is made that *DPYD* genotyping should be performed for all patients prior to treatment with fluoropyrimidines, as the clinical implication score for *DPYD* is essential. Then, in chapter 5, *DPYD* genotyping is applied prospectively in a nationwide clinical trial.[45] Patients with an intention to treatment with fluoropyrimidines are genotyped for *DPYD**2A, *DPYD**13, c.2846A>T and c.1236G>A. Heterozygous carriers are treated with an initially reduced dose of fluoropyrimidines according to the DPWG PGx guidelines at the start of the study. The goal of the study is to show that *DPYD* genotyping improves patient safety. In chapter 6 we show a cost analysis of prospective *DPYD* genotyping of four *DPYD* variants.[46] In chapter 7, we look into severe toxicity in patients who receive fluoropyrimidines as part of chemoradiation therapy.[47] Fluoropyrimidine dosages in chemoradiation therapy are substantially lower compared to fluoropyrimidine dosages in other treatment regimens. Current PGx guidelines do not distinguish fluoropyrimidine dosing recommendations between treatment regimens. Therefore, in this chapter we compare severe toxicity between wild-type patients and *DPYD* variant allele carriers, either treated with standard or reduced fluoropyrimidine dosages, who receive chemoradiation therapy. In chapter 8, the first 21 months of implementation of *DPYD* genotyping at Leiden University Medical Center is evaluated, to study the feasibility of *DPYD* genotyping in daily clinical care.[48] Clinical acceptance of *DPYD* genotyping as well as adherence to the genotyping results are the main objectives of this study. In chapter 9 we look into the aspect of quality control of genotyping in the laboratory, in specific confirmation practice.[49] We use *DPYD* genotyping as an example. We discuss if it should be required to have two independent genotyping assays to correctly determine a genotype. Implementation of *DPYD* genotyping in clinical practice can improve if there is consensus on laboratory requirements.

In the first part of this thesis we describe how to reduce severe fluoropyrimidine-induced toxicity by *DPYD* genotyping of *DPYD**2A, *DPYD**13, c.2846A>T and c.1236G>A. Yet, is it known that not all severe fluoropyrimidine-induced toxicity can be predicted using *DPYD* genotyping of these four variants. Therefore, we investigate other options, beyond genotyping of the current four *DPYD* variants, to reduce severe fluoropyrimidine-induced toxicity. This is shown in the second part of this thesis, entitled “beyond current *DPYD* pharmacogenetics”.

In chapter 10 we investigate four DPD phenotyping assays. The goal of the study is to determine the clinical value of each DPD phenotyping assay, by assessing clinical validity parameters (e.g. sensitivity and specificity) for DPD deficiency and the onset of severe fluoropyrimidine-induced toxicity. In the following chapters, we focus on future application of genetics. In chapter 11 we investigate a special group of *DPYD* variant allele carriers, i.e.

the compound heterozygous patients.[50] These patients carry multiple *DPYD* variants and the effect of the *DPYD* variants on the DPD enzyme activity cannot be predicted using the gene activity score. We determine the prevalence of these patients using several publicly available databases. In addition, we describe a few patient cases and apply additional genotyping assays to determine the location of the *DPYD* variants on the alleles (phasing), in order to determine a gene activity score and predict the DPD phenotype. In chapter 12 we describe a genome-wide association study. It is expected that other enzymes besides DPD, and thus other genes besides *DPYD,* are involved in the onset of severe fluoropyrimidine-induced toxicity. With the genome-wide approach we aim to discover other variants, outside the *DPYD* gene, which are associated to the onset of severe fluoropyrimidine-induced toxicity.

This thesis ends with a general discussion, including future perspectives (chapter 13), followed by an English and Dutch summary (chapter 14).

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