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Chapter 8

General discussion and future perspectives
Introduction

Since its approval in the 1960s, the fluoropyrimidine 5 fluorouracil (5-FU) has been extensively used, either as single agent or in combination with other drugs or radiotherapy in the treatment of many types of cancer such as breast, anal, vulvar, head and neck, and gastrointestinal cancer. In the last decades, 5-FU is increasingly used in an oral formulation, as the pro-drug capecitabine. The use of fluoropyrimidines often results, like other cytotoxic chemotherapeutic drugs, in undesired toxicity. The incorporation of the 5-FU metabolite fluorouridine triphosphate (FUTP) into RNA and inhibition of thymidylate synthase by FdUMP, underlying the antineoplastic effect in tumour cells, also interfere with the metabolism of normal, rapidly proliferating cells like the gastrointestinal mucosa, bone marrow, hair follicles and nail beds leading to leukopenia, alopecia, mucositis and stomatitis [1]. The first sign of 5-FU systemic toxicity is often stomatitis, varying from mild erythema to haemorrhagic ulceration of the oral cavity. Frequently, stomatitis is accompanied by diarrhoea. The effect of 5-FU on the brain stem can cause nausea and vomiting during administration and its effect on the skin can cause hyperpigmentation and hand-foot syndrome or when used in combination with radiotherapy skin inflammation and ulceration.

Besides these expected therapy related toxic effects, in a small proportion of fluoropyrimidine treated patients, extreme toxicity is observed. It has become clear that a large part of this severe toxicity is caused due to a partial or complete dihydropyrimidine dehydrogenase (DPD) deficiency and hence, a strongly reduced capacity to degrade 5-FU into metabolites [2-6]. DPD deficiency, however, can not explain the toxicity in all patients. There may be several reasons for this. Fluoropyrimidines are often used in combination treatments with other cytotoxic drugs or radiotherapy, and a contributory effect of non-fluoropyrimidines in the toxicity observed. Alternatively, polymorphisms involving genes other than DPYD (gene encoding for DPD) that play a role in fluoropyrimidine metabolism may cause non-DPD related toxicity. For example variants in the TYMS gene will lead to reduction of the enzyme thymidylate synthase. In breast cancer patients homozygous for the TYMS 3RG allele, have a significantly higher incidence of toxicity and a lower response was observed when compared to heterozygous patients, and patients in which the TYMS 3RG allele was not present [7]. In addition, the presence of the TYMS 2R/2R variant was also associated with higher toxicity [8]. Variants in the dihydropyrimidinase coding gene, DPYS, were likely to cause structural destabilization and protein misfolding [9] and patients with a partial deficiency of dihydropyrimidinase are at risk to develop severe 5-FU related toxicity.
For the β-ureidopropionase enzyme which catalyzes the last step in the 5-FU and uracil-degradation pathway, it is suggested that variants of the UPB1 gene coding for β-ureidopropionase are likely to be associated with increased toxicity although the role of these variants are less significant than alterations in DPYD [11-13].

Besides its role in 5-FU pharmacokinetics and toxicity, the level of DPD present in tumour cells is likely to be correlated to 5-FU resistance and treatment response. Indeed, prospective studies in colorectal cancer patients showed that overexpression of thymidylate synthase and DPD in certain types of tumours may explain the resistance to 5-FU therapy [14-16]. A similar correlation was found for bladder cancer [17], and for lung cancer where a high DPD expression in NSCLC tumour cells is correlated with EGFR mutations [18].

The examples given in the previous paragraphs show that the individual’s genotype greatly influences the behaviour of a drug. Pharmacogenetics, the heritability of drug response, may help to explain some of the variability in drug response between individuals [19]. However, besides the variation in drug response caused by the genotype of an individual, non-genetic factors such as age, organ function, food, smoking status, concomitant therapy, drug interactions and nature of the disease may also influence the drug’s effect in an individual [20].

DPD is encoded by the gene DPYD for which 567 coding genetic variants are currently known [21], some of them being pathogenic since they reduce enzyme function or stability [22, 23]. We hypothesized that variability in DPD activity and its effect on DPD related toxicity could be best examined by a phenotyping approach, since current DPYD genetic testing only explains part of the fluoropyrimidine-related toxicity. Tests based on phenotyping approaches are already known and used in pharmacokinetics research, such as probes for phase I and phase II metabolic enzymes and drug transporters [24]. In this thesis, uracil being used as an oral loading dose is studied as a probe for DPD deficiency in cancer patients treated with fluoropyrimidines and our aim was to develop a test procedure that is suitable to be incorporated broadly into daily practice in hospital care.

Status of DPD testing

Several tests have been developed aiming to predict or explain DPD related fluoropyrimidine toxicity [25] (Chapter 2). These tests include both genotyping and phenotyping-based assays. To date, in daily practice, DPD testing is often used as counselling for family members of
DPD deficient subjects or in a retrospective setting to provide an explanation for severe fluoropyrimidine related toxicity. In our view this is not useful, as the toxicity then already has taken place and the aim is to avoid toxicity. The prospective screening of DPD deficiency is only used sparsely internationally, but was recently successfully implemented in a number of hospitals in the Netherlands (Chapter 7). The severity of DPD related toxicity is illustrated by prolonged hospitalization and by its rare, but potential lethal outcome [26, 27]. This observation raises the question why prospective DPD testing is not yet adopted as standard care in the field of oncology.

The successfullness of a diagnostic test or screening method

There are numerous guidelines and criteria for appraising diagnostic test studies and diseases [28-30]. It is interesting to investigate to what extent these criteria for diagnostic tests are met for routinely DPD screening in patients with an indication for fluoropyrimidine containing therapy. One of the criteria for a diagnostic test state that there should be a suitable test or examination to detect DPD related toxicity. The term suitable can be interpreted in multiple ways, and makes it unclear to what extent a test has to be validated or studied to be considered as suitable. In recent history, there are several well examples of diagnostic tests in the field of oncology that have been accepted by oncologists and were implemented broadly within a short period. Interestingly, for these tests, no prospective clinical trial was performed. This is illustrated by the discovery of Kirsten rat sarcoma viral oncogene (KRAS) mutations and its role in the treatment of colorectal cancer with cetuximab and panitumumab. It was first discovered that cetuximab had little or no effect in colorectal tumours harbouring KRAS mutation [31, 32]. Patient selection by analysis of KRAS mutations has been a fundamental event to increase efficiency and reduce cost after multiple retrospective studies all showed the same results [33]. Recently this was extended to RAS wild type tumours. Apparently a strong biological rationale and effect (shown by multiple retrospective studies all showing the same direction of effect) doesn’t need a prospective randomised study to change clinical practice.

It is remarkable that KRAS testing was successfully implemented while DPD testing is still under discussion. With KRAS testing, there is a very clear correlation between the test result and the treatment outcome. This is not fully the case with prospective DPD testing. It is obvious that DPD testing can prevent 5-FU related toxicity, however, not all 5-FU toxicity can be explained by reduced DPD activity. This is illustrated by the fact that approximately 30–50% of all patients suffering from 5-FU related toxicity have no decreased DPD enzyme
activity and [34, 35] and it is thus important to realize that prospective testing for DPD deficiency will not exclude all 5-FU related toxicity.

An important criterion for a diagnostic test is that the cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole. Prospective screening for DPD deficiency can reduce treatment costs by preventing toxicity initiated by low DPD activity. Severe Common Toxicity Criteria (CTC) grade III or IV toxicity might lead to hospitalization of patients for several days at high care units or even death (grade V). Ideally to be cost effective, the costs of prospective testing must be in favour to the costs of treatment of toxicity due to DPD deficiency. The prevalence of partial DPD deficiency in Caucasian population is approximately at least 3% [36-38]. As a result, many patients with an indication for fluoropyrimidine-containing therapy need to be screened prospectively in order to diagnose the minority with DPD deficiency. Nevertheless, it was described that the average total treatment cost per patient was lower with a prospective DPD screening strategy compared to nonscreening [39].

Importantly, the test result of a diagnostic test should lead to an actionable, clinical recommendation, or in the case of DPD deficiency, to a fluoropyrimidine dose adjustment or advice for alternative therapy. The test result of DPD deficiency measured in PBMCs can present as a complete or partial malfunction of the enzyme and there is a linear correlation between DPD activity and 5-FU clearance [40]. For the genotyping approach in which \textit{DPYD} variants are determined, there are clear dosing advices that are related to the presence of \textit{DPYD} variants, though there is data available that the toxicity risk of certain \textit{DPYD} variants is also influenced by factors such as gender, mode of administration and co-treatment with folinic acid [41]. Chapter 7 shows, that it is possible to implement prospective \textit{DPYD} screening effectively in a hospital setting, with the result that 90–100% of all patients with an indication for a fluoropyrimidine containing therapy are being screened.

**Evaluation of the oral uracil loading dose**

Uracil is not registered for human use, but the quality of commercially available uracil is very high without impurities as is described in Chapter 3 and can be used safely in patients. The safety of oral uracil is further demonstrated by the fact that there were no side effects of any form observed in all subjects following the administration of the uracil loading dose. Uracil in plasma can be determined by High Pressure Liquid Chromatography (HPLC) of
with HPLC-mass spectrometry. This equipment is present in clinical or pharmaceutical laboratories in hospitals. The uracil dose that was used in this thesis is 500 mg/m². There is a disadvantage of a uracil dose that is based on the body surface area (BSA) of patients since this requires individual doses of uracil have to be on stock or prepared by the pharmacy. In Chapter 4 and 5 an intensive blood-sampling scheme was used in order to establish comprehensive plasma-concentration curves of uracil following oral intake. Because a full sampling scheme and the associated test length of 4 hours can be considered as patient unfriendly, it was replaced by a limited sampling schedule in which blood is taken after 60 and 120 minutes following uracil ingestion. Is this feasible in daily clinical practice and patient-friendly enough? There are other diagnostic tests based on the same blood sampling principle that are accepted and implemented broadly, and used successfully. As an example, the oral glucose tolerance test is used to diagnose diabetes and gestational diabetes mellitus defined as glucose intolerance identified during pregnancy [42, 43]. Diabetes may be diagnosed based on HbA1C criteria or plasma glucose criteria, either the fasting plasma glucose or the 2-hour plasma glucose value after a 75-g oral glucose tolerance test. The use of this glucose test shows that it is indeed possible that the oral uracil loading in its present form is suitable to be incorporated into daily practice in hospitals.

Patient selection and uracil pharmacokinetics

In this thesis, uracil was orally administrated to healthy volunteers and cancer patients with, and without DPD deficiency. All study subjects who were tested, were Caucasian which is interesting, since differences are reported in DPD activity among different populations. The prevalence of DPD deficiency is 3–5% in a European/Caucasian population, but is estimated at approximately 8% for the African-American population [37, 44]. This difference in prevalence will not influence the test result of the oral uracil loading dose. Genetic variations in DPYD might be unique depending on race and were investigated for different ethnical groups in which several DPYD variants showed different distributions [45-48]. In addition, differences in pyrimidine catabolism have been reported between men and women. In general women suffer more from side effects during fluoropyrimidine-containing therapy [49-52]. A possible explanation may be the lower clearance of 5-FU in women compared to men [53]. Moreover, differences have been observed in endogenous uracil and dihydrouracil levels between men and women, but the mean uracil/dihydrouracil ratio was comparable [54]. For the oral uracil loading dose, it was not investigated if uracil pharmacokinetics and the uracil/dihydrouracil ratio differed between men and women.
In Chapter 5 we showed that the presence of metastatic disease in colorectal cancer patients has no effect on uracil pharmacokinetics. This is in line with the observation that extensive hepatic replacement due to liver metastases had no effect on 5-FU pharmacokinetics indicating that the amount of DPD is probably not influenced by moderate reduction in liver function [55]. The gastrointestinal absorption of uracil is a pharmacokinetic first order process and the elimination follows a saturable Michaelis-Menten kinetics [56]. Of note, all patients that were included in the studies described in this thesis had an intact gastrointestinal (GI) tract. Surgical alteration of the structure of the GI tract such as gastric or bowel resection, may alter its function which can have impact on drug absorption [57]. For this reason, the oral uracil loading dose is not suitable for patients with such GI alterations and alternative testing has to be performed.

In this thesis, the oral uracil loading dose was not used in a prospective setting. All patients already received one or multiple dose of 5-FU or capecitabine before they received the oral uracil test dose. They were included based on the DPD activity that was assessed by measuring the DPD activity in peripheral blood mononuclear cells (PBMCs) [58] and in Chapter 6 severe toxicity occurred before DPD activity was measured. Despite the fact the uracil loading was not investigated in a true prospective setting, the test was applied to a variable group of patients with and without toxicity, variable DPD activity and with and without metastatic disease.

**Future perspectives**

The results in this thesis indicate that the oral uracil loading dose is suitable as a phenotyping probe for DPD deficiency and can be incorporated into daily practice in hospital care. As mentioned before the equipment for analysing the plasma samples is already present in most hospitals, so that the turnaround-time of the test can be short with 1 or 2 days. The local pharmacy can order or prepare the uracil that is needed for the test as long as there is no commercially uracil solution available. Most hospitals in the Netherlands have special wards that are used for short stay of patients or the clinical laboratories can handle the patient logistic that is needed to perform the test.

Nevertheless, the test still might be optimised to simplify the test procedure in order to further increase acceptance and applicability. Since the uracil/dihydouracil ratio after uracil ingestion is determined by DPD enzyme activity and not so much by the dose administered, a fixed dose uracil will not influence the test result and is for practical reasons preferred
over the BSA determined dose of a subject that was used in this thesis. Subsequently, a
dried blood spot sampling might be candidate to replace the venous sampling method that
is currently used.

Pharmacokinetics can be used as a tool to optimise 5-FU therapy. With PK guided 5-FU
dosing, the start dose of 5-FU is based on BSA and titrated during following administration
based on 5-FU plasma concentration and AUC [59, 60]. Besides its role in diagnosing DPD
deficiency, the oral uracil loading dose might be useful to establish a more specific 5-FU
starting dose when therapy is started close to the desired plasma level and Area Under the
Curve if a clear relation exists between uracil and 5-FU PK.

Although not studied in this thesis, it may be interesting to investigate if the oral uracil loading
dose can play a role in individual pharmacokinetically (PK) guided 5-FU dosing. This 5-FU
dosing approach leads to higher efficacy and tolerability compared to 5-FU dosing based on
BSA. It has already been suggested that the use of 5-FU Michaelis-Menten pharmacokinetic
models might be suitable to predict a-priori 5-FU plasma concentrations [61].

The genotyping strategy that was evaluated in Chapter 7 can be further improved. There
are more DPYD variants than the four tested that alter DPD enzyme activity, and novel
variants are still discovered. The presence of one of these non-tested variants potentially
will lead to a false negative test result with the risk to develop toxicity. Since the costs for
genotyping continues to decrease [62, 63], the number of DPYD variants tested could be
expanded to improve sensitivity. The variants that are tested in the current test strategies are
mostly coding variants, but there have been pathogenic variants described in the noncoding
DPYD gene regions that are not routinely tested [64]. This can be resolved by sequencing
the entire DPYD gene, but although more informative, this is far more expensive than the
strategy that was used in Chapter 7.

This thesis does not answer the question which DPD test strategy is the most efficient one
to prevent and predict DPD related toxicity. This requires the multiple test strategies being
compared to each other in a prospective, head to head study in which the outcome should
be the prevention of fluoropyrimidine related toxicity. At this moment a large prospective,
multicentre study is performed in which different strategies to prevent DPD related toxicity
are evaluated (EudraCT registration number: 2014-005064-15).

Despite the fact that the cost effectiveness of DPD screening should be investigated more
thoroughly and not all fluoropyrimidine related toxicity can be prevented, pre-treatment
DPD testing, irrespective of what specific test is used, should be standard care and incorporated in oncology guidelines. We advocate that all patients who are first time treated with a fluoropyrimidine containing therapy should be screened for DPD deficiency.

REFERENCES


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