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Chapter 1

General introduction
Fluoropyrimidines in the treatment of cancer

Fluoropyrimidines have been applied in the treatment of cancer for decades and are extensively used worldwide. The most known fluoropyrimidine is 5-fluorouracil (5-FU). 5-FU was developed in 1954 after the discovery that liver tumours absorbed radioactive uracil more readily than normal liver cells. Previously, Charles Heidelberger discovered that fluorine in fluoroacetic acid inhibited the vital enzyme aconitase that catalyzes the stereo-specific isomerization of citrate to isocitrate via cis-aconitate in the tricarboxylic acid cycle. Based on this finding that a fluorine atom causes a profound alteration in biological effect, and the fact that uracil in tumours is incorporated into RNA, Heidelberger substituted a hydrogen atom in the uracil molecule for a fluorine atom with the idea that if 5-FU were to have biological activity, it should block DNA synthesis [1]. These two scientific results led to the development of 5-FU as an anticancer drug [2].

In a clinical setting, 5-FU is administered intravenously, as a bolus or prolonged infusion or as a topical cream and orally. The intravenous and oral presentations of 5-FU are widely used in the treatment of a range of cancers such as breast cancer, cancers of the gastrointestinal and urogenital tract and head and neck cancer. Topical 5-FU is used in the treatment of actinic keratosis and Bowen’s disease. Meta-analysis of the efficacy of intravenous continuous infusion of 5-FU compared with bolus administration in advanced colorectal cancer showed that continuous infusion is superior compared to bolus infusion in terms of tumour response and resulted in a slightly increase in overall survival [3]. The morbidity associated with indwelling catheters and infusion pumps and patient inconvenience with regard to the length of continue infusion schemes of 5-FU led to the development of the oral fluoropyrimidines. Oral administration of 5-FU results in wide intra- and interpatient variability in 5-FU plasma levels [4]. The oral fluoropyrimidines can be divided into 3 groups, 5-FU prodrugs; 5-FU combined with a DPD inhibitor, and 5-FU prodrugs combined with a DPD inhibitor [5]. The most frequently used 5-FU prodrug is capecitabine. This drug is first converted to 5-deoxyfluorocytidine in the liver by carboxylesterase and then converted to doxifluridine by cytidine deaminase, which is found in liver, plasma, and tumour tissue. The toxic intermediary doxifluridine is then converted to 5-FU by thymidine phosphorylase, that is more abundant in tumours than in normal tissue, resulting in tumour 5-FU concentrations that far exceed plasma levels and produce greater antineoplastic effects with lower toxicity [6, 7]. The availability of capecitabine resulted in the Netherlands in a massive shift from intravenously therapy to oral therapy in the treatment of colorectal
cancer. In the Netherlands, approximately 90% of all fluoropyrimidine-containing therapy contains capecitabine. Published cost-effectiveness analysis regarding the treatment of stage III colon cancer shows that capecitabine is less costly and more effective than 5-FU treatment [8, 9], which indicates that huge cost savings are established in the treatment of colon cancer.

**Fluoropyrimidine mechanism of action and toxicity**

*In vivo*, 5-FU is partly converted intracellular to the active metabolites fluorodeoxyuridine monophosphate (FdUMP) and fluorouridine triphosphate (FUTP) [10]. FUTP disrupts RNA synthesis and FdUMP inhibits thymidylate synthase (TS), which is the key enzyme in the de novo synthesis of thymidylate that is necessary for DNA replication and repair. TS catalyzes the reductive methylation of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (TMP). The TS protein functions as a dimer with one nucleotide-binding site. The 5-FU metabolite FdUMP binds to this nucleotide-binding site forming a stable complex and thereby blocking the binding of dUMP, which results in inhibition of TMP synthesis [11, 12].

The metabolite FUTP is incorporated into RNA leading to dysfunction [3]. In cancer cell lines a correlation was observed between FUTP misincorporation into RNA and loss of clonogenic potential [13, 14]. Several *in vitro* studies indicated that 5-FU misincorporation disrupt many aspects of RNA processing leading to profound effects on cellular metabolism and viability [10]. With intravenous administration, the length of infusion is correlated with the mechanism of action of 5-FU since the mechanism of action of bolus administration is mainly inhibition of TS, while continuous infusion is cytotoxic by misincorporation into RNA [15]. These two mechanisms of action lead to differences in type of toxicity. The results of a meta-analysis showed that, with 5-FU bolus, hematologic toxicity was more frequent than with continuous infusion (31% and 4%, p < 0.0001). On the other hand, continuous infusion resulted in higher incidence of hand-foot syndrome compared with bolus infusion (13% and 34%, p < 0.0001) [3]. Incidence and proportions of all other toxicities were identical for bolus and continuous infusion. Independent prognostic factors were age, sex, and performance status for nonhematologic toxicities, performance status, and treatment for hematologic toxicities, and age, sex, and treatment for hand-foot syndrome.
**Fluoropyrimidine metabolism**

The rate-limiting enzyme in 5-FU catabolism is dihydropyrimidine dehydrogenase (DPD). More than 80% of the amount of 5-FU administered is normally catabolized primarily in the liver where DPD is abundantly expressed [16, 17]. DPD is also present in normal and tumour cells. 5-FU is converted by DPD into 5,6-dihydrofluorouracil (DHFU). Subsequently, DHFU is degraded into fluoro-β-ureidopropionic acid (FUPA) and fluoro-β-alanine (FBAL). In general, fluoropyrimidines are tolerated well although approximately 10% of the patients treated with fluoropyrimidines suffer from CTC grade III or IV toxicity. Interindividual variability in the activity of DPD can be the cause of this severe toxicity. DPD is encoded by *DPYD* and polymorphisms in *DPYD* have shown to be related to toxicity in colorectal patients treated with capecitabine and 5-FU [18-20]. In patients with a near complete DPD deficiency this can even lead to death [21]. Knowledge of the clinical impact of reduced DPD activity on the pharmacokinetics and pharmacodynamics of fluoropyrimidines may lead to dose individualized therapy. Therapeutic drug monitoring of 5-FU has been shown to result in reduced intra- and inter-individual variability in 5-FU plasma levels and pharmacokinetic guided dose adjustments of 5-FU-containing therapy results in a significantly improved efficacy and tolerability [22]. In addition, pharmacokinetic Michaelis-Menten models allows the use a limited sampling strategy and offer the opportunity to predict a priori the 5-FU plasma concentrations in patients receiving adapted doses of 5-FU [23].

**Aim and outline of this thesis**

The general aim of this thesis is to study the use of an oral uracil loading dose as probe for DPD deficiency in cancer patients treated with fluoropyrimidines and to develop a test procedure that is suitable to be incorporated broadly into daily practice in hospital care. With regard to this latter aspect, it will be studied if a prospective DPD testing strategy can be successfully incorporated into routine clinical healthcare as a standard procedure for all patients using a fluoropyrimidine.

In **CHAPTER 2** a review is presented of studies that describe predictive tests developed for screening for DPD. This chapter outlines the status of methods for testing for DPD deficiency and their use in daily practice.

In order to perform clinical tests, uracil as an investigational medicinal product must be approved by the competent authorities since is not registered for human use. The document
required for this approval is called ‘Investigational Medicinal Product Dossier’ (IMPD) and includes summaries of information related to the quality, manufacture and control of the Investigational Medicinal Product, data from non-clinical studies and data from its clinical use. **CHAPTER 3** is the Investigational Medical Product Dossier of uracil for the oral uracil loading test that was used for approval by the Medical Ethical committees of clinical trials described in Chapter 5, 6 and 7.

In **CHAPTER 4** the pharmacokinetics of the oral uracil loading dose in healthy volunteers and cancer patients is studied. Part of this study is a dose finding strategy in order to determine which uracil dose is optimal. This study can be considered as a phase 1 study in order to determine optimal dose, safety and compare results between healthy volunteers and patients. In this study oral uracil is administered in dosages of 500 and 1000 mg/m² Body Surface Area (BSA) to healthy volunteers. This study is important to determine the most effective dose and to determine if disease status will influence the results of the uracil loading test.

**CHAPTER 5** describes a study in which the oral uracil loading dose is administered to colorectal cancer patients with and without metastasis, all with normal DPD status. This study is performed to investigate if presence of metastases will influence the pharmacokinetics of oral uracil. Since the objective of the oral uracil loading dose is that is will be used prospectively in all patients with different types of cancer, disease status ideally does not influence pharmacokinetics. This is the first study in which the potential effect of metastatic disease on uracil pharmacokinetics in colorectal cancer patients will be investigated.

The EURABEL2 study is described in **CHAPTER 6**. The aim of this study is to develop a limited sampling strategy, to detect decreased uracil elimination in patients with a DPD deficiency and to perform a more in-depth quantitative compartmental pharmacokinetic analysis of uracil plasma concentrations.

In this study patients with toxicity will be included and divided in two groups based on the results of the measurement of DPD activity in peripheral blood mononuclear cells (PBMCs), which in this study is considered to be the gold standard. The performance of the oral uracil loading dose in the two groups will be compared with the results of the gold standard and specificity and sensitivity will be calculated.

In **CHAPTER 7** the results are described of a study that evaluated the clinical acceptance and adherence of a prospective *DPYD* genotyping strategy that was implemented at Leiden University Medical Center. The objective of this genotyping strategy is that all patients who
have an indication for first time treatment with 5FU or CAP are routinely prospectively screened for the presence of four pathogenic variants to prevent 5FU related toxicity caused by DPYD genetic variations.

This thesis ends with a general discussion and future perspectives in CHAPTER 8. A summary of this thesis is presented in CHAPTER 9.

REFERENCES


