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The role of pharmacogenetics in capecitabine efficacy and toxicity

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ABSTRACT

Capecitabine is an oral prodrug of 5-fluorouracil (5-FU) and approved for treatment of various malignancies. Hereditary genetic variants may affect a drug's pharmacokinetics or pharmacodynamics and account for differences in treatment response and adverse events among patients. In this review we present the current knowledge on genetic variants, commonly single-nucleotide polymorphisms (SNPs), tested in cohorts of cancer patients and possibly useful for prediction of capecitabine efficacy or toxicity. Capecitabine is activated to 5-FU by *CES*, *CDA* and *TYMP*, of which SNPs in *CDA* and *CES2* were found to be associated with efficacy and toxicity. In addition, variants in genes of the 5-FU metabolic pathway, including *TYMS*, *MTHFR* and *DPYD* also influenced capecitabine efficacy and toxicity. In particular, well-known SNPs in *TYMS* and *DPYD* as well as putative *DPYD* SNPs had an association with clinical outcome as well as adverse events. Inconsistent findings may be attributable to factors related to ethnic differences, sample size, study design, study endpoints, dosing schedule and the use of multiple agents. Of the SNPs described in this review, dose reduction of fluoropyrimidines based on the presence of *DPYD* variants *2A (rs3918290), *13 (rs55886062), −2846A>T (rs67376798) and −1236G>A/HapB3 (rs56038477) has already been recommended. Other variants merit further validation to establish their definite role in explanation of interindividual differences in the outcome of capecitabine-based therapy.

Introduction

Capecitabine, a prodrug of the antimetabolite 5-fluorouracil (5-FU), has been registered for treatment of colon cancer in the adjuvant setting as well as for treatment of advanced colon, breast and gastric cancer. The drug is active as single agent, but can also be combined with other cytotoxic agents, such as oxaliplatin [1,2], irinotecan [2], a taxane [3] or cisplatin [1]. In colon cancer, a pooled analysis of randomized trials has shown equivalence in efficacy between infusional 5-FU- and capecitabine-containing regimens [4]. In advanced esophago-gastric cancer, meta-analysis of two randomized trials in which patients received infusional 5-FU or capecitabine combinations, overall survival (OS) was even superior for the latter treatment regimen [5]. The convenience of an oral formulation given daily for a particular period mimicking continuous 5-FU infusion makes capecitabine an attractive treatment option, although regular monitoring of patient's adherence to oral anticancer medication balanced by tolerability is important to ensure optimal drug exposure. Of interest, some tumors express high levels of thymidine phosphorylase (TYMP), the rate-limiting enzyme activating capecitabine to 5-FU, enabling high and sustained intratumoral levels of active drug [6].

Although the efficacy of capecitabine is considered to be equivalent to 5-FU, their toxicity profiles vary. Both drugs induce gastrointestinal adverse events (AEs), of which the incidence of nausea is not different among comparative treatment groups [4]. In case of capecitabine, the incidence of stomatitis is significantly lower [4], while that of diarrhea is significantly increased especially when combined with irinotecan [7]. In comparison with intermittent 5-FU, capecitabine is associated with a lower rate of neutropenia, but hand-foot syndrome (HFS) occurs far more frequently [4]. Both drugs are known for a low prevalence of cardiovascular toxicity [8].

The incidence and severity of AEs of capecitabine depend on therapy-related factors, such as dosing schedule, duration, previous treatment and overlapping toxicity when combined with cytotoxic agents. Dosing usually consists of administration twice daily for two weeks followed by a rest period of one week in a three-week cycle. The starting dose is 1,250 mg/m^2 twice daily when given as single agent, but dose reductions are frequently required to improve tolerability [2,3]. In breast cancer, a lower starting dose of 1,000 mg/m^2 or dose-adjusting capecitabine during treatment

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does not seem to compromise efficacy [9]. In combination regimens, initial doses vary between 825–1,000 mg/m^2 twice daily.

Host-related factors of influence on capecitabine-induced AEs are dihydropyrimidine dehydrogenase (DPD) enzymatic activity, renal dysfunction, gender and age, body weight, regional differences, and drug-drug interactions [2,10–12]. The DPD enzyme is required to convert 5-FU to 5-fluorodihydrouracil. Deficient or low DPD activity due to alterations in the *DPYD* gene is estimated to occur in 3–5% of individuals, which may lead to increased toxicity from 5-FU as well as capecitabine [11]. Another important factor of influence on interindividual differences in AEs is renal function. A 50% decrease in creatinine clearance is associated with a 50% reduction in clearance of the toxic catabolite fluoro-beta-alanine (FBAL) [12]. Concentration-effect analyses have shown a positive relationship between the area under the curve (AUC) of FBAL and treatment-related grade $\geqslant$3 diarrhea [13]. For that reason, tailored doses of capecitabine are recommended in case of reduced creatinine clearance, while therapy is withheld if clearance is less than 30 mL/min [12]. For gender, the clearance of FBAL is less in women [12]. The age-related increase in concentration of FBAL might be explained by a physiological decrease in renal function in the elderly [2,12]. A high body weight results in a high body surface area, which is associated with a high volume of distribution and a decreased clearance of FBAL [12]. Regional variations in the tolerability of capecitabine as well as 5-FU have been reported in studies in which patients were included from US and East-Asia [2], but underlying reasons for the differences are not clear. For drug-drug interactions, some drugs are mentioned to be of influence on metabolism, while caution is required with concomitant use of nephrotoxic agents [2,12].

Research in pharmacogenetics has gained interest with respect to its contribution to our understanding of the interindividual variation in drug effects. Genetic polymorphisms, primarily single nucleotide polymorphisms (SNPs), may affect expression and/or activity of various proteins including drug-metabolizing enzymes, drug transporters and targets, or transcription factor binding sites resulting in altered gene expression, i.e. encoding for proteins involved in detoxification or excretion. Extensive studies have been carried out on SNPs linked to the 5-FU metabolic pathway for prediction of treatment response and/or toxicity. The well-known example is *DPYD* of which the *DPYD**2A variant results in a catalytic inactive form of the enzyme leading to excessive toxicity [14]. Given similarities between capecitabine and 5-FU in terms of their mechanism of action and elimination, these genetic variations also affect the outcome of capecitabine. Moreover, novel genetic variants might be identified in the key enzymes of capecitabine activation to 5-FU. In this comprehensive review, we summarized the information available on SNPs in the capecitabine-activating pathway as well as 5-FU-metabolizing genes in order to determine, whether these genetic variants play a role in the differential efficacy and toxicity from capecitabine among individuals.

Capecitabine metabolic pathway

Capecitabine is activated to 5-FU through a three-step enzymatic process consecutively requiring carboxylesterase (CES), cytidine deaminase (CDA) and TYMP (Fig. 1) [15]. After rapid intestinal absorption, the first step of activation primarily occurs in the liver and involves enzymatic hydrolysis by CES producing 5′-deoxy-5-fluorocytidine (5′-DFCR). Among three 60-kDa CES isoenzymes, CES1A2 and CES2 exert highest catalytic efficiencies in the hydrolysis of capecitabine *in vitro* [16]. 5′-DFCR is converted to 5′-deoxy-5-fluorouridine (5′-DFUR) by CDA, which is a ubiquitous enzyme mainly expressed in the liver. High CDA activity in cancer cells has been associated with increased sensitivity to capecitabine [17,18]. Moreover, a potential role of CDA in capecitabine toxicity has been suggested in patients that developed severe life-threatening AEs in the presence of high serum activity of CDA [19,20]. It is of note that while CDA is involved in the activation of capecitabine, it functions as a major detoxifying enzyme for other antimetabolites, such as gemcitabine and cytarabine [17,18]. The final conversion of 5′-DFUR to 5-FU is mediated by TYMP. Given the relatively higher TYMP expression in some tumors compared to healthy tissue, preferential activation of capecitabine to 5-FU might lead to tumor selectivity [6,21,22]. *TYMP* expression is elevated in the palm compared with the back of the hand, which was hypothesized to be a major causative mechanism for capecitabine-related HFS [23].

The mechanism of action of 5-FU has been described elsewhere [24] and entails, briefly, misincorporation of 5-FU metabolites into RNA and DNA and inhibition of thymidylate synthase (TYMS). In particular, TYMS inhibition by 5-fluoro-2′-deoxyuridine 5′-monophosphate (FdUMP) triggers a cascade of molecular alterations that lead to misincorporation of 5-FU metabolites into DNA, impaired DNA replication, synthesis and repair, which eventually leads to DNA breaks. Preclinical findings in human cancer cell lines have demonstrated that high TYMS activity was associated with 5-FU resistance [25]. Methylene tetrahydrofolate reductase (MTHFR) is one of the many enzymes that play a role in the metabolism of folates, their primary source is diet. MTHFR carries out a central reaction by irreversibly catalyzing the conversion of 5,10-methylene tetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate, the primary circulating form of folate, which serves as a methyl-group for DNA methylation reactions [26]. An elevated level of 5,10-MTHF, such as in low MTHFR activity, might theoretically lead to greater inhibition of TYMS and enhanced cytotoxicity of 5-FU.

The catabolism of 5-FU is mainly controlled by DPD, which is a rate-limiting enzyme in the liver responsible for conversion of 80% of 5-FU into dihydrofluorouracil (DHFU) [15]. DPD levels vary considerably among individuals with consequences for efficacy and toxicity during 5-FU therapy [11,14]. Low DPD activity results into severe AEs due to accumulation of active 5-FU metabolites [11,14]. DHFU is then converted to fluoro-β-ureidopropionate (FUPA) and subsequently to FBAL by dihydropyrimidinase and β-ureidopropionase, respectively [15]. Excretion of the metabolites occurs by the kidney [22]. Mean urinary recovery of the administered dose amounts to 71–87% and mainly consists of FBAL (51–62%), followed by 5′-DFUR (7–11%) and 5′-DFCR (6–7%) and small percentages of other compounds.

Genetic polymorphisms and functionality

Several candidate SNPs involved in capecitabine efficacy and/or toxicity have been investigated for functionality in the past. A brief overview is provided here for better interpretation of pharmacogenetic results.

TYMS genetic variants located in the regulatory regions have shown to influence the transcription rate. Higher intratumoral TYMS levels may translate into relative resistance to 5-FU [27–29]. Of particular interest is *TYMS* 2R or 3R (rs45445694) constituting double or triple tandem repeats of 28 base pairs (bp) in the 5'untranslated region (UTR). An enhancer box (E-box) sequence containing a binding site for upstream stimulating factors (USFs) is located in the first of the double tandem repeats of the 2R allele and the two first of the triple tandem repeats of the 3R allele. Binding of USFs to the E-box enhances the *TYMS* transcription rate and, consequently, 3R compared to 2R will result in greater enzyme activity as demonstrated *in vitro* [27,29]. Furthermore, a glycine to