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Influence of metastatic disease on the usefulness of uracil pharmacokinetics as a screening tool for DPD activity in colorectal cancer patients

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ABSTRACT

Purpose: Dihydropyrimidine Dehydrogenase (DPD) deficiency can lead to severe toxicity in patients treated with a standard dose of a fluoropyrimidine such as 5-fluorouracil (5-FU) or capecitabine (CAP). Administration of oral uracil and subsequent measurement of uracil and dihydrouracil (DHU) plasma concentrations has been used to identify patients with DPD deficiency. Liver metastasis might influence systemic DPD activity. The aim of the study is to investigate the effect of metastatic disease on the pharmacokinetics of uracil and DHU after oral administration of uracil.

Methods: 500 mg/m^2 uracil was administered orally to 12 subjects with stage II-III colorectal cancer (CRC) who were treated in the adjuvant setting and to 12 subjects with stage IV metastasized CRC, all treated with capecitabine containing therapy. All subjects had a normal DPD activity defined as >6 nmol/mg/hr determined in peripheral blood mononuclear cells (PBMCs).

Results: The mean uracil clearance (CL 51.7 (SD 6.4) versus 46.7 (SD 13.0) l/h), Area under the curve ($AUC_{0\text{-}220min}$ 20.6 (SD 6.4) versus 21.0 (SD 5.7) h*mg/l), elimination half life (t1/2 21 (SD 7) vs 21 (SD 8) min), maximum concentration time (T_{max} 27 (SD 9) vs 25 (SD 9) min), Volume of distribution (V 26.58 (SD 10.11) vs 21.10 (SD 8.48) l) and the elimination constant (k_{el} 2.01 (SD 0.56) vs 2.41 (SD 0.72) h^{-1}) did not differ significantly ($p>0.05$) non-metastatic CRD versus metastatic CRC.

Conclusions: Uracil pharmacokinetics is similar in CRC patients with and without metastasis. Therefore, the uracil test dose could be used as a DPD phenotype test in both adjuvantly treated and metastatic CRC patients using similar cut off criteria to identify patients with DPD deficiency.

INTRODUCTION

Capecitabine is an oral prodrug of 5-fluorouracil (5-FU). Both drugs are extensively used for the treatment of patients with colorectal, breast, gastric and head and neck cancer. Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme in the catabolism of capecitabine and 5-FU, converting >80% of an administered dose of 5-FU to inactive metabolites, a process mainly occurring in the liver. Patients with a partial or complete DPD deficiency have a strongly reduced capacity to degrade 5-FU which may thus result in severe toxicity [1-5]. Several methods have been proposed to identify patients with reduced DPD activity [6]. Since uracil is a non-toxic structural analogue of 5-FU, the metabolism of uracil is similar to that of 5-FU and can therefore be used as a phenotype probe for DPD activity. Like fluoropyrimidines, uracil is metabolized initially by DPD and subsequently degraded by other enzymes into eventually beta-alanine [7] (Figure 8.1). In a previous study we described the use of an oral uracil loading dose to assess

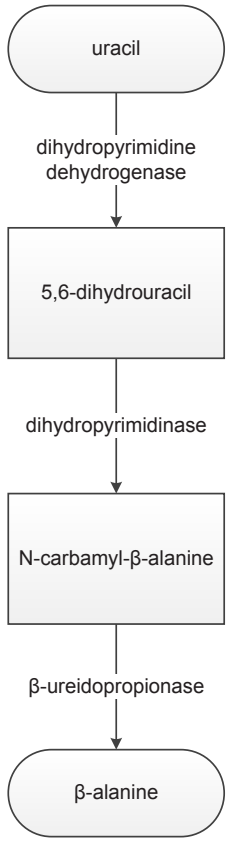

Figure 8.1 Catabolic pathway of uracil.

the DPD status in healthy volunteers and in DPD-deficient CRC patients [8]. The purpose of the current study was to investigate whether or not the presence of metastases might influence the pharmacokinetics of orally administered uracil. The catabolism of 5-FU by DPD occurs mainly in the liver and contributes substantially to the metabolism of 5-FU [9,10]. Liver metastases might alter uracil pharmacokinetics since in cancer patients, metastases in the liver and steatosis, caused by systemic chemotherapy, have shown to reduce drug metabolism [11]. Secondly, concomitant inflammatory responses have been observed during initiation, invasion, and metastasis of tumors. Components of cancer inflammation like chemokines, prostaglandins, and cytokines have shown to down-regulate cytochrome P450 enzyme activity [12]. Indeed, for CYP2C19, a discordant slow metabolizer phenotype compared to the predictive genotype was found in patients with advanced metastatic cancer [13]. For this reason it might be possible that the presence of a significant metastasis burden might alter DPD activity and uracil pharmacokinetics as well. Therefore, to further validate the oral uracil loading test, we performed a study in colorectal cancer patients treated with capecitabine to compare uracil pharmacokinetics in patients with metastatic disease and patients who were treated in the adjuvant setting without metastatic disease.

MATERIALS AND METHODS

Calculation of sample size

Based on the pharmacokinetic analysis of the data from 11 healthy volunteers enrolled a previous study [8] we calculated a mean uracil clearance of 50.6 l/hour with a variance of 21%. We considered empirically that a difference in uracil clearance >25% was clinically relevant. Based on this consideration, to achieve 80% power at a 0.05 significance level in order to detect a difference in uracil clearance in subjects with and without metastasizes, the calculated sample size is 24 (12 +12).

Study subjects

Twelve subjects with metastasized CRC and 12 subjects with CRC in the adjuvant setting were included in this study. All subjects, were treated with capecitabine containing therapy, had a normal DPD activity >6 nmol/mg/hour measured in PBMCs to avoid an effect on uracil pharmacokinetics caused by inactivating *DPYD* mutations. The value of >6 nmol/mg/hour is a threshold level to distinguish individuals with and without DPD deficiency [14]. All subjects were aged >18 years

and had adequate renal and liver function. Three hospitals in the Netherlands participated in this study that was approved by the Medical Ethics Committee BEBO in Assen, The Netherlands. Informed consent was obtained from all individual participants included in the study. Prior to uracil administration, blood samples were obtained to measure creatinine clearance, alanine transaminase (ALAT) and gamma-glutamyl transpeptidase (gamma-GT) as markers for renal and liver damage.

Uracil administration

Uracil (Pharma Waldhof GmbH, Düsseldorf, Germany) was administered orally at a test dose of 500 mg/m^2 body surface area, calculated by the Dubois and Dubois formula, after an overnight fast (last food intake >8 h earlier). All subjects had to abstain from food during 2 hours after oral administration of uracil. Administration took place at least 48 hours after the last administration of capecitabine. All the test doses were administered between 08:00 AM and 09:00 AM to avoid circadian effects. The uracil powder was mixed with 100–200 mL of tap water and immediately after preparation the suspension was ingested within a few minutes.

Collection of blood samples

Blood samples were obtained at t = 0, 15, 30, 45, 60, 80, 100, 120, 150, 180 and 220 min from an intravenous indwelling catheter.

Samples were immediately placed on ice and subsequently centrifuged at 2,500 x g for 10 min. The plasma was stored at -20°C until analysis.

DPD activity

The activity of DPD was determined in PBMCs using radiolabeled thymine followed by separation of radiolabeled thymine from radiolabeled dihydrothymine using reversed-phase HPLC and online detection of radioactivity, as described before [15].

Analytical method for uracil and dihydrouracil

Uracil and DHU plasma concentrations were measured by a validated HPLC method described by Maring et al. [16]. Calibration samples were prepared by spiking human heparinised plasma obtained from volunteers with appropriate amounts of uracil and 5,6-DHU (Sigma Chemical Co,

Zwijndrecht, The Netherlands). Uracil was quantified at 266 nm and DHU at 205 nm. The internal standard chlorouracil was quantified at both wavelengths.

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters area under the curve ($AUC_{0\text{-}220min}$), uracil clearance (Cl), C_{max} and T_{max} were calculated with'KINFIT module' of MwPharm version 3.50 (Mediware, Groningen, the Netherlands)®. KINFIT is a Bayesian curve fitting module in which we used a one compartment model. The AUC was calculated by a logarithmic trapezoidal rule. Statistical analysis was performed by using SPSS version 19.0 (SPSS inc, Chicago, IL). To examine whether the uracil and DHU plasma concentrations and derived pharmacokinetic parameters differed between the two study groups, an independent-samples Student's t-test was performed. Levene's test for equality was used to determine if the variance of each pharmacokinetic parameter was equal.

RESULTS

Table 8.1 displays the characteristics of the patients included in this study. In the metastasized group all patients had liver metastasis. Length, weight, age, BSA, leukocyte count, renal- and liver function were inventoried in all patients and did not differ significantly ($p>0.05$) between the two study groups. DPD activity was equally distributed between the two groups (9.5 (SD 2.9) and 10.3 (SD 1.8) nmol/mg/hour respectively). The patients in the adjuvant and metastatic group were using capecitabine as monotherapy (n=12), combined with oxaliplatin (n=7) or combined with oxaliplatin and bevacizumab (n=5) for treatment of CRC. Capecitabine and bevacizumab was only used in the metastatic group. Mean age of subjects adjuvantly treated for CRC was 63 (SD 10 years) and 69 (SD 6 years) for those with metastatic disease. Mean Body Surface Area (BSA) did not differ between the patients in treated in the adjuvant setting ($p=0.601$) compared to patients with metastatic CRC.

Table 8.2 displays the pharmacokinetic parameters of the two study groups. Clearance of uracil was lower in the group with metastatic disease (46.7 (SD 5.7) l/hr) compared to clearance in adjuvantly treated patients (51.7 (SD 11.7) l/hr), but the difference was not statistically significant ($p=0.327$). Figure 8.2 shows the concentration-time curves for uracil and DHU in both study groups. The mean exposure to uracil was not different ($p=0.889$) between the two groups ($AUC_{0\text{-}220min}$ 20.6 (SD 6.4) h*mg/l) for adjuvantly treated patients and for metastatic patients ($AUC_{0\text{-}220min}$ 21.0 (SD 5.7) h*mg/l). The time to reach T_{max} did not differ but the maximum concentration of uracil was

Table 8.1 Patient characteristics of the study population with standard deviation between brackets

Baseline characteristic	Adjuvant	Metastatic
DPD activity (nmol/mg/l)	9.5 (2.9)	10.3 (1.8)
Creatinin (µmol/l)	76.6 (15.8)	79.8 (18.1)
ALAT (U/l)	27.4 (8.1)	22.7 (11.6)
GammaGT (U/l)	39.8 (11.5)	52.7 (39.3)
Leukocytes (mmol/L)	4.9 (1.6)	5.9 (1.8)
weight (kg)	82 (13)	84 (11)
length (cm)	174 (7)	177 (10)
BSA (m^2)	1.97 (0.20)	2.01 (0.19)
Age (y)	63 (10)	69 (6)

Adjuvant, adjuvantly treated CRC patients; metastatic, study group with metastatic CRC; ALAT, Alanine Amino Transferase; GammaGT, Gamma-glutamyltransferase; BSA, Body Surface Area.

Table 8.2 Pharmacokinetic parameters of the two study groups with standard deviation between brackets

Pharmacokinetic parameter	Adjuvant	Metastasis	p value
AUC220 (h*mg/l)	20.6 (6.4)	21.0 (5.7)	0.889
CL (l/h)	51.7 (11.7)	46.7 (13.0)	0.327
t1/2 (min)	21 (7)	21 (8)	0.927
T_{max} (min)	27 (9)	25 (9)	0.600
C_{max} (mg/l)	19.9 (4.0)	25.8 (5.7)	0.008
V (l)	26.58 (10.11)	21.10 (8.48)	0.164
K_{el} (h^{-1})	2.01 (0.56)	2.41 (0.72)	0.136

Adjuvant, adjuvantly treated CRC patients; metastasis, study group with metastatic CRC; AUC, Area Under the Curve; thalf, elimination half time; T_{max}, time point of maximum concentration; C_{max}, maximum concentration; V, volume of distribution; k_{el}, elimination constant.

significantly different between the two groups with 19.9 mg/l (SD 4.0) in the adjuvantly treated group and 25.8 mg/l (SD 5.7) in the group with metastatic disease (p=0.008). Also, the half-life of uracil did not differ and was 21 minutes for both groups, as was the case for the volume of distribution and elimination. No adverse events related to uracil administration of uracil were identified in the study.

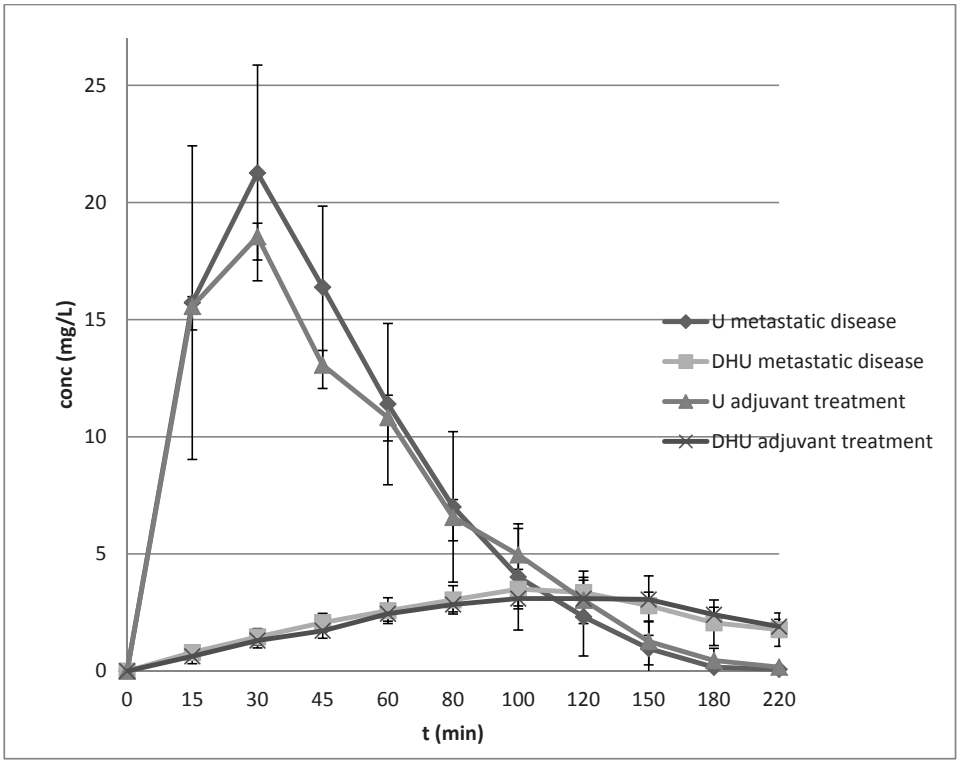

Figure 8.2 Time versus concentration curve of the mean ± SD values of U and DHU in both study groups.

DISCUSSION

This is the first study investigating the potential effect of metastatic disease on uracil pharmacokinetics in CRC patients with a normal DPD activity. Our results show that metastasis has no effect on uracil pharmacokinetics. Hypothetically, the extent of metastatic disease might influence DPD activity and uracil pharmacokinetics. In our study the patients with metastatic disease all had a good health performance. Liver enzymes were normal in both study groups and no patients with extreme cachexia have been identified. Therefore, we cannot fully exclude that the presence of cachexia, commonly seen in metastatic disease, might influence uracil PK.

This study did not reveal a difference in uracil pharmacokinetics between patients adjuvantly treated for CRC and those with metastatic disease with the exception of C_{max}. The C_{max} observed for the adjuvantly treated group is in line with C_{max} observed in a previous study [9]. C_{max} is determined by dose, absorption/degradation rate of a drug and volume of distribution, which are

considered to be equal between the two study groups. Based on the pharmacokinetic profile of capecitabine and the fact that both groups were treated with capecitabine, we find it not likely that capecitabine migh contribute to this observation.In the metastatic group however, patients were treated with bevacizumab. The test was performed more than 14 days after bevacizumab administration, but since the long elimination half time of approximately 20 days of this drug, we cannot exclude the possibility that bevacizumab or the presence of metastatic disease might influence the gastrointestinal absorption and hence C_{max}. C_{max} however does not play a role of interest if the test is used to discriminate between DPD-deficient individuals and individuals with a normal DPD status. In this context we focused on uracil and DHU levels at t=120 min.

In this study, we did not perform a pharmacokinetic analysis of dihydrouracil. Dihydrouracil has its own unique elimination pathway (Figure 8.1). Dihydropyrimidinase deficiency is very rare and since dihydropyrimidinase deficiency is very rare and its effect on the toxicity of fluoropyrimidines is not known, we dit not investigate the pharmacokinetics off dihyrouracil.

We enrolled only patients with a normal DPD activity in the study. This study setup was chosen to exclude the effect of *DPYD* polymorphisms that have a large effect on uracil pharmacokinetics [17]. Such as large decrease of DPD enzyme activity caused by DPD polymorphisms would have excluded the detection of a smaller effect of metastatic disease on uracil PK. In this study, concomitant use of DPD-inhibiting medication such as cimetidine was not allowed and could therefore not have confounded the results.

The orally administered uracil did not result in any adverse events in our patients and can be used safely. Orally administered uracil 500 mg/m^2 is considered to saturate DPD fully during the period that plasma concentration levels are above the Michaelis constant [8]. Because of this, differences in DPD activity between individuals will become more profoundly clear than the determination of physiological levels of uracil/DHU ratios, which show high variation between individuals [5,18-21]. Our oral uracil loading test is useful to be introduced into clinical practice. However, the test can be further optimized with a limited sample strategy in combination with the dried blood spot method for sample selection and patient convenience. In conclusion, in patients with a normal DPD activity, with the exception of C_{max} we found no evidence for different uracil pharmacokinetics in patients with metastatic CRC as compared to patients treated adjuvantly. Since C_{max} is not used as a discriminating parameter, orally administered uracil could therefore be used as a DPD phenotype test in both adjuvantly treated and metastatic CRC patients using the same cut off criteria. The results of this study look very promising to use the oral uracil loading dose as a easy and robust test to evaluate DPD activity in a clinical setting.

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