Assessment of Pharmacokinetic Interaction Between Capecitabine and Cetuximab in Metastatic Colorectal Cancer Patients

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Abstract. Aim: This study focuses on the plasma disposition and metabolic activation of capecitabine (CCB) when administered alone or when combined with cetuximab (CTX). Patients and Methods: Twenty-four chemo-naïve patients with KRAS wild-type colorectal cancer were randomized into two arms and received either CCB alone (1,000 mg/m² bid p.o.), followed by CCB plus CTX (loading dose (LD)=400 mg/m² followed by 250 mg/m² weekly i.v. maintenance dose) (Arm A; n=12 patients (patients)) or CCB plus CTX followed by CCB alone (Arm B; n=12 patients). Plasma samples were collected from the cubital vein and CCB, 5'-desoxy-5-fluorocytidine (5'-DFCR) and 5'-desoxy-5 fluorouridine (5'-DFUR) were quantified by a sensitive, selective reversed phase high-performance liquid chromatography (HPLC) assay. Non-compartment pharmacokinetic parameters have been calculated by Phoenix WinNonlin. Results: No clinically relevant impact of CTX on CCB pharmacokinetic parameters and metabolic conversion could be detected in both arms after statistical evaluation (ANOVA). Conclusion: From the pharmacokinetic point of view, co-administration of CTX to CCB seems to be safe.

Today, the first-line treatment for metastatic colorectal cancer (mCRC) comprises a combination of 5-fluoruracil (5-FU),

folinic acid or the 5-FU prodrug capecitabine (CCB) or irinotecan with oxaliplatin (OX) (1). The fluoropyrimidine carbamate CCB has been developed to generate 5-FU within the tumor and to mimic the therapeutic effect of a 5-FU infusion but with the advantage of being orally administered, which leads to easier treatment in therapy. It is absorbed through the gastrointestinal wall as an intact molecule and, then, rapidly metabolized to 5-FU via a three-step enzymatic cascade as depicted in Figure 1.

In recent studies, these first-line regimens, called FOLFIRI (5-FU, folinic acid and irinotecan) or FOLFOX (5-FU, folinic acid and OX), have improved the response rate and progression-free survival (PFS) of patients with mCRC. A median survival time of about 20 months is observed (2, 3).

Since the positive effects of a targeted chemotherapy using monoclonal antibodies, such as bevacizumab or cetuximab (CTX), have become obvious, several new concepts have undergone clinical scrutiny. Treatment with CTX, a monoclonal antibody directed against the epidermal growth factor receptor, improved overall survival (OS) and PFS and preserved the quality of life in patients with KRAS wild type mCRC (4). In several preclinical models, CTX has been proven to increase tumor cell apoptosis, suppress invasion and metastasis, inhibit proliferation and down-regulate the production of pro-angiogenic factors (5, 6). Investigators have found that the addition of CTX to 5-FU demonstrated significant synergistic growth inhibition in colorectal xenograft tumors (7). Although there has not been sufficient research on the combination of CCB plus CTX, several studies have demonstrated the feasibility and efficacy of the triple-combination of XELOX (CCB and OX) with CTX (5, 8). The CELIM trial focused on FOLFIRI plus CTX and FOLFOX plus CTX for patients with non-resectable

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Figure 1. Metabolic activation of capecitabine (CCB).

liver metastases without hepatic disease. Resectability rates were evaluated after 8 and 12 cycles of therapy. The confirmed overall response rates were 57% (FOLFIRI arm) and 68% (FOLFOX arm) with 38% and 30% R0 resections, respectively (9). However, recently analyzed data from a subgroup analysis of the phase III COIN study showed inferior outcome and tolerability of CTX plus XELOX to CTX plus FOLFOX (10). A pharmacokinetic study concerning a possible interaction between CCB and OX demonstrated a lack of influence of OX on plasma disposition and metabolic activation of CCB (11). As a consequence, it is yet to be proven whether there exists a pharmacokinetic interaction between CCB and CTX or not.

The objective of this phase II pharmacokinetic trial was to assess the pharmacokinetics and the metabolic activation of the prodrug CCB when CTX was co-administered in two different sequences (Arm A and Arm B).

Patients and Methods

Patients' eligibility. Male and female patients older than 18 years with histologically confirmed KRAS wild type metastatic colorectal carcinoma were screened for this study. They needed to be eligible for therapy with CCB and CTX. Other key inclusion criteria were Karnofsky performance status of ≥ 80 at study entry, no known or suspected brain metastasis, no concurrent chronic systemic immune therapy or hormone therapy and no previous chemotherapy for metastatic disease for at least 6 months. Adequate bone marrow function (leucocytes $\geq 3.0 \times 10^9/l$ and neutrophils $\geq 1.5 \times 10^9/l$, platelets $\geq 100 \times 10^9/l$ and hemoglobin ≥ 8 g/dl), renal function (serum creatinine $\leq 1.5 \times$ the upper limit of normal (ULN)) and hepatic function (serum bilirubin $\leq 1.5 \times$ ULN, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) levels $\leq 2.5 \times$ ULN or $\leq 5.0 \times$ ULN if liver metastases are present) were required. Women of reproductive

potential were required to use effective contraception. Study-specific exclusion criteria included stage 3 or 4 heart failure defined according to the New York Heart Association (NYHA), uncontrolled angina, any concurrent malignancy other than non-melanoma skin cancer or carcinoma *in situ* of the cervix (patients with a previous malignancy but without evidence of disease for ≥ 5 years were acceptable), administration of any investigational agents within 4 weeks prior to entry, previous exposure to epidermal growth factor receptor (EGFR) pathway targeting therapy, known grade 3 or 4 allergic reaction to any of the components of the treatment, pregnancy, lactation and/or known drug or alcohol abuse. This study was approved by the ethics committee of the Medical University of Vienna (EudraCT Number 2011-002921-23) and all patients provided written informed consent. Table I characterizes the two collectives.

Study design and treatment. The study was designed as a multi-center, parallel group phase II trial. Randomization of 24 chemo-naïve patients into two arms (arm A and arm B) allowed the exclusion of potential carry-over effects. Within each arm, patients served as their own controls to minimize problems associated with inter-patient variability. CCB and/or CTX were given for 9 weeks; in week 10, the therapy was switched to the continuous standard regimen of XELOX plus CTX. As described in Table II, in Arm A, patients received either CCB alone (1st cycle), followed by CCB plus CTX (2nd cycle). In Arm B, the 1st cycle consisted of CCB and CTX, followed by CCB alone (2nd cycle).

CCB (Xeloda®) was obtained from Roche Austria GmbH (Vienna, Austria) and given p.o. 1 h after standard breakfast or evening meal at doses of $1,000 \text{ mg/m}^2$ bidaily. CTX (Eribitux®) was supplied by Merck Austria GmbH (Vienna, Austria) and administered intravenously as a loading dose of 400 mg/m^2 , followed by weekly maintenance doses of 250 mg/m^2 . During the study period no dose modifications were allowed.

Blood samples. Blood samples of 5 ml each were drawn from the cubital vein in week 1 (day 1) in week 4 (day 1) at the following times: pre-dose, 30, 60, 90, 120, 150, 180, 240, 300 and 360 min

Table I. *Patients' characteristics.*

	Gender female/male	Age (years) mean	Weight (kg) mean±SD	Height (cm) mean±SD	Body surface area (m ²) mean±SD
Arm A (n=12)	5/7	64.2	72.4±10.6	169.8±6.9	1.8±0.1
Arm B (n=12)	4/8	64.3	79.3±14.9	175.4±10.7	1.9±0.2

Table II. *Dose schedule.*

	Cycle 1			Cycle 2			Dose
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	
A n=12	CCB	CCB	CTX	CCB	CCB	CTX	1,000 mg/m ² bidaily <i>p.o.</i> LD=400 mg/m ² followed by 250 mg/m ² wkly <i>i.v.</i>
B n=12	CCB	CCB		CCB	CCB		1,000 mg/m ² bidaily <i>p.o.</i> LD=400 mg/m ² followed by 250 mg/m ² wkly <i>i.v.</i>
	CTX						

LD, Loading dose; *p.o.*, orally; *i.v.*, intravenous; wkly, weekly; CCB, capecitabine; CTX, cetuximab.

after the first oral morning CCB dose was administered. In order to obtain information about a probable accumulation of CCB in the blood, additional plasma samples were collected in week 1, in week 4 and in week 7 on day 5 of therapy at 30, 60, 90 and 120 min after morning intake of Xeloda® tablets.

Samples were collected in heparinized vacutubes and red blood cells were separated by centrifuging at 2,500 rpm for 10 min. From the supernatant, two portions each of 1.0 ml were put into a 1.5 ml Eppendorf tube and frozen immediately at -80°C until high-performance liquid chromatography (HPLC) analysis.

Sample preparation. A solid phase extraction (SPE) was used to remove the matrix components from the analytes. For preconditioning, the cartridges (Oasis HLB C18, 30 m, 1ccm packing volume; Waters Corporation, Milford, MA, USA) were activated with 1.0 ml of methanol and pre-conditioned with 1.0 ml of water. After applying 1.0 ml of plasma sample, 5'-desoxy-5-fluorocytidine (5'-DFCR) and 5'-desoxy-5-fluorouridine (5'-DFUR) were eluted from the cartridge by 1.0 ml of water-methanol (95:5 %, v/v). From the eluate, an aliquot of 30 µl was injected into the HPLC to quantify 5'-DFCR and 5'-DFUR. Subsequently, CCB was eluted from the same cartridge with 1.0 ml of methanol and 10 µl of the eluate was injected into the HPLC apparatus for the quantification of CCB.

Analytical procedure. Quantification of CCB, 5'-DFCR and 5'-DFUR in plasma samples was performed by two different reversed-phase HPLC assays, which were established in our laboratories not long ago and published in full detail recently (12, 13). For CCB, the limit of quantification (LOQ) was 156 ng/ml, while the limit of detection (LOD) was 78 ng/ml; signal to noise ratio of 1:5. For both metabolites, the LOQ was 156 ng/ml and the LOD was found to be 39 ng/ml referring to a signal-to-noise ratio of at least 1:5. Quantitation of CCB and its metabolites in unknown plasma samples was performed by external standard method using as calibration graph.

Biometric calculations. Curve fitting of drug and metabolite plasma concentration-time data was performed by the pharmacokinetic (PK) scientific software Phoenix WinNonlin version 6.0 (Certara Inc., Princeton, NJ, USA) using Nelder-Mead nonlinear iterative least square curve fitting algorithm. A non-compartment model for extravascular input (model 3 of the WinNonlin library) was chosen for PK modeling of CCB and its metabolites.

The following parameters were analyzed for CCB: *t*_{max}, time to reach peak plasma concentration (min); *c*_{max}, peak plasma concentration (µg/ml); AUC₀₋₃₆₀, area under the concentration-time-curve from 0 to 360 min (µg/ml*min); AUC₀₋₁₂₀, area under the concentration-time-curve from 0 to 120 min (µg/ml*min); MRT_{last}, mean residence time from 0 to *t*_{last} (min); *V*_z: volume of distribution for the central compartment (l); Cl_{tot}, total body clearance (l/h); *t*_{1/2} λ_z, half-life of terminal elimination (min). *V*_z and Cl_{tot} were not calculated for 5'-DFCR and 5'-DFUR.

Additionally, an apparent formation rate (*R*) of the metabolites catalyzed by carboxylesterase (*hCES*) and cytidine-deaminase (*CytDA*), respectively, has been calculated by dividing the metabolite AUC by its precursor AUC. In order to be able to compare the enzyme activity of day 1 with the enzyme activity of day 5, we used the AUC₀₋₁₂₀ for the calculations. The area from 0 to 120 min has been chosen because, within this time span, most of the metabolites are formed. A high *R* value indicates high activity of the responsible metabolizing enzyme.

$$R_{hCES} = \frac{AUC_{0-120} DFUR}{AUC_{0-120} CCB} \quad R_{CytDA} = \frac{AUC_{0-120} DFUR}{AUC_{0-120} DFCR}$$

All statistical analyses were performed using GraphPad Prism® Version 6.0 and StatMate 2.0 (GraphPad Software Inc., La Jolla, CA, USA) with a minimum significance level of *p*<0.05. Probable statistical outliers were identified by the Grubb's or Route test using

Figure 2. Mean plasma concentration-time curves of capecitabine (CCB). A (Arm A, week 1) and D (Arm B, week4) show the CCB regimen, B (Arm A, week 4) and C (Arm B, week1) the combination therapy of CCB plus cetuximab (CTX).

the extreme studentized deviate method, with these outlying subjects being excluded from the data set for all further calculations. Depending on the characteristics of the data and the tested subgroups, the unpaired or the paired two sided Student's *t*-test, the analysis of variance (ANOVA) or the Kruskal-Wallis H test were performed to reveal a potential significant difference between the groups.

Results

Plasma concentrations. The plasma concentration-time curves of CCB, 5'-DFCR and 5'-DFUR obtained after administration of 1,000 mg/m² CCB on day 1 in week 1 and 4 are depicted in Figures 2-4, respectively.

As can be seen from Figure 2, peak plasma concentrations (and area under the concentration-time curve (AUC_{last})) of CCB remained unaffected by CTX administration. The sequence of either CCB followed by CTX or CTX followed by CCB administration also shows no effect on plasma concentrations time curves of CCB. Maximum CCB concentrations were reached within 30 to 60 min. CCB concentrations were detectable at 360 min after ingestion in almost all blood

samples. Concentration-time-profiles of CCB were very similar for all subgroups. However, in all subgroups of CCB, higher peak concentrations (C_{max} =9-15 µg/ml) were observed compared to the literature (C_{max} =3-7 µg/ml) (14). Mean plasma concentrations of CCB were somehow lower in the CCB plus CTX group in Arm A.

Concentration-time profiles of the subsequent metabolite 5'-DFCR (Figure 3) were very similar to the profiles of the parent compound with peak concentrations occurring slightly later than those of CCB. This delay in time to reach peak concentrations of 5'-DFCR gives evidence for a fast conversion of CCB into the subsequent metabolite 5'-DFCR. 5'-DFCR concentrations were measurable in the blood over the whole investigation time. Comparable to CCB, the administration sequence has no impact on the concentration-time curves of 5'-DFCR.

The plasma concentrations of the 5-FU precursor 5'-DFUR were of a similar order of magnitudes in the CCB regimen and when administered in combination with CTX. C_{max} of 5'-DFUR and of its two precursors occurred in all groups within 90 min after CCB administration.

Figure 3. Mean plasma concentration-time curves of 5'-deoxy-5-fluorocytidine (5'-DFCR). A (Arm A, week 1) and D (Arm B, week 4) show the distribution of 5'-DFCR when capecitabine (CCB) is administered alone, B (Arm A, week 4) and C (Arm B, week 1) show the combination therapy of CCB plus cetuximab (CTX).

5'-DFUR plasma concentrations were comparable to those of 5'-DFCR. Peak concentrations occurred 60 min after administration and the metabolite was detectable in the blood over the whole time period.

As can be seen from all Figures, the inter-patient variability was rather low and the mean plasma concentration-time curves of CCB and both metabolites were overall similar with low standard deviations.

Pharmacokinetics. The PK parameters of all compounds are listed in Table III as their geometric mean plus 95% confidence interval (CI) with the exception of $t_{\max}$; this parameter is calculated as its median value ($\pm$ SD).

No significant differences in $c_{\max}$ or AUC_{last} between control group and combination regimen plus CTX could be observed for CCB. CTX was associated only with a non-significant prolongation of $t_{\max}$ and MRT_{last} for CCB ($t_{\max} = +0.25\%$, $MRT_{\text{last}} = +3.20\%$). $t_{1/2}$ λ_z showed a weak non-significant increase for CCB in the combination regimen

of CCB plus CTX. Plasma clearance (Cl_{tot}) and volume of distribution (V_z) of CCB were in a similar order of magnitude without significant differences when CTX was added to the therapy.

$C_{\max}$ and AUC_{last} of 5'-DFCR remained unaffected by adding CTX to the therapy. However, CTX was also associated with a non-significant prolongation of $t_{\max}$ and MRT_{last} for 5'-DFCR.

A weak non-significant increase of $t_{1/2}$ λ_z for 5'-DFCR can be seen in the CCB plus CTX group compared to the control group.

$C_{\max}$ and AUC_{last} of 5'-DFUR were decreased in the combination therapy with CTX but without significance. Minor alterations of MRT_{last} and a slight reduction of $t_{\max}$ for 5'-DFUR were observed in the combination regimen. The half-life of terminal elimination of 5'-DFUR was comparable for all subsets.

All observed differences in the other PK data of CCB and its metabolites were not statistically significant.

Figure 4. Mean plasma concentration-time curves of 5'-deoxy-5 fluorouridine (5'-DFUR). A (Arm A, week 1) and D (Arm B, week 4) show the plasma concentration-time curve of 5'-DFUR in the capecitabine (CCB) regimen, B (Arm A, week 4) and C (Arm B, week 1) show the combination therapy of CCB plus cetuximab (CTX).

In addition, we explored the PK of CCB, when given in different sequences of administration: CCB administered alone (Arm A, week 1) followed by CCB plus CTX (Arm A, week 4) or CCB plus CTX (Arm B, week 1) followed by CCB (Arm B, week 4). C_{max} , AUC_{last} and t_{max} of CCB appear to be slightly higher in Arm A but they did not differ significantly. No alterations of MRT_{last} could be observed in both therapy regimens. Plasma clearance and volume of distribution of CCB increased non-significantly when CTX was added to the therapy before the CCB cycle started (Arm B).

As for 5'-DFUR, C_{max} , AUC_{last} and t_{max} appear to be slightly non-statistically higher in Arm A as well. No alterations of MRT_{last} could be observed for 5'-DFUR.

In contrast, C_{max} , AUC_{last} and t_{max} of the 5-FU precursor 5'-DFUR seem to increase non-significantly when the subject was randomized in Arm B. Despite this, all other PK parameters of 5'-DFUR show no statistically significant differences in both groups.

Overall, our findings, even when research focuses at the different sequences of co-administering CTX, do not have a significant impact on CCB pharmacokinetics.

Dividing the product AUC_{last} by the precursor AUC_{last} makes it possible to estimate a specific activity of the enzyme that catalyses the product formation. AUC_{0-120} values were used to calculate these apparent formation rates of CCB metabolites. Table IV presents the R-factors for hCES and CytDA.

The activity of RhCE seemed to be increased by CTX by about 6% in arm A and by about 38% in arm B ($p=0.18$). This result is favorable because a high R value indicates a high activity of the responsible converting enzyme.

The activity of RcytDA was slightly increased by CTX by about 7% in arm A, while, contrarily, in Arm B was non-significantly ($p=0.16$) reduced by about 22%. As an overall conclusion, changes in R values for both enzymes showed no statistically significant difference and were negligible.

Table III. Pharmacokinetic parameters (expressed as their geometric mean plus 95% confidence interval, t_{max} median value $\pm$ SD) of capecitabine (CCB), 5'-desoxy-5-fluorocytidine (5'-DFCR) and 5'-desoxy-5 fluorouridine (5'-DFUR) for both arms of the study.

CCB	ARM A		ARM B	
	CCB	CCB+CTX	CCB+CTX	CCB
c_{max} [μ g/ml]	11.05 (8.30-13.80)	8.74 (6.70-10.78)	10.43 (2.39-18.46)	9.70 (6.13-13.28)
t_{max} [min]	45 $\pm$ 43	60 $\pm$ 52	30 $\pm$ 41	30 $\pm$ 30
AUC _{last} [min* μ g/ml]	563 (428-699)	479 (392-566)	552 (175-928)	550 (402-699)
$t_{1/2} \lambda_z$ [min]	22 (16-29)	22 (15-28)	28 (14-41)	24 (14-34)
MRT _{last} [min]	67 (49-84)	84 (54-114)	69 (47-92)	67 (50-85)
V _z [l]	101 (73-129)	118 (84-149)	137 (40-233)	120 (41-200)
Cl _{tot} [l/h]	3.2 (2.3-4.1)	3.8 (3.0-4.5)	3.4 (1.9-5.0)	3.5 (2.4-4.6)
5'-DFCR	CCB	CCB+CTX	CCB+CTX	CCB
c_{max} [μ g/ml]	6.35 (4.40-8.20)	6.25 (4.08-8.42)	7.03 (4.46-9.59)	6.28 (4.72-7.83)
t_{max} [min]	60 $\pm$ 45	75 $\pm$ 52	60 $\pm$ 35	45 $\pm$ 35
AUC _{last} [min* μ g/ml]	550 (430-670)	575 (306-844)	720 (409-1031)	613 (482-745)
$t_{1/2} \lambda_z$ [min]	43 (33-53)	50 (17-82)	53 (21-84)	45 (39-52)
MRT _{last} [min]	92 (73-111)	116 (87-144)	105 (84-126)	96 (80-113)
5'-DFUR	CCB	CCB+CTX	CCB+CTX	CCB
c_{max} [μ g/ml]	6.52 (3.69-9.36)	5.39 (2.40-8.38)	5.84 (4.34-7.35)	7.28 (4.87-9.69)
t_{max} [min]	60 $\pm$ 52	105 $\pm$ 50	60 $\pm$ 45	60 $\pm$ 39
AUC _{last} [min* μ g/ml]	513 (367-658)	480 (330-631)	567 (458-677)	653 (507-799)
$t_{1/2} \lambda_z$ [min]	43 (33-53)	43 (18-68)	46 (29-63)	49 (30-67)
MRT _{last} [min]	94 (67-122)	118 (89-148)	103 (83-124)	93 (74-110)

c_{max} , Peak plasma concentration; t_{max} , time to reach peak plasma concentration; AUC_{last}, area under the concentration-time-curve; $t_{1/2} \lambda_z$, half-life of terminal elimination; MRT_{last}, mean residence time from 0 to tlast; V_z: volume of distribution for the central compartment; Cl_{tot}, total body clearance.

Discussion

In this pharmacokinetic study, we report the metabolic activation of CCB when given in combination with CTX in two different sequences of both drugs.

CCB and CTX are metabolized *via* different biochemical pathways and, therefore, a PK drug-drug interaction seems rather unlikely. CCB is metabolized *via* human carboxylesterase; CTX, in contrast, is thought to be metabolized by the reticuloendothelial system, without undergoing hepatic or renal metabolism. Therefore, monoclonal antibodies can be administered in patients with renal or hepatic dysfunction (15-18).

As can be seen from Figures 2-4, the inter-patient variability was rather low and the mean plasma concentration-time curves of CCB and both metabolites were overall similar with low standard deviations. This lets us conclude that the study protocol, which specified that CCB had to be ingested 1 h after standard breakfast or meal, was followed at a high degree of compliance.

The peak concentrations of 5'-DFCR and 5'-DFUR appeared slightly later than those of CCB, which confirms a fast conversion of CCB into the first metabolite.

Table IV. Apparent formation coefficients (R_{enzyme}) for 5'-desoxy-5-fluorocytidine (5'-DFCR) and 5'-desoxy-5 fluorouridine (5'-DFUR)-metabolizing enzymes.

	ARM A		ARM B	
	CCB	CCB+CTX	CCB+CTX	CCB
RhCES	0.83 $\pm$ 0.49	0.89 $\pm$ 0.32	1.35 $\pm$ 1.27	0.97 $\pm$ 0.49
RcytDA	1.11 $\pm$ 0.50	1.18 $\pm$ 0.74	0.90 $\pm$ 0.41	1.12 $\pm$ 0.32

CCB, Capecitabine; CTX, cetuximab; R, formation rate; hCES carboxylesterase, cytDA; cytidine-deaminase.

Table III comprises the pharmacokinetic parameter of CCB, 5'-DFCR and 5'-DFUR. In summary, all observed differences in the PK data of CCB and its metabolites were not statistically significant (Student's *t*-test, ANOVA). Changes of R values of hCES and CytDA were not significantly different in both arms of the study and negligible in regard to metabolic activation. However, higher C_{max} values for all subgroups of CCB were observed as compared to the literature. CCB is a high-affinity substrate

for hCES; therefore, the metabolic conversion into DFCR occurs rapidly and at a high extent.

The degree to which the stomach is full plays an essential role in the absorption of drugs: ingestion of CCB after continental breakfast is recommended for rapid absorption of the drug from the gut into the blood. As studies exposed before fasted conditions, increase both the rate and the extent of CCB absorption and, therefore, the intake of a standard meal prior to the administration of CCB is important (19, 20). Consequently, the poor health status of the patients and the, thereby, reduced appetite can explain the increase of C_{max} of CCB and its metabolites. Another important factor is the pH value in the gastrointestinal tract because it determines the grade of ionization of a compound, which, consequently, is responsible for the extent of absorption into the blood (21). CCB is absorbed faster in a stomach with low pH (19). *In vitro* studies have revealed that CCB is unstable under strongly acidic conditions. Degradation of CCB in a stomach at very low pH might result in a decrease of the amount of unchanged drug being available for absorption (14).

Further, the co-administration of other drugs may increase the variability of plasma concentrations of CCB and metabolites (22). It has been demonstrated that increases in the C_{max} and AUC_{0-last} values up to 30% could be obtained for CCB and 5'-DFCR when the over the counter antacid Maalox® was given together with CCB (23).

The health status of the patients often required the administration of co-medication (up to 10 drugs per patient) that may have had an impact on the disposition of CCB and its metabolites in the body. It is essential to know all drugs of medication during the validation procedure and for the pharmacokinetic concept of the clinical proposal. We recently gave evidence for an analytical interference (peak overlap) between CCB and pantoprazole, a widely prescribed proton-pump inhibitor (PPI) (20). Such interferences may lead to false pharmacokinetic results and have to be excluded prior to initialization of the clinical study.

Many studies have examined the combination therapy of CCB with first-line treatment for metastatic colorectal cancer. Some years ago, we investigated the impact of two of these drugs (OX and of bevacizumab) on the metabolic activation of CCB. In both studies, absolutely no indices for a PK drug-drug interaction could be shown (11, 24).

In some pharmacokinetic studies, the potential of monoclonal antibodies to modulate the pharmacokinetics of cytostatic drugs has been demonstrated. We could show that CTX, when combined with irinotecan, also has no impact on the pharmacokinetics of the cytostatic drug and its main metabolites (25-27).

The data obtained here from our phase II pharmacokinetic study are in line with the above mentioned studies. Although an increase of plasma concentration of metabolites is

favorable, no clinically relevant drug-drug interaction between CCB and CTX could be detected. We provided evidence that an amount of 6-8 µg/ml of 5'-DFUR, the immediate precursor of 5-FU, is formed independently whether CCB is administered alone or combined with CTX. From the pharmacokinetic point of view, the combination therapy CCB plus CTX is a safe regimen without modified plasma concentration-time profiles.

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