A Rapid and Simple HPLC Assay for Quantification of Capecitabine for Drug Monitoring Purposes

ANDRE FARKOUH[1], DAGMAR ETTLINGER[1], JOHANNES SCHUELLER[2], APOSTOLOS GEORGOPOULOS[3], WERNER SCHEITHAUER[4] and MARTIN CZEJKA[1]

[1]Department of Clinical Pharmacy and Diagnostics, Faculty of Life Sciences, University of Vienna, A-1090 Vienna, Austria;
[2]Department of Internal Medicine and Oncology, Rudolfstiftung Hospital, A-1030 Vienna, Austria;
Departments of [3]Internal Medicine and [4]Oncology, University Clinic of Internal Medicine I, Medical University Vienna, Vienna General Hospital, A-1090 Vienna, Austria

Abstract. *Aim: Capecitabine, a 5-fluorouracil prodrug, has been integrated into the management of multiple cancer types. In order to obtain information about plasma levels of capecitabine in patients who had drug intake at home during chemotherapy, a simple HPLC method for capecitabine monitoring has been developed and validated. Patients and Methods: Capecitabine levels were quantified by a simple reversed-phase HPLC system with an external standard method. Plasma samples were obtained from 12 colorectal cancer patients who underwent chemotherapy with capecitabine alone (1000 mg/m^2) or combined with oxaliplatin (130 mg/m^2). Results: Although there was evidence that capecitabine had not been taken according to the chemotherapeutic schedule in two cases, the study demonstrated that its combination with oxaliplatin showed no significant drug–drug interactions. Conclusion: Due to its robustness, specificity and sensitivity, the method is also well-suited for capecitabine analysis in other pharmacokinetic studies.*

The fluoropyrimidine carbamate capecitabine (CCB), a 5-fluorouracil (5-FU) prodrug, represents an antitumour agent for the treatment of multiple cancer types, with a special emphasis on gastrointestinal tumours (1). This drug is at least as effective as 5-FU/leucovorin, with significant superiority in relapse-free survival and a trend towards improved disease-free and overall survival (2, 3). After resorption from the gut, CCB is first hydrolysed in the liver into 5'deoxy-5-fluorocytidine (5'-DFCR) by a 60 kDa human carboxylesterase (hCE) (4). Subsequently 5'-DFCR is metabolised by cytidine deaminase into 5'-deoxy-5-fluorouridine (5'-DFUR), which itself is converted finally by pyrimidine-nucleoside phosphorylase inside the tumour cell, giving the cytotoxic agent 5-FU (5) (Figure 1). Inside the tumour cell, the enzyme dihydropyrimidine dehydrogenase hydrogenates 5-FU to the much less toxic 5-fluoro-5,6-dihydro-fluorouracil. Dihydropyrimidinase cleaves the pyrimidine ring to yield 5-fluoro-ureido-propionic acid, which is finally cleaved to α-fluoro-β-alanine by β-ureido-propionase and eliminated in the urine (6).

As CCB is administered in tablet form (Xeloda®; Hoffmann-La Roche Ltd., Basle, Switzerland), it represents a major advance in the treatment of colorectal cancer, allowing patients to take their medication at home and minimising the disruption to their lives. Standard dose is 1000 mg/m^2 body surface area given twice daily at equal doses approximately 12 h apart (7).

Because of home medication, it is essential to determine patients' compliance in order to maximise the therapeutic outcome. By using the HPLC assay described below, plasma samples may be analysed quickly, with low cost and without time-consuming analytical processes, compared to other analytical assays (8).

The combination of CCB and oxaliplatin (OxPt) has demonstrated promising synergistic activity in advanced colorectal cancer therapy (9, 10). Because of the rather complex enzymatic conversion of CCB, its metabolism may be easily influenced by other co-administered drugs. Therefore this pharmacokinetic (PK) study was designed to evaluate whether CCB is metabolised to the same extent when administered together with OxPt.

Correspondence to: André Farkouh, Department of Clinical Pharmacy and Diagnostics, Faculty of Life Sciences, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria. E-mail: andre.farkouh@univie.ac.at

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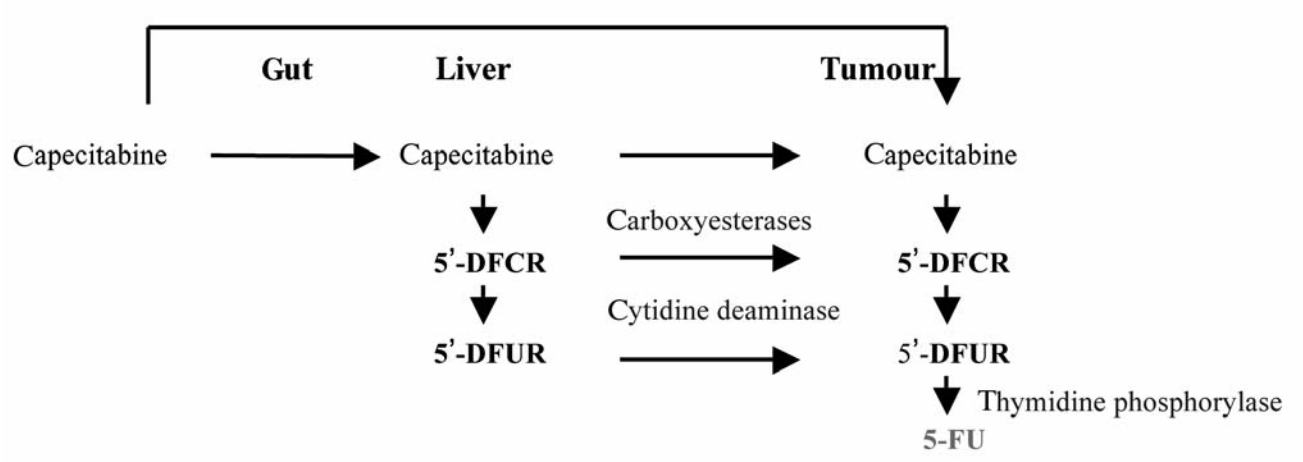

Figure 1. *Three-step in vivo activation of capecitabine.*

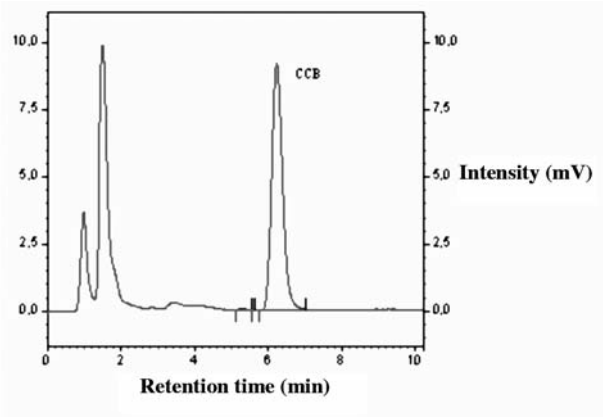

Figure 2. *HPLC chromatogram of capecitabine (CCB) after SPE out of human plasma samples (retention time: 6.223 min).*

Table I. *Characterization of calibration graph.*

Slope	347.2±1.341
Y-intercept when X=0.0	–1.246±2.737
X-intercept when Y=0.0	0.003588
1/slope	0.002880
95% Confidence intervals	
Slope	343.9 to 350.4
Y-intercept when X=0.0	–7.943 to 5.452
X-intercept when Y=0.0	–0.01580 to 0.02275
Goodness of Fit	
r^2	0.9999
Sy.x	6.135
F	67030.0
DFn, DFd	1.000, 6.000
p-Value	0.000097
Deviation from zero?	Significant
Number of X values	8

Materials and Methods

Materials. CCB (pure chemical substance, batch No.W0129 MFD 02 2002) was supplied by Hoffmann-La Roche Ltd. for preparation of standard plasma samples. Water and methanol of HPLC grade purity were obtained from Baker (Deventer, the Netherlands).

Sample preparation. Matrix components were removed from plasma samples by solid-phase extraction (SPE). The samples were passed under vacuum (pressure of –20 kPa, flow rate of approximately 1 ml/min) through SPE cartridges (Oasis HLB 1cm^3, 30 mg packing volume; Waters, Milford, MA, USA). After washing the cartridges with 1 ml of methanol, 1 ml of water was applied for preconditioning. A total of 1 ml plasma sample was forced through the cartridge subsequently. After washing-out of the matrix components by 1 ml of water-methanol (95:5%, v/v), CCB was eluted from the cartridge with 1 ml of methanol and vortexed for 5 s. An aliquot of 30 μl was injected into the HPLC.

Due to the high polarity of the CCB metabolites (5'-DFCR, 5'-DFUR and 5-FU), they eluted from the SPE cartridge during loading with plasma sample. This eluate may be used for the determination of all metabolites by a separate HPLC run. In the present study, only the last eluate (after matrix components were removed) was used in order to shorten the analytical time for CCB monitoring of human plasma.

Chromatography. An La Chrom liquid chromatograph system (Merck Hitachi Ltd., Tokyo, Japan) was used, consisting of an L-7100 pump, an L-7200 autosampler, an L-7300 column oven, an L-7400 UV-detector and an L-7450 diode array detector. HPLC modules were connected by a D7000 interface to a Compaq computer (Compaq Computer Corp., Harris County, TX, USA).

Table II. *Stability and recovery of capecitabine.*

Initial sample concentration (ng/ml)	Week 1		Week 2		Week 3	
	Concentration (ng/ml)	Recovery (%)	Concentration (ng/ml)	Recovery (%)	Concentration (ng/ml)	Recovery (%)
312	305	97.8	290	92.9	322	103.2
625	666	106.6	597	95.5	611	97.8
1250	1323	105.8	1280	102.4	1157	92.6
2500	2455	98.2	2521	100.9	2595	103.8

Module control and data collection were performed by using the HSM control and integration software (Merck Hitachi Ltd.).

Separation of CCB from matrix components was performed on a LiChrospher® 100 (Merck, Darmstadt, Germany) column RP-18 endcapped (5 μm), protected by a guard column 10×4 mm ID., 5 μm RP-18 (LiChrocart 4-4; Merck). The mobile phase consisted of water and methanol of HPLC grade purity (50 + 50%, v/v); flow rate was 0.5 ml/min (pressure, 91 bar at 36˚C). No buffer was required in the mobile phase system. CCB was detected at 305 nm, with high sensitivity and standard response. Retention time of CCB was 6.2±0.5 min. No interferences with matrix peaks or pre-/co-administered compounds were observed (Figure 2).

The column efficiency was determined by injection of standard solutions as described in the manufacturer's manual. The number of theoretical plates ($NW_{1/2}$ for Europe) was calculated at half peak height ($W_{1/2}$) by the following equation: $NW_{1/2}=5.54 \times (tR/W_{1/2})^2$ (W and tR are expressed in seconds). The tailing factor (T) was determined by the following equation: $T= W_{0.05}/2 \times b_{0.05}$, where W represents the peak width at 5% of the peak height and b is the peak width from the centre to the leading edge at 5% of the peak height. The capacity factor (k') was determined by the following equation: $k'= (tR-t_0)/t_0$, where tR is the retention time of CCB and t_0 is the retention time of the non-eluting peak.

Calibration. Quantitation of CCB in unknown plasma samples was performed by using an external standard method. To obtain the calibration graph, pooled plasma samples from healthy volunteers were spiked with CCB (stock solution of 1 mg/ml water) giving the following final concentrations: 20000, 10000, 5000, 2500, 1250, 625, 312 and 156 ng/ml (Table I).

The plasma calibration samples were solid-phase extracted and chromatographed identically as the patient samples. For determination of LOQ and LOD, additional standard plasma samples were prepared with CCB concentrations of 156, 78, 39 and 20 ng/ml. The limit of CCB quantification was 156 ng/ml, while the limit of CCB detection was 78 ng/ml by injection of 30 μl samples.

Stability. A set of four standard plasma samples containing 312, 625, 1250 and 2500 ng/ml was prepared and stored at –80˚C. Samples were thawed after one, two and three weeks and analysed as described above (Table II). The intention of this assay was to obtain data within one week after sampling; therefore, freezing-thawing cycles were investigated for only three weeks.

Therapeutic drug monitoring during CCB monotherapy and CCB/OxPt polychemotherapy. As CCB is a prodrug, it is essential to know whether there is any drug–drug interaction when combined with other cytostatic agents. By using the described HPLC method, a possible influence of OxPt on CCB plasma levels was investigated. For this purpose, plasma samples were obtained from two groups of 12 colorectal cancer patients each. In the first group, the patients received a standard chemo-therapeutic regimen (1000 mg/m^2 Xeloda®, perorally twice daily over two weeks), while in the second group the patients received a dual-chemotherapy regimen (Xeloda® as in the previous group plus 130 mg/m^2 OxPt infused on day 1). All patients had given written, informed consent according to the guidelines of the Ethics Committee of the Rudolfstiftung Hospital. Plasma samples were obtained on day 1 (Monday) and day 5 (Friday) over a specific time period of two weeks, when patients had their weekly check-up at the Department of Oncology (Rudolfstiftung Hospital). According to the study treatment protocol, samples should be taken approximately two hours after ingestion of Xeloda® together with a standard continental breakfast. Therefore, they were taken at 10 a.m. The samples were centrifuged immediately after collection (five minutes at 6000 rpm) and the supernatant was analysed within one hour. Plasma concentration data of monitored patients were available promptly within one hour after sample collection.

Results

Figure 3 depicts the mean plasma concentration-time curves of CCB monotherapy and CCB/OxPt dual-chemotherapy. The rapid quantification method, applied in the analysis of the human plasma samples, was able to detect immediately that Xeloda® had not been taken according to the chemo-therapeutic schedule in two cases.

Analysis of the CCB monochemotherapy group showed that t_{max} occurred after 46.1±26.9 min, c_{max} was 5405.9±2357.9 ng/ml, AUC_{last} was 274543±72287 ng/ml/min and $t_{1/2el}$ was 25.5±8.2 min. In the CCB/OxPt dual-chemotherapy regime, t_{max} occurred after 59.1±30.5 min, c_{max} was 7286.75±5276.1 ng/ml, AUC_{last} was 315875±102824 ng/ml/min and $t_{1/2el}$ was 29.4±17.3 min. All values represent mean±standard deviation.

No significant differences (AUC_{last}) between the mono- and dual-chemotherapy were observed (p=0.28986, Student's t-test). Moreover as shown in Figure 3, no statistically significant differences in plasma levels were measured. These results demonstrated that OxPt has no influence on the three-step metabolic pathway of CCB, most probably because of the non-enzymatic conversation of OxPt.

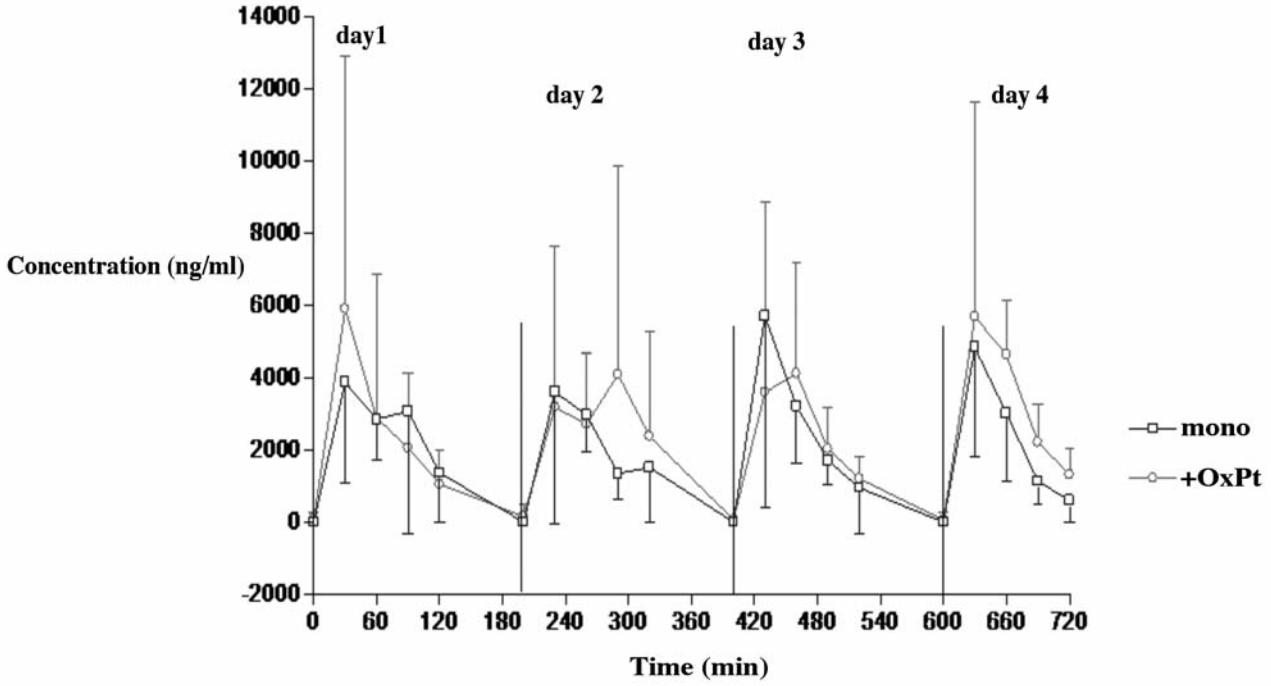

Figure 3. *Capecitabine mono (mono) versus capecitabine combined with oxaliplatin (+OxPt). The individual times when blood samples were taken (0, 30, 60, 90 and 120 minutes) are shown on the x-axis in the consecutive order of the days on which samples were taken. The time between the sampling days is excluded. Day 1 = First sampling day, Monday (first week), 0-120 minutes; Day 2 = Second sampling day, Friday (first week), is shown as 200-320 minutes; Day 3 = Third sampling day, Monday (second week), 400-520 minutes; Day 4 = Fourth and final sampling day, Friday (second week), 600-720 minutes. Data are presented as the mean ± standard deviation.*

Discussion

CCB is a thymidine phosphorylase (TP)-activated oral fluoropyrimidine, rationally designed to generate 5-FU *via* a three-step enzymatic cascade, preferentially within tumours. This tumour selectivity is achieved by the significantly higher activity of TP in tumour compared to healthy tissue.

The described analytical method was applied to monitor CCB in plasma samples of patients who were treated with Xeloda® in an outpatient regimen against colorectal cancer. Therefore a simple, robust, specific and sensitive HPLC method, using the simplest laboratory equipment, was developed.

The rationale for rapid drug monitoring is based on a possible relationship between the toxicity and efficacy of CCB and polymorphisms in the carboxylesterase 2 (CES 2) enzyme (11). Moreover, food intake may reduce the rate and the extent of absorption with a decrease of $AUC_{0\rightarrow\infty}$ (−60%) and c_{max} (−35%), and prolongation of t_{max} (+1.5 h) (12). Furthermore it should be noted that patient compliance contributes essentially to a better clinical outcome. The described method was successfully used for monitoring CCB in an outpatient chemotherapeutic regimen in order to check patient compliance immediately, at a low cost and without time-consuming analytical processes.

Moreover, by using this method, it was possible to address the question regarding the possible influence of OxPt on CCB plasma levels when administered together. No significant difference between these mono- and dual-chemotherapies was observed.

Additionally, it should be noted that CCB may potentially interact with other antitumor agents or co-administrated drugs, such as irinotecan (CPT-11) (13-14). This constellation increases the risk of potential side-effects. Using the described method, possible alterations of CCB plasma levels may be revealed quickly and easily.

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