Impact of Co-administered Drugs on Drug Monitoring of Capecitabine in Patients with Advanced Colorectal Cancer

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Abstract. Background: Drug monitoring is a useful tool for obtaining detailed information about the disposition of a drug in an individual patient during chemotherapy. According to the international guidelines, the analytical assay for quantification of a compound in biological samples must be validated. Among a number of parameters, peak purity is an important requirement. Materials and Methods: We analyzed pharmacokinetics in patients who received chemotherapy with capecitabine and up to 10 various co-medications. Results: Out of seven investigated co-administered drugs, we found evidence that the proton pump inhibitor pantoprazole causes peak interferences with capecitabine during high-performance liquid chromatography analysis. Therefore quantification of capecitabine in plasma samples can be inaccurate. Conclusion: We recommend an altered time schedule for co-administered drugs or changing the mobile phase used in the assay.

Patients with cancer commonly receive multiple medications including cytotoxic chemotherapy, hormonal agents, targeted therapies and supportive care drugs. Additionally, most of the patients are elderly, and so require medications for comorbid conditions such as cardiovascular, gastrointestinal, and rheumatological diseases (1, 2).

Today, the first-line treatment for metastatic colorectal carcinoma comprises of a combination of 5-fluoruracil (5-FU), folinic acid, or the 5-FU pro-drug capecitabine and irinotecan or oxaliplatin (3). In recent studies, these regimes, called FOLFIRI or FOLFOX, have improved the response rate and progression-free survival of patients with colorectal cancer (4). Capecitabine, an oral fluoropyrimidine carbamate, is an equally effective and tolerable, but more convenient alternative to i.v. 5-FU. It is rapidly absorbed through the gastrointestinal wall as an intact molecule. Capecitabine is first metabolized in the liver into 5-doexy-5-fluorocytidine (5’-DFCR) by human carboxyesterase isoenzyme-2 (hCES2). 5’-DFCR is then converted to 5-deoxy-5-fluorouridine (5’-DFUR) by cytidine deaminase in tumor cells and in the liver. Finally, 5’-DFUR is metabolized by thymidine phosphorylase into the cytotoxic agent 5-FU inside tumor cells (5).

The quantification of capecitabine, 5’-DFCR and 5’-DFUR in patients, especially outpatients, undergoing a chemotherapeutic treatment receiving capecitabine alone, or in combination therapy, is a very commonly used method in pharmacokinetic and drug-monitoring studies (6, 7).

In our trial, patients suffering from advanced colorectal cancer received capecitabine in a new combination chemotherapy. In addition, they were given several drugs against side-effects of the chemotherapeutics or other symptoms caused by illness, which included metamizole, pantoprazole, lornoxicam, dexamethasone, metformin, pioglitazone, or enoxaparin sodium.

The health status of patients often necessitates the inclusion of other new drugs during therapy that are not listed in the study protocol and therefore are not checked for peak interferences during the validation procedure of the analytical assay subsequently used.

The objective of the present study was to examine the impact of such concurrent medications on the high-performance liquid chromatography (HPLC) assay for analysis of capecitabine, 5’-DFCR and 5’-DFUR.
Materials and Methods

Chemicals. The pure chemical standard of capecitabine (Xeloda®) was obtained from Roche Austria GmbH (Mannheim, Germany). 5’-DFCR and 5’-DFUR, also as pure chemical standards, were purchased from AK Scientific, Inc. (Union City, CA, USA) and TCI Europe (Eschborn, Germany), respectively. HPLC-grade water, methanol and acetonitrile were supplied by Merck Chemicals (Darmstadt, Germany) and ammonium acetate was purchased from Aldrich-Chemie (Steinheim, Germany). Commercially available in metamizole (Novalgin®) and enoxaparin sodium (Lovenox®) were obtained from SanofiAventis (Vienna, Austria). Dexamethasone (Dexamethason Nycomed), pantoprazole (Pantoloc®) and lornoxicam (Xefo®) were supplied by Nycomed Austria (Linz, Austria), and pioglitazone and metformin were purchased from Stada (Vienna, Austria) and Hexal (Holzkirchen, Germany), respectively.

Patients and therapy. In this investigation, we analyzed plasma samples from 24 patients who were treated with capecitabine (1000 mg/m² bid in week 1, 4 and 7) in a new combination chemotherapy against advanced colorectal cancer. Positive vote by the ethics committee of the Medical University of Vienna (EudraCT Number 2011-002921-23). As listed in Table I, they received different combination of drugs to improve their poor health.

Chromatography. The HPLC apparatus (Merck VWR®Hitachi Chromaster) consisted of the following components: a Chromaster 5110 quaternary pump, a solvent degasser model 2003 (VWR), a Chromaster 5210 autosampler, a Chromaster 5410 UV/VIS detector and a Chromaster 5310 column oven. The system was operated according to the GLP guidelines of the European Medicines Agency (8).

Quantification of capecitabine, 5’-DFCR and 5’-DFUR in plasma samples was performed by two different reversed-phase HPLC assays, which were established in our laboratories eight years ago and were published in full detail recently (6, 7).

For the quantification of capecitabine, a Rp-18 column (EcoCart® 125-3, LiChrospher® 100, 5 μm) protected by a guard column (LiChroCART® 4-4, LiChrospher® 100 Rp-18, 5 μm, Merck) was used. The mobile phase consisted of water-methanol, 50:50 v/v and the flow rate was 0.6 ml/min (back pressure 63 bar at 36°C). However, we changed the detection wavelength from 305 nm to 280 nm. The LOQ was 156 ng/ml referring to a signal-to-noise ratio 1:5.

The two metabolites 5’-DFCR and 5’-DFUR were simultaneously analyzed on a reversed-phase Amide C16 column (15 cm x 4.6 mm, 5 μm, Supelco, USA) protected by a guard column (LiChroCART® 4-4, LiChrophter® 100 RP-18, 5 μm). Gradient elution was performed using a mobile phase containing methanol-ammonium acetate (1mmol, pH 4.0) (10:90, v/v), for solvent A and methanol-ammonium acetate (1 mmol, pH 4.0) (90:10 v/v), for solvent B. The gradient started with 100% solvent A for 5 min, then changed from 100% solvent A to 100% solvent B within 22 min; thereafter, it remained at 100% solvent B for 2 min and finally re-equilibrated to 100% solvent for 8 minutes. The flow rate was 0.8 ml/ min (65 bar back pressure, 36°C) and the UV detection wavelength was sat at 280 nm. 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.

Table I. Medication dose, time of intake and maximum plasma concentration of all tested drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg)</th>
<th>Time of intake</th>
<th>Cmax (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone</td>
<td>Various</td>
<td>Before breakfast</td>
<td>66</td>
</tr>
<tr>
<td>Enoxaparin sodium</td>
<td>20-40</td>
<td>Various</td>
<td>4</td>
</tr>
<tr>
<td>Lornoxicam</td>
<td>16-18</td>
<td>Semidaily before meal</td>
<td>1</td>
</tr>
<tr>
<td>Metamizole</td>
<td>20 drops</td>
<td>As needed</td>
<td>8</td>
</tr>
<tr>
<td>Metformin</td>
<td>500</td>
<td>Semidaily with meal</td>
<td>5</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>40</td>
<td>30 min before breakfast</td>
<td>2.5</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>30</td>
<td>With meal</td>
<td>1.05</td>
</tr>
</tbody>
</table>

Sample preparation. Solid-phase extraction was used to remove the matrix components from the samples. For pre-conditioning, the cartridges (Oasis HLB, 1 cm³, 30 mg packing volume; Waters Corporation, Milford, MA, USA) were washed with 1 ml of methanol and activated with 1.0 ml of water. Then 1.0 ml of plasma sample was forced through the cartridges under vacuum (-5 bar) followed by 1.0 ml of water–methanol (95:5 v/v). An aliquot of 30 μl was injected into the HPLC to quantify 5’-DFCR and 5’-DFUR. Capecitabine was eluted from the cartridge with 1 ml of methanol and this time 10 μl were used in a separate HPLC run for the determination of capecitabine.

Pre-medication. Drugs of pre-medication were solved in pooled plasma to obtain a concentration that corresponds to the maximum plasma concentration (Cmax) as reported in the literature after administration of standard doses (Table I). In order to evaluate the potential impact of the premedication on the quantification of capecitabine and its metabolites, the same sample preparation method was used as for the patient plasma sample. In addition, the conditions of the HPLC system were identical to the chromatographic methods used for the analysis of the plasma samples.

Results

Figure 1A shows a typical chromatogram of a patient’s plasma sample obtained 90 min after capecitabine ingestion. The retention time of capecitabine was 6.2 min. The matrix peaks in front of the chromatogram eluate from 0.4 to 2.0 min and do not interfere with the capecitabine peak.

As can be seen in Table II, seven different drugs were extracted and analyzed for their potential peak interference with the determination of capecitabine. For dexamethasone, a peak was observed with a retention time of 9.6 min. The metamizole peak can be seen at 0.7 min. The dexamethasone and metamizole peaks did not interfere with the capecitabine peak.

Enoxaparin sodium, lornoxicam, metformin and pioglitazone showed no peaks when using the described HPLC method.

What this study shows is that the peak of pantoprazole (Figure 2A and B) overlaps with that from capecitabine as they both have retention times of 6.2 min. This is of great clinical importance for further pharmacokinetic studies focusing on...
colorectal cancer research because if a patient is given pantoprazole, it is impossible to determine the exact plasma capecitabine concentration using this method without modification. Figure 2C and D depict the interference in the HPLC assay when capecitabine and pantoprazole are purposely injected into the HPLC machine together. It is clear that these two drugs cannot be separated using our standard HPLC method. For a clinical study, this could pose a significant problem because both pantoprazole and capecitabine are taken in the morning, pantoprazole 30 min before breakfast and capecitabine immediately after breakfast (9, 10). This situation is represented in Figure 2E and F. It shows the results of analysis of plasma samples 90 min after drug administration, in which it is not possible to quantitate capecitabine due to the interference with pantoprazole. The exact influence of pantoprazole on the determination of capecitabine differs between different patient plasma samples. Figure 2E and F illustrate two separate cases. In the first, we were able to suggest a possible solution, which, however, do not apply to the second case. Figure 2E shows a clear capecitabine peak overlapping the pantoprazole peak. We would recommend to subtract the pantoprazole standard chromatogram from the patient’s chromatogram by peak area subtraction. By this the pantoprazole peak is erased from the pantoprazole peak in the plasma sample chromatogram. This procedure makes it possible to obtain the exact peak area of capecitabine in patient’s plasma samples. Figure 2F shows the results for another patient who received pantoprazole as a pre-medication to the usual capecitabine dose. This time the pantoprazole peak is even higher than the capecitabine peak. It is not possible to properly separate these two substances using the aforementioned method. This suggests that the best course would be to regulate the time of intake of pantoprazole and capecitabine. Normally, pantoprazole is administered at least 30 min before breakfast and the C<sub>max</sub> is reached after 2.5 h, with a concentration of 2-3 μg/ml (11). Concerning capecitabine, it is important that the drug is administered with food, and it is therefore taken 30 min after breakfast (9). The C<sub>max</sub> for capecitabine appears after 1.5 h at a concentration of 3-4 μg/ml (12). As shown in Figure 3A, this leads to overlapping concentration–time curves of both drugs. Therefore a delayed administration of capecitabine is recommended. When capecitabine is taken 4 h after the administration of pantoprazole, the concentration–time curves do not overlap, as a consequence an exact determination of CCB is possible (Figure 3B).

However, we established a modified HPLC assay in order to separate the two drugs because the compliance of some patients in outpatient situations can be difficult in regard to drug intake. In the validated HPLC assay, the mobile phase consists of water and methanol, 50:50 v/v. We changed the eluent to water and acetonitrile, 40:60 v/v and as can be seen in Figure 4, capecitabine and pantoprazole no longer overlap. The retention time of capecitabine is changed to 2.1 min and that of pantoprazole to 3.7 min. Therefore an exact
determination of capecitabine is possible and the matrix peaks do not interfere with the peak of interest.

Figure 1B represents a chromatogram of the two metabolites in the patient’s plasma sample taken 90 min after administration of capecitabine. 5'-DFCR peaks at around 5.8 min and 5'-DFUR at 6.3 min. No interference by the matrix peaks was observed.

This time the seven drugs were extracted and analyzed for potential interference with the determination of the two metabolites. The results and retention times are summarized in Table II.

The present study illustrates that dexamethasone, enoxaparin sodium, lornoxicam, metformin, pantoprazole and pioglitazone show no peaks when using this HPLC
method. As a result, 5’-DFCR and 5’-DFUR can be properly determined in patients that take these drugs.

Two metamizole peaks were observed, with retention times of 13.4 min and 14.9 min. Given that the two capecitabine metabolites are first seen after around 5.8 and 6.4 minutes, it is clear that the quantification of 5’-DFCR and 5’-DFUR is not affected by metamizole.

**Patient Profiles**

In the last two years we analyzed approximately 200 concentration-time profiles in blood samples of patients receiving capecitabine in a phase III clinical study against advanced colorectal cancer. In about 30% out of these 200 samples a peak overlap between capecitabine and pantoprazole was observed. Figure 5 depicts the mean concentration-time curve of 12 patients receiving capecitabine and pantoprazole as a pre-medication.

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**Figure 3.** Plasma concentration–time curves of capecitabine and pantoprazole after simultaneous intake (A) and sequential intake (B).

**Figure 4.** Peaks for capecitabine and pantoprazole clearly separated when using the modified high-performance liquid chromatography assay.

**Figure 5.** Concentration–time profile for 12 patients after administration of 1,000 mg capecitabine. The individual times when blood samples were taken (0-360 min) are shown on the x-axis. A: Mean concentration±standard deviation of capecitabine. B: Mean concentration±standard deviation of 5’-DFCR (green circles) and 5’-DFUR (blue squares).
Discussion

The aim of this investigation was to evaluate the impact of co-administered drugs in patients with colorectal cancer on the quantification of capecitabine and its metabolites, which has been successfully-tested and documented. The health status of these patients often requires the addition of co-administered drugs, which have not been included for peak interferences during the validation procedure of the analytical assay. In our opinion, it is important to highlight this problem because it can complicate the quantification of capecitabine, 5'-DFCR and 5'-DFUR and may lead to false pharmacokinetic results.

The results indicated that enoxaparin, lornoxicam, metformin and pioglitazone do not affect the quantification of capecitabine because under our HPLC conditions no peaks were observed. Due to the fact that the observed retention times of dexamethasone and metamizole are different from that of capecitabine, co-administration of these two drugs has no effect on capecitabine determination. On the other hand, pantoprazole exhibited a peak with identical retention time to that of capecitabine, which is of importance for pharmacokinetic studies concerning new capecitabine regimens. This interaction is problematic because an accurate determination of the capecitabine concentration is difficult. In cases with only minor interference, it is possible to use pantoprazole baseline subtraction. Thereby the pantoprazole peak is subtracted from the capecitabine–pantoprazole peak in order to determine the exact concentration of capecitabine. In most cases, however, this solution is not applicable. Simultaneous administration of capecitabine and pantoprazole may lead to concentration–time curves as depicted in Figure 3A. Taking into account the similar pharmacokinetics of both drugs, delayed administration of capecitabine is recommended to allow more accurate determination.

Due to the problematic compliance of some patients in outpatient situations, we established a modified HPLC assay that makes it possible to separate both drugs sensitively and selectively. In the validated assay, the mobile phase consists of water and methanol 50:50 v/v. We changed the eluent to water and acetonitrile 60:40 v/v. By this means, exact quantification of capecitabine is possible, the retention time of capecitabine is 2.1 min and that of pantoprazole is 3.7 min and there is no interference by matrix peaks.

From the pharmacokinetic point of view, the following procedure is recommended: Verification of peak overlap of capecitabine and pantoprazole; pass on this information to the oncologic unit: in a clinical setting, it is easy to modify the sequence of drug administration; for patients on outpatient regimens, we prefer use of the modified HPLC assay.

The aforementioned results show that even a method that has been used for years for therapeutic drug monitoring, sometimes needs modification when new medications are integrated into the therapy regimen. This is especially important when the co-medication in question is not accounted for in the study protocol.

References