Abstract. Background: The effect of biweekly cetuximab (CTX) on the pharmacokinetics of CPT-11 and its metabolites was evaluated in this prospective, paired, crossover study. Patients and Methods: Patients with epidermal growth factor receptor-positive advanced colorectal cancer received infusions of CPT-11 (180 mg/m²; FOLFIRI schedule) every second week. CTX (500 mg/m² for 120 min) was infused on day 2, followed by biweekly infusions (500 mg/m² for 120 min). Plasma samples were analysed on day 1 of cycle 1 (CPT-11 monotherapy) and on day 1 of cycle 3 or 4 (CPT-11 plus CTX). The endpoint of this study was to evaluate differences in plasma concentrations of CPT-11 and metabolites between cycle 1 and cycle 3 (or 4). Results: Generally, there was little difference in CPT-11 pharmacokinetics when combined with CTX in the 11 enrolled patients. However, a significantly lower area under the concentration-time curve from 0-48 hours was observed for the SN-38 glucuronide metabolite with combination therapy versus monotherapy (by 27%, p<0.05). Conclusion: CTX has no clinically relevant impact on the pharmacokinetics of CPT-11 or its activation into SN-38; however, there may be a delay in detoxification of SN-38 by β-D-glucuronidation.

The topoisomerase-I inhibitor irinotecan (CPT-11) is a highly potent agent against advanced colorectal cancer (ACRC) and is used successfully in combination with other chemotherapeutic agents, such as 5-fluorouracil and folinic acid (1, 2). CPT-11 has complex metabolism, with activation into the potent topoisomerase-I inhibitor, SN-38, catalysed by the human carboxyesterases and elimination of SN-38 by β-D-glucuronidation into SN-38 glucuronide (SN-38gluc) catalysed by UDP-glucuronosyltransferase (UGT1A1). Moreover, biotransformation of CPT-11 into non-toxic metabolites, such as aminopentane carboxylic acid (APC), and a primary amine metabolite (7-ethyl-10-[4amino-1-piperidino]-carbonyloxycamptothecin) are catalysed by the cytochrome P-450 isoenzyme, CYP3A4 (3, 4). As these metabolic pathways are sensitive to drug-drug interactions, there is a need to fully evaluate pharmacokinetics when combining CPT-11 with other agents. A further characteristic of CPT-11 is the protein- and pH-dependent equilibrium of the active lactone and the inactive, toxic carboxylate form of CPT-11 and of all metabolites. A change in pH values or protein concentration in the blood and tissues may lead to a variation of efficacy and pharmacokinetics of CPT-11.

Cetuximab (CTX) is an epidermal growth factor receptor (EGFR)-targeted IgG1 monoclonal antibody (mAb). The fact that the transmembrane glycoprotein EGFR is frequently expressed in various carcinomas and a high EGFR activity is correlated with a poor tumour prognosis led to the introduction of CTX for cancer treatment (5-7). CTX is approved for use in combination with CPT-11 chemotherapy or as monotherapy in the treatment of ACRC (8-11). CTX is also approved for use as a single agent for the treatment of EGFR-expressing, recurrent metastatic colorectal carcinoma in patients who are intolerant to CPT-11-based chemotherapy (12). Preclinical and clinical studies have demonstrated synergistic activity between CPT-11 and CTX (12-14); however, only few pharmacokinetic studies describe the disposition of CPT-11 when given in combination with CTX in ACRC (15, 16).

Although CTX is typically administered on a weekly schedule, pharmacokinetic data demonstrate that CTX has a long terminal half-life (110 hours), allowing administration of a biweekly schedule. Indeed, active serum concentrations of CTX were maintained throughout the 2-week dosing period with such a regimen (15, 17). A CTX dose of 500 mg/m² every 2 weeks exhibited predictable pharmacokinetics that were similar to those of the approved weekly dosing regimen. Results from phase I...
studies show that a biweekly schedule of CTX is well tolerated and exhibits similar pharmacodynamics to conventional weekly dosing, and does not appear to compromise efficacy (18-21). The development of a biweekly dosing regimen for CTX would provide treatment flexibility when combined with biweekly or longer chemotherapy regimens. Furthermore, the advantage of being able to synchronize the administration of CTX and concomitant chemotherapy is desirable for both patients and health care professionals.

CTX, as a mAb against EGFR, is unlikely to interact with other agents, although its rather long half-life and subsequent retention in the body has resulted in investigations of potential interactions between weekly CTX and CPT-11 (15, 22). To date, only few regulatory guidelines are available concerning the evaluation of drug-drug interactions between mAbs and antineoplastic drugs (23). Therefore, pharmacokinetic investigations regarding potential drug interactions between mAbs and antineoplastic drugs are necessary to assess the efficacy and safety of new regimens. The objective of this study was to investigate the pharmacokinetics of CTP-11 in combination with biweekly CTX in patients with ACRC.

Patients and Methods

Study design and patients. This pharmacokinetic study had a prospective crossover design, with patients serving as their own controls to minimize interpatient variability. Inclusion criteria included ACRC; Karnofsky performance status >80% and prior chemotherapy with CPT-11 plus 5-fluorouracil/folinic acid. In addition, tumours were EGFR positive as confirmed by immunohistochemistry (EGFR pharmDx™ Kit; Dako). Eligible patients (3 female, 8 male) had no renal impairment as judged by standard biochemical parameters (plasma creatinine <1.5 mg/dl) and no hepatic impairment (bilirubin <0.6 mg/dl, γ-glutamyl-transferase <100 U/l) and alanine aminotransferase <30 U/l). Written informed consent was obtained from each patient according to the specifications of the Ethics Committee of the University Vienna. The mean age was 61.5±4.38 (range 54-66) years, mean body mass 82.3±22.3 (range 60-125) kg and mean body surface area was 1.92±0.27 (range 1.62-2.51) m².

Treatment. CPT-11 (Campto®) was supplied in vials containing 100 mg CPT-11 in 5 ml sterile solution (Pfizer Inc., Vienna, Austria) and was infused through a central venous catheter at a constant rate for one hour every second week (180 mg/m²) on day 1 (FOLFIRI schedule with folinic acid, 5-fluorouracil and irinotecan). Patients received premedication with tropisetron and atropine one hour before CPT-11 infusion, and ranitidine and dexamethasone before CTX infusion. CTX (Erbitux®) was supplied as a sterile infusion containing 2 mg/ml CTX in 50 ml vials (Merck, Vienna, Austria) and was administered through a central venous catheter at a constant rate (500 mg/m²) for 120 min as a loading dose on day 2 (the day after the first CPT-11 infusion). Subsequent CTX infusions were performed biweekly at a constant rate of 500 mg/m² for 120 min, and CPT-11 was infused immediately after CTX administration. Plasma samples were analysed on day 1 of cycle 1 to provide data about the monotherapy regimen (referred to as MONO) and on day 1 of cycle 3 or 4 to provide data regarding the CPT-11 plus CTX combination (referred to as CTX).

Analytical procedure. Samples were split and the first analysis was performed under neutral analytical conditions to differentiate between lactone and carboxylate amounts. Analysis was repeated using the second sample under acidic analytical conditions (1.0 ml supernatant with 40 μl phosphoric acid 8.5%) to obtain total lactone amounts. The assay for quantification of CPT-11, SN-38, SN-38gluc and APC by isocratic reversed–phase HPLC using fluorometric detection has been described in detail elsewhere (4, 24).

Biometric calculations. WinNonlin® Professional Version 5.1 (Pharsight Corporation, USA) was used for curve fitting of plasma concentration data of CPT-11 and metabolites. A non-compartmental model of the WinNonlin library was used (model 202). From data obtained under acidic HPLC conditions, the following pharmacokinetic parameters were calculated: cmax: peak plasma concentrations (ng/ml); tmax: time to reach maximum plasma concentration (h); AUClast area under the concentration time curve from 0 to 48 h (ng/ml/h); AUCinf: area under the concentration–time curve from 0 to infinity (ng/ml/h); t1/2λz: half-life of terminal elimination (h); MRTlast: mean residence time from 0 to last (h); Vzpred: predicted volume of distribution (l); VDSS: volume of distribution at steady state (l); Cltot: total body clearance (l/h); Vd, VDSS and Cltot were not calculated for CPT-11 metabolites. AUClast values each of CPT-11, SN-38, SN-38gluc and APC (AUCmet in both groups were compared with the sum of AUCCPT-11 + SN-38 + SN-38gluc + APC and the percentage AUCmet was calculated:

\[
\text{AUCmet} = \frac{\text{AUCCPT-11 + SN-38 + SN-38gluc + APC}}{\text{AUCCPT-11 + SN-38 + SN-38gluc + APC}} \times 100
\]

Apparent formation coefficients (Rf) of metabolites catalysed by CYP3A4, hCE and UGT1A1 were calculated by dividing the formed metabolite AUClast by the precursors’ AUClast (RfCYP3A4=AUCAPC/AUCCTX; RfCE=AUCSN-38/AUCCPT-11; RfUGT1A1=AUCSN-38gluc/AUCSN-38). A high Rf value indicates high activity of the responsible metabolizing enzyme.

Statistical evaluation of differences in plasma concentrations and pharmacokinetic parameters were performed using paired, two-sided Student’s t-tests. Descriptive statistics were performed using GraphPad Prism® Version 5.0 and Graph Pad Instat® Version 3.0 (GraphPad Software Inc., San Diego, CA, USA).

Results

Plasma concentrations. Mean CPT-11 plasma concentrations were similar during MONO and combination CTX treatment, with only small decreases observed at 0.75 and 1.0 hour after the start of the infusion (Figure 1A). Compared with CPT-11 measurements, larger decreases in plasma concentrations were observed with SN-38 between 1.0 to 3.0 hours in the combination arm (Figure 1B). A weak rebound effect occurring from 2 to 6 hours after the start of CPT-11 infusion was observed for SN-38. This effect is based on the degradation of SN-38 glucuronide by enterobacterial enzymes and redistribution of SN-38 from the intestinal mucosa into the central systemic blood circulation. For both SN-38gluc and APC, plasma disposition was lower throughout the whole
sampling period with the CTX schedule, although there was a high degree of patient intervariability (Figure 1C and 1D).

The effects of CPT-11 plus CTX on the equilibrium of the active CPT-11 lactone and the inactive carboxylate form were investigated (Figure 2). For CPT-11, mean plasma concentration-time curves were higher for the lactone form than the carboxylate form, particularly with combination therapy in the first 5 hours after the start of the infusion (Figure 2A). In contrast, SN-38 carboxylate plasma concentrations were higher than lactone concentrations in the CTX group, as well as in the MONO group (Figure 2B). Plasma concentrations of APC lactone and carboxylate differed from the observed trend for CPT-11 and SN-38 profiles (Figure 2C); however, as APC only contributes to SN-38 formation to a very small extent, this observation is considered clinically irrelevant.

Pharmacokinetics. Pharmacokinetic parameters are listed in Table I and were calculated from plasma data obtained under acidic analytical conditions representing total amounts of each compound. In general, pharmacokinetic differences between the MONO and combination CTX therapy groups were small and statistically insignificant. When pharmacokinetic parameters of CPT-11 were analyzed, $C_{\text{max}}$ of CPT-11 was 19% lower with CTX versus MONO (not significant, ns); however, there was with little difference in AUC parameters between the groups. The pharmacokinetic parameters $V_{\text{dss}}$ and $Cl_{\text{tot}}$ remained unaffected, indicating that combination CTX therapy had no distinct influence on CPT-11 disposition when given biweekly. The only significant difference was for $t_{1/2\lambda_z}$ ($p < 0.05$), which was prolonged by 24% with the CTX regimen, but $MRT_{\text{last}}$ values were similar for both groups. Although not significant, it was noted that $C_{\text{max}}$ was also lower with the CTX regimen for all analyzed metabolites, with reductions of 14% for SN-38, 37% for SN-38gluc and 42% for APC (Table II). In addition, CTX reduced the $AUC_{\text{last}}$ and $AUC_{\text{inf}}$ of SN-38gluc 27% ($p < 0.05$) and 21% (ns), respectively and APC 32% and 33%, respectively (both ns), but not SN-38. Other pharmacokinetic parameters of CPT-11 metabolites were largely unaffected (Table I).

$AUC_{\text{last}}$ values were used to calculate apparent formation rates of CPT-11 metabolites (Table II). Significantly lower RFs were observed in the CTX group compared with the MONO group when calculating $Rf_{\text{CYP3A4}}$ for APC (–30%, $p=0.010$) and $Rf_{\text{UGT1A1}}$ for SN-38gluc (–29%, $p=0.041$). These data provide evidence for a probable lower activity of CYP3A4 and UGT1A1. From $AUC_{\text{last}}$ data, the percentage distribution was calculated for all compounds in both regimens. The percentage $AUC_{\text{inf}}$ for CPT-11:SN-38:SN-38gluc:APC was...
71.5:1.7:11.2:15.7% in the MONO regimen and 79.9:1.9:9.7:11.5% in the CTX regimen, indicating a slight shift to CPT-11 from APC with the CTX regimen.

Discussion

In this study of patients with ACRC, high doses of CTX administered biweekly with CPT-11 had little impact on the drug disposition of CPT-11 and its metabolites. Although assessing tolerability was not an objective of the study, general observations suggest that the toxicity of CPT-11 was not increased and there were no unexpected adverse events with biweekly CTX.

Concentration-time profiles for CPT-11 and SN-38 were very similar for both groups. Lower plasma disposition of SN-38gluc and APC was observed with the combination therapy versus MONO, although there was high interpatient variability. The reduction occurred over the analysis period, indicating a continuous effect of CTX on disposition rather than a distribution effect that would have ended after a few hours. As both metabolites are biotransformed via enzymatic catalysis of different enzymes, lower plasma concentrations are probably a systemic effect and not the result of inhibition or induction of enzymes. The results of the present study are consistent with two earlier studies concerning the weekly administration of CTX with CPT-11 (15, 16).

No statistically significant difference for the key pharmacokinetic parameters, such as $C_{\text{max}}$, $C_{\text{last}}$, or $V_d$ were observed. With CTX, there was no change in AUC$_{\text{last}}$ for CPT-11 and SN-38, a non-significant decrease for APC (32%) and a significant decrease for SN-38gluc (27%, $p<0.05$). In our previous study, similar observations were found with the combination regimen of CPT-11 and the mAb, bevacizumab (25). Compared with CPT-11 monotherapy, CPT-11 plus bevacizumab resulted in reductions in AUC$_{\text{last}}$ of CPT-11 (9%), SN-38 (29%), SN-38gluc (19%) and APC (17%).

By our previous study we investigated the plasma disposition and metabolism of CPT-11 (350 mg/m$^2$) in combination with weekly CTX (16). Peak concentrations of SN-38 (the pharmacologically active compound) were identical compared to our study: 0.9% of CPT-11 $C_{\text{max}}$ in both MONO regimens and 1.0% of CPT-11 in both CTX regimens.

When the equilibrium between active lactone and inactive carboxylate forms was assessed in the present study, the active lactone form dominated for CPT-11, particularly in combination with CTX, while the inactive carboxylate form dominated for SN-38 with both regimens. The reason for this is unclear. The equilibrium between lactone and carboxylate forms is very sensitive to pH and protein concentration in blood and tissue. Analytical errors can be excluded because both compounds were analyzed within the same analytical procedure simultaneously.

When apparent formation rates were analysed, administration of CTX did not appear to alter human carboxylesterase activity responsible for the formation of the active metabolite, SN-38; however, activity of UGT1A1 responsible for β-D-glucuronidation of SN-38 was significantly lower (29%, $p=0.041$). In addition, activity of CYP3A4 responsible for the formation of the inactive APC metabolite was significantly reduced. The calculated $R_f$ values obtained in the present study are similar to results we obtained in our previous study with weekly CTX (0.025 and 0.140 respectively), but $R_f$ values were approximately three-fold higher in the present study (weekly CTX $R_f=1.750$) (16).
It is also worth considering that following the presence of high levels of vascular endothelial growth factor that may occur in cancer, vasculature becomes excessively permeable and leaky. This leads to an uneven delivery of nutrients, oxygen and therapeutic agent to the tumor and might cause an increase in free hydroxyl radicals. Accordingly, it might be possible that leaky membranes do not retain CPT-11 and metabolites completely, and therefore a certain amount of administered drug may be lost to unknown compartments.

In summary, no significant pharmacokinetic interaction was observed between CPT-11 and biweekly CTX in patients with ACRC. From the pharmacokinetic point of view, biweekly CTX appears to be a safe and efficient schedule. Comparing to weekly administration of CTX, typical pharmacological side-effects such as skin reactions, asthenia, nausea and diarrhea occurred at the same extent. The results support the clinical use of biweekly application CTX, which is more convenient for patients and hospital personnel.

**References**

Table II. Apparent formation coefficients (Rf) with irinotecan (CPT-11) when given as monotherapy (MONO) and in combination with cetuximab (CTX).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rf&lt;sub&gt;MONO&lt;/sub&gt;</th>
<th>Rf&lt;sub&gt;CTX&lt;/sub&gt;</th>
<th>Rf&lt;sub&gt;UGT1A1&lt;/sub&gt;</th>
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<tr>
<td>Mean</td>
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<td>SD</td>
<td>0.006</td>
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<td>-29</td>
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<tr>
<td>P-value</td>
<td>0.000</td>
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APC, Aminopentane carboxylic acid; CYP3A4, cytochrome P450 3A4; hCE, human carboxyesterase; SD, standard deviation, CV, coefficient of variation; UGT1A1, UDP-glucuronosyltransferase 1A1.


