Factors Involved in Prolongation of the Terminal Disposition Phase of SN-38: Clinical and Experimental Studies[1]

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ABSTRACT

The active metabolite of irinotecan (CPT-11), 7-ethyl-10-hydroxycamptothecin (SN-38), is either formed through enzymatic cleavage of CPT-11 by carboxyl esterases (CEs) or through cytochrome P-450 3A-mediated oxidation to 7-ethyl-10-[4-(1-piperidino)-1-amino] carbonyloxycamptothecin (NPC) and a subsequent conversion by CE. In the liver, SN-38 is glucuronidated (SN-38G) by UGT1A1, which also conjugates bilirubin. Fourteen patients were treated with 350 mg/m^2 CPT-11, and we performed pharmacokinetic analysis during a 500-h collection period. The half-life and area under the plasma concentration-time curve of SN-38 were 47 ± 7.9 h and 2.0 ± 0.79 μM·h, respectively, both representing a 2-fold increase as compared with earlier reported estimates (A. Sparreboom *et al.*, Clin. Cancer Res., *4:* 2747–2754, 1998). As an explanation for this phenomenon, we noted substantial formation of SN-38 from CPT-11 and NPC by plasma CE, consistent with the low circulating levels of NPC observed. In addition, transport studies in Caco-2 monolayers indicated that nonglucuronidated SN-38 could cross the membrane from apical to basolateral, indicating the potential for recirculation processes that can prolong circulation times. Interestingly, individual levels of fecal β-glucuronidase, which is known to mediate SN-38G hydrolysis, were not related to any of the SN-38 kinetic parameters ($r = 0.09$; $P = 0.26$), suggesting that interindividual variation in this enzyme is unimportant in explaining SN-38 pharmacokinetic variability. We have also found, in contrast to earlier data, that SN-38G/SN-38 plasma concentration ratios decrease over time from ~7 (up to 50 h) to ~1 (at 500 h). This decrease could be explained by the fact that glucuronidation of SN-38 and bilirubin is increasingly competitive at lower drug levels. In addition, no evidence was found for SN-38G transport through the Caco-2 cells. Our findings indicate that until now the circulation time of SN-38 has been underestimated. This is of crucial importance to our understanding of the clinical action of CPT-11 and for future pharmacokinetic/pharmacodynamic relationships.

INTRODUCTION

CPT-11,[3] a water-soluble derivative of camptothecin, is currently registered for use in patients with metastatic colorectal cancer refractory to 5-fluorouracil therapy as well as first-line therapy and has shown clinical activity against several other types of solid tumors (1, 2). CPT-11 itself has weak, if any, pharmacological activity *in vitro* and is thought to exert its antitumor activity *in vivo* after enzymatic cleavage by a CE that generates the active metabolite, SN-38, which is at least 100-fold more cytotoxic than CPT-11 (3). Peripheral converting enzyme activity in animals has been characterized in serum (4), liver (5), and the small intestine (6), and preliminary evidence indicates CE activity within the tumor as well (7–9). SN-38 can be metabolized further very efficiently by UDP glucuronosyltransferase 1A1 (10) and 1A7 (11) to an inactive β-glucuronide derivative, SN-38G (12, 13). Another metabolic pathway of CPT-11 consists of a cytochrome P-450 3A4 and 3A5-mediated oxidation of the bipiperidine side chain attached to the core structure (14, 15). The main metabolites resulting from this pathway have been identified as APC (16) and NPC (17). Although APC has been shown to be a poor substrate of CE in *in vitro* models (16), NPC can be converted into SN-38 by liver CE and, as such, may contribute to the overall production of the pharmacologically active species (17). We reported previously that CPT-11 is predominantly eliminated in feces after hepatobiliary and intestinal secretion, with unchanged drug as the major excretion product followed by smaller amounts of SN-38 and APC (18). Interestingly, SN-38G concentrations in feces were very low, presumably as a result of hydrolysis of the glucuronic acid moiety by bacterial β-glucuronidases (18, 19). We hypothesized, based also on the long terminal disposition half-life and extensive biliary secretion, that CPT-11 and its metabolites can undergo enterohepatic recirculation, and that

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[3] The abbreviations used are: CPT-11, irinotecan; CE, carboxylesterase; SN-38, 7-ethyl-10-hydroxycamptothecin; SN-38G, 7-ethyl-10-[3,4,5-trihydroxy-pyran-2-carboxylic acid]camptothecin (the β-glucuronic acid conjugate of SN-38); APC, 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin; NPC, 7-ethyl-10-[4-(1-piperidino)-1-amino]carbonyloxycamptothecin; AUC, area under the concentration-time curve; HPLC, high-performance liquid chromatography; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase.

this might play a role in the variability of pharmacokinetic parameters observed earlier. In the present study, we have reexamined the plasma disposition of CPT-11 and its metabolites using an extended sampling time period of 500 h in a group of patients with colorectal cancer receiving the drug as single agent at a dose level of 350 mg/m^2 and performed various *in vitro* experiments to explain the observed phenomena.

PATIENTS AND METHODS

Patients and Treatment. Patients with a histologically or cytologically confirmed diagnosis of colorectal cancer refractory to standard therapy or for whom other treatment options were not available were eligible for the present study. Additional eligibility criteria included: age between 18 and 70 years; Eastern Cooperative Oncology Group performance status $\leq$1; no previous treatment with antineoplastic agents for at least 4 weeks (or 6 weeks in case of nitrosoureas or mitomycin C); no prior treatment with CPT-11 or other topoisomerase I inhibitors; adequate hematopoietic (WBC count $>3.0 \times 10^9$/liter, absolute neutrophil count $>2.0 \times 10^9$/liter, and platelet count $>100 \times 10^9$/liter), renal (serum creatinine concentration $\leq$135 μM or creatinine clearance $\geq$60 ml/min) and hepatic function (total serum bilirubin $\leq 1.25 \times$ upper normal limit, and ASAT and ALAT concentrations $\leq 3.0 \times$ upper normal limits); and no unresolved bowel obstruction or chronic colic disease. The current clinical protocol was approved by the Rotterdam Cancer Institute Review Board, and all patients signed informed consent before study entry.

Vials that contained 40 or 100 mg of CPT-11 (as a hydrochloride trihydrate form) formulated as a concentrated sterile solution (active drug concentration, 20 mg/ml) in *d*-sorbitol and a lactic acid-sodium hydroxide buffer system of pH 3.5–4.5 were provided by Rhône-Poulenc Rorer (Antony Cedex, France). The CPT-11 dose of 350 mg/m^2 was administered as a 90-min i.v. infusion, after dilution of the pharmaceutical preparation in 250 ml of isotonic sodium chloride. In all patients, premedication consisted of 8 mg of ondansetron i.v. combined with 10 mg of dexamethasone i.v., administered 30 min before the start of chemotherapy.

Sample Collection and Handling. Blood samples for pharmacokinetic analysis were drawn from a vein in the arm opposite to that used for drug infusion and collected in 10-ml glass tubes containing lithium heparin as anticoagulant. Samples were obtained at the following time points: before drug administration; at 0.5, 1, and 1.5 h during infusion; and 0.17, 0.33, 0.5, 1, 1.5, 2, 4, 5, 8.5, 24, 32, 48, 56, 196, 360, and 504 h after the end of infusion. Blood was immediately processed to plasma by centrifugation for 5 min at 2500 rpm (4°C), which was then stored at −80°C until the time of analysis (see below). A pretreatment feces sample was collected from all patients 1 day prior to drug administration in a polystyrene container and stored immediately at −80°C. After thawing, these samples were homogenized individually on ice (at 0°C) to prevent enzyme degradation in one or two volumes of a 0.1 M sodium acetate buffer (pH 7.0), depending on the water content of the sample, using an Ultra-Turrax T25 homogenizer (IKA-Labortechnik, Dottingen, Germany). The homogenates were centrifuged for 5 min at 15,000 rpm, and the clear supernatants were diluted 1-fold with 50% glycerol in water (v/v). The dilutions were stored at −80°C until analysis for β-glucuronidase activity (see below).

Drug Analysis. Pure reference standards of CPT-11 hydrochloride trihydrate (batch, KO16), and the metabolites SN-38G trifluoroacetate (batch, YEO265), NPC trifluoroacetate (batch, YEO304), and SN-38 hydrochloride (batch, LIE783) were kindly provided by Rhône-Poulenc Rorer and were used as received. Drug concentrations in plasma were determined as the total of lactone and carboxylate forms by a validated HPLC method with fluorescence detection as described previously (20). This method was further modified, as reported, to allow determinations of SN-38 and SN-38G at the low femtomol level (21). Because of straying fluorescence characteristics, an incomplete extraction, and the compound's low relative retention on the reversed-phase HPLC column (Hypersil ODS with 5 μm particles), the unchanged parent drug could not be detected with this latter method (21). Likewise, attempts to separately measure SN-38 lactone levels and lactone to total ratios of SN-38 with similar sensitivity characteristics as described above for SN-38 total drug (21) have not yet been successful because of interference from endogenous plasma constituents.

Pharmacokinetic Analysis. Individual plasma concentrations of CPT-11 and its metabolites were fitted to a three-compartment model using the Siphar version 4.0 software package (SIMED, Créteil, France), as described (18). The rate constants of the various disposition phases and the AUC were estimated with a weighted-least squares method (weighting factor, 1/*y*) using the fitted model, whereas the total plasma clearance of CPT-11 was calculated by dividing dose (expressed in mg base equivalents per squared meter of body surface area) and the observed AUC. The C_{max} values (peak drug level) were determined graphically (as observed values) in a concentration-time scatter plot. Metabolic ratios were calculated as defined (22) and included the relative extent of conversion of CPT-11 to SN-38 (*i.e.*, $AUC_{SN\text{-}38}$:$AUC_{CPT\text{-}11}$) and the relative extent of glucuronidation of SN-38 (*i.e.*, $AUC_{SN\text{-}38G}$:$AUC_{SN\text{-}38}$). The latter was also evaluated as a function of time after drug administration. The systemic SN-38 glucuronidation rate in individual patients was estimated by calculation of the biliary index values (23), expressed as $AUC_{CPT\text{-}11} \times (AUC_{SN\text{-}38}$:$AUC_{SN\text{-}38G})$.

Measurement of Fecal β-Glucuronidase Activity. The enzyme activity was determined by a miniaturized colorimetric assay using phenolphthalein glucuronic acid as an artificial substrate, based on a procedure described for β-glucuronidase in bile of mini pigs (24). Briefly, 20-μl sample aliquots of feces homogenate were mixed with 20 μl of 0.1 M sodium acetate buffer (pH 7.0) containing 0.2% (w/v) BSA and 10 μl of the same buffer in the presence of 0.03 M phenolphthalein glucuronic acid (Sigma-Aldrich Co., Zwijndrecht, the Netherlands) and incubated for 1 h at 37°C in a shaking water bath. The enzymatic reaction was terminated by the addition of 200 μl of 0.1 M sodium phosphate buffer (pH 12.0), and the reaction product phenolphthalein was determined by measurement of the absorbance at 550 nm against a reagent blank on a Bio-Rad Model 550 automated microplate reader (Bio-Rad Laboratories, Hercules, CA). A calibration standard curve of phenolphthalein was constructed on the day of analysis, and concentration *versus* absorbance data were fitted by linear regression analysis. The

mean regression equation had slope and *y*-intercept values of 0.292 ± 0.021 and -0.004 ± 0.013 ($n = 15$), respectively, with a Pearson's moment correlation coefficient >0.9943. Enzyme activity levels in unknown feces samples were calculated in triplicate using interpolation of the corresponding regression analysis and expressed as micrograms of phenolphthalein liberated per h at 37°C per milligrams of feces based on dry-weight measurements (μg/h/mg). A formal method validation was performed as described (25) by replicate analysis of quality control samples spiked to contain three different concentrations on several occasions in the presence of a duplicate 7-point calibration curve and reference samples containing 1.6 units/h of lyophilized type IX-A β-glucuronidase (EC 3.2.1.31) from *Escherichia coli* (Sigma Chemical Co., St. Louis, MO) dissolved in 50% glycerol in 0.1 M sodium acetate buffer (v/v). The within-run and between-run precision, as determined by one-way ANOVA, ranged from 2.18 to 3.55% and 2.84 to 4.89% ($n = 22$ at each of the concentrations), respectively, with a mean percentage deviation from nominal values of less than ±6.14% for phenolphthalein data and ±3.30% for the β-glucuronidase reference standard.

***In Vitro* Metabolism of CPT-11 and NPC.** Biotransformation of CPT-11 and NPC into SN-38 was studied in freshly prepared aliquots of human plasma, following a 5-min centrifugation step at $3000 \times g$ of whole blood samples obtained from healthy volunteers. Prior to incubation, plasma samples were placed in a shaking water bath at 37°C for 5 min. Aliquots of 50 μl of the lactone forms of CPT-11 and NPC (from stock solutions containing 1.00 and 1.77 mg/ml in DMSO, diluted in a mixture of methanol-0.01 M hydrochloric acid) were then added to 450 μl of plasma to yield the desired final concentrations (~0.2 to 200 μ*M*), followed by slight agitation by vortex mixing. To determine the reaction velocity (*i.e.*, *V* expressed in nM/h/l of plasma), sample aliquots were taken at a fixed time interval of 24 h, which was determined in preliminary experiments to be sufficiently long to achieve steady-state (not shown), and analyzed for the presence of total SN-38 as described above for plasma samples. The Michaelis-Menten kinetics of the maximum process rate (*i.e.*, V_{max}) and the drug concentration associated with $0.5 \times V_{max}$ (*i.e.*, K_m) were determined by a nonlinear regression analysis implemented on the Number Cruncher Statistical System software package (version 5.X; Jerry Hintze, East Kaysville, UT, 1992).

Drug Transport by Caco-2 Cells. The human colon adenocarcinoma cell line Caco-2 (American Type Culture Collection, Rockville, MD) was grown as monolayers in DMEM containing 10% heat-inactivated fetal bovine serum, 100 μg/ml penicillin and streptomycin, and 2 mM freshly added L-glutamine (all from Life Technologies, Inc., Breda, the Netherlands) according to procedures recommended by the American Type Culture Collection. Cells were grown at 37°C in a humidified atmosphere in 5% CO_2/95% air as stock cultures in 75-cm^3 flasks and split at ~80% confluency using trypsin-EDTA. Caco-2 cells were then seeded at a density of 2.5×10^4 cells/insert in Transwell 12-well plates containing 1-cm^2 permeable polycarbonate inserts with a 0.4-μm pore size (Costar Corp., Cambridge, MA). The Caco-2 cells were maintained to monolayer growth by medium change every 3 days, until use in transport experiments at 28 days after seeding. The apical side of the cell layer (insert) contained 0.5 ml, whereas the basolateral side (well) contained 1.5 ml. Transport studies were initiated with CPT-11, SN-38, and SN-38G [all dissolved in a mixture of methanol-0.01 M hydrochloric acid (1:1, v/v) and diluted further in medium at final concentrations of 1.7 and 17 μM (CPT-11), 2.5 and 25 μM (SN-38), and 1.5 μM (SN-38G), respectively], added to either the apical or basolateral side. The final concentration of methanol in the dosing medium was always <1%. At the end of the experiment (1, 2, 6, or 24 h continuous exposure times), the entire apical and basolateral side solutions were collected separately in 1.5-ml polypropylene tubes (Eppendorf, Hamburg, Germany) and then centrifuged for 5 min at 15,000 rpm (4°C) to remove residual particulate matter. The supernatants were transferred to clean tubes and immediately stored at −80°C until analysis by HPLC as described (20). Mean transport fractions were calculated as the fraction of the total drug transported after exposure.

The β-glucuronidase content in the Caco-2 cells was measured using the phenolphthalein assay as described above for feces samples (detection limit, 0.3 μg/h/mg of protein), and total protein levels with the Coomassie-brilliant blue G-250 assay (26).

RESULTS

Patient Characteristics and Toxicity. A total of 14 patients (4 females and 10 males) with various solid tumors was studied, with a median performance status of 0 (range, 0–1). The median age was 53 years (range, 37–71 years), and all patients had normal hematopoietic and liver functions at the time of the study; the median clinical chemistry values for all 14 patients included a total bilirubin level of 9 μM (range, 4–12 μM); a serum creatinine level of 103 μM (range, 72–132 μM); ASAT and ALAT levels of 34 units/l (range, 17–185 units/l) and 22 units/l (range, 6–225 units/l), respectively; a total protein concentration of 79 g/dl (range, 69–87); and a serum albumin level of 45 g/dl (range, 38–51 g/dl). All 14 patients were assessable for complete pharmacokinetics of CPT-11 and metabolites. Overall, the treatment was very well tolerated, with neutropenia being the main hematological toxicity, although neutropenia graded ≥2 (on a 4-point scale according to National Cancer Institute Common Toxicity Criteria version 2.0) was encountered in only three patients (20%). Gastrointestinal toxicity was most prominent among nonhematological side effects, with diarrhea graded ≥2 occurring in eight patients (53%) and graded ≥3 in only 1 patient. One patient developed severe liver dysfunction immediately after CPT-11 administration, with total bilirubin levels rising to levels >250 μM within 200 h and ASAT graded 3. This patient died eventually 9 days after CPT-11 administration after experiencing grade 4 leukocytopenia, grade 4 neutropenia, and grade 4 diarrhea. Autopsy revealed an obstructed biliary tree by pigment stones.

Pharmacokinetics. The plasma concentration-time profiles of SN-38 after CPT-11 treatment were very similar for all patients studied (displayed in Fig. 1). In line with previous findings (18, 22), plasma concentrations gradually increased to reach peak levels within 1.5–3 h after start of the i.v. administration and slowly began to decline thereafter. SN-38 concentrations still remained detectable at 500 h after drug adminis-

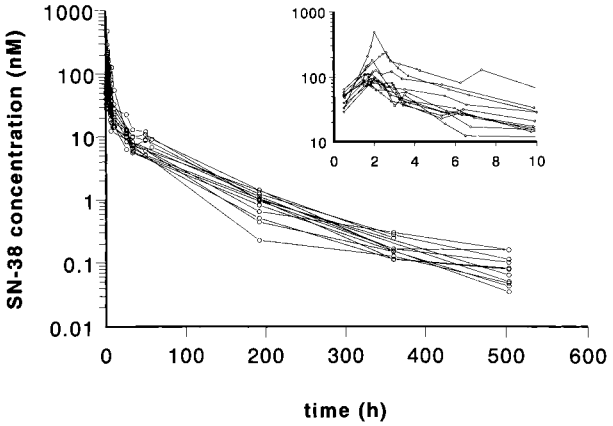

Fig. 1 Plasma concentration-time profiles of SN-38 in 14 patients treated with a 90-min i.v. infusion of CPT-11 at a dose level of 350 mg/m^2. *Inset,* concentration-time data up to 10 h after start of drug infusion.

tration [lower limit of quantitation of the HPLC assay, ~13 pM (21)]. As a result, values for $AUC_{0\text{-inf}}$ and $T_{1/2}$ were significantly higher as compared with estimates based on standard sampling time periods (Table 1). SN-38G was the principal metabolite of CPT-11 (detected in plasma of most patients), with an estimated $T_{1/2}$ slightly decreased as compared with unconjugated SN-38. The time profile of the molar concentration ratios of SN-38G and SN-38 was also relatively consistent between patients (Fig. 2), showing peak values at ~6 h and a gradual decrease toward the end of the sampling time period. Data obtained from the patient with liver dysfunction and biliary obstruction showed aberrant pharmacokinetic profiles (Fig. 3), with substantially increased plasma concentrations of both SN-38G and SN-38.

***In Vitro* Studies.** Because SN-38G is susceptible to the effects of bacterial β-glucuronidases after hepatobiliary secretion (18, 19), pretherapy values of fecal β-glucuronidase activity were evaluated to gain insight into potential enterohepatic recirculation mechanisms involved in the prolonged circulation times of SN-38. Individual levels of enzyme activity were found to vary enormously between patients (range, 0.56–44.6 μg/h/mg of feces) and were not related to any of the SN-38 kinetic parameters ($r = 0.09$; $P = 0.26$), suggesting that interindividual variation in this enzyme is unimportant in explaining SN-38 pharmacokinetic variability.

The potential of oxidative metabolites of CPT-11 taking part in the overall production of SN-38 was evaluated in experiments in freshly prepared human plasma at fixed concentrations of NPC and CPT-11 (17). The formation of SN-38 from NPC and CPT-11 by serum CE is shown in Fig. 4. For NPC, the mean values of K_m and V_{max} obtained using the Michaelis-Menten equation were 74 μM and 76 pmol/h/ml plasma, respectively, which is within the same range as described for this conversion previously in an experimental setting using human liver microsomes or purified hepatic CE (17). We also confirmed the possibility of CPT-11 transformation to SN-38 in human plasma with K_m and V_{max} values of 126 μM and 52 pmol/h/ml plasma, respectively, which agree very well with data of a previous study (27).

We have also studied the transepithelial flux of CPT-11, SN-38, and SN-38-G using the human colonic cell line Caco-2, a well-established model of human intestinal absorption (28–30), to further define the potential role of enterohepatic circulation in the kinetic profile of SN-38. The total transport fraction was clearly time dependent because of increased transport rates of the carboxylate forms of both compounds. CPT-11 and SN-38 were found to be transported to the apical side in a concentration-dependent manner with the 24-h exposure times, with transport fraction values of 0.404 and 0.405 at low concentrations and 0.269 and 0.399 at the highest concentrations tested, respectively. The flux from the basolateral to the apical side was substantially greater (4–9-fold) than that from the apical to the basolateral side. Interestingly, SN-38G could not be transported to the basolateral side after exposure at the apical side at the tested concentration. In addition, SN-38 could not be detected after exposure to SN-38G in either compartment, consistent with undetectable levels of β-glucuronidase in the Caco-2 cells (not shown).

DISCUSSION

By prospectively measuring concentrations of CPT-11 and its metabolites in plasma of cancer patients over an extended sampling time period of 500 h, we demonstrated in the present work that the terminal disposition half-life and AUC of the active metabolite SN-38 are ~2-fold increased as compared with estimates reported in earlier studies (18, 22). In addition, we found that the SN-38-G:SN-38 plasma concentration ratio decreases in time from ~7 (at 50 h) to ~1 (at 500 h). These data not only emphasize the need to apply appropriate kinetic models with sufficient sampling time points for the accurate estimation of complete concentration-time profiles but may also have direct significant clinical relevance in view of the fact that relationships between drug exposure and effect (*i.e.*, toxicity and efficacy) are still poorly defined.

Several possible explanations can account for the increase in disposition half-life of SN-38 and the time-varying SN-38G:SN-38 concentration ratios after i.v. administration of CPT-11. Previously, biliary secretion of CPT-11, SN-38, and SN-38G has been described and quantitated in a rat model, whereas intestinal reabsorption of biliary secreted radioactivity in that same study already suggested recirculation of at least some of the compounds (31). This, in combination with numerous clinical data, has led to the proposed enterohepatic recirculation of CPT-11 and its metabolites. It has been suggested in clinical studies that the enterohepatic recirculation is so prominent that its effects can be measured in the systemic circulation by a short rise of concentration of SN-38 at ~8 h after i.v. administration of CPT-11 (23). As described in earlier reports (18, 32) and again in this study, we could not confirm a consistent rise in the SN-38 plasma concentration shortly after systemic drug exposure. Direct proof of a recirculation process of any of the compounds has, to our knowledge, not yet been published. For this reason, we investigated the intestinal absorption and transepithelial flux of CPT-11, SN-38, and SN-38-G *in vitro* in Caco-2 cell monolayers, an established model of human intestinal drug absorption resembling the small bowel. In these *in vitro* experiments, we found a time and concentration-dependent

Table 1 Summary of plasma pharmacokinetics[a]

	CPT-11	SN-38	SN-38G
C_{max}[b] (μM)	7.53 ± 2.20	0.168 ± 0.115	0.639 ± 0.258
AUC_{0-56} (μM·h)	41.7 ± 13.9	1.12 ± 0.570	11.0 ± 9.26
AUC_{0-inf} (μM·h)	ND[c]	1.99 ± 0.790	26.9 ± 18.2
$T_{1/2,\ 0-56}$ (h)	13.9 ± 3.20	29.4 ± 18.6	26.6 ± 9.23
$T_{1/2,\ 0-inf}$ (h)	ND[c]	47.0 ± 7.90	39.2 ± 24.3
CL (l/h/m^2)	15.6 ± 4.32		
V_{ss} (l/m^2)	147 ± 55.5		
MRT (h)	10.4 ± 2.89		
REC (AUC_{SN-38}:AUC_{CPT-11})		0.020 ± 0.011 (range, 0.008–0.045)	
REG (AUC_{SN-38G}:AUC_{SN-38})		10.4 ± 8.20 (range, 2.10–28.0)	
BI [AUC_{CPT-11} × (AUC_{SN-38}:AUC_{SN-38G})]		2015 ± 1359 (range, 439–5160)	

[a] Data were obtained from 14 patients treated with a 1.5-h i.v. infusion of CPT-11 at a dose level of 350 mg/m^2. Data are expressed as mean values ± SD.

[b] C_{max}, peak plasma concentration; *AUC*, area under the plasma concentration-time curve; $T_{1/2}$, half-life of the terminal disposition phase; *CL*, total plasma clearance; V_{ss}, volume of distribution at steady-state; MRT, mean residence time; REC, relative extent of conversion of CPT-11 into SN-38; REG, relative extent of glucuronidation of SN-38 into SN-38G; BI, biliary index.

[c] CPT-11 could not be detected in plasma samples obtained at 196, 360 and 504 h after the end of infusion with the applied HPLC assay (lower limit of quantitation, ~3.4 nM; Ref. 20).

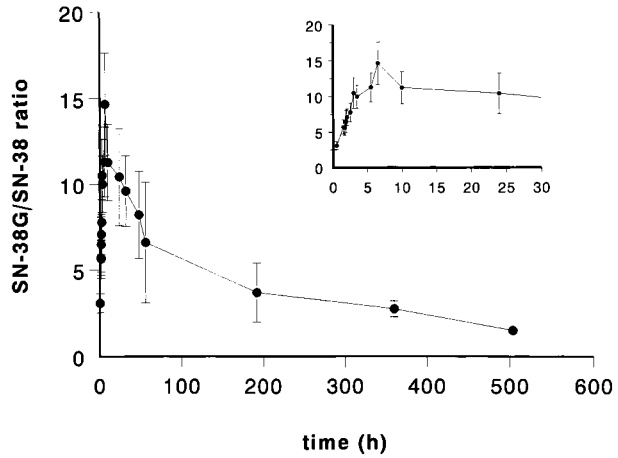

Fig. 2 Concentration ratios of SN-38G and nonglucuronidated SN-38 (SN-38G:SN-38) as a function of time after CPT-11 infusion. Data were obtained from 14 patients treated with a 90-min i.v. infusion of CPT-11 at a dose level of 350 mg/m^2 and are displayed as mean values (●) ± SD (*bars*). *Inset*, concentration ratio time data up to 30 h after start of infusion.

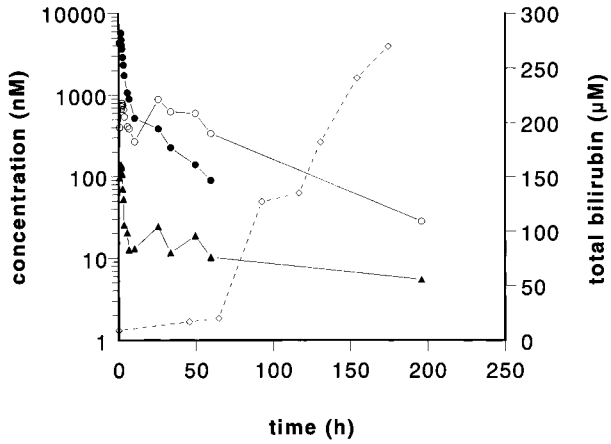

Fig. 3 Plasma concentration time profiles of CPT-11 (●), SN-38G (○), SN-38 (▲) (all *left y-axis*) and total bilirubin (±, *dotted line*; *right y-axis*) in a single patient with progressively impaired liver function treated with a 90-min i.v. infusion of CPT-11 at a dose level of 350 mg/m^2.

transport fraction for both CPT-11 and SN-38. Although the transport fractions from basolateral to apical were higher as compared with that from apical to basolateral, it is still possible that *in vivo* transport from the intestinal lumen to the circulation of CPT-11 and SN-38 is of clinical relevance, especially when intraluminal concentration of either one of the compounds is relatively high. SN-38G on the other hand could not be transported from the apical to basolateral side, consistent with its increased aqueous solubility resulting from the highly polar nature of the glucuronic acid group. In addition, the difference in absorption pattern between SN-38 and SN-38G is in accordance with the decreasing SN-38G:SN-38 ratio in time. Furthermore, we could not detect SN-38 after exposure of SN-38G in any compartment of this model, consistent with the undetectable levels of β-glucuronidase activity in the Caco-2 cells. There was also no metabolism of CPT-11 in the Caco-2 cells, despite the fact that cytochrome P-450 3A4, the major isoform in the human intestine (33, 34), has been indicated to be present at low levels in the Caco-2 cell line (35). To further examine the potential contribution of cytochrome P-450 3A4, if any, to the transport of CPT-11 using the Caco-2 cell system, experiments using cells with increased expression of this isozyme induced with addition of 1α,25-dihydroxyvitamin D_3 (36) to the growth medium are currently under investigation.

Because we found a concentration-dependent uptake of SN-38 in the above-described model, high fecal SN-38 concentrations can be of clinical significance, in a sense that a potential recycling of SN-38 reduces the effective clearance and may add a distributional compartment by way of the enteric circuit. Many glucuronides are susceptible to the effects of enterohepatic recirculation after hydrolysis through the action of bacterial and

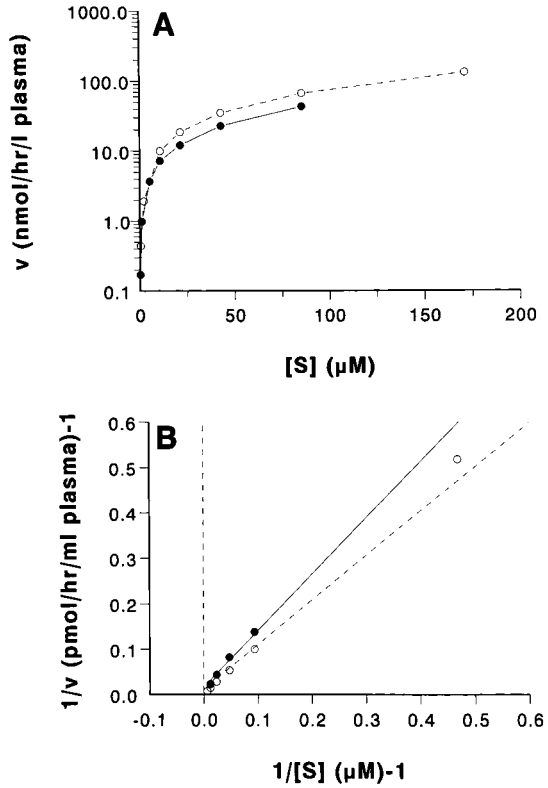

Fig. 4 Kinetics of the biotransformation of SN-38 (*V*, velocity) from CPT-11 (●, *solid line*) and NPC (○, *dotted line*) by human plasma as a function of the substrate (*S*) concentration (*A*), and the same data in a Michaelis-Menten plot [$V = V_{max} \times [S]/([S] + K_m)$] (*B*).

enteric β-glucuronidases (37). Indeed, conversion of SN-38G to SN-38 by bacterial intestinal β-glucuronidase has been shown to occur in animal models (38) as well as in humans (18). In addition, high intraluminal concentrations of SN-38 in combination with prolonged retention and subsequent structural and functional injuries to the intestinal tract is considered to be one of the mechanisms underlying the late-onset form of intestinal toxicity encountered in patients treated with CPT-11 (39). To assess the importance of SN-38G deconjugation with respect to potential SN-38 recycling, we therefore also evaluated the levels of β-glucuronidase activity in fecal pretreatment specimens of individual patients. Interpatient enzyme activity varied up to a 100-fold and did not correlate with any of the plasma pharmacokinetic parameters of SN-38. Overall, this finding shows very clearly that interindividual variation in this enzyme is unimportant in explaining SN-38 pharmacokinetic variability. This is also consistent with our recent observation that modulation of fecal β-glucuronidase activity by neomycin coadministration has no significant influence on systemic (plasma) concentrations of SN-38 (40). A potential approach for reducing CPT-11-induced intestinal toxicity may therefore be to reduce bacterial β-glucuronidase-mediated deconjugation of SN-38G to limit local accumulation of SN-38 and subsequent mucosal destruction. A clinical trial to evaluate the toxicological consequences of pretreatment with neomycin before the administration of CPT-11 is in progress.[4] The paradox between the concentration-dependent absorption process of SN-38 in the Caco-2 cells and the lack of relationship of fecal β-glucuronidase activity with SN-38 pharmacokinetics is presumably caused by the lack of enzyme activity in the luminal contents of the entire small intestine, where reabsorption of drug is most likely to occur (39).

Another possibility that could lead to prolongation of the terminal disposition phase of SN-38 is its continuous formation out of the oxidative metabolites of CPT-11. In an *in vitro* model, conversion of APC to SN-38 by rabbit liver CE has been described (41). However, rabbit liver CE, although very similar to human liver CE with respect to amino acid sequence, is ~100-fold more efficient in activating APC then the human enzyme *in vitro* (42). Indeed, *in vitro* conversion of APC to SN-38 by human CE or human liver microsomes could not be demonstrated (16), suggesting that APC is not a prodrug of SN-38 in humans. NPC, on the other hand, could be metabolized into SN-38 *in vitro* by human liver microsomes and human liver CE to SN-38 after enzymatic cleavage of the 4-*N*-(1-piperidino)-1-amino group at C10 (17). We have reported recently that peak plasma concentrations and AUC values of NPC are very low after CPT-11 administration (32), which could point to rapid and virtually complete conversion of this compound to SN-38 in the systemic circulation. To test this possibility, we evaluated the *in vitro* production of SN-38 from NPC and CPT-11 in freshly prepared human plasma and found substantial formation from both compounds. These data appear to indicate that this metabolic pathway is underestimated in relationship to what extent it adds to the total amount of SN-38 formed. Although this route could clearly contribute to the prolonged disposition phase of SN-38 and may be an important determinant of the substantial interpatient variability in CPT-11 pharmacokinetics observed here and elsewhere (32), the overall quantitative aspects of this source of SN-38 remain unknown.

Another potential contributing factor to the prolonged circulation time of SN-38 may come from competitive binding of SN-38 and bilirubin to UDP glucuronosyltransferases (43). Thus, in the lower concentration regions of SN-38, competitive binding with bilirubin may inhibit glucuronidation and prolong circulation times of the active metabolite. Although we do not have direct proof for this mechanism in the clinical situation, it is known that even minor liver enzyme disturbances and/or slight hyperbilirubinemia can give significant rise in both hematological and intestinal toxicity (44–46). In patients with (slightly) elevated bilirubin levels, competitive binding will influence the early plasma SN-38 concentration only to a minor extent but will give a disproportional prolongation of the terminal disposition phase of SN-38 and is thus likely to affect SN-38G:SN-38 concentration ratios. The importance of this competitive interaction between SN-38 and bilirubin is further underscored by our observations made in a single patient who developed very severe toxicity. This patient developed liver failure during treatment with CPT-11 accompanied with a dramatic rise in serum bilirubin concentrations. Compared with

[4] D. F. S. Kehrer and A. Sparreboom, unpublished data.

other patients, this patient experienced at least a 10-fold increase in the SN-38 plasma concentrations, which is in accordance with this hypothesis and consistent with other published data indicating substantial increases in systemic exposure to CPT-11 and SN-38 in patients with liver dysfunction (45).

In addition to the processes mentioned, a variety of other factors may influence SN-38 pharmacokinetics, including binding of the compound to plasma proteins [principally human serum albumin and gamma globulin (18)]. It has been well established that for drugs with very high protein binding [*e.g.,* SN-38, which is 94–96% bound (47)], prolonged sampling may demonstrate a relatively slow redistribution of drug into plasma and thus prolong the apparent half-life. For drugs undergoing enterohepatic recirculation (*e.g.,* SN-38), the times that patients ingest food may also impact upon plasma drug concentrations. However, although the times that the patients under study took any food were not noted and its potential impact on kinetics were not investigated here, a previous clinical study with the related compound topotecan has shown that food intake does not affect the extent of drug absorption (48).

In conclusion, we have shown by applying an extended sampling time period of 500 h that until now the circulation time of SN-38 in cancer patients treated with CPT-11 has been greatly underestimated. Because of the poorly defined relationships between pharmacokinetic parameters and pharmacodynamic outcome of CPT-11 treatment, the presently observed prolonged terminal disposition phase of SN-38 should be taken into consideration in future studies attempting to identify kinetic correlates that would assist in prediction of both hematological and intestinal toxicity. Further investigation to reveal the clinical importance of our findings is clearly warranted.

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