Irinotecan (CPT-11) Metabolism and Disposition in Cancer Patients

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ABSTRACT

The objective of this study was to determine the metabolic fate and disposition of the antitumor camptothecine derivative irinotecan (CPT-11). Ten patients with histological proof of malignant solid tumor received 200 mg/m² CPT-11 as a 90-min i.v. infusion, followed by a 1.5-h i.v. infusion of cisplatin (60 or 80 mg/m²). Plasma, urine, and feces were collected for 56 h and analyzed by a specific reversed-phase high-performance liquid chromatographic assay for the parent drug and all four metabolites positively identified to date: SN-38; its β-glucuronide conjugate, SN-38β-glucuronide (SN-38G); 7-ethyl-10-[4-N-(5-aminoantipic acid)]-1-piperidino-carbonyloxycamptothecine (APC); and 7-ethyl-10-[4-N-(1-piperidino)-1-amino-carbonyloxycamptothecine (NPC).

A three-exponential decline was observed in plasma for all compounds, with a clear predominance of the parent drug [25.6 ± 5.71 μM · h (CPT-11) versus 15.8 ± 3.51 μM · h (total metabolites)]. Total urinary excretion was 28.1 ± 10.6% of the dose, with unchanged CPT-11 and SN-38G as the main excretion products. Whereas renal clearance of SN-38 was only a minor route of drug elimination, fecal concentrations of this compound were unexpectedly high (on average, 2.45% of the dose), suggestive of intestinal hydrolysis of SN-38. Total urinary excretion was 28.1 ± 10.6% of the dose, with unchanged CPT-11 and SN-38G as the main excretion products.

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INTRODUCTION

The topoisomerase I poison irinotecan [CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecine is one of the most promising antitumor agents to have entered clinical trials in recent years, displaying a broad range of clinical activity against several neoplastic disorders, including gynecological cancers and 5-fluorouracil-refractory colorectal cancer (reviewed in Refs. 1 and 2). Structurally, CPT-11 is unique among camptothecines drugs because of a bulky 1,4'-bipiperideine-1'-carboxylate side chain located at the C-10 position, with an ethyl group at the C-7 position (Fig. 1). The former side chain can be cleaved enzymatically by a carboxylesterase converting enzyme that generates the active metabolite SN-38 (7-ethyl-1-hydroxycamptothecine) (3; see Fig. 1). Because SN-38 is >100-fold more potent as a topoisomerase I inhibitor in various in vitro systems, CPT-11 is thought to function in vivo as a prodrug of SN-38. Peripheral converting enzyme activity in animals has been characterized in serum (4), liver (5), and small intestine (6), and preliminary evidence indicates that carboxylesterase activity within the tumor may also be an important factor in drug activity (7, 8). SN-38 undergoes further metabolism, mediated by UDP glucuronosyltransferase 1A1, to an inactive β-glucuronide derivative, SN-38G (9–11).

Another pathway of CPT-11 metabolism consists of a cytochrome P-450 3A-mediated oxidation of the terminal piperidine group on the C-10 side chain, which gives rise to the formation of several compounds (12, 13). The structures of the major metabolites of CPT-11 resulting from this pathway have recently been established by Rivory and coworkers as APC (14) and NPC (15; see Fig. 1). Other, minor metabolites have not yet been positively identified. Although APC was recently shown to be less biologically active than SN-38 in in vitro culture, the contribution of this and other metabolites to biological effects in vivo is still unknown. In addition, there have been no reports on the quantitative urinary and fecal elimination of NPC and APC.

Thus, there is an urgent need to establish a mass balance for CPT-11 and to determine the complete metabolic fate of this drug in humans. Thus, here, we have examined the plasma disposition, metabolism, and urinary and fecal excretion path-
ways of CPT-11 in a group of patients with solid tumors using a recently developed analytical method based on HPLC that detects all currently identified metabolites (16, 17).

MATERIALS AND METHODS

Patients and Treatment. The metabolism and disposition of CPT-11 were studied in 10 adult patients participating in a Phase I study of CPT-11 in combination with cisplatin in various nonhematological malignancies (18). All patients had a histologically confirmed diagnosis of a malignant solid tumor that was refractory to standard forms of therapy and had adequate hematopoietic, hepatic, and renal functions at the time of study. All patients had no more than one prior combination chemotherapy regimen or two single-agent regimens, excluding prior treatment with CPT-11 or other topoisomerase I inhibitors and platinum derivatives. Vials that contained 40 or 100 mg of CPT-11 (as a hydrochloride trihydrate form) were provided by Rhône-Poulenc Rorer (Antony Cedex, France). The CPT-11 dose of 200 mg/m² was administered as a 90-min i.v. infusion, followed immediately by a 3-h i.v. infusion of cisplatin (60 or 80 mg/m²). Premedication was uniform for all patients and consisted of (a) ondansetron (8 mg i.v.) or tropisetron (3 mg i.v.) and (b) dexamethason (10 mg i.v.) just prior to start of the CPT-11 infusion, and it was repeated after 24 h. The clinical protocol was approved by the Rotterdam Cancer Institute Review Board, and all patients signed informed consent forms before entering the study.

Sample Collection. In each patient, sufficient plasma, urine, and feces was obtained before CPT-11 administration to evaluate possible interfering peaks in the HPLC analysis. Blood samples for analysis of CPT-11 and its metabolites were obtained at the following time points: before infusion; 0.5, 1, and 1.5 h during infusion; and 0.17, 0.33, 0.5, 1, 1.5, 2, 4, 5, 8.5, 11, 24, 32, 48, and 56 h after the end of infusion. All blood samples were drawn from a vein in the arm opposite to that used for CPT-11 infusion. Samples were collected in glass tubes containing lithium heparin and centrifuged immediately for 5 min at 3000 x g (4 °C) to yield plasma, which was stored frozen in polystyrene vials (Eppendorf, Hamburg, Germany) at -80 °C until the time of analysis. Complete urine collections were obtained for the duration of the study during hospitalization (i.e., up to 56 h after start of drug administration), and 0.5-ml aliquots were diluted 1:1 (v/v) with drug-free human plasma and stored frozen in microtubes. Complete collections of feces were also obtained in polystyrene containers and stored immediately at -80 °C to prevent continued degradation of SN-38G (17). Weighted feces samples were homogenized individually in five volumes of 5% (w/v) aqueous perchloric acid using five 1-min bursts of an Ultra-Turrax T25 homogenizer (IKC-Labortechnik, Dottingen, Germany) operating at 20,500 rpm. Aliquots of the feces homogenates were diluted with human plasma prior to further sample processing as described above for urine.

Determination of CPT-11 and Metabolites. Pure reference standards of CPT-11 hydrochloride trihydrate (batch KO16) and the metabolites SN-38G trifluoroacetate (batch YEO265), NPC trifluoroacetate (batch YEO304), APC hydrochloride (batch EBO1143; Ref. 19), and SN-38G hydrochloride (batch LIE783) were kindly supplied by Rhône-Poulenc Rorer and were used as received. CPT-11 and SN-38 in plasma were measured by a previously described HPLC method (16), whereas for simultaneous quantitation of CPT-11 and SN-38G and of APC and SN-38 in urine and feces, the methodology was further modified as described (17). The latter method proved also suitable for concurrent analysis of NPC.4

Briefly, 250-μl aliquots of plasma, plasma-diluted urine, or feces homogenate were acidified with 500 μl of a mixture of methanol-5% (w/v) aqueous perchloric acid to allow the estimation of total, i.e., lactone plus carboxylate, concentrations of the drug and its metabolites simultaneously. The samples were rocked on a multitube vortex mixer for 5 min, followed by centrifugation at 24,000 x g for 5 min (4 °C). The clear supernatant from the extracts was diluted 2-10-fold with methanol-0.1 M ammonium acetate containing 10 mM tetrabutylammonium sulfate (3:7, v/v), adjusted to pH 5.3 with hydrochloric acid. The diluted extracts were transferred to limited volume
inserts, and 100–200-μl aliquots were injected into the HPLC system. The system was composed of a constaMetric 4100 solvent delivery system, an autoMetric 4100 autosampling device, and a fluoriMonitor 4100 fluorescence detector (LDC Analytical, Riviera Beach, FL). Chromatographic separations were achieved using a Hypersil ODS column (100 × 4.6 mm inner diameter, 5 μm particle size; LC Service, Emmen, the Netherlands), protected by a LiChroCART guard column (4 × 4 mm inner diameter, 5 μm particle size; Merck, Darmstadt, Germany). The column temperature was maintained at 50°C using a SpH99 column oven (Spark Holland, Meppel, the Netherlands). Injected samples were isocratically eluted with methanol-0.1 M ammonium acetate containing 10 mM tetrabutylammonium sulfate [35:65 (v/v)] for CPT-11 and SN-38 in human plasma only or 30:70 (v/v) for other compounds in plasma, urine, and feces homogenate. The mobile phase was delivered at 1.0 mL/min, and the column effluent was monitored at excitation and emission wavelengths of 355 and 515 nm, respectively. The detector signal was processed with a Chrom-Card data analysis software system implemented on an IBM personal computer running under Microsoft Windows 3.0 (Fisons, Milan, Italy). The qualitative and quantitative determination of each compound was based on HPLC retention times and peak area measurements, respectively, in comparison with injected standards, typically over ranges of 2–200 ng/ml (CPT-11 and SN-38) and 10–400 ng/ml (SN-38G, NPC, and APC) in plasma or 100-5000 ng/ml in urine and feces homogenate. Calibration curves were prepared in blank samples of the appropriate biological matrix and fitted by a least squares linear regression function with proportional weighting using the Lotus Version 2.4 software package (Lotus Development Co., New York, NY). The mean overall extraction efficiencies for CPT-11 were 85.3 ± 5.3% in plasma, 89.2 ± 12.4% in urine, and 90.0 ± 5.0% in feces homogenate. Recoveries observed for the metabolites ranged between 82.6 and 99.3% and were not significantly different from CPT-11. The percentage deviation from nominal values and the between-run and within-run variabilities at various concentration levels for each compound were always <15%.

Pharmacokinetic Data Analysis. The plasma concentration-time curves were analyzed using the pharmacokinetic program Siphar Version 4.0 (SIMED, Créteil, France), by determination of slopes and intercepts of the plotted curves with multieponential functions. Initial parameter estimates were obtained by an automated peeling-algorithm procedure, with an integrated numerical algorithm based on the Powell method to minimize any objective function by the following criteria:

\[ F = \sum_{i=1}^{n} \left( \frac{Y_{o,i} - Y_{c,i}}{\sigma_{i}} \right)^{2} \]

where \( n \) is the number of observations; \( Y_{o,i} \) and \( Y_{c,i} \) are the observed and calculated \( Y \) values, respectively, for the \( i \)th observation; and \( \sigma_{i} \) is the SD for the \( i \)th observation. The statistical best fit was determined by application of (a) Akaike’s information criterion with the \( \chi^2 \) test to discriminate between models and (b) the coefficient of correlation, defined as the ratio of the SD computed using the variance-covariance matrix and the parameter value. Both weighted least squares and extended least squares methods were evaluated to estimate model parameters minimizing the sum of squared differences between experimental and computed values and the log-likelihood function. The drug disposition half-lives (\( t_{1/2} \)), AUCs from time 0 to infinity, total body clearance, and steady-state volume of distribution (\( V_{ss} \)) were determined on the basis of the best fitted curves, whereas the peak plasma concentration (\( C_{max} \)) and the time to the peak plasma concentration (\( T_{max} \)) were determined graphically.

Cell Cultures. The human colon carcinoma cell line WiDr and the ovarian adenocarcinoma cell line IGROV-1 were grown and maintained in RPMI (Brunschwig, Amsterdam, the Netherlands). Cells were kept in continuous logarithmic growth at 37°C in a humidified atmosphere in 5% CO₂/95% air in medium supplemented with 10% (w/v) heat-inactivated bovine calf serum (Hyclone, Logan, UT), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM freshly added L-glutamine (all from Life Technologies, Inc., Gaithersburg, MD). Exponentially grown cells were trypsinized and plated (2000 cells/well) in 96-well culture plates (Costar, Cambridge, MA), 48 h before drug exposure. CPT-11 and the metabolites were dissolved separately in DMSO to obtain concentrations of ~2 μg/ml (SN-38) and ~20 μg/ml (CPT-11, SN-38G, NPC, and APC). The compounds were added to the cells by serial dilution in the medium, followed by an incubation period of 5 days. After fixation with 10% (w/v) aqueous trichloracetic acid, inhibition of cell proliferation was assessed using sulforhodamine B staining as described (20), with minor modifications (21). Each compound was tested in quadruplicate in at least three independent experiments. Cell survival was plotted relative to controls incubated in medium in the absence of drug.

RESULTS

Complete pharmacokinetic studies were performed in 10 patients entered into a Phase I clinical trial of CPT-11 given as a 90-min i.v. infusion in combination with cisplatin (18). Full clinical toxicities and treatment responses will be reported in detail elsewhere. The group consisted of six men and four women, ranging in age from 36 to 66 years. Each patient had a refractory solid malignancy, with colorectal cancer being the predominant disease type, present in 7 of 10 cases. The median clinical chemistry values for all 10 patients included a total bilirubin level of 8 μM (range, 7–11 μM); a serum creatinine level of 87 μM (range, 72–115 μM); aspartate aminotransferase and alanine aminotransferase levels of 19 units/liter (range, 15–42 units/liter) and 18 units/liter (range, 13–27 units/liter), respectively; and total protein concentrations of 73 g/liter (range, 69–78 g/liter). Only two of the participants in the study were previously treated according to conventional protocols, typically with a 5-fluorouracil-containing chemotherapeutic regimen.

Analytical Method. To gain a preliminary insight into the composition of the CPT-11 metabolites in plasma, urine, and feces, samples from patients were analyzed by our initial HPLC procedure (16). This assay procedure was subsequently modified, as described (17), so that baseline resolution of all chromatographic peaks observed could be achieved [Fig. 2. A (plas-
Table 1: Plasma pharmacokinetic parameters for CPT-11 and the metabolites NPC and APC. The retention times of the suspected metabolites and of pure reference standards of SN-38G, NPC, APC, and SN-38 were identical in all matrices (means ± SD: 6.95 ± 0.21, 9.50 ± 0.92, 11.8 ± 1.27, and 28.6 ± 1.77 min, respectively). Attempts to obtain positive structural identification of the metabolites by tandem mass spectrometry and nuclear magnetic resonance spectroscopy have not yet been successful because of interference from endogenous, nonfluorescent, polar urinary, and fecal constituents. Analysis of the HPLC chromatograms did not reveal any other major compounds with similar characteristics that might represent CPT-11 metabolites in any matrix. In addition, treatment of whole-sample aliquots or isolated metabolite peaks with 1000 units of β-glucuronidase (EC 3.2.1.31) failed to release the parent drug or CPT-11 metabolite, with the exception of SN-38 from its hydrophilic glucuroconjugate, as predicted previously (17).

Plasma Disposition. The plasma concentration versus time profiles of CPT-11 and the metabolites were similar for the 10 patients studied, with a typical example shown in Fig. 3. All of the concentration-time profiles were best fitted simultaneously to a three-compartmental model after zero-order input using the Powell minimization algorithm and weighted least squares analysis with the weighting factor of 1/Y. The mean plasma pharmacokinetic parameters for CPT-11 and the metabolites, as calculated by this triexponential model, are listed in Table 1. Plasma concentrations of CPT-11 decreased rapidly immediately after cessation of the infusion, followed by a more prolonged terminal phase with a half-life of ~13.5 h, which is within the same range as described for this compound previously (22). The concentration-time course of the two cytochrome P-450-mediated metabolites NPC and APC followed the same general pattern as the parent drug, although concentrations were always well below corresponding CPT-11 levels. Consequently, the relative plasma AUC value of CPT-11 was >35 and 4 times greater than those of NPC and APC, respectively. The terminal half-life estimate for NPC could not be determined accurately in some patients due to constraints in sensitivity of the HPLC procedure (lower limit of quantitation, 10 ng/ml) but was overall still within the same range as that observed for CPT-11 and APC.

All metabolites consistently peaked within 1–2 h after start of the i.v. infusion, with APC predominating up to ~25 h postinfusion (Fig. 3). At this time, SN-38G became increasingly more important due to a 1.75-fold increased terminal elimination half-life relative to CPT-11 and APC. As a result of this extended elimination phase, which was also observed for unconjugated SN-38, SN-38G was the principal metabolite detected in plasma in most patients. Overall, however, the mean AUC value for unchanged CPT-11 was >40% larger than the summation of the AUCs of the measured metabolites.

Urinary and Fecal Recovery. The time course of the cumulative urinary and fecal elimination of CPT-11 and its metabolites for a representative patient is depicted in Fig. 4. A and B, respectively. The urinary excretion pattern was virtually identical in all 10 patients, with ~30% (range, 10.7–40.1%) of the dose excreted in the first 15 h; after this time, only very little was excreted. The time course of the fecal excretion was more variable, with most of the compounds excreted from 5 to 24 h after the CPT-11 infusion in 9 of 10 patients. Data of fecal excretion from one patient were excluded in the pharmacokinetic calculations because of incomplete stool collection. Although patients were kept in the study for only ~56 h after start of drug administration, the cumulative excretion in either urine or feces is unlikely to have changed after this time period.

The total cumulative urinary excretion of CPT-11 and the metabolites accounted for 28.1 ± 10.6% (mean ± SD) of the dose in the 10 patients. Surprisingly, fecal excretion represented only 24.4 ± 13.3% of the dose (Table 2), leading to a total excretion of ~52% of the dose. As in plasma, unchanged CPT-11 could be distinguished in urine and feces of all patients as the predominant species. SN-38G was the major metabolite in
Table 1  Summary of pharmacokinetic parameters of CPT-11 and metabolites in plasmaa

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CPT-11</th>
<th>SN-38G</th>
<th>NPC</th>
<th>APC</th>
<th>SN-38</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.52 ± 0.11</td>
<td>2.32 ± 0.21</td>
<td>2.66 ± 0.47</td>
<td>2.86 ± 0.54</td>
<td>2.08 ± 0.15</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µM)</td>
<td>3.90 ± 0.484</td>
<td>0.475 ± 0.265</td>
<td>0.057 ± 0.019</td>
<td>0.524 ± 0.292</td>
<td>0.090 ± 0.023</td>
</tr>
<tr>
<td>$t_{1/2}(\alpha)$ (h)</td>
<td>0.197 ± 0.273</td>
<td>0.720 ± 0.320</td>
<td>0.382 ± 0.208</td>
<td>1.07 ± 0.335</td>
<td>0.684 ± 0.183</td>
</tr>
<tr>
<td>$t_{1/2}(\beta)$ (h)</td>
<td>2.35 ± 0.64</td>
<td>2.11 ± 0.41</td>
<td>0.85 ± 0.48</td>
<td>2.92 ± 0.96</td>
<td>1.34 ± 0.45</td>
</tr>
<tr>
<td>$t_{1/2}(\gamma)$ (h)</td>
<td>3.15 ± 2.06</td>
<td>23.5 ± 10.6</td>
<td>9.67 ± 5.12</td>
<td>15.1 ± 2.30</td>
<td>23.8 ± 7.70</td>
</tr>
<tr>
<td>AUC (µM·h)</td>
<td>25.6 ± 5.71</td>
<td>8.01 ± 2.95</td>
<td>0.731 ± 0.329</td>
<td>5.90 ± 1.20</td>
<td>1.14 ± 0.357</td>
</tr>
<tr>
<td>CL (liters/h/m²)</td>
<td>14.0 ± 3.15</td>
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<td></td>
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<tr>
<td>MRT (h)</td>
<td>10.7 ± 0.62</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$V_{ss}$ (liters/m²)</td>
<td>138 ± 24.0</td>
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</table>

a Data were obtained from 10 cancer patients after the first treatment course of a 90-min i.v. infusion of CPT-11 at a dose level of 200 mg/m². The parameters were calculated by compartmental analysis, and data represent mean values ± SD. $T_{\text{max}}$, time to maximum concentration; $C_{\text{max}}$, maximum concentration; $t_{1/2}(i)$, half-life of the ith disposition phase; CL, total body clearance; MRT, mean residence time; $V_{ss}$, steady-state volume of distribution.

Fig. 4  Representative urinary (A) and fecal (B) excretion versus time profiles of CPT-11 (○) and its metabolites SN-38G (●), NPC (●), APC (■), and SN-38 (□) in a single patient given irinotecan by a 90-min i.v. infusion at a dose level of 200 mg/m².

urine, consistent with the highly polar nature of the glucuronic acid group and increased aqueous solubility with subsequent rapid renal excretion. Whereas renal clearance of SN-38 was only a minor route of drug elimination, fecal concentrations of this compound were unexpectedly high with, on average, 2.45% of the dose excreted unconjugated in the feces. The two cytochrome P-450-mediated metabolites NPC and APC were mainly excreted in the feces, reaching mean total percentages of 1.68 and 5.08% of the dose, respectively, probably due to their lower water solubility favoring a hepatobiliary secretion pathway.

In Vitro Cytotoxicity. Preliminary insight into the cytotoxic properties of the metabolites relative to CPT-11 and SN-38 was obtained by the exposure of the IGROV-1 ovarian carcinoma and the WiDr colon carcinoma cell lines to various concentrations of each test compound for a period of 5 days. Similar to the recent data obtained for APC in an analogous cell growth inhibition assay using the KB human epidermoid cell line (14), all metabolites (i.e., SN-38G, NPC, and APC) were found to be weak inhibitors of cell proliferation (Fig. 5). The mean concentration of each compound to produce a 50% inhibition of normal cell growth (IGROV-1 and WiDr cell lines, respectively) were 1850 and 2210 nM for CPT-11, 1010 and 1520 nM for SN-38G, 1860 and 1610 nM for NPC, 930 and 1580 nM for APC, and 1.54 and 3.63 nM for SN-38. Overall, these data indicate that SN-38G, NPC, and APC are up to 1000-fold less potent than the active metabolite SN-38. This is in line with previous data (14) and structure-activity studies demonstrating loss of biological activity with (bulky) substitutions at the C-10 position of the camptothecine structure (23).

DISCUSSION

Here, we have described, for the first time, the human pharmacokinetics of CPT-11 and metabolites in plasma, urine, and feces. The data complement previous knowledge of the clinical pharmacology of CPT-11 and have important practical implications for its optimal use. Previous studies of CPT-11 metabolism in cancer patients were able to recover only 25–50% of the administered dose in urine and bile, expressed as the summation of CPT-11, SN-38, and SN-38G, during the first 48 h postinfusion (24). This gave rise to the hypothesis that a substantial portion of the dose is eliminated through other metabolic and (intestinal) secretory pathways. The use of a recently developed specific analytical method (17) helped to resolve this
Irinotecan Metabolism in Humans

CPT-1 treatment is the disposition of CPT-1 and its potentially active metabolites in plasma. The pharmacokinetic data are not very likely, because: (a) in human liver microsomal experiments, cisplatin had no statistically significant effect on CPT-1 metabolism (25); and (b) comparison of the pharmacokinetics and metabolism of CPT-1 in clinical combination therapy with cisplatin to single agent therapy did not reveal an apparent kinetic interaction (26).

Of the greatest importance for the antitumor activity of CPT-1 treatment is the disposition of CPT-1 and its potentially active metabolites in plasma. The pharmacokinetic model presented here accurately describes the plasma concentration versus time profile of both CPT-1 and all four metabolites simultaneously. The disappearance of CPT-1 and SN-38 from the central plasma compartment was characterized by terminal elimination half-lives of ~13.5 and 23.5 h, respectively, which are in good agreement with recent data from Rivory et al. (22), obtained with the drug administered as a single agent. Previously, the use of simpler noncompartmental or linear biexponential models to describe CPT-1 disposition have consistently underestimated the elimination phase and failed to yield dose-independent parameter estimates (27, 28). Thus, the results of this study emphasize the need to apply appropriate kinetic models with sufficient sampling time points for the accurate estimation of complete CPT-1 (and metabolite) concentration-time profiles. In our patients, we observed that the total amount of NPC and APC in plasma accounted for only ~1/4 to 1/3 of the total CPT-1 AUC. The metabolites were only detectable in plasma during times of relatively high concomitant CPT-1 concentrations. The parallel decline of NPC and APC with the parent drug suggests that metabolite elimination is formation rate limited. Terminal elimination phases of SN-38G and unconjugated SN-38 were also very similar, as observed previously with CPT-1 administered as a 90-min i.v. infusion (9), and declined at a constant SN-38G to SN-38 ratio of ~7.

The basis for the delayed elimination of these metabolites relative to CPT-1 is not completely understood but may involve (saturable) rate-limited elimination processes or differences in binding affinity for plasma proteins in addition to preferential enterohepatic recycling of SN-38 and conversion of CPT-1 to SN-38 by carboxylesterases during intestinal reabsorption. Overall, the plasma AUC of the metabolites constituted only ~40% of the total AUC, indicating a clear predominance of the parent drug. The potential contribution of CPT-1 metabolites to antitumor activity, it will be essential to determine their plasma protein binding (preliminary data are reported in Ref. 29). The expected lower plasma binding of the metabolites compared

Table 2  Cumulative urinary and fecal excretion of CPT-11 and metabolites

<table>
<thead>
<tr>
<th>Compound</th>
<th>( f_{\text{ure}} ) (%a)</th>
<th>( f_{\text{feca}} ) (%)</th>
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<tbody>
<tr>
<td>CPT-11</td>
<td>20.9 ± 7.38</td>
<td>14.9 ± 10.3</td>
</tr>
<tr>
<td></td>
<td>(12.4-33.4)</td>
<td>(5.68-25.9)</td>
</tr>
<tr>
<td>SN-38G</td>
<td>3.39 ± 2.31</td>
<td>0.317 ± 0.306</td>
</tr>
<tr>
<td></td>
<td>(0.86-7.13)</td>
<td>(0.103-0.678)</td>
</tr>
<tr>
<td>NPC</td>
<td>0.308 ± 0.198</td>
<td>1.68 ± 0.200</td>
</tr>
<tr>
<td></td>
<td>(0.181-0.735)</td>
<td>(1.49-1.90)</td>
</tr>
<tr>
<td>APC</td>
<td>2.80 ± 1.68</td>
<td>5.08 ± 1.98</td>
</tr>
<tr>
<td></td>
<td>(0.853-6.06)</td>
<td>(2.28-6.88)</td>
</tr>
<tr>
<td>SN-38</td>
<td>0.389 ± 0.260</td>
<td>2.45 ± 1.16</td>
</tr>
<tr>
<td></td>
<td>(0.075-0.807)</td>
<td>(1.45-3.77)</td>
</tr>
<tr>
<td>Total compounds</td>
<td>28.1 ± 10.6</td>
<td>24.4 ± 13.3</td>
</tr>
<tr>
<td></td>
<td>(11.8-42.2)</td>
<td>(11.0-38.0)</td>
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</tbody>
</table>

" Data were obtained from 10 (urine) or 9 (feces) cancer patients after the first treatment course of a 90-min i.v. infusion of CPT-11 at a dose level of 200 mg/m². The data represent mean values ± SD, with ranges in parentheses. fe, percent of the absolute CPT-11 dose excreted as indicated drug.

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Fig. 5 Cell survival curves of the IGROV-1 ovarian adenocarcinoma (A) and the WiDr colon carcinoma cell lines (B) after a 5-day continuous exposure to different concentrations of CPT-11 (○), SN-38G (□), NPC (●), APC (■), or SN-38 (□), as assessed by the sulforhodamine B assay. Data points, mean values of at least three independent experiments performed in quadruplicate; bars, SD.
with that of SN-38, which is 94–96% bound, principally to human serum albumin and gamma globulin.\(^5\) could denote a greater ability to interact with topoisomerase I or other essential binding sites.

The cumulative urinary excretion of unchanged CPT-11, SN-38, and SN-38G of 20.9, 0.389, and 3.39%, respectively, agrees very well with the data of previous studies in which CPT-11 was administered without cisplatin (24, 30–33). In addition, only small amounts of the major cytochrome P-450 metabolites APC and NPC were detected in urine. The mean renal clearance of CPT-11, i.e., the product of the dose-fraction excreted unchanged in urine and total body clearance, was estimated to be 2.93 liters/h/m\(^2\). This value is less than the glomerular filtration rate in humans, presumably due to binding of CPT-11 to plasma proteins (~58–68% in the studied concentration range),\(^5\) and suggests that CPT-11 is neither reabsorbed nor actively secreted into the tubular lumen to a great extent. It also indicates that as much as ~80% of the overall clearance can be attributed to nonrenal processes, including metabolic degradation of CPT-11. Part of the nonrenal elimination was accounted for by fecal excretion of unchanged CPT-11 and the four metabolites, as suggested previously (17), leading to a total recovery of ~50% of the dose. This is highly surprising because it would suggest that half of the dose in urine and feces may constitute some further unknown nonextractable or, more likely, nonfluorescent metabolites. These may include compounds formed after decarboxylation of the carboxylate form of CPT-11, metabolites resulting from oxidation of the camptothecine nucleus or from combinations of these pathways, CPT-l 1. Part of the nonrenal elimination of CPT-11, i.e., the product of the dose-fraction excreted unchanged in urine and total body clearance, was estimated to be 2.93 liters/h/m\(^2\). This value is less than the glomerular filtration rate in humans, presumably due to binding of CPT-11 to plasma proteins (~58–68% in the studied concentration range)\(^5\) and suggests that CPT-11 is neither reabsorbed nor actively secreted into the tubular lumen to a great extent. It also indicates that as much as ~80% of the overall clearance can be attributed to nonrenal processes, including metabolic degradation of CPT-11. Part of the nonrenal elimination was accounted for by fecal excretion of unchanged CPT-11 and the four metabolites, as suggested previously (17), leading to a total recovery of ~50% of the dose. This is highly surprising because it would suggest that half of the dose in urine and feces may constitute some further unknown nonextractable or, more likely, nonfluorescent metabolites. These may include compounds formed after decarboxylation of the carboxylate form of CPT-11, metabolites resulting from oxidation of the camptothecine nucleus or from combinations of these pathways, as described by Lokiec et al.\(^{12}\). Further investigation is required to quantitate the contribution of the formation of these compounds and potentially other, as yet unidentified, metabolites to the overall renal and nonrenal clearance. Chemical or microflora-induced degradation of CPT-11 within the gut lumen following biliary or intestinal secretion, as observed with anthracycline antineoplastic drugs (34), is unlikely to play an important role in the overall drug elimination, in view of the extended stability of this compound in feces homogenates (17). In addition, concentrations of CPT-11 in sweat, pleural fluid, and saliva were recently shown to be fairly lower than corresponding plasma concentrations (35, 36), suggesting that excretion into these fluids are of subordinate importance.

As predicted by earlier studies (12, 17), there was no indication of glucuronic acid conjugates of CPT-11 or any other metabolite (except SN-38) in either urine or feces. This may be related to the large size of these molecules relative to SN-38, preventing them from interaction with the glucuronosyltransferases. However, great caution has to be exercised with respect to the fecal metabolites, as endogenous (biliary or enteric) and bacterial \(\beta\)-glucuronidase expressed by the intestinal microflora may together with other enzymes have led to modification of the CPT-11 metabolites excreted by this route. Indirect evidence for this was obtained from the observation of unexpectedly high fecal concentrations of SN-38 accompanied by a virtual disappearance of SN-38G in all nine patients. This notion has been described previously by Kaneda and Yokokura with CPT-11 administered to rats (37).

The finding of increased local concentrations of the active metabolite SN-38 released from SN-38G in the intestines may have considerable ramifications with respect to the clinical use of CPT-11. The major dose-limiting toxicities of CPT-11 include diarrhea and, to a lesser extent, myelosupression (1). The diarrhea is characterized by an unexpected onset and significant incidence (~60–70%) and does not respond adequately to conventional antidiarrheal agents. Although the mechanism for the observed toxicity is still controversial, it is thought to be related to structural and functional injuries to the intestinal tract that result from the mitotic inhibitory activity of SN-38 (38). In line with this hypothesis, it is of interest to note that the only patient in our study experiencing delayed diarrhea graded >2 (National Cancer Institute common toxicity criteria) had also the highest fecal excretion of unconjugated SN-38 and the lowest fecal SN-38G to SN-38 excretion ratio. Previous studies designed to establish pharmacokinetic-dynamic relationships for CPT-11 have shown a correlation between systemic SN-38 glucuronidation rates, expressed as a biliary index (i.e., the product of the plasma AUC of CPT-11 and the ratio of the plasma AUCs of SN-38 to SN-38G) and diarrhea incidences (30, 33). However, similar to recent findings by Canal et al. (39), such a relationship was not observed in our patient population.\(^6\) In all, these data suggest that consideration of interindividual differences of fecal \(\beta\)-glucuronidase activity would assist in deriving more accurate prediction of CPT-11-induced intestinal side effects and may provide a basis for the modulation of the experienced toxicity. This need is intensified in light of recent findings of Takasuna and coworkers (40) that cotreatment of CPT-11 with baicalin, an inhibitor of \(\beta\)-glucuronidase, or penicillin plus streptomycin (38) markedly ameliorated the severity of diarrhea in rats. A clinical trial to evaluate the effects of pretreatment with the antibiotic neomycin before the administration of CPT-11 is in progress at our institute.

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