

Minireview

Clinical Pharmacokinetics and Metabolism of Irinotecan (CPT-11)

Ron H. J. Mathijssen,¹ Robbert J. van Alphen,¹
Jaap Verweij, Walter J. Loos, Kees Nooter,
Gerrit Stoter, and Alex Sparreboom²

Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, 3008 AE Rotterdam, the Netherlands

Abstract

CPT-11 belongs to the class of topoisomerase I inhibitors, and it acts as a prodrug of SN-38, which is approximately 100–1000-fold more cytotoxic than the parent drug. CPT-11 has shown a broad spectrum of antitumor activity in preclinical models as well as clinically, with responses observed in various disease types including colorectal, lung, cervical, and ovarian cancer. The pharmacokinetics and metabolism of CPT-11 are extremely complex and have been the subject of intensive investigation in recent years. Both CPT-11 and SN-38 are known in an active lactone form and an inactive carboxylate form, between which an equilibrium exists that depends on the pH and the presence of binding proteins. CPT-11 is subject to extensive metabolic conversion by various enzyme systems, including esterases to form SN-38, UGT1A1 mediating glucuronidation of SN-38, as well as CYP3A4, which forms several pharmacologically inactive oxidation products. Elimination routes of CPT-11 also depend on the presence of drug-transporting proteins, notably P-glycoprotein and canalicular multispecific organic anion transporter, present on the bile canalicular membrane. The various processes mediating drug elimination, either through metabolic breakdown or excretion, likely impact substantially on interindividual variability in drug handling. Strategies to individualize CPT-11 administration schedules based on patient differences in enzyme or protein expression or by coadministration of specific agents modulating side effects are under way and may ultimately lead to more selective chemotherapeutic use of this agent.

Introduction

Camptothecin, a plant alkaloid isolated from *Camptotheca acuminata* (family Nyssaceae), was first discovered in the early 1960s (1). Because of severe and unpredictable side effects of

camptothecin in early clinical studies, clinical development was halted in the 1970s (2–5). It was later revealed that the water insolubility of camptothecin was an important factor mediating the unpredictable toxic effects (6). The elucidation of the mechanism of action of camptothecin, *i.e.*, inhibition of topoisomerase I, a nuclear enzyme which relaxes torsionally strained (supercoiled) DNA (7–9), resulted in renewed interest in this agent, and subsequently, numerous derivatives have been synthesized and tested clinically (10–12). Some of these agents, including CPT-11³ (13), topotecan (14), 9-aminocamptothecin and 9-nitrocamptothecin (15–17), lurtotecan (GI147211, GG211; Ref. 18), and DX-8951f (19), have a broad spectrum of antitumor activity both *in vitro* and *in vivo* and show more predictable and clinically manageable toxicities than the originally isolated structure. Two of these agents, topotecan and CPT-11, have recently been introduced into clinical practice for the treatment of ovarian and colorectal cancer, respectively. Of the currently available camptothecin analogues, CPT-11 has an extremely complex pharmacological profile, which is dependent on a host of enzymes involved in metabolic transformation and active transport proteins, regulating intestinal absorption and hepatobiliary secretion mechanisms (Fig. 1). Furthermore, CPT-11 is chemically unique because of the presence of a bulky side chain on the core structure, the enzymatic cleavage of which is a requirement for pharmacological activity. In addition, CPT-11 is known in two distinguishable forms, an active α -hydroxy- δ -lactone ring form and an inactive carboxylate form, between which a pH-dependent equilibrium exists (12) that significantly impacts on the kinetic profile of the compound. In recent years, a wealth of information has become available that has substantially aided in our understanding of the clinical actions of this agent. Here, we will review the clinical pharmacokinetic properties of CPT-11 and its metabolites that appear to be crucial for optimal anticancer chemotherapeutic use.

Pharmacokinetic Properties

Plasma Disposition. The plasma pharmacokinetics of CPT-11 in humans has now been addressed in several studies, and taking into account that these data are from different laboratories, the results are fairly comparable. Most of the initial studies have been performed with the drug administered as a short i.v. infusion (0.5–1.5 h). After such administration, peak plasma concentrations were reached at the end of infusion

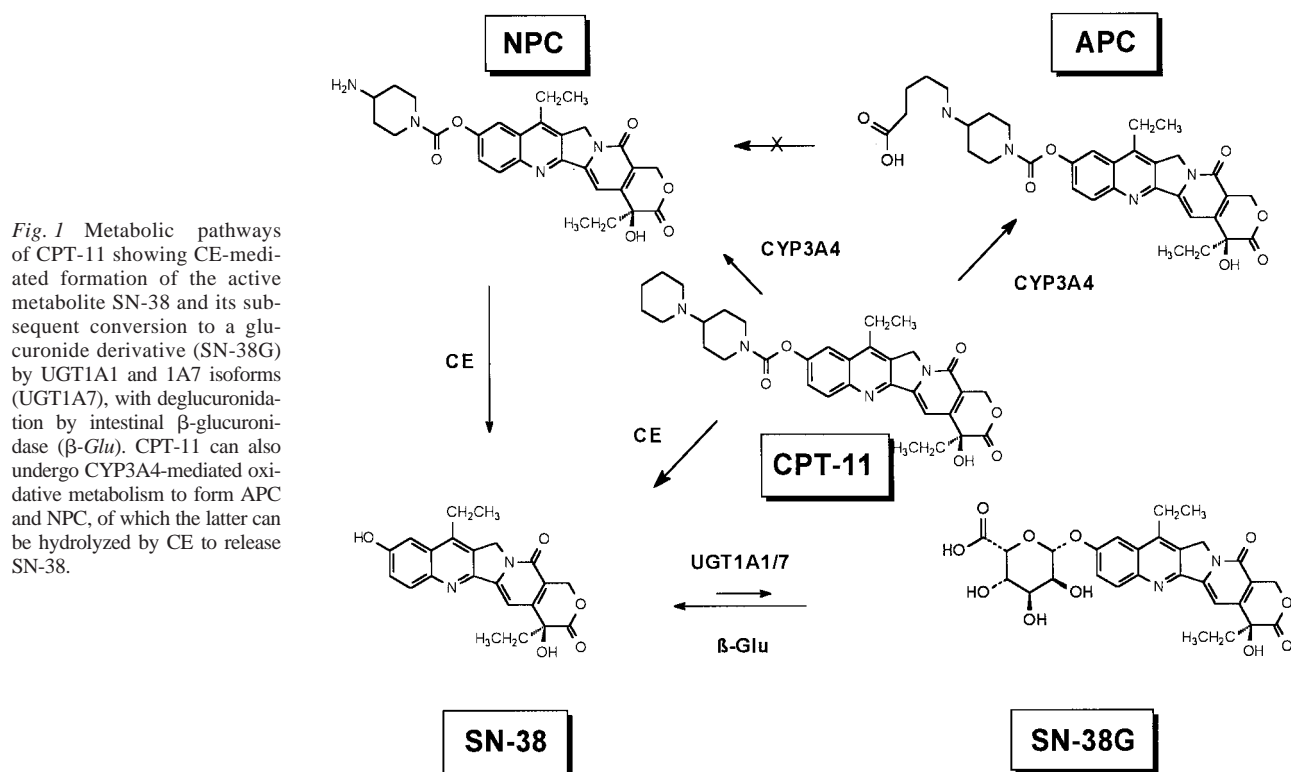
Received 3/6/01; revised 5/7/01; accepted 5/10/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ These authors contributed equally to this work.

² To whom requests for reprints should be addressed, at Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, Groene Hilledijk 301, 3075 EA Rotterdam, the Netherlands. Phone: 31-10-4391112; Fax: 31-10-4391053; E-mail: sparreboom@onch.azr.nl.

³ The abbreviations used are: CPT-11, irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin); SN-38, 7-ethyl-10-hydroxycamptothecin; SN-38G, 7-ethyl-10-[3,4,5-trihydroxy-pyran-2-carboxylic acid]-camptothecin (the β -glucuronide conjugate of SN-38); APC, 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin; NPC, 7-ethyl-10-[4-(1-piperidino)-1-aminocarbonyloxycamptothecin]; CE, carboxylesterase; UGT, UDP glucuronosyltransferase; CYP3A, cytochrome P-450 isoform 3A; AUC, area under the plasma concentration *versus* time curve; cMOAT, canalicular multispecific organic anion transporter; BCRP, breast cancer resistance protein; P-gp, P-glycoprotein.



(20–36), with a rapid decrease thereafter as a result of multiple distribution and elimination pathways (32, 36). A rebound peak in the concentration-time curve has been noticed in some studies (22, 26, 31, 36) and was initially ascribed to enterohepatic recirculation (22, 26). More recently, it has been suggested that this phenomenon is related to substantial uptake of CPT-11 lactone by erythrocytes and its subsequent release, followed by accumulation of the carboxylate form in the plasma compartment (37). Equilibrium between the two drug forms is rapidly established, and contrary to CPT-11, the principal metabolite SN-38 predominates in its active lactone form (33, 34). The lactone form of SN-38 accounts for approximately 60–70% of total plasma SN-38 after the end of a CPT-11 infusion, declining to of 33–66% at equilibrium (23, 33). The mean lactone:total AUC ratio of SN-38 is high, \sim 64%, albeit with large interpatient variability (23, 24, 33, 34, 38). This ratio remains constant for the whole dose range measured. Examples of concentration-time profiles for the lactone and carboxylate forms of CPT-11 and SN-38 are shown in Fig. 2.

The peak concentration of CPT-11 appears to be dose-proportional in a large dose range (100–750 mg/m²), although substantial interpatient variability has been noted (21–23, 25, 26, 39, 40). This variability increases at later time points (41). CPT-11 systemic exposure or AUC also increases in a dose-independent way at doses ranging from 33 to 180 mg/m² (24–26, 39, 40, 42), indicating linear pharmacokinetics.

The ratio of the lactone:total AUC remains relatively constant over the entire dose range of CPT-11, with mean values ranging from 34 \pm 5% up to 44 \pm 4% (33, 34). The conversion of the lactone into the carboxylate form of CPT-11 is rapid, with

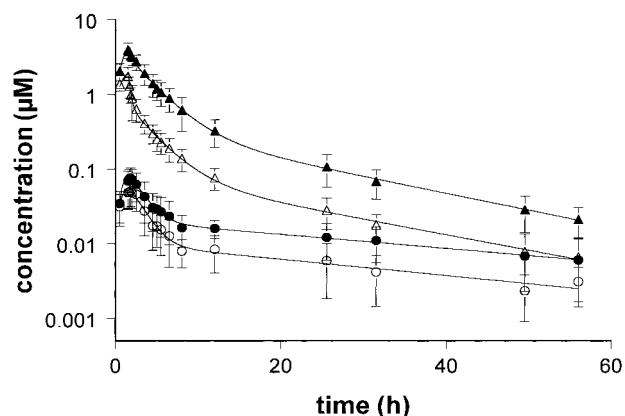


Fig. 2 Plasma concentration-time profiles of the lactone (Δ , \circ) and total drug (*i.e.*, lactone plus carboxylate) forms of CPT-11 (Δ , \blacktriangle) and SN-38 (\circ , \bullet) in a group of 17 patients receiving CPT-11 at 200 mg/m² as a 90-min i.v. infusion. All pharmacokinetic curves were fit to a three-exponential equation, using Siphar version 4.0 (InnaPhase, Philadelphia, PA), assuming a three-compartmental model for distribution and elimination of the compounds. Data are presented as means; bars, SD. Data were obtained from Mathijssen *et al.* (38).

a mean half-life of 9.5 min (34). The plasma profiles of the two forms of CPT-11 are comparable and can best be fitted in a bi- or tri-exponential equation (22, 25, 43), with mean terminal half-life values of about 9.3–14.2 h (22, 25, 35). More recently, it has been suggested that the terminal disposition half-life of

CPT-11 is much longer, which may be related to the fact that prolonged sampling schedules were applied in combination with a highly sensitive detection method (20, 43).

The volume of distribution at steady state of CPT-11 is large, suggesting extensive tissue distribution, and remained unchanged with an increase in dose (20, 22, 24, 31). Similarly, the total plasma clearance of CPT-11 was found to be dose independent, with a value of 13.5 ± 3.5 L/h/m² for the total drug (*i.e.*, lactone plus carboxylate forms) and 45.6 ± 10.8 L/h/m² for the lactone form (35). The clearance is unaltered during repeated cycles, despite a mean interpatient variability of ~30% (21, 35), and an intra-patient variability of ~13.5%.

Plasma Protein Binding. An important factor in the pharmacology of drugs is its binding to plasma proteins. In accordance with the hydrophilic nature of CPT-11, in blood, 80% of the drug is mainly bound to and/or localized in erythrocytes, whereas SN-38 is bound for at least 99%, mainly to albumin and lymphocytes, but also to erythrocytes and neutrophils (44). Although binding to plasma proteins appears to be of subordinate importance for CPT-11, binding of the principal metabolite SN-38 to plasma proteins in adults and pediatric patients is thus substantial and independent of (pretherapy) serum albumin levels (~94–96%; Refs. 44–46). In the presence of albumin, the lactone forms of CPT-11 and SN-38 are more stable (47), with higher percentages of the lactone forms available, compared with the situation without this protein. The protein binding is not significantly different for the lactone and carboxylate form of CPT-11. In contrast, SN-38 lactone binds significantly stronger to albumin than its corresponding carboxylate form, which could explain the better stability of SN-38 *in vivo* compared with CPT-11 (47). This, in turn, may play a role in the differential terminal disposition half-lives and volumes of distribution between these molecules. In *in vitro* incubations in the presence of human serum albumin, the apparent half-life of the interconversion of CPT-11 is more than three times higher than that in patients (34). This suggests that there is an early preferential uptake and/or metabolism of the lactone form of CPT-11, thereby altering the ratio of CPT-11 lactone *versus* carboxylate (34).

Metabolism

Several studies have shown that hepatic metabolism and biliary secretion are major pathways of CPT-11 elimination in both animals and humans, with major contributions from various classes of enzymes, including CEs, UGTs, CYP3As, and β -glucuronidases.

Esterase-mediated Biotransformation. The conversion of CPT-11 to SN-38 through cleavage of the ester-bond at C10 by CEs has been studied extensively in recent years. It has been suggested that two human isozymes of liver CE, *i.e.*, hCE-1 and hCE-2, have been characterized that might catalyze this conversion (48, 49). No tumor reduction in SQ20b cells was seen after incubation of CPT-11 with hCE-1, compared with a 60% reduction for hCE-2 (49). Therefore, hCE-2 probably plays the most important role in CPT-11 conversion by CEs in cancer patients. The plasma of several species, including mouse and horse, contains high levels of esterases other than CE and it converts CPT-11 much better into SN-38 than human plasma

does. It has been suggested that butyrylcholinesterase, which has CPT-11 converting activity, might be responsible for this phenomenon (50, 51).

SN-38 concentrations have been shown to increase with the CPT-11 dose over the dose range studied (100–750 mg/m²; Ref. 35). The terminal disposition half lives of SN-38 lactone and total drug are significantly longer than those of CPT-11 (38, 43). In an *in vitro* study of human liver microsomes, twice as much SN-38 was formed when CPT-11 was present in its lactone form in comparison with CPT-11 in its carboxylate form (52). Because CPT-11 lactone is converted easier, this phenomenon might contribute to the predominance of SN-38 in its lactone form in plasma (52).

A clear relationship between CE levels and the chemosensitivity of proliferating tissues and cell lines in general has been suggested (53–55). CPT-11 was significantly more active in five human small-cell lung cancer cell lines than in four non-small cell lung cancer cell lines, and this was attributed to a higher CE activity in the former case (55). CPT-11-converting CE activity in human tumors has also been studied *in vivo* (56). A wide range of variability in enzyme activity in primary colorectal tumors was seen, with no significant difference between primary and secondary tumors. Relatively high CE activity in these tumors, as compared with normal liver tissue has been noticed, which might suggest a local activation of CPT-11 in tumor tissues (56). There were no significant differences between enzyme activity in human liver and colon tissues.

Selective upgrading of CE levels in tumors, thereby producing tumor-specific activity may clearly have substantial clinical implications; it will maximize the exposure of tumors to SN-38 and limit systemic drug concentrations and therefore also limit adverse effects of CPT-11. This upgrading has been studied for several enzyme/prodrug combinations, using antibodies or viruses to attack tumor cells. For the CE/CPT-11 combination, a recombinant replication-deficient adenovirus vector coding for human CE cDNA has been developed (57). This vector effectively suppressed A549 cell growth *in vitro* in the presence of CPT-11 by 7–17-fold. Because SN-38 diffuses from the tumor cell in which it has been produced to its neighbor cells in growth-suppressive concentrations, only 10% of the A549 cells need to be infected for a cytotoxic effect in 48% of the cells (57). In CPT-11-resistant A549 cells, which had a 6-fold resistance and 42% CE activity compared with normal A549 cells, functional CE expression could be accomplished by infection with this vector (57, 58), and these resistant cells could be suppressed efficiently *in vitro* and *in vivo* (58). This sensitivity has also been described for two other human tumor cell lines, although in another eight human cell lines, only a minimal effect was seen (59).

In various studies, the activity of CE isozymes from animal species has been compared with the human enzyme(s), and the latter show consistently lower activity than those of the other species investigated (60, 61). Although the active site amino acids of rabbit and human CEs are almost identical, human CE converts CPT-11 100–1000-fold less efficiently (59). In Rh30 human rhabdomyosarcoma cells, expression of the rabbit enzyme was associated with more rapid tumor regression and a better prevention of tumor recurrence *in vitro* (59, 62). In addition, immune-deprived mice, carrying rabbit CE-expressing

Rh30 cells, were more sensitive to CPT-11 than human CE-expressing cells or control cells. No recurrent tumors occurred in the rabbit-enzyme group, in contrast to 29 and 100% recurrence in the two other groups, respectively (59). The clinical utility of this approach is currently under further investigation.

UGT-mediated Biotransformation. SN-38 itself is further metabolized in human liver by UGT1A1 to an inactive compound, SN-38G (63–65). The lactone functionality of SN-38G could also be hydrolyzed after a pH-dependent equilibrium (65). Mostly, the plasma concentrations of SN-38G are related to SN-38 plasma concentrations, with peak values at ~1.2 h (range, 15 min to 4.5 h) after the end of infusion (31, 43, 66). SN-38G concentrations increase linearly with the administered CPT-11 dose, suggesting that hepatic glucuronidation is not saturated in the dose ranges studied (up to 600 mg/m²; Refs. 36, 42). The AUC of SN-38G is ~7-fold higher than that of SN-38, suggesting extensive glucuronidation of SN-38 into SN-38G (32, 66), with high interpatient variability (41).

Considerable variation in the conversion of SN-38 into SN-38G in human liver cells has also been noticed *in vitro* (63). This may be attributable to different enzyme activities of the various UGT isoforms. Two distinct gene families of UGT enzymes exist, *UGT1* and *UGT2*, which are both being subclassified into several isoforms. Results obtained from transfection studies have shown that isoform UGT1A1 is primarily responsible for the SN-38 conversion (63), although it was shown more recently that isoform 1A7 is ~21-fold more efficient at physiological pH than UGT1A1 (67).

A genetic polymorphism has been reported in the TATA box sequences of UGT1A1 (68). Normally, the box contains (TA)₆TAA in its promoter region, and sometimes a box contains an extra TA repeat (68, 69). An extra TA repeat in the TATA box has been associated with Gilbert's syndrome (70). The metabolic ratio (SN-38:SN-38G) for these patients is significantly higher compared with the common genotype, suggesting less SN-38 glucuronidation capacity (69). In addition, it has been reported that in patients with the Crigler-Najjar type I syndrome, UGT1 activity is totally lacking (63, 71, 72). As a result, SN-38 cannot be inactivated adequately into SN-38G, and therefore patients with these disorders are at increased risk for severe CPT-11-induced toxicity (63). On the basis of the relative importance of the systemic glucuronidation and the existence of genetic polymorphism, it has been proposed that UGT1A1 genotyping might be an approach to individualize CPT-11 treatment schedules (68, 73).

A significant correlation was observed recently between SN-38 glucuronidation rates and bilirubin glucuronidation by human liver microsomes (63). Interestingly, UGT1A1 is the isozyme that also conjugates bilirubin (72). Also for bilirubin glucuronidation, an inverse correlation between glucuronidation rate and number of TA repeats has been reported (68–70). It has been shown subsequently that baseline values of unconjugated bilirubin correlate significantly with both neutropenia and the AUCs of CPT-11 and SN-38, and this relationship might thus be useful in individual dose prescription or adaptation (72). No relationship has been observed between the glucuronidation of SN-38 and that of para-nitrophenol, which is glucuronidated by several UGTs, including UGT1A1 (63). An intriguing observation has been that valproic acid, an inhibitor of glucuronidation,

given 5 min before CPT-11 in rats, caused 99% inhibition in SN-38G formation (74), resulting in a mean increase in SN-38 AUC of 270%. Pretreatment with phenobarbital, an inducer of UGT, resulted in a 72% enhancement in the AUC of SN-38G, with a concomitant reduction in the AUCs of CPT-11 and SN-38 of 31 and 59%, respectively (74). These findings are consistent with data indicating that a patient with Gilbert's syndrome could be treated successfully with simultaneous administration of CPT-11 and phenobarbital (31). CPT-11 and SN-38 were formed in concentrations comparable with those achieved in normal patients, although the AUC of SN-38G appeared to be substantially altered (31).

In human lung cancer cell lines, the role of SN-38 glucuronidation in cytotoxicity profiles has been studied extensively (75). PC-7/CPT cell lines showed an increased glucuronidation in comparison with normal PC-7 cells, and when UGT activity was inhibited, the cells became more sensitive to SN-38. Thus, an up-regulation of the UGT activity may lead to SN-38 resistance in the tumor (75). BCRP or mitoxantrone-resistance half-transporter (MXR) expression was also found to result in 400–1000-fold resistance to SN-38 as compared with parental cell lines (76). Because MXR cells were highly capable to resist drugs that are susceptible to glucuronidation, UGT activity in resistant and normal cell lines were compared. In resistant cells, higher SN-38G levels were seen than in normal cells. Thus, in cancer cells glucuronidation might contribute to drug resistance, but the lack of high UGT levels in resistant MXR cells suggests that BCRP alone is sufficient to induce drug resistance patterns (76).

CYP3A-mediated Biotransformation. Recently, other quantitatively important metabolites of CPT-11 have been identified, the formation of which is dependent of CYP3A. Of these, APC is a major metabolite detectable in plasma and is formed by a CYP3A-mediated oxidation of the distal piperidine group at C10 of CPT-11 (77–79). NPC is also formed through this pathway, by cleavage of the distal piperidino group of CPT-11 (77, 79–82). APC peaks at ~2 h after the end of infusion, and AUC values increase linearly with increasing CPT-11 dose, despite important interpatient variation (36). At equimolar concentrations (as compared with CPT-11), APC did not inhibit the conversion of CPT-11 into SN-38 (83).

Similar to CPT-11, APC shows little cytotoxic activity, although it has some inhibitory effect on acetylcholinesterase, an enzyme involved with the acute cholinergic syndrome observed directly following CPT-11 administration (83). It is likely that APC does not contribute directly to the total activity and toxicity after CPT-11 administration *in vivo*, because it shows minor antitumor activity *in vitro* compared with SN-38 and less toxicity compared with CPT-11. NPC is also a poor inhibitor of cell growth and a poor inducer of topoisomerase I-DNA cleavable complexes, with less antitumor activity in cell lines than CPT-11 (80, 84). In addition, formation of APC and NPC is unlikely to take place in human (colorectal) tumors, where CYP3A levels are generally very low (85). It is of particular interest, though, that human liver microsomes and human liver CE are able to hydrolyze NPC into SN-38 though still to a lesser extent than CPT-11 (80, 84), whereas for APC this conversion has not been demonstrated conclusively (80, 86). Nevertheless, patients with high AUCs of SN-38, also had

high AUCs of CPT-11 and APC, suggesting that some degree of correlation might be present (36). In addition, NPC cannot be formed from APC, in contrast to a formerly suggested conversion (81, 84).

The recognition that CPT-11 is a CYP3A substrate is an important finding, because it makes this agent subject to a host of enzyme-mediated drug interactions, even with commonly prescribed comedication (81, 87). For example, the prototypical CYP3A inhibitors, ketoconazole and troleandomycine, inhibit the conversion of CPT-11 into APC and NPC almost completely (77–79, 81). In addition, both loperamide and racecadotril inhibit APC and NPC formation by >50%, whereas ondansetron inhibits these formations by 25 and 75%, respectively (78, 81). APC and NPC formation correlated significantly with testosterone 6 β hydroxylation, which is also mediated by CYP3A (78, 81). It has been shown recently that the subtype CYP3A4 is the main isozyme involved in formation of both APC and NPC (79, 82), although it was suggested previously that cytosolic aldehyde dehydrogenase might also be involved in APC formation (81). CYP3A5 also showed catalytic activity, but neither APC nor NPC was formed in its presence (79, 82). Instead, a new (unidentified) metabolite was found (79, 82). Because this enzyme is expressed in only 25–30% of adult livers, this might explain some of the interpatient variability in CPT-11 metabolism (82). Preliminary evidence has also been generated indicating that SN-38 might be metabolized by CYP3A4, although the clinical implications of this finding have not yet been evaluated.

β -Glucuronidase-mediated Biotransformation. When SN-38G is excreted in bile and intestines, several bacteria including *Escherichia coli*, *Bacteroides* species, and *Clostridium perfringens* can convert this compound back to the active metabolite SN-38 by producing the enzyme β -glucuronidase (88–90). Various studies have described (microflora-derived) β -glucuronidase activity in nonhuman (91–94) and human intestines (95–97). In rats and athymic mice, within 1 h after the end of infusion, acute diarrheal symptoms with impaired water absorption and increased prostaglandin E₂ levels have been found (92, 93). Histopathological changes in the descending colon, cecum, and ileum were observed after the daily administration of CPT-11 (91–93). Recently, these mucosal abnormalities were confirmed in human colon (97). A good correlation between this histological damage and β -glucuronidase activity in the intestinal lumen has been noticed, whereas a poor correlation was observed with CE activity (91). The lesser damage found in small intestines compared with damage in the colon could be explained by a lower exposure of this tissue to SN-38. This may be attributable to a smaller amount of β -glucuronidase in the lumen of small intestines (94).

Because many drugs could affect the functioning of the intestinal bacteria, comedication may influence the chemotherapeutic treatment of patients with CPT-11 (98). Because bacteria producing β -glucuronidase activity will be killed by antibiotics, it is anticipated that this will lead to a reduction in acute and delayed diarrhea and cecal damage (91). It has been demonstrated that antibiotics had no effects on the plasma concentrations of CPT-11, SN-38, or SN-38G in mice (penicillin/streptomycin; Ref. 94) and in humans (neomycin; Ref. 99). This combined treatment also resulted in reduced SN-38 concentra-

Table 1 Cumulative urinary and fecal excretion of CPT-11 and metabolites

Compound	Urine	Feces	Total
CPT-11	22.4 \pm 5.50	32.3 \pm 4.47	54.7
SN-38G	3.02 \pm 0.77	0.27 \pm 0.17	3.29
SN-38	0.43 \pm 0.12	8.24 \pm 2.51	8.67
APC	2.23 \pm 1.53	8.29 \pm 2.95	10.5
NPC	0.14 \pm 0.08	1.36 \pm 0.94	1.50
Total compounds	30.2 \pm 6.60	62.0 \pm 7.60	92.2
Not extracted	1.25 \pm 1.55	9.86 \pm 3.77	11.1

Parent drug and metabolite abundance are expressed as percentages of administered dose and were determined by quantitative radiometric high-performance liquid chromatography. Data were obtained from Slatter *et al.* (20).

tions in the intestinal contents with concomitantly increased SN-38G levels (94, 99). A potential approach for modulating CPT-11-induced intestinal toxicity may therefore be to reduce bacterial β -glucuronidase-mediated deconjugation 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 at our institute.

Excretion

Several studies have examined the excretion of CPT-11 in bile, feces, and urine in animals and humans. In humans, only 52% of the given dose has been recovered using nonradiometric HPLC methods (32, 100), whereas in a recent radiometric study quantitative recovery (95.8 \pm 2.7% of radioactivity) was obtained (20). CPT-11 and its three most common metabolites (SN-38, SN-38G, and APC) were responsible for almost all (~93%) detected material (20).

Urinary Elimination. In urine, CPT-11, APC, and SN-38G are the main compounds detected within 24 h after CPT-11 administration (20, 32), with CPT-11 accounting for about 10–22% of the administered dose and SN-38 contributing only 0.18–0.43% (20, 22, 25, 31, 66, 100). Consistent with the highly polar nature of the glucuronic acid group and increased aqueous solubility, SN-38G may be excreted rapidly by the kidneys (32). The cumulative excretion of APC and NPC was very low, amounting to <1% of the dose (32). This suggests that the bulk of CPT-11 and its metabolites is excreted during the first 24 h after infusion, whereas assessing urinary recovery of radiolabeled CPT-11 over 196 h also showed that the excretion was almost complete within 48 h (Table 1; Ref. 20). Similar findings were obtained with daily administration schedules of CPT-11, indicating unchanged urinary excretion profiles (26, 29).

Biliary Secretion. In a few patients, CPT-11 and SN-38 concentrations in bile have been measured (20, 24, 25, 100). CPT-11, SN-38, and SN-38G biliary secretion varies between approximately 3–22, 0.1–0.9, and 0.6–1.1%, respectively (100). NPC was also detected in bile at very low concentrations (80). In a female patient, carrying a biliary T-tube and receiving radiolabeled CPT-11, 30.1% of the administered dose was recovered in bile as radioactive compounds (20).

In rats, a cMOAT located on the bile canalicular membrane was shown to be responsible for the transport of CPT-11 car-

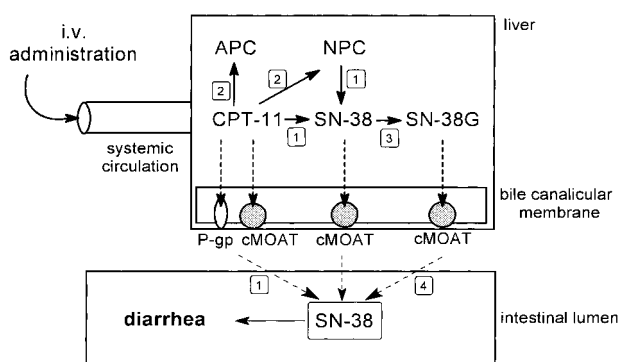


Fig. 3 Primary active transport systems for CPT-11 and its metabolites in the bile canalicular membrane of humans indicating the hypothesis for the mechanism of toxicity caused by i.v. administration of CPT-11 [modified from Sugiyama *et al.* (105)]. 1, carboxylesterase-mediated conversion; 2, CYP3A-mediated conversion; 3, UGT1A-mediated conversion; 4, β -glucuronidase-mediated conversion.

boxylate, SN-38 carboxylate, and both SN-38G carboxylate and lactone (101). For CPT-11 carboxylate, cMOAT appeared to be a low-affinity transporter, whereas a high-affinity transporter is still unknown (102). For SN-38G lactone and carboxylate, cMOAT is the high-affinity transporter, whereas a low-affinity transporter most likely also exists, presumably P-gp. It has also been found that cMOAT transports SN-38G carboxylate more efficiently than its lactone form (102). In humans, the same transport mechanisms by cMOAT of CPT-11 carboxylate, SN-38 carboxylate, and SN-38G were found as in rats (Fig. 3; Ref. 103).

Cyclosporin A, an inhibitor of the biliary secretion (104), and a known substrate and inhibitor of P-gp (105), significantly increased plasma concentrations of CPT-11, SN-38, and SN-38G in rats (104). At a cyclosporin A dose of 60 mg/kg, the AUCs of CPT-11, SN-38, and SN-38G increased 2–4-fold. This is most likely the result of reduced renal and nonrenal clearance, which simultaneously was reduced by 55 and 81%, respectively. Simultaneously, the terminal disposition half-lives of CPT-11 and its metabolites were substantially prolonged. The conversion of CPT-11 into SN-38 was not altered, but the glucuronidation of SN-38 decreased in comparison to CPT-11 infusion without cyclosporin A (104). In view of these results, it has been suggested that P-gp is one of the high-affinity transporters of CPT-11 carboxylate (106). However, similar to P-gp, cMOAT function may also be inhibited by cyclosporin A or other substrates and modulators (106). Therefore, inhibition has been studied at various concentrations of CPT-11. Because the high-affinity component is most active at substrate concentrations of 5 μM and cMOAT is most active at 250 μM , inhibitors of P-gp and cMOAT were compared at these concentrations. It appeared that P-gp substrates or modulators such as verapamil, PSC-833 (valsopodar), or cyclosporin A inhibited the uptake of CPT-11 carboxylate most at concentrations of 5 μM . This suggests that P-gp might be the high-affinity component in CPT-11 carboxylate transport (106).

As an adverse effect, cyclosporin A also inhibits bilirubin excretion, and this may lead to hyperbilirubinemia (104). In the

human Dubin-Johnson syndrome, chronic hyperbilirubinemia is seen as a result of a mutation in the *cMOAT* gene (107), and variability in the expression of this protein might play a role in the variability in experienced toxicity during CPT-11 treatment (103).

Fecal Excretion. In a mass balance study using radiolabeled CPT-11, fecal excretion was the major route of drug elimination, with 63.7% of the administered drug recovered (20). The unexpectedly high SN-38 concentrations and relatively low SN-38G concentrations in fecal specimens are suggestive for substantial β -glucuronidase activity in human intestinal contents, and consequently, the SN-38:SN-38G ratio was relatively high in all patients (20, 32). SN-38, APC, and NPC are mainly excreted in feces, 2.5 and 1.7% for SN-38 and NPC, respectively (32).

Considerations of Schedule and Route of Administration

Despite all kinds of dosing schedules, the total CPT-11 dose that can be tolerated in any time period is the same (23–25). Prolonged infusion times might theoretically improve the efficacy of CPT-11, because cytotoxicity of topoisomerase I inhibitors is S-phase specific (10), although the relevance of this principle for CPT-11, given the prolonged terminal disposition half-life of SN-38, is unclear. From xenograft models, it was known that low-dose protracted schedules of CPT-11 administration were more effective and less toxic than the usual higher dose, short infusion periods (108, 109). Chronic low-dose exposure of CPT-11 in patients has been studied recently (29, 39, 45, 110, 111) and revealed that the AUC ratios of lactone to total drug for CPT-11 and SN-38 were in the same range as for the shorter infusion periods (29). The maximal and recommended tolerated doses in these studies, 10–30 mg/m²/day, are much lower for the prolonged infusions than for the shorter durations. Surprisingly, the SN-38 AUC levels reached similar values as compared with those of the shorter infusion periods, presumably reflecting saturation of enzymatic biotransformation with the latter schedules (29, 39, 45, 110).

Oral Administration. Clearly, the availability of a suitable oral formulation of CPT-11 would allow more convenient use of prolonged dosing schedules. When human liver and intestine S9 fractions were incubated with CPT-11, the agent was mostly hydrolyzed into SN-38 by liver, duodenal, jejunal, and ileal fractions and less by colon and rectal fractions (91, 112). Because it has been discovered that CEs are widely expressed in the human liver and gastrointestinal tract, it may be possible to effectively administer CPT-11 p.o. (112, 113), with the knowledge that substantial presystemic metabolism could take place. The low pH value of the stomach may be favorable for the retention of CPT-11 in its lactone form (113).

Oral administration of CPT-11 in mice resulted in peak concentrations of CPT-11 and SN-38 in plasma within 1 h after administration (114, 115), and the bioavailability of CPT-11 increased with increasing dose and amounted to 10–20% (115). In contrast, and of concern, the SN-38 AUC after oral administration did not correlate linearly with the dose, and in fact even decreased with increasing CPT-11 dose. It has been suggested that saturation of CE might cause this nonlinear correlation

(115), but the high levels of CE expressed in human intestinal tissues do not render this suggestion very likely (113). The higher SN-38 total:CPT-11 total ratios, compared with i.v. administration, suggest a presystemic CPT-11 conversion (116). Lactone *versus* carboxylate CPT-11 ratio was comparable with i.v. infusions, but the SN-38 ratio was much higher (113), suggesting a longer persistence of SN-38 lactone when it is given p.o. When CPT-11 was given orally to nude mice bearing human tumor xenografts, the agent retained its cytotoxic properties (53). Furthermore, in early clinical trials initial antitumor activity has been observed (113), suggesting that oral CPT-11 might be an attractive therapeutic option for the treatment of several human malignancies.

The uptake rates of [¹⁴C]CPT-11 and [¹⁴C]SN-38 from hamster intestinal epithelial cells and human colon carcinoma HT29 cells were recently published, providing important insight on the absorption characteristics of these agents (117). The lactone forms of CPT-11 and SN-38 were transported mainly by a passive diffusion, whereas the carboxylate forms were absorbed as a result of an active transport mechanism. Both compounds were transported significantly more in their lactone form, and the uptake rates showed a clear pH dependency, because the uptake decreased to 68% when the pH values were higher than 6.8. Lower SN-38 uptake rates also correlated with lower cytotoxicity (117). It was suggested that raising pH values in human intestines might decrease the reabsorption of the drug and as a result it will lower the intestinal side effects (117). The clinical utility of this concept is currently under further investigation. Clearly, demonstration of unaltered pharmacokinetics of SN-38 in the presence of intestinal alkalization is of crucial importance. Thus, although these investigators have shown that this concept can reduce CPT-11-induced intestinal toxicity, this may be a pyrrhic victory if a simultaneously altered metabolic clearance (by way of a decreased enterohepatic recirculation of SN-38) results in reduced antitumor activity.

i.p. Administration. i.p. administration of CPT-11 has also been studied recently and may have some potential advantages over the i.v. route. It appeared that the therapy was more effective and less toxic in mice bearing C26 colon cancers (118). In mice bearing P388 leukemia ascites, substantially elevated peritoneal AUCs of CPT-11 and SN-38 were found, whereas the achieved plasma levels were comparable with those after i.v. dosing (118). If the i.p. administration of CPT-11 does not prove to have major local toxicities, this administration pathway might be an asset for the adjuvant therapy of colorectal cancer.

Pharmacokinetic-Pharmacodynamic Relationships

Relationships between pharmacokinetic parameters and CPT-11-induced side effects have been studied extensively. For abdominal cramps, nausea, vomiting, and anorexia, no correlation with any parameter has been found (23). For myelosuppression and diarrhea in relation to the AUCs of CPT-11 and SN-38, respectively, the findings are highly variable, and final conclusions cannot yet be drawn (Table 2; Refs. 21, 29, 45).

The product of the plasma AUC ratio of SN-38:SN-38G and the plasma CPT-11 AUC (*i.e.*, the biliary index) may be an important kinetic variable because it is thought to be correlated with SN-38 bile concentrations (66). Patients with high biliary

Table 2 Correlation between toxicities and the AUCs of CPT-11 and SN-38

Observed toxicity	CPT-11 AUC	SN-38 AUC	Refs.
Neutropenia	No	No	26, 29, 40, 45
	No	Yes	23, 39
	Yes	Yes	21, 22, 25, 27
Diarrhea	No	No	21, 23, 29, 45
	No	Yes	40
	Yes	No	26, 39
	Yes	Yes	22, 25, 27

indices, suggestive of high SN-38 biliary concentrations, in some studies experienced more severe diarrhea (grades 3 and 4 on the National Cancer Institute-Common Toxicity Criteria scale) than those with low biliary indices (31, 66), but this could not be confirmed in other studies (21, 119, 120). It has been hypothesized that consideration of interindividual differences in fecal β -glucuronidase activity would likely assist in a more accurate prediction of CPT-11-induced intestinal side effects and may provide a basis to modulate the experienced toxicity (32).

Evaluation of Drug Interactions

Drug interactions may arise as a result of altered pharmacodynamics or pharmacokinetics of the drugs involved. In the case of pharmacokinetic interactions, this is usually attributable to modification of tissue disposition and metabolism of the drugs. These phenomena are of particular importance in cancer chemotherapy when cytotoxic agents are used, because of the increased risks of severe toxicity. Most of the data available currently to evaluate potential drug interactions with CPT-11 come from clinical trials of CPT-11 given in combination with one or more other anticancer agents (Table 3). Although in several cases only limited information is available, some (preliminary) conclusions can be drawn.

Pharmacokinetic interaction studies have been performed with cisplatin either given alone or in combination with vindesine (121, 122) or ifosfamide (123), and indicated unaltered disposition profiles of both CPT-11 and its metabolites at any dose level or sequence tested (124–126). Likewise, the pharmacokinetics of carboplatin were not influenced by CPT-11, although in one study, the AUC of both CPT-11 and SN-38 was lower than expected, suggesting increased clearance of CPT-11 when given together with carboplatin (127, 128). The basis for this apparent change in pharmacokinetic behavior is unknown, although an effect on CYP3A expression attributable to prior exposure to carboplatin cannot be excluded (129).

In a Japanese study, altered pharmacokinetics were also found when 5-fluorouracil was administered immediately after CPT-11 infusion (130). Compared with an earlier CPT-11 monotherapy study conducted by the same group, the AUC of CPT-11 substantially increased whereas the AUC of SN-38 decreased. An inhibition of CE by 5-FU was suggested to explain these unexpected results (130). Other studies did not find altered pharmacokinetics for this combination therapy or eventually with the further addition of leucovorin (folinic acid;

Table 3 Evaluation of pharmacokinetic interactions between CPT-11 and anticancer agents

Combination drug(s)	Dose (CPT-11) (mg/m ²)	Kinetic alterations	Refs.
Carboplatin	5 ^a (60)	AUC _{CPT-11} ↓; AUC _{SN-38} ↓	127, 128
Cisplatin	80 (260)	None	32, 119, 124, 125
Cisplatin/Vindesine	100/3.0 (37.5)	None	120, 121
Cisplatin/Ifosfamide	70/1.5 (60)	None	123
Docetaxel	75 (200)	None	135
	70 (250)	None	149
	50 (60)	None	150
Etoposide	80 (80)	None	30
5-Fluorouracil	500 (125)	AUC _{CPT-11} ↑; AUC _{SN-38} ↓	130
	2600 (80)	None	131, 132, 134
Oxaliplatin	85 (200)	None	148
Paclitaxel	175 (150)	AUC _{CPT-11} ↑; AUC _{SN-38} ↑	136, 137
	75 (50)	None	139
Raltitrexed	3.0 (350)	None	151

^a Target AUC in mg·min/ml.

Table 4 Factors contributing to variability in response to CPT-11

Parameter	Source of variability	Example(s)
Dose selection	Patient's condition	Performance status
	Host sensitivity	Previous treatments
Systemic exposure	Concomitant drugs	Cyclosporin A (P-glycoprotein inhibitor)
		Valproic acid (UGT1A1 inhibitor)
		Phenobarbital (UGT1A1 inducer)
		Phenytoin (CYP3A4 inducer)
		Ketoconazole (CYP3A4 inhibitor)
	Pharmacogenetics	Gilbert's syndrome (UGT1A1 mutation)
		Crigler-Najjar type 1 syndrome (UGT1A1 deficiency)
		Dubin-Johnson syndrome (cMOAT mutation)
	Altered organ functions	Hepatic dysfunction
Active site levels	Resistance mechanisms	P-glycoprotein
		BCRP
		Topoisomerase I mutations
		CYP3A4 and/or UGT1A1 overexpression

Refs. 131–134). The conclusion from the Japanese study has been criticized in view of the large interpatient variation and noncomparable patient populations (134).

Although no comparative pharmacokinetic data are available, it has also been suggested that an interaction or sequence-dependent effect is present for the combination of CPT-11 and docetaxel. Because both drugs are metabolized by CYP3A, competition might occur when these drugs are given sequentially, and as a result, the clearance of docetaxel might be decreased (135). A significant pharmacokinetic interaction has been observed between CPT-11 and paclitaxel, which is characterized by increased plasma levels of both CPT-11 and SN-38 (136). Similar reactions have also been reported in rats (137) and Cremophor EL, the vehicle used for paclitaxel formulation seems to play a major role (104). This type of interaction appears to be related to micellar encapsulation of certain agents in this vehicle and has also been demonstrated to occur with paclitaxel itself and anthracyclines (138). In any event, because this interaction occurs during the terminal disposition phase of CPT-11, in currently applied dosing schedules the interaction is likely of only minor importance. The combination of weekly administration of CPT-11 with a fixed dose of paclitaxel (75

mg/m²) indicated that the sequence of drug administration did not affect elimination of CPT-11 (139). There was also no sequence-dependent, chemotherapy-related toxicity. To date, no other cytotoxic drug has been shown to affect the pharmacokinetics of CPT-11 or was affected itself by CPT-11.

Anticonvulsants, phenytoin and phenobarbital in particular, are known to induce several metabolic pathways relevant to xenobiotics. CYP3A4, in particular, has increased expression when patients are treated with these compounds. Indeed, a recent study in patients with recurrent or progressive malignant glioma receiving CPT-11 and phenytoin indicated that AUCs of CPT-11, SN-38, and SN-38G were approximately 40, 25, and 25%, respectively, of those determined previously in patients with metastatic colorectal cancer not receiving antiepileptics (140). Similarly, enzyme-inducing anticonvulsants significantly lowered systemic exposure to CPT-11 and SN-38 in children with newly diagnosed high-grade glioma (141). This interaction is of major importance because it suggests that anticonvulsants thereby largely reduce the potential antitumor effects of CPT-11. In addition to modulation of CYP3A4, another possible explanation for the interaction is the induction of membrane transporters that enhance drug excretion (142).

Conclusions and Perspectives

CPT-11, because of its broad spectrum of antitumor activity, is clearly one of the most important new anticancer drugs developed in the last few decades. The clinical pharmacokinetic behavior of CPT-11 has been explored extensively in recent years, and the generated information has been of fundamental importance in our understanding of the clinical effects of this agent (Table 4). In addition, a wealth of information has become available that has yielded valuable insight into the mechanism of action, the mechanisms of tumor resistance, toxicities, and considerations of dosage and schedule and route of drug administration. Many of these studies have been made possible by the development of selective analytical methodologies to specifically monitor the parent drug and individual metabolites, with sufficient sensitivity to detect the compounds at levels achieved after therapeutic dosing. However, only through further investigations that may allow better definition of the biochemistry and pharmacokinetics of CPT-11 can the rational optimization of therapy involving this agent be achieved. This need has become even more important in light of the current clinical use of CPT-11 in combination with other antineoplastic drugs or agents specifically administered to modify CPT-11-induced toxicity profiles (143). In this respect, the use of mathematical models to predict systemic exposure measures for CPT-11 and its metabolites by application of limited-sampling strategies (38, 41, 144–147), coupled with continued investigations into the role of individual enzyme expression levels and detection of enzyme polymorphism, will allow more rational and selective chemotherapy with this agent.

References

- Wall, M. E., Wani, M. C., Cook, C. E., Palmer, K. H., McPhail, A. T., and Sim, G. A. Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. *J. Am. Chem. Soc.*, **88**: 3888–3890, 1966.
- Muggia, F. M., Creaven, P. J., Hansen, H. H., Cohen, M. H., and Selawry, O. S. Phase I clinical trial of weekly and daily treatment with camptothecin (NSC-100880): correlation with preclinical studies. *Cancer Chemother. Rep.*, **56**: 515–521, 1972.
- Moertel, C. G., Schutt, A. J., Reitemeier, R. J., and Hahn, R. G. Phase II study of camptothecin (NSC-100880) in the treatment of advanced gastrointestinal cancer. *Cancer Chemother. Rep.*, **56**: 95–101, 1972.
- Schaepfi, U., Fleischman, R. W., and Cooney, D. A. Toxicity of camptothecin (NSC-100880). *Cancer Chemother. Rep.*, **5**: 25–36, 1974.
- Creemers, G. J., Lund, B., and Verweij, J. Topoisomerase I inhibitors. Topotecan and irinotecan. *Cancer Treat. Rev.*, **20**: 73–96, 1994.
- Rajkumar, S. V., and Adjei, A. A. A review of the pharmacology and clinical activity of new chemotherapeutic agents in lung cancer. *Cancer Treat. Rev.*, **24**: 35–53, 1998.
- Hsiang, Y.-H., Hertzberg, R., Hecht, S., and Liu, L. F. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J. Biol. Chem.*, **260**: 14873–14878, 1985.
- Hsiang, Y.-H., and Liu, L. F. Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. *Cancer Res.*, **48**: 1722–1726, 1988.
- Hsiang, Y.-H., Lihou, M. G., and Liu, L. F. Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. *Cancer Res.*, **49**: 5077–5082, 1989.
- Gerrits, C. J. H., de Jonge, M. J. A., Schellens, J. H. M., Stoter, G., and Verweij, J. Topoisomerase I inhibitors: the relevance of prolonged exposure for clinical development. *Br. J. Cancer*, **76**: 952–962, 1997.
- O'Leary, J., and Muggia, F. M. Camptothecins: a review of their development and schedules of administration. *Eur. J. Cancer*, **34**: 1500–1508, 1998.
- Gelderblom, H. A., de Jonge, M. J. A., Sparreboom, A., and Verweij, J. Oral topoisomerase I inhibitors in adult patients: present and future. *Investig. New Drugs*, **17**: 401–415, 1999.
- Kunimoto, T., Nitta, K., Tanaka, T., Uehara, N., Baba, H., Takeuchi, M., Yokokura, T., Sawada, S., Miyasaka, T., and Mutai, M. Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. *Cancer Res.*, **47**: 5944–5947, 1987.
- Kingsbury, W. D., Boehm, J. C., Jakas, D. R., Holden, K. G., Hecht, S. M., Gallagher, G., Caranfa, M. J., McCabe, F. L., Faucette, L. F., and Johnson, R. K. Synthesis of water-soluble (aminoalkyl)camptothecin analogues: inhibition of topoisomerase I and antitumor activity. *J. Med. Chem.*, **34**: 98–107, 1991.
- Pantazis, P., Harris, N., Mendoza, J., and Giovannella, B. The role of pH and serum albumin in the metabolic conversion of 9-nitrocamptothecin to 9-aminocamptothecin by human hematopoietic and other cells. *Eur. J. Haematol.*, **55**: 211–213, 1995.
- Loos, W. J., Sparreboom, A., Verweij, J., Nooter, K., Stoter, G., and Schellens, J. H. M. Determination of the lactone and lactone plus carboxylate forms of 9-aminocamptothecin in human plasma by sensitive high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. B*, **694**: 435–441, 1997.
- Blaney, S. M., Takimoto, C., Murry, D. J., Kuttesch, N., McCully, C., Cole, D. E., Godwin, K., and Balis, F. M. Plasma and cerebrospinal fluid pharmacokinetics of 9-aminocamptothecin (9-AC), irinotecan (CPT-11), and SN-38 in nonhuman primates. *Cancer Chemother. Pharmacol.*, **41**: 464–468, 1998.
- Emerson, D. L., Besterman, J. M., Brown, H. R., Evans, M. G., Leitner, P. P., Luzzio, M. J., Shaffer, J. E., Sternbach, D. D., Uehling, D., and Vuong, A. *In vivo* antitumor activity of two new seven-substituted water-soluble camptothecin analogues. *Cancer Res.*, **55**: 603–609, 1995.
- Vey, N., Giles, F. J., Kantarjian, H., Smith, T. L., Beran, M., and Jeha, S. The topoisomerase I inhibitor DX-8951f is active in a severe combined immunodeficient mouse model of human acute myelogenous leukemia. *Clin. Cancer Res.*, **6**: 731–736, 2000.
- Slatter, J. G., Schaaf, L. J., Sams, J. P., Feenstra, K. L., Johnson, M. G., Bombardt, P. A., Cathcart, K. S., Verburg, M. T., Pearson, L. K., Compton, L. D., Miller, L. L., Baker, D. S., Pesheck, C. V., and Lord, R. S. Pharmacokinetics, metabolism, and excretion of irinotecan (CPT-11) following i.v. infusion of [¹⁴C]CPT-11 in cancer patients. *Drug Metab. Dispos.*, **28**: 423–433, 2000.
- Canal, P., Gay, C., Dezeuze, A., Douillard, J. Y., Bugat, R., Brunet, R., Adenis, A., Herait, P., Lokiec, F., and Mathieu-Boue, A. Pharmacokinetics and pharmacodynamics of irinotecan during a Phase II clinical trial in colorectal cancer. *J. Clin. Oncol.*, **14**: 2688–2695, 1996.
- Abigeres, D., Chabot, G. G., Armand, J.-P., Herait, P., Gouyette, A., and Gandia, D. Phase I and pharmacologic studies of the camptothecin analog irinotecan administered every 3 weeks in cancer patients. *J. Clin. Oncol.*, **13**: 210–221, 1995.
- Rowinsky, E. K., Grochow, L. B., Ettinger, D. S., Sartorius, S. E., Lubejko, B. G., Chen, T. L., Rock, M. K., and Donehower, R. C. Phase I and pharmacological study of the novel topoisomerase I inhibitor 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin (CPT-11) administered as a ninety-minute infusion every 3 weeks. *Cancer Res.*, **54**: 427–436, 1994.
- Rothenberg, M. L., Kuhn, J. G., Burris, H. A., Nelson, J., Eckardt, J. R., Tristan-Morales, M., Hilsenbeck, S. G., Weiss, G. R., Smith, L. S., and Rodriguez, G. I. Phase I and pharmacokinetic trial of weekly CPT-11. *J. Clin. Oncol.*, **11**: 2194–2204, 1993.
- de Forni, M., Bugat, R., Chabot, G. G., Culine, S., Extra, J. M., Gouyette, A., Madelaine, I., Marty, M. E., and Mathieu-Boue, A. Phase

I and pharmacokinetic study of the camptothecin derivative irinotecan, administered on a weekly schedule in cancer patients. *Cancer Res.*, 54: 4347–4354, 1994.

26. Catimel, G., Chabot, G. G., Guastalla, J. P., Dumortier, A., Cote, C., Engel, C., Gouyette, A., Mathieu-Boue, A., Mahjoubi, M., and Clavel, M. Phase I and pharmacokinetic study of irinotecan (CPT-11) administered daily for three consecutive days every three weeks in patients with advanced solid tumors. *Ann. Oncol.*, 6: 133–140, 1995.
27. Sasaki, Y., Hakusui, H., Mizuno, S., Morita, M., Miya, T., Eguchi, K., Shinkai, T., Tamura, T., Ohe, Y., and Saijo, N. A pharmacokinetic and pharmacodynamic analysis of CPT-11 and its active metabolite SN-38. *Jpn. J. Cancer Res.*, 86: 101–110, 1995.
28. Pitot, H. C., Goldberg, R. M., Reid, J. M., Sloan, J. A., Skaff, P. A., Erlichman, C., Rubin, J., Burch, P. A., Adjei, A. A., Alberts, S. A., Schaaf, L. J., Elfring, G., and Miller, L. L. Phase I dose-finding and pharmacokinetic trial of irinotecan hydrochloride (CPT-11) using a once-every-three-week dosing schedule for patients with advanced solid tumor malignancy. *Clin. Cancer Res.*, 6: 2236–2244, 2000.
29. Herben, V. M. M., Schellens, J. H. M., Swart, M., Gruia, G., Vernillet, L., Beijnen, J. H., and ten Bokkel Huinink, W. W. Phase I and pharmacokinetic study of irinotecan administered as a low-dose, continuous intravenous infusion over 14 days in patients with malignant solid tumors. *J. Clin. Oncol.*, 17: 1897–1905, 1999.
30. Masuda, N., Fukuoka, M., Kudoh, S., Matsui, K., Kusunoki, Y., Takada, M., Nakagawa, K., Hirashima, T., Tsukada, H., and Yana, T. Phase I and pharmacologic study of irinotecan and etoposide with recombinant human granulocyte colony-stimulating factor support for advanced lung cancer. *J. Clin. Oncol.*, 12: 1833–1841, 1994.
31. Gupta, E., Mick, R., Ramirez, J., Wang, X., Lestingi, T. M., Vokes, E. E., and Ratain, M. J. Pharmacokinetic and pharmacodynamic evaluation of the topoisomerase I inhibitor irinotecan in cancer patients. *J. Clin. Oncol.*, 15: 1502–1510, 1997.
32. Sparreboom, A., de Jonge, M. J. A., de Bruijn, P., Brouwer, E., Nooter, K., Loos, W. J., van Alphen, R. J., Mathijssen, R. H. J., Stoter, G., and Verweij, J. Irinotecan (CPT-11) metabolism and disposition in cancer patients. *Clin. Cancer Res.*, 4: 2747–2754, 1998.
33. Sasaki, Y., Yoshida, Y., Sudoh, K., Hakusui, H., Fujii, H., Ohtsu, T., Wakita, H., Igarashi, T., and Itoh, K. Pharmacological correlation between total drug concentration and lactones of CPT-11 and SN-38 in patients treated with CPT-11. *Jpn. J. Cancer Res.*, 86: 111–116, 1995.
34. Rivory, L. P., Chatelut, E., Canal, P., Mathieu-Boue, A., and Robert, J. Kinetics of the *in vivo* interconversion of the carboxylate and lactone forms of irinotecan and of its metabolite SN-38 in patients. *Cancer Res.*, 54: 6330–6333, 1994.
35. Chabot, G. G., Abigeres, D., Catimel, G., Culine, S., de Forni, M., Extra, J. M., Mahjoubi, M., Herait, P., Armand, J. P., and Bugat, R. Population pharmacokinetics and pharmacodynamics of irinotecan (CPT-11) and active metabolite SN-38 during Phase I trials. *Ann. Oncol.*, 6: 141–151, 1995.
36. Rivory, L. P., Haaz, M.-C., Canal, P., Lokiec, F., Armand, J. P., and Robert, J. Pharmacokinetic interrelationships of irinotecan (CPT-11) and its three major plasma metabolites in patients enrolled in Phase I/II trials. *Clin. Cancer Res.*, 3: 1261–1266, 1997.
37. Loos, W. J., Verweij, J., Gelderblom, H. A., de Jonge, M. J. A., Brouwer, E., Dallaré, B. K., and Sparreboom, A. Role of erythrocytes and serum proteins in the kinetic profile of total 9-amino-20(S)-camptothecin in humans. *Anti-Cancer Drugs*, 10: 705–710, 1999.
38. Mathijssen, R. H. J., van Alphen, R. J., de Jonge, M. J. A., Verweij, J., de Bruijn, P., Loos, W. J., Nooter, K., Vernillet, L., Stoter, G., and Sparreboom, A. Sparse-data set analysis for irinotecan and SN-38 pharmacokinetics in cancer patients co-treated with cisplatin. *Anti-Cancer Drugs*, 10: 9–16, 1999.
39. Ohe, Y., Sasaki, Y., Shinkai, T., Eguchi, K., Tamura, T., Kojima, A., Kunikane, H., Okamoto, H., Karato, A., and Ohmatsu, H. Phase I study and pharmacokinetics of CPT-11 with 5-day continuous infusion. *J. Natl. Cancer Inst. (Bethesda)*, 84: 972–973, 1992.
40. Rothenberg, M. L., Eckardt, J. R., Kuhn, J. G., Burris, H. A., Nelson, J., Hilsenbeck, S. G., Rodriguez, G. I., Thurman, A. M., Smith, L. S., Eckhardt, S. G., Weiss, G. R., Elfring, G. L., Rinaldi, D. A., Schaaf, L. J., and Von Hoff, D. D. Phase II trial of irinotecan in patients with progressive or rapidly recurrent colorectal cancer. *J. Clin. Oncol.*, 14: 1128–1135, 1996.
41. Mick, R., Gupta, E., Vokes, E. E., and Ratain, M. J. Limited-sampling models for irinotecan pharmacokinetics-pharmacodynamics: prediction of biliary index and intestinal toxicity. *J. Clin. Oncol.*, 14: 2012–2019, 1996.
42. Rothenberg, M. L., Kuhn, J. G., Schaaf, L. J., Eckhardt, S. G., Villalona-Calero, M. A., Petit, R. G., Miller, L. L., Elfring, G. L., Murphy, T. C., and Von Hoff, D. D. Phase I and pharmacokinetic study of intravenous irinotecan (CPT-11) administered once every 2 weeks +/- G-CSF. *Ann. Oncol.*, 10: 63, 1998.
43. Kehrer, D. F. S., Yamamoto, W., Verweij, J., de Jonge, M. J. A., de Bruijn, P., and Sparreboom, A. Factors involved in prolongation of the terminal disposition phase of SN-38: clinical and experimental studies. *Clin. Cancer Res.*, 6: 3451–3458, 2000.
44. Combes, O., Barré, J., Duché, J.-C., Vernillet, L., Archimbaud, Y., Marietta, M. P., Tillement, J. P., and Urien, S. *In vitro* binding and partitioning of irinotecan (CPT-11) and its metabolite, SN-38, in human blood. *Investig. New Drugs*, 18: 1–5, 2000.
45. Ma, M. K., Zamboni, W. C., Radomski, K. M., Furman, W. L., Santana, V. M., Houghton, P. J., Hanna, S. K., Smith, A. K., and Stewart, C. F. Pharmacokinetics of irinotecan and its metabolites SN-38 and APC in children with recurrent solid tumors after protracted low-dose irinotecan. *Clin. Cancer Res.*, 6: 813–819, 2000.
46. Burke, T. G., and Mi, Z. H. The structural basis of camptothecin interactions with human serum albumin: impact on drug stability. *J. Med. Chem.*, 37: 40–46, 1994.
47. Burke, T. G., Munshi, C. B., Mi, Z. H., and Jiang, Y. The important role of albumin in determining the relative human blood stabilities of the camptothecin anticancer drugs. *J. Pharm. Sci.*, 84: 518–519, 1995.
48. Slatter, J. G., Su, P., Sams, J. P., Schaaf, L. J., and Wienkers, L. C. Bioactivation of the anticancer agent CPT-11 to SN-38 by human hepatic microsomal carboxylesterases and the *in vitro* assessment of potential drug interactions. *Drug Metab. Dispos.*, 25: 1157–1164, 1997.
49. Humerickhouse, R., Lohrbach, K., Li, L., Bosron, W. F., and Dolan, M. E. Characterization of CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2. *Cancer Res.*, 60: 1189–1192, 2000.
50. Wierdl, M., Morton, C. L., Danks, M. K., and Potter, P. M. Isolation and characterization of a cDNA encoding a horse liver butyrylcholinesterase: evidence for CPT-11 drug activation. *Biochem. Pharmacol.*, 59: 773–781, 2000.
51. Morton, C. L., Wadkins, R. M., Danks, M. K., and Potter, P. M. The anticancer prodrug CPT-11 is a potent inhibitor of acetylcholinesterase but is rapidly catalyzed to SN-38 by butyrylcholinesterase. *Cancer Res.*, 59: 1458–1463, 1999.
52. Haaz, M.-C., Rivory, L. P., Riché, C., and Robert, J. The transformation of irinotecan (CPT-11) to its active metabolite SN-38 by human liver microsomes. *Naunyn Schmiedeberg Arch. Pharmacol.*, 356: 257–262, 1997.
53. Kawato, Y., Furuta, T., Aonuma, M., Yasuoka, M., Yokokura, T., and Matsumoto, K. Antitumor activity of a camptothecin derivative, CPT-11, against human tumor xenografts in nude mice. *Cancer Chemother. Pharmacol.*, 28: 192–198, 1991.
54. Takaoka, K., Ohtsuka, K., and Jin, M. Conversion of CPT-11 to its active form SN-38, by carboxylesterase of non-small cell lung cancer. *Proc. Am. Soc. Clin. Oncol.*, 16: 252, 1997.
55. van Ark-Otte, J., Kedde, M. A., van der Vijgh, W. J. F., Dingemans, A. M., Jansen, W. J., Pinedo, H. M., Boven, E., and Giaccone, G. Determinants of CPT-11 and SN-38 activities in human lung cancer cells. *Br. J. Cancer*, 77: 2171–2176, 1998.
56. Guichard, S., Terret, C., Hennebelle, I., Lochon, I., Chevreau, P., Fretigny, E., Selves, J., Chatelut, E., Bugat, R., and Canal, P. CPT-11 converting carboxylesterase and topoisomerase I activities in tumour and normal colon and liver tissues. *Br. J. Cancer*, 80: 364–370, 1999.

57. Kojima, A., Hackett, N. R., Ohwada, A., and Crystal, R. G. *In vivo* human carboxylesterase cDNA gene transfer to activate the prodrug CPT-11 for local treatment of solid tumors. *J. Clin. Investig.*, *101*: 1789–1796, 1998.
58. Kojima, A., Hackett, N. R., and Crystal, R. G. Reversal of CPT-11 resistance of lung cancer cells by adenovirus-mediated gene transfer of the human carboxylesterase cDNA. *Cancer Res.*, *58*: 4368–4374, 1998.
59. Danks, M. K., Morton, C. L., Krull, E. J., Cheshire, P. J., Richmond, L. B., Naeve, C. W., Pawlik, C. A., Houghton, P. J., and Potter, P. M. Comparison of activation of CPT-11 by rabbit and human carboxylesterases for use in enzyme/prodrug therapy. *Clin. Cancer Res.*, *5*: 917–924, 1999.
60. Satoh, T., Hosokawa, M., Atsumi, R., Suzuki, W., Hokusui, H., and Nagai, E. Metabolic activation of CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, a novel antitumor agent, by carboxylesterase. *Biol. Pharm. Bull.*, *17*: 662–664, 1994.
61. Inaba, M., Ohnishi, Y., Ishii, H., Tanioka, Y., Yoshida, Y., Sudoh, K., Hokusui, H., Mizuno, N., Ito, K., and Sugiyama, Y. Pharmacokinetics of CPT-11 in rhesus monkeys. *Cancer Chemother. Pharmacol.*, *41*: 103–108, 1998.
62. Danks, M. K., Morton, C. L., Pawlik, C. A., and Potter, P. M. Overexpression of a rabbit liver carboxylesterase sensitizes human tumor cells to CPT-11. *Cancer Res.*, *58*: 20–22, 1998.
63. Iyer, L., King, C. D., Whittington, P. F., Green, M. D., Roy, S. K., Tephly, T. R., Coffman, B. L., and Ratain, M. J. Genetic predisposition to the metabolism of irinotecan (CPT-11). *J. Clin. Investig.*, *101*: 847–854, 1998.
64. Rivory, L. P., and Robert, J. Identification and kinetics of a β -glucuronide metabolite of SN-38 in human plasma after administration of the camptothecin derivative irinotecan. *Cancer Chemother. Pharmacol.*, *36*: 176–179, 1995.
65. Atsumi, R., Suzuki, W., and Hokusui, H. Identification of the metabolites of irinotecan, a new derivative of camptothecin, in rat bile and its biliary excretion. *Xenobiotica*, *21*: 1159–1169, 1991.
66. Gupta, E., Lestingi, T. M., Mick, R., Ramirez, J., Vokes, E. E., and Ratain, M. J. Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. *Cancer Res.*, *54*: 3723–3725, 1994.
67. Ciotti, M., Basu, N., Brangi, M., and Owens, I. S. Glucuronidation of 7-ethyl-10-hydroxycamptothecin (SN-38) by the human UDP-glucuronosyltransferases encoded at the *UGT1* locus. *Biochem. Biophys. Res. Commun.*, *260*: 199–202, 1999.
68. Ando, Y., Saka, H., Asai, G., Sugiura, S., Shimokata, K., and Kamataki, T. *UGT1A1* genotypes and glucuronidation of SN-38, the active metabolite of irinotecan. *Ann. Oncol.*, *9*: 845–847, 1998.
69. Iyer, L., Hall, D., Das, S., Mortell, M. A., Ramirez, J., Kim, S., di Rienzo, A., and Ratain, M. Phenotype-genotype correlation of *in vitro* SN-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human liver tissue with *UGT1A1* promoter polymorphism. *Clin. Pharmacol. Ther.*, *65*: 576–582, 1999.
70. Monaghan, G., Ryan, M., Seddon, R., Hume, R., and Burchell, B. Genetic variation in bilirubin UDP-glucuronosyltransferase gene promoter and Gilbert's syndrome. *Lancet*, *347*: 578–581, 1996.
71. Wasserman, E., Myara, A., Lokiec, F., Goldwasser, F., Trivin, F., Mahjoubi, M., Misset, J. L., and Cvitkovic, E. Severe CPT-11 toxicity in patients with Gilbert's syndrome: two case reports. *Ann. Oncol.*, *8*: 1049–1051, 1997.
72. Wasserman, E., Myara, A., Lokiec, F., Riofro, M., Imadalou, P., Bleuzen, P., Santoni, J., Trivin, F., Herait, P., Mahjoubi, M., Armand, J. P., Misset, J. L., and Cvitkovic, E. Bilirubin (BIL) and SN-38: pharmacokinetic/pharmacodynamics correlation. *Ann. Oncol.*, *10*: 58, 1998.
73. Ando, Y., Saka, H., Ando, M., Sawa, T., Muro, K., Ueoka, H., Yokoyama, A., Saitoh, S., Shimokata, K., and Hasegawa, Y. Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res.*, *60*: 6921–6926, 2000.
74. Gupta, E., Wang, X., Ramirez, J., and Ratain, M. J. Modulation of glucuronidation of SN-38, the active metabolite of irinotecan, by valproic acid and phenobarbital. *Cancer Chemother. Pharmacol.*, *39*: 440–444, 1997.
75. Takahashi, T., Fujiwara, Y., Yamakido, M., Katoh, O., Watanabe, H., and Mackenzie, P. I. The role of glucuronidation in 7-ethyl-10-hydroxycamptothecin resistance *in vitro*. *Jpn. J. Cancer Res.*, *88*: 1211–1217, 1997.
76. Brangi, M., Litman, T., Ciotti, M., Nishiyama, K., Kohlhagen, G., Takimoto, C., Robey, R., Pommier, Y., Fojo, T., and Bates, S. E. Camptothecin resistance: role of the ATP-binding cassette (ABC), mitoxantrone-resistance half-transporter (MXR), and potential for glucuronidation in MXR-expressing cells. *Cancer Res.*, *59*: 5938–5946, 1999.
77. Lokiec, F., Monegier du Sorbier, B., and Sanderink, G.-J. Irinotecan (CPT-11) metabolites in human bile and urine. *Clin. Cancer Res.*, *2*: 1943–1949, 1996.
78. Haaz, M.-C., Rivory, L. P., Riché, C., Vernillet, L., and Robert, J. Metabolism of irinotecan (CPT-11) by hepatic microsomes: participation of cytochrome P-450 3A and drug interactions. *Cancer Res.*, *58*: 468–472, 1998.
79. Santos, A., Zannetta, S., Cresteil, T., Deroussent, A., Pein, F., Raymond, E., Vernillet, L., Risse, M. L., Boige, V., Gouyette, A., and Vassal, G. Metabolism of irinotecan (CPT-11) by CYP3A4 and CYP3A5 in humans. *Clin. Cancer Res.*, *6*: 2012–2020, 2000.
80. Dodds, H. M., Haaz, M.-C., Riou, J.-F., Robert, J., and Rivory, L. P. Identification of a new metabolite of CPT-11 (irinotecan): pharmacological properties and activation to SN-38. *J. Pharmacol. Exp. Ther.*, *286*: 578–583, 1998.
81. Haaz, M.-C., Riché, C., Rivory, L. P., and Robert, J. Biosynthesis of an aminopiperidino metabolite of irinotecan [7-ethyl-10-[4-(1-piperidino)carbonyloxycamptothecine] by human hepatic microsomes. *Drug Metab. Dispos.*, *26*: 769–774, 1998.
82. Dodds, H. M., Wunsch, R. M., Gillam, E. M. J., and Rivory, L. P. Further elucidation of the pathways involved in the catabolism of the camptothecin analogue irinotecan. *Proc. Am. Assoc. Cancer Res.*, *40*: 110, 1999.
83. Rivory, L. P., Riou, J.-F., Haaz, M.-C., Sable, S., Vuilhorgne, M., Commerçon, A., Pond, S. M., and Robert, J. Identification and properties of a major plasma metabolite of irinotecan (CPT-11) isolated from the plasma of patients. *Cancer Res.*, *56*: 3689–3694, 1996.
84. Dodds, H. M., Haaz, M.-C., Riou, J. F., Robert, J., and Rivory, L. P. Identification of a new metabolite of CPT-11 (irinotecan): pharmacological properties and activation to SN-38. *J. Pharmacol. Exp. Ther.*, *286*: 578–583, 1998.
85. Massaad, L., de Waziers, I., Ribrag, V., Janot, F., Beaune, P. H., Morizet, J., Gouyette, A., and Chabot, G. G. Comparison of mouse and human colon tumors with regard to Phase I and Phase II drug-metabolizing enzyme systems. *Cancer Res.*, *52*: 6567–6575, 1992.
86. Rivory, L. P., Bowles, M. R., Robert, J., and Pond, S. M. Conversion of irinotecan (CPT-11) to its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), by human liver carboxylesterase. *Biochem. Pharmacol.*, *52*: 1103–1111, 1996.
87. Dresser, G. K., Spence, J. D., and Bailey, D. G. Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clin. Pharmacokinet.*, *38*: 41–57, 2000.
88. Skar, V., Skar, A. G., and Stromme, J. H. β -Glucuronidase activity related to bacterial growth in common bile duct bile in gallstone patients. *Scand. J. Gastroenterol.*, *23*: 83–90, 1988.
89. Yokoi, T., Narita, M., Nagai, E., Hagiwara, H., Aburada, M., and Kamataki, T. Inhibition of UDP-glucuronosyltransferase by aglycons of natural glucuronides in kampo medicines using SN-38 as a substrate. *Jpn. J. Cancer Res.*, *86*: 985–989, 1995.
90. Sperker, B., Backman, J. T., and Kroemer, H. K. The role of β -glucuronidase in drug disposition and drug targeting in humans. *Clin. Pharmacokinet.*, *33*: 18–31, 1997.
91. Takasuna, K., Hagiwara, T., Hirohashi, M., Kato, M., Nomura, M., Nagai, E., Yokoi, T., and Kamataki, T. Involvement of β -glucuronidase in intestinal microflora in the intestinal toxicity of the antitumor camptothecin derivative irinotecan hydrochloride (CPT-11) in rats. *Cancer Res.*, *56*: 3752–3757, 1996.

92. Kase, Y., Hayakawa, T., Togashi, Y., and Kamataki, T. Relevance of irinotecan hydrochloride-induced diarrhea to the level of prostaglandin E2 and water absorption of large intestine in rats. *Jpn. J. Pharmacol.*, 75: 399–405, 1997.
93. Araki, E., Ishikawa, M., Iigo, M., Koide, T., Itabashi, M., and Hoshi, A. Relationship between development of diarrhea and the concentration of SN-38, an active metabolite of CPT-11, in the intestine and the blood plasma of athymic mice following intraperitoneal administration of CPT-11. *Jpn. J. Cancer Res.*, 84: 697–702, 1993.
94. Takasuna, K., Hagiwara, T., Hirohashi, M., Kato, M., Nomura, M., Nagai, E., Yokoi, T., and Kamataki, T. Inhibition of intestinal microflora β -glucuronidase modifies the distribution of the active metabolite of the antitumor agent irinotecan hydrochloride (CPT-11) in rats. *Cancer Chemother. Pharmacol.*, 42: 280–286, 1998.
95. Fujisawa, T., and Mori, M. Influence of various bile salts on β -glucuronide activity of intestinal bacteria. *Appl. Microbiol.*, 25: 95–97, 1997.
96. Cole, C. B., Fuller, R., Mallet, A. K., and Rowland, I. R. The influence of the host on expression of intestinal microbial enzyme activities involved in metabolism of foreign compounds. *J. Appl. Bacteriol.*, 59: 549–553, 1985.
97. Van Huyen, J. P., Bloch, F., Attar, A., Levoir, D., Kreft, C., Molina, T., and Bruneval, P. Diffuse mucosal damage in the large intestine associated with irinotecan (CPT-11). *Dig. Dis. Sci.*, 43: 2649–2651, 1998.
98. Mallett, A. K., Bearne, C. A., and Rowland, I. R. The influence of incubation pH on the activity of rat and human gut flora enzymes. *J. Appl. Bacteriol.*, 66: 433–437, 1989.
99. Planting, A. S. T. Modulation of irinotecan pharmacokinetics/pharmacodynamics by combined treatment with neomycin. *Proc. Am. Soc. Clin. Oncol.*, 19: 1007, 2000.
100. Lokiec, F., Canal, P., Gay, C., Chatelut, E., Armand, J. P., Roche, H., Bugat, R., Goncalves, E., and Matieu-Boue, A. Pharmacokinetics of irinotecan and its metabolites in human blood, bile and urine. *Cancer Chemother. Pharmacol.*, 36: 79–82, 1995.
101. Chu, X-Y., Kato, Y., Niinuma, K., Sudo, K. I., Hakusui, H., and Sugiyama, Y. Multispecific organic anion transporter is responsible for the biliary excretion of the camptothecin derivative irinotecan and its metabolites in rats. *J. Pharmacol. Exp. Ther.*, 281: 304–314, 1997.
102. Chu, X-Y., Kato, Y., and Sugiyama, Y. Multiplicity of biliary excretion mechanisms for irinotecan, CPT-11, and its metabolites in rats. *Cancer Res.*, 57: 1934–1938, 1997.
103. Chu, X-Y., Kato, Y., Ueda, K., Suzuki, H., Niinuma, K., Tyson, C. A., Weizer, V., Dabbs, J. E., Froehlich, R., Green, C. E., and Sugiyama, Y. Biliary excretion mechanism of CPT-11 and its metabolites in humans: involvement of primary active transporters. *Cancer Res.*, 58: 5137–5143, 1998.
104. Gupta, E., Safa, A. R., Wang, X., and Ratain, M. J. Pharmacokinetic modulation of irinotecan and metabolites by cyclosporin A. *Cancer Res.*, 56: 1309–1314, 1996.
105. Sugiyama, Y., Kato, Y., and Chu, X-Y. Multiplicity of biliary excretion mechanisms for the camptothecin derivative irinotecan (CPT-11), its metabolite SN-38, and its glucuronide: role of canalicular multispecific organic anion transporter and P-glycoprotein. *Cancer Chemother. Pharmacol.*, 42: S44–S49, 1998.
106. Chu, X-Y., Kato, Y., and Sugiyama, Y. Possible involvement of P-glycoprotein in biliary excretion of CPT-11 in rats. *Drug Metab. Dispos.*, 27: 440–441, 1999.
107. Paulusma, C. C., Kool, M., Bosma, P. J., Scheffer, G. L., ter Borg, F., Scheper, R. J., Tytgat, G. N., Borst, P., Baas, P., and Oude Elferink, R. P. A mutation in the human canalicular multispecific organic anion transporter gene causes the Dubin-Johnson syndrome. *Hepatology*, 25: 1539–1542, 1997.
108. Houghton, P. J., Cheshire, P. J., Hallman, J. D., Lutz, L., Friedman, H. S., Danks, M. K., and Houghton, J. A. Efficacy of topoisomerase I inhibitors, topotecan and irinotecan, administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors. *Cancer Chemother. Pharmacol.*, 36: 393–403, 1995.
109. Thompson, J., Zamboni, W. C., Cheshire, P. J., Lutz, L., Luo, X., Li, Y., Houghton, J. A., Stewart, C. F., and Houghton, P. J. Efficacy of systemic administration of irinotecan against neuroblastoma xenografts. *Clin. Cancer Res.*, 3: 423–431, 1997.
110. Takimoto, C. H., Morrison, G., Harold, N., Quinn, M., Monahan, B. P., Band, R. A., Cottrell, J., Guemei, A., Llorens, V., Hehman, H., Ismail, A. S., Flemming, D., Gosky, D. M., Hirota, H., Berger, S. J., Berger, N. A., Chen, A. P., Shapiro, J. D., Arbuck, S. G., Wright, J., Hamilton, J. M., Allegra, C. J., and Grem, J. L. Phase I and pharmacologic study of irinotecan administered as a 96-hour infusion weekly to adult cancer patients. *J. Clin. Oncol.*, 18: 659–667, 2000.
111. Furman, W. L., Stewart, C. F., Poquette, C. A., Pratt, C. B., Santana, V. M., Zamboni, W. C., Bowman, L. C., Ma, M. K., Hoffer, F. A., Meyer, W. H., Pappo, A. S., Walter, A. W., and Houghton, P. J. Direct translation of a protracted irinotecan schedule from a xenograft model to a Phase I trial in children. *J. Clin. Oncol.*, 17: 1815–1824, 1999.
112. Ahmed, F., Vyas, V., Cornfield, A., Goodin, S., Ravikumar, T. S., Rubin, E. H., and Gupta, E. *In vitro* activation of irinotecan to SN-38 by human liver and intestine. *Anticancer Res.*, 19: 2067–2072, 1999.
113. Drengler, R. L., Kuhn, J. G., Schaaf, L. J., Rodriguez, G. I., Villalona-Calero, M. A., Hammond, L. A., Stephenson, J. A., Hodges, S., Kraynak, M. A., Staton, B. A., Elfring, G. L., Locker, P. K., Miller, L. L., Von Hoff, D. D., and Rothenberg, M. L. Phase I and pharmacokinetic trial of oral irinotecan administered daily for 5 days every 3 weeks in patients with solid tumors. *J. Clin. Oncol.*, 17: 685–696, 1999.
114. Zamboni, W. C., Houghton, P. J., Thompson, J., Cheshire, P. J., Hanna, S. K., Richmond, L. B., Lou, X., and Stewart, C. F. Altered irinotecan and SN-38 disposition after intravenous and oral administration of irinotecan in mice bearing human neuroblastoma xenografts. *Clin. Cancer Res.*, 4: 455–462, 1998.
115. Stewart, C. F., Zamboni, W. C., Crom, W. R., and Houghton, P. J. Disposition of irinotecan and SN-38 following oral and intravenous irinotecan dosing in mice. *Cancer Chemother. Pharmacol.*, 41: 259–265, 1997.
116. Radomski, K. M., Stewart, C. F., Panetta, J. C., Houghton, P. J., and Furman, W. L. Phase I and pharmacokinetic study of oral irinotecan in pediatric patients with solid tumors. *Proc. Am. Soc. Clin. Oncol.*, 41: 592a, 2000.
117. Kobayashi, K., Bouscarel, B., Matsuzaki, Y., Ceryak, S., Kudoh, S., and Fromm, H. pH-dependent uptake of irinotecan and its active metabolite, SN-38, by intestinal cells. *Int. J. Cancer*, 83: 491–496, 1999.
118. Guichard, S., Chatelut, E., Lochon, I., Bugat, R., Mahjoubi, M., and Canal, P. Comparison of the pharmacokinetics and efficacy of irinotecan after administration by the intravenous *versus* intraperitoneal route in mice. *Cancer Chemother. Pharmacol.*, 42: 165–170, 1998.
119. de Jonge, M. J. A., Sparreboom, A., Planting, A. S. T., van der Burg, M. E. L., de Boer-Dennert, M. M., ter Steeg, J., Jaques, C., and Verweij, J. Phase I study of 3-week schedule of irinotecan combined with cisplatin in patients with advanced solid tumors. *J. Clin. Oncol.*, 18: 187–194, 2000.
120. de Jonge, M. J. A., Verweij, J., de Bruijn, P., Brouwer, E., Mathijssen, R. H. J., van Alphen, R. J., de Boer-Dennert, M. M., Vernillet, L., Jacques, C., and Sparreboom, A. Pharmacokinetic, metabolic, and pharmacodynamic profiles in a dose-escalating study of irinotecan and cisplatin. *J. Clin. Oncol.*, 18: 195–203, 2000.
121. Shinkai, T., Arioka, H., Kunikane, H., Eguchi, K., Sasaki, Y., Tamura, T., Ohe, Y., Oshita, F., Nishio, M., and Karato, A. Phase I clinical trial of irinotecan (CPT-11), 7-ethyl-10[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin, and cisplatin in combination with fixed dose of vindesine in advanced non-small cell lung cancer. *Cancer Res.*, 54: 2636–2642, 1994.
122. Mogi, H., Hasegawa, Y., Watanabe, A., Nomura, F., Saka, H., and Shimokata, K. Combination effects of cisplatin, vinorelbine and irinotecan in non-small-cell lung cancer cell lines *in vitro*. *Cancer Chemother. Pharmacol.*, 39: 199–204, 1997.
123. Fujita, A., Takabatake, H., Tagaki, S., and Sekine, K. Phase I/II study of cisplatin, ifosfamide and irinotecan with rhG-CSF support in

- patients with stage IIIB and IV non-small-cell lung cancer. *Cancer Chemother. Pharmacol.*, *45*: 279–283, 2000.
124. DeVore, R. F., Johnson, D. H., Crawford, J., Garst, J., Dimery, I. W., Eckardt, J., Eckhardt, S. G., Elfring, G. L., Schaaf, L. J., Hanover, C. K., and Miller, L. L. Phase II study of irinotecan plus cisplatin in patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.*, *17*: 2710–2720, 1999.
125. Kudoh, S., Fukuoka, M., Masuda, N., Yoshikawa, A., Kusunoki, Y., Matsui, K., Negoro, S., Takifuji, N., Nakagawa, K., and Hirashima, T. Relationship between the pharmacokinetics of irinotecan and diarrhea during combination chemotherapy with cisplatin. *Jpn. J. Cancer Res.*, *86*: 406–413, 1995.
126. de Jonge, M. J. A., Verweij, J., Planting, A. S. T., van der Burg, M. E. L., Stoter, G., de Boer-Dennert, M. M., de Bruijn, P., Brouwer, E., Vernillet, L., and Sparreboom, A. Drug-administration sequence does not change pharmacodynamics and kinetics of irinotecan and cisplatin. *Clin. Cancer Res.*, *5*: 2012–2017, 1999.
127. Okamoto, H., Nagatomo, A., Kunitoh, H., Kunikane, H., and Watanabe, K. A Phase I clinical and pharmacologic study of a carboplatin and irinotecan regimen combined with recombinant human granulocyte-colony stimulating factor in the treatment of patients with advanced non-small cell lung carcinoma. *Cancer (Phila.)*, *82*: 2166–2172, 1998.
128. Fukuda, M., Oka, M., Soda, H., Terashi, K., Kawabata, S., Nakatomi, K., Takatani, H., Tsurutani, J., Tsukamoto, K., Noguchi, Y., Fukuda, M., Kinoshita, A., and Kohno, S. Phase I study of irinotecan combined with carboplatin in previously untreated solid cancers. *Clin. Cancer Res.*, *5*: 3963–3969, 1999.
129. LeBlanc, G. A., Sundseth, S. S., Weber, G. F., and Waxman, D. J. Platinum anticancer drugs modulate P-450 mRNA levels and differentially alter hepatic drug and steroid hormone metabolism in male and female rats. *Cancer Res.*, *52*: 540–547, 1992.
130. Sasaki, Y., Ohtsu, A., Shimada, Y., Ono, K., and Saijo, N. Simultaneous administration of CPT-11 and fluorouracil: alteration of the pharmacokinetics of CPT-11 and SN-38 in patients with advanced colorectal cancer. *J. Natl. Cancer Inst. (Bethesda)*, *86*: 1096–1097, 1994.
131. Saltz, L. B., Kanowitz, J., Kemeny, N. E., Schaaf, L., Spriggs, D., Staton, B. A., Berkery, R., Steger, C., Eng, M., Dietz, A., Locker, P., and Kelsen, D. P. Phase I clinical and pharmacokinetic study of irinotecan, fluorouracil, and leucovorin in patients with advanced solid tumors. *J. Clin. Oncol.*, *14*: 2959–2967, 1996.
132. Vanhoefer, U., Harstrick, A., Köhne, C.-H., Achterrath, W., Rustum, Y. M., Seeber, S., and Wilke, H. Phase I study of a weekly schedule of irinotecan, high-dose leucovorin, and infusional fluorouracil as a first-line chemotherapy in patients with advanced colorectal cancer. *J. Clin. Oncol.*, *17*: 907–913, 1999.
133. Benhammouda, A., Bastian, G., and Rixe, O. A Phase I and pharmacokinetic study of CPT-11 and 5-FU (F) combination. *Proc. Am. Soc. Clin. Oncol.*, *16*: 202, 1997.
134. Saltz, L., Shimada, Y., and Khayat, D. CPT-11 (irinotecan) and 5-fluorouracil: a promising combination for therapy of colorectal cancer. *Eur. J. Cancer*, *32A*: S24–S31, 1996.
135. Adjei, A. A., Klein, C. E., Kastrissios, H., Goldberg, R. M., Alberts, S. R., Pitot, H. C., Sloan, J. A., Reid, J. M., Hanson, L. J., Atherton, P., Rubin, J., and Erlichman, C. Phase I and pharmacokinetic study of irinotecan and docetaxel in patients with advanced solid tumors: preliminary evidence of clinical activity. *J. Clin. Oncol.*, *18*: 1116–1123, 2000.
136. Asai, G., Yamamoto, N., and Sakai, S. Paclitaxel (Taxol) affects pharmacokinetics of irinotecan and its metabolites. *Proc. Am. Soc. Clin. Oncol.*, *18*: 201a, 1999.
137. Yamamoto, N., Negoro, S., Chikazawa, H., Shimizu, T., and Fukuoka, M. Pharmacokinetic interaction of the combination of paclitaxel and irinotecan *in vivo* and clinical study. *Proc. Am. Soc. Clin. Oncol.*, *18*: 186a, 1999.
138. van Zuylen, L., Verweij, J., and Sparreboom, A. Role of formulation vehicles in taxane pharmacology. *Investig. New Drugs*, *19*: 125–141, 2001.
139. Murren, J. R., Peccerillo, K., DiStasio, S. A., Li, X., Leffert, J. J., Pizzorno, G., Burtneess, B. A., McKeon, A., and Cheng, Y. Dose escalation and pharmacokinetic study of irinotecan in combination with paclitaxel in patients with advanced cancer. *Cancer Chemother. Pharmacol.*, *46*: 43–50, 2000.
140. Friedman, H. S., Petros, W. P., Friedman, A. H., Schaaf, L. J., Kerby, T., Lawyer, J., Parry, M., Houghton, P. J., Lovell, S., Rasheed, K., Cloughsey, T., Stewart, E. S., Colvin, O. M., Provenzale, J. M., McLendon, R. E., Bigner, D. D., Cokgor, I., Haglund, M., Rich, J., Ashley, D., Malczyn, J., Elfring, G. L., and Miller, L. L. Irinotecan therapy in adults with recurrent or progressive malignant glioma. *J. Clin. Oncol.*, *17*: 1516–1525, 1999.
141. Gajjar, A. J., Radomski, K. M., Bowers, D. C., Chintagumpala, M. M., Thompson, S. J., Houghton, P. J., and Stewart, C. F. Pharmacokinetics of irinotecan (IRN) and metabolites in pediatric high-grade glioma patients with and without co-administration of enzyme-inducing anticonvulsants. *Proc. Am. Soc. Clin. Oncol.*, *19*: 162a, 2000.
142. Grossman, S. A., Hochberg, F., Fisher, J., Chen, T.-L., Kim, L., Gregory, R., Grochow, L. B., and Piantadosi, S. Increased 9-amino-camptothecin dose requirements in patients on anticonvulsants. *Cancer Chemother. Pharmacol.*, *42*: 118–126, 1998.
143. Kehrer, D. F. S., Sparreboom, A., Verweij, J., de Bruijn, P., Nierop, C. A., van der Schraaf, J., Ruijgrok, E. J., and de Jonge, M. J. A. Modulation of irinotecan-induced diarrhea by cotreatment with neomycin in cancer patients. *Clin. Cancer Res.*, *7*: 1136–1141, 2001.
144. Chabot, G. G. Limited sampling models for simultaneous estimation of the pharmacokinetics of irinotecan and its active metabolite SN-38. *Cancer Chemother. Pharmacol.*, *36*: 463–472, 1995.
145. Sasaki, Y., Mizuno, S., Fujii, H., Ohtsu, T., Wakita, H., Igarashi, T., Itoh, K., Sekine, I., Miyata, Y., and Saijo, N. A limited sampling model for estimating pharmacokinetics of CPT-11 and its metabolite SN-38. *Jpn. J. Cancer Res.*, *86*: 117–123, 1995.
146. Nakashima, H., Lieberman, R., Karato, A., Aroka, H., Ohmatsu, H., Nomura, N., Shiraishi, J., Tamura, T., Eguchi, K., and Shinkai, T. Efficient sampling strategies for forecasting pharmacokinetic parameters of irinotecan (CPT-11): implication for area under the concentration-time curve monitoring. *Ther. Drug Monit.*, *17*: 221–229, 1995.
147. Yamamoto, N., Tamura, T., Karato, A., Uenaka, K., Eguchi, K., Shinkai, T., Ohe, Y., Oshita, F., Arioka, H., and Nakashima, H. CPT-11: population pharmacokinetic model and estimation of pharmacokinetics using the bayesian method in patients with lung cancer. *Jpn. J. Cancer Res.*, *85*: 972–977, 1994.
148. Wasserman, E., Cuvier, C., Lokiec, F., Goldwasser, F., Kalla, S., Mery-Mignard, D., Ouldacaci, M., Besmaïne, A., Dupont-Andre, G., Mahjoubi, M., Marty, M., Missset, J. L., and Cvitkovic, E. Combination of oxaliplatin plus irinotecan in patients with gastrointestinal tumors: results of two independent Phase I studies with pharmacokinetics. *J. Clin. Oncol.*, *17*: 902–906, 1999.
149. Couteau, C., Risse, M. L., Ducreux, M., Lefresne-Soulas, F., Riva, A., Lebecq, A., Ruffie, P., Rougier, P., Lokiec, F., Bruno, R., and Armand, J. P. Phase I and pharmacokinetic study of docetaxel and irinotecan in patients with advanced solid tumors. *J. Clin. Oncol.*, *18*: 3545–3552, 2000.
150. Masuda, N., Negoro, S., Kudoh, S., Sugiura, T., Nakagawa, K., Saka, H., Takada, M., Niitani, H., and Fukuoka, M. Phase I and pharmacologic study of docetaxel and irinotecan in advanced non-small-cell lung cancer. *J. Clin. Oncol.*, *18*: 2996–3003, 2000.
151. Ford, H. E., Cunningham, D., Ross, P. J., Rao, S., Aherne, G. W., Benepal, T. S., Price, T., Massey, A., Vernillet, L., and Gruija, G. Phase I study of irinotecan and raltitrexid in patients with advanced gastrointestinal tract adenocarcinoma. *Br. J. Cancer*, *83*: 146–152, 2000.