

Minireview

Current Perspectives on the Clinical Experience, Pharmacology, and Continued Development of the Camptothecins

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Abstract

The camptothecins are a maturing class of anticancer agents. In this article, we review the pharmacology and antitumor activity of the camptothecin analogues that are approved for clinical use and those investigational agents undergoing clinical evaluation. Camptothecin is a naturally occurring cytotoxic alkaloid that has a unique intracellular target, topoisomerase I, a nuclear enzyme that reduces the torsional stress of supercoiled DNA during the replication, recombination, transcription, and repair of DNA. Topotecan and irinotecan are synthetic analogues designed to facilitate parenteral administration of the active lactone form of the compound by introducing functional groups to enhance solubility. They are now well-established components in the chemotherapeutic management of several neoplasms. Topotecan has modest activity in patients treated previously with ovarian and small cell lung cancer and is currently approved for use in the United States as second-line therapy in these diseases. Preliminary evidence of activity against hematological malignancies is also promising. Irinotecan is a prodrug that undergoes enzymatic conversion to the biologically active metabolite 7-ethyl-10-hydroxy-camptothecin. It is presently the treatment of choice when used in combination with fluoropyrimidines as first-line therapy for patients with advanced colorectal cancer or as a single agent after failure of 5-fluorouracil-based chemotherapy. Encouraging preliminary results suggest that irinotecan may have an increasing role in the treatment of other solid tumors, including small and non-small cell lung cancer, cervical cancer, ovarian cancer, gastric cancer, and malignant gliomas. Several additional camptothecin analogues are in various stages of clinical development, including 9-aminocamptothecin, 9-nitrocamptothecin, 7-(4-methylpiperazinomethylene)-10,11-ethylenedioxy-20(S)-camptothecin, exatecan mesylate, and karenitecin. Efforts to further optimize therapeutic effectiveness through drug delivery strategies that prolong tumor exposure to these S phase-specific

agents, such as improving oral bioavailability through structure modification and innovative formulation approaches, alternative parenteral dosage forms, and administration schedules, are being actively pursued. Combining camptothecins with other anticancer drugs and treatment modalities, as well as gaining a better understanding of the factors contributing to tumor sensitivity and resistance, continues to be the object of considerable interest.

Introduction

Inhibitors of topoisomerase I have proven to be among the most promising new classes of antineoplastic agents introduced into the clinic in recent years. Wall *et al.* (1) isolated the lead compound in this class, camptothecin, from the Chinese bush *Camptotheca acuminata* in 1966. However, it was not until 1985 that the nuclear enzyme topoisomerase I was identified as its molecular target (2). The poor solubility of camptothecin, conferred by the unusually weak basicity of its quinoline nitrogen atom, precluded direct parenteral administration to patients. Instead, the less active water-soluble carboxylate salt of camptothecin was used for the initial phase I clinical trials performed in the early 1970s. Although some evidence of antitumor activity was observed, additional clinical evaluation was compromised by its severe and unpredictable toxicity, particularly hemorrhagic cystitis (3, 4). In the following 10 years, an improved understanding of the mechanism of action, chemistry, and pharmacology of the compound led to the development of analogues with properties that were more suitable for clinical development.

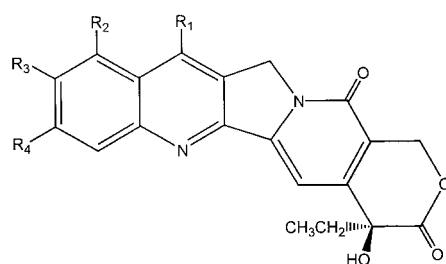
Two compounds in this class, topotecan² (Hycamptin) and irinotecan (Camptosar), have been approved for clinical use as anticancer drugs in the United States by the FDA. Topotecan is presently indicated as a second-line therapy for advanced ovarian cancer and SCLC. Irinotecan is approved for use in the treatment of advanced colorectal cancer, both as first-line therapy in combination with 5-FU and as salvage treatment in 5-FU refractory disease. There are several other camptothecin analogues in various stages of clinical evaluation, including 9-AC,

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² The abbreviations used are: topotecan, (S)-9-*N,N*-dimethylaminoethyl-10-hydroxycamptothecin; FDA, Food and Drug Administration; 9-AC, 9-amino-20(S)-camptothecin; SN-38, 7-ethyl-10-hydroxy-camptothecin; 9-NC, 9-nitrocamptothecin; GI-147211, 7-(4-methylpiperazinomethylene)-10,11-ethylenedioxy-20(S)-camptothecin; ABC, adenosine triphosphate binding cassette; MDR, multidrug-resistance; MRP, multidrug resistance-associated protein; cMOAT, canalicular multispecific organic anion transporter; NFκB, nuclear factor κB; AUC, area under the curve; CL, clearance; CNS, central nervous system; CL_{CR}, creatinine clearance; CV, coefficient of variation; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; irinotecan, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin; CSF, cerebrospinal fluid; CYP3A4, 3A4 isoform of hepatic cytochrome P450; UGT, uridine diphosphate glucuronosyl transferase; 5-FU, 5-fluorouracil.



Compound	Molecular weight	R ₁	R ₂	R ₃	R ₄
Camptothecin	348.36	-H	-H	-H	-H
Topotecan	421.46 ^a	-H	-CH ₂ N(CH ₃) ₂	-OH	-H
Irinotecan	586.69 ^a	-CH ₂ CH ₃	-H	-O-C(=O)-N(CH ₂) ₂ -N(CH ₂) ₂	-H
SN-38	392.42	-CH ₂ CH ₃	-H	-OH	-H
9-Aminocamptothecin	363.38	-H	-NH ₂	-H	-H
9-Nitrocamptothecin	393.36	-H	-NO ₂	-H	-H
GI-147211	518.57 ^a	-CH ₂ -N(CH ₃) ₂ -CH ₃	-H	-OCH ₂ CH ₂ O-	-H
Exatecan	435.46 ^a	-CH ₂ -CH ₂ -CH ₂ -NH ₂	-H	-CH ₃	-F
Karenitecin	448.60	-H	-CH ₂ CH ₂ Si(CH ₃) ₃	-H	-H

^a Free base.

Fig. 1 Chemical structures of the clinically important camptothecins.

9-NC, GI-147211, exatecan mesylate, and karenitecin. This review presents an overview of the camptothecins from the perspective of the clinically relevant aspects of their pharmacology, toxicology, and antitumor efficacy. In particular, recent advances in understanding their pharmacokinetic behavior, interactions with other drugs, pharmacogenetics, mechanisms of resistance, and status of their clinical application and development have been summarized.

Camptothecins: Structure, Chemistry, and Mechanism of Action

Structure and Chemistry. The characteristic structural features of the camptothecins include a five-ring backbone comprised of a quinoline subunit fused through two interposed rings to a terminal α -hydroxy- δ -lactone ring with a chiral center at position C-20 (Fig. 1). The naturally occurring 20S-isomer of camptothecin inhibits purified topoisomerase I 10–100 times more potently than the 20R-isomer (5). Potency can be enhanced by appropriate substitutions at positions C-9 and C-10 of the aromatic A ring of the quinoline moiety (6).

The intrinsic chemical reactivity of the lactone is necessary for the biological activity of the camptothecins. However, it is also susceptible to spontaneous reversible hydrolysis such that the intact lactone form predominates at acidic pH, and the inactive opened-ring carboxylate species is favored at neutral and alkaline pH (7). This reaction is reversible, pH-dependent,

and influenced by solution composition. The lactone predominates at acidic pH, and the carboxylate at neutral and alkaline pH. The carboxylate form of camptothecin has superior water solubility but it is ~ 10 -times less potent than the intact lactone structure (8). In the absence of plasma proteins, the rate and extent of lactone hydrolysis is not substantively affected by chemical substitution on the opposing quinoline terminus of the molecule, with the carboxylate form predominating at equilibrium in pH 7.4 aqueous solution (7). However, the presence of substituent groups on the A and B rings can significantly modulate binding affinity to serum albumin, resulting in a marked effect on the relative concentrations of the carboxylate and lactone forms in plasma at equilibrium. For example, the carboxylate forms of camptothecin and 9-AC bind to human serum albumin with 200-fold greater affinity than the lactone, resulting in a predominance of the former in the presence of this blood protein *in vitro* (9). In contrast, the lactone form of SN-38 preferentially binds to albumin, thus shifting the equilibrium in favor of the active form of the compound (9). The binding affinity of camptothecin carboxylate for purified albumin is significantly higher for human albumin than the protein of other species (10). However, the binding affinity of the carboxylate to human serum albumin is reduced in the presence of other blood proteins such as γ -globulin, α_1 -acidic glycoprotein, fibrinogen, and hemoglobin. In addition, camptothecin displays higher stability in whole blood than in plasma (11). This enhanced stability results from partitioning of the compound into the lipid bilayers of erythrocytes, locating the lactone ring in a hydrophobic environment protected from hydrolysis.

The reversible hydrolysis of the lactone ring also has major implications for the interpretation of pharmacokinetic data for this class of compounds. Early analytical methods for camptothecin and its derivatives provided only a measure of the total drug concentration, defined as the additive concentrations of the lactone and carboxylate forms, because samples were acidified to quantitatively convert the carboxylate species to the lactone form before analysis. Subsequently, several isocratic reversed-phase high-performance liquid chromatography methods with fluorescence detection were developed to facilitate the selective determination of the intact lactone form of the drug (12, 13). Rapidly precipitating plasma samples with methanol chilled to -70°C stabilizes interconversion between the lactone and carboxylate forms of the drug before their chromatographic analysis. Solid phase extraction performed promptly after sample collection has also been used for preliminary separation of the carboxylate and lactone species (14). Therefore, the nature of the assay and form of the drug measured should be taken into consideration whenever comparing pharmacokinetic data from different studies of a camptothecin.

The pharmacological consequences of administering camptothecin as a sodium salt, as was done during the early clinical trials of the drug (3, 4), were not fully appreciated until several nonclinical pharmacokinetic studies were undertaken some 20 years later. These studies followed the development of analytical methods permitting the selective quantitation of the lactone form of camptothecin in the presence of the carboxylate species in plasma samples (15, 16). The extent of conversion of the camptothecin sodium salt to the active lactone form was found to be only 11.5% in animal pharmacokinetic studies (16). A

significant fraction of the dose is eliminated by urinary excretion, presumably as the carboxylate species. The ensuing reformation of the lactone ring, attributable to the lower pH within the urinary tract, is thought to be responsible for the severe hemorrhagic cystitis experienced by patients treated in this manner (15).

Mechanism of Action. The DNA topoisomerases are nuclear enzymes that reduce the torsional stress of supercoiled DNA. This action enables selected regions of DNA to become sufficiently exposed and relaxed to facilitate essential cellular processes such as DNA replication, recombination, and transcription to occur (17). Topoisomerase I is a M_r 100,000 protein that covalently binds to double-stranded DNA through a reversible transesterification reaction. This reaction yields an intermediate in which a tyrosine moiety of the enzyme is linked to the 3'-phosphate end of the DNA strand, thereby creating a single-strand break (18). This so-called "cleavable complex" facilitates the relaxation of torsional strain in supercoiled DNA, either by allowing passage of the intact single strand through the nick or by free rotation of the DNA about the uncleaved strand (19). Once the torsional strain has been relieved, the enzyme rejoins the cleaved strand of DNA and dissociates from the relaxed double helix.

The camptothecins bind to and stabilize the normally transient DNA-topoisomerase I cleavable complex (2, 20). Although the drug does not affect the initial cleavage action of topoisomerase I, the religation step is inhibited, leading to the accumulation of single-stranded breaks in the DNA. These lesions are not in themselves toxic to the cell, because the strands readily religate on drug removal. However, collision of the DNA replication fork with the ternary drug-enzyme-DNA complex produces an irreversible double-strand break that ultimately leads to cell death (21). The camptothecins are, therefore, S phase-specific drugs, because ongoing DNA synthesis is a necessary condition to induce the above sequence of events leading to cytotoxicity. This has important implications for the clinical use of these agents, because optimal therapeutic efficacy of S phase-specific cytotoxic drugs generally requires prolonged exposure of the tumor to concentrations exceeding a minimum threshold. In fact, recent studies of low-dose, protracted administration of camptothecin analogues in mice bearing xenografts of human tumors have shown less toxicity and equal to or better antitumor activity than shorter, more intense dosing schedules (22). However, camptothecin-induced cytotoxicity has also been observed in cells that are not actively synthesizing DNA. Replication-independent mechanisms of cytotoxicity may involve the induction of serine proteases and endonucleases (23).

Topoisomerase I is constitutively expressed throughout the cell cycle in all mammalian cells. Its expression is regulated at the transcriptional, translational, and post-translational levels (24, 25). Catalytic activity of the enzyme *in vitro* is enhanced by protein kinase C-mediated phosphorylation (26) and decreased by polyadenosine diphosphate ribosylation (27). Some malignant tissues contain higher levels of topoisomerase I than their normal counterparts (28, 29). There are also significant differences in topoisomerase I expression between different tumor types (30). For instance, higher expression of the enzyme has been detected in colon and cervical cancers than in lung and breast tumors (30). Whether or not differential expression of the

enzyme contributes to the selective antitumor effects exhibited by the camptothecins remains to be established.

The precise sequence of events that transpire from drug-induced DNA damage leading to cell death have not been fully elucidated. *In vitro* studies have shown that camptothecin-induced DNA damage abolishes the activation of the p34^{cdc2}/cyclin B complex and results in cell cycle arrest at the G₂ phase (31). It has also been observed that treatment with camptothecins can induce transcription of the c-fos and c-jun early response genes, which occurs in association with internucleosomal DNA fragmentation, a characteristic of programmed cell death (32). In addition, noncytotoxic concentrations of camptothecins can induce the differentiation of human leukemia cells (33). Finally, recent reports have suggested that the camptothecins may also have an antiangiogenesis effect (34, 35).

Mechanisms of Resistance

A variety of mechanisms of resistance to topoisomerase I-targeted agents have been characterized *in vitro*, although relatively little is known about their significance in the clinical setting. These mechanisms involve either pretarget events, such as drug accumulation, metabolism, and intracellular drug distribution, or drug-target interactions. More recently, post-target events, such as DNA synthesis or repair, cell cycle progression, and regulation of cell death, have also been shown to play an important role in the sensitivity to these drugs.

Several multidrug efflux proteins that belong to the ABC transmembrane transport superfamily have been implicated in the resistance of cancer cells to the camptothecins. Topotecan is the only clinically important derivative with unambiguous susceptibility to the classic MDR phenotype associated with the expression of P-glycoprotein (36). Whereas the results of some initial studies were inconsistent, it now appears that camptothecin, 9-AC, and SN-38 are not substrates of P-glycoprotein (36). However, this transporter has been implicated in the biliary excretion of the carboxylate form of irinotecan (37). The clinical relevance of P-glycoprotein-mediated transport from cells as a mechanism of resistance against topotecan remains unclear (36). This is because the magnitude of *in vitro* resistance to topotecan is substantially lower than observed with other MDR substrates such as the *Vinca* alkaloids, epipodophyllotoxins, anthracyclines, and taxanes. Furthermore, MDR-overexpressing tumor models are not significantly resistant to topotecan *in vivo*, and the drug has not been shown to induce P-glycoprotein-associated MDR. In contrast, expression of MRP appears to have a markedly greater effect on the sensitivity of cancer cells to topotecan, irinotecan, and camptothecin (38, 39). Furthermore, the hepatic cMOAT, an MRP homologue also designated MRP2, is responsible for the biliary excretion of SN-38 carboxylate and the lactone and carboxylate forms of its glucuronidated metabolite (37). More recently, overexpression of another ABC family transporter, the BCRP/MXR/ABCP gene coded protein, has been correlated with *in vitro* resistance to a number of topoisomerase I and topoisomerase II inhibitors (40).

Drug metabolism may also play a role in the resistance of tumors to the prodrug irinotecan. Cell lines lacking carboxylesterase activity are unable to convert irinotecan to SN-38 and demonstrate reduced sensitivity to treatment with the prodrug *in*

vitro (41). However, because hepatic conversion most likely predominates *in vivo*, local carboxylesterase activity within tumor cells may not have a major role in determining clinical sensitivity to this agent (42). Cellular localization of topoisomerase I has been postulated as being another potential mechanism of resistance for drugs targeting this enzyme. Topoisomerase I must be present in the nucleus to exert its function. Subcellular redistribution of the enzyme from the nucleoli to other regions within the nucleus or to the cytoplasm has been observed after treatment with camptothecin derivatives *in vitro* (42). The specific relationship between this phenomenon and the development of resistance remains to be defined.

Camptothecin resistance may also result from decreased expression of topoisomerase I. There is a good correlation between *in vitro* sensitivity to camptothecin analogues and topoisomerase I levels for certain tumor cell lines (43, 44). However, the limited data available presently from clinical studies has failed to confirm this relationship (45). The absence of a simple correlation between clinical response and expression of the putative molecular target of the drug may not be entirely unexpected in consideration of the multitude of pharmacological factors and interdependent cellular events that are involved. Chromosomal deletions or hypermethylation of the topoisomerase I gene are possible mechanisms resulting in decreased topoisomerase I expression in resistant cells (46). A transient down-regulation of topoisomerase I has been demonstrated after prolonged exposure to camptothecins both *in vitro* and *in vivo* (47). Consistent with this, a progressive decrease in the number of copies of topoisomerase I in peripheral blood mononuclear cells isolated from ovarian cancer patients was detected during the course of a 21-day continuous i.v. infusion of topotecan (48). How this effect relates to clinical outcome remains to be defined.

Mutations leading to reduced topoisomerase I enzyme catalytic activity or DNA binding affinity have also been described *in vitro* in association with camptothecin resistance (49, 50). In addition, some post-translational events, such as enzyme phosphorylation (26) or poly-ADP ribosylation (27), may have a significant impact on the activity of topoisomerase I and on its susceptibility to inhibition. Finally, an observation of potential clinical interest is the up-regulation of topoisomerase II in human tumor cells after exposure to a camptothecin analogue *in vitro* (51), providing a rationale for sequential therapy with topoisomerase I and II inhibitors. However, clinical optimization of this therapeutic strategy may prove to be extremely difficult, because scheduling issues such as the duration of treatment and time interval between the administration of the two agents cannot be directly extrapolated from *in vitro* models.

Despite intensive investigation, the specific events comprising the cellular response to the stabilized DNA-topoisomerase complexes have still not been elucidated in much detail. An enzyme with 3'-specific tyrosyl-DNA phosphodiesterase activity has been described recently, which may be involved in the repair of topoisomerase I-DNA complexes (52). Ubiquitin/26S proteasome-dependent degradation of topoisomerase I may also play a role in the repair response to topoisomerase I-mediated DNA damage (53). The fact that cell cycle arrest in the G₂ and S phases has been correlated with drug resistance to topoisomerase I inhibitors in colon cancer and leukemia cell lines *in vitro*

(54) suggests that enhanced DNA repair activity may lead to camptothecin resistance. It has also been observed that abrogation of camptothecin-induced S phase arrest by 7-hydroxystaurosporine (UCN-01), a selective protein kinase C inhibitor, enhances the antitumor activity of camptothecin (55). Wild-type p53 status has been associated *in vitro* with increased sensitivity to topoisomerase I inhibitors (56). Nevertheless, it has been shown that cells without functional p53 can undergo apoptosis after exposure to camptothecins (46). In common with other DNA-damaging agents, the camptothecins induce p53 expression in damaged cells (57). Prolongation in the duration of the cell cycle has been associated with resistance to camptothecins, presumably by reducing the proportion of cells in S phase at any given time (58). Finally, up-regulation of NFκB has been detected in cancer cells exposed to irinotecan, which may mediate resistance to chemotherapy-induced apoptosis. In fact, inhibiting NFκB through the adenoviral delivery of a modified form of IκBα, the endogenous inhibitor of NFκB, markedly sensitizes chemoresistant tumors to irinotecan in animal models (59). Some reports also suggest that preventing NFκB activation by proteasome-inhibiting agents may enhance the antitumor efficacy of or circumvent resistance to topoisomerase-targeted agents (60). Other potential mechanisms of decreased sensitivity to camptothecins involving events that occur downstream from the generation of DNA damage to the triggering of apoptosis and cell death are as yet very poorly understood.

Camptothecins Approved for Use as Anticancer Drugs

Topotecan

Clinical Pharmacokinetics. Topotecan (Hycamtin; Smith-Kline Beecham Pharmaceuticals, Philadelphia, PA) is a semisynthetic derivative of camptothecin with a basic *N,N*-dimethylaminomethyl functional group at C-9 that confers water solubility to the molecule (Fig. 1). A considerable amount of the drug is converted to the carboxylate form on reconstitution in normal saline, whereas at the lower pH of 5% dextrose for injection, the maximum extent of conversion is 10% with equilibrium being achieved within 30 min (61). A new parenteral formulation has been introduced that contains tartaric acid in the infusion diluent to provide a sufficiently low pH to maintain essentially all of the drug in the lactone form indefinitely (62).

The pharmacokinetic behavior of topotecan has been studied extensively in adult and pediatric cancer patients during phase I and phase II clinical trials, both as a single agent and in combination with other chemotherapeutic agents. Pharmacokinetic parameters of the drug determined in cancer patients treated with single agent topotecan are summarized in Table 1 (63–72). Topotecan is most commonly administered as a 30-min i.v. infusion. Plasma concentrations of the inactive carboxylate form of the drug exceed the lactone species within 5–10 min after completing the infusion. The ratio of the lactone:total drug AUC values ranges from 0.3 to 0.4 when given as a 30-min infusion, which is similar to the ratio of their concentrations in plasma during more prolonged infusions after steady-state has been achieved. Plasma levels of topotecan lactone and total drug decline in a biexponential manner after i.v. infusion with similar

Table 1 Pharmacokinetic parameters of the camptothecins in adult cancer patients with solid tumors^a

Compound	Administration	Chemical form assayed	C _{max} (D) ^b (nM)	CL (liter/h/m ²)	V _{ss} (liter/m ²)	t _{1/2,z} (h)	AUC ratio (L:T)	Urinary excretion (%)	Oral bioavailability (%)	Ref.
Camptothecin sodium	Bolus i.v.	T	21,300 (20)	0.34	7.0	15.3	NR ^c	16.5		3, 4
Topotecan	30 min i.v.	L	78 (1.5)	27.4	75.4	2.8	0.37			64–66
		T	142 (1.5)	13.4	68.6	4.1		30.2		64, 72
	24 h ci	L	5.8 (1.5)	27.5	92.0	4.1	0.33			67–69
		T	10.7 (1.5)	14.4	NR	3.5		50.0		67
	21 d ci	L	1.5 (0.6)	40.2	NR	NR	0.33			70, 71
Irinotecan	p.o.	T	4.2 (0.6)	13.1	NR	NR		40.0		70, 71
		L	12.8 (1.5)				0.33	NR	30	66
	90 min i.v.	L	975 (150)	53.5	NR	5.7	0.44			115, 116
		T	2,304 (150)	17.0	142.0	7.3		25.7		115, 116, 119
	96 h ci	T	28.6 (10)	24.9	NR	NR	NR	NR		120
SN-38 ^e	p.o.	L	78.9 (66)	262.0 ^d		11.4	0.39	NR	8	117, 118
		L	33.4 (150)			11.5	0.51			115, 116
	90 min i.v.	T	93.5 (150)			10.2		0.26		115, 116, 119
		T	6.4 (10)			NR	NR	NR		120
	p.o.	L	18.4 (66)			16.9	0.75	NR		117
9-AC	72 h ci	L	2.6 (2.5)	27.4	259.8	16.8	0.09			154, 158
		T	59.1 (2.5)	2.0	23.6	8.4 ^f		32.1		159
	p.o.	L	12.7 (0.84)			9.72	0.08		49	160, 161
		T	116 (1.5)			NR		NR	63	161
	p.o.	L	35 (1.5)			NR	0.11			171–174
GG-211	30 min i.v.	T	432 (1.5)			14.4		5.0	NR	171–174
		L	25.6 (1.5)	65.5	311	5.6	0.27			177–178
	72 h ci	T	96.5 (1.5)	21.0	149	9.6		11.0		177
		L	2.2 (1.75)	64.4	NR	7.5	NR	11.4		179
	21 d ci	L	0.7 (0.4)	50.3	NR	NR	NR	NR		180
Exatecan	p.o.	L	7.9 (6)			6.8	0.43		11.3	181
		T	NR			NR		NR	11.8	181
	30 min i.v.	T	177 (0.5)	1.8	14.3	7.3	NR	6.10		187, 188
		T	144 (2.4)	2.9	8.9	13.9	NR	8.9		191

^a The information presented in this table represents a comprehensive evaluation of the published data for each compound from single agent clinical studies in adult cancer patients. In cases where pharmacokinetic data was reported for multiple studies of the same administration schedule, data was selected from studies that were considered to be most reliable, based on an assessment of the methodology described in the report and agreement with other published data for the drug. Parameters for which more than one reference has been cited are the average of values from the individual studies.

^b The abbreviations used are: ci, continuous i.v. infusion; C_{max} (D), peak plasma concentration (dose in mg/m²); CL, total plasma clearance; V_{ss}, apparent volume of distribution at steady state; t_{1/2,z}, half-life of the terminal disposition phase; L, intact lactone form of the camptothecin; T, total camptothecin (lactone + carboxylate).

^c NR, parameter was either not reported or could not be estimated from reported data.

^d CL/bioavailable fraction.

^e SN-38 pharmacokinetic parameters obtained after irinotecan administration.

^f Reported value is considered to be unreliable.

terminal phase half-lives of 2.4 to 4.3 h. The biological half-life of topotecan in humans is much shorter than that of camptothecin sodium and other analogues that have been clinically evaluated (Table 1). As a consequence, drug accumulation does not occur when five daily doses are given by 30-min i.v. infusion at an interval of 24 h, which is the approved dosing regimen for topotecan.

Topotecan exhibits linear pharmacokinetic behavior, as indicated by proportionality between the administered dose and AUC for both the total drug and intact lactone species, over a relatively wide range of doses (0.4–22.5 mg/m²). Furthermore, there are no significant changes in its pharmacokinetics on repeated daily dosing (62). The disposition of the drug in pediatric patients is comparable with adults (73). The total body CL of topotecan averages 27.4 liter/h/m² for the lactone form and 13.4 liter/h/m² for the total drug in various clinical studies.

Topotecan has a moderate steady-state apparent volume of distribution, being only ~2-times body weight for the lactone species and total drug, consistent with the hydrophilic character of the compound. The fraction of topotecan bound to plasma proteins, which has been reported as ranging from 7 to 35% based on total drug determinations, is much lower than that of other camptothecins (74, 75). This may partially account for its greater CNS penetration in comparison to other analogues as determined in nonclinical studies (76). The CSF:plasma AUC ratio ranged from 29 to 42% in pediatric patients treated with continuous i.v. infusions of topotecan (77).

The elimination of topotecan is thought to predominantly result from its conversion to the carboxylate species followed by renal excretion. The percentage of the administered dose recovered as unchanged drug in the urine ranges from 30 to 50% (Table 1). A clear relationship between CL_{CR} and topotecan CL

has been documented (78). In comparison to normal patients with a CL_{CR} of ≥ 60 ml/min, the CL of total topotecan is decreased by 33 and 75% in patients with CL_{CR} ranging from 40–59 and 20–39 ml/min, respectively. Topotecan disposition and hematological toxicity is not significantly altered in patients with hepatic dysfunction presenting as an elevation in total bilirubin up to 10 mg/dl (79). Population pharmacokinetic studies suggest that patient characteristics and laboratory values, including sex, height, weight, and serum creatinine concentration, have at best a moderate ability to predict topotecan CL in an individual patient (80). A significant correlation between topotecan CL and patient age was not identified. This is a somewhat unexpected finding in consideration of the association between CL_{CR} and age. However, only 8 of the 82 patients in the study population of this retrospective analysis were older than 72 years. Pharmacokinetic data from a greater number of elderly patients is needed to more conclusively assess the effect of patient age on topotecan CL.

Three metabolites of the drug, *N*-desmethyl-topotecan, topotecan-*O*-glucuronide, and *N*-desmethyl-topotecan-*O*-glucuronide, were identified recently in plasma, urine, and bile at low concentrations (81). Thus, whereas hepatic cytochrome P450-dependent metabolism does not seem to be a major pathway of drug elimination, topotecan CL nevertheless appears to be enhanced in pediatric patients receiving concomitant treatment with dexamethasone, phenobarbital, or phenytoin (82), and reduced in a patient receiving terfenadine (82). Sequence-dependent pharmacokinetic interactions have also been observed when topotecan is combined with other antineoplastic agents such as cisplatin. In particular, the AUC of topotecan and drug-related toxicities are both significantly greater when topotecan is administered after cisplatin rather than before. This effect is believed to result from subclinical renal tubular toxicity induced by cisplatin leading to a decrease in the CL of topotecan (83). Aside from this, there has been no evidence of a clinically significant pharmacokinetic interaction between topotecan and any of the other chemotherapeutic agent with which it has been combined (75, 84), including paclitaxel, anthracyclines, etoposide, cytarabine, and cyclophosphamide.

Bioavailability after oral administration ranges from 30 to 40% in humans (66, 85). Peak plasma concentrations are achieved within 1 h after ingestion, and the ratio of lactone:total drug AUC values is comparable with the i.v. route of administration. Coadministration of topotecan with food results in a small decrease in the absorption rate but does not affect the extent of absorption (85). The variability in the AUC of topotecan is relatively high after oral administration both between patients (CV, 40–73%) and within the same patient (CV, 25–96%; Ref. 86). Relatively low and highly variable bioavailability may be potentially problematic for a drug with a narrow therapeutic index that is given p.o. at its MTD.

There is an expanding volume of literature suggesting that dose individualization may be beneficial for drugs with narrow therapeutic indices such as antineoplastic agents. The best candidates for dose-individualization are drugs that exhibit highly variable pharmacokinetics among patients when treated according to the same dose and schedule, and that display a good correlation between AUC and response or toxicity. Individualizing dosages based on a precise determination of AUC values

by frequent blood sampling on a routine basis is generally impractical, being labor-intensive, expensive, and inconvenient for patients. Accordingly, limited sampling strategies have been developed for topotecan and a host of other chemotherapeutic agents to provide an estimate of the AUC by measuring drug concentration in one or two plasma samples obtained at predetermined times (62). Population pharmacokinetic models can also potentially be used to individualize dosing regimens for optimal therapeutic benefit. However, despite the relatively high degree of interpatient variability in the AUC of topotecan, even after i.v. administration (CV, 35–60%; Ref. 62), the real benefit derived from routine drug-level monitoring and dose individualization is questionable. This is because the principal severe toxicities are easily managed hematological effects, and no studies have demonstrated the existence of a relationship between topotecan dose or AUC and therapeutic response.

Administration Schedules and Toxicity. The schedule of topotecan most extensively investigated, and the one that has been approved for clinical use is a 1.5 mg/m² dose given as a 30-min i.v. infusion on 5 consecutive days, repeated every 3 weeks (62, 65). Many clinical investigations have been undertaken to explore the administration of topotecan as a continuous i.v. infusion, based on *in vitro* studies demonstrating that prolonged exposure to low concentrations of the drug enhances chemosensitivity relative to short-term exposure to high drug concentrations (22). A wide variety of schedules have been evaluated, including 24-h infusions administered at intervals of 1 or 3 weeks, 72-h infusions given every 7, 14, or 21 days, a 5-day infusion repeated every 3 weeks, and a 21-day continuous infusion (75). The dose intensity of the 21-day continuous i.v. infusion exceeds that achieved with any other dosing regimen, although the higher doses that can be administered with this schedule lead to an increased incidence of thrombocytopenia and cumulative anemia (70). The infusion rate recommended for phase II testing of the 21-day continuous i.v. infusion schedule when given every 4 weeks, 0.53 mg/m²/day, provides a dose intensity of 2.8 mg/m²/week, which is ~10% greater than that achieved with the daily times 5 schedule approved by the FDA for clinical use. Continuous infusion schedules have also been extensively studied in pediatric populations. The MTDs for children are considerably lower than those achieved in adult patients (*i.e.*, 0.3 mg/m²/day for the 21-day continuous i.v. infusion regimen; Ref. 87). Administration of topotecan by the oral route has also been assessed using once daily dosing for 5 or 10 days every 3 weeks and a 21-day uninterrupted schedule (85, 86). The recommended dose for phase II studies is 2.3 mg/m²/day for the daily times 5 every 3 week schedule (85), which is only 50% higher than the dose given by the i.v. route according to this same schedule, although the oral bioavailability is only 30–40%.

Neutropenia has proven to be the DLT for all of the administration schedules of topotecan. It is often accompanied by dose-limiting thrombocytopenia. The incidence of grade 4 neutropenia for the approved dosing regimen is as high as 81%, with a 26% incidence of febrile neutropenia (88). There is a good correlation between the degree of neutropenia and AUC values based on intact lactone or total drug concentrations (61–72). Severe neutropenia has been observed in patients with moderate to severe renal dysfunction when treated at a third of

Table 2 Single agent activity of topotecan

Disease	Dose and schedule	No. of patients	Previous chemotherapy	Response rate	Median survival time (months)	Grade 3–4 toxicities (% of patients)	Ref.
Ovarian cancer	1.5 mg/m ² /d, 30 min i.v. inf. × 5d q3w	112	Yes	20.5%	15.3	Neutropenia: 52% Thrombopenia: 3% Anemia: 6% Vomiting: 10% Diarrhea: 6%	90
Small-cell lung cancer	1.5 mg/m ² /d, 30 min i.v. inf. × 5d q3w	107	Yes	24.3%	6.3	Neutropenia: 89% Thrombopenia: 58% Anemia: 42% Vomiting: 5% Mucositis: 2%	91
Myelodysplastic syndromes	2 mg/m ² /d, 120 h i.v. ci q3–4w	30	Yes (50% of patients)	37%	NR ^a	Neutropenia: NR Thrombopenia: NR Anemia: NR	93
Chronic myelomonocytic leukemia	2 mg/m ² /d, 120 h i.v. ciq3–4w	30	Yes (25% of patients)	27%	NR	Neutropenic fever: 85% Vomiting: 2% Mucositis: 19% Diarrhea: 13%	93

^a NR, not reported.

the recommended dose (78). An initial 50% reduction of the daily dose to 0.75 mg/m² is recommended for untreated or minimally pretreated patients with moderate renal dysfunction, defined as a CL_{CR} of 20–40 ml/min. The daily dose should be decreased even more to 0.5 mg/m² for such patients who have received extensive previous therapy. An acceptable dose has not been established for patients with severe renal impairment (*i.e.*, CL_{CR} < 20 ml/min; Ref. 78). Dose reduction is not necessary in patients with hepatic dysfunction (79).

The MTD for the daily 30-min i.v. infusion times 5 schedule of topotecan in patients with hematological malignancies is 4.5 mg/m²/day (89). Gastrointestinal side effects such as mucositis and diarrhea become dose limiting at these higher doses. Other less frequently encountered toxicities of the drug are nausea and vomiting, mucositis, elevated serum transaminase activities, fever, fatigue, and rash. However, most of these nonhematological side effects are generally manageable. Diarrhea is uncommon, and hemorrhagic cystitis does not occur.

Antitumor Activity. The primary indication of topotecan is second-line therapy against advanced ovarian carcinoma in patients who have failed previous treatment with platinum compounds or paclitaxel-containing chemotherapy regimens. This is supported by the results of a phase III trial in which patients with advanced ovarian carcinoma, who had progressed during or after treatment with a single platinum-based regimen, were randomized to receive either 30-min infusions of topotecan 1.5 mg/m²/day for 5 days or paclitaxel 175 mg/m² given as a 3-h i.v. infusion (Table 2; Ref. 90). Objective response rates were not significantly different between the two groups, being 20.5% for patients treated with topotecan and 13.2% for patients that received paclitaxel ($P = 0.14$). The median time to disease progression in the topotecan arm, 23 weeks, was significantly greater than the 14 weeks observed in the cohort receiving paclitaxel ($P = 0.002$). However, overall survival was similar in both treatment groups, and myelosuppression was significantly greater in the group of patients treated with topotecan. Because

the use of paclitaxel as first-line therapy in combination with platinum compounds is now well established, topotecan has become one of the most widely used agents for salvage therapy in patients with ovarian carcinoma.

Topotecan was also granted FDA approval recently as a therapeutic option for recurrent SCLC. Its use for this indication was established in a randomized trial that compared single agent topotecan against combination therapy with cyclophosphamide, doxorubicin and vincristine in 211 patients who had relapsed after completing first-line chemotherapy. Topotecan proved to be just as effective as the cyclophosphamide/doxorubicin/vincristine combination with regard to response rate, time to disease progression, and overall survival, but provided better control of disease-associated symptoms (Table 2; Ref. 91). A subsequent randomized study of topotecan in comparison to best supportive care for patients with extensive-disease SCLC, as upfront therapy after treatment with cisplatin plus etoposide, showed that topotecan modestly increased the time to disease progression from a median of 2.3 to 3.4 months but failed to improve survival (92).

In addition, topotecan has shown some interesting activity against hematological malignancies. Complete response rates of 27 and 37% were achieved in phase II studies involving patients with chronic myelomonocytic leukemia and myelodysplastic syndromes, respectively, with the drug given as a 5-day continuous i.v. infusion (Table 2; Ref. 93). Objective responses have also been observed in phase I clinical trials in patients with acute myelogenous leukemia (94).

Some evidence of antitumor activity has also been documented against several pediatric malignancies including rhabdomyosarcoma, neuroblastoma, retinoblastoma, osteosarcoma, and soft tissue sarcomas (95, 96). Case reports describe objective responses against refractory parenchymal brain metastases of ovarian and SCLC (97, 98), and against primary CNS non-Hodgkin lymphoma (99). However, activity against primary CNS tumors is poor, although the drug appears to readily

penetrate the blood brain barrier (100). Topotecan has not shown significant activity in patients with most other common tumor types, as indicated by response rates of 10–15% against breast cancer (101, 102), 0–10% against NSCLC (103, 104), and 0–10% colorectal cancer (70, 105).

The single agent activity of topotecan against ovarian carcinoma and SCLC has provided the rationale for developing combination regimens of the drug with other anticancer agents active in these diseases, such as cisplatin and paclitaxel. Incorporating topotecan into multiagent chemotherapy regimens has been difficult because of severe hematological toxicity, requiring a significant reduction of the single agent therapeutic doses. Nevertheless, encouraging preliminary results have been described for the topotecan/cisplatin and topotecan/carboplatin/paclitaxel combinations, given as front line therapy for patients with advanced ovarian cancer, with overall response rates of 80–90% and complete response rates of 24–46% (106, 107). Both regimens are currently being evaluated in phase III clinical trials against the standard of care carboplatin/paclitaxel combination in advanced ovarian cancer patients. Similarly, response rates of 60–90% have been observed in chemo-naïve patients with SCLC treated with topotecan/paclitaxel and topotecan/carboplatin/paclitaxel (108, 109). Topotecan in combination with cisplatin has also been explored in SCLC patients treated previously. Objective responses were observed in 28% and 16% of sensitive and refractory patients, respectively (110). These treatment regimens are presently being prospectively tested in randomized trials. An unexpectedly high rate of treatment-related fatal sepsis from topotecan/cisplatin and topotecan/paclitaxel combinations in patients with extensive SCLC led to the temporary suspension of patient accrual in these two arms of a three-arm randomized Cancer and Leukemia Group B trial, which also included a paclitaxel/cisplatin arm. These treatment arms were later reopened using lower drug doses, and results of this study are still pending. Finally, topotecan has been combined with agents that are active against hematological malignancies, particularly cytarabine. Phase II studies have shown promising activity for this combination, with complete remissions documented in 61% of the patients with myelodysplastic syndromes and 44% of those with chronic myelomonocytic leukemia (111). A randomized study comparing topotecan/cytarabine *versus* cytarabine/idarubicin in patients with myelodysplastic syndromes is currently ongoing.

Whereas the theoretical basis for protracted drug administration is firmly supported by preclinical studies, superior efficacy of such schedules in the clinical setting remain unproven. A response rate of 35% was observed with the 21-day i.v. infusion schedule in ovarian cancer patients that had progressed after receiving one previous platinum-containing regimen (48); however, this finding has not been confirmed in other phase II studies. Moreover, a small randomized phase II study showed a lower response rate with a 24-h continuous i.v. infusion schedule than with the 30-min infusion given daily for 5 days, although the latter was associated with a significantly higher incidence of severe neutropenia (112). However, conclusive evaluation of this therapeutic approach will require undertaking appropriately designed and powered randomized trials, particularly considering the heterogeneity of the ovarian cancer popu-

lation and the limited number of patients included in the few studies reported to date.

Irinotecan

Clinical Pharmacokinetics. Irinotecan (Camptosar; Pharmacia and Upjohn Co., Kalamazoo, MI) is a water-soluble prodrug designed to facilitate parental administration of the potent 7-ethyl-10-hydroxy analogue of camptothecin (SN-38). It contains a dibasic bispiperidine substituent, linked through a carbonyl group to the hydroxyl at C-10, to confer water solubility for parenteral administration. Enzymatic cleavage of this promoiety on presentation to the systemic circulation affords SN-38, the biologically active compound, which is a 1000-fold more potent inhibitor of purified topoisomerase I *in vitro* than irinotecan (75). Conversion to SN-38 is mediated by carboxylesterases (113), predominantly in the liver, although recent studies have also shown that butyrylcholinesterase present in human serum has irinotecan-activating activity (114). The significance of intratumoral generation of SN-38 in tumors that exhibit sensitivity to irinotecan remains to be demonstrated.

The main pharmacokinetic parameters of irinotecan and SN-38 obtained in clinical studies performed in adult cancer patients with solid tumors have been summarized in Table 1 (115–120). In the majority of studies, the peak plasma concentration and AUC of irinotecan were found to increase proportionally with the administered dose, indicative of linear pharmacokinetic behavior (115–120). Both the lactone and the open-ring carboxylate form of irinotecan and SN-38 are detectable in plasma shortly after i.v. infusion. However, the AUC of SN-38 is only ~4% of the irinotecan AUC, suggesting that only a relatively small fraction of the dose is ultimately converted to the active form of the drug (116). However, the biological half-life of the lactone form of SN-38, 11.5 h, is much longer than that of topotecan, thereby representing a potential pharmacological advantage of irinotecan (Table 1). The CL of irinotecan lactone is approximately two-times greater than that of topotecan, being 53.5 liter/h/m² on average (Table 1). Irinotecan has a moderate apparent volume of distribution, with mean values at steady state of 142 liter/m² for the total drug, which is approximately four times total body weight.

In comparison to other camptothecin derivatives, a relatively large percentage of the intact lactone form of both irinotecan and SN-38 persists in the plasma of patients after drug administration, attributable to the preferential binding of the lactone form to serum albumin. The lactone:total compound AUC ratio ranges from 40 to 44% for irinotecan and from 51 to 75% for SN-38 (115–120). Total drug plasma protein binding is 30–43% for irinotecan and significantly higher for SN-38, in the 92–96% range (75). Penetration of the drug into the CNS has not been characterized in humans, although in nonhuman primates the CSF:plasma AUC ratio for irinotecan lactone was only 14%, which is significantly lower than the ratio for topotecan (76). Nevertheless, promising activity has been observed in several recent clinical trials of irinotecan in patients with malignant gliomas (121, 122).

Biliary excretion appears to be the major route of irinotecan elimination, because urinary excretion of the parent compound accounts for only 26% of the administered dose in patients (Table 1). Aside from conversion to SN-38, irinotecan

is subject to biotransformation by several other pathways. Presently, at least two oxidative metabolites have been identified, namely 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin (123) and 7-ethyl-10-(4-amino-1-piperidino)-carbonyloxycamptothecin (124). As with the parent compound, both metabolites are poor inhibitors of topoisomerase I, and they are not significantly converted to SN-38. CYP3A4 appears to be responsible for the production of these two metabolites. Because this enzyme is involved in the biotransformation of many commonly used drugs, the potential exists for clinically relevant pharmacokinetic drug interactions. In fact, phase I studies performed in brain cancer patients requiring concomitant treatment with anticonvulsants and glucocorticoids on a chronic basis revealed that irinotecan CL was significantly greater than that observed in groups of comparable patients who did not receive medications known to induce CYP3A4 (121). In contrast to topotecan and consistent with evidence that irinotecan elimination is predominantly mediated by metabolism and biliary excretion, rather than by urinary excretion, cisplatin does not seem to alter irinotecan CL. No relevant pharmacokinetic interactions have been apparent either with different irinotecan drug combinations with other cytotoxic agents, such as 5-FU, etoposide, or oxaliplatin. The existence of multiple metabolic pathways for irinotecan, many of which are probably unidentified, is underscored by the fact that only ~50% of the total administered dose is recovered in urine (28%) and feces (25%) as unchanged irinotecan or its known metabolites (125). However, 95% of drug-related radioactivity was recovered in urine and feces after i.v. administration of ^{14}C -labeled irinotecan to patients, with fecal excretion being the predominant route of elimination (64%; Ref. 126).

The major mechanisms of SN-38 elimination appear to be glucuronidation and biliary excretion. Renal excretion of unchanged SN-38 represents only 0.26% of the irinotecan dose (Table 1). The cMOAT is believed to be responsible for the biliary excretion of irinotecan, SN-38, and SN-38 glucuronide (38). P-glycoprotein (MDR1) may also be involved in the biliary transport of the high-affinity component of the carboxylate form of irinotecan (38). A slight elevation in the SN-38 plasma concentration occurring several hours after the end of drug infusion, suggestive of some degree of enterohepatic recycling, has been observed in a few studies (127, 128). At this stage in the clinical development of the drug, it may be difficult to conclusively establish the existence of a rebound peak in the plasma profile because of practical limitations imposed on subjecting patients to the prolonged and intensive sampling schedules that would be required. The presence of β -glucuronidase activity in the bacterial microflora of the intestinal tract could potentially contribute to the gastrointestinal toxicity of irinotecan by releasing unconjugated SN-38 after biliary excretion of its glucuronide conjugate.

The 1A1 isoform of UGT predominantly catalyzes SN-38 glucuronidation (129). Nonclinical pharmacokinetic studies demonstrated that the concurrent administration of irinotecan and valproic acid, a competitive inhibitor of UGT, increased systemic exposure to SN-38 (130). The importance of this particular interaction has not been established in humans. The opposite effect of a decrease in the AUC of SN-38 resulted when irinotecan was given to female rats together with pheno-

barbital (130). However, the nature of this interaction may not be as straightforward as implied, because in addition to potentiating glucuronidation, phenobarbital may also induce cytochrome P450.

It has been suggested that the extent of SN-38 glucuronidation may be inversely related to the risk of severe diarrhea resulting after irinotecan therapy (127). UGT1A1 is also the isozyme responsible for bilirubin glucuronidation (131). Polymorphisms of this enzyme are associated with several familial hyperbilirubinemia conditions, namely Crigler-Najjar syndrome type I and II, as well as Gilbert's disease. Crigler-Najjar syndromes are rare, occurring in only 1 in a million births, whereas Gilbert's disease occurs in up to 15% of the general population. Gilbert's disease is caused by the presence of an additional thymidine-adenine repeat in the promoter region of the UGT1A1 gene, resulting in a mild hyperbilirubinemia that may be clinically silent (131). It has been suggested that the existence of UGT enzyme polymorphisms could significantly impact the clinical use of irinotecan. Cancer patients with Gilbert's disease may be at increased risk for irinotecan-induced diarrhea because of decreased glucuronidation of SN-38 (132). Positive correlations have been found between baseline serum levels of unconjugated bilirubin and the severity of neutropenia in patients treated with irinotecan, as well as the AUC of irinotecan and SN-38 (133). Genetic deficiency in UGT1A1 activity has also been observed in certain ethnic groups, such as the Inuit Indian population in Canada (134). Studies assessing the correlation between the UGT1A1 promoter genotype and irinotecan pharmacokinetics have shown that the extent of SN-38 glucuronidation was significantly lower in patients with the (TA)₇TAA mutation. Specifically, the SN-38 glucuronide:SN-38 AUC ratio was 9.3 in patients with the wild-type allele (6/6), 4 in heterozygotes (6/7), and 2.4 homozygotes (7/7), respectively. Consistent with this, none of the patients with the wild-type allele (6/6) developed significant toxicity when treated with 300 mg/m² of irinotecan given as a 90-min i.v. infusion, whereas 36% of heterozygotes (6/7) and 45% of homozygotes (7/7) experienced grade 2 or more severe diarrhea or neutropenia. Therefore, screening for the UGT1A1 promoter polymorphism may be predictive of the extent of SN-38 glucuronidation and irinotecan toxicity, and may lead to individualized treatment with irinotecan based on pharmacogenetics (135).

The absolute bioavailability of irinotecan after oral administration is only 8% in humans based on lactone measurements (118). However, the SN-38: irinotecan AUC ratio, when expressed on a molar basis, is three times greater after oral administration than when identical doses are given parenterally. Thus, despite the low apparent bioavailability, oral dosing may be a convenient way to maintain sustained plasma levels of SN-38 lactone, at concentrations that are comparable with those provided by i.v. infusions. First-pass conversion of irinotecan to SN-38 in the intestine and liver may be a potential explanation for this effect.

Population pharmacokinetic studies have not shown any relationships of significance between irinotecan CL and demographic characteristics, including age, sex, height, weight, and body surface, or renal function (119). However, significant negative correlations have been reported between irinotecan CL and some liver function markers, such as bilirubin and γ -

glutamyl transpeptidase (119). Also of interest, serum bilirubin, glutamic-oxaloacetic transaminase, and glutamic-pyruvic transaminase levels are positively correlated with the SN-38:irinotecan AUC ratio. It was hypothesized that a greater fraction of the prodrug could be converted to SN-38 in patients exhibiting lower irinotecan CL because of a prolongation of its exposure to hepatic carboxylesterases. Pharmacokinetic/pharmacodynamic relationships have been identified between irinotecan AUC and myelosuppression, and between SN-38 pharmacokinetic variables and diarrhea (115–120). Some studies have also found an association between SN-38 AUC and myelosuppression (125). As observed with topotecan, pharmacokinetic variables derived from the time course of SN-38 lactone have not proven to be superior to those based on the more conveniently measured total SN-38 plasma concentration in their predictive value for severity of toxicity.

Schedule of Administration and Toxicity. The approved administration schedule of irinotecan in the United States is 125 mg/m² given as a 90-min i.v. infusion once weekly for 4 of 6 weeks (116). In Europe, the most widely used dosing regimen is 350 mg/m² given as a 60-min i.v. infusion once every 3 weeks (128), whereas in Japan, where the drug was initially developed, 100 mg/m² every week or 150 mg/m² every other week are the schedules more commonly used (136). Protracted or repeated dosing regimens have been explored with irinotecan based on the same premise advanced for other camptothecin analogues that continuous exposure may confer a therapeutic advantage. These include short infusions repeated on 5 consecutive days, a 4-day continuous infusion given weekly for 2 weeks every 21 days, and a 14-day continuous i.v. infusion every 3 weeks (75). Oral delivery of irinotecan has also undergone phase I clinical testing given once a day for 5 days every 3 weeks (117). As opposed to the experience with topotecan, the maximum dose intensity achieved with protracted infusion schedules of irinotecan is actually two to three times lower than that obtained with short-infusion regimens. However, irinotecan appears to be more effectively converted to SN-38 during prolonged continuous i.v. infusion, with SN-38:irinotecan AUC ratios ranging from 16 to 24% (75). As mentioned earlier in this review, similar findings are observed on oral administration, in this case potentially because of intestinal and hepatic first-pass biotransformation of irinotecan to SN-38 (117).

The principal DLT observed for all of the dosing regimens is delayed diarrhea, with or without neutropenia (116, 128, 136). The frequency of grade 3 or 4 diarrhea was as great as 35% of the treated patients in early clinical studies. Adoption of an intensive loperamide regimen, consisting of a 4-mg dose starting at the onset of any loose stool occurring more than a few hours after therapy, followed by 2 mg every 2 h for up to 12 h after diarrhea resolves, has effectively reduced the incidence of this side effect by more than half (137). However, standard doses of antidiarrheal agents tend to be ineffective when intervention is initiated after severe diarrhea develops. Episodes of diarrhea generally resolve within a week and are rarely fatal unless associated with fever and neutropenia. Another antidiarrheal agent, acetorphan, has shown improved control of irinotecan-induced delayed diarrhea when combined with loperamide (138). Alternative strategies to reduce irinotecan-associated diarrhea have been evaluated in nonclinical studies with some

success. These include the use of β -glucuronidase inhibitors or antibiotics to decrease intestinal activity of the enzyme (139) and cyclosporin A (140) to presumably inhibit cMOAT-mediated biliary excretion of SN-38 (38). Preliminary results obtained in humans suggest that the coadministration of neomycin with irinotecan can significantly reduce fecal β -glucuronidase activity, as measured by a colorimetric phenolphthalein microassay, and decrease the amount of SN-38 present in the feces (141). A phase I study undertaken to assess the coadministration of irinotecan with cyclosporin A revealed a decrease in irinotecan CL and prolongation of the apparent biological half-lives of irinotecan, SN-38, and SN-38 glucuronide (142). However, these effects could also result from the inhibition of CYP3A4 and P-glycoprotein by cyclosporin A; thus, the nature of this interaction remains unclear. The potential of these approaches to modulate irinotecan-induced diarrhea is currently undergoing prospective clinical evaluation.

Myelosuppression is the second most commonly encountered toxicity of irinotecan (116, 128, 136). Grade 3 or 4 neutropenia occurs in 14–47% of the patients treated by the once every 3 week schedule (143–145) and is somewhat less frequently encountered among patients treated with the weekly schedule (12–19%; Refs. 137, 146). Febrile neutropenia occurs in 3% of the patients and may be life threatening, particularly when associated with concomitant diarrhea.

A cholinergic syndrome resulting from inhibition of acetylcholinesterase activity by irinotecan is frequently evident within the first 24 h after dosing (75, 116, 128, 136). Symptoms include acute diarrhea, diaphoresis, hypersalivation, abdominal cramps, visual accommodation disturbances, lacrimation, rhinorrhea, and less often, asymptomatic bradycardia. These effects are short lasting and respond within minutes to atropine. Atropine may be prophylactically given to patients who have experienced previously a cholinergic reaction before treatment with additional cycles of irinotecan. Other common and generally manageable nonhematological toxicities are nausea and vomiting, fatigue, vasodilatation or skin flushing, mucositis, serum transaminase elevations, and alopecia (75, 116, 128, 136). Finally, there have been case reports of dyspnea and interstitial pneumonitis associated with irinotecan therapy in Japanese lung cancer patients.

Antitumor Activity. The major therapeutic indication for irinotecan is the treatment of colorectal cancer. Phase II trials conducted in Japan, the United States, and France consistently found response rates in the 10–35% range for single agent therapy in patients with previously treated and untreated metastatic colorectal cancer, including those with 5-FU-resistant tumors (145, 146). The efficacy of irinotecan as second-line therapy for colorectal cancer was confirmed in two phase III studies performed in Europe. The first trial randomized patients with advanced colorectal cancer who had progressed during previous treatment with 5-FU-based chemotherapy regimens to receive either irinotecan 300–350 mg/m² every 3 weeks or best supportive care (143). The 1-year survival rate of 36% for the irinotecan-treated group was significantly greater than the 14% observed for the control group ($P < 0.01$). The second study compared irinotecan treatment against three different continuous i.v. infusion regimens of 5-FU in patients with advanced colorectal cancer treated previously (144). This trial also

Table 3 Single agent activity of irinotecan

Disease	Dose and schedule	No. of patients	Previous chemotherapy	Response rate	Median survival time (months)	Grade 3–4 toxicities (% of patients)	Ref.
Colorectal cancer	300–350 mg/m ² , 90 min i.v. inf. q3w	189	Yes	NR ^a	9.2	Neutropenia: 22% Diarrhea: 22% Vomiting: 14% Cholinerg. syn.: 12%	143
	300–350 mg/m ² , 90 min i.v. inf. q3w	127	Yes	NR	10.8	Neutropenia: 14% Diarrhea: 22% Vomiting: 14%	144
	300–350 mg/m ² , 30 min i.v. inf. q3w	48	No	19%	12	Neutropenia: 48% Diarrhea: 35% Vomiting: 13%	145
		165	Yes	18%	10	Neutropenia: 47% Diarrhea: 39% Vomiting: 22%	145
	125 mg/m ² , 90 min i.v. inf. qw × 4/6 w	223	No	29%	NR	Neutropenia ^b : 11% Diarrhea: 30% Vomiting: 12%	149
	125 mg/m ² , 90 min i.v. inf. qw × 4/6 w	64	Yes	14%	10.6	Neutropenic fever: 4% Neutropenia: 19% Diarrhea: 33% Vomiting: 22%	146
Small-cell lung cancer	100 mg/m ² , 90 min i.v. inf. qw	35	Yes (27 pts)	37%	NR	NR	147
Non-small-cell lung cancer	100 mg/m ² , 90 min i.v. inf. qw	122	No	21%	11.5	Neutropenia ^b : 8% Diarrhea: 15%	151
		26	Yes	0%	NR	NR	147
Ovarian cancer	100 mg/m ² , qw or 150 mg/m ² , q2w 90 min i.v. inf.	55	Yes	24%	NR	Leucopenia ^c : 87% Diarrhea ^c : 44% Vomiting ^c : 60%	147
Cervical cancer	125 mg/m ² , 90 min i.v. inf. weekly × 4/6 w	42	Yes	21%	6.4	Anorexia ^c : 67% Neutropenia: 36% Diarrhea: 24% Vomiting: 45% Anemia: 62%	147

^a NR, not reported.^b Only grade 4.^c Grade ≥2.

showed a survival advantage for the patients receiving irinotecan as compared with the 5-FU treatment group, with 1-year survival rates of 45% and 32%, respectively ($P < 0.05$; Table 3).

Promising antitumor activity has also been observed against several other types of solid tumors (Table 3). Phase II trials performed in patients with SCLC have shown response rates of 16–33% in patients treated previously and of up to 50% in untreated patients, including some responses in patients with brain metastases (147). Single-agent activity of irinotecan against NSCLC is similar to that reported for several other new chemotherapeutic drugs, such as paclitaxel, docetaxel, gemcitabine, and vinorelbine, with response rates in the 15–32% range (147). Encouraging results have also been reported against gynecological malignancies, including response rates of 14–26% in patients with cervical cancer and 21–30% in patients with ovarian cancer treated previously (147). Finally, modest activity has been reported against gastric cancer (response rate, 20–30%), breast cancer (8–25%), pancreatic cancer (9–11%), and gliomas (5–15%; Ref. 147).

The evaluation of irinotecan against hematological malignancies is limited to a few phase II trials conducted in Japan.

These studies were performed by the same investigators and included a very heterogeneous population of patients with a broad range of tumor types and histologies, from low and intermediate grade non-Hodgkin lymphoma to Hodgkin's disease and acute leukemias. Subgroup analysis revealed a response rate of 42% in patients treated previously with non-Hodgkin lymphoma and of 38% in patients with refractory or relapsed adult T-cell leukemia-lymphoma (147). However, these encouraging results need to be confirmed.

The use of irinotecan together with 5-FU has received considerable interest in that colorectal cancer is the principle indication for both agents. Preliminary results from two phase III trials involving previously untreated patients with metastatic colorectal cancer have shown that the irinotecan/5-FU combination is more effective than either drug given alone in regard to response rate, progression-free survival, and overall survival (148, 149). Randomized trials evaluating the role of this combination in the adjuvant setting are ongoing. Clinical studies to assess the antitumor activity of irinotecan when combined with several other cytotoxic agents that are active against colorectal cancer, such as oxaliplatin or oral fluoropyrimidines, are cur-

rently in progress. Cisplatin is another chemotherapeutic agent that has been extensively evaluated in combination with irinotecan. Results from the initial phase II studies of this combination in patients with SCLC were most encouraging, because objective responses were observed in >80% of treated patients. Early reports of a phase III trial using irinotecan/cisplatin as first-line therapy for patients with extensive-disease SCLC yielded a highly significant improvement in the 1-year survival rate over the standard therapy with etoposide/cisplatin (60% *versus* 40%; $P = 0.005$; Ref. 150). More modest results have been reported for this combination in patients with NSCLC, with response rates in the 30–50% range. Randomized trials performed in Japan have shown that single agent irinotecan is equally effective as the irinotecan/cisplatin or cisplatin/vindesine combinations, when used as first-line therapy against advanced NSCLC (Table 3; Ref. 151). However, none of these multiagent chemotherapy regimens against which single agent irinotecan was compared are currently considered standard of care in Western countries for this patient population. The irinotecan/cisplatin combination is also undergoing phase II testing against other tumor types, including ovarian, cervical, or gastric cancer, among others. Studies to evaluate additional irinotecan drug combinations of potential clinical interest, such as those with taxanes, anthracyclines, *Vinca* alkaloids, or alkylating agents, are in progress. Clinical trials to assess the efficacy of irinotecan administered p.o. or as a prolonged continuous i.v. infusion have been initiated, the results of which are awaited with interest.

Investigational Camptothecin Analogues in Clinical Development

9-AC. 9-AC (Fig. 1; National Cancer Institute, Bethesda, MD) is a semisynthetic camptothecin analogue with potent antitumor activity against a wide spectrum of human tumor xenograft models (152). Preclinical studies found that optimal antitumor efficacy was realized when the 9-AC lactone plasma concentration was maintained above a threshold concentration near 10 nM for at least 72 h (153). Accordingly, the initial phase I studies of 9-AC involved administration by continuous i.v. infusion for durations ranging from 24 h to 21 days (154–156). Subsequently, daily treatment with the drug given as a short i.v. infusion on 5 consecutive days every 3 weeks was also evaluated (157). Pharmacokinetic studies performed during these clinical trials revealed that <10% of the total drug was present in plasma as the active lactone form. This differed markedly from the pharmacokinetic behavior of 9-AC in mice, in which plasma levels of the intact lactone and opened-ring carboxylate forms of the drug were very similar after bolus i.v. injection, such that 9-AC lactone accounted for 62% of the total drug AUC (16). Other pharmacokinetic parameters of the drug in cancer patients are summarized in Table 1 (154, 158–161). The extent of CSF penetration has not been assessed in humans, and it is only 3.5% in nonhuman primates (76). Nevertheless, a phase I trial was performed in patients with malignant gliomas, which indicated that the CL of 9-AC was increased in patients receiving stable regimens of anticonvulsant drugs known to induce CYP450 enzymes (162). This finding was rather unexpected, because there had been no previous evidence suggesting

that 9-AC was subject to significant hepatic metabolism. Biliary excretion appears to be the primary route of drug elimination as suggested by a preclinical study in which 55% of the radioactivity originating from the injection of [^3H]9-AC to mice was recovered in the feces (16). Consistent with preclinical pharmacokinetic studies, urinary excretion of the unchanged drug accounts for approximately one-third of the total dose of 9-AC administered to cancer patients (158).

Regardless of the route and schedule of administration, the most frequently encountered DLT of 9-AC is neutropenia, although thrombocytopenia and diarrhea may also prove to be dose-limiting in a minority of patients (154–161). Other commonly encountered toxicities include nausea and vomiting, mucositis, anemia, fatigue, and alopecia. In phase I studies, there was a relationship between the steady-state plasma concentration or AUC of 9-AC and the degree of myelosuppression (155).

Phase II studies using the 72-h i.v. infusion schedule have been conducted in patients with various types of malignancies with disappointing results. Objective response rates were 25% against non-Hodgkin lymphoma (163), 13% against breast cancer (164), 0% against colon cancer (165), and 9% in patients with NSCLC (166). Dose-limiting hematological toxicity has precluded achieving plasma concentrations of 9-AC lactone in humans comparable with the levels provided by doses affording optimal activity against human tumor xenografts in nude mice (167). However, evidence of antileukemic activity, as indicated by bone marrow hypoplasia, was observed during a phase I trial of 9-AC given as a 7-day continuous i.v. infusion to adults with refractory or relapsed acute leukemias (156).

Phase II clinical trials evaluating the efficacy of the drug given by more prolonged i.v. infusions are presently ongoing. Oral administration is also being evaluated as a more convenient method to provide prolonged systemic exposure to 9-AC than continuous i.v. infusion. Absolute oral bioavailability of the drug formulated as a colloidal dispersion, which had been developed as an alternative parenteral dosage form (154), was only 13% in dogs (168). Similarly, a phase I study of oral 9-AC using the colloidal dispersion formulation was discontinued before identifying the MTD because of poor bioavailability and apparent saturable absorption of the drug (169). Subsequently, a rather interesting solid oral dosage form was developed by incorporating 9-AC into poly(ethylene)glycol-1000, a wax-like material that melts at 39°C, which yielded bioavailabilities of 10% in dogs (168) and 49% in cancer patients (161). Thus, the oral bioavailability of 9-AC in cancer patients is far superior to that observed with any other camptothecin to date and may represent a real clinical advantage of this drug over the other camptothecin analogues. Consistent with this, peak plasma concentrations of 9-AC lactone provided by doses of 0.84 mg/m²/day that are tolerated when administered once daily for 7–14 days every 3 weeks are considerably greater than those achieved with the MTD of the 72-h i.v. infusion schedule (Table 1). Phase II efficacy studies using this dosage form have been initiated in Europe.

9-NC. 9-NC (Fig. 1; SuperGen, San Ramon, CA), a potent but poorly soluble camptothecin analogue, is being developed exclusively for oral administration. Although it undoubtedly has intrinsic cytotoxic and topoisomerase I-inhibiting activities, the compound undergoes pH-dependent metabolic

conversion to 9-AC in blood *in vitro*, with maximal conversion occurring at pH 6.0 (170). 9-NC has proven to be extremely difficult to measure in biological fluids, contributing to a notable paucity of reliable pharmacokinetic data in cancer patients. A pharmacokinetic study performed in healthy volunteers treated with oral 9-NC disclosed that 9-AC total drug plasma concentrations well above the putative therapeutic threshold concentration of 10 nM could be readily achieved (171). However, the AUC of 9-AC was only 12% of the parent drug AUC, implying that the extent of conversion of 9-NC to 9-AC in patients was relatively minor and of questionable significance in contributing to the pharmacological effects of the drug. The absolute bioavailability of 9-NC in humans has not been determined, because the poor solubility of the drug precludes parenteral administration. Notwithstanding, the rate and extent of absorption appears to be comparable with the solid oral dosage form of 9-AC, based on similar plasma concentrations of the parent compound provided by a 1.5 mg/m² dose.

The DLT is myelosuppression when given on a continual basis according to a 5-days on/2-days off weekly regimen (172, 173). Diarrhea and hemorrhagic cystitis are other commonly encountered toxicities (172–174). Promising antitumor activity has been described in a phase II clinical trial in patients with pancreatic carcinoma, with a reported response rate of 32% in 60 evaluable patients with advanced disease treated with single agent 9-NC, including responses in patients with progressive disease despite previous treatment with gemcitabine (175). However, it should be noted that minor responses and disease stabilizations were categorized as response in this study. A subsequent phase II trial using conventional response criteria documented a response rate of only 9% in pancreatic cancer patients treated previously (176). An ambitious phase II program to evaluate the clinical efficacy of the agent against a broad spectrum of tumor types was initiated recently. Given the close similarities in the chemical structure, physicochemical properties, and biological activities of 9-NC and 9-AC, it is difficult to perceive that 9-NC could offer any significant clinical advantage over oral 9-AC.

GI-147211. GI-147211 (GlaxoWellcome, Research Triangle Park, NC), also referred to as GG-211 in the literature, is a water soluble synthetic analogue of camptothecin (Fig. 1). In common with topotecan, this compound has a basic functional group at C-7, which renders it positively charged at physiological pH. Phase I studies have explored several i.v. dosing schedules, including treatment with a short i.v. infusion on 5 consecutive days every 3 weeks, a 72-h i.v. infusion, and continuous i.v. infusion for 21 days (177–181). As indicated in Table 1, pharmacokinetic studies in cancer patients have shown that the intact lactone:total drug AUC ratio seems somewhat greater when the drug is p.o. administered (0.43) than when given by the i.v. route (0.27). The CL of GI-147211 is greater than that of topotecan, which may be a consequence of enhanced tissue distribution as suggested by the substantially greater apparent volume of distribution. Therefore, the terminal phase half-life of GI-147211, which ranges from 5.6 to 9.6 h, is longer than that of topotecan despite its higher CL (Table 1). Renal excretion of unchanged drug accounts for 11–12% of the administered dose, and oral bioavailability is low (11.3%; Table 1).

As observed with other camptothecins, the DLT of GI-

147211 has invariably proven to be myelosuppression. Neutropenia predominates in the repeated daily dosing schedules, and thrombocytopenia becomes more prominent with protracted infusions. Nonhematological toxicities, including nausea and vomiting, fatigue, headache, and alopecia, were mild and relatively infrequent. Preliminary reports on the results of several phase II studies indicate only modest activity in patients with ovarian cancer (182) and NSCLC (183).

Interest in the continued clinical development of this drug eventually declined because the toxicity profile, pharmacokinetic behavior, and spectrum of antitumor activity did not offer any clear advantage over topotecan. Subsequently, a liposomal formulation was identified that markedly prolonged the systemic duration of GI-147211 (184). Preliminary results from a phase I clinical trial indicated that i.v. administration of the liposomally encapsulated drug afforded a 70-fold greater AUC and a 50–150-times lower apparent volume of distribution for total GI-147211 relative to infusion of the free drug (185). This effect on drug disposition is rather remarkable, because a simple unstabilized unilamellar liposome was used. Moreover, tumor responses in patients with ovarian cancer were observed in this phase I clinical trial (185). Continued clinical evaluation of liposomal GI-147211, which has been referred to as NX211 and also liposomal lurotecane (NeXstar Pharmaceuticals, San Dimas, CA), is planned on the basis of these encouraging findings.

Other New Compounds in Clinical Trials. Several additional camptothecin analogues have been introduced recently into phase I clinical trials. These compounds include exatecan mesylate (DX-8951f) and karenitecin, the chemical structures of which are shown in Fig. 1. Exatecan (Daiichi Pharmaceuticals, London, United Kingdom) is a synthetic hexacyclic water-soluble derivative of camptothecin that potently inhibits the growth of human tumor cell lines *in vitro* and tumor xenografts *in vivo*, including tumors resistant to topotecan or irinotecan (186). When evaluated in the National Cancer Institute's *in vitro* anticancer drug screen, analysis using the COMPARE program revealed that exatecan had a spectrum of activity that was similar to that of SN-38, but the antiproliferative effects of exatecan were 6 to 28 times greater than SN-38 or topotecan on a molar basis. Phase I clinical trials have been initiated to evaluate the administration of exatecan according to a variety of schedules, including a 30-min i.v. injection given once every 3 weeks, on a weekly basis, once a day for 5 consecutive days, and by a 24-h continuous i.v. infusion (187). Neutropenia proved to be dose limiting for the single dose and daily times 5 schedules of the 30-min i.v. infusion (187, 188). When given once every 3 weeks, diarrhea was frequently encountered but mild in all of the instances, which may represent a potential clinical benefit over irinotecan (186). The daily times 5 schedule has been selected for additional clinical development based on its superior antitumor activity. These studies and several preliminary reports of phase II clinical trials suggest that exatecan may have significant activity against SCLC and NSCLC (188, 189), and pancreatic carcinoma (190). The pharmacokinetic behavior of exatecan appears to be linear (Refs. 187, 188, 191; Table 1). The C_{max} values achieved with the recommended dose for phase II evaluation of the 30-min i.v. infusion schedules exceed the concentrations required to inhibit tumor cell growth in the human tumor cloning assay (0.01 ng/ml or 23 pM).

Karenitecin (BioNumerik Pharmaceuticals, San Antonio, TX) is a very lipophilic compound that exhibits more potent cytotoxic activity than camptothecin both *in vitro* and *in vivo*. In addition to superior *in vitro* potency, its increased lactone stability and enhanced oral bioavailability are potential clinical advantages (192). In fact, a preliminary report of a phase I clinical trial of karenitecin administered by short i.v. infusion on a daily times 5 schedule in patients with advanced solid tumors showed a very favorable lactone:total drug AUC ratio of 0.87. Despite its greater *in vitro* potency, the MTD achieved in patients (1 mg/m²/day) was not much different from that of topotecan for this administration schedule. Again, myelosuppression was the DLT (192).

Preliminary results of phase I studies of two novel, soluble prodrugs of camptothecin were also reported recently (193, 194). These compounds, PEG-camptothecin (Ref. 193; Enzon Inc., Piscataway, NJ) and MAG-camptothecin (Ref. 194; Pharmacia and Upjohn, Milan, Italy), consist of a high molecular weight water soluble polymer covalently linked to camptothecin through the C-20 hydroxyl group. The presence of the polymeric moiety ostensibly confers water solubility, decreased protein binding, and the potential for tumor-specific drug targeting. Pharmacokinetic studies performed in cancer patients after i.v. administration of these derivatives demonstrated prolonged plasma levels of free camptothecin. However, it remains to be demonstrated whether any of these newer derivatives portend a clear therapeutic advantage over the two camptothecin analogues currently approved for clinical use.

Conclusions

The camptothecins are a maturing class of anticancer agents that have shown significant clinical activity against a broad range of malignancies. A better understanding of their molecular targets and mechanism of action have led to the development of several analogues with improved physicochemical and pharmacological properties. Topotecan and irinotecan are already well-established components in the chemotherapeutic management of several neoplasms. Topotecan has modest but definite activity in previously treated patients with ovarian and SCLC. However, its use in combination with other cytotoxic agents, such as cisplatin, has been limited by hematological toxicity. Ongoing studies will further define the role of topotecan in the treatment of hematological malignancies, particularly chronic myelomonocytic leukemia and myelodysplastic syndromes.

Irinotecan is the most promising of the clinically evaluated camptothecin analogues. It is the only new cytotoxic drug approved for the treatment of colorectal cancer in several decades. Irinotecan is presently the treatment of choice in combination with fluoropyrimidines as first-line therapy for patients with advanced colorectal cancer or as a single agent after 5-FU-based chemotherapy failure. Studies evaluating its role in the adjuvant setting are ongoing. Encouraging results from various phase II studies suggest that irinotecan may have an increasing role in the treatment of other solid tumors, including SCLC and NSCLC, cervical cancer, ovarian cancer, gastric cancer, and malignant gliomas. Experience gained in controlling gastrointestinal toxicity with an intensive loperamide regimen has

greatly improved the tolerability of the drug, making it more suitable for incorporation into multiagent chemotherapy regimens. Recent advances in understanding the influence of genetic polymorphisms in the glucuronyl transferase that acts on SN-38 may facilitate additional optimization of irinotecan therapy through pharmacogenetically guided dose individualization. Although the biotransformation of the camptothecins is not completely understood, hepatic metabolism does appear to contribute significantly to the elimination of these compounds from the body, as suggested by alterations in their pharmacokinetic behavior in patients that are concurrently receiving medications that modulate cytochrome P450 enzymes.

Additional efforts to optimize drug delivery strategies may also help to improve the therapeutic effectiveness of the camptothecins. Concordant with their S phase specificity, preclinical studies have consistently shown better antiproliferative activity with prolonged exposure to drug concentrations above a minimum threshold as compared with shorter exposure to high drug concentrations. Current experience derived from phase II clinical studies suggest that protracted drug administration schedules are at least as effective and less toxic than intermittent administration of higher doses given as short i.v. infusions. Accordingly, dosing regimens that prolong drug exposure such as chronic oral delivery, continuous i.v. infusion, or liposomal encapsulation warrant continued clinical evaluation. Administering chemotherapeutic agents by prolonged continuous infusion on an outpatient basis has become a routine practice. Nevertheless, continuous i.v. infusion suffers from several disadvantages, including inconvenience of the ambulatory infusion devices to the patient and the medical complications associated with central venous catheters, most commonly thrombotic events or infection, the latter being particularly worrisome when dealing with myelosuppressive chemotherapeutic agents. Although oral drug delivery represents a much more convenient method for continuous drug administration and is much preferred by patients, the bioavailability of the camptothecins tends to be relatively low and highly variable. These are undesirable characteristics for any drug that needs to be delivered at or near its MTD because of the potential risk of an accidental overdose if a larger fraction of the dose happens to be absorbed for some reason. Efforts to improve the absorption characteristics of these compounds through structure modification and innovative formulation approaches are being actively pursued. Parenteral liposomal formulations may avoid the necessity for frequent repeated dosing, because drug encapsulation prolongs the duration of the agent in systemic circulation. In addition, liposomal encapsulation has the potential to direct more drug to the tumor and at the same time decrease systemic toxicity associated with the free drug.

Enhancing the therapeutic effectiveness of the camptothecins by combining them with other anticancer drugs and treatment modalities, such as radiation and biological agents, continues to be the focus of considerable interest to clinical investigators. Preclinical studies indicate that appropriate sequencing with drugs that modulate cell cycle checkpoints could greatly enhance camptothecin activity. Another important property that supports the use of camptothecins in combination regimens is that the sensitivity of neoplastic cells appears to be independent of p53 status. Additional insight into the mecha-

nisms responsible for clinical sensitivity and resistance to the camptothecins will certainly benefit efforts to optimize their clinical effectiveness.

Whereas new camptothecin analogues are still being developed, the next generation of topoisomerase I inhibitors, compounds that are not structurally related to camptothecin, are beginning to enter clinical trials. The principal distinguishing characteristic of these compounds is the greater chemical stability of the biologically active entity as compared with the labile lactone ring of the camptothecins. Whether any of these noncamptothecin topoisomerase I inhibitors prove to be more clinically efficacious than topotecan or irinotecan will become evident during the next several years.

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