

REVIEW

Lalitha Iyer · Mark J. Ratain

Clinical pharmacology of camptothecins

Abstract Camptothecins (CPTs) are a unique class of chemotherapeutic agent which inhibit DNA synthesis by inhibiting topoisomerase I activity. Structure-activity studies on the original CPT alkaloid led to the development of the new analogues irinotecan (CPT-11), topotecan, and 9-aminocamptothecin, which have improved water solubility and lower toxicity. CPT analogues exhibit interesting pharmacokinetic/pharmacodynamic and metabolic properties that are of major research and clinical interest. This review describes the clinical pharmacology of these 3 CPT analogues. Specific areas such as absorption after extravascular administration, pharmacokinetic/pharmacodynamic variability, metabolism, and administration in special populations are discussed.

Key words Camptothecins · Irinotecan · Topotecan · 9-Aminocamptothecin · Clinical pharmacology

to isolate the alkaloid and demonstrate its promising anti-neoplastic activity [131]. Despite encouraging results in the initial clinical trials [39, 81] with camptothecin sodium (NSC-100880), its development was hampered by severe toxicities such as hemorrhagic cystitis and diarrhea [78]. Subsequently, there was a renewed interest in the clinical development of CPT as a result of the understanding of the role of topoisomerase I enzyme in its antitumor activity [50, 54] and the synthesis of structural analogues of CPT with greater water solubility and lower toxicities [34, 134, 135]. These analogues, irinotecan, topotecan, 9-aminocamptothecin (9-AC, NSC 603071), 9-nitrocamptothecin (9-NC), and lurtotecan (GI 147211) (Fig. 1), contain structural modifications to the A- and B-rings of CPT. This review focuses on the clinical pharmacology of some of these improved CPTs: irinotecan, 9-AC, and topotecan.

Introduction

Camptothecin (CPT) (Fig. 1) is a cytotoxic alkaloid extracted from the bark, fruit, and leaves of the Chinese tree *Camptotheca acuminata*. Wall and coworkers were the first

Mechanism of action

CPTs inhibit the activity of topoisomerase I and are primarily active in the S-phase of the cell cycle [69, 71]. Topoisomerase I is critical for cell growth and proliferation. It makes a transient single-strand break in the supercoiled DNA duplex resulting in relaxation of supercoiled DNA [2]. CPTs stabilize the covalent cleavable DNA–topoisomerase I complex causing arrest of the replication fork, which in turn results in inhibition of DNA synthesis and ultimately cell death [54, 55].

An intact lactone group in the E-ring of CPT is required for optimal inhibition of topoisomerase I activity [51]: opening of the lactone (closed) form to the hydroxy-acid (open) form leads to a loss of pharmacologic activity. In addition, the (S) stereochemistry and -OH group in position 20 are essential for optimal anticancer activity [51, 134]. Topoisomerase I is overexpressed in certain tumor types, including colorectal and cervical tumors [38, 75], when compared with corresponding nonmalignant cells. This finding has generated a special interest in developing CPT analogues to target tumors such as colorectal cancer.

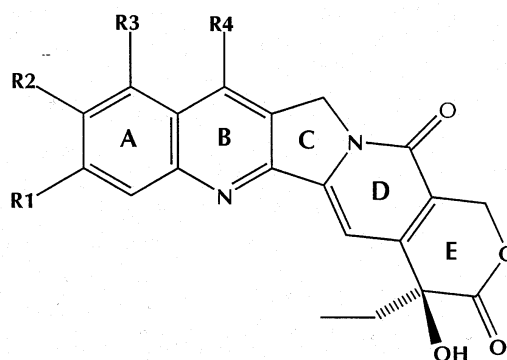
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L. Iyer · M. J. Ratain
Committee on Clinical Pharmacology, University of Chicago,
Chicago, IL, USA

M. J. Ratain
Department of Medicine and Cancer Research Center,
University of Chicago, Chicago, IL, USA

M. J. Ratain (✉)
University of Chicago, 5841 South Maryland Avenue, MC 2115,
Chicago, IL 60637, USA
e-mail: mjratain@mcis.bsd.uchicago.edu
Tel. 1 773 702 4400; Fax: 1 773 702 0963

Fig. 1 Structures of CPT and its analogues irinotecan, topotecan, lurtotecan, 9-AC, and 9-nitro-camptothecin



<u>Compound</u>	<u>R1</u> (C-11)	<u>R2</u> (C-10)	<u>R3</u> (C-9)	<u>R4</u> (C-8)
Camptothecin	H	H	H	H
Irinotecan	H		H	CH ₂ CH ₃
Topotecan	H	OH		H
Lurtotecan		H	H	
9-Aminocamptothecin	H	H	NH ₂	H
9-Nitrocamptothecin	H	H	NO ₂	H

Irinotecan

During the search for water-soluble analogues of CPT, several derivatives with an intact δ -lactone ring were investigated. This led to the development of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11) or irinotecan (Fig. 1) [22]. In preclinical studies, irinotecan exhibited greater antitumor activity than CPT or doxorubicin in a variety of murine tumors, both in ascites and in solid forms, when administered by various routes [4, 68]. Irinotecan also demonstrated significant antitumor activity against colorectal, small cell and non-small cell lung, cervical, breast, and ovarian cancers in human tumor cloning assays [9, 111].

Bioanalytical method

CPT and its analogues undergo reversible hydrolysis from the lactone to the open form at elevated pH (>7.0). As the lactone form is the pharmacologically active entity, efforts have been made to introduce additional steps in the analytical procedure to separate lactone and open forms of irinotecan and its active metabolite 7-ethyl-10-hydroxy camptothecin (SN-38) [94], as well as topotecan [8] and 9-AC [119]. However, these methods include sample treatment with acids to estimate total drug, with the open form subsequently being estimated by subtraction. This can be cumbersome and requires immediate processing of plasma samples. Variations in hydrolysis rates could lead to instability of the lactone forms. In addition, lactone forms of CPT analogues are generally bound to plasma proteins to a greater extent than the corresponding hydroxy acid forms, and there may be variations in free lactone concentrations due to interindividual differences in plasma protein concentrations and lactone-protein binding capacity. If the

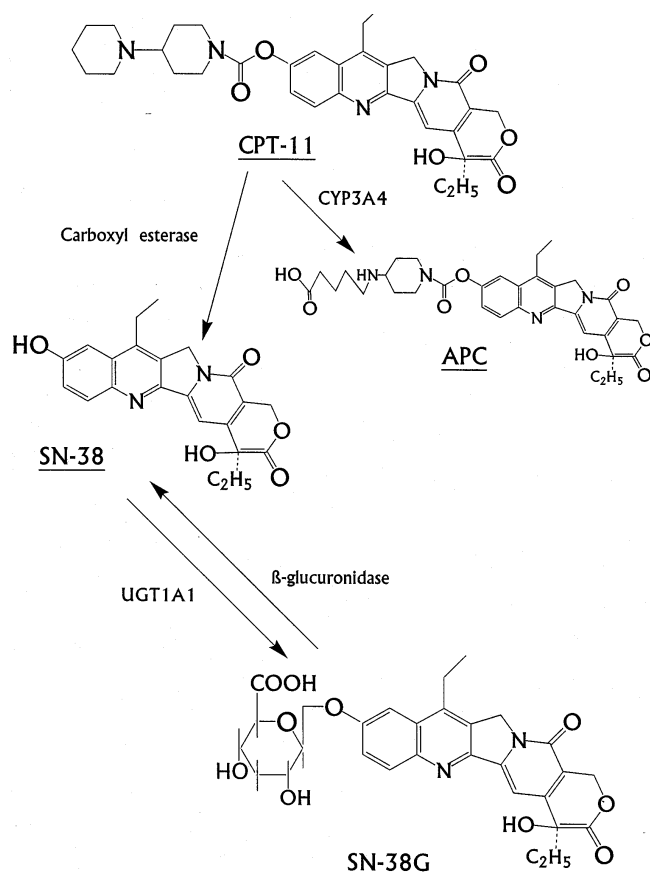


Fig. 2 Metabolism of irinotecan to APC, SN-38, and SN-38G

interconversion between lactone and hydroxy acid forms is predominantly determined by pH, the ratio of free lactone to free open forms must be relatively constant. Hence, quantitation of total concentrations of CPT and its analogues should be adequate for correlation of pharmacokinetics with clinical outcomes. Moreover, certain studies have shown that total drug may be more predictive of drug effect in the case of topotecan [40], and the areas under the curve (AUCs) for total forms correlate significantly with the AUC of lactone forms, as seen with irinotecan and SN-38 [106].

Reverse-phase high-performance liquid chromatography (HPLC) methods coupled with fluorescence detection have been used for the quantitation of irinotecan and its metabolites in biological fluids. Rivory and Robert [94] introduced the use of an ion-pairing agent, tetrabutylammonium phosphate (TBAP), for the simultaneous determination of open and closed forms of irinotecan using fluorescence detection at 355 nm (excitation) and 515 nm (emission). Barilero et al. [5] used a solid-phase extraction method to determine SN-38 and irinotecan simultaneously in plasma samples at 380 nm (excitation) and 500 nm (emission). Most clinical studies have used slight modifications of the above methods, using CPT as the internal standard. For the determination of 10-O-glucuronyl-SN-38 (SN-38G), samples are incubated with β -glucuronidase enzyme, and then subjected to HPLC assay. SN-38G concentrations are then

determined as "SN-38 equivalents" calculated from the increase in SN-38 concentrations due to treatment with β -glucuronidase. SN-38G may also be quantitated directly using fluorescence measurements at 355 nm (excitation) and 515 nm (excitation) [56].

Absorption

The pharmacokinetics of irinotecan after oral administration are being studied extensively [71]. In mice, molar ratios of SN-38 AUC/irinotecan AUC were at least 3-fold greater after oral irinotecan administration compared to equivalent intravenous doses, indicating a higher exposure of SN-38 due to first-pass metabolism [116]. This ratio decreased at higher doses of irinotecan, possibly due to saturation of conversion of irinotecan to SN-38 by carboxylesterase (CE) [116]. Significant grade 4 diarrhea and neutropenia were observed after oral irinotecan 100 mg/m²/day on a daily $\times$ 5 every 3 weeks schedule [32].

Metabolism

Irinotecan undergoes sequential metabolism to SN-38 by tissue and serum CEs and to SN-38G by hepatic uridine diphosphate glucuronosyltransferases (UGTs) (Fig. 2). UGT1A1 has recently been shown to glucuronidate SN-38 to SN-38G [58–60]. K_m values of 0.28 μ M and 4.1 μ M have been reported for CE [122] and UGT [56], respectively.

SN-38 is the active metabolite of irinotecan, with 100- to 1000-fold greater antitumor activity than irinotecan [64, 65]. Recently, an additional metabolite of irinotecan was identified in plasma from irinotecan-treated patients [96]. This metabolite {7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin; APC is formed from irinotecan by cytochrome P450 3A4 (CYP3A4) (Fig. 2) [47]. APC has been shown to be about 500-fold less potent than SN-38 in terms of antitumor activity [96].

SN-38 and SN-38G undergo significant biliary excretion and enterohepatic circulation. SN-38G may be deconjugated to form SN-38 by intestinal β -glucuronidase [61, 118].

The severe diarrheal episodes caused by irinotecan are related to its metabolism, as the active metabolite, SN-38, has been shown to accumulate in the intestine after intraperitoneal irinotecan administration to athymic mice [3]. Gupta et al. [41] have shown that glucuronidation of SN-38 may protect against irinotecan-induced gastrointestinal toxicity. The clinical significance of UGT1A1 polymorphism in the metabolism of irinotecan is discussed below.

Pharmacokinetics

Irinotecan entered clinical trials in the USA in 1991 after undergoing some clinical investigation in Japan (1986) and France (1990) [43, 97, 98, 100]. Most trials have used the continuous infusion (CI) route at weekly intervals for 4

Table 1 Pharmacokinetic parameters of irinotecan (total) from major clinical trials

Dose (mg/m ²)	Schedule	<i>n</i>	Terminal <i>t</i> _{1/2} (h)	<i>C</i> _{max} (μg/ml)	Cl _{sys} (l/h/m ²)	<i>V</i> _{dss} (l/m ²)	Reference
50–150	90-min i.v. infusion, weekly	17	2.8 (1.5–4.3)	0.61–2.97	16.75 (13.3–21.5)	–	82
25–40	5-day i.v. infusion	24	26.55 (16.1–37.7)	0.12–0.20	–	–	83
50–180	30–90-min i.v. infusion, weekly × 4, every 6 weeks	17	7.9 ± 2.8	0.89–1.97	15.3 ± 3.5	–	99
50–145	30–90-min i.v. infusion, weekly × 3, every 5 weeks	47	9.3 ± 0.5	0.67–2.58	15 ± 0.8	–	31
100–345	90-min i.v. infusion, every 3 weeks	31	5.2	1.8–4.5 μmol/l	0.35 ± 0.03	148 ± 20	102
100–750	30-min i.v. infusion, every 3 weeks	64	14.2 ± 0.9	2.3–17.3	15 ± 1.0	157 ± 8.0	1
100	90-min i.v. infusion, weekly × 4, every 6 weeks	36	7.2	0.74–2.31	–	–	105
145	90-min i.v. infusion, weekly × 4, every 6 weeks	40	8.8 ± 4.3	0.83–3.58	14.6 ± 6.4	136 ± 73.9	46

Table 2 Pharmacokinetic parameters of SN-38 (total) from major clinical trials

Dose (mg/m ²)	Schedule	<i>n</i>	Terminal <i>t</i> _{1/2} (h)	<i>C</i> _{max} (μg/ml)	AUC (μg.h/ml)	Reference
50–150	90-min i.v. infusion, weekly	17	6.75 (3.0–10.2)	0.018–0.032	0.12–0.35	82
25–40	5-day i.v. infusion	24	29.6 (24 ± 39)	0.007–0.01	0.86–1.08	83
50–180	90-min i.v. infusion, weekly × 4, every 6 weeks	17	13 ± 5.8	0.26–0.039	0.215–0.449	99
50–145	30–90-min i.v. infusion, weekly × 3, every 5 weeks	47	7.7 ± 0.9	0.01–0.075	0.036–0.443	31
100–345	90-min i.v. infusion, every 3 weeks	31	5.9	75–191 μmol/l	51 ± 3 μmol-min/l	102
100–750	30-min i.v. infusion, every 3 weeks	64	13.8 ± 1.4	0.032–0.299	0.160–2.370	1
100	90-min i.v. infusion, weekly × 4, every 6 weeks	36	10.5	0.01–0.07	0.10–0.35	105
145	90-min i.v. infusion, weekly × 4, every 6 weeks	40	11.6 ± 8.2	0.013–0.060	0.053–2.422	46

weeks followed by a rest period of 2 weeks, while other trials have used a once every 3 weeks dosage schedule (Table 1). The various dosage schedules and pharmacokinetic parameter estimates of irinotecan and SN-38 determined in major clinical trials are listed in Tables 1 and 2, respectively.

Terminal elimination half-life (*t*_{1/2}) values of about 5–14 h have been reported for irinotecan in clinical trials using the 30- or 90-min CI schedules. When infused continuously for 5 days [83] a much higher *t*_{1/2} of about 27 h was obtained. Attempts have been made to fit the plasma irinotecan concentration-time data to 2- and 3-compartment models. For example, Rowinsky et al. [104] determined α and β half-lives of 4.9 min and 3.9 h, respectively, after fitting the data to a 2-compartment model equation. A triexponential model was described by de Forni et al. [31] and α , β , and γ half-lives of 6.7 min, 2 h, and 9.3 h, respectively, were obtained. The large volumes of distribution (> 140 l/m²) reported for irinotecan (Table 1) indicate significant binding to the tissue compartment.

There is considerable variation in the half-lives obtained for SN-38 (Table 2), perhaps due to the significant variability in its rate of glucuronidation. In general, SN-38 *C*_{max}

is >100-fold lower than the corresponding values for irinotecan. One study has reported a statistically significant correlation between the irinotecan AUC and the corresponding SN-38 AUC [31]. Plasma concentrations of SN-38G were higher than corresponding concentrations of SN-38 in these studies [41, 95], with SN-38G AUCs being at least 10-fold higher than those for SN-38.

The dose dependency of irinotecan clearance is unclear because conflicting data have been published. Dose escalation from 100 to 750 mg/m² resulted in a >50-fold reduction in CPT clearance from 26 to 12 l/m²/h in one study [1]. Decreases in clearance estimates have also been reported by Negoro et al. [84] (0.35 to 0.22 l/m²/h) and by Gupta et al. [41] (20 to 13 l/m²/h). Several other studies have reported constant rates of clearance over a 4-fold irinotecan dose range [31, 99, 102]. Irinotecan has been reported to exhibit nonlinear pharmacokinetics in rats [62]. The nonlinearity in irinotecan disposition and the conflicting literature reports are not surprising, and saturation of irinotecan metabolic pathways and biliary transport mechanisms may explain these findings.

Modulation of irinotecan pharmacokinetics by coadministration of cyclosporine A (CSA) was studied in rats by

Table 3 Major toxicities of irinotecan reported at MTD/RPTD in clinical trials (MTD maximum tolerated dose, RPTD recommended phase II dose)

Dose (mg/m ²)	Schedule	MTD/RPTD (mg/m ²)	Grade 3–4 diarrhea (%)	Grade 3–4 neutropenia (%)	Grade 3–4 leukopenia (%)	Reference
50–150	90-min i.v. infusion, weekly	100	25.0	–	–	82
25–40	5-day i.v. infusion	40	66.7	–	33.3	83
50–180	90-min i.v. infusion, weekly × 4, every 6 weeks	150	16.7	16.7	–	99
50–145	30–90-min i.v. infusion, weekly × 3, every 5 weeks	145	57.1	14.2	57.1	31
100–345	90-min i.v. infusion, every 3 weeks	240	7.7	23.1	–	102
100–750	30-min i.v. infusion, every 3 weeks	600	14.2	14.2	14.2	1
100	90-min i.v. infusion, weekly × 4, every 6 weeks	100	22.2	–	13.8	105

Gupta et al. [42]. In these studies, clearance of irinotecan and its metabolites was reduced due to CSA-induced decreased biliary excretion. Based upon these results, a phase I trial of irinotecan administered with CSA has been initiated at the University of Chicago [35]. Preadministration of CSA resulted in a 30% decrease in plasma clearance of irinotecan at a 100 mg/m² dose administered weekly for 4 weeks. The maximum tolerated dose (MTD) was determined to be 60 mg/m², with leukopenia being the dose-limiting toxicity [Fagbemi S et al., unpublished results].

Considerable interpatient variability in irinotecan and SN-38 pharmacokinetics and toxicity is characteristic of irinotecan therapy. This could largely be due to pharmacogenetic differences between individuals in the metabolism of irinotecan. Our recent finding that UGT1A1 glucuronidates SN-38 is a major breakthrough in understanding these variations [L. Iyer et al., unpublished results]. SN-38 glucuronidation may be pharmacologically manipulated. For example, in a recent study, irinotecan administration in rats pretreated with valproic acid and phenobarbital resulted in inhibition and induction of SN-38G formation, respectively [45].

Pharmacogenetic differences in UGT1A1 expression and activity may lead to variations in SN-38 glucuronidation rates and variations in irinotecan pharmacokinetics and toxicity. UGT1A1 is also responsible for bilirubin glucuronidation in humans [14], and is absent in individuals with Crigler-Najjar type I (CN-I) syndrome [13, 28]. Patients exhibiting UGT1A1 polymorphism, such as those with Gilbert's syndrome [37, 79], may be at increased risk for irinotecan-induced gastrointestinal toxicity [133]. The specific molecular defect in Gilbert's syndrome is the presence of a A(TA)₇TAA fragment in the UGT1A1 promoter instead of the A(TA)₆TAA segment that is present in healthy individuals [15, 79]. There appears to be wide variation in the incidence of Gilbert's syndrome, ranging from 0.5% to 15% in various groups. A genotyping test has recently been developed to identify polymorphisms in the promoter region of UGT1A1 [79; L. Iyer et al., unpublished results]. Preliminary results from in vitro metabolism studies indicate a significant correlation between SN-38 glucuronidation rates and UGT1A1 genotype [L. Iyer et al.,

unpublished results]. The incidence of A(TA)₇TAA homozygotes in the donor livers tested ($n = 29$) was 14%. These findings may enable the individualization of irinotecan therapy using pharmacogenetically derived doses, which may improve the narrow therapeutic index of this agent.

Pharmacodynamics and toxicity

The major dose-limiting toxicities of irinotecan are diarrhea and myelosuppression. Other toxicities reported include nausea, emesis, alopecia, asthenia, abdominal pain, and anemia [31]. Table 3 lists the incidence of diarrhea, leukopenia, and neutropenia at the MTD or recommended phase II dose (RPTD) determined in some of the major clinical trials.

Irinotecan-induced diarrhea usually occurs in 2 stages: an early stage which is due to a cholinergic syndrome produced by CPTs by inhibition of acetylcholine esterase [66]; and delayed diarrhea which is hypothesized to be due to direct enteric injury caused by SN-38 and irinotecan [3]. There appears to be a dose-dependent increase in the incidence of diarrhea, as 34%, 50%, and 100% of patients developed grade 3–4 diarrhea at dose levels of 100 mg/m², 125 mg/m², and 175 mg/m² [41]. Gupta et al. [41] introduced a surrogate measure of biliary SN-38 excretion, known as the biliary index (BI), which is determined from $AUC_{\text{irinotecan}} \times AUC_{\text{SN-38}} / AUC_{\text{SN-38G}}$. A significant correlation was found between the BI and severity of diarrhea in irinotecan-treated patients.

Several investigators have used pharmacodynamic models to relate irinotecan pharmacokinetic parameters with toxicities [42]. Sigmoidal E_{max} models have been used to describe relationships between percentage decreases in absolute neutrophil counts and diarrhea versus irinotecan and SN-38 AUCs [1, 23, 102]. Other investigators have used linear models to describe these relationships [31, 105].

Limited sampling models

Limited sampling models (LSMs) have been used to estimate pharmacokinetic parameters and the toxicity of irinotecan and SN-38 [21, 76, 107]. Sasaki et al. [107] used 2 sampling points at 2.5 h and 13.5 h to predict the irinotecan and SN-38 AUC accurately. Other investigators have used a trivariate model based on sampling times of 0.5 h, 1 h, and 6 h to predict the SN-38 and irinotecan AUC [21]. Mick et al. [76] have used LSMs with time points of 3.0 h and 11.5 h, 3.5 h and 11.5 h, and 9.5 h and 11.5 h to estimate the AUC for irinotecan, SN-38, and SN-38G adequately.

Using a Bayesian algorithm, Yamamoto et al. [137] have recently described LSMs using sampling times of 4 and 8 h after irinotecan infusion on days 1 and 3. In this study, irinotecan (60 mg/m²) was administered as a 90-min CI after etoposide 60 mg/m² infusion for 60 min for 3 consecutive days. The irinotecan AUC correlated significantly with degree of leukopenia, neutropenia, and nausea/vomiting, but not with severity of diarrhea or response.

Special populations

Vassal et al. [128] have recently established the efficacy of irinotecan in peripheral primitive neuroectodermal tumor (pPNET) and neuroblastoma xenografts in mice. This finding indicates that irinotecan may be an interesting new drug in pediatric oncology because neuroblastomas account for 6–8% of all malignancies in children [128]. Irinotecan also produced significant growth delays in human tumor xenografts derived from pediatric central nervous system malignancies, such as gliomas, medulloblastomas, and ependymomas, transplanted into mice [48]. No major differences in pharmacokinetic parameters of irinotecan have been reported between colorectal cancer patients ≥ 65 and < 65 years of age [108]. No significant gender or race (Caucasian vs black) differences have been reported for irinotecan pharmacokinetics or toxicity [92].

9-AC

9-AC has an amino group substituted in position C-9 in the A-ring of CPT (Fig. 1). It has demonstrated high antitumor activity against lung, breast, and melanoma xenografts, hepatic metastases of primary colon tumors, and intraperitoneally implanted leukemias and sarcomas [80, 86–89, 135]. 9-AC has been shown to induce disease-free remissions in mice with colon cancer xenografts [38].

The major disadvantage with 9-AC relates to its formulation: it is the most poorly water-soluble analogue of CPT. 9-AC was initially formulated in dimethylacetamide (DMA) using a special diluent (NSC 651935) consisting of 51% polyethylene glycol 400 and 49% 0.01 M phosphoric acid [24]. Due to incompatibility with aqueous solutions and adherence to plastic surfaces, a new lipid colloidal

dispersion (CD) formulation of 9-AC in dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol has recently been developed which has a particle size of $< 3 \mu\text{m}$ and is compatible with aqueous solutions and more stable [70]. More recent clinical trials have been employing the CD formulation of 9-AC [33, 73].

Bioanalytical method

Measurement of 9-AC in biological fluids is usually performed using reverse-phase HPLC separation and fluorescence detection [117, 119]. Supko and Malspeis [117] introduced a method to improve the relatively poor fluorescence of 9-AC lactone (in comparison with CPT) using postcolumn acidification with trifluoroacetic acid, with a lower limit of quantification of 13 nm in human plasma samples. Takimoto et al. [119] have introduced a solid-phase extraction method to separate the 9-AC lactone form from the carboxylate form which has an improved sensitivity of 0.25 nm.

Metabolism

The results from clinical studies such as the CI studies in humans indicate no major metabolism of 9-AC [29]. No glucuronide formation, similar to SN-38, was detected by incubating plasma samples from 9-AC-treated patients with β -glucuronidase [120]. However, systematic studies designed to investigate possible 9-AC metabolism have not been performed *in vitro* using hepatic cellular or subcellular fractions.

Pharmacokinetics and toxicity

The DMA formulation of 9-AC was administered as a 72-h continuous intravenous infusion in a phase I clinical trial [103] at doses of 5 to 60 $\mu\text{g}/\text{m}^2/\text{h}$ ($n = 31$). A terminal $t_{1/2}$ of about 36 h was determined at the 45 and 60 $\mu\text{g}/\text{m}^2/\text{h}$ doses, indicating a longer half-life than that of the more water-soluble CPT analogues irinotecan and topotecan. Significant variations in plasma 9-AC lactone levels and volumes of distribution were noted; these were attributed to possible interindividual differences in protein binding [103]. Minimal gastrointestinal toxicities were reported in this trial. Neutropenia was the dose-limiting toxicity and the MTD was 45 $\mu\text{g}/\text{m}^2/\text{h}$. Minor responses were observed in 3 patients.

Results from another ongoing phase I 9-AC (72-h CI) clinical trial from the Harvard/Phase I Oncology program [33] suggest at least a 20% increase in MTD for the CD formulation of 9-AC. In addition, a shorter $t_{1/2}$ of about 14 h was found in this study, which may be due to the use of the revised assay methodology [119] and is in agreement with results from other investigations [29].

The clinical pharmacology of 9-AC administered as a 72-h CI every 14 days in 48 patients at a dose range of 5 to

47 $\mu\text{g}/\text{m}^2/\text{h}$ was studied by Takimoto et al. [120, 121]. Only about 10% of total plasma 9-AC circulated in the active lactone form, which is lower than that reported for SN-38 and topotecan. Total 9-AC clearance increased more than 2-fold at higher doses, indicating nonlinear pharmacokinetics [121]. However, clearance of 9-AC lactone was uniform with increasing doses (24.5 ± 7.3 $\text{l}/\text{h}/\text{m}^2$) [29]; terminal $t_{1/2}$ values of 11.1 ± 4.5 h were determined in this study using the DMA formulation of 9-AC. Dose-limiting myelosuppression was observed, with 9-AC doses of 35 $\mu\text{g}/\text{m}^2/\text{h}$ without G-CSF or 47 $\mu\text{g}/\text{m}^2/\text{h}$ with G-CSF support being recommended for phase II studies [29]. Repeated administration of 9-AC in 3 patients showed no evidence of drug accumulation after multiple cycles of 2 weeks each [121].

Sixteen patients were treated with oral 9-AC (CD formulation) at the University of Chicago using doses of 0.2 to 0.68 $\text{mg}/\text{m}^2/\text{day}$ for 5 days repeated every other week [73]. There was significant interpatient variability in C_{max} and $\text{AUC}_{0-\infty}$ at each dose level, and these parameters exhibited poor correlations with dose ($r^2 = 0.57$ and $r^2 = 0.34$, respectively). Fatigue and anorexia were the most common side effects noted. This study was discontinued for ethical reasons as the formulation was considered unsuitable for further clinical development. The considerable intersubject variations in 9-AC pharmacokinetics and low 9-AC exposure after oral administration may have been due to a combination of factors such as saturable absorption and/or elimination, polymorphism in gut enzymes leading to differential formation of any unknown metabolites of 9-AC, and differences in protein-binding capacities. Newer formulations such as soft gelatin capsules are being developed for oral administration of 9-AC [114]. Preliminary results indicate oral bioavailability of 27–49% using this formulation [114].

Pharmacodynamics

Pharmacodynamic analysis in the 72-h CI study [121] indicated a strong correlation between dose-limiting neutropenia and steady-state plasma 9-AC lactone concentrations (C_{ss}) ($r^2 = 0.77$) and 9-AC dose administered ($r^2 = 0.71$). The relationship between degree of hematologic toxicity and C_{ss} values was best described using a sigmoidal E_{max} model [121]. Weaker correlations were obtained between C_{ss} (9-AC lactone) and thrombocytopenia ($r^2 = 0.36$). Total bilirubin, albumin, and age were also found to be predictive of neutropenia and thrombocytopenia in a sigmoidal E_{max} model developed by Minami et al. [77]. 9-AC concentrations were not significantly better than dose in predicting toxicity in this study.

Special populations

The fate of nonrenally cleared 9-AC is unknown. If most 9-AC is renally cleared, it is logical to expect that dose reductions will be necessary in patients with compromised

renal function, although clinical studies addressing this issue have not yet been designed. In the phase I clinical studies by Takimoto et al. [121], there was no correlation between either renal or hepatic function and any pharmacokinetic or pharmacodynamic parameter, although the sample size was small due to the exclusion of patients with severe organ dysfunction from this study. Age and gender differences in the clinical pharmacology of 9-AC have not yet been determined.

Topotecan

Topotecan [(S)-9-dimethyl-aminomethyl-10-hydroxy-camptothecin hydrochloride] has a stable basic chain (dimethyl-aminomethyl) substituted in the 9th position of the A-ring of camptothecin (Fig. 1) [7, 26, 49, 60]. Topotecan exhibited activity against tumor xenografts in mice as well as significant in vitro activity against breast, colon, kidney, lung, and ovarian tumor colony-forming units [18, 130]. It has now been approved in the USA for clinical use as second-line therapy in metastatic ovarian cancer [6].

Bioanalytical method

Similar to irinotecan, SN-38, and 9-AC, topotecan concentrations are measured in biological fluids using a reverse-phase HPLC assay method. Beijnen and coworkers [8] developed a method to deproteinize plasma with cold methanol, which stabilizes the lactone and acid forms of topotecan. Fluorescence detection at 381 nm (excitation) and 527 nm (emission) was used after HPLC separation, with a lower limit of quantitation of 0.05 ng/ml . Other investigators have utilized solid-phase extraction to isolate the lactone form, followed by acidification to measure total topotecan [43].

Absorption

The bioavailability and efficacy of oral administration of topotecan have been studied extensively [16, 20, 27, 67, 74, 110]. Using murine tumor models, McCabe and Johnson [74] demonstrated an oral topotecan bioavailability of 28%, using AUC values from intraperitoneal topotecan administration for comparison. In this study, oral topotecan was as effective as parenteral topotecan in inhibiting tumor growth and was tolerated at about 1.7-fold the MTD obtained after parenteral administration. Oral topotecan had a bioavailability of 44% in humans and exhibited efficacy comparable to that of topotecan administered by continuous intravenous infusion [67]. Schellens et al. [110] have reported an oral bioavailability of 21–45% after single-dose (1.5 mg/m^2) administration in humans. However, the same group had earlier reported significant diarrhea and myelosuppression after chronic oral dosing of topotecan at doses of 0.4–0.6 mg/m^2 , indicating significant differences in safety

Table 4 Pharmacokinetic parameters of topotecan (total) from major clinical trials

Dose (mg/m ²)	Schedule	n	Terminal t _{1/2} (h)	C _{max} (ng/ml)	Cl _{sys} (l/h/m ²)	Vd _{ss} (l/m ²)	Reference
2.5–22.5	30-min i.v. infusion, every 3 weeks	17	4.3 ± 1.8	185–778	8.0 ± 3.1	40 ± 1.8	132
0.5–2.5	30-min i.v. infusion, daily × 5, every 3 weeks	12	2.9 ± 0.7	–	30 ± 11	25 ± 19	40
1.25–1.5	30-min i.v. infusion, daily × 5	10	3.4*	59–65*	21*	84.5*	104
1.2–2.0	24-h i.v. infusion, weekly	21	3.5	3.7–6.7	14.0	–	46
0.5–1.5	24-h i.v. infusion, daily × 5, every 3 weeks	34	2.25	33.9–73.4 ^a	33	72.68	126

* Median value

^a nM

profiles between single-dose and repetitive oral dosing of topotecan [109].

Compared with other camp to the cins, topotecan has also shown measurable penetration into the cerebrospinal fluid (>30%) in nonhuman primates, and therefore has been suggested as a potential alternative in refractory meningeal tumors [11, 12]. Intraperitoneal administration has been suggested for the treatment of ovarian cancer confined to the peritoneal cavity due to its promising antitumor activity in mice with ovarian carcinoma xenografts [91].

Metabolism

No metabolites of topotecan have been identified to date, although some demethylation has been suggested to occur [49, 93]. There is speculation that topotecan may undergo (CYP 3A-catalyzed metabolism [49], as its clearance is altered significantly by coadministration of CYP3A inhibitors and inducers in pediatric patients [115]. Topotecan has been reported to be excreted unchanged into the urine in amounts ranging from 25% to 90% of the administered dose depending upon the dosing schedule [49, 104, 129]. Despite large urinary excretion, topotecan, unlike CPT, does not produce urinary toxicity due to its greater water solubility and consequent lack of precipitation in urine [49]. There also appears to be some biliary excretion of topotecan, with peak biliary concentrations being about 1.5-fold greater than corresponding plasma concentrations [132].

Pharmacokinetics

The pharmacokinetics of topotecan have been studied in humans using various infusion schedules. Table 4 lists the pharmacokinetic data obtained from clinical trials. Topotecan has a relatively short half-life (2 to 4 h) compared with irinotecan and 9-AC. Longer infusions for 3, 5, and 21 days were designed to increase the t_{1/2} of topotecan and to achieve a higher dose intensity [19, 52, 63], but resulted in little influence on the dispositional characteristics of topotecan [43].

Steady-state and maximal concentrations of topotecan and AUC values correlate linearly with dosage, indicating linear pharmacokinetics [10, 49, 52, 126, 132]. The ratio of lactone to total topotecan AUC was also relatively constant (about 0.3) over a dose range of 2.5 to 22.5 mg/m² [49, 132]. However, nonlinear increases in C_{ss} and AUC values have been reported in a phase I study of topotecan administered as a 24-h CI [125]. No significant accumulation of topotecan was observed during 21-day CI [49] or 30-min infusion for 5 days [49, 101, 104, 126].

Steady-state volumes of distribution of topotecan range from about 25 to 75 l/m², indicating extensive binding to tissue components (Table 4) [49]. Relatively less plasma protein binding (21%) has been reported for topotecan in humans [132] compared with 9-AC, irinotecan, and SN-38. Erythrocytes act as a depot for topotecan (lactone), with C_{ss} values almost 1.7-fold those obtained in plasma [25]. The total body clearance of total topotecan from plasma ranged from 8 to 33 l/h/m² (Table 4), and has been reported to be independent of the administered dose [101, 126].

Pharmacodynamics and toxicity

The major dose-limiting toxicities of topotecan are neutropenia and to a lesser extent thrombocytopenia [17, 26, 131]. Reported nonhematologic toxicities include nausea (12%), vomiting (10%), fatigue (3%), and alopecia (11%) [26].

Pharmacokinetic/pharmacodynamic models have been used to predict hematological toxicities using pharmacokinetic measurements. Grochow et al. [40] have used a sigmoidal E_{max} model to relate change in absolute neutrophil count to topotecan dosage, using a 30-min intravenous infusion for 5 days. A dose of 0.9 mg/m²/day was found to produce a 50% decrease in absolute neutrophil count. The sigmoidal E_{max} model could also predict changes in neutropenia in patients who received topotecan dose escalations or reductions [40, 101], and indicated a lack of cumulative toxicity. This was confirmed by the results from a 24-h CI once-weekly dosing schedule, which showed a correlation between total and lactone AUC and C_{new} Ha values and decrease in absolute neutrophil counts on days 15, 22, and 29 [46]. The derived pharmacodynamic parameters from sigmoidal E_{max} models on these days were

predictive and suggested a lack of alteration of topotecan pharmacokinetics after repetitive dosing. Other studies that have adequately predicted topotecan toxicity using sigmoidal $E_{\max}$ models include the daily $\times 5$, 30-min CI for 3 weeks study [126], the 24-h CI study [125], and the 72-h CI study in children [115]. In general, total topotecan AUC and C_{ss} were better predictors of hematological toxicity than lactone AUC and C_{ss} [49].

LSMs

LSMs have been developed to describe the pharmacokinetics/pharmacodynamics of topotecan. These allow reliable estimation of topotecan AUC and clearance values based upon a single plasma sampling time of 2 h after a 30-min intravenous infusion [124, 127].

Special populations

Topotecan exhibits significant growth inhibitory activity in pediatric xenograft models [37, 54]. Encouraged by these results, clinical trials have been conducted to evaluate the use of topotecan in children [90, 115, 123]. A dose of 1 mg/m²/day (72-h CI) with G-CSF support has been recommended for phase II trials in children [90], with myelosuppression being the dose-limiting toxicity [90, 123]. Significantly higher levels of urinary drug recovery have been reported in children compared to adults, the mechanism for which is unclear. Prior therapy with nephrotoxic agents has been proposed to be a possible reason [115].

The safety of topotecan administration in the presence of renal and hepatic dysfunction has also been studied [84, 85, 112]. Dose adjustments have been suggested for patients with moderate renal dysfunction (creatinine clearance of 20–39 ml/min), with recommended starting doses of 0.75 mg/m²/day for 5 days every 3 weeks [84]. Topotecan disposition and toxicity were not altered in patients with impaired hepatic function when the drug was administered at daily $\times 5$ doses of 1.5 mg/m²/day [85].

Conclusions

CPTs are a classic example of the impact of traditional medicine on modern therapeutics. CPT and its analogues have exhibited promising anticancer activity in a variety of tumors and are undergoing extensive clinical development [30, 113, 136], with the result that 2 CPT analogues (irinotecan and topotecan) have already received Food and Drug Administration approval for use in colorectal and ovarian cancers in the USA.

Many issues regarding the clinical use of CPTs are unclear, including the significant variability in pharmacokinetics, toxicity, pharmacodynamic relationships, and mechanisms of drug interactions. Investigations are now

focusing on the development of different dosing schedules and coadministration with pharmacokinetic modulators that may improve the efficacy and decrease the toxicity of CPTs. There is also considerable interest in the development of oral and extravascular formulations of CPTs, which have the advantages of more convenient administration and prolonged exposure. Recent advances in our understanding of the role of metabolic enzyme polymorphism in the disposition of irinotecan are expected to lead to more individualized dosing of irinotecan in the future. The use of CPT analogues in patients with renal and hepatic dysfunction, and in elderly and pediatric populations, needs to be more clearly delineated. Combination therapy with CPTs and other chemotherapeutic agents, such as cisplatin, as well as with radiation treatment is being investigated.

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