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EDITORIAL

Current perspectives on camptothecins in cancer treatment

J Dancey and EA Eisenhauer

National Cancer Institute (Canada) Clinical Trials Group, Queen's University, 82-84 Barrie Street, Kingston, Ontario, Canada K76 3N6.

Summary The camptothecins are a new class of chemotherapeutic agents which have a novel mechanism of action targeting the nuclear enzyme topoisomerase I. Knowledge of the structure-activity relationships of the parent compound camptothecin has led to the development of effective soluble analogues with manageable toxicities. Broad anti-tumour activity shown in preclinical studies has been confirmed in phase I/II studies for irinotecan and topotecan. Two other derivatives, 9-aminocamptothecin and GI 147211C, are undergoing phase I and early phase II evaluation. Although camptothecin is a plant extract, it and most of its derivatives are not affected by the classic P-gp^{MDR1} mechanism of resistance which may allow the development of novel combination chemotherapeutic regimens. Important areas of future endeavour will include the development of rational combination regimens and the pursuit of randomised trials. Based on single agent data, colorectal cancer and non-small-cell lung cancer should be the focus for future irinotecan studies. Small-cell lung cancer and ovarian carcinoma are logical tumour types to pursue with topotecan. Both 9-aminocamptothecin and GI 147211C are too early in their clinical evaluation to make recommendations about their future roles. Finally, the unfolding story of camptothecin analogue development will give important insights into the predictive value of preclinical observations on relative efficacy, schedule dependency, combination strategies and resistance mechanisms which have helped determine the strategies for clinical evaluation of these agents.

Keywords: camptothecin; irinotecan; 9-aminocamptothecin; topoisomerase I inhibitor; topotecan

More than 30 years ago, an extract from the Chinese tree *Camptotheca acuminata* was found to have anti-tumour activity in experimental systems (Wall *et al.*, 1966). The active compound, camptothecin, being insoluble in aqueous solution, was modified and its water-soluble sodium salt was evaluated in clinical studies in the late 1960s and early 1970s. Leucopenia and thrombocytopenia were dose-limiting toxicities (Muggia *et al.*, 1972). Despite promising anti-tumour activity in phase I studies, results in phase II trials in patients with gastrointestinal malignancies (Moertel *et al.*, 1972) and melanoma (Gottlieb, 1972) indicated the drug was ineffective and highly toxic. Myelosuppression, vomiting, diarrhoea and sterile haemorrhagic cystitis were often severe and, as a result, further clinical testing of camptothecin ceased.

Several developments in the late 1980s renewed interest in camptothecin: topoisomerase I was identified as the cellular target of the drug (Hsiang *et al.*, 1988); the structure-activity relationship was determined for camptothecin (Jaxel *et al.*, 1989), leading to the development of effective, water-soluble synthetic and semisynthetic derivatives (Wall *et al.*, 1993); and topoisomerase I levels were found to be higher in some tumour tissues compared with the normal tissue counterpart (Giovanella *et al.*, 1989; Van der Zee *et al.*, 1991). Currently camptothecin and four analogues: topotecan, irinotecan (CPT-11), 9-aminocamptothecin and GI 147211C (GG211) are undergoing clinical evaluation. During the past 25 years, knowledge of topoisomerase biochemistry, genetics, molecular biology and interaction with inhibitors has increased exponentially and these will be reviewed with the results of preclinical and clinical evaluations of camptothecin and its derivatives.

DNA topoisomerase I

Topoisomerases are nuclear enzymes that modulate the three-dimensional structure of DNA by inducing transient breaks that allow unwinding of supercoiled DNA (reviewed in Pommier, 1993). Topoisomerase I is a 100 000 kDa protein which relaxes positive and negative supercoils of DNA arising during DNA and RNA synthesis by making transient single-stranded breaks in DNA (Champoux, 1976). Intense research has clarified the camptothecin-topoisomerase I-DNA interaction. The drug binds to and stabilises the topoisomerase I enzyme-DNA cleavable complex after DNA cleavage preventing resealing of DNA and causing an accumulation of cleavable complexes (Hsiang *et al.*, 1988, 1989). The subsequent interaction between the advancing replication fork of DNA and the drug-stabilised cleavable complex results in an arrest of DNA replication with formation of double-strand breaks. These in turn activate endonucleases, triggering further DNA fragmentation and ultimately cell death (Zhang *et al.*, 1990). Thus, cytotoxicity is dependent on the expression of topoisomerase I and on DNA replication. Compared with the levels of the enzyme in normal tissues, a significant increase of topoisomerase I has been detected in surgical specimens of colon adenocarcinoma, ovarian and oesophageal carcinoma, in cultures of non-Hodgkin's lymphoma and leukaemia cells and in xenograft lines of human colon adenocarcinoma, carcinoma of the stomach, breast, lung and malignant melanoma (Potmesil, 1994). Cell lines which have high levels of enzyme are hypersensitive to camptothecin-induced cytotoxicity (Madden, 1992). Conversely, cell lines resistant to camptothecin may contain qualitatively or quantitatively altered forms of the target enzyme (Pommier, 1993). Although topoisomerase I is expressed throughout the cell cycle, cells in S-phase are 1000 times more sensitive than cells in G_1 or G_2-phase to the cytotoxicity of camptothecins reflecting the need for DNA replication for drug efficacy (Del Bino *et al.*, 1991). Although much is known, our understanding of the mechanism of activity of these agents might be incomplete. These agents are active in human tumour xenografts that typically have low S-phase fractions and studies have shown that the fraction of cells killed by a brief exposure to camptothecin is sometimes larger than the S-phase fraction of the cell population, thus other cellular effects of camptothecins may be linked to cytotoxicity (O'Connor *et al.*, 1991).

Structure-activity experiments have defined the features of the molecule critical for cytotoxicity. Camptothecin has a heterocyclic five-ring structure with a lactone moiety and an

Correspondence: E Eisenhauer
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S-hydroxyl moiety on ring E (Figure 1). Camptothecin lactone exists in a pH-dependent equilibrium with an open ring carboxylate form. At physiological pH 90% of the drug exists as carboxylate. Both the pentacyclic ring structure of camptothecin, and the lactone and hydroxyl moieties are required for cytotoxicity as molecules with fewer than five rings, or bearing either a 20(R) hydroxyl or the open ring carboxylate are biologically inactive (Hertzberg *et al.*, 1989*a*,*b*). Substitutions at positions 9 or 10 by amino or hydroxyl groups lead to compounds with equal or greater *in vivo* activity than the parent compound (Wani *et al.*, 1980, 1987). Knowledge of these features has led to the development of analogues of camptothecin which are both water soluble and effective. Four analogues are now undergoing clinical evaluation: irinotecan, topotecan, 9-aminocamptothecin and GI 147211 (GG211).

Irinotecan (CPT-11)

Preclinical studies

The first of the water-soluble analogues is irinotecan (CPT-11) or 7-ethyl-10-(4-[1-piperidino]-1-piperidino)methyl-10-hydroxycamptothecin. Irinotecan, a prodrug with limited activity, is converted in plasma by de-esterification into SN-38 which has 1000 times the potency of the parent compound (Kawato *et al.*, 1991*a*).

Irinotecan is active against a diverse array of tumour cell lines *in vitro* and *in vivo*. The SN-38 metabolite is a more effective inhibitor of topoisomerase I and more cytotoxic toward HT-29 human colon carcinoma cells in culture than camptothecin, 9-aminocamptothecin and topotecan (Tanizawa *et al.*, 1994). Irinotecan, when given by intraperitoneal, intravenous or oral routes, showed substantial activity against a broad spectrum of mouse and human tumour xenografts including human cancer xenograft lines resistant to topotecan, vincristine or melphalan (Kunimoto *et al.*, 1987). Interestingly, sensitivities of some tumour cell lines to irinotecan were independent of their ability to produce SN-38 suggesting that cytotoxicity is not solely dependent on the production of the metabolite (Kawato *et al.*, 1991*b*). Unlike 9-aminocamptothecin and topotecan, the efficacy of irinotecan was not substantially influenced by administration schedule in preclinical studies (Furuta, 1990).

Clinical studies of irinotecan

Clinical evaluation of irinotecan is well advanced. Phase I trials (Table I) were conducted in Japan and more recently in the United States and Europe on several schedules: 30 min infusion every week and every 3 weeks; 30–90 min infusion daily for 3 days every 3 weeks; 90 min infusion every week and 3 weeks; and 120 h continuous intravenous infusion every 3–4 weeks. Dose-limiting toxicities were somewhat dependent on the treatment schedule. Dose-limiting leucopenia, neutropenia and diarrhoea were prominent in single-dose regimens, while gastrointestinal toxicities prevailed with c.i.v. schedules. Diarrhoea is the most significant gastrointestinal toxicity and may occur early or late following treatment. The early syndrome begins during or shortly after the infusion of irinotecan and is often associated with flushing, sweating, nausea, vomiting and abdominal cramps. Both inhibition of acetylcholinesterase at muscarinic receptors (Kawato *et al.*, 1993) and stimulation of the nicotinic receptors in autonomic ganglion cells (Gandia *et al.*, 1993) have been postulated as mechanisms. It can be managed by the administration of diphenhydramine or atropine with a serotonin antagonist such as ondansetron. Late diarrhoea begins 1–3 weeks after treatment and may last 5–7 days. Its occurrence is unpredictable and it may be severe. It is refractory to most antidiarrhoeal agents including opiates, atropine and octreotide but it may respond to high-dose loperamide with or without the enkephalinase inhibitor acetorphan (Hagipantelli *et al.*, 1995). In one small study, early aggressive treatment with loperamide 2 mg every 2 h (24 mg per 24 h) until 12 h without a bowel movement reduced the incidence of severe diarrhoea to 6% (Abigerges *et al.*, 1994). Other toxic effects of irinotecan included thrombocytopenia, eosinophilia, anaemia, alopecia, fatigue, transient elevation of liver function tests, rash and mucositis. Rarely, cases of

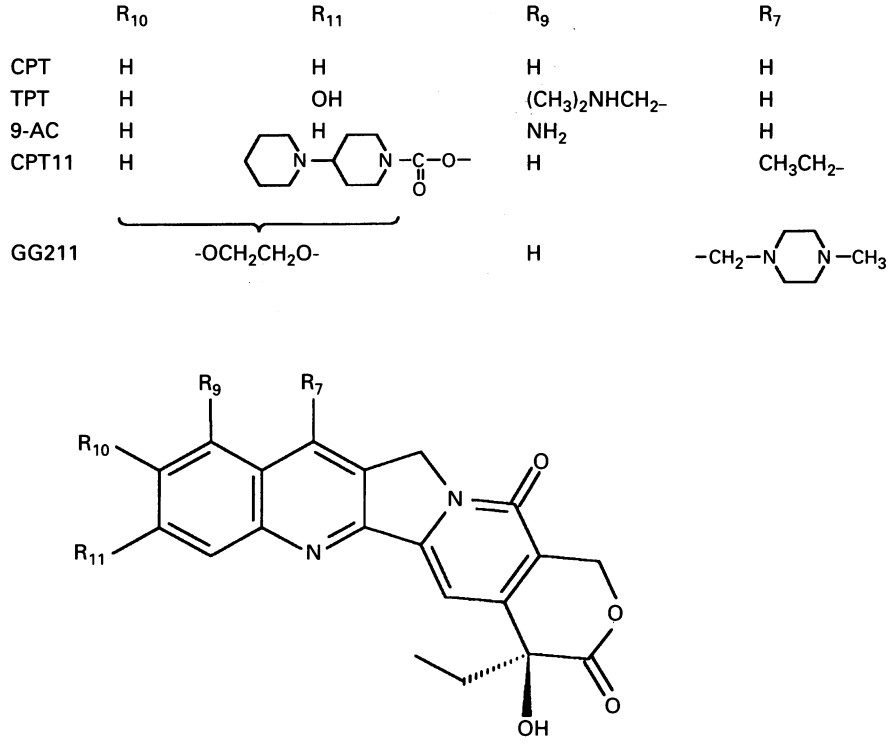

Figure 1 CPT, camptothecin; TPT, topotecan; 9-AC, 9-aminocamptothecin; CPT-11, irinotecan; GG211, GI147211C.

interstitial pneumonitis have occurred in previously treated patients with lung cancer.

The pharmacokinetics of irinotecan are complex. In plasma, carboxylesterases rapidly convert the prodrug into SN-38 and both irinotecan and SN-38 are converted by pH-dependent hydrolysis from lactone to carboxylate forms. Concentrations of the irinotecan lactone in plasma is almost two orders of magnitude higher than that of SN-38 and both lactone forms represent 44% and 50% of the total drug and metabolite detectable in plasma (Rowinsky *et al.*, 1994*a*). Peak plasma levels and AUC of irinotecan correlate well with dose (De Forni *et al.*, 1994). The AUC of SN-38 correlates with the AUC but not the dose of irinotecan. The reported terminal half-life of irinotecan is 5.2–9.3 h, and the mean residence times for it and SN-38 are 9.1 and 10.0 h. Hydrolysis of irinotecan and SN-38 lactone is less than for topotecan and 9-aminocamptothecin with 33–66% remaining intact at 24 h after infusion of irinotecan. The maintenance of biologically relevant concentrations of SN-38 for long durations may explain the observation that anti-tumour efficacy of irinotecan is not schedule dependent. There is significant interpatient variability in the conversion of irinotecan to SN-38, implying that dose increases may not lead to proportional increases in cytotoxicity.

Biliary and urinary excretion are both important routes of elimination. In humans 37±4% of the drug is detected in urine in 48 h (Rowinsky, 1994*a*). Both irinotecan and SN-38 undergo glucuronic acid conjugation and are eliminated in bile (Narita *et al.*, 1993; Gupta *et al.*, 1994). β-Glucuronidase of intestinal microflora can cleave the glucuronide and free intestinal SN-38 which may play a role in producing the late diarrhoea. Indirect estimates of biliary concentration of SN-38 and its glucuronide have shown good correlation between the concentration of SN-38 and the occurrence of late diarrhoea (Araki *et al.*, 1993).

In single agent phase II trials (Table II), irinotecan was active against a wide range of carcinomas and lymphomas. Activity was observed using schedules of 100–150 mg m^{-2} $week^{-1}$ and 350 mg m^{-2} every 3 weeks. The response rates appear to favour weekly administration but direct comparisons between the two schedules have not been made. Of particular interest are the results of five trials in patients with metastatic colorectal cancer where response rates have ranged from 14% to 32%. Similar levels of activity have been seen in untreated colorectal patients, patients previously treated with 5-FU and patients who were treated after progressing on 5-FU (Bugat *et al.*, 1995). Response rates of 34% and 36% were observed in untreated patients with non-small-cell lung cancer. Complete and partial remissions were seen in 24% of ovarian cancer and 23% of breast cancer patients who had received prior chemotherapy. Results of three studies in patients with cervical cancer have been mixed, perhaps reflecting different schedules and patient populations. On the weekly schedule response rates of 24–27% were observed in patients previously treated with cisplatin but 0% in patients who were refractory to cisplatin. A response rate of 15% was observed in chemotherapy-naive patients on the 3 weekly schedule but 24% in the subset of 21 patients who had measurable disease outside previously irradiated fields. Partial responses were seen in 23% of patients with advanced gastric cancer, 40% of patients with small-cell lung cancer and in 39% with non-Hodgkin's lymphoma. Thus, irinotecan has impressive activity in many malignancies particularly colorectal carcinoma, non-small-cell lung cancer and cervical carcinoma. Toxic effects are usually manageable but late diarrhoea may be severe despite maximal medical therapy. Because of this it may be challenging to combine this drug with other cytotoxic agents particularly those with similar toxicities. A direct comparison of the weekly and 3 weekly schedule is an obvious question to be addressed in a comparative trial.

Future directions

Future study of irinotecan will be likely to fall into three major areas: the pursuit of effective (preferably mechanism-based) methods of overcoming the late diarrhoea, the development of rational, safe combination regimens (see section on Combination treatment) and the randomised comparison of irinotecan-based regimens with standard therapy. The tumour types which ought to be the focus of the initial group of comparative trials include both colorectal cancer and non-small-cell lung cancer based on the single agent data in these diseases.

Secondary areas of endeavour which merit evaluation include further phase II testing, particularly in those tumour types with supportive preclinical data such as sarcoma and glioma, and the development of a better understanding of the pharmacokinetic/dynamic relationship of the drug to toxic and efficacy outcomes.

Topotecan

Preclinical studies

Topotecan (9-(dimethylamino)methyl-10-hydroxycamptothecin) is a camptothecin derivative having aqueous solubility conferred by the charged amino group on the 9-substituent. When tested against a variety of transplantable mouse and human tumours, topotecan demonstrated anti-tumour

Table I Phase I studies of irinotecan (CPT-11)

Reference	*Schedule*	*MTD (mg m^{-2} day^{-1})*	*Phase II (mg m^{-2})*	*Limiting toxicity*	*Responses*
Taguchi *et al.* (1990)	60 min i.v. q28d	250	200	Neutropenia	Not reported
Rowinsky *et al.* (1994*a*)	90 min i.v. q21d	290	240	Neutropenia, nausea, vomiting	1 PR rectal 1 PR cervix 1 PR RCC
Abigerges *et al.* (1994)	30 min i.v. q21d	>600	ND	ND	4 PR colon 1 PR cervix
De Forni *et al.* (1994)	30 min i.v. qwk	145	115	Neutropenia	1 PR oesophagus
Negoro *et al.* (1991*a*)	90 min i.v. qwk	150	100	Leucopenia, diarrhoea	2 PR NSCLC
Rothenberg *et al.* (1993)	90 min i.v. qwkx4 q6wk	180	150	Diarrhoea	2 PR colon
Lestingi *et al.* (1995)	90 min i.v. qwkx4 q6wk	175	145*	Neutropenia, diarrhoea	1 PR gastric
Ohe *et al.* (1992)	120 h c.i.v. q3–4wk	40	30	Diarrhoea	Not reported
Clavel *et al.* (1992)	30–90 min i.v. × 3d q21d	145	100	Neutropenia	1 PR mesothelioma 1 PR breast

MTD, maximum tolerated dose; PR, partial response; ND, not determined; RCC, renal cell carcinoma; NSCLC, non-small-cell ling carcinoma; c.i.v., continuous intravenous infusion; *G-CSF and aggressive antidiarrhoeal support.

activity when administered intravenously, intraperitoneally, subcutaneously or orally (McCabe, 1994). Preclinical testing indicated that, in topotecan-sensitive tumours, longer exposure to the drug increased the magnitude of response (Burris *et al.*, 1992; Friedman *et al.*, 1994). This could indicate that, in the clinic, more prolonged schedules of administration might be superior to short infusions.

Clinical studies of topotecan

Based on the results or preclinical screening, topotecan entered phase I studies in the United States and Europe (Table III). Short, intermediate and prolonged infusion schedules have been studied including: single intravenous injection every 21 days; 30 min infusion on 5 consecutive days every 21–28 days; 24, 72, 96 and 120 h continuous intravenous infusions every 21–28 days; and a 21 day continuous intravenous infusion every 28 days. In all studies myelosuppression was dose-limiting although the pattern of myelosuppression varied with the method of administration. Intermittent bolus and short infusion schedules resulted in non-cumulative neutropenia as the predominant toxicity, whereas prolonged continuous infusions were followed by neutropenia, thrombocytopenia and anaemia. Non-haematological toxic effects were generally mild and included alopecia, nausea, vomiting, diarrhoea, elevations in hepatic enzymes, mucositis, skin rash and fatigue.

Pharmacokinetic studies of topotecan show the drug is rapidly hydrolysed in plasma to the open-ring form following intravenous administration (Rowinsky *et al.*, 1992). The plasma clearance is biexponential with a terminal half-life of 3 h. Renal elimination is important as 40% of the drug is excreted in the urine within the first 24 h of treatment. There is a good correlation between the AUC of total topotecan (lactone plus hydroxyacid) and the grade of neutropenia in patients with normal or impaired renal or hepatic function (Grochow *et al.*, 1994). Patients with reduced creatinine clearance require dose adjustment as they are at increased risk of toxicity from topotecan. However, hyperbilirubinaemia does not alter topotecan disposition or toxicity and no dose adjustment is required in patients with serum bilirubin as high as 170 μmol l^{-1}.

Although the dose-limiting toxicity of topotecan was neutropenia, attempts to improve dose intensity on the daily × 5 schedule by using haematopoietic growth factors were not successful (Murphy *et al.*, 1992; Rowinsky *et al.*, 1992; Janik *et al.*, 1993).

Since several anti-tumour responses were seen in the daily × 5 phase I trial, this schedule was selected for phase II evaluation. Following this decision, the 21-day c.i.v. phase I trial was completed and also appeared active, thus a limited number of phase II trials have been initiated with the more prolonged schedule.

Because of its broad activity in phase I studies, phase II studies of topotecan were initiated for many different tumour types (Table IV). In a randomised phase II study comparing the daily × 5 day schedule to 72 h c.i.v. schedule in untreated patients with advanced NSCLC, response rate, median time to progression and median survival favoured the daily for 5 days schedule (Weitz *et al.*, 1995). In untreated colorectal cancer an 8% response rate was seen using the 21-day c.i.v. schedule. This response rate was similar to that observed in colorectal carcinoma with the daily × 5 day schedule, but the c.i.v. administration was associated with significant cumulative myelosuppression and pronounced anaemia (Creemer *et al.*, 1995). Activity in other tumour types on the daily × 5 schedule included response rates of 40% and 21% in untreated and previously treated SCLC respectively, 33% in breast cancer and 27% in head and neck cancer. In two trials involving heavily pretreated patients

Table II Irinotecan (CPT-11) phase II trials

Reference	*Tumour site*	*Evaluable patients*	*Prior Rx*	*Dose schedule (mg m^{-2} $dose^{-1}$)*	*Response CR*	*PR*	*%*
Ohno *et al.* (1990)[a]	Lymphoma	66	All	40 daily × 3d qwk	9	17	39
Ota *et al.* (1994)	Leukaemia	41	All	15–20 b.i.d. × 7d q3–4wk	0	2	5
Sakata *et al.* (1994)	Pancreas	35	Some	100 qwk 150 q2wk	0	4	11
Wagener *et al.* (1994)	Pancreas	18	None	350 q3wk	0	3	15
Takeuchi *et al.* (1991)[a]	Ovary	55	52 CDDP 4 XRT	100 qwk	0	13	24
	Cervix	55	30 CDDP 52 XRT	150 q2wk	5	8	24
Kavanagh *et al.* (1994)	Cervix	11	All CDDP	150 qwk × 4wks q6wks	1	2	27
Potkul *et al.* (1995)	Cervix	14	All CDDP resistant	125 qwk × 4wk q6wk	0	0	0
Chevallier *et al.* (1995)	Cervix	34	No CT	350 q3wks	1	4	15[b]
Douillard *et al.* (1995)	NSCLC	11	No CT	350 q3ks	0	4	36
Negoro *et al.* (1991*b*)[a]	NSCLC	67	None	100 qwk	0	23	34
Masuda *et al.* (1992)	NSCLC	26	All	100 qwk	0	0	0
	SCLC	8	None	100 qwk	0	4	50
	SCLC	27	All	100 qwk	2	7	33
Bonneterre *et al.* (1993)	Breast	12	All	350 q3wk	1	0	8
Taguchi *et al.* (1994)[a]	Breast	65	46 CT	100 qwk	1	14	23
Shimada *et al.* (1993)	Colon	63	51 CT	100 qwk 150 q2wk	0	17	27
Pitot *et al.* (1994)	Colon	34	21 CT	125 weekly × 4 q6wk	0 0	5 2	24 15
Rothenberg *et al.* (1994)	Colon	44	All	125–150 weekly × 4 q6wk	1	10	25
Bugat *et al.* (1994)	Colon	85	All	350 q3wk	2	10	14
Rougier *et al.* (1994)	Colon	35	None	350 q3wk	0	7	20
Conti *et al.* (1994)	Colon	19	None	125 weekly × 4 q6wk	0	6	32
Futatsuki *et al.* (1994)	Gastric	60	45 CT	100 qwk 150 q2wk	0	14	23

[a]Studies with early and late results. [b]1 CR and 3 PR (24%) in 21 patients with measurable disease outside previously irradiated areas.

with carcinoma of the ovary, response rates of 14% and 25% were seen. In both studies, the majority of non-responding patients had prolonged disease stabilisation, an observation reminiscent of results with taxoids in platinum-treated patients. Minimal activity was observed against carcinomas of the pancreas, prostate, kidney, NSCLC, melanoma, mesothelioma, soft-tissue sarcoma and glioma.

Future directions

Based on its promising single agent phase II results, further studies of topotecan in combination with other effective cytotoxic agents are warranted in SCLC, breast cancer, head and neck cancer and ovarian cancer (see section on Combination treatment). Small-cell lung cancer and ovarian

Table III Topotecan phase I studies

Reference	*Schedule*	*MTD mg m^{-2} dose^{-1}*	*Phase II mg m^{-2} dose^{-1}*	*Limiting toxicity*	*Objective responses*
Wall *et al.* (1992)	30 min i.v. q21d	22.5	20	Neutropenia	None
Hasegawa *et al.* (1993)	30 min i.v. q21d	22.5	20	Leucopenia	Not reported
Blaney *et al.* (1993)[a]	24 h c.i.v. q21d	7.5[b]	5.5	Leucopenia, thrombocytopenia	None
Abbruzzese *et al.* (1993)	24 h c.i.v. q21d	12.5	10[c]	Neutropenia	None
ten Bokkel Huinink *et al.* (1992)	24 h c.i.v. q21d	10.5	8.4	Neutropenia, thrombocytopenia	None
Haas *et al.* (1994)	24 h c.i.v. qwk	1.75	1.5	Neutropenia	1 PR colon
Pratt *et al.* (1994)[a]	72 h c.i.v. q21d G-CSF	3.9	3.0	Neutropenia, thrombocytopenia	1 CR neuroblastoma
Sabiers *et al.* (1993)	72 h c.i.v. qwk 72 h c.i.v. q2wk	2 2.6	2	Myelotoxicity	Not reported
Burris *et al.* (1994)	72 h c.i.v. q21d 120 h c.i.v. q21d	4.8 3.4	4.8[d]	Neutropenia, thrombocytopenia	None
Kantarjian *et al.* (1993)[e]	120 h c.i.v. q21–28d	11.8	10	Mucositis	2 CR AML 1 CR CML-BC 2 PR AML
Rowinsky *et al.* (1992)	30 min i.v. × 5d q21d	2	1.5	Neutropenia	1 CR NSCLC 1 CR ovary 2 PR NSCLC
Saltz *et al.* (1993)	30 min i.v. × 5d q28d	1.75	1.5	Neutropenia	1 PR oesophagus 1 PR CUP
Verweij *et al.* (1993)	30 min i.v. × 5d q21d	1.5	1.5	Leucopenia	1 PR SCLC 1 PR NSCLC 1 PR pancreas
Tubergen *et al.* (1994)	30 min i.v. × 5d q21d G-CSF	2.4	2.0	Neutropenia, thrombocytopenia	None
Hochster *et al.* (1994)	21d c.i.v. q28d	14.7	11.3	Neutropenia, thrombocytopenia	2 PR ovary 1 PR NSCLC 1 PR breast
Plaxe *et al.* (1993)	24 h c.i.p. q28d[f]	4	3	Neutropenia	5 reduction of ascites

[a]Paediatric patients; [b]for c.i.v. schedules dose cited is total dose over the total time of infusion; [c]dose for previously untreated patients without G-CSF; MTD, 15 mg m^{-2} with G-CSF; [d]recommended schedule for higher dose intensity; [e]all leukaemia patients; [f]c.i.p, continuous intraperitoneal infusion.

Table IV Topotecan phase II studies

Reference	*Tumour site*	*Evaluable patients*	*Prior Rx*	*Dose schedule mg m^{-2} dose^{-1}*	*Response CR*	*PR*	*%*
Giantonio *et al.* (1993)	Prostate	28	Hormones	1.5 × 5 days q21d	0	1	5
Ilson *et al.* (1993)	Renal cell	15	No CT	1.5 × 5 days q21d	0	0	0
Kudelka *et al.* (1993)	Ovary	28	All CT	1.5 × 5 days q21d	0	4	14
Armstrong *et al.* (1995)	Ovary	16	All CDDP refractory	1.5 × 5 days q21d	1	3	25
Chang *et al.* (1995)	Breast	15	0–1 regimens	1.5 × 5 days q21d	0	5	33
Eisenhauer *et al.* (1994)	Sarcoma	29	None	1.5 × 5 days q21d	0	3	10
Eisenhauer *et al.* (1994)	Glioma	31	CT 12 XRT 24	1.5 × 5 days q21d	1	1	7
Creemers *et al.* (1994)	Colon	28	None	1.5 × 5 days q21d	0	2	7
Sugarman *et al.* (1994*a*)	Colon	19	Unknown	1.5 × 5 days q21d	0	0	0
Creemers *et al.* (1995)	Colon	16	None	0.6 c.i.v. × 21d q 28d	1	0	6
Robert *et al.* (1994)	Head and neck	15	No CT	1.5 × 5 days q21d	0	4	27
Schiller *et al.* (1994) Hutson *et al.* (1995)	SCLC	35	No CT	2.0 × 5 days q 21d G-CSF	0	14	40
Wanders *et al.* (1995)	SCLC	57	All CT	1.5 × 5 days q21d	2	7	21
Perez-Soler *et al.* (1995)	SCLC	25	All refractory to etoposide	1.25 × 5 days q21d	0	3	12
Weitz *et al.* (1995)	NSCLC	38	No CT	1.5 × 5 days q21d	0	5	18
		36		1.3 c.i.v. × 3 d q 28d	0	2	8
Perez-Soler *et al.* (1994)	NSCLC	37	None	1.5 × 5 days q21d	0	5	14
Lynch *et al.* (1994)	NSCLC	20	None	2.0 × 5 days q21d	0	0	0
Maksymiuk *et al.* (1995)	Mesothelioma	22	None	1.5 × 5 days q21d	0	0	0
Scher *et al.* (1994)	Pancreas	34	No CT	1.5 × 5 days q21d	0	4	12
Sugarman *et al.* (1994*b*)	Pancreas	15	None	1.5 × 5 days q21d	0	0	0

CT, chemotherapy; XRT, radiation therapy; CR, complete response; PR partial response.

cancer have already been identified as tumour types for randomised studies. In the former, topotecan is being evaluated in a front-line setting, while in ovarian cancer, a randomised comparison with paclitaxel in platinum pretreated patients has been completed, although results are not yet available. The findings of this ovarian trial and that of a phase II study of topotecan in paclitaxel failures will be important in determining if front-line regimens incorporating this new drug should be developed further.

At the present time the daily for 5 days schedule appears to offer the best balance of efficacy and toxicity compared with 72 h or 21-day c.i.v. schedules, despite preclinical data favouring prolonged drug exposures. However, the optimal schedule of administration of topotecan may not yet be defined and several trials evaluating the activity of the 21-day infusion are ongoing. The results of these studies may lead to an interest in chronic oral dosing strategies.

Finally, the evidence of a relationship between total topotecan AUC and neutropenia (Grochow *et al.*, 1994) together with recent publication of a limited sampling model for determining topotecan AUC (Minami *et al.*, 1996) should lead to prospective studies assessing the pharmacokinetic/dynamic behaviour of this agent.

9-Aminocamptothecin

Among the many semisynthetic or totally synthetic camptothecin analogues screened, 9-aminocamptothecin was selected for advanced testing and clinical development primarily because of its ability to induce complete remissions in mice bearing human colonic adenocarcinoma and malignant melanoma cell lines known to be resistant to standard chemotherapeutic agents (Giovanella *et al.*, 1989, 1991; Pantazis *et al.*, 1992). Like topotecan, pharmacokinetic and efficacy studies of 9-aminocamptothecin suggested that maintaining the lactone plasma concentration above a threshold level for a prolonged period was required for optimal therapeutic effect (Supko *et al.*, 1993).

The innate aqueous insolubility of 9-aminocamptothecin resulted in difficulty devising a suitable formulation and delayed initiation of phase I studies. Two studies of 9-aminocamptothecin, formulated in polyethylene glycol 400, phosphoric acid and dimethylacetamide, have been completed. In both, the drug was given as a 72 h continuous intravenous infusion either every 2 weeks or every 3 weeks in patients with advanced solid tumours (Dahut *et al.*, 1994; Rubin *et al.*, 1994). The 72 h infusion was selected to try to achieve the prolonged drug concentrations above a 'threshold' level known to be of importance to anti-tumour effect in animal model systems. Preliminary reports indicate the drug formation and schedule were well tolerated. Leucopenia was dose-limiting in both trials. In one study the maximum tolerated dose (MTD) was 59μg m^{-2} h^{-1} and the dose was escalated to 74 μg m^{-2} h^{-1} with granulocyte colony-stimulating factor (G-CSF). With the highest doses, grade 3 thrombocytopenia as well as nausea/vomiting (controlled with antiemetics), total alopecia, stomatitis and, infrequently, diarrhoea were seen. In the 19 patients evaluated in this phase I study of 9-AC every 2 weeks, there were no objective responses; minimal responses were evident in patients with colon, lung and gastric carcinomas (Dahut *et al.*, 1994).

Preliminary pharmacokinetic studies of 9-aminocamptothecin given as a 72 h infusion were done as part of the phase I evaluation (Takimoto *et al.*, 1994). Steady-state plasma concentrations increased linearly from 0.89 ± 0.63 nM to 5.6 ± 0.6 nM over the dose range of 5 to 59 μg m^{-2} h^{-1} and total body clearance was 26.5 ± 8.6 ml min^{-1} m^{-2}. Nonlinear regression analysis demonstrated biphasic pharmacokinetics for 9-aminocamptothecin lactone with a $t_{1/2\alpha}$ of 1.5–2.5 h and a $t_{1/2\beta}$ of 10.7–12.9 h. Mean steady-state plasma levels of 9-aminocamptothecin lactone correlated well with the percentage decrease in granulocyte and leucocyte counts. Phase II testing of 9-aminocamptothecin as a 72 h infusion every 2 weeks is ongoing. Furthermore, clinical testing of a colloid dispersion formulation which improves the aqueous solubility of 9-aminocamptothecin 20-fold is under way. Definitive comments on schedule, formulation and efficacy await the results of these studies.

GI 147211C (GG211)

GI 147211C (recently renamed GG211) is the synthetic water-soluble camptothecin analogue, 7-(4-methylpiperazinomethylene)-10,11-ethylenedioxy-20(s)-camptothecindihydrochloride. In comparison with topotecan *in vitro*, GI 147211C is a more potent inhibitor of topoisomerase I and has greater cytotoxicity (Kang *et al.*, 1993; Emerson *et al.*, 1995). Anti-tumour activity was assessed in xenograft models and its anti-tumour effect was dose schedule-dependent with a greater reduction in tumour volume achieved by prolonged dosing (2× week for 5 weeks). Concurrent experiments demonstrated that GI 147211C was slightly more effective than topotecan in suppressing tumour growth. Preliminary reports of phase I clinical trials of daily times 5 days and 72 h infusion schedules are available. On the daily times 5 every 21 day schedule, the maximal tolerated dose was 1.75 mg m^{-2} day^{-1} in minimally pretreated patients and 1.2 mg m^{-2} day^{-1} in heavily pretreated patients (Eckardt *et al.*, 1995). Dose-limiting toxicities were neutropenia and thrombocytopenia with no evidence of cumulative toxicity. With the 72 h c.i.v. schedule, both neutropenia and thrombocytopenia were dose-limiting at the maximum tolerated dose in pretreated patients of 2.0 mg m^{-2} day^{-1} (O'Dwyer *et al.*, 1995). Non-haematological toxicities seen with both schedules were mild and included alopecia, anorexia, fatigue, nausea, vomiting, headache and phlebitis. Responses were seen in patients with breast, ovary and colorectal cancer who received the drug on the 72 h c.i.v. schedule.

Combination treatment

Extensive preclinical investigation has led to specific strategies for combining camptothecins with chemotherapeutic agents and radiation. *In vitro* and, for some drugs, *in vivo* studies show that the efficacy of camptothecins is synergistic or additive when compared sequentially with alkylating agents (cisplatin and cyclophosphamide) (Kano *et al.*, 1992), topoisomerase II inhibitors (doxorubicin, daunorubicin and etoposide) (Del Bino *et al.*, 1992) but antagonistic when combined with the antimetabolite methotrexate. Efficacy of drug combinations depended not only on choice of drug but also on schedule as the administration of camptothecins concurrently with some chemotherapeutic agents leads to antagonistic rather than synergistic effects (Bertrand *et al.*, 1992; Kaufmann, 1991).

The combination of irinotecan and cisplatin was superior to combinations of cisplatin with vindesine or etoposide against human lung adenocarcinoma cell lines (Kuraishi *et al.*, 1992). The scheduling of the drugs was critical for success as sequential administration of camptothecins followed by cisplatin led to synergistic cytotoxicity while concurrent administration led to antagonism. Camptothecins may inhibit topoisomerase I-mediated repair of alkylating agent-induced DNA damage. There is clinical evidence to support these laboratory observations. In a phase I trial, toxicity of topotecan and cisplatin was schedule-dependent. The administration of cisplatin on day 1 followed by topotecan daily for 5 days resulted in greater neutropenia and thrombocytopenia than administration of cisplatin after topotecan (Rowinsky *et al.*, 1994*b*). Studies are underway to determine which regimen possesses superior antineoplastic effect. In a phase I study of irinotecan with cisplatin, the partial remission rate was 54% in patients with NSCLC (Masuda *et al.*, 1992) and in a phase II study of the same drugs in untreated patients with extensive and limited SCLC

the response rates were 79% and 78% respectively. Although these two studies do not answer the question of appropriate timing of drug administration, these results are similar to standard therapies and suggest that the combination is effective (Fujiwara *et al.*, 1994).

Synergy was also seen *in vitro* when camptothecins were administered sequentially but not concurrently with topoisomerase II inhibitors. Pretreatment with irinotecan has been shown to increase in topoisomerase II mRNA in cells and cellular overexpression of topoisomerase II is likely to increase cytotoxicity of topoisomerase II inhibitors (Kim *et al.*, 1992). In a phase I study of topotecan given by continuous infusion on days 1–3 and etoposide given over 2 h on days 7–9, sequential sampling of tumours in five patients was performed. Topoisomerase II levels were markedly increased immediately before etoposide was given on day 7 and levels decreased by day 9 in the tumour cells of one patient who had resolution of malignant ascites (Eckardt *et al.*, 1994). The concurrent administration of irinotecan and etoposide yielded a response rate of 21% in a phase II study of 61 untreated patients with NSCLC (Goto *et al.*, 1995), which was less than that previously reported in two phase II trials of irinotecan alone, suggesting that the *in vitro* data on scheduling is clinically relevant.

Scheduling effects were also seen when camptothecins were combined with radiation in tissue culture cell lines. Synergy was seen only when the drugs were administered shortly after irradiation suggesting low-dose radiation triggers cells to enter S-phase rendering them sensitive to the cytotoxic effects of camptothecins (Mattern *et al.*, 1991; Kim *et al.*, 1992). The clinical relevance of the synergistic effects of camptothecins with radiation has not been determined.

Drug resistance and camptothecins

The development of cellular resistance to chemotherapeutic agents is an important cause of treatment failure in cancer patients. In the laboratory, at least three well-defined mechanisms of resistance to topoisomerase I inhibitors have been described: alteration of topoisomerase I structure or function; P-glycoprotein (P-gp)-mediated resistance; and, for irinotecan, reduction of conversion of the prodrug to its active metabolite.

Qualitative and quantitative alterations of topoisomerase I are the most significant phenomena causing resistance to camptothecins. In several normal and malignant tissue culture lines relative resistance, measured as the increase in the dose of campotothecin required to produce a given level of survival compared with parental cells, was between 2- and 350-fold (Andoh *et al.*, 1987; Tanizawa *et al.*, 1993). Point mutations (Benedetti *et al.*, 1993), deletions (Sugimoto *et al.*, 1990*b*) and rearrangements (Tan *et al.*, 1989) in the topoisomerase I gene have been reported and may be associated with decreased topoisomerase I levels or activity. The mutations were contained in well-conserved regions of topoisomerase I gene and the domains around the mutations were likely to be critical for enzyme activity and interaction with camptothecins. Deletions and rearrangements may lead to structural and functional alterations of the enzyme and can be accompanied by reduced transcription and enzyme production. Intriguingly, some cell lines which had alterations in topoisomerase I levels and activity were more sensitive to the effects of radiation and topoisomerase II inhibitors (Sugimoto *et al.*, 1990*a*). Preliminary experiments indicated a pattern of cross-resistance among available camptothecins; however, cross-resistance was not absolute as some cell lines resistant to topotecan were sensitive to the cytotoxic effects of irinotecan (Houghton *et al.*, 1993).

Unlike water-insoluble camptothecin and 9-aminocamptothecin, water-soluble derivatives topotecan and irinotecan may be substrates for P-glycoprotein (Chen *et al.*, 1991). Both drugs show reduced cytotoxicity measured by IC_{50} values against MDR_1 cell lines expressing P-gp (Tsuro *et al.*, 1988; Hendricks *et al.*, 1992). However, the relative resistance to the water-soluble camptothecins was modest compared with resistance to doxorubicin, vinblastine and etoposide in the same cell lines.

Two other potentially important mechanisms of resistance have been described. Reduced conversion of the prodrug irinotecan to its active metabolite SN-38 caused loss of efficacy in a cell line selected for resistance to camptothecins (Niimi *et al.*, 1992). Finally, molecular inhibitors of apoptosis such as overexpression of bcl-2 decreased cytotoxicity of camptothecins (Walton *et al.*, 1993). The clinical relevance of all of these mechanisms of resistance remains to be established.

Discussion

Topoisomerase I inhibitors represent a promising new class of chemotherapeutic agents with a novel mechanism of action. Renewed interest in their study after the initial failure of the parent compound in clinical trials 20 years ago has been driven not only by the understanding of their mechanism of action, but also by an appreciation of structure–activity relationships. The broad anti-tumour activity shown in cell culture and animal studies has been confirmed in clinical phase I/II evaluation of irinotecan and topotecan.

Irinotecan has activity in an array of solid tumours but because of the impressive results in NSCLC and colorectal carcinoma these two tumour types should be the primary focus for the development of combination therapy and randomised trials, at least initially. Preclinical studies have provided helpful information for the development of combination regimens and favour sequential administration of irinotecan with DNA-damaging agents such as cisplatin and topoisomerase II inhibitors such as etoposide. Irinotecan and 5-FU in colorectal carcinoma are also an obvious combination for evaluation and clinical studies are ongoing. In terms of drug delivery, both the weekly and 3-weekly schedules have been shown to be effective; which of the two provides the best therapeutic index is also a question for comparative trials. In addition to the goal of improving efficacy in these and other tumour types, attention must be paid to the toxic effects, especially diarrhoea. Despite maximal therapy diarrhoea remains problematic and will need new solutions before irinotecan can be easily assimilated into routine practice.

Topotecan has a much more favourable toxicity profile than irinotecan but its spectrum of activity in phase II trials is somewhat less impressive. Clearly further studies of topotecan in combination with other effective cytotoxic agents are warranted in SCLC, head and neck cancer, ovarian cancer and possibly breast cancer. Its activity in previously treated ovarian cancer is of particular interest. The results of the recently completed phase III trial comparing the efficacy of topotecan with paclitaxel will be important in determining the enthusiasm for incorporating topotecan into front-line ovarian cancer regimens. In SCLC, the role of topotecan in first-line treatment should be explored and a study addressing this question is currently ongoing in the United States. Phase I/II studies are also underway with topotecan in combination with alkylating agents and topoisomerase II inhibitors similar to those described for irinotecan. At the present time the daily for 5 days schedule appears to offer the best balance of efficacy and toxicity compared with 72 h or 21 day c.i.v. schedules, despite preclinical data favouring prolonged drug exposure. However, clinical studies examining the question of prolonged administration have been limited to tumour sites in which topotecan has not shown impressive activity on the daily × 5 day schedule so these may not have been good models in which to study alternative schedules.

There is no doubt that the clinical data have confirmed that the camptothecins represent an exciting new class of

chemotherapeutic agents. The role each analogue will play in improving survival or palliative treatment of specific malignancies is evolving with the present generation of randomised trials but this will take several years to unfold. The data on topotecan and irinotecan have shown how modifications of the parent molecule lead to substantially different efficacy and toxicity profiles. Thus, results of phase II studies with 9-aminocamptothecin and GG 211 will be of great interest.

An additional aspect of the story of camptothecin development deserves comment. It is to point out the critical role that preclinical experiments played in resurrecting the interest in a class of compounds that would otherwise have remained abandoned. The identification of a unique mechanism of action and the chemical studies to determine structure–activity relationships permitted the synthesis of new molecules which were appropriate for clinical evaluation. Furthermore, the clinical trials themselves have been shaped to accommodate new schedules or end points, such as critical blood levels, when preclinical data suggested these factors might play an important role in efficacy. It will be of interest to see if these predictions prove to be accurate as clinical experience matures. Meanwhile the preclinical–clinical dialogue must continue to further our understanding of the determinants of toxicity, resistance and efficacy. Such data will allow optimisation of the use of these agents and permit the development of better analogues in this class.

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