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Topoisomerase I inhibitors: the relevance of prolonged exposure for present clinical development

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Summary Topoisomerase I inhibitors constitute a new class of anti-cancer agents. Recently, topotecan and irinotecan were registered for clinical use in ovarian cancer and colorectal cancer respectively. Cytotoxicity of topoisomerase I inhibitors is S-phase specific, and in vitro and in vivo studies have suggested that, for efficacy, prolonged exposure might be more important than short-term exposure to high concentration. Clinical development of those topoisomerase I inhibitors that have reached this stage is also focused on schedules aiming to achieve prolonged exposure. In this review, we summarize all published preclinical studies on this topic for topoisomerase I inhibitors in clinical development, namely 20-S-camptothecin, 9-nitro-camptothecin, 9-amino-camptothecin, topotecan, irinotecan and GI147211. In addition, preliminary data on clinical studies concerning this topic are also reviewed. The data suggest that prolonged exposure may indeed be relevant for anti-tumour activity. However, the optimal schedule is yet to be determined. Finally, clinical data are yet too immature to draw definitive conclusions.

Keywords: topoisomerase I; camptothecin; prolonged exposure

Topoisomerase 1–3′ is a nuclear enzyme abundantly present in all eukaryotic cells (Roca, 1995). Human topoisomerase 1–3′ is a monomeric 100-kDa polypeptide encoded by a single-copy gene located on chromosome 20q12–13.2 (Juan et al, 1988). Like all topoisomerases, topoisomerase I relaxes torsionally strained (supercoiled) duplex DNA. A tyrosine group of topoisomerase I becomes covalently bound to the 3′-phosphate at the DNA break site (cleavable complex). To accomplish DNA relaxation, topoisomerase I introduces a single-strand nick in the phosphodiester backbone of the DNA, allows the intact strand to pass through the nick and then rejoins the nicked strand. DNA relaxation results from swiveling at this nick and plays an important role in DNA replication and RNA transcription. The enzyme-bridged breaks are then resealed by topoisomerase I (religation) (Champoux, 1976; D'Arpa et al, 1989; Roca, 1995).

Topoisomerase enzymes provide an essential function in solving topological problems encountered in DNA replication and DNA transcription. Topoisomerase may also be involved in recombinational processes and chromatin assembly, however their roles in these processes are less well defined (D'Arpa et al, 1989).

As long ago as the 1970s, camptothecin (CPT), an extract from the chinese tree *Camptotheca acuminata*, was showed to have antitumour activity against several tumours. However, in phase I and II studies, unpredictable severe toxicities occurred that led to the discontinuation of further development (Wall et al, 1966; Gottlieb et al, 1970; Creaven et al, 1972; Moertel et al, 1972; Muggia et al, 1972). In the late 1980s, studies revealed that camptothecin induced single-strand DNA breaks in the presence of topoisomerase I, thus identifying this enzyme as a major target for the antitumour effect (Hsiang et al, 1988). The cellular effects of camptothecin can be entirely attributed to its action on topoisomerase I as has been proven in genetic studies with yeast and mammalian cells (Andoh et al, 1987; Gupta et al, 1988; Kjeldsen et al, 1988; Nitiss et al, 1988; Eng et al, 1989). Topoisomerase I cleavable complexes occur preferentially within expressed genes (Stewart et al, 1987; D'Arpa et al, 1989).

The lactone form of camptothecin (CPT) and all CPT analogues appears to reversibly stabilize the cleavable complex, which results in single-strand DNA breaks and inhibition of religation in the presence of the drug. DNA synthesis is arrested in the presence of topoisomerase I inhibitors – religation does not occur, resulting in irreversible inhibition of DNA synthesis with double-strand DNA breaks. These events lead to the arrest of the cell cycle in the S-G_2 phase and ultimately cell death (Hsiang et al, 1989). A S-phase specific cytotoxicity for topoisomerase I inhibitors has been observed, as S-phase cells are up to 1000-fold more sensitive than G_1 or G_2/M-phase cells after brief exposure to the drug (Liu et al, 1972; Drewinko et al, 1974; D'Arpa et al, 1980). Analysis of the distribution of RNA polymerase molecules indicates that CPT-stabilized cleavable complexes block elongation by impeding the progression of the RNA polymerase molecules along the transcription unit (Zhang et al, 1988). Inhibition of RNA synthesis is rapidly reversible after removal of CPT from cultured cells, probably as a result of the dissociation of topoisomerase I cleavable complexes from transcription units. Thus, camptothecin demonstrates inhibition of DNA and RNA synthesis with fragmentation of nuclear DNA but, upon removal of the drug, nucleic acid synthesis inhibition and DNA fragmentation are reversible and, only at higher dose and longer exposure times, do these effects become irreversible (Horwitz et al, 1971–1973; Kessel, 1971; Horwitz, 1974). Cytotoxicity of topoisomerase I drugs in the absence of detectable DNA synthesis has also been found in some

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cell lines, such as human lymphocytes. The mechanism of this non-S-phase cytotoxicity is unknown but could be due to transcription inhibition (Bruno et al, 1992). Other effects of camptothecin analogues are induction of maturation in a variety of leukaemia cell lines, expression of proto-oncogenes and endonucleolytic DNA damage characteristic of apoptosis (Kaufmann, 1989; Chou et al, 1990; Del Bino et al, 1991; Kharbanda et al, 1991; Ling et al, 1991; McSheehy et al, 1991; Aller et al, 1992).

Topoisomerase I inhibitors are active both in slowly and rapidly proliferating tumours (Liu et al, 1981; Hwang et al, 1989). Sensitivity of tumour cells to these drugs is related to the topoisomerase I level, topoisomerase I catalytic activity and the interaction between topoisomerase I and its inhibitor, hence the importance of intracellular drug concentration.

Topoisomerase I is present at relatively high levels in both proliferating and quiescent cells, suggesting that its function may be independent of cellular growth rate. In proliferating cells, topoisomerase I mRNA levels are significantly higher than in quiescent cells. However topoisomerase I protein is increased much less, which may be due to a shorter half-life of the protein in proliferating cells than in resting cells (Hwang et al, 1989, 1993). The catalytic activity of topoisomerase I also depends on the phosphorylation state of the enzyme, and phosphorylation has been shown to increase during mitogenic stimulation of quiescent cells (Samuels et al, 1992).

The regulation of topoisomerase I is altered in neoplastic cells. Colon cancer cells, for example, contain a five- to sixteen-fold higher level of topoisomerase I than normal colon mucosa cells (Giovanella et al, 1989; Sabiers et al, 1993).

Despite high levels of topoisomerase I, some human tumour cell lines are nevertheless resistant to topoisomerase I inhibitors, which may be attributed to a low specific activity of this form of topoisomerase I (Sugimoto et al, 1990; Tanizawa et al, 1990; Takeda et al, 1992). The effect of topoisomerase I inhibitors on the enzyme can also be influenced by point mutations or deletions within the topoisomerase I genes that affect protein or enzyme activity levels (Saijo et al, 1994). An absolute low level of topoisomerase I is another mechanism of resistance to topoisomerase I inhibitors (Andoh et al, 1987; Gupta et al, 1988; Kanzawa et al, 1990; Sugimoto et al, 1990).

In order to exert inhibitory effects, topoisomerase I inhibitors have first to enter tumour cells, while anti-tumour activity is only achieved with the lactone form of the compounds. This lactone form has a relatively short half-life and, at physiological pH, the hydroxyl moiety will predominate. Topoisomerase I inhibitors show readily reversible interaction with the target enzyme and do not form an intracellular reservoir (Covey et al, 1989; Subramanian et al, 1995). Therefore exposure of only limited duration of tumour cells to the active lactone form of topoisomerase I inhibitors will be achieved in dose schedules with short-lasting infusions. Related to cell entry, Ma et al (1996) reported an ovarian cancer cell line that is resistant to topotecan and SN38 because of a decreased influx of the drug (Ma et al, 1996). In a CPT-11 multidrug-resistant cell line, the cellular concentration of the drug appeared dependent on the plasma transmembrane potential (Aogi et al, 1994).

P-glycoprotein overexpression does not influence the intracellular drug concentration of camptothecin, and many of its non-charged derivatives, MDR-1-overexpressing cells, are more resistant to the positively charged camptothecin-derivative topotecan (Chen et al, 1991; Hendricks et al, 1992; Liu et al, 1992).

In vitro studies with topoisomerase I inhibitors suggest that cytotoxicity increases upon prolonged exposure to the drug.

This review will further summarize the preclinical and clinical studies of continuous or long-term exposure of topoisomerase I inhibitors in cancer research.

20-S-CAMPTOTHECIN

20-S-camptothecin (20-S-CPT) has been identified as the active agent in the extract of the *Camptotheca* tree (Wall et al, 1966). 20-S-CPT is water insoluble.

Stereochemistry and the positions of substituents have been found to be crucial in CPT and its analogues for the presence or absence of effects on topoisomerase I, indicating that the compounds interact with an asymmetrical receptor site on the enzyme or enzyme–DNA complex (Jaxel et al, 1989). The R-camptothecin isomer has little or no effect on topoisomerase I, in contrast to the natural S-isomer which has a single asymmetrical carbon located at position 20.

Interaction with the receptor is influenced by configurational alterations causing little change in general chemical properties of topoisomerase I inhibitors but producing marked changes in topoisomerase I interaction (Jaxel et al, 1989).

The lactone form of the topoisomerase I inhibitor, which predominates at pH < 4.0, is the more potent inhibitor of the enzyme and therefore a much more potent anti-tumour agent than the inactive open-ring compound.

Preclinical studies

In vivo studies

Antineoplastic and toxic effects in L1210 leukaemia of intraperitoneal administration of the 20-S-CPT sodium (20-S-CPT-Na^+) formulation were found to vary with the schedule of administration (Venditti, 1971). An intermittent schedule (days 1,5 and 9) of administration appeared superior on the resulting lifespan compared with any of the alternative treatment schedules studied, being a day 1–9 single daily dosing; dosing every 3 h on day 1, 5 and 9; every 3 h on day 1 or dosing with a single dose on day 1 (Guarino et al, 1973; Schaeppi et al, 1974).

The sodium salt of 20-S-CPT is not the optimal formulation of administration. Prolonged administration of water-insoluble formulations of 20-S-CPT were recently studied in nude mice bearing human cancer xenografts. To test the efficacy of the lipophilic moiety 20-S-CPT was dispersed in intralipid 20% and injected intramuscularly (i.m.) at a dose of 0.1 mg per 25 g of body weight. The same formulation was also administered orally and intravenously. Intravenous 20-S-CPT resulted in toxic deaths without inhibitory effects. Tested against 13 human cancer xenografts resistant to the most commonly available chemotherapeutic agents, 20-S-CPT given i.m. at a dose of 4 mg kg^{-1} twice weekly induced complete regression in the majority of the animals in 10 out of 13 xenografts. Only one melanoma and two colon cancers showed a poor response. Daily oral administration of 20-S-CPT at a dose of 4–8 mg kg^{-1} resulted in complete tumour regression in mice carrying SPA lung carcinoma. After 6 months of continuous treatment, regrowth was observed in five of the seven xenografts, suggesting 20-S-CPT resistance under prolonged treatment (Giovanella et al, 1991). 20-S-CPT given i.m. at a dose of 4 mg kg^{-1} twice daily also induced complete regression in BRO

melanoma xenografts. In vitro cell proliferation of the same cell line was inhibited at a remarkably low concentration of 1 ng ml^{-1}, and it was demonstrated that a period of 20–24 h of drug exposure was required for complete growth inhibition (Pantazis et al, 1992). In this model, 20-S-CPT i.m. (2 mg kg^{-1}) appeared to be the most effective mode of drug administration to induce tumour inhibition compared with i.v or i.p administration. 20-S-CPT at a dose of 2 mg kg^{-1} $day^{-1} \times 2$ intragastrically followed by one day of rest was more effective in inducing complete tumour inhibition than 1 mg kg^{-1} $day^{-1} \times 5$ (intragastrically) with two days rest (Pantazis et al, 1994).

Nude mice bearing intracranial human brain tumour xenografts were treated with intraperitoneal (i.p.) 20-S-CPT in different schedules. Single doses of CPT did not prolong survival, but CPT i.p. twice per week for 6 weeks or daily oral 20-S-CPT induced 10 weeks' survival in 40% or 60% of animals respectively (Phillips et al, 1994). In addition, 20-S-CPT administered intragastrically at an intermittent weekday schedule for 10 weeks was well tolerated and induced tumour responses in human cancer xenografts of malignant melanoma and colon carcinoma (Potmesil et al, 1995). In order to bypass the insolubility of 20-S-CPT lactone, the compound can also be incorporated into a liposome-based delivery system for i.m. administration. Release studies of liposomal 20-S-CPT show an initial rapid 50% loss of the drug in 4 h, followed by a slow leakage of the remaining drug over a period of 20 h. Complete tumour regression occurred after a single i.m. injection of this formulation at 10 mg kg^{-1} in nude mice xenografted with CLO breast carcinoma or BRO melanoma, with minimal host toxicity (Daoud et al, 1995).

Lipid-complexed 20-S-CPT bypasses its insolubility and makes prolonged low-dose exposure possible.

These preclinical studies suggest that intermittent intraperitoneal or more convenient daily oral administration of 20-S-CPT for a prolonged period is well tolerated and may have anti-tumour effects. Anti-tumour effects seem to be dose and schedule dependent. The intramuscular or oral administration of camptothecin seems to enable protracted dose scheduling.

Clinical studies with camptothecin and prolonged exposure

Daily × 5 i.v. administration

In the early 70s, three phase I studies with intravenous administration of the sodium 20-S-camptothecin (20-S-CPT-Na^+) were performed in which 20-S-CPT-Na^+ (0.5–10 mg kg^{-1}) was administered as single i.v. bolus every 2–4 weeks. Myelosuppression with leucopenia and thrombocytopenia was the dose-limiting toxic effect. Diarrhoea, reversible haemorrhagic cystitis and alopecia were observed at higher dose levels (Gottlieb et al, 1970). Muggia et al (1972) studied i.v. 20-S-CPT-Na^+ at a once weekly and daily × 5 schedule every 3 weeks. On the weekly schedule, dose-limiting toxicities (DLTs) were leuco- and thrombocytopenia, while haemorrhagic cystitis occurred in several patients who received multiple doses. Cumulative leuco- and thrombocytopenia were also dose limiting with the daily × 5 schedule, resulting in haemorrhagic cystitis in 3 of 17 patients (Muggia et al, 1972). Phase II trials with 20-S-CPT-Na^+ have been performed in patients with melanoma and advanced gastrointestinal carcinomas. Melanoma patients were treated with 20-S-CPT-Na^+ every 2 weeks (Gottlieb et al, 1972). Patients with gastrointestinal carcinoma were treated with either single-dose 20-S-CPT-Na^+ (90–180 mg m^{-2} every 3 weeks or a daily × 5 schedule (11–55 mg m^{-2} day^{-1}) every 4 weeks (Moertel et al, 1972). Both treatment schedules showed equal toxicity. Because of severe and unpredictable myelosuppression, haemorrhagic cystitis and diarrhoea, the sodium salt formulation of camptothecin was then disregarded.

Prolonged exposure

However, results of the above mentioned preclinical studies recently renewed the interest in new formulations of camptothecin, and the drug is again undergoing phase I evaluation. 20-S-CPT in gelatin capsules administered orally once a day for 21 days followed by 1 week rest was studied in 52 patients. Doses were escalated from 0.3 to 15.4 mg m^{-2} day^{-1} (Stehlin et al, 1995). DLT of 20-S-CPT over a 3-week period was diarrhoea. Loose stools occasionally occurred in all patients at doses above 6.5 mg m^{-2} day^{-1} with a 32% incidence of persistent diarrhoea. Anti-diarrhoeal medication generally solved this problem. The maximum tolerated dose was 8.7 mg m^{-2} day^{-1}. Chemical cystitis, resulting in dysuria and occasional haematuria occurred in 20% of patients. It resolved within a week of drug discontinuation but sometimes reappeared with continued administration. Only two extensively pretreated patients experienced severe haematological toxicity, recovering within 10–14 days. In 12 patients, the oral administration of 20-S-CPT could be continued for 6–12 months, in five patients for more than 1 year. No long-term toxicities were reported. Partial responses occurred in two patients with breast cancer and two patients with melanoma, and one patient with non-Hodgkin lymphoma achieved a complete remission.

Thus, it is possible to administer orally 20-S-CPT to patients with solid tumours for a long period of time without inducing long-term cumulative haematological or non-haematological toxicity. Presently, 20-S-camptothecin has entered a phase II study.

9-NITRO-CAMPTOTHECIN, 9-AMINO-CAMPTOTHECIN

9-Nitro-camptothecin (9NC) is a semisynthetic derivative of the natural product camptothecin and is water insoluble. 9NC is a precursor required for the synthesis of 9-amino-camptothecin (9AC) from CPT. 9NC is chemically more stable than 9AC, which is oxidized readily, generating toxic degradation products (Hinz et al, 1994; Pantazis et al, 1994). An additional finding is that 9NC is converted to 9AC by human cells of solid tissue of origin. Conversion of 9NC is less in haematopoietic cells. Cellular conversion of the lactone form of 9NC to 9AC is maximal in a slightly acidic environment (pH 6.0) (Hinz et al, 1994; Pantazis et al, 1994*a*). Because of this relationship, results of preclinical and clinical studies of both compounds will be discussed under one heading.

Preclinical studies

In vivo studies

In vivo studies of 9NC and 9AC in the malignant melanoma BRO xenograft showed that, after 40 days of treatment with i.m. 9NC or 9AC at 4 mg kg^{-1} twice a week, all engrafted mice were tumour free and did not experience significant toxicity. Growth inhibition of BRO cells in vitro occurred at a low 9NC concentration of 1 ng ml^{-1} and was complete after a period of 20–24 h of exposure (Pantazis et al, 1992).

Nude mice inoculated with three tumorigenic breast cancer cell lines developed complete tumour regression when treated with

Table 1 Topoisomerase I inhibitors – continuous/prolonged administration in solid tumours (9-amino-camptothecin)

Drug	Dose schedule	Number of patients	Cp-ss	MTD	DLT	Reference
9-Amino-CPT i.v	5–59 μg m^{-2} h^{-1} 72 h q 14 d	48	0.9–10.6 nM	35 μg m^{-2} h^{-1}	Neutropenia	Dahut et al (1996)
	47–74 μg m^{-2} 72 h q 14 d + G-CSF			47 μg m^{-2} h^{-1}	Neutropenia Thrombopenia	
9-Amino-CPT i.v.	– 72 h	19	–	–	Neutropenia	Rubin et al (1994)
9-Amino-CPT i.v.	36–62 μg m^{-2} h^{-1} 72 h q 14 d	18	2.23 ng ml^{-1}	Not yet reached	Myelosuppression	Langevin et al (1996)
9-Amino-CPT i.v.	6.2–9.4 μg m^{-2} h^{-1} 21 d q 28 d	19	–	> 9.4 μg m^{-2} h^{-1}	Not yet reached	Hochster et al (1996*a*)
9-Amino-CPT i.v.	17–25 μg m^{-2} h^{-1} 120 h/wk × 3 q 4 wk	20	2.9 ± 1.6 (17 μg m^{-2} h^{-1})	Not yet reached	Not yet reached	Takimoto et al (1996)

i.v., intravenous; i.p., intraperitoneal; p.o., oral; MTD, maximum-tolerated dose; DLT, dose-limiting toxicity; Cp-ss; plasma–concentration steady state; – not stated.

9NC i.m. at a dose of 4 mg kg^{-1} twice a week (Pantazis et al, 1993*a*). No tumour regrowth nor toxicity occurred during prolonged 9NC administration.

Cell cultures of non-tumorigenic breast cancer cells (MDA-MB-134) and tumorigenic cells (MDA-MB-231) were exposed to 9NC. The non-tumorigenic cells accumulated in G_2/M without significant changes in S-fraction. Removal of 9NC from the cultures of non-tumorigenic cells after 120 h resulted in regrowth at a rate similar to untreated cells. In tumorigenic cells exposed to 9NC, there was a marked increase in cells containing a reduced DNA content and going into apoptosis. Removal of 9NC from the cultures of tumorigenic cells after 120 h did not result in regrowth after 120 h (Pantazis et al, 1993*a*).

Experiments with 9NC and 9AC at an i.m. dose of 4 mg kg^{-1} twice weekly in various human breast cancer xenografts resulted in complete tumour regression but, regardless of 9NC continuation or discontinuation, tumorigenic MDA-MB-231 tumours regrew after a period of 50 days of complete tumour regression (Pantazis et al, 1993*b*). This indicates that drug resistance occurs.

Protracted i.v. administration of 9AC to mice innoculated with CLO human breast cancer cells was studied in various schedules. 9AC i.v. daily × 3 every 21 days at dose levels of 0.75 and 1 mg kg^{-1} day^{-1} resulted in tumour regression but, ultimately, with regrowth. This i.v. schedule had no inhibitory effect on tumour progression, unlike the i.m. 9AC 1 mg kg^{-1} administration described earlier. A 5-day period of continuous 9AC administration followed by 2 days' rest was highly effective in tumour inhibition and regression even at a dose of 0.5 mg kg^{-1} day^{-1}. 9AC doses of 1 mg kg^{-1} day^{-1} or above were toxic for the animals. Intragastric administration of 9NC and 9AC was studied at different doses and schedules in mice with CLO xenografts. The optimal 9NC and 9AC dose and schedule was 1 mg kg^{-1} day^{-1} for 5 days followed by 2 days' rest. The authors conclude that, for practical reasons, oral administration is the route of choice for 9NC (Pantazis et al, 1993*b*).

Intramuscular administration of 9NC 4 mg kg^{-1} twice a week was efficacious in nude mice bearing human 2774 ovarian cancer (Pantazis et al, 1993*c*). Prolonged exposure of tumorigenic (2774) and non-tumorigenic (DUN) ovarian cancer cells in vitro to a concentration of 1 ng ml^{-1} of 9NC resulted in accumulation of non-tumorigenic cells in G_2/M and accumulation of tumorigenic cells containing reduced DNA content and going into apoptosis (Giovanella et al, 1994).

In a human melanoma xenograft model intramuscular administration gave the best anti-tumour effects of 9NC, 9AC and CPT. A dose schedule of 2 mg kg^{-1} day^{-1} ×2 with 1 day rest compared with 1 mg kg^{-1} day^{-1} ×5 with 2 days' rest was more effacicious for CPT and equally effective for 9NC (Pantazis et al, 1994*b*).

Intragastric application of 9AC on a 5-day/week schedule for 3–6 weeks induced complete remission in subcutaneous human xenografts of malignant melanoma and non-small-cell lung carcinoma, and its efficacy was better than that of 20-S-camptothecin (Rubin et al, 1994).

Two observations can be made on these preclinical studies: lower 9NC or 9AC concentrations applied for long periods of treatment are more effective in inducing apoptosis than higher concentrations for short periods. When 9NC initiates the process of apoptosis in tumorigenic cells, this is not reversible, even after removal of the drug. Non-tumorigenic cells are reversibly inhibited as long as drug exposure continues.

Route of administration and dose scheduling of 9NC and 9AC seem to be crucial for optimal anti-tumour responses. Prolonged or intermittent administration of a lower dose of these drugs is most efficacious.

Clinical studies with prolonged or continuous exposure

72-h infusion

Phase I studies of 9-amino-camptothecin in adult patients with solid tumours have been performed initially with continuous i.v. infusion over 72 h (Table 1). Leucopenia appeared to be the dose-limiting toxicity, together with modest thrombocytopenia. Nausea and vomiting, alopecia, stomatitis and diarrhoea were less frequently reported (Rubin et al, 1994; Takimoto et al, 1994; Dahut et al, 1996). Steady-state plasma concentrations increased linearly with the dose and ranged from 0.9 to 10.6 nm and correlated well with percentage decrease of granulocyte count (Takimoto et al, 1994). In a similar phase I study in children, side-effects were similar, but the MTD in children exceeded that in adults (Langevin et al, 1996).

Prolonged exposure

Phase I studies with longer infusion durations of 9-AC in adults are ongoing. A continuous i.v. infusion for 120 h weekly for 3 out of every 4 weeks is feasible, with DLT not yet being reached at the dose level of 20 μg m^{-2} h^{-1}. The resulting dose intensity is already higher than the dose intensity of the recommended phase II dose of 35 μg m^{-2} h^{-1} over 72 h when given every 2 weeks (Takimoto et al, 1996).

The same holds for continuous infusion of 9AC for 21 consecutive days every 28 days (Hochster et al, 1996*a*). The latter phase I studies suggest that with prolonged infusion a higher dose intensity of 9AC can be achieved. A phase I study with oral 9-NC given for 5 consecutive days every week revealed haematological toxicity as being dose limiting. Non-haematological toxicity was substantial with nausea/vomiting, diarrhoea and haemorrhagic cystitis. An interesting level of anti-tumour activity was reported (Verschraegen et al, 1996).

Further studies on prolonged dosing of oral 9NC and i.v. 9AC are presently on-going.

In summary, dose intensities are higher for 9AC when administered with longer infusion duration. Oral administration of 9NC for 5 consecutive days gives substantial non-haematological toxicity.

TOPOTECAN

Topotecan (TPT, 9-dimethylaminomethyl-10-hydroxycamptothecin) is a water-soluble potent camptothecin analogue with activity against various tumour types in in vitro and in vivo studies.

Preclinical studies

In vitro studies

In vitro effects of topotecan against cells from biopsy specimens of colorectal, breast, lung, ovarian, renal, gastric cancer and cancers of unknown primary origin were studied with 1-h and with continuous exposure in a human tumour clonogenic assay. With 1-h TPT exposure in vitro, responses were seen in 10% and 25% of assessable tumour specimens at TPT concentrations of 1.0 and 10.0 μg ml^{-1} respectively. Response rates were 34% and 76% at concentrations of 0.1 and 1.0 μg ml^{-1} TPT with continuous exposure (Burris et al, 1992), suggesting that TPT was more active with long-term incubation. Continuous exposure of TPT in vitro showed an initial decrease of the active lactone form of TPT, followed by a stable ratio up to 72 h, which corresponded to 19% of the initial value. The fraction of the lactone form during 1-h exposure is not known, but nevertheless it is very likely that the concentration–time product (dose intensity) is greater for continuous exposure than for 1-h exposure (Burris et al, 1992). This implies that the time period of exposure to topotecan is an even greater determinant of cytotoxicity than anticipated.

In vivo studies

Different TPT schedules were studied in female CBA/CaJ immune-deprived mice engrafted with seven colon carcinoma cell lines, six juvenile rhabdomyosarcomas and three osteosarcoma cell lines (Houghton et al, 1991). Initially, TPT was administered intraperitoneally (i.p.) using a schedule of four doses of TPT every 4 days (q4d × 4 schedule). The maximum-tolerated dose (MTD) with this schedule was 12.5 mg kg^{-1} per administration, and TPT caused significant regression in four of five rhabdomyosarcoma xenografts. Subsequently, the effect of TPT was studied as a daily ×5 dose given for 3 consecutive weeks by oral gavage (2 mg kg^{-1} per administration) or daily × 5 for 3 weeks intraperitoneally. Intraperitoneal administration was at least as efficacious as oral dosing but more toxic (Houghton et al, 1991). Intraperitoneal TPT 2 mg kg^{-1} per dose was lethal in more than 15% of the mice, the MTD with intraperitoneal administration was 1.5–1.75 mg kg^{-1} per dose. The effect of prolonged topotecan administration was studied in two moderately responsive xenografts, Rh 12 rhabdomyosarcoma and VRC_5 colon adenocarcinoma. Mice bearing Rh 12 rhabdomyosarcoma xenografts were treated with TPT 2.0 or 1.75 mg kg^{-1} per dose day^{-1} × 5 for three courses or a lower dose (1.25 mg kg^{-1} per dose) for up to 20 courses. The prolonged low-dose regimen resulted in complete remission of all tumours without regrowth. The same effect was seen at an even lower dose level of 1.0 mg kg^{-1} per dose, also without significant toxicity. Mice with VCR_5 colon adenocarcinoma showed significant tumour reduction with prolonged oral administration of TPT at a dose of 1.0 mg kg^{-1} per dose × 5 for 20 cycles. However, regrowth occurred after 16 weeks.

Additional studies with prolonged exposure schedules in mice bearing xenografts of colon adenocarcinoma, rhabdomyosarcoma and brain tumours showed less toxicity and better anti-tumour activity than dose-intensive short-exposure schedules (Houghton et al, 1995). These in vivo studies show that oral administration is as efficacious as parental application, although the AUC is lower with oral administration.

Furthermore, prolonged intraperitoneal and oral (p.o.) TPT administration resulted in responses of xenografts not responsive to a short-term parental intermittent high-dose schedule (Houghton et al, 1991, 1995).

From these preclinical data, prolonged exposure to topotecan seems a treatment schedule with a potentially higher benefit with regard to anti-tumour activity.

Clinical studies with prolonged or continuous exposure

Daily × 5 i.v. administration

Phase I studies with single i.v. bolus daily for 5 days repeated every 3–4 weeks show a maximum-tolerated dose of 1.5–2.5 mg m^{-2} day^{-1}. The dose-limiting toxicity was myelosuppression, in particular neutropenia (Rowinsky et al, 1992; Saltz et al, 1992; Verweij et al, 1993).

Non-haematological toxicities were usually mild and reversible and consisted of nausea, vomiting, fatigue, alopecia and sometimes diarrhoea.

Phase II studies with this daily ×5 TPT regimen every 21 days showed promising response rates in patients with small-cell lung cancer (10–39%) and in pretreated patients with ovarian cancer with response rates ranging from 9.5% to 25% (Kudelka et al, 1993; Schiller et al, 1994; Armstrong et al, 1995; Wanders et al, 1995; Carmichael et al, 1996; Malmstrom et al, 1996; Creemers et al 1997). Other solid tumours, such as melanoma, colon carcinoma, head and neck cancer, renal cell carcinoma, cervix and prostate carcinoma, appear to be much less sensitive to this regimen (Ilson et al, 1993; Roethke et al, 1993; Lynch et al, 1994; Robert et al, 1994; Sugarman et al, 1994; Kraut et al, 1995; Puc et al, 1995; Noda et al, 1996; Perez Soter et al, 1996; Smith et al, 1996). In these phase II studies, CTC grade III–IV neutropenia (32–81%) was reported as being the major toxicity. Thrombocytopenia CTC grade III–IV is infrequent. Anaemia greater than CTC grade II was reported in 27–60%.

Table 2 Topoisomerase I inhibitors – continuous/prolonged administration in solid tumours (topotecan)

Drug	Dose (mg m^{-2}) schedule	Number of patients	Cp-ss	MTD	DLT	Reference
Topotecan i.v.	2.5–5.0 24 h q 3 wk	15	–	4 mg m^{-2} 24 h^{-1}[a] 5 mg m^{-2} 24 h^{-1}[b]	Neutropenia Thrombopenia	Recondo (1991)
Topotecan i.v.	2.5–5.0 24 h q 3 wk	10	4–10 ng ml^{-1}	–	Neutropenia	Reid (1992)
Topotecan i.v.	2.5–10.5 24 h q 3 wk	22	20 ng ml^{-1}	8.4 mg m^{-2} 24 h^{-1}	Neutropenia	ten Bokkel Huinink (1992)
Topotecan i.p.	3–4 24 h q 4 wk	12	–	4 mg m^{-2} 24 h^{-1}	Neutropenia	Plaxet et al (1993)
Topotecan i.v.	–/72 h q 1 wk	12	–	2 mg m^{-2} 72 h^{-1}	Neutropenia	Sabiers et al (1993)
	–/72 h q 2 wk	7	–	2.6 mg m^{-2} 72 h^{-1} 72 h	Neutropenia	
Topotecan i.v. + G-CSF	10–15 24 h q 3 wk	13	–	4 mg m^{-2a} 10 mg m^{-2b}	Neutropenia (+G-CSF: Thrombopenia)	Abbruzzese (1993)
Topotecan i.v.	2.0–7.5 24 h q 3 wk	29	18.2 ± 3.7 nM	7.5 mg m^{-2}	Neutropenia Thrombopenia	Blaney (1993)
Topotecan i.v.	1.0–2.0 24 h q 1 wk	32	4.7–11.4 nM	1.75 mg m^{-2} 24 h^{-1}	Neutropenia	Haas (1994)
Topotecan i.v.	0.75–1.9 day^{-1} 72 h q 3 wk	27	3.1 ± 1.4 ng ml^{-1} 1.0 mg m^{-2} d^{-1}	1.3 mg m^{-2}	Neutroperia	Pratt (1994)
Topotecan i.v.	0.17–0.68 day^{-1} 120 h q 3 wk	14	5.5 ng ml^{-1}	0.68 mg m^{-2} day^{-1}	Thrombopenia	Burris (1994)
	0.68–1.6 day^{-1} 72 h q 3 wk	32	2.0 ng ml^{-1}	1.6 mg m^{-2} day^{-1}	Neutropenia	
Topotecan i.v.	0.2–0.7 21 d q 28 d	44	–	0.53 mg m^{-2} day^{-1}	Thrombopenia + Neutropenia	Hochster et al (1994)
Topotecan i.v.	0.6 day^{-1} 21 d	9	–	–	Neutropenia Thrombopenia	Khater (1995)
Topotecan i.v.	0.4 day^{-1} 21 d q 28 d	16	–	– (Phase II)	–	Hochster et al (1996)
Topotecan i.v.	0.8–1.1 day^{-1} 21 d q 28 d	12 Pediatric	–	0.8 mg m^{-2} day^{-1}	Thrombopenia	Bowman et al (1996)

[a]Chemotherapy pretreated. [b]Non-pretreated. i.v., intravenous; i.p., intraperitoneal, p.o. oral; MTD, maximum-tolerated dose; DLT, dose-limiting toxicity; CP-ss, plasma–concentration steady state; – not stated.

Prolonged exposure

Continuous infusion of topotecan has been studied in various schedules: a 24 h infusion weekly and every 3 weeks; a 72 h infusion administered weekly, every 14 days and every 21 days; a 120-h infusion every 3–4 weeks; and a 21-day continuous infusion administered every 28 days (Table 2).

In one study, TPT was administered intraperitoneally for 24 h every 4 weeks (Plaxe et al, 1993). Studies with continuous infusion of topotecan of 72 h or more show mild non-haematological toxicities (nausea, vomiting, alopecia). Dose-limiting toxicity is always leucocytopenia, more often with associated thrombocytopenia than with the daily × 5 i.v. bolus. Anaemia requiring blood transfusions and thrombocytopenia with platelet transfusions are particular problems related to these schedules. In phase II studies in paediatric patients and adults with acute leukaemia, continuous infusion of TPT for 120 h resulted in severe mucositis as DLT (Kantarjian et al, 1993; Furman et al, 1996).

In a phase I study with continuous intravenous topotecan administration for 21 days every 28 days in 44 patients with solid tumours, the MTD was 0.53 mg m^{-2} day^{-1}, with myelosuppression as DLT (Hochster et al, 1994). The steady-state lactone TPT concentration was low, approximately 4 ng ml^{-1}. No consistent relationship was found between drug level and haematological toxicity. Partial tumor responses were noted in two patients with ovarian cancer, one patient with breast cancer, one patient with renal cell cancer and one patient with non-small-cell lung cancer (NSCLC) (Hochster et al, 1994). Blood transfusions and platelet transfusions were necessary in 45% and 11% of patients respectively. The authors concluded that a 21-day infusion of TPT is generally well tolerated with minimal non-haematological toxicity. In a phase II study with this regimen in patients with progressive ovarian cancer after platinum-containing chemotherapy, response rate was 37% and neutropenia was the major toxicity (31%). Blood transfusions needed to be given to 50% of patients (Hochster et al, 1996*b*). Further phase II studies with the 21-day continuous infusion of TPT are ongoing.

The bioavailability of oral TPT varies from 32% to 44% with relatively limited intrapatient variation (Kuhn et al, 1995; Schellens et al, 1996). Oral TPT was studied in paediatric patients with solid tumours in a phase I study with two different dose schedules. In one dose schedule, TPT was administered orally every day for 21 days out of every 28 days; in the second schedule, oral TPT was given 5 days on and 2 days off for 15 total doses. In the 21-day schedule oral bioavailability was 46 ± 22% at 0.8 mg m^{-2} and 34 ± 14% at dose level 1.1 mg m^{-2}. DLT of both schedules is thrombocytopenia, and myelosuppression is well correlated with systemic exposure to oral TPT (Bowman et al, 1996). Thus, in vitro studies show that time period of exposure to

Table 3 Topoisomerase I inhibitors – continuous/prolonged administration in solid tumours (CPT-11)

Drug	Dose (mg m²) Schedule	Number of patients	Cp-ss	MTD	DLT	Reference Year
CPT-11 i.v.	125–225 every other wk	20	–	≥ 200 mg m^{-2}	Not yet reached	Rothenberg (1996)
CPT-11 i.v. bolus.	33–115 day^{-1} 3 d q 3 wk	46	2034 ng ml^{-1}	115 mg m^{-2}	Neutropenia + diarrhoea	Catimel et al (1995)
CPT-11 i.v.	5–40 day^{-1} 120 h q 3 wk	36	6.8–10.5 ng ml^{-1} (SN 38)	40 mg m^{-2} day^{-1}	Neutropenia diarrhoea	Ohe et al (1992)

i.v., Intravenous; i.p., intraperitoneal; p.o, oral; MTD, maximum-tolerated dose; DLT, dose-limiting toxicity; Cp-ss, plasma–concentration steady state; –, not stated.

topotecan is an important determinant of cytotoxicity. In vivo studies with human xenografts with prolonged administration of topotecan show better anti-tumour activity. In patients with solid tumours, continuous infusion of TPT is well tolerated, and tumour responses are being reported. Phase I studies with an oral formulation of TPT in adult patients with solid tumours are ongoing.

IRINOTECAN (CPT-11)

CPT-11 (7-ethyl-10 [4-(piperidino)-1-piperidino]carboxyloxy-camptothecin) is a water-soluble analogue of camptothecin. CPT-11 has little inherent anti-tumour activity in vitro, but it is converted to SN-38, a metabolite that is 1000-fold more potent than the parent compound in vitro (Kawato et al, 1991; Kanzawa et al, 1993).

Preclinical studies

In vivo studies

CPT-11 has been studied in human tumour xenografts with chemorefractory colon-carcinoma, chemoresponsive rhabdomyosarcoma and sublines of rhabdomyosarcoma with in vivo resistance to vincristine, melphalan and topotecan, as well as with three paediatric brain tumours (Houghton et al, 1993, 1995). As a single i.v. administration at the maximum-tolerated dose (50 mg kg^{-1}), CPT-11 had no inhibitory effect on any colon carcinoma xenograft; however, when administered for one cycle i.v. at a dose of 10–40 mg kg^{-1} per dose daily ×5 for 2 consecutive weeks, it demonstrated significant activity against five of eight colon carcinoma models. Rhabdomyosarcomas and two xenografts (Rh 18 rhabdomyosarcoma and VRC_5 colon adenocarcinoma), resistant in vivo to topotecan, were also highly responsive to this schedule (Houghton et al, 1993). To determine whether prolonged periods of treatment were more effective CPT-11 was administered as before as a daily ×5 schedule for 2 weeks, but the cycles were repeated every 21 days for a total of three cycles. The MTD was 10 mg kg^{-1} day^{-1}. Complete regression of all VRC_5 colon tumours was achieved at 5–10 mg kg^{-1} per dose. CPT-11, given as a protracted schedule at 5 mg kg^{-1} day^{-1}, showed greater activity than a shorter intense therapy at 40 mg kg^{-1} per dose.

A single cycle of CPT-11 was only modestly active at a dose of 40 mg kg^{-1} in 4 of 25 Rh 12 rhabdomyosarcoma xenografts whereas three cycles of therapy at 10 mg kg^{-1} day^{-1}, daily × 5, resulted in complete regression in 12 of 13 tumours. Similar results were obtained in colon carcinoma and human brain tumour xenografts (Houghton et al, 1993).

Thus, protracted therapy with low-dose CPT-11 has increased therapeutic efficacy compared with more toxic short-term schedules.

Clinical studies with prolonged or continuous administration

In a phase I study with CPT-11 given as a 5-day continuous infusion every 3 weeks, the dose was escalated from 5 to 40 mg m^{-2} day^{-1} (Ohe et al, 1992) (Table 3). Dose-limiting toxicity consisted of CTC grade III–IV diarrhoea. Toxic effects greater than CTC grade II included diarrhoea (69%), nausea and vomiting (58%), leucopenia (25%), anaemia (25%), thrombocytopenia (6%) and hepatic dysfunction (14%). Diarrhoea was dose dependent, in contrast to the white blood cell nadir, which was not dose dependent (Ohe et al, 1992). In another phase I study, CPT-11 was administered intravenously over 30 min for 3 consecutive days every 3 weeks. Both leucopenia and diarrhoea were dose limiting at a dose of 115 mg m^{-2} day^{-1} (Catimel et al, 1995).

In limited studies with low-dose schedules of CPT-11 once daily × 3, once daily × 5 and twice daily × 7, anti-tumour responses were reported in patients with leukaemia and lymphomas (Ohno et al, 1990; Tsuda et al, 1992).

From small studies in ovarian and cervical cancer, it was suggested that there were no significant differences between schedules concerning efficacy, but clearly these data need further confirmation (Takeuchi et al, 1991*a* and *b*). Response rates in patients with NSCLC treated with CPT-11 at a dose of 200 mg m^{-2} every 3–4 weeks or 100 mg m^{-2} weekly do not seem to differ (Nakai et al, 1991; Negoro et al, 1991). In patients with solid tumours, the dose schedule apparently does not seem to be crucial in efficacy of the drug. However, CPT-11 may have more efficacy when administered at lower doses for a longer time to patients with malignant lymphoma.

An oral formulation of CPT-11 has been tested on a daily × 5 schedule every 3 weeks with diarrhoea and neutropenia as dose-limiting toxicities (Drengler et al, 1996).

GI147211

GI147211, (7-(4-methyl piperazinomethylene)10,11-ethylenedioxy-20-(S)-camptothecin) is a water-soluble analogue of camptothecin. The water-solubilizing groups were introduced on position 7 in the B ring.

Preclinical studies

GI147211 appeared to have anti-tumour activity in vitro as well as in vivo (Emerson et al, 1995). In these studies, the dose schedule of twice a week administration for 5 weeks did not appear optimal. Recent data demonstrate that GI147211 is more active

Table 4 Topoisomerase I inhibitors – continuous/prolonged administration in solid tumours (GI147211)

Drug	Dose (mg m^{-2}) Schedule	Number of of patients	Cp-ss	MTD	DLT	Reference
GI147211 i.v.	0.3–0.5 day^{-1} 7–21 d q 28 d	38	0.1–0.35 ng ml^{-1}	0.5 mg m^{-2} d^{-1} × 21	Neutropenia Thrombopenia	Khater et al (1996)
GI147211 i.v.	72 h q 28 d?	36	–	1.5 mg m^{-2} d^{-1}[a] 2.0 mg m^{-2} d^{-1}[b]	Myelosuppression	O'Dwyer et al (1995)

[a]Chemotherapy pretreated. [b]Non-pretreated. i.v., Intravenous; i.p., intraperitoneal, p.o, oral; MTD, maximum-tolerated dose; DLT, dose-limiting toxicity; Cp-ss, plasma–concentration steady state; –, not stated.

when administered at higher doses using an every 4 days schedule for a total of three doses (Emerson et al, 1994). Again, dose scheduling seems to be important for an optimal anti-tumour effect.

Clinical studies with prolonged or continuous administration

Daily ×5 i.v. administration

Three phase I studies with intravenous GI14721 have been performed, two studies with a 30-min GI147211 infusion once daily for 5 consecutive days every 3 weeks, a third study with GI147211 given as a 72-h continuous infusion (Eckardt et al, 1995; O'Dwyer et al, 1995; Gerrits et al, 1996). In all studies, AUC increased with dose in a linear fashion, and dose-limiting toxicity consisted of leucocytopenia as well as thrombocytopenia. Non-haematological toxicity was mild and there was no diarrhoea or haemorrhagic cystitis. Preliminary results of phase II studies show anti-tumour activity in ovarian cancer and small-cell lung cancer (Wanders et al, 1996).

Prolonged exposure

A phase I study with continuous infusion of GI147211 has been performed with doses ranging from 0.3 to 0.5 mg m^{-2} day^{-1} for 7, 14 and 21 days. DLT reached at 0.5 mg m^{-2} day^{-1} consisted of neutropenia and thrombocytopenia. Non-haematological toxicities CTC grade ≥ II consisted of nausea, vomiting, dyspepsia, fatigue and diarrhoea. Pharmacokinetics of GI147211 showed mean steady-state concentrations ranging from 0.1 to 0.35 ng ml^{-1}. The total body clearance was similar to the clearance with shorter infusions (Khater et al, 1996) (Table 4).

DISCUSSION AND CONCLUSION

Topoisomerase I inhibitors are a class of drugs with a broad anti-tumour activity, even against previously chemotherapy-resistant tumours. The issues concerning drugs scheduling are many, and one of the conclusions from this review could be that there is no true consistency in the use of schedules and models in preclinical studies. It would be worthwhile to try to achieve this consistency in the development of drugs such as these. Clearly, many of the relevant questions on scheduling can already be answered in in vitro studies, such as the ones that have been performed with topotecan. With appropriate in vitro studies, one could easily mimic potential clinical application schedules. Following in vitro studies, in vivo studies could be performed taking the data from the in vitro studies into account. Obviously, long-term infusional application in animal models is difficult to achieve but, on the other hand, many of the performed in vivo studies, because of their diversity, do not result in conclusive evidence. With a consistent approach in preclinical studies, one could also avoid the need to perform too many clinical studies on scheduling. We also recommend performing the clinical phase I and II studies with inclusion of pharmacokinetic/pharmacodynamic (PK/PD) relationship studies. A good example of this can be found in a yet unpublished study relating levels of topoisomerase I inhibitors to parameters such as decreased cleavable complex formation. Making use of the appropriate combinations of clinical studies with PK/PD studies, the number of studies necessary could easily be reduced. Also, such studies would answer the question of whether thresholds exist for the effect of topoisomerase I inhibitors in conjunction with exposure duration. The preliminary results from the above reviewed phase I and phase II studies indicate that prolonged administration with topoisomerase I inhibitors is feasible in patients with cancer. However, unfortunately, the optimal dose and schedule of the various agents available remain to be elucidated. Although preliminary results are encouraging and warrant further clinical exploration, the concept should still be considered as being investigational.

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