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Review

Camptothecin Delivery Methods

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Camptothecin has shown significant antitumor activity to lung, ovarian, breast, pancreas, and stomach cancers. Camptothecin, however, like a number of other potent anticancer agents such as paclitaxel, is extremely water insoluble. Furthermore, pharmacology studies have determined that prolonged schedules of administration given continuously are required. Thus, this insolubility has restricted its clinical application. For these reasons, a number of water-soluble analogs have been synthesized and a number of different formulation approaches have been investigated. In this review, we examine each of these approaches and discuss their advantages and limitations.

KEY WORDS: camptothecin; antitumor activity; solubility; insolubility.

INTRODUCTION

Compounds found in nature display a wide range of diversity in terms of their structures and physical and biologic properties. The function of these compounds in plants, fungi, and marine organisms are still not widely understood. Currently, it is believed that many of these compounds act in defense of the detrimental effects of toxins and/or carcinogens found in the plant or attack by outside predators (1). During the period 1950–1959, Wall and Wani (1) saved thousands of plant ethanolic extracts. One of the extracts saved and stored was prepared from the leaves of *Camptotheca acuminata*, Nyssaceae, a tree that grows in southeastern provinces of China. Camptothecin (the extract) is an indole alkaloid with a pentacyclic ring, which is highly unsaturated (Fig. 1). On treatment with alkali, the compound readily opens forming an open lactone sodium salt shown in Fig. 1 (2). On acidification, the extremely water-soluble sodium salt is readily re-converted to the lactone. However, the parent compound is extremely water-insoluble in virtually all organic compounds except dimethyl sulfoxide (DMSO) in which it exhibits moderate solubility. This insolubility in most biocompatible solvents has made it very difficult to deliver this drug into the body through the conventional routes such as oral, and intravenous or intramuscular injection.

The antitumor activity of camptothecin was demonstrated in a CA-755 (Adenocarcinoma 755) assay (1). This test involves the implantation of a measurable solid tumor into a host mouse and measuring the survival time and inhibition of the tumor growth in response to the anticancer drug. Later, it was shown that camptothecin possesses the ability to halt the growth of a wide range of animal and human tumors and is remarkably active in the life prolongation of mice treated with L1210 leukemia cells (2).

In preliminary pharmacologic and clinical evaluation of camptothecin sodium (1970, Baltimore Cancer Research Center), sixteen patients with various advanced solid tumors were chosen. At intervals of 2–4 weeks they received 35 single injections of 0.5–10.0 mg/kg of camptothecin. In five patients, partial remissions (>50% mass decrease) and in six others objective responses were noted. Most of the responses occurred in patients with advanced and often refractory gastrointestinal carcinoma. Toxicity consisted of alopecia, mild gastrointestinal symptoms, hemorrhagic cystitis, and dose-limiting myelosuppression. These side effects were generally tolerable (3).

Dozens of clinical trials are being conducted worldwide using camptothecin and its derivatives with broad application against a wide variety of tumors. For example, camptothecin treatment of tumor-bearing mice (i.e., lung, ovarian, breast, pancreas, and stomach cancers) resulted in complete remissions in nearly 80% of the lines examined (4). These studies also showed that CPT was more effective than any of the clinically available anticancer drugs tested, which included such drugs as 5-fluorouracil, doxorubicin, methotrexate, vincristine, and vinblastine.

Camptothecin thus appears to be a promising drug. However, it has experienced several barriers in making it into clinical use. In this review, we discuss the mechanism of action and the stability problems of camptothecin, and side effects associated with it. Different delivery strategies that have been examined to solve these problems are discussed in terms of their potential for success.

METHODS

Mechanism of Action

Camptothecin (CPT) induces its cytotoxity by inhibiting both DNA and RNA synthesis in mammalian cells. The in-

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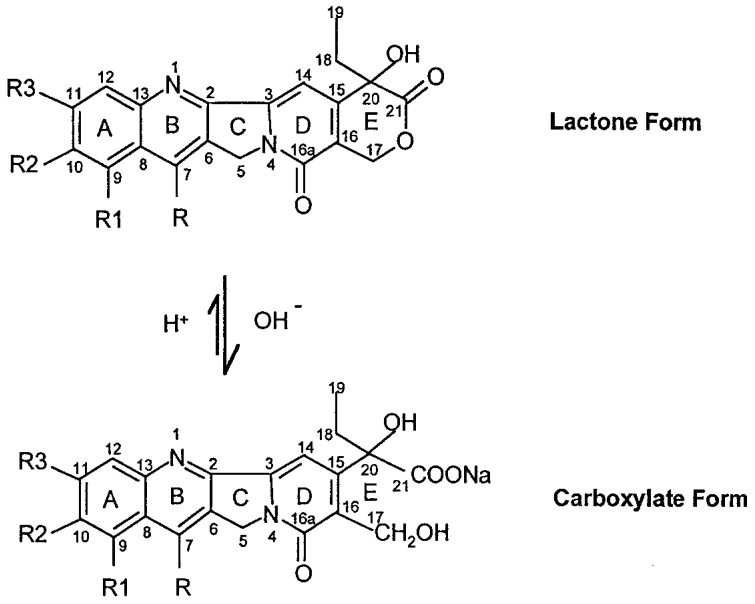

Camptothecin: R=R1=R2=R3=H

10-Hydroxycamptothecin: R=R1=R3=H, R2=OH

10-Methoxycamptothecin: R=R1=R3=H, R2=OMe

9-Nitrocamptothecin: R=R2=R3=H, R1=NO_2

9-Aminocamptothecin: R=R2=R3=H, R1=NH_2

Topotecan: R=R3=H, R1=$CH_2NH(CH_3)_2$

Irinotecan (CPT-11): R1=R3=H, R=C_2H_5, R2=$C_{11}H_{19}O_2$

Fig. 1. Camptothecin (lactone and carboxylate form) and its analogs.

hibition of RNA synthesis results in shortened RNA chains and is rapidly reversible upon drug removal (5). The inhibition of DNA synthesis, on the other hand, is only partially reversible upon drug removal. Another prominent effect of camptothecin is the rapid and reversible fragmentation of cellular DNA in cultured mammalian cells. This is accomplished by stabilization of the binding of topoisomerase I to DNA, leading to DNA fragmentation. Camptothecin blocks the rejoining step of the breakage-rejoining reaction of mammalian DNA topoisomerase I (Topo I).

The various mechanisms of resistance to anticancer drugs reported in the literature have been described (6). Upon exposure to natural product drugs, tumor cells can acquire resistance to structurally and functionally unrelated drugs. The classical form of drug resistance is caused by P-glycoprotein, a protein inserted in the plasma membrane that acts as an ATP driven efflux pump. Acquisition of resistance to CPT does not correlate with the presence of P-glycoprotein. CPT has been shown to bypass the P-glycoprotein mediated multidrug resistance phenotype (7), which limits the long-term usefulness of many antineoplastic agents. With regard to camptothecin, some mammalian cell lines selected for resistance to CPT exhibit decreased levels of topo I, which in turn, results in a decreased number of DNA single-strand breaks compared with wild-type cells. Decreases in topo I levels have also been associated with rearrangement and hypermethylation of the topo I gene, as well as the presence of mutations in the topo I gene (8).

Most recently, camptothecin and camptothecin derivatives are being combined with other chemotherapeutic agents and other therapeutic modalities. Preclinical experimental studies suggest that when combined with other agents, radiation or hyperthermia, camptothecin may demonstrate a synergistic antitumor activity (9). The involvement of topoisomerase I in DNA repair suggests that camptothecins may have clinical application as radiation sensitizers. It has been shown *in vitro* that camptothecins can enhance radiation-induced cytotoxicity (10). At present, phase-I clinical trials are in progress to assess combinations of radiation therapy and topoisomerase I targeting agents.

Other Clinical Applications of CPT

Other than as anticancer agents, another utility for topoisomerase I inhibitors may be their antiviral activity as reported by Takahashi *et al.* (11). Topoisomerase I activity is involved in HIV-reverse transcriptase function. Priel *et al.* (12), showed that inhibition of topo I by CPT can block the development of retroviral-induced malignancies in mice.

Tests of antitumor and antiviral activity of camptothecins in the treatment of HIV-associated malignancies such as Kaposi's sarcoma and HIV-associated lymphoma are in clinical trials. Vollmer-Haase *et al.* (13) also observed that in one patient with progressive multifocal leukoencephalopathy, CPT showed anti-infective activity against the JC papovavirus. In a study by Clements *et al.* (14), it was shown that CPT is not only capable of inhibiting the endothelial cell growth in a non-toxic manner, but also it can inhibit angiogenic growth. This observation demonstrates that besides the tumoricidal activity, CPT may have indirect antitumor activity due its anti-angiogenic activity. This anti-angiogenic activity may have clinical relevance in treating other conditions such as restenosis and psoriasis.

Another application of camptothecin entrapped in liposomes was recently explored against Leishmaniasis by Proulx *et al.* (15). The main reservoirs of parasites in visceral leishmaniasis are macrophages of the liver and spleen. These macrophages have a tendency to take up liposomes. Thus, the use of liposomes represents a strategic approach for accumulation of drugs within these tissues to more efficiently treat this parasitic infection.

Physical Properties of Camptothecin

Camptothecin possesses a high melting point (264–267°C), and has a molecular weight of 348.11 obtained by high-resolution mass spectroscopy, corresponding to the formula $C_{20}H_{16}N_2O_4$ (2). It gives an intense blue fluorescence under UV and is optically active ($[\alpha]^{20}_D$, +31.3°) [chloroform-methanol (8:2)].

Structure-Activity Relationship

One important structural requirement for successful interaction with the topoisomerase I target and antitumor potency *in vivo* is a closed α-hydroxylactone moiety (4). Unfortunately, this functionality hydrolyzes under physiological conditions, i.e., at pH 7 or above, with the lactone ring readily opening to yield the inactive carboxylate form of the drug (16). Ring opening of camptothecin is thought to result in a loss of activity due to the following three reasons. First, the carboxylate form displays decreased membrane associations. Comparison of the membrane affinity of camptothecin with that of its carboxylate form indicates that ring opening produces a greater than two-fold reduction in membrane associations. Second, ring opening results in a charged drug species and charged species exhibit limited diffusibility through cell lipid bilayer domains of low dielectric constant; hence, ring opening of camptothecins results in agents with altered diffusibility characteristics. Third, evidence from cell-free experiments indicates that ring opening results in significantly attenuated activity towards the topoisomerase-I target (16). In summary, decreased cell membrane binding, decreased membrane diffusibility, and decreased intrinsic potency against the topoisomerase target all contribute to explain the reduction in cytotoxic activity that accompanies lactone ring opening of camptothecin.

The second structural requirement for successful interaction with the topoisomerase I target and antitumor potency *in vivo* is for the compound to be in its 20-(S) form. Camptothecin occurs in two different enantiomeric forms 20-S and 20-R indicating the particular arrangement of atoms and groups in space around the chiral center (C_{20}) of this molecule. Wani *et al.* (17) demonstrated that the 20-(R) form of the compound was inactive both in topoisomerase I inhibition and in *in vivo* assays, while the 20-(S) form of the compound had great potency in inhibiting human colon cancer xenografts in nude mice.

Chemical Modification

Camptothecin has also been chemically modified with the aim of finding a derivative with improved chemotherapeutic activity. Modified camptothecins examined with this goal in mind include 9-aminocamptothecin (9-AC) and 9-nitrocamptothecin (9-NC). Substitutions at carbons 9 and 10 (Fig. 1) by amino groups lead to compounds with greater *in vivo* activity. 9-AC is a water-insoluble camptothecin derivative with impressive preclinical activity (18). The results observed in experiments with 9-AC were comparable to those observed in native CPT experimental treatments. However, 9-AC achieved the onset of a complete remission with lower total dose and within a shorter period, and no pattern of emerging resistance was observed in tumor-bearing mice (19). 9-NC is an intermediate of CPT synthetic conversion into 9-AC. This camptothecin analog can also be metabolically converted *in vivo* to 9-AC. In preclinical studies, this analog has shown excellent anticancer activity in nude mice bearing human tumor xenografts (20).

Two other camptothecin derivatives that have been approved in the United States for use in solid tumors are irinotecan (CPT-11) and topotecan (21). CPT-11 and topotecan are both water-soluble and potent derivatives of camptothecin. CPT-11 is a prodrug that possesses limited antitumor activity, but is converted by the enzyme carboxylesterase to a very active compound (SN-38) *in vivo*. SN-38 is reported to be anywhere from 200 to 1000 times more potent than CPT-11 (22). Topotecan was made water-soluble by the presence of a stable, basic side chain at carbon 9 of the A ring. It can be administered without the severe and unpredictable side effects that are associated with camptothecin sodium. Like camptothecin, the lactone ring in topotecan is sensitive to pH change and hydrolyzes into inactive carboxylate form in basic environment. Another analog of CPT is 10-hydroxycamptothecin (HCPT). In a topo I inhibition assay, HCPT has been shown to be more active than either CPT or topotecan. Its IC_{50} (the minimum drug concentration that inhibits cleavable complex formation by 50%) is 0.106 μM, as compared to 0.677 μM (0.236 μg/ml) for CPT, and 1.110 μM for topotecan (23).

Progress has been made via chemical modification of camptothecin to improve its solubility in aqueous media. This is important in terms of delivering this drug in an effective dosage form. However, despite all the chemical modifications specified above, the camptothecin derivatives still contain a terminal lactone ring that makes them susceptible to ring opening in aqueous solutions by undergoing a rapid, pH-dependent, non-enzymatic hydrolysis to form an open ring hydroxy carboxylic acid (16). In each case, the open ring carboxylate form is inactive.

BN 80915 a new camptothecin homologue, wherein a seven-membered β-hydroxylactone replaces the six-membered α-hydroxylactone of the parent compound, has

been reported recently (24). BN 80915 has shown enhanced plasma stability and activity in animal models and represents the only lactone ring modification known that conserves both the capacity to inhibit topo-I and the antitumor activity. This new derivative is not a substrate for P-glycoprotein and MDR[2] protein; the two drug efflux pumps most commonly associated with resistance of tumor cells to antineoplastic agents. BN 80915 hails a new generation of camptothecin derivatives and can be used as a template for the elaboration of new anticancer agents.

Pharmacokinetics

As discussed previously, the equilibrium between lactone and open salt forms of camptothecins is pH-dependent, favoring ring closure with increasing acidity. In addition, this equilibrium between open and closed forms is also affected by the preferential binding of serum albumin to the salt form (25). This preference of albumin for the carboxylate form results in a rapid ring opening of circulating CPT. Other blood components, such as erythrocyte membranes and lipoproteins, bind to CPTs, favoring the closed lactone form (25).

Very little of the chemotherapeutic activity of CPT is associated with the open salt, as illustrated by experiments with this form. A closed lactone ring is an essential pharmacophore for activity against cancer cells. The low anticancer activity of the open salt form in animals also is consistent with the failure of clinical trials with the sodium salt of CPT (26). Owing to the association of the chemotherapeutic activity of CPT with lactone and of mainly toxic responses with the open salt form, the goal of pharmacokinetic and metabolic studies must be to assist in the development of dosing regimens optimal for tumor uptake of CPTs.

In an attempt to establish the optimal schedule of administration numerous trials have been conducted (27). It appears that large doses of camptothecin and its derivatives given at large intervals are not greatly effective. It has been shown that the camptothecins require a prolonged schedule of administration given continuously at low doses or frequently fractioned dosing schedules to spare normal hematopoietic cells and mucosal progenitor cells with low topoisomerase-I levels while preserving efficacy (28). To achieve this type of prolonged schedule of administration, different delivery systems have been designed. In the following we review these systems and discuss the advantages and disadvantages associated with them.

Delivery Systems

The development of new drug delivery systems such as liposomes, microspheres, micelles, and injectable pastes, has received considerable attention in the field of cancer therapy. This interest has been ignited by the advantages these delivery systems possess, such as ease of application, localized delivery for a site-specific action and prolonged delivery periods. Moreover, decreased body drug dosage with concurrent reduction in possible undesirable side effects common to most forms of systemic delivery, and improved patient compliance and comfort have put them in the spotlight. In this part of the review, we outline the different strategies used to stabilize camptothecin in its lactone form. These strategies help to preserve the drug's anticancer activity with a view towards reducing its adverse effects.

Polymer Conjugated Camptothecin

As discussed earlier, camptothecin is extremely insoluble in water and is also insoluble in many organic solvents. This feature has severely restricted its clinical application (29). However, there has been increased interest in pursuing water-soluble CPT derivatives, because of their superior antitumor activity against *in vitro* human cancers and *in vivo* animal xenografts. Most attempts at producing water-soluble derivatives of CPT have been limited to making substitutions on the A and B rings (e.g., 9-AC and CPT-11) (30). These temporary chemical modifications are devised to alter the aqueous solubility and biodistribution of the parent drug, while keeping its inherent pharmacological properties intact. Some of these derivatives show quite good pharmacokinetics and efficacy, but the toxicological effects of these substitutions are controversial (31). These prodrugs are designed to dissociate *in vivo*, in a predictable fashion, to the active drug by either an enzymatic mechanism or simple hydrolysis initiated under physiological pH conditions. Another strategy would be to attach the camptothecin to a polymer carrier.

Greenwald *et al.* (32), attempted to solubilize 20 (S)-camptothecin as a non-ionic α-alkoxy ester transport form (prodrug) and demonstrated enhanced circulatory retention as well as sustained therapeutic release of native drug. This was achieved by condensation of camptothecin with poly(ethylene glycol) (PEG) 40 kDa dicarboxylic acid in the presence of diisopropylcarbodiimide. The result of this synthesis was a mixture of camptothecin mono and disubstituted esters in a ratio of approximately 2:1. All their studies utilized the mixture of mono and disubstituted esters. Examination of the physical properties of the mono and disubstituted esters mixture provided rates of hydrolysis in water, buffer, and rat and human plasma. It was demonstrated that aqueous formulations were able to stand for 24 h with less than 10% hydrolysis occurring due to the low rate of hydrolysis at room temperature. Furthermore, less than 5% loss of PEG camptothecin was observed in aqueous solutions (pH = 5.6) maintained at 4°C for over 6 months. This finding indicates easy formulation and storage of the transport form. *In vitro* P388 cell toxicity for the mixture (IC_{50} = 27nM) revealed no sign of cytotoxicity. *In vivo* studies employing P388-treated mice with this transport form (administered i.p. as an aqueous solution) produced a remarkable rate of 200% increase in life expectancies (ILS) and a cure rate of 80% at a dose of 16 mg/kg camptothecin equivalents with no acute toxicity. Pharmacokinetic studies of the transport form were done (i.v.) using CD-1 mice and displayed a blood $t_{1/2\alpha}$ of less than 15 m, but more significantly a $t_{1/2\beta}$ of 3.6 h, with detectable amounts still present after 24 h. This result confirms that the transport form circulates to release camptothecin over a substantial period of time. Finally, in an experiment performed with the transport form, in PBS buffer containing human serum albumin, results revealed that at physiologic pH the modified lactone ring structure does not engage in hydrolytic ring opening.

Conover *et al.* (33), continued their studies to assess the efficacy of PEG-α-conjugated camptothecin (prodrug, Fig. 2). Circulatory retention studies were performed in non-tumor bearing mice injected intravenously with 300 mg/kg of PEG-

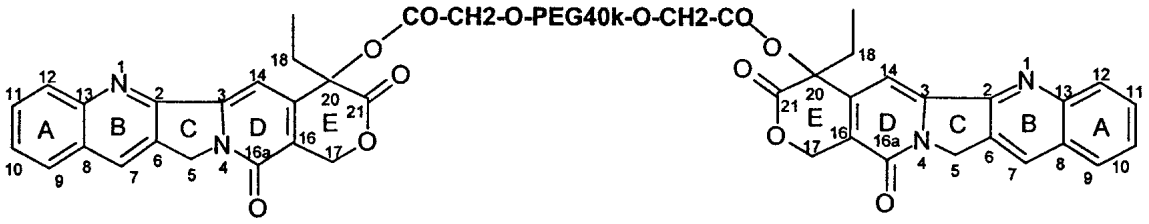

Fig. 2. Chemical structure of PEG-α-CPT. Reproduced from reference 33.

α-camptothecin. The results revealed that PEG-α-CPT was retained in the circulation for a prolonged period and this was attributed to the high molecular weight of PEG. Therapeutic efficacy was evaluated in both a murine P388/0 leukemia and a colorectal HT-29 carcinoma xenograft model. Five intraperitoneal injections of 3.2 mg/kg/day 20-(S)-camptothecin equivalents of PEG-α-camptothecin in their leukemia model resulted in significant survival over untreated controls ($p < 0.001$), with a mean time to death of treated vs. control of 2.94 and a cure rate of 80% ($n = 20$). The colorectal carcinoma xenograft model demonstrated that 2–3 mg/kg/day 20-(S)-camptothecin equivalents of PEG-α-camptothecin given 5 days a week for 5 weeks could reduce an initial tumor burden of 300 mm^3 by more than 90% without any signs of overt toxicity. It was concluded that a water-soluble polymeric transport carrier for CPT, based on poly(ethylene glycol) esters, both stabilizes and extends the circulatory exposure of CPT. In addition, PEG may function to decrease the toxicity and increase the therapeutic efficacy of CPT by a combination of lactone stabilization and slow release. In both studies mentioned earlier a heterogeneous mixture of mono and disubstituted ester prodrug of PEG-α-camptothecin were used and therefore are not clinically suitable. Moreover, with respect to patient compliance, daily injection of this type of dosage form may be considered as a disadvantage.

Conover *et al.* (34), prepared a new homogeneous form of disubstituted CPT, PEG-β-camptothecin. The employment of a bifunctional spacer group (glycine) in the PEG prodrug strategy yielded a water-soluble non-ionic α-amidoester prodrug. *In vitro* P388 (Leukemia cells) cell toxicity for PEG-β-camptothecin (IC_{50} = 12 nM) was in the expected range for a prodrug that releases CPT (IC_{50} = 7 nM). The *in vitro* half-life of hydrolysis of PEG-β-CPT to CPT at 37°C is 40 h in pH 7.4 phosphate buffer and 6 h in rat plasma. In this study, the *in vivo* circulatory retention, antitumor activity, and tissue biodistribution of PEG-conjugated camptothecin-20-O-glycinate was evaluated. Nontumor-bearing mice injected intravenously with 875 mg/kg of PEG-β-CPT were employed for circulatory retention studies. Antitumor activity of the prodrug in nude mouse xenograft models was determined both intraperitoneally and intravenously. Biodistribution studies were performed in nude mice bearing colorectal carcinoma xenografts with tritium-labeled PEG-β-CPT and CPT injected intravenously. PEG-β-CPT showed a blood $t_{1/2\alpha}$ of approximately 6 m and a $t_{1/2\beta}$ of 10.2 h and significant antitumor activity was seen in all treated xenograft models. Ideally, it would be desirable for release of the drug to occur only in the vicinity of tumor cells, thereby sparing normal cells from concomitant destruction. By using labeled CPT, it appeared that more labeled CPT accumulated in solid tumors when delivered in the PEG-β-CPT form. This greater preference for tumor tissue was at least ten times more than normal tissue. It has already been demonstrated that macromolecules greater than roughly 50 kDa, circulating for extended periods, show substantial tumor accumulation (35). One reason for this enhanced accumulation of drug is the greater permeability of neovasculature in tumors and the second reason is the absence of effective lymphatic drainage in tumor tissue (36). The combination of increased tumor vascular permeability with insufficient tissue drainage results in what is termed "the enhanced permeability and retention effect", which is thought to be a universal solid tumor phenomenon for macromolecular drugs (35). Therefore, by conjugating PEG to CPT-20-O-glycinate a homogenous water-soluble prodrug was produced with an extended circulatory life and altered biodistribution. This modification generates greater tumor accumulation as compared to unconjugated CPT, and produces significant antitumor activity. Since the amount and form of the CPT reaching the tumor site can be affected by the use of different amino acids within the PEG-CPT conjugate, further studies are necessary.

An investigation was undertaken by Conover *et al.*, (37) to determine the impact of various amino acid spacers on the activity of PEG-CPT conjugates. Using the P388/0 murine leukemia cell line, the *in vitro* biologic efficacy of the PEG-conjugated CPT compounds was tested. The kinetic properties for the PEG conjugate series and the cytotoxicities of CPT and the PEG-amino acid conjugates are shown in Table I. In PBS (pH 7.4) at room temperature, among the PEG-amino acid conjugates tested, the proline, alanine, and leucine derivatives appeared quite stable. A murine ascite model

Table I. Rates of Hydrolysis and IC50 Values of PEG-Amino Acid-CPTs. Reproduced from Reference 37

Amino acid spacer	IC_{50} (nM)[a]	% buffer hydrolysis in 24 h[b]	t1/2 (h)[c] Rat plasma	t1/2 (h)[c] Human plasma
None (native CPT)	7			
Glycine	34	>10	9	4
Alanine	16	<10	15	7
Phenylalanine	27	>10	9	2
Methionine	7	>10	6	3
Glutamate (Ome)	16	>10	21	9
Leucine	358	<10	25	4
Proline	3000	<10	113	6

[a] All experiments were carried out in duplicate on P388/0 cells:STD of measurement ± 10.

[b] PBS buffer (pH 7.4) at 37°C.

[c] These results represent the half lives (±10) by disappearance of the PEG-conjugates.

against mouse lymphoid neoplasm cells (P388/0) was used to monitor and assess the *in vivo* anticancer activity of the synthesized PEG-amino acid conjugates. Mice were injected with P388/0 cells and then treated i.p. for five consecutive days. The *in vivo* screen results are shown in Table II. The 20 mg/kg total dose level of native CPT was toxic with a mean time to death of 8.1 days. This resulted in an ILS (Increase in Life Span) of –35.2% with no cures. Significant antitumor activity was observed against HT-29 (human colon cancer cells), A549 (human lung carcinoma cells), SKOV3 (human ovary adenocarcinoma cells), PC-3 (human prostate carcinoma cells), LS174T (human colon adenocarcinoma cells), MIA-PaCa-2 (human pancreas carcinoma cells), and M109 (Madison lung carcinoma cells) tumors in mice when treatment regimens of PEG-alanine-CPT were used. The results showed that both the PEG-CPT conjugates' degradation and *in vivo* activity were altered by using specific amino acid spacers. Different mechanisms such as simple changes in circulatory dissociation rates to more complicated intratumoral, extracellular or even intracellular release can be responsible for differences in *in vivo* activities of PEG-CPT conjugates.

Recently, Caiolfa *et al.* (38), synthesized two soluble N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers to contain CPT (5 wt % and 10 wt %). The α-hydroxyl group of CPT was linked to the polymers through a Glycine-Phenyl alanine-Leucine-Glycine spacer. This arrangement first makes it possible for esterases and proteases at the tumor site to cleave the drug from the polymer and release it slowly, and second prevents this water-soluble derivative of CPT in plasma from deactivating. To determine the tumor and tissue distribution, nude mice bearing HT29 human colon carcinoma were injected intravenously with radiolabeled free and bound CPT. Antitumor activity of the CPT-conjugates was also followed *in vivo* on the same animal model.

Results showed that, *in vitro*, CPT-conjugates were fairly stable in simulated physiologic fluids and plasma and native body enzymes such as elastase and cysteine-proteases were able to release the active drug. Total cell exposure to the drug released from polymer conjugates and free unconjugated CPT was measured. It was shown that the cell exposure to CPT was always 3- to 7-fold lower in the case of polymer conjugate than that measured after administration of unconjugated CPT. To assess the biodistribution of the conjugates, HT29 human colon carcinoma bearing mice were injected intravenously with [^{3}H]CPT-conjugate and free [^{3}H]CPT. More than 90% tumor inhibition, some complete tumor regressions, and no toxic deaths were observed after repeated intravenous administration of CPT-conjugates.

In a series of studies by Harada *et al.* (39,40), therapeutic efficacy, tumor targeting, drug release kinetics, and dose dependent pharmacokinetics of T-0128 were evaluated. T-0128 is a macromolecular prodrug comprised of T-2513 (7-ethyl-10-aminopropyloxy-CPT) bound to carboxymethyl dextran through a Gly-Gly-Gly linker. This molecule has a molecular weight of 130 kDa with a weak anionic charge, which is big enough not to be excreted from the kidneys. Macromolecules greater than 70 kDa with weak anionic charges are shown to circulate in the blood for a long time. It was shown that the triplet glycine (Gly) linker could be cleaved by lysosomal cathepsin B to release T-2513 slowly and steadily, which resulted in improved therapeutic efficacy.

To increase the stability of lactone ring and efficacy of CPT *in vivo*, Singer *et al.* (41,42) investigated the conjugation of hydroxyl group positioned at carbon 20 in CPT structure to poly(L-glutamic acid). Poly(L-glutamic acid) has many advantages over the other currently available polymers. Due to its anionic characteristic it enhances the solubility of CPT. It is biodegradable and has multiple available conjugation sites for drugs allowing for higher drug loading concentration. This conjugate showed an increased maximum tolerated dose and substantial antitumor activity. Application of conjugated polymers to increase the solubility of camptothecins has not only been limited to CPT, but other derivatives of CPT such as 9-AC have been explored as well (43). In a study by Leu *et al.* (44), glucoronide derivative of 9-AC was prepared, which was 80 times more soluble than CPT in pH 4 (Fig. 3). 9-AC and glucuronidic acid were linked via a self-immolative carbamate spacer. Glucuronide prodrug was selected due to two important reasons. First, the hydrophilicity of this functional group and second the glucuronide prodrug can be selectively

Table II. Activity of PEG-CPT Derivatives against P388/0 Leukemia *in Vivo*. Reproduced from Reference 37

Test compound	Total dose[a] (mg/kg)	Medium time to death (days) [cures/group]	Mean time to death (days)[b]	%ILS[c]
Control		12.0 [0/10]	12.5 ± 1.3	
CPT	20[d]	8.5 [0/10]	8.1 ± 1.3	–35.2
PEG-gly-CPT	20	40.0 [6/10]	28.2 ± 15.3	125.6
PEG-ala-CPT	20	22.0 [0/10]	21.7 ± 1.6	73.6
PEG-phe-CPT	20	27.0 [1/10]	26.8 ± 8.6	114.4
PEG-met-CPT	20	32.0 [4/10]	31.1 ± 10.0	148.8
PEG-glu-CPT	20	20.0 [0/10]	19.7 ± 0.7	57.6
PEG-leu-CPT	20	22.0 [0/10]	22.4 ± 1.0	79.2
PEG-pro-CPT	20	13.0 [0/10]	13.1 ± 1.2	4.8

Derivatives were given daily (i.p. ×5), following an injection of P388/0 cells into the abdominal cavity with survival monitored for 40 days.
Animals surviving at 40 days were considered cured.
[a] Equivalent dose of CPT
[b] Kaplan-Meier estimates with survivors uncensored.
[c] ILS is (T/C-1) × 100
[d] Toxic dose level

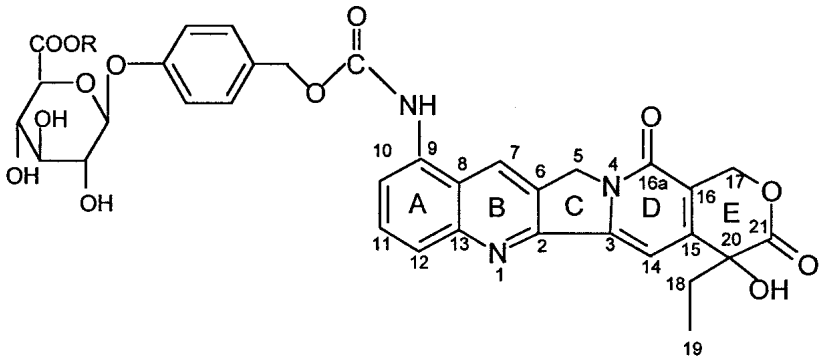

Fig. 3. Glucuronidic acid conjugated to 9-AC (glucuronide prodrug). Reproduced from reference 44.

activated at tumor cells targeted with β-glucuronidase antibody conjugates.

Overall, polymer bound camptothecin has shown a clear advantage in terms of improving drug stability, tumor accumulation, and sustained release over the unconjugated free drug. Toxicity data on some of the conjugated polymers and spacers is still required. Further studies to determine the pharmacological characteristics and therapeutic window of these CPT-conjugates are necessary. One drawback is that they still have the requirement for frequent administration to be effective (once a day for 5 days) and although enhanced tumor accumulation occurs there is still a significant amount of the drug not reaching the tumor.

Micro- and Nanoparticle Encapsulated Camptothecin

The development of controlled release formulations which consist of encapsulated CPT seems to be a promising strategy, especially with the use of biodegradable and biocompatible poly(lactide-co-glycolide) (PLGA). These polymer devices are designed to be implanted or injected directly at the tumor site. Possibilities for the local administration of microspheres or nanoparticles containing CPTs are direct injection into a tumor (45), or implantation during surgery (46). There are a few tumors that can be accessible by the first route. Examples of these are those found in the prostate, skin, and oral cavity. Additionally, there are times that the complete removal of cancer cells is not possible even after surgical excision. In this case, local application of microspheres/nanoparticles that are capable of slowly releasing a drug for an extended period after surgery may increase the probability of complete regression of residual cancer cells (47,48).

CPT is released from these polymer devices by a combination of diffusion and polymer degradation. PLGA is composed of lactic and glycolic acids and can be obtained in many different molecular weights. A free carboxylic end group can be found at the end of each PLGA polymer chain. When hydrolyzed, PLGA is degraded into acidic oligomers and monomers. This hydrolysis step involves the imbibition of water into the polymer system, which in turn causes the device to swell. As a result of the degradation and imbibition of water, the microenvironmental pH within large specimens of PLGA is acidic (49). Since CPT is in its active lactone form at low pH, this phenomenon may favor the stabilization of camptothecin within the delivery device.

Shenderova *et al.* (50), utilized PLGA microspheres as 10-Hydroxycamptothecin slow-release carriers. An oil-in-water emulsion-solvent evaporation method was used to encapsulate 10-HCPT in PLGA 50:50 (50-mole % lactic acid) microspheres. They observed that the lactone form of the drug was retained within PLGA microspheres for more than 10 weeks (>95% lactone) under a simulated physiological environment and it was concluded that PLGA microspheres have the ability to stabilize 10-HCPT within the device prior to being released. Application of PLGA for the stabilization of 10-HCPT has been demonstrated in another work by Mallery *et al.* (51) in a murine human oral squamous cell carcinoma regression model. Therefore, PLGA has the potential to stabilize other analogues within this class of chemotherapeutic agents such as CPT, topotecan, and irinotecan.

Based on this work, Ertl *et al.* (52), developed CPT-containing-microspheres with sustained release properties by varying the method of solvent evaporation to study the influence of drug-loading on microsphere size, encapsulation efficiency and especially the release profile of the incorporated drug. In this study a special type of PLGA, the H-series, containing more carboxylic end chains than the non-H-series, was selected for encapsulation of CPT. Stabilization of the CPT-lactone during release from H-PLGA microspheres as well as during the encapsulation process was investigated. The results showed that CPT was molecularly dispersed in the PLGA matrix at 1.2% drug load and above this concentration, CPT crystals started to form in the polymer matrix. The release profile of CPT from the PLGA microspheres was biphasic, comprising a burst delivering 20–35% of the total drug mass within the first 5 h, the amount of drug released in this burst phase increasing with drug loading. The burst phase was followed by exponentially declining delivery of CPT releasing a total of 40–75% of the originally loaded drug within 100 h. It was determined that the CPT-lactone form was maintained during preparation and storage.

Another possible strategy is to use CPT-loaded nanoparticles as injectable and targetable delivery systems. For example, Yang *et al.* (53), investigated the specific passive drug targeting of CPT after intravenous injection by incorporation into solid lipid nanoparticles (SLN). A CPT loaded SLN suspension was prepared by high-pressure homogenization. This suspension consisted of 0.1% (w/w) camptothecin, 2.0% (w/w) stearic acid, 1.5% (w/w) soybean lecithin and 0.5% (w/w) poloxamer™ 188. *In vitro* drug release was investigated in pH 7.4 phosphate buffer saline at 37°C and the results showed that this system was capable of releasing the drug for up to a week. In tested organs such as the liver, heart, spleen, lung,

kidney, and brain the mean residence times of CPT-SLN were much higher than those of a camptothecin control solution (more than 10%), especially in the brain and heart. The release of CPT from CPT-SLN exhibited a diffusional release profile and the CPT-lactone form was protected from hydrolyzing to the carboxylate form. It was concluded that the SLN may allow a reduction in dosage and a decrease in systemic toxicity, and thus may be a promising carrier and drug targeting system for lipophilic antitumor drugs. Unfortunately, a lack of toxicity data and the non-selective accumulation of drug in vital organs are some important disadvantages associated with this system.

In an attempt to improve the dissolution rate and cytotoxicity of camptothecin, it was incorporated into oxidized-cellulose (OC) microspheres by a spray drying method (54). Oxidized cellulose with different carboxylic content (7%, 13%, and 20%) was used to prepare microspheres. The size of microspheres were reported to be in the range of 1.25–1.52 ± 0.4 μm. The results of release studies in buffer pH 7.4 revealed that the dissolution of CPT was faster from microsphere formulations compared to physical mixtures and free CPT. In *in vitro* cytotoxicity studies against human derived RPMI-8402 lymphoid and THP-1 myeloid leukemia cell lines, more effective results were observed from OC microsphere formulations of CPT in comparison to free CPT. Unfortunately, the time to release 50% CPT from OC-microspheres was between 19–37 h, which may not satisfy the need for long-term drug release for optimum antineoplastic activity *in vivo*.

Liposomes, Micelles, Miniemulsions

Early studies of CPT and lipid based formulations demonstrated that the insoluble parent compound CPT, was readily soluble in various lipids while maintaining its biologic activity (55,56). These findings suggest that liposomes or emulsions may be an effective delivery vehicle for these drugs.

It has been demonstrated that camptothecin binds to membranes by intercalating between the acyl chains of the phospholipid membrane while at the same time it remains stable (57). To prove this, stability profiles were determined for CPT and its analogues both free in solution and bound to dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG) bilayers. Due to the drug's lactone ring penetration into the liposome's lipid bilayer, and hence reduced contact with water molecules, the lipid bilayer can keep CPT in its active lactone form. This approach may be applied for increasing the half-life of the biologically active lactone form of the intercalated agent in blood circulation (58,59).

Lundberg (1998) devised oleic acid esters of CPT and analogues such as 10-HCPT and SN-38, which were capable of being incorporated into liposome bilayers and submicron lipid emulsions (60). This esterification process with oleic acid was chosen to make more lipophilic derivatives of the parent drugs. Small unilamellar liposomes and submicron lipid emulsions were prepared from DPPC (dipalmitoyl phosphatidylcholine), PEG and polysorbate 80 and characterized for size and colloidal stability. Subsequently, the esterified CPT and its analogues were incorporated into the liposomes with the mean efficiency ranging from 1.4% to 10.5%. The *in vitro* cytotoxic action of the parent drugs added as DMSO-ethanol solutions and their ester derivatives in liposomes and lipid emulsions was evaluated. In terms of activity, both parent drugs and the corresponding fatty acid derivatives were very similar. The esters in lipid carriers showed more activity against T-47D (breast cancer cells) and Caco 2 cells (colon adenocarcinoma cells) than the parent drugs, with IC_{50} values of ~1 and 3μM, respectively.

Application of the 9-NC derivative delivered by a liposome aerosol has previously been reported in the treatment of human breast, colon, and lung cancer xenografts in nude mice (61,62). Following this work, Koshkina *et al.* (63), analyzed the pharmacokinetics and tissue distribution of inhaled CPT formulated in dilauroylphosphatidylcholine (DLPC) liposomes. It was speculated that by depositing CPT within the lungs where there is little albumin (64) and with rapid transit to sites of cancer, more of the active lactone form of the drug would reach the tumor cells. Table III shows a quantitative comparison of aerosol dosing with previously reported oral, intravenous, and intramuscular dosing of CPT in mice (65). The aerosol consisted of CPT in liposomes, while for the other routes of administration CPT was dispersed as a fine emulsion in Intralipid 20 (soybean oil + egg yolk phospholipids + glycerol). Table III shows that the tissue concentrations of CPT were considerably greater after aerosol treatment at 30 min than after administration by the other routes. At 120 min, the drug concentrations in mice treated by the non-

Table III. Concentration of CPT in Five Organs and Tumors of Mice Treated for 30 min with CPT Liposome Aerosol at a Dose of 0.081 mg/kg and in Five Organs and Tumors of Mice Treated by the Oral, Intravenous and Intramuscular Routs with Tritiated CPT in a Lipid Dispersion at a Dose of 4 mg/kg. Reproduced from Reference 63

	Aerosol		Oral		Intravenous		Intramuscular	
Organ	30 min	90 min	30 min	90 min	30 min	90 min	30 min	90 min
Lungs	310	17	43	5.3	32	36	44	170
Tumor	26	6.8	31	6.0	36	56	42	57
Brain	61	9.0	37	2.7	12	4	27	74
Blood	26	3.2	39	5.3	23	19	60	144
Liver	103	42.9	137	21.3	50	54	105	118
Kidney	95	7.2	243	210	109	146	176	294
Mean	104	14.4	88	42	44	53	76	143
SD	106	14.7	86	83	35	50	56	85

aerosol routes were three to ten times greater in the non-aerosol treated animals. Therefore, it was concluded that despite the 50-fold greater dose of the drug given in non-aerosol groups, the aerosol provided quicker penetration and relatively larger concentrations in the five organs and tumors of the mice that were examined. This study clearly suggests that liposomal aerosol delivery of CPT has advantages over the other routes of administration (i.e., oral, i.m., and i.v.) due to two reasons. First, the low concentration of albumin in tracheal and bronchial surface liquids is 1–2% of the albumin concentration in plasma (0.5–0.7 mg/ml). Second, the faster penetration of drug into the tumor tissues may help to preserve a more lactone form of the drug.

Following their study, Koshkina *et al.* (66), modulated the respiratory physiology through the addition of 5% CO_2 to the air source used to generate CPT entrapped liposome aerosols. The CO_2 enriched aerosol not only increased pulmonary ventilation but also increased deposition of the inhaled particles. Results of this study showed that a significantly higher concentration of CPT was found in mice organs exposed to 5% CO_2-air aerosols compared to organs of mice exposed to normal air aerosols.

Other advantages of using liposomes for CPT delivery to the body are: (a) enhancement of drug cellular internalization, (b) decreased unwanted systemic toxicity, and (c) increased drug solubility in biologic fluids. Despite all these advantages, these systems still suffer from a lack of selectivity in targeting tumor cells and although the efficiency of CPT delivery into the tumor tissues has been improved, the need for sustained delivery of the drug in low dosages for a long period of time is still unsatisfactory. The leakiness of liposomes, their clearance by the circulatory macrophages, and relatively short half-life is a major disadvantage of this type of delivery system.

In addition to liposomes, micelle solutions and microemulsions have also been proposed as efficient strategies for drug delivery. The solubilization capacity, simple method of preparation, and potential increase in the permeability of the drug through biologic membranes by the micellar solutions and microemulsions offer some potential advantages as delivery systems (67).

Different formulations of CPT were designed by Cortesi *et al.* (67), first to increase the solubility of camptothecin in body fluids and second to reduce the toxicities associated with the administration of this drug. In their work they described (a) the preparation and characterization of liposome-associated CPT, (b) the preparation of micellar solutions and microemulsions containing CPT, and (c) the *in vitro* performances of these three delivery systems on cultured human leukemic K562 cells. The results revealed that the prepared microemulsion composed of a surfactant (Labrasol®), oil (isostearyl-isostearate), and water was stable, single phase, transparent liquid system, and optimal formulation for CPT. All three micellar solutions, liposomes, and microemulsion were able to release CPT effectively and showed antiproliferative activity on K562 cells. Their efficacy was similar or slightly enhanced in comparison with that exerted by the free drug.

Recently, amphiphilic diblock copolymer micelles have received considerable attention among fellow scientists due to their capability to solubilize hydrophobic drugs and increase their circulation time (68). The hydrophobic drugs can be either covalently attached to block copolymers to form micellar structures or can be physically incorporated inside the hydrophobic moiety of polymeric micelles (69). For example, Kowan *et al.* (69), used the diblock copolymer, poly(aspartic acid)-block-polyethylene glycol as a micellar carrier for the anticancer drug adriamycin for intravenous administration. In another study by Zhang *et al.* (70), a diblock copolymer of poly(DL-lactide)-block-(methoxy polyethylene glycol) was investigated as a potential micellar solubilizer and carrier for taxol. It was shown that factors such as higher poly(DL-lactide) (PDLLA) content and higher molecular weight of the copolymer have a significant role in the solubilization of taxol. This result places emphasis on the fact that taxol interacts strongly with the hydrophobic PDLLA segment of the micelle. These studies point to the future direction of new camptothecin delivery systems using micelles, which may emerge in near future.

CONCLUSIONS

Recently, numerous studies have focused on designing new drug delivery systems for camptothecin and other antitumor drugs. This effort is mainly done because of the scarce selectivity and high toxicity that characterize many antitumor drugs. Due to this fact, many of these drugs, which are presently in clinical use, are limited in their dosage and effectiveness. In this respect, the preparation and characterization of specialized delivery systems, such as liposomes, microspheres, microemulsions, micellar solutions, and polymer conjugated CPT, were proposed. This could be taken as a starting point for future utilization in experimental therapy.

REFERENCES

1. M. E. Wall and M. Wani. Camptothecin and Taxol: Discovery to clinic. *Cancer Res.* **55**:753–760 (1995).
2. M. E. Wall and M. Wani. Plant antitumor agents I. The isolation and structure of camptothecin, a novel alkaloid leukemia and tumor inhibitor from Camptotheca acuminata. *J. Am. Chem. Soc.* **88**:3888–3890 (1966).
3. Y. Sugimoto and S. Tsukahara. Decreased expression of topoisomerase I in camptothecin-resistant tumor cell lines as determined by a monoclonal antibody. *Cancer Res.* **50**:6925–6930 (1990).
4. B. C. Giovanella and H. R. Cheng. Complete growth inhibition of human cancer xenografts in nude mice by treatment with 20-S-camptothecin. *Cancer Res.* **51**:3052–3055 (1991).
5. H. T. Abelson and S. Penman. Selective interruption of high molecular weight RNA synthesis in Hela cells by camptothecin. *Nature New Biol.* **237**:144–146 (1972).
6. W. B. Pratt and R. W. Ruddon. *The anticancer drugs*, The Oxford University Press, New York, 1994.
7. M. Potmesil. Camptothecins: From bench research to hospital wards. *Cancer Res.* **54**:1431–1439 (1994).
8. I. Madelaine and S. Prost. Sequential modifications of topoisomerase I activity in a camptothecin resistant cell line established by progressive adaptation. *Biochem. Pharmacol.* **45**:339–348 (1993).
9. P. Pantazis. Preclinical studies of water insoluble camptothecin congeners: cytotoxicity, development of resistance and combination treatments. *Clin. Cancer Res.* **1**:1235–1244 (1995).
10. M. R. Mattern, G. A. Hofmann, F. L. McCabe, and R. K. Johnson. Synergistic cell killing by ionizing –radiation and topo-I inhibitor topotecan. *Cancer Res.* **51**:5813–5816 (1991).
11. H. Takahashi, M. Matsuda, A. Kojima, T. Sata, T. Andoh, T. Kurata, K. Nagashima, and W. W. Hall. Human immunodeficiency virus type-I reverse transcriptase-Enhancement of activity by interaction with cellular topo-I. *Proc. Natl. Acad. Sci.* **92**:5694–5698 (1995).
12. E. Priel, E. Aflalo, G. Chechelnitsky, D. Benharroch, M. Aboud,

and S. Segal. Inhibition of retrovirus-induced disease in mice by camptothecin. *J. Virol.* **67**:3624–3629 (1993).
13. J. Vollmer-Haase, P. Young, and E. B. Ringelstein. Efficacy of camptothecin in progressive multifocal leucoencephalopathy. *Lancet* **349**:1366–1370 (1997).
14. M. K. Clements, C. B. Jones, M. Cumming, and S. S. Daoud. Antiangiogenic potential of camptothecin and topotecan. *Cancer Chemotherp. Pharmacol.* **44**:411–416 (1999).
15. M. E. Proulx, A. Desormeaux, J-F. Marquis, M. Olivier, and M. G. Bergeron. Treatment of visceral leishmaniasis with sterically stabilized liposomes containing camptothecin. *Antimicrob. Agents Chemother.* **45**:2623–2627 (2001).
16. C. Jaxel and K. W. Kohn. Structure activity study of the actions of camptothecin derivatives on mammalian topoisomerase I. *Cancer Res.* **49**:5077–5082 (1989).
17. M. C. Wani and A. W. Nicholas. Plant antitumor agents 28. Resolution of a key tricyclic synthon: total synthesis and antitumor activity of 20-(S)- and 20-(R)-camptothcin. *J. Med. Chem.* **30**: 2317–2319 (1987).
18. C. H. Takimoto and J. Wright. Clinical applications of the camptothecins. *Biochem. Biophys. Acta* **1400**:107–119 (1998).
19. M. Potmesil, M. E. Wall, M. Wani, R. Silber, J. S. Stehlin, and B. C. Giovanella. 9-amino and 10,11-methylenedioxy camptothecins in the treatment of human cancer xenografts. *Proc. Am. Assoc. Cancer Res.* **32**:337 (1991).
20. P. Pantazis, J. A. Early, A. J. Kozielski, J. T. Mendoza, H. R. Hinz, and B. C. Giovanella. Regression of human breast carcinoma tumors in immunodeficient mice treated with 9-nitrocamptothecin: Differential response of nontumorigenic and tumorigenic human breast cells in vitro. *Cancer Res.* **53**:1577–1582 (1993).
21. G. J. Creemers, B. Lund, and J. Verweij. Topoisomerase I inhibitors: topotecan and irenotecan. *Cancer Treatment Rev.* **20**:73–96 (1994).
22. Y. Kawato, M. Aonuma, and Y. Hirota. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res.* **51**:4187–4191 (1991).
23. R. Zhang, Y. Li, Q. Cai, T. Liu, H. Sun, and B. Chambless. Preclinical pharmacology of the natural product anticancer agent 10-hydroxycamptothecin, an inhibitor of topo I. *Cancer Chemother. Pharmacol.* **41**:257–267 (1998).
24. A. K. Larsen, C. Gilbert, G. Chyzak, S. Y. Plisov, and I. Naguibneva. Unusual potency of BN 80915, a novel fluorinated E-ring modified camptothecin, toward human colon carcinoma cells. *Cancer Res.* **61**:2961–2967 (2001).
25. Z. Mi and T. G. Burke. Differential interactions of camptothecin lactone and carboxylate forms with human blood components. *Biochemistry* **33**:10325–10336 (1994).
26. F. M. Muggia and P. J. Creaven. Phase I clinical trial of weekly and daily treatment with camptothecin: Correlation with preclinical studies. *Cancer Chemother. Rep.* **56**:515–521 (1972).
27. E. Rowinsky, L. Grochow, C. B. Hendricks, D. Ettinger, A. A. Forastier, L. A. Horowitz, and R. C. Donehower. Phase I and pharmacologic study of Topotecan, a novel topoisomerase I inhibitor. *J. Clin. Oncol.* **10**:647–656 (1992).
28. J. O'Leary and F. M. Muggia. Camptothecins: A review of their development and schedules of administration. *Eur. J. Cancer* **34**: 1500–1508 (1998).
29. M. Wall and M. Wani. Camptothecin and taxol: from discovery to clinic. *J. Ethnopharmacol*. **51**:239–254 (1996).
30. W. Kingsbury and J. Boehm. Synthesis of water soluble (aminoalkyl)camptothecin analogues: Inhibition of topoisomerase I and antitumor activity. *J. Med. Chem.* **34**:98–107 (1991).
31. J. Supko and L. Malspeis. Pharmacokinetics of the 9-amino and 10,11-methylenedioxy derivatives of camptothecin in mice. *Cancer Res.* **53**:3062–3069 (1993).
32. R. B. Greenwald and C. W. Gilbert. Drug delivery systems: water soluble Taxol-2-poly(ethylene glycol) ester prodrugs-design and in vivo effectiveness. *J. Med. Chem.* **39**:424–431 (1996).
33. C. D. Conover, A. Pendri, and R. B. Greenwald. Camptothecin delivery systems: antitumor activity of a camptothecin-20-0-polyethylene glycol ester transport form. *Anticancer Res.* **17**: 3361–3368 (1997).
34. C. D. Conover and R. B. Greenwald. Camptothecin delivery systems: enhanced efficacy and tumor accumulation of camptothecin following its conjugation to polyethylene glycol via a glycine linker. *Cancer Chemother. Pharmacol.* **42**:407–414 (1998).
35. H. Maeda and L. Seymour. Conjugates of anticancer agents and polymers: advantages of macromolecular therapeutics in vivo. *Bioconjug. Chem.* **3**:351–358 (1992).
36. H. Dvorak and J. Nagy. Identification and characterization of the blood vessels of solid tumors that are leaky to circulating macromolecules. *Am. J. Pathol.* **138**:95–104 (1988).
37. C. D. Conover and R. B. Greenwald. Camptothecin delivery systems: the utility of amino acid spacers for the conjugation of camptothecin with polyethylene glycol to create prodrugs. *Anti-Cancer Drug Design* **14**:499–506 (1999).
38. V. R. Caiolfa, M. Zamai, A. Fiorino, E. Frigerio, C. Pellizzoni, R. d'Argy, A. Ghiglieri, MG. Castelli, M. Farao, E. Pesenti, M. Gigli, F. Angelucci, and A. Suarato. Polymer-bound camptothecin: initial biodistribution and antitumour activity studies. *J. Control. Release* **65**:105–119 (2000).
39. M. Harada, H. Sakakibara, T. Yano, T. Suzuki, and S. Okuno. Determinants for the drug release from T-0128, camptothecin analogue-carboxymethyl dextran conjugate. *J. Control. Release* **69**:399–412 (2000).
40. M. Harada, J.-I. Murata, Y. Sakamura, H. Sakakibara, S. Okuno, and T. Suzuki. Carrier and dose effects on the pharmacokinetics of T-0128, a camptothecin analogue-carboxymethyl dextran conjugate, in non-tumor and tumor-bearing rats. *J. Control. Release* **71**:71–86 (2001).
41. J. W. Singer, P. de Vries, R. Bhatt, J. Tulinsky, P. Klein, C. Li, L. Milas, R. A. Lewis, and S. Wallace. Conjugation of camptothecin to poly-(L-glutamic acid). *Annal. NY Acad. Sci.* **922**:136–150 (2000).
42. J. W. Singer, R. Bhatt, J. Tulinsky, K. R. Buhler, E. Heasley, P. Klein, and P. de Vreis. Water-soluble poly-(L-glutamic acid)-Glycamptothecin conjugates enhance camptothecin stability and efficacy in vivo. *J. Control. Release* **74**:243–247 (2001).
43. S. Sakuma, Z.R, Lu, P. Kopeckova, and J. Kopecek. Biorecognizable HPMA copolymer-drug conjugates for colon-specific delivery of 9-aminocamptothecin. *J. Control. Release* **75**:365–379 (2001).
44. Y.-L. Leu, S. R. Roffler, and J.-W. Chern. Design and synthesis of water-soluble glucuronide derivatives of camptothecin for cancer prodrug monotherapy and antibody-directed enzyme prodrug therapy. *J. Med. Chem.* **42**:3623–3628 (1999).
45. D. F. Emerich, S. R. Winn, P. Snodgrass, D. LaFreniere, M. Agostino, T. Wiens, and H. Xiong. Injectable chemotherapeutic microspheres and Glioma II: Enhanced survival following implantation into deep inoperable tumors. *Pharm. Res.* **17**:776–781 (2000).
46. A. Roalland. *Pharmaceutical particulate carriers: Therapeutic applications*, Marcel Dekker, New York, 1993.
47. H. Brem and L. Piatadosi. Placebo controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. *Lancet* **345**: 1008–1012 (1995).
48. L. K. Fung and M. Shin. Chemotherapeutic drugs released from polymers: Distribution of 1,3-bis (2-chloroethyl)-1-nitrosurea in the rat brain. *Pharm. Res.* **13**:671–682 (1996).
49. S. Cohen and H. Bernstein. *Microparticulate systems for the delivery of proteins and vaccines*, Marcel Dekker, New York, 1996.
50. A. Shenderova, T. G. Burke, and S. P. Schwendeman. Stabilization of 10-Hydroxycamptothecin in poly(lactide-co-glycolide) microsphere delivery vehicles. *Pharm. Res.* **14**:1406–1414 (1997).
51. S. R. Mallery, A. Shenderova, P. Pei, S. Begum, J. R. Ciminieri, R. F. Wilson, B. C. Casto, D. E. Schuller, and M. A. Morse. Effects of 10-hydroxycamptothecin, delivered from locally injectable poly(lactide-co-glycolide) microspheres, in a murine human oral squamous cell carcinoma regression model. *Anticancer Res.* **21**:1713–1722 (2001).
52. B. Ertl and P. Platzer. Poly(D,L-lactide-co-glycolide) microspheres for sustained delivery and stabilization of camptothecin. *J. Control. Release* **61**:305–317 (1999).
53. S. C. Yang and L. F. Lu. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. *J. Control. Release* **59**:299–307 (1999).
54. V. Kumar, J. Kang, and R. J. Hohi. Improved dissolution and cytotoxicity of camptothecin incorporated into oxidized-cellulose

microspheres prepared by spray drying. *Pharm. Dev. Technol.* **6**:459–467 (2001).
55. S. M. Sugarman. Lipid-complexed camptothecin: formulation and initial biodistribution and antitumor activity studies. *Cancer Chemother. Pharmacol.* **37**:531–538 (1996).
56. T. G. Burke. Liposomal stabilization of camptothecin's lactone ring. *J. Am. Chem. Soc.* **114**:8318–8319 (1992).
57. T. G. Burke and A. K. Mishra. Lipid bilayer partitioning and stability of camptothecin drugs. *Biochem.* **32**:5352–5364 (1993).
58. D. L. Emerson. Liposomal delivery of camptothecins. *Pharm. Sci. Tech. Today* **3**:205–209 (2000).
59. T. G. Burke and X. Gao. Stabilization of topotecan in low pH liposomes composed of Distearylphosphatidylcholine. *J. Pharm. Sci.* **83**:967–969 (1994).
60. B. B. Lundberg. Biologically active camptothecin derivatives for incorporation into liposome bilayers and lipid emulsions. *Anticancer Drug Design* **13**:453–461 (1998).
61. V. Knight and N. V. Koshkina. Anticancer effect of 9-nitrocamptothecin liposome aerosol on human cancer xenografts in nude mice. *Cancer Chemother. Pharmacol.* **44**:177–185 (1999).
62. V. Knight, E. S. Kleinerman, J. C. Waldrep, B. C. Giovanella, B. E. Gilbert, and N. V. Koshkina. 9-nitrocamptothecin liposome aerosol treatment of human cancer subcutaneous xenografts and pulmonary cancer metastases in mice. *Ann. NY Acad. Sci.* **922**: 151–163 (2002).
63. N. V. Koshkina and B. E. Gilbert. Distribution of camptothecin after delivery as a liposome aerosol or following intramuscular injection in mice. *Cancer Chemother. Pharmacol.* **44**:187–192 (1999).
64. J. C. Patton. Mechanisms of macromolecule absorption by the lungs. *Adv. Drug Deliv. Rev.* **19**:3–20 (1996).
65. E. A. Ahmed and S. Jacob. Influence of route of administration on [3H]-camptothecin distribution and tumor uptake in CASE-bearing nude mice: whole body autoradiographic studies. *Cancer Chemother. Pharmacol.* **39**:122–129 (1996).
66. N. V. Koshkina, V. Knight, B. E. Gilbert, E. Golunski, L. Roberts, and J. C. Waldrep. Improved respiratory delivery of the anticancer drugs, camptothecin and paclitaxel, with 5% CO_2-enriched air: pharmacokinetic studies. *Cancer Chemother. Pharmacol.* **47**:451–456 (2001).
67. R. Cortesi and E. Esposito. Formulation study for the antitumor drug camptothecin: liposomes, micellar solutions and a microemulsion. *Int. J. Pharm.* **159**:95–103 (1997).
68. G. Kwon, M. Yokoyama, T. Okano, Y. Sakurai, and K. Kataoka. Biodistribution of micelle-forming polymer drug conjugates. *Pharm. Res.* **10**:970–974 (1993).
69. G. Kwon, S. Suwa, M. Yokoyama, T. Okano, Y. Sakurai, and K. Kataoka. Enhanced tumor accumulation and prolonged circulation times of micelle-forming poly(ethyleneoxide-aspartate) block copolymer-adriamycin conjugates. *J. Control. Release* **29**: 17–23 (1994).
70. X. Zhang, J. K. Jackson, and H. M. Burt. Development of amphiphilic diblock copolymers as micellar carriers of taxol. *Int. J. Pharm.* **132**:195–206 (1996).