in vivo *19*: 283-292 (2005)

Review

Novel Camptothecin Derivatives

KATHLEEN LEGARZA[1] and LI-XI YANG[1,2]

[1]Radiobiology Laboratory, Integrated Radiation Oncology Graduate Medical Education Program, Department of Radiation Oncology, California Pacific Medical Center Research Institute, San Francisco, CA; [2]St. Mary's Medical Center, San Francisco, CA, U.S.A.

Abstract. *Efforts continue to be made in the field of oncology to find new and effective chemotherapeutic agents against cancer. From these efforts the camptothecins have emerged as a promising group of agents. Camptothecin, first discovered in 1958, was found to be an effective anti-chemotherapeutic agent, but the toxicities were too great to be used in a clinical setting. Derivatives of the original camptothecin molecule have been created by modifying one or more rings in an effort to improve the pharmacokinetics and toxicity profiles of the parent compound. This article reviews both the in vivo and in vitro characteristics of these novel agents.*

Camptothecin was originally extracted from a native tree of Tibet and China called *Camptotheca acuminata* in latin and xi shu in chinese. Camptothecin is found in all organs of the tree and is thought to provide the tree protection against herbivores. Extracts from the tree were used in traditional Chinese medicine for a variety of ailments including psoriasis, viral illnesses and cancer (1). It was not until 1958 that two western scientists, Monroe E. Wall and Manskukh C. Wani, actually isolated the compound camptothecin as the active anti-cancer agent (2).

Further investigation of camptothecin led to the elucidation of its mechanism of action as a topoisomerase I inhibitor. Topoisomerase I is an enzyme that binds to DNA, forming a phosphotyrosine bond and causing single strand cleavage in preparation for DNA synthesis during S-phase (3). It is thought that camptothecins stabilize the enzyme/DNA complex, thus inhibiting religation and release of the enzyme (4,5). Topoisomerase I levels may be higher in some tumor cells than in normal cells, which may allow for some specificity of camptothecins for tumor cells (6,7).

Although camptothecin was shown to be highly effective in killing cancer cells, it was unsuccessful in clinical trials due to unpredictable pharmacological properties and severe toxic effects, including myelosuppression, diarrhea and hemorrhagic cystitis (8). More recently, modifications have been made to the parent compound in order to design semi-synthetic and synthetic analogues with better pharmacological properties and fewer side-effects. Topotecan and irinotecan, the first analogues approved by the FDA, are now widely used as chemotherapeutic agents. Topotecan has been approved as a second-line agent for ovarian and small cell lung cancer, while irinotecan has been approved for metastatic colorectal carcinoma. Irinotecan is a prodrug that is converted to the active metabolite form 10-hydroxy-7-ethylcamptothecin (SN38). Unfortunately, several mechanisms of drug resistance to these compounds have been elucidated, including changes in the topoisomerase enzyme and increase in efflux proteins such as multidrug-resistant proteins (MRPs) and breast cancer-resistant protein (BRCP) (9-11). Scientists continue to develop analogues in order to improve the pharmaco-kinetics, drug resistance, clinical efficacy and toxicity profiles of the original camptothecin molecule. This paper summarizes some of the modifications that have been made and the trials that test their efficacy.

Rubitecan

Rubitecan (RFS2000) was created by adding a nitro group in the nine position of the A-ring of the parent camptothecin (CPT) molecule. Like CPT, this analog continues to demonstrate lactone ring instability. The drug can be administered orally or intravenously. Oral bioavailability is influenced by food intake, thus the drug needs to be taken under fasting conditions (12). The metabolic conversion from the lipophilic prodrug,

Correspondence to: Li-Xi Yang, M.D., Ph.D., Radiobiology Laboratory, California Pacific Medical Center Research Institute, #602, OPR Bldg., 3801 Sacramento Street, San Francisco, CA 94118, U.S.A. Tel: 415-600-6203, Fax: 415-600-6215, e-mail: Yang@cooper.cpmc.org

Key Words: Camptothecin analogs, topoisomerase I inhibitor, anticancer drugs.

9-nitrocamptothecin (9-NC), into 9-aminocamptothecin (9-AC), is not well defined or quantified. The cytotoxicity of 9-NC and 9-AC is not affected by P-glycoprotein, MDR 1, or MDR 2. However, 9-AC but not 9-NC is susceptible to cellular efflux and resistance associated with BCRP (13).

Despite some susceptibility to resistance, phase I studies indicated growth inhibition in human tumor xenografts including lung, colorectal, breast, pancreatic, ovarian, prostate, stomach, melanoma and leukemia treated with rubitecan. The maximum tolerated dose (MTD) in mice was determined to be 1mg/m^2/day (14). It has also been demonstrated that 9-NC activates the apoptotic pathway in a human ovarian cancer cell line (SKOV-3) (15).

Based on these preclinical results, the drug was tested in phase I, II and III trials. Rubitecan, administered at a starting dose of 0.5 mg/m^2 along with capcitabine in a phase I trial, showed no objective response. However, disease was stabilized in 9 patients (43%) with refractory solid tumors (16). In phase II trials, rubitecan was ineffective in treating patients with cutaneas melanoma, uveal melanoma, glioblastoma multiforme and GI leiomyosarcoma, but minimally effective in treating other soft-tissue sarcomas (17-19). In patients with pancreatic cancer treated with 9-NC, an objective response was demonstrated in 4 patients (28.6%) and a subjective response (pain control, body weight and performance status) in 13 patients (92.9%) (20). Rubitecan was tested against best care (89% alternative chemotherapy, 11% supportive care) in 309 patients with refractory pancreatic cancer in a phase III trial. Patients in the best care group were crossed over to the rubitecan group at the time of failure. The median survival of 147 days in the cross-over group was significantly better than 60 days in the non cross-over group ($p<0.0001$). Toxicities, including grade 3/4 neutropenia, anemia, nausea, vomiting and diarrhea, occurred more frequently in the patients treated with rubitecan (21).

In addition to the promising results of this phase III trial, rubitecan has been shown to enhance radiation. A study by Amorino *et al.* showed that human NSCL cancer cells (H460) treated with 5, 10 and 15 nM of RFS-2000 followed by 2.38 Gy/min resulted in dose enhancement ratios (DER) of 1.22, 1.54 and 2.0, respectively. In mouse xenograft models, tumor growth delay was greatest in the tumors treated with RFS-2000 and radiation with a growth delay value of 9.2±0.5 days compared to those treated with RFS-2000 (5.1±0.5 days) alone or radiation alone (2.5±0.4 days). The mechanism of action for this synergistic effect was attributed, at least in part, to inhibition of SLD (22). Human lung carcinoma cells (H460) responded best to combinations of either RFS-2000 or CPT-11 with etoposide and radiation therapy, demonstrating the enhancement of radiation effect when both a topoisomerase I inhibitor and a topoismerase II inhibitor were used in combination (23).

Since several aspects, including radiation enhancement, are promising for the use of rubitecan, further modifications have been made in an effort to decrease the dose and increase clinical response. This has led to the development of an aerosolized liposomal formulation of rubitecan, known as dilauroylphosphatidylcholine-9-nitro-20(S)-camptothecin (DLPC-9-NC). Preliminary data showed equivalent tumor response at lower doses compared to other routes of administration. The recommended dose was 13.3 μg/kg/day on a 60-minute exposure 5 days a week for 8 weeks at a concentration of 0.4 mg/ml in the nebulizer. Out of the 25 patients treated in the study, 2 patients with uterine cancer had a partial response and 3 patients with primary lung cancer had stabilized disease. The dose-limiting toxicity was chemical pharyngitis. Other side-effects included cough, sore throat, nausea, vomiting, anorexia, dysgeusia, fatigue and neutropenia. A decrease in pulmonary function tests while on treatment was observed, but the end of treatment FEV1 value was not significantly different from baseline (24).

Exatecan

Exatecan (DX-8951f) is a totally synthetic, water soluble analogue that does not require enzymatic activation like some of the other prodrugs such as irinotecan. It has modifications at both the A-ring and B-ring as indicated by the molecular name 7-(4-methylpiperazinomethylene)-10,11-ethylenedioxy-20(S)-camptothecin. It is a more potent inhibitor of topoisomerase I than camptothecin, topotecan and SN38. The IC_{50} in murine p388 leukemia cells of DX-8951f was 0.975 μg/ml, which was superior to SN38 (2.71 μg/ml), topotecan (9.52 μg/ml) and camptothecin (23.5 μg/ml) (25). Exatecan has broad activity in multiple cell lines and/or xenografts including human breast, gastric, renal, colon, ovarian, cervical and lung (25,26). Part of the effectiveness may come from the fact that the efflux pump P-glycoprotein multidrug transporter does not recognize exatecan as a substrate. It is equally effective in both human lung cancer PC-6 and the cell variant that over expresses P-glycoprotein (27).

A phase I trial by Rowinsky *et al.* defined the pharmacokenetics of exatecan using a starting dose schedule of 0.1 mg/m^2/d daily 30-minute intravenous infusion for 5 days in 3-week cycles. The results showed an MTD of 0.3 mg/m^2/d for heavily pretreated and 0.5 mg/m^2/d for minimally pretreated patients. Objective tumor response or lack of progression was noted in 36% of patients with a variety of tumor types which were resistant to or recurred after prior treatments with other chemotherapeutic agents (28).

A phase II study showed moderate activity against previously treated breast cancer patients, with 7.7% partial response, 10% minor response and 41% with stable disease (29). Exatecan stabilized disease in patients with ovarian

cancer that was resistant to platinum, taxanes and topotecan, but caused significant hematological or GI toxicities in 25% of patients (30). Exatecan was not successful in untreated advanced NSCLC or previously treated metastatic colorectal carcinoma (31-32). A phase III trial has been conducted in patients with locally advanced or metastatic pancreatic cancer. The trial showed that exatecan used with gemcitibine was not superior to gemcitibine alone (33). The dose-limiting toxicity was myelosuppression, most commonly neutropenia, but thrombocytopenia and anemia were also commonly experienced. Nausea, vomiting and diarrhea were usually grade 1 or 2, which could be managed with medication. Other less common side-effects included mild to moderate stomatitis, malaise, weakness, headache, anorexia, elevation in liver enzymes, altered taste sensation and dizziness (28-33).

DE-310

DE-310 was created by covalently linking a carrier, carboxymethydextran polyalcohol, to exatecan. This modification was made in an effort to improve the biodistribution and reduce the toxicity of exatecan. One study showed that, in tumor-bearing mice, the concentration of released DX-8951 from the conjugated form (DE-310) was 30 times greater in tumor tissue than in plasma. This suggests there may be some tumor selectivity of the carrier form (34). In a phase I trial testing the efficacy in a range of adult solid tumors, DE-310 stabilized disease in five patients and produced no complete or partial responses. The terminal half-life of the conjugate form and released form was 208.8 hours and 175.1 hours, respectively. Toxicities included grade 3/4 myelosuppression, transient elevations in liver transaminases, and mild nausea, vomiting and anorexia. The pharmacokinetics and clinical efficacy of DE-310 seem promising in these early trials. The results of ongoing clinical trials will provide more information about this analogue (35).

Gimatecan

Gimatecan is a lipophilic analogue also known as ST1481, which has an oxyiminomethyl at position seven of the B-ring (36). Several features of gimatecan make it superior to camptothecin and topotecan in laboratory studies. The lipophilic properties of the molecule allow for oral administration and increase cellular accumulation, but at the same time give it a higher affinity for albumin. Experimental data using non-small cell lung cancer NCI-H460 and ovarian A2780/DX cells lines showed that the concentration required for 50% cell growth inhibition (IC_{50}) of gimatecan (0.01±0.006 μM) is less than camptothecin (0.33±0.05 μM), topotecan (1.38±0.19 μM) and SN38 (0.21+ 01 μM). In the presence of albumin the potency is less (0.063±0.004 μM), but was still superior to the other drugs (37). The lipophilic nature also helps to stabilize the bond between the analogue and topoisomerase I complex. After NaCl disruption of the complex the DNA remains cleaved, indicating that the bond formed by gimatecan is more stable compared to other camptothecins (38). In addition to inhibiting topoisomerase I, gimatecan may also have antiangiogenic effects (39). Another beneficial property of gimatecan is the ability of the drug to overcome resistance. In several studies, there was a lack of recognition of the novel analogue by both the MDR-1 and BCRP transport systems (36,37,40).

Gimatecan has superior efficacy in mouse models to topotecan. In both daily and q8-10day cycles, gimatecan produced a higher CR rate at lower concentrations (0.5 mg/kg and 5-6 mg/kg, respectively) compared to topotecan (1.2 mg/kg and 18-22 mg/kg, respectively) (37, 41). The novel analogue not only has improved efficacy, but also has a lower toxicity. The TI (LD_{10}/ED_{90}) is 3 for gimatecan compared to 1.7 for topotecan. After oral administration in mice the highest concentrations were seen in the liver, but it can also be isolated from the brain, indicating a possible clinical role for liver metastasis and CNS tumors (41).

Phase I trials confirmed that, in humans, gimatecan maintained its properties of rapid absorption and slow elimination. These trials indicate that the analogue has some activity in recurrent malignant glioma, melanoma and metastatic colorectal cancer (42,43). In an ongoing phase II trial of patients with metastatic colorectal cancer, there has been 1 complete response and 3 stabilized disease (44). Toxicities in these early trials included myelosuppression, mild diarrhea and mucositis (42-44). It will be interesting to see if the promising results of the preclinical trials translate into a significant clinical benefit.

Belotecan

Belotecan, or CKD-602, is a novel water soluble camptothecin analog with the molecular name (7-[2-(N-isopropylamino)ethyl]-(20S)-camptothecin). The stability of the molecule has been confirmed in both plasma and methanol for at least 3 months (45). Conversion from the lactone to carboxylate form occurs and is dependent on pH. This can be considered an unfavorable characteristic because the conversion is enzyme-dependent, which can be variable from patient to patient (46). In xenograft models, CKD-602 induced regression of 88% in SKOV-3 ovarian tumor, 87% in MX-1 breast tumor and 67% in LX-1 lung tumor. In HT-29, WIDR and CX-1 colon tumors, the regression rate was 80%, 94% and 76%, respectively (47).

The results of human phase I and II trials have also been promising. In a phase I trial, CKD-602 was found to be effective against gastric and ovarian tumors at a MTD of 0.7

mg/m^2/day. The fractionated renal clearance was determined to be 33-50% (48). Two phase II trials were completed testing CKD-602 at a starting dose of 0.5mg/m^2/day for 5 days every 3 weeks, one in patients with ovarian cancer and the other in patients with small cell lung cancer (SCLC). The overall response rate in the ovarian cancer patients was 45% (9/20) with 4 patients experiencing partial response and 5 patients experiencing a CA125 response. Another 5 patients had stable disease. In the patients with SCLC, there was 1 complete responder and 8 partial responders. In all these trials, the DLT was neutropenia. In the studies, 75-100% of the patients experienced grade 3/4 neutropenia and 16.7-30% experienced thrombocytopenia. Grade 3/4 GI toxicity was very uncommon (49,50).

Karenitecin

This analogue, also known as BNP1350, is a modification of the 7 position of the B-ring with the molecular name (7-[(2-trimethylsilyl)ethyl]-20(S)-camptothecin. Karenitecin and the silitecan class of derivatives are highly lipophilic, remain in the lactone form under physiological conditions, and do not require hepatic conversion. The drug can be administered intravenously or orally with good oral bioavailability seen in mice (51). Penetration into the central nervous system of non human primates was found to be low with the ratio of cerebral spinal fluid area under the curve (AUC) to the plasma AUC being less than 5% (0.4%-3.0%) (52).

In preliminary trials, karenitecin was found to be cytotoxic to pediatric cell lines as well as adult lung, prostate, pancreatic, breast, colon, ovarian and head and neck cell lines (53, 54). The antiproliferative effects of karenitecin on human ovarian and colon cancer (SW1398, WiDr) cell lines were superior to SN-38 and topotecan (55, 51). Treatment of colon cancer xenograft models with karenitecin resulted in ≥50% growth inhibition. Tumor growth inhibition of 3 ovarian cancer xenografts ranged from 81-91%, which was significantly ($p<0.05$) superior to the results produced by topotecan (51). The silitecan in general have been shown to inhibit tumor growth in U87 glioma xenografts (56). Karenitecin was not a substrate for p-glycoprotein, multidrug-resistant protein, or lung-resistant protein (51). In addition, it was not susceptible to cellular efflux by BCRP, but did induce the overexpression of the protein (55).

Due to the success of karenitecin against pediatric tumors and a variety of adult tumors in phase I trials, it was tested in a phase II trial. In this trial, 1mg/m^2/day for 5 days over 3 weeks of karenitecin was delivered intravenously to patients with metastatic melanoma. There was one complete response, no partial responses and 33% disease stabilization (57). Other phase II trials are currently underway to define the role of karenitecin in other tumor types.

Lurtotecan

Lurtotecan (GI-147211) was created by the addition of an N-methyl piperazinomethylene group at the C7 position of the B-ring and an ethylenedioxy group to the A-ring. The modification does not protect against the hydrolysis of the lactone ring into its inactive form in the serum. Given as an oral medication the bioavailability was found to be low, thus intravenous administration was recommended (58). In preclinical studies, lurtotecan was found to be more water soluble and have a higher affinity for topoisomerase than topotecan. It also had superior potency to topotecan in melanoma, colon cancer, breast cancer and ovarian cancer cell lines. Even in the SKVLB multidrug-resistant ovarian cell line, lurtotecan with an IC_{50} of 99 nM had superior potency to topotecan with an IC_{50} of 149 nM. When tested on HT-29 and SW-48 human colon xenografts, lurtotecan produced tumor regression, while topotecan only slowed tumor growth compared to controls (59). In phase I trials, responses were seen in patients with breast, ovarian, colon and liver neoplasms (60, 61). In a phase II trial, lurtotecan administered as 1.2 mg/m^2/day for 5 days every 3 weeks produced 3 partial responses in patients with breast cancer and 2 partial responses in patients with non-small cell carcinoma (62). Lurtotecan was tested in two groups of patients with small cell lung cancer, one group with refractory disease and one group with chemosensitive disease. The drug was administered at 1.2 mg/m^2/day as a 30-minute infusion for 5 consecutive days every 3 weeks. Only partial responses were seen. The response rate was 16.6% in the refractory group and 21.1% in the chemosensitive group. The major toxicities included neutropenia, thrombocytopenia, nausea, vomiting, fatigue and anorexia (60-62).

Modifications of the original lurtotecan molecule produced a liposomal form called OSI-211 (formerly known as NX211) with the hope of improving the therapeutic index by decreasing clearance and allowing for accumulation of the drug in tumor cells. This hypothesis was supported by a KB xenograft model that showed that the therapeutic index of NX211, lurtotecan and topotecan was 0.5, 1 and 2.9, respectively. The maximum plasma concentration (Cmax) of NX211 was 20.1 μg/ml, which was 122-fold higher than the Cmax of lurtotecan. The area under the curve (AUC) was 127 μg·h/ml for NX211 and 0.0672 for lurtotecan (63). A phase I trial showed a partial response in one patient with breast cancer and in one patient with ovarian cancer. In humans renal clearance was low, as the main route of elimination is biliary. In one study the DLTs were neutropenia and thrombocytopenia; however, in another study the DLTs were stomatitis, esophagitis and diarrhea (64, 65).

DRF-1042

DRF-1042 [5(RS)-(2-hydroxyethoxy)-20(S)-CPT] is somewhat water soluble and has an affinity for protein. The analogue is susceptible to reversible pH-dependent hydrolysis converting the lactone ring into the inactive carboxylate form (66). Preclinical studies confirmed good oral bioavailability and stability in human plasma (67).

A phase I trial using the analogue in patients with a variety of solid tumors demonstrated a therapeutic response in 67% (8/12) patients, 2 with a CR, 2 with a PR and 4 with stabilized disease. The tumors that responded included osteosarcoma, renal cell carcinoma and breast carcinoma. The half-life was 8.8 hours for the lactone form and 21.1 hours for the total form. The MTD was 120 mg/m^2/day given orally for 5 days over 2 consecutive weeks every 3 weeks and the recommended dose for phase II trials was 80 mg/m^2/day. The DLTs were myelosuppression, especially neutropenia and diarrhea (66).

Diflomotecan

Diflomotecan (BN 80915) is a member of a group called the homocamptothecins. It is distinguished from other homocamptothecins because it is fluorinated at the 9 and 10 positions of the A-ring. Homocamptothecins have an ethylene spacer between the alcohol moiety and the carboxyl group on the E-ring of camptothecin, creating a 7-membered lactone ring that is more stable in the plasma compared to other camptothecin analogues. The six-membered ring, characteristic of most camptothecin analogues, undergoes an equilibrium reaction through hydrolysis which results in an inactive carboxylate form in the serum. Acidic environments reverse this process thus activating the six-member ring, which can lead to hemorrhagic cystitis. In the seven-member ring analogues, the hydrolysis is irreversible even under acidic conditions (68, 69). Thus, diflomotecan is more stable in the serum and is potentially less toxic, but still maintains a high affinity for topoisomerase I.

The efficacy of diflomotecan has been proven in various studies. In preclinical studies, BN80915 was shown to have a higher affinity for topoisomerase I, more DNA cleavage sites and increased tumor cytoxicity compared to camptothecin (70,71). Difomotecan was found to be a potent inducer of apoptosis in HL-60 human promyelocytic cells, thought to be partially due to increased activation of caspase-3 and 8 (69). Results show that BN-80915, with an IC_{50} of 1.5±0.8 nM, has stronger antiproliferative effects compared to topotecan (70±20 nM) and SN-38 (80±20 nM) at 6 hours in HT-29 human colon cancer cells. This same pattern was seen in other colon cancer cell lines including CaCo-2 and HCT-116. In addition, the multidrug-resistant cell lines HL-60/Vinc and HL-60/AR did not show cross resistance toward BN 80915 (70). However, in humans, the ABCG2 421C>A genotype was found to adversely affect the pharmacokinetics of diflomotecan efflux from cells thus increasing the plasma levels of the drug (72).

In a phase I trial, involving patients with adult solid tumors, the mean oral bioavailability was 72.24±59.2% and there was a low rate of urinary excretion. After two cycles 6 out of 22 patients had stabilization of disease. The recommended phase II dose schedule was determined to be 0.27 mg/day for 5 days every 3 weeks. Toxicities were mainly hematological, although patients also experienced alopecia, gastrointestinal toxicity and fatigue (73).

Prothecan

Prothecan is a water soluble, stable prodrug which was developed through the conjugation of camptothecin-(20) O-glycinate with polyethelene glycol (PEG). PEG-camptothecin (CPT) is released when it is hydrolyzed to CPT in human tissues. It is thought that the high molecular weight of this molecule may allow for better vascular permeation, intravascular retention and, possibly, selective tumor distribution (74, 75). PEG-alpha-CPT has been tested in a colorectal xenograft model and was found to reduce tumor burden by 90% without severe toxicity (75). Conover *et al.* further conjugated PEG-CPT with different amino acids and found that the specific amino acids influenced the pharmacokinetics and tumor efficacies of the conjugates. PEG-alanine-CPT in particular had moderate activity against a range of human cells lines and significant antitumor activity in xenograft models of lung, breast, prostate, colon, ovarian and pancreatic cancers (76).

In a phase I trial, the MTD was determined to be 7,000 mg/m^2 in both heavily and minimally pretreated patients with solid tumors. Three patients, one with lung cancer, one with peritoneal carcinomatosis of unknown primary, and one with osteosarcoma, experienced a partial response to treatment. The half-life value of PEG-CPT was 77.46±36.77 hours and the fractional excretion of CPT in urine was 1.29%±0.67% at 48 hours. Toxicities included myelosuppression, with neutropenia being the dose-limiting toxicity, cystitis, nausea, vomiting, diarrhea and alopecia. Cystitis occurred in 35% of patients and was mild in the majority of cases, consisting of isolated microscopic hematuria. The study did not show a relationship between the severity of side-effects and dose of PEG-CPT or exposure to CPT, thus there may be variability in the lactone ring opening. This would be a down-side to this analog compared to some of the others (74).

MAG-CPT

Camptothecin (CPT) can be covalently conjugated to methacryloylglycinamide (MAG) with an amino acid spacer at the C-20 position to make it water soluble. CPT is released into the plasma through an enzyme- and pH-dependent process. In one study, 10 patients with colorectal cancer received 60 mg/m^2 of MAG-CPT for 24 hours, 3 days or 1 week prior to surgery. At the time of surgery, tumor, plasma and adjacent normal tissue samples were collected simultaneously. Contrary to previous xenografts studies, which showed preferential delivery of CPT into tumor tissue, the results of this study showed that the ratios of MAG-CPT to CPT were equal in plasma and in tumor tissue from 24 hours to 7 days after dosing (77). In a phase I trial, MAG-CPT was given as a 30-minute infusion over 3 days every 4 weeks in patients with solid tumors and showed no activity in the small number of evaluable patients. The DLT was bladder toxicity, presenting in some cases as prolonged hemorrhagic cystitis. Pharmacokinetic studies indicated that the free CPT was dependent on the release rate of the carrier due to equal half-lives of 11.2 hours (78). In another trial, MAG-CPT was given to adult patients with solid tumors as a weekly intravenous schedule 3 weeks every 4 weeks. The conclusion of the trial was that MAG-CPT should not be given in a weekly schedule because of variable CPT urinary excretion and severe bladder toxicity (79). Adding a carrier molecule to CPT does appear to improve the pharmacokinetic properties, but the bladder toxicity remains severe.

T-0128

T-0128 is another analogue created by the addition of a carrier molecule linked by amino acids to CPT. One study showed that the release of the carboxymethyl dextran carrier from T2513 (7-ethyl-10-aminopropyloxy-CPT) may be dependent on the macrophage concentration since the efficacy of T-0128 was improved in the presence of macrophages (80). Another study suggested that the release was facilitated by lysosomal enzymes and that the triple glycine linker allows for slow liberation of T-0128. When this analogue was tested in mouse xenografts, it had maximum efficacy against MX-1 human mammary tumors (81). One study compared the plasma half-life of T-0128 in rats with and without tumor. The results showed that the plasma half-life was reduced in rats bearing tumor, suggesting a preferential uptake by tumor cells (82). In Walker-256 carcinoma, T-0128 shrunk the tumor by 50% with an ED_{50} of 2.3mg/kg, which was 10 times less than the ED_{50} of T-2513. The therapeutic index (TI = ED_{50}/MTD) of T-0128 was 43, which was superior to the TI of T-2513(2.6), CPT-11(1.4) and topotecan (4.6). T-0128 was also found to be more effective than CPT-11 and topotecan in refractory xenograft models (83).

BAY 38-3441

Another prodrug, BAY 38-3441, was created by conjugating camptothecin to a carbohydrate moiety with a peptide spacer. The purpose of the conjugate was to stabilize the lactone ring allowing for prolonged exposure of cells to the active form. In preclinical studies, BAY 38-3441 was found to inhibit tumor growth in xenograft models of MX-1 breast, LXFL529 lung, CXF280 and HT29 colon cancer cell lines (84). A phase I trial testing a variety of dose schedules concluded that 320 mg/m^2/day as 30-minute infusions given daily for 3 days every 3 weeks would be the best schedule to use in phase II trials. The major toxicities produced by this schedule were diarrhea and thrombocytopenia (85). In another phase I study, the dose-limiting toxicity was elevated serum creatinine levels related to the Cmax (86).

Oxyalkanoic Acid Esters

Only preclinical studies have been done on novel oxyalkanoic acid esters of camptothecin. The hypothesis behind this modification on the E-ring was that it would stabilize the lactone ring, thereby decreasing toxicity and increasing tumor cell death. Several of these derivatives were tested in multiple cancer cell lines and the results were compared to camptothecin, irinotecan and taxol. The results indicated the antitumor activity was improved in the derivatives that had shorter carbon chains between the carbonyl and oxygen. The antitumor effects were similar to camptothecin, but superior to irinotecan and taxol, even in MDR1-expressing cells. Further trials are currently underway to confirm the properties of these novel agents (87).

Conclusion

The camptothecin analogues are emerging as a promising group of chemotherapeutic agents. The analogues were created by modifying the different rings of the original camptothecin molecule, giving each analogue unique properties. These modifications have resulted in various improvements in the parent molecule, including changes in bioavailability, stabilization of the lactone ring, and/or a decrease in the substrate recognition by drug-resistant proteins. These changes have translated into greater tumoricidal effects with improvements in the toxicity profile in preclinical studies. In some cases, the preclinical success translated into moderate response rates in clinical trials, while in other cases it did not. As a better understanding of these analogues develops through these clinical trials, progress is being made toward creating more effective and less toxic chemotherapeutic agents.

Acknowledgements

This work was supported in part by grants from The Susan G. Komen Breast Cancer Foundation, from the U. S. Army Medical Research Acquisition Activity (USAMRAA), and from the Tobacco-Related Disease Research Program, University of California, U.S.A.

References

1 Yau X-F, Wang Y, Yu T, Zhang YH and Dai SJ: Variations in camptothecin content in *Camptotheca acuminata* leaves. Bot Bull Acad Sin *44*: 99-105, 2003.
2 Wall ME, Wani MC, Cook CE, Palmer KH, McPhail HT and Sim GA: The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. J Am Chem Soc *88*: 3888-3890, 1966.
3 Tse YC, Kirgaard K and Wang JC: Covelant bonds between protein and DNA. Formation of phosphotyrosine linkage between certain DNA topoisomerases and DNA. J Biol Chem *255*: 5560-5565, 1980.
4 Hsiang Y-H, Hertzberg R, Hecht S and Liu LF: Camptothecin induces protein-linked DNA breaks *via* mammalian DNA topoisomerase I. J Biol Chem *260*: 14873-14878, 1985.
5 Hsiang YH, Lihou MG and Liu LF: Arrest of DNA replication by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. Cancer Res *49*: 5077-5082, 1989.
6 Van der See AG, Hollema H, De Jong S, Boonstra H, Gouw A, Willemse PH, Sijlstra JG and De Vries EG: P-glycoprotein expression and DNA topoisomerase I and II activity in benign tumors of the ovary and in malignant tumors of the ovary, before and after platinum/cyclophosphamide chemotherapy. Cancer Res *51*: 5915-5920, 1991.
7 Giovanella BC, Stehlin JS, Wall ME, Wani MC, Nicholas AW, Liu LF, Silber R and Potmesil M: DNA topoisomerase I-targeted chemotherapy of human colon cancer xenografts. Science *246*: 1046-1048, 1989.
8 Muggia FM, Creaven PJ, Hansen HH, Cohen MH and Selawry OS: Phase I clinical trial of weekly and daily treatment with camptothecin (NSC-100880): correlation with preclinical studies. Cancer Chemother Rep *56*: 515-521, 1972.
9 Cherencik JE, Staker BL, Burgin AB and Pourquier P: Mechanism of resistance by human topoisomerase I mutations. J Mol Biol 339: 773-784, 2004.
10 Hoki Y, Fujimori A and Pommier Y: Differential cytotoxicity of clinically important camptothecin derivatives in P-glycoprotein-overexpressing cell lines. Cancer Chemother Pharmacol *40*: 433-438, 1997.
11 Maliepaard M, van Gastelen MA, de Jong LA, Pluim D, van Waardenburg RC, Ruevekamp-Helmers MC, Floot BG and Schellens JH: Overexpression of the BCRP/MXR/ABCP gene in a topotecan-selected ovarian tumor cell line. Cancer Res *59*: 4559-4563, 1999.
12 Schoffski P, Herr A, Vermorken JB, Van den Brande J, Beijnen JH, Rosing H *et al*: Clinical phase II study and pharmacological evaluation of rubitecan in non-pretreated patients with metastatic colorectal cancer-significant effect of food intake on bioavailability of the oral camptothecin analogue. Eur J Cancer *38*: 807-813, 2002.
13 Rajendra R, Gounder MK, Saleem A, Schellens JH, Ross DD, Bates SE *et al*: Differential effects of breast cancer resistance protein on cellular accumulation and cytoxicity of 9-aminocamptothecin and 9-nitrocamptothecin. Anticancer Drugs *14*: 427-436, 2003.
14 Giovanella BC, Stehlin JS, Hinz HR, Kozielski AJ, Harris NJ and Vardeman DM: Preclinical evaluation of the anticancer activity and toxicity of 9-nitro-20(S)-camptothecin (Rubitecan). Int J Oncol *20*: 81-88, 2002.
15 Hu X, Balan KV, Ramos-DeSimone N, Wyche JH, Han Z and Pantazis P: Differential susceptibility to 9-nitrocamptothecin (9-NC)-induced apoptosis in clones derived from a human ovarian cancer cell line: possible implications in the treatment of ovarian cancer patients with 9-NC. Anticancer Drugs *14*: 427-436, 2003.
16 Michealson MD, Ryan Dp, Fuchs CS, Supko JG, Garcia-Carbonero R, Paul Eder J and Clark JW: A Phase I study of 9-nitrocamptothecin given concurrently with capecitibine in patients with refractory, metastatic solid tumors. Cancer *97*: 148-154, 2003.
17 Patel SR, Beach J, Papadopoulos N, Burgess MA, Trent J, Jenkins J and Benjamin RS: Results of a 2-arm phase II study of 9-nitrocamptothecin in patients with advanced soft tissue sarcomas. Cancer *97*: 2848-2852, 2003.
18 Raymond E, Campone M, Stupp R, Menten J, Chollet P, Lesimple T *et al*: Multicenter phase II and pharmacokinetic study of RFS2000 (9-nitro-camptothecin) administered orally 5 days a week in patients with glioblastoma multiforme. Eur J Cancer *38*: 1348-1350, 2002.
19 Ellerhorst JA, Bedikian AY, Smith TM, Papadopoulos NE, Plager C and Eton O: Phase II trial of 9-nitrocamptothecin (RFS 2000) for patients with metastatic cutaneous or uveal melanoma. Anticancer Drugs *13*: 169-172, 2002.
20 Konstadoulakis MM, Antonakis PT, Tsibloulis BG, Stathopoulos GP, Manouras AP, Mylonaki DB and Golematis BX: A phase II study of 9-nitrocamptothecin in patients with advanced pancreatic adenocarcinoma. Cancer Chemother Pharmacol *48*: 417-420, 2001.
21 Jacobs AD, Burris HA, Rivkin S, Ritch PS, Eisenberg PD and Mettinger KL: A randomized phase III study of rubitecan (ORA) *vs.* best choice (BC) in 409 patients with refractory pancreatic cancer report from a North-American multi-center study. Proc Am Soc Clin Oncol *22*: 316, 2004.
22 Amarino G, Hercules SK, Mohr P, Pyo H and Choy H: Preclinical evaluation of the orally active camptothecin analog, RFS-2000 (9-nitro-20(S)-camptothecin) as a radiation enhancer. Int J Radiation Oncology Biol Phys *47*: 503-509, 2000.
23 Kim JS, Amorino GP, Pyo H, Cao Q and Choy H: Radiation enhancement by the combined use of topoisomerase I inhibitors, RFS-2000 or CPT-11, and topoisomerase II inhibitor etoposide in human lung cancer cells. Radiother Oncol *62*: 61-67, 2002.
24 Carson JP, Zhang N, Frampton GM, Gerry NP, Lenburg ME and Christman MF: Pharmacogenomic identification of targets for adjuvant therapy with the topoisomerase poison camptothecin. Cancer Res *64*: 2096-2104, 2004.
25 Mitsui I, Kumazawa E, Hirota Y, Aonuma M, Sugimori M, Ohsuki S *et al*: A new water-soluble camptothecin derivative, DX-8951f, exhibits potent antitumor activity against human tumors *in vitro* and *in vivo*. Jpn J Cancer Res *86*: 776-782, 1995.

26 Van Hattum AH, Pinedo HM, Schluper HM, Erkelens CA, Tohgo A and Boven E: The activity profile of the hexacyclic camptothecin derivative DX-8951f in experimental human colon cancer and ovarian cancer. Biochem Pharmacol *64*: 1267-1277, 2002.

27 Royce ME, Rowinsky EK, Hoff PM, CoyleJ, DeJager R, Pazdur R and Saltz LB: A phase II study of intravenous exatecan mesylate (DX-8951f) administered daily for five days every three weeks to patients with metastatic adenocarcinoma of the colon or rectum. Invest New Drugs *22*: 53-61, 2004.

28 Rowinsky EK, Johnson TR, Geyer CE Jr, Hammond LA, Eckhardt SG and Drengler R: DX-8951f, a hexacyclic camptothecin analog, on a daily-times-five schedule: phase I and pharmacokinetic study in patients with advanced solid malignancies. J Clin Oncol *18*: 3151-3163, 2000.

29 Esteva FJ, Rivera E, Cristofanilli M, Valero V, Royce M, Duggal A, Colucci P, DeJager R and Hortobagyi GN: A phase II study of intravenous exatecan mesylate (DX-8951f) administered daily for 5 days every 3 weeks to patients with metastatic breast carcinoma. Cancer *98*: 900-907, 2003.

30 Verschraegen CF, Kudelka AP, Hu W, Vincent M, Kavanagh JJ, Loyer E, Bastein L, Duggal A and De Jager R: A phase II study of intravenous exatecan mesylate (DX-8951f) administered daily for 5 days every 3 weeks to patients with advanced ovarian, tubal, or peritoneal cancer resistant to platinum, taxane and topotecan. Cancer Chemother Pharmacol *53*: 1-7, 2004.

31 Royce ME, Rowinsky EK, Hoff PM, Coyle J, DeJager R, Pazdur R and Saltz LB: A phase II study of intravenous exatecan mesylate (DX-8951f) administered daily for five days every three weeks to patients with metastatic adenocarcinoma of the colon or rectum. Invest New Drugs *22*: 53-61, 2004.

32 Baybrook JP, Ranson M, Manegold C, Mattson K, Thatcher N, Cheverton P *et al*: Phase II study of exatecan mesylate (DX-8951f) as first line therapy for advanced non-small cell lung cancer. Lung Cancer *41*: 215-219, 2003.

33 O'Reilly EM, Abou-Alfa GK, Letourneau R, Harker WG, Madiano M, Hurwitz H, Tchekmedyian NS, Ackerman J, DeJager RL and Eckhardt SG: A randomized phase III trial of DX-8951f (exatecan mesylate; DX) and gemcitabine (GEM) *vs.* gemcitabine alone in advanced pancreatic cancer (APC). Proc Am Soc Clin Oncol *23*: 315, 2004.

34 Masubuchi N: Pharmacokinetics of DE-310, a novel macromolecular carrier system for the camptothecin analog DX-8951f, in tumor-bearing mice. Pharmazie *59*: (abstract only), 2004.

35 DE-310 Takimoto CHM, Forero L, Schwartz GH, Tolcher AW, Hammond LA, Patnaik A, Ducharme M, Cooke B, DeJager R and Rowinsky EK: A phase I and pharmacokinetic study of DE-310 administered as a 3 hour infusion every 4 weeks (wks) to patients (pts) with advanced solid tumors or lymphomas. Proc Am Soc Clin Oncol *22*: 130, 2003.

36 Pratesi G, Beretta GL and Zunino F: Gimatecan, a novel captothecin with a promising preclinical profile. Anticancer Drugs *15*: 545-552, 2004.

37 De Cesare M, Pratesi P, Perego P, Carenini N, Tinelli S, Merlini L *et al*: Potent antitumor activity and improved pharmacological profile of ST1481, a novel 7-substituted camptothecin. Cancer Res *61*: 7189-7195, 2001.

38 Dallavalle S, Ferrari A, Biasotti B, Merlini L, Penco S, Gallo G, Marzi M, Tinti MO, Martinelli R, Pisano C, Carminati P, Carenini N, Baretta G, Perego P, De Cesare M, Partesi G and Zunino F: Novel 7-oximinomethyl derivatives of camptothecin with potent *in vitro* and *in vivo* antitumor activity. J Med Chem *44*: 3264-3274, 2001.

39 Petrangolini G, Pratesi G, De Cesare M, Supino R, Pisano C, Marcellini M, Giordano V, Laccabue D, Lanzi C and Zunino F: Antiangiogenic effects of the novel camptothecin ST1481 (gimatecan) in human tumor xenografts. Mol Cancer Res *12*: 863-870, 2003.

40 Croce AC, Bottiroli G, Supino R, Favini E, Zuco V and Zunino F: Subcellular localization of the camptothecin analogues, topotecan and gimatecan. Biochem Pharmacol *67*: 1035-1045, 2004.

41 Dallavalle S, Delsoldato T, Ferrari A, Merlini L, Penco S and Carenini N: Novel 7-substituted camptothecins with potent antitumor activity. J Med Chem *43*: 3963-3969, 2000.

42 Supko JG, Alderson L, Wen P, Cassidy K, Pace S, Obrocea M and Hochberg F: Pharmacokinetics of gimatecan, and orally administered camptothecin analogue, in patients with malignant gliomas. Proc Am Soc Clin Oncol *23*: 136, 2004.

43 Zhu AX, Ready NE, Clark JW, Safran H, Lynch TJ, Carminati P, Zanna C, Chabner BA, Calabresi P and Supko JG: Phase I trial of gimatecan given orally once a week for 3 of 4 weeks in patients with advanced solid tumors. Proc Am Soc Clin Oncol *22*: 139, 2003.

44 Boni C, Gamucci T, Bonetti A, Bisagni G, Dall'o' E, Zanna C, Marsoni S and Sessa C: A phase II study of the novel oral camptothecin ST1481 in pretreated metastatic colorectal cancer (CRC). Proc Am Soc Clin Oncol *22*: 290, 2004.

45 Cho Y, Seo HB, Yu KS, Bae KS, Yi SY, Jang IJ and Shin SG: Simple and sensitive determination of the new antitumor drug CKD-602 in human plasma by liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci *784*: 25-31, 2003.

46 Kim JH, Lee SK, Lim JL, Shin HJ and Hong CI: Performulation studies of a novel camptothecin anticancer agent, CKD-602: physicochemical characterization and hydrolytic equilibrium kinetics. Int J Pharm *239*: 207-211, 2002.

47 Lee JH, Lee JM, Kim JK, Ahn SK, Lee SJ, Kim MY, Jew SS, Park JG and Hong CI: Antitumor activity of 7-[2-(N-isopropylamino) ethyl]-(20S)-camptothecin), CKD-602, as a potent DNA topoisomerase I inhibitor. Arch Pharm Res *21*: 581-590, 1998.

48 Kim HK, Bang Y-J, Heo DS, Shin S-G and Kim NK: Phase I trial of CKD-602, a novel camptothecin derivative, in patients with advanced solid tumors. Proc Am Soc Clin Oncol *21*: 99a, 2002.

49 Song Y, Seo S-S, Bang Y-J, Kang S-B, Nam J-H, Ryu S-Y, Lee K-H, Park SY, Hong CI and Lee HP: Phase II evaluation of ckd-602, a camptothecin analog, administered on a 5-day schedule in patients with recurrent or refractory ovarian cancer. Proc Am Soc Clin Oncol *22*: 467, 2003.

50 Lee JH, Lee SJ, Kim JS, Yeol RB, Park K, Kim NK, Heo SD, Kim SW, Suh C and Lee JS: Phase II trial of CKD-602, a new camptothecin analogue, in patients with small cell lung caner (SCLC). Proc Am Soc Clin Oncol *22*: 668, 2004.

51 Hausheer FH, Cao S, Kanter P, Haridas K, Zhao M, Murali D, Seetharamulu P, Yao S, Reddy D, Pavankumar P, Saxe J, Huang Q, Chen X, Parker A, Wu M, Martinez N and Rustum YM: Karenitecins: New preclinical developments with BNP1350, a novel, potent highly lipophilic camptothecin. Proc Am Assoc Cancer Res *40*: 111, 1999.

52 Thompson PA, Berg Sl, Aleksic A, Kerr JZ, McGuffey L, Dauser R, Nuchtern JG, Hausheer F and Blaney SM: Plasma and cerebrospinal fluid pharmacokinetics study of BNP1350 in nonhuman primates. Cancer Chemother Pharmacol *53*: 527-532, 2004.

53 Kerr JZ, Berg SL, Hausheer F and Blaney SM: Karenitecins: cytotoxicity studies in pediatric tumor cell lines. Proc Am Assoc Cancer Res *40*: 112, 1999.

54 Van Huttun AH, Pinedo HM, Schluper HMM, Hausheer FH and Bovis E: New highly lipophilic camptothecin BNP1350 is an effective drug in experimental human cancer. Int J Cancer *88*: 260-266, 2000.

55 Yin M-B, Guo B, Vanhoefer U, Azrak RG, Minderman H, Frank C, Wrzosek C, Slocum HK and Rustum YM: Characterization of protein kinase chk1 essential for the cell cycle checkpoint after exposure of human head and neck cancer A253 cells to a novel topoisomerase I inhibitor BNP-1350. Mol Pharmacol *57*: 453-459, 2000.

56 Pollak IF, Erff M, Bom D, Burke TG, Strode JT and Curran DP: Potent topoisomerase I inhibition by novel silitecans eliminates glioma proliferation *in vitro* and *in vivo*. Cancer Res *59*: 4898-4905, 1999.

57 Hausheer FH, Berhorn E, Liu Z, Sullivan D, Centeno B, Derderian J, Deconti R, Daud A, Sullivan P and Andrews S: Phase II trial of karenitecin (BNP1350): Clinical and translational study. Proc Am Soc Clin Oncol *22*: 723, 2004.

58 Gerrits CJ, Schellens JH, Creemers GJ, Wessel P, Planting AS, Pritchard JF, DePee S, de Boer-Dennert M, Harteveld M and Verweij J: The bioavailability of oral GI147211 (GG211), a new topoisomerase I inhibitor. Br J Cancer *76*: 946-951, 1997.

59 Sessa C, Wanders J, Roelvink M, Dombernowsky P, Neilsen D, Morant R, Drings P, Wissel P and Hanauske AR: Second-line treatment of small-cell lung cancer with the camptothecin-derivative GI147211: a study of the EORTC Early Clinical Studies Group (ECSG). Ann Oncol *2*: 207-210, 2000.

60 Stevenson JP, DeMaria D, Sludden J, Kaye SB, Paz-Ares L, Grochow LB, McDonald A, Selinger K, Wissel P, O'Dwyer PJ and Twelves C: Phase I/pharmacokinetic study of the topoisomerase I inhibitor GG211 administered as a 21-day infusion. Ann Oncol *10*: 339-344, 1999.

61 Paz-Ares L, Kunka R, DeMaria D, Cassidy J, Alden M, Beranek P, Kaye S, Littlefield D, Reilly D, Depee S, Wissel P, Twelves C and O'Dwyer P: A phase I clinical and pharmacokinetic study of the new topoisomerase inhibitor GI147211 given as a 24-hour continuous infusion. Br J Cancer *78*: 1329-1336, 1998.

62 Gamucci T, Paridaens R, Heinrich B, Schellens JH, Pavlidis N, Verweij J, Sessa C, Kaye S, Roelvink M, Wanders J and Hanauske A: Activity and toxicity of GI147211 in breast, colorectal and non-small-cell lung cancer patients: an EORTC-ECSG phase II clinical study. Ann Oncol *7*: 793-797, 2000.

63 Emerson D, Bendele R, Brown E, Chiang S, Desjardins JP, Dihel LC, Gill SC, Hamilton M, LeRay JD, Moon-McDermott L, Moynihan K, Richardson FC, Tomkinson B, Luzzio MJ and Baccanari D: Antitumor efficacy, pharmacokinetics, and biodistribution of NX211: a low-clearance liposomal formulation of Lurotecan. Clin Cancer Res *6*: 2903-2912, 2000.

64 Gelmon K, Hirte H, Fisher B, Walsh W, Ptaszynski M, Hamilton M, Onetto N and Eisenhauer E: A phase I study of OSI-211 given as an intravenous infusion days 1, 2, and 3 every three weeks in patients with solid cancers. Invest New Drugs *22*: 263-275, 2004.

65 Giles FJ, Tallman MS, Guillermo GM, Cortes JE, Thomas DA, Wierda WG *et al*: Phase I and pharmacokinetic study of a low-clearance, unilamellar liposomal formulation of Lurtotecan , a topoisomerase I inhibitor, in patients with advanced leukemia. Cancer *100*: 1449-1458, 2004.

66 Chatterjee A, Digumarti, Mamidi RN, Katneni K, Upreti VV, Surath A, Srinivas ML, Uppalapati S, Jiwatani S, Subramaniam S and Srinivas NR: Safety, tolerability, pharmacokinetics, and pharmacodynamics of an orally active novel camptothecin analog, DRF-1042, in refractory cancer patients in a phase I dose escalation study. J Clin Pharmacol *44*: 723-736, 2004.

67 Upreti VV, Mamidi RN, Katneni K and Srinivas NR: Quantitative determination of DRF-1042 in human plasma by HLPC: validation and application in clinical pharmacokinetics. Biomed Chromatogr *17*: 385-390, 2003.

68 Lavergne O, Lesueur-Ginot L, Rodas FP and Bigg DCH: BN 80245: an E-ring modified camptothecin with potent antiproliferative and topoisomerase I inhibitory activities. Bioorg Med Chem Lett *7*: 2235-2238, 1997.

69 Lansiaux A, Facompre M, Wattez N, Hildebrand MP, Bal C, Demarquay D, Lavergne O, Bigg DCH and Bailly C: Apoptosis induced by the homocamptothecin anticancer drug BN80915 in HL-60 cells. Mol Pharmacol *60*: 450-461, 2001.

70 Larsen AK, Gilbert C, Chyzak G, Plisov SY, Naguibneva I, Lavergne O, Lesueur-Ginot L and Bigg DCH: Unusual potency of BN 80915, a novel fluorinated E-ring modified camptothecin, toward human colon carcinoma cells. Cancer Res *61*: 2961-2967, 2001.

71 Bailly C, Lansiaux A, Dassonneville L, Demarquay D, Coulomb H, Huchet M, Lavergne O and Bigg DCH: Homocamptothecin, an E-ring modified camptothecin analog, generates new topoisomerase I-mediated DNA breaks. Biochemistry *38*: 15556-15563, 2001.

72 Sparreboom A,Gelderblom H, Marsh S, Ahluwalia R, Obach R, Principe P, Twelves C, Verweij J and McLeod HL: Difomotecan pharmacokinetics in relation to ABCG2 412C>A genotype. Clin Pharmacol Ther *76*: 38-44, 2004.

73 Gelderblom H, Salazar R, Verweij J, Pentheroudakis G, de Jonge MJ, Devlin M, van Hooije C, Seguy F, Obach R, Prunonosa J, Principe P and Twelves C: Phase I pharmacological and bioavailability study of oral diflomotecan (BN80915), a novel E-ring-modified camptothecin analogue, in adults with solid tumors. Clin Cancer Res *9*: 4101-4107, 2003.

74 Rowinsky EK, Rizzo J, Ochoa L, Takimoto CH, Forouzesh B, Schwartz G, Hammond LA, Patnaik A, Kwiatek J, Goetz A, Denis L, McGuire J and Tolcher AW: A phase I and pharmacokinetic study of pegylated camptothecin as a 1-hour infusion every 3 weeks in patients with advanced solid malignancies. J Clin Oncol *21*: 148-157, 2003.

75 Conover CD, Greenwald RB, Pendri A, Gilbert CW and Shum KL: Camptothecin delivery system: enhanced efficacy and tumor accumulation of camptothecin following its conjugation to polyethylene gycol *via* a glycine linker. Cancer Chemother Pharmacol *42*: 407-414, 1998.

76 Conover CD, Greenwald RB, Pendri A and Shum KL: Camptothecin delivery systems: the utility of amino acid spacers for the conjugation of camptothecin with polyethylene glycol to create prodrugs. Anticancer Drug Des *14*: 499-506, 1999.

77 Sarapa N, Britto MR, Speed W, Jannuzzo M, Breda M, James CA, Porro M, Rocchetti M, Wanders A, Mahteme H and Nygren P: Assessment of normal and tumor tissue uptake of MAG-CPT, a polymer-bound prodrug of camptothecin, in patients undergoing elective surgery for colorectal carcinoma. Cancer Chemother Pharmacol *52*: 424-430, 2003.

78 Schoemaker NE, van Kesteren C, Rosing H, Jansen S, Swart M, Lieverst J, Fraier D, Breda M, Pellizzoni C, Spinelli R, Grazi Porro M, Beijnen JH, Schellens JHM and ten Bokkel Huinink WW: A phase I and pharmacokinetic study of MAG-CPT, a water-soluble polymer conjugate of CPT. Br J Cancer *87*: 608-614, 2002.

79 Wachters FM, Groen HJ, Maring JG, Gietema JA, Porro M, Dumez H, de Vries EG and van Oosterom AT: A phase I study with MAG- camptothecin intravenously administered weekly for 3 weeks in a 4-week cycle in adult patients with solid tumours. Br J Cancer *90*: 2261-2267, 2004.

80 Harada M, Imai J, Okuno S and Suzuki T: Macrophage-mediated activation of camptothecin analogue T-2513-carboxymethyl dextran conjugate (T-0128): possible cellular mechanism for antitumor activity. J Control Release *69*: 389-397, 2000.

81 Harada M, Sakakibara H, Yano T, Suzuki T and Okuno S: Determinants for the drug release from T-0128, camptothecin analogue-carboxymethyl dextran conjugate. J Control Release *69*: 399-412, 2000.

82 Harada M, Murata J, Sakamura Y, Sakakibara H, Okuno S and Suzuki T: 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, 2000.

83 Okuno S, Harada M, Yano T, Yano S, Kiuchi S, Tsuda N, Sakamura Y, Imai J, Kawaguchi T and Tsujihara K: Complete regression of xenografted human carcinomas by camptothecin analogue-carboxymethyl dextran conjugate (T-0128). Cancer Res *60*: 2988-2995, 2000.

84 Mross K, Richly H, Schleucher N, Korfee S, Tewes M, Scheulen ME, Seeber S, Beinert T, Schweigert M, Sauer U, Unger C, Behringer D, Brendel E, Haase CG, Voliotis D and Strumberg D: A phase I clinical and pharmacokinetic study of the camptothecin glycoconjugate, BAY-38-3441, as a daily infusion in patients with advanced solid tumors. Ann Oncol *15*: 1284-1294, 2004.

85 Mross K, Sauer U, Haring B, Behringer D, Brendel E and Unger C: Phase I dose escalation pharmacokinetics (PK) study of BAY 38-3441 administered as a short infusion once every three weeks. Proc Am Soc Clin Oncol *21*: 100a, 2002.

86 Siu LL, Batist G, Bangash N, MacLean M, McIntosh L, Miller WH, Oza AM, Petrenciuc O and Seymour L: A phase I study of BAY 38-3441 given as a short infusion daily for five days every three weeks: A National Cancer Institute of Canada Clinical Trials Group study. Proc Am Soc Clin Oncol *21*: 99a, 2002.

87 Yang L-X, Pan X and Wang H-J: Novel camptothecin derivatives. Part 1: Oxyalkonoic acid esters of camptothecin and their *in vitro* and *in vivo* antitumor activity. Bio Med Chem Let *12*: 1241-1244, 2002.

Received November 13, 2004
Accepted December 16, 2004