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A phase I clinical and pharmacokinetic study of the topoisomerase I inhibitor topotecan (SK&F 104864) given as an intravenous bolus every 21 days

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Topotecan (SK&F 104864) is a novel antitumor agent whose mechanism of action is inhibition of the DNA unwinding protein topoisomerase I. An analog of camptothecin, topotecan was designed to be more water soluble in an effort to decrease the severe and sporadic toxicities experienced during phase I/II trials of the parent compound. In this phase I clinical and pharmacological trial, topotecan was given as a bolus intravenous (i.v.) infusion over 30 min every 21 days. A total of 42 patients entered the study, receiving doses ranging from 2.5 to 22.5 mg/m^2. The maximum tolerated dose (MTD) of topotecan given in this schedule was 22.5 mg/m^2. Myelosuppression, primarily neutropenia, was dose-limiting. The extent of prior therapy did not predict for more severe neutropenia. Non-hematologic toxicities were mild and included low-grade to moderate fever, nausea, vomiting, alopecia, diarrhea and skin rashes. There were no objective partial or complete responses, although there was a suggestion of antitumor activity in three patients. Topotecan undergoes pH-dependent hydrolysis of the lactone ring; only the closed, lactone form is active. The lactone form predominated during infusion, with hydrolysis occurring rapidly following the end of infusion. There were linear relationships between dose administered and peak plasma lactone concentrations as well as AUC lactone to AUC total. The lactone was rapidly cleared from plasma with a total body clearance of 25.7 (±6.7) l/h/m^2. The plasma lactone concentration declined rapidly with a harmonic mean terminal half-life of 3.4 (±1.1) h. Lactone hydrolysis and renal excretion were the major routes of elimination. Topotecan given as an i.v. bolus every 21 days proved to be a well-tolerated drug with primarily hematological toxicity.

Key words: Pharmacokinetic study, phase I trial, topoisomerase I inhibitor, topotecan.

Introduction

The DNA topoisomerases are enzymes which relax supercoiled DNA by transiently breaking either one or two strands of the double helix, which allows sufficient uncoiling so that replication and transcription can proceed. Two types of topoisomerases are found in all eukaryotic cells: type I topoisomerase (Topo I), which produces single-strand breaks, and type II topoisomerase (Topo II), which produces double-strand breaks. Together, they facilitate relaxation and uncoiling of DNA supercoils by enabling cleavage and then rejoining of the DNA helix during replication and RNA transcription. By covalently binding to DNA, the enzymes form a 'cleavable complex' which spans the nick but allows relative rotation of the broken ends of DNA. The

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break is then resealed after the free strand (or strands) have passed.[1-3] In view of their intimate association with DNA replication, Topo I and II are attractive targets for anticancer agents. It is now known that Topo II is the target for such diverse drugs as the anthracyclines, anthracenediones, amsacrine, actinomycin D, and the epipodophyllotoxins, etoposide and teniposide.[1-4] Topo I inhibition is currently the focus of much attention.

The plant alkaloid camptothecin, a product of the Asian tree *Camptotheca acuminata*, was screened in the 1960s and found to have antitumor activity in *in vitro* assays.[5,6] However, hematological, gastrointestinal and urinary bladder toxicities were found to be severe and unpredictable during phase I and II trials. Despite objective responses in patients with colorectal cancer, non-small cell lung cancer, gastric cancer, melanoma and acute non-lymphocytic leukemia, its development was abandoned.[7-10] When camptothecin was found to be a specific inhibitor of Topo I,[11-13] interest was sparked to investigate less toxic analogs.

Due to the insolubility of camptothecin lactone, the drug used in the initial clinical trials (NSC 100880) was the sodium salt of camptothecin, which was inactive unless in an acid pH environment where the lactone form was reconstituted. Topotecan (SK&F 104864), (*S*)-9-dimethylaminomethyl-10-hydroxycamptothecin hydrochloride, is a semisynthetic analog of camptothecin with improved water solubility (Figure 1).[14,15] It incorporates a stable basic side chain at the 9-position of the A-ring of 10-hydroxycamptothecin, which provides water solubility at acidic pH without requiring hydrolysis of the E-ring lactone. In addition, it has much reduced binding to plasma proteins. Greater than 99% of camptothecin is bound to human plasma proteins.[7,16] Topotecan binding to plasma proteins varies somewhat between species, but in humans the percent bound (mean $\pm$ SD) is 21.32 $\pm$ 1.62.[17] The water solubility and diminished protein binding are felt to lessen the risk of cystitis. Topotecan is known to undergo pH-dependent hydrolysis of the E-ring lactone; only the closed lactone form of the drug is active.[14,15] At pH levels below 7.0, the closed form of topotecan predominates. At pH 6.0, for example, the lactone accounts for more than 80% of the total compound.

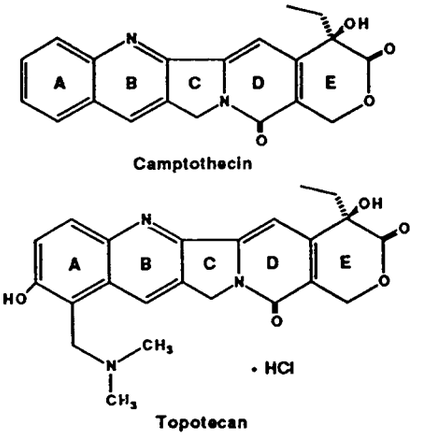

Figure 1. Structures of camptothecin and topotecan (SK&F 104864).

Topo I inhibitors cause cytotoxicity by interfering with enzyme function. Therefore, their activity is proportional to the amount of enzyme present in target tissues. Unlike Topo II, whose level varies during the cell cycle, the amount of Topo I appears to remain constant across the cell cycle.[18] However, Topo I inhibitors are highly cell-cycle specific. Cells in S phase are 1000-fold more sensitive to camptothecin than cells in G_1 or G_2, when the exposure time is brief.[19,20] Recently, assays for Topo I in surgical specimens from patients with colon cancer have shown an elevated level (up to 14- to 16-fold) compared with adjacent normal colonic mucosa.[21] This finding suggests a possibility of an improved therapeutic index beyond that afforded by the activity predicted against proliferating tissues.

Topotecan is active against a variety of tumors in animal models.[22] It is highly effective against murine leukemias and such chemorefractory tumors as colon carcinomas 38 and 51, Lewis lung carcinoma, and B16 melanoma. After toxicology studies demonstrated acceptable toxicity in animals (reversible, dose-related toxicity to proliferating tissues such as bone marrow and gastrointestinal epithelium), topotecan advanced to phase I trials.[23] We describe the phase I experience with topotecan given as an intravenous (i.v.) bolus over 30 min every 21 days.

Materials and methods

Patient Selection

All patients had a microscopically confirmed diagnosis of metastatic solid tumors refractory to all known forms of effective therapy, a SWOG performance status of 2 or less and an anticipated life expectancy of at least 12 weeks. Additional eligibility criteria included: white blood cell count of at least 3000 cells/μl (granulocytes greater than or equal to 1500 cells/μl), platelets greater than 100 000/μl, hemoglobin greater than 10 g/dl, serum creatinine 1.5 mg/dl or less and bilirubin 1.5 mg/dl

or less. Patients with a prior history of hemorrhagic cystitis, prior pelvic irradiation or urinalysis indicating greater than five red blood cells per high power field on microscopic examination were excluded. Patients must have been off previous anticancer therapy for at least 3 weeks (6 weeks if a nitrosourea or mitomycin C were used). All patients signed informed consent documents approved by the Institutional Review Board of the treating institution.

Study design

A minimum of three patients were treated at each dose level of topotecan. Once WHO grade 2 or greater toxicity was observed, additional patients were treated to further define the nature and severity of the toxicity before progressing to the next dose level. Patients who tolerated topetocan without serious toxicity continued to receive the drug without dose escalations or reductions as long as their tumor remained stable or decreased in size. Patients with evidence of tumor progression were removed from study. Patients experiencing severe or life-threatening toxicity, defined as WHO grade 4 hematologic or grade 3 non-hematologic toxicity, were removed from study unless tumor response status or extenuating concomitant medical conditions allowed for individual decisions regarding continuation on study.

Drug administration

Topotecan was supplied in 2 ml (5 mg base/ml) ampules as a solution with 0.1 M gluconate buffer at pH 3.0. The drug was diluted in 100 ml of normal saline (pH 5.6, range 4.5–7.0) and administered i.v. via an infusion pump over 30 min. In mice the LD_{10} was 74.9 mg/m^2. However, when the compound was tested in dogs at 0.1 $MELD_{10}$, marked leukopenia occurred. Therefore, the starting dose in this phase I clinical trial was 2.5 mg/m^2, which was 1/30 the $MELD_{10}$ in mice. Subsequent doses were escalated using a modified Fibonacci scheme. All patients were hospitalized for at least 24 h after receiving their first dose of topotecan. Subsequent doses were given in the outpatient clinic. Patients returned weekly for physician visits to record history and physical examinations. Chemistry profiles, hemograms, urinalyses and stool specimens for occult bleeding were checked weekly. Chest X-rays and electrocardiograms were repeated prior to each cycle and tumor measurements were performed every two cycles.

Blood sampling and urine collections

Blood and urine specimens for pharmacokinetic studies were obtained from 17 patients during their first course of treatment. Urine samples were collected at baseline and every 6 h for 12 h then every 12 h up to 48 h and stored at −20°C until analysis. Blood samples were collected prior to drug administration, midway through the 30 min infusion, at the end of infusion, and 5, 15, 30 min and 1, 2, 3, 4, 6, 8, 12, 16 and 24 h post-infusion. The blood samples were immediately centrifuged at 5000 r.p.m. for 3 min and the plasma immediately subjected to solid phase extraction.

Sample preparation

Plasma or bile extraction of topotecan was accomplished by adding 1 ml of 0.1 M NH_4PO_4:1 mM dioctylsulfosuccinate buffer (pH 6.5) to 0.5 ml of plasma spiked with a structurally related analog, SKF105107 ([*S*)]-9-morpholinomethyl-10-hydroxycamptothecin). The mixture was vortexed and applied to a pre-conditioned C_{18} solid phase extraction cartridge (Fisher PrepSep). The sample cartridges were pre-conditioned with 2 ml of HPLC grade methanol followed by 1 ml of 0.01 M $NaPO_4$, pH 6.3, then by 1 ml of the 0.1 M NH_4PO_4 buffer. Immediately following the addition of the sample mixture, the column was washed with 1 ml of the 0.1 M NH_4PO_4 buffer followed by two additional 1 ml volumes of the 0.01 M $NaPO_4$. The absorbed topotecan was eluted with 0.75 ml of methanol, followed by 0.25 ml of the 0.01 M $NaPO_4$ and immediately analyzed by HPLC. The extraction efficiency of topotecan from plasma was 85.4%. Urine samples were centrifuged for 10 min at 2000 r.p.m. to separate any insoluble materials and filtered through a 0.45 μm filter.

HPLC analysis

This HPLC method is specific for the lactone (closed) form of topotecan. The chromatographic procedure consisted of injecting (U6K, Water Associates, Milford, MA) 10–80 μl of the plasma or bile extract onto an ODS cartridge column (Ultrasphere XL, 75 mm × 4.6 mm, 3μ, Beckman) preceded by a Nova-Pak® C_{18} pre-column (Guard Pak, Waters Associates). The helium purged mobile phase consisted of methanol:250 mM dioctylsulfosuccinate : 1 M NH_4PO_4 : triethylamine

(65:4:2.3:0.3) pH to 6.0 with H_3PO_4 and pumped (model 600; Water Associates) at a flow rate of 1.0 ml/min. Fluorescence was monitored with a Model LS-1 LC fluorescence detector (Perkin-Elmer, Norwalk, CT) with the excitation wavelength set at 375 nm and the emission wavelength set at 470 nm. Chromatograms and peak height areas were stored and analyzed on a Waters 740 data module (Waters Associates). Retention times for topotecan and internal standard in plasma were 2.9 and 2.2 min, respectively. The amount of topotecan in each sample was calculated by comparison of the peak area ratios with that of the standard curve analyzed on the same day. Standard curves constructed in blank donor plasma were linear ($r^2 > 0.999$) over the range of 2–1000 ng/ml. Following quantitation of the lactone, the plasma extracts were acidified with 2% phosphoric acid and analyzed by the above HPLC methodology. Acidification converts any of the hydrolyzed open form of topotecan which might be present to the lactone form and is a measure of the total (lactone plus carboxylate) of both forms. The chromatographic system (Waters Associates) for quantitation of topotecan as total in the urine consisted of a model 600 E gradient pump, a model 740 data module, an automatic sample injector (WISP model 710B) and a model 470 fluorescence detector. The excitation and emission wavelengths were set at 385 and 525 nm, respectively. A Novapak® C_{18} pre-column (GuardPak, Waters Associates) preceded the C_{18} (Novapak, 3.9 mm × 150 mm, 4μ) analytical column. The helium purged mobile phase consisted of solvent A, acetonitrile:acetic acid:triethylamine (98.25%:1%:0.75%) and solvent B, ultrapure H_2O:acetic acid:triethylamine (98.25%:1%:0.75%). A linear gradient of an initial concentration of 7%A:93%B to a final concentration of 25%A:75%B was run at a flow rate of 2.5 ml/min over 15 min. Retention times for topotecan and the internal standard were 14 and 17 min, respectively. Samples were diluted (1:5 to 400) in mobile phase (7%A:93%B) as needed to produce concentrations within the linear ($r^2 > 0.99$) range (5–500 ng/ml) of the standard curve.

Pharmacokinetic analysis

The pharmacokinetic parameters were calculated using model independent methods.[24] The terminal rate constant (k) was determined by log-linear regression analysis of the terminal phase of the plasma concentration–time curves. The terminal plasma half-lives were calculated by the equation $t_{1/2} = 0.693/k$. The area under the plasma concentration–time curve (AUC) and the area under the first moment curve (AUMC) were calculated by the linear trapezoidal rule up to the last measurable data points with extrapolation to infinity. Clearance was calculated by dividing the total dose of topotecan received by the AUC. The apparent volume of distribution at steady state (V_{dss}) was determined by the following relationships: $V_{dss} = (\text{dose} \times \text{AUMC}/\text{AUC}^2) - (\text{dose} \times \text{duration of infusion})/(2 \times \text{AUC})$.

Results

Patient characteristics

A total of 42 patients were enrolled in this trial. The patient characteristics are outlined in Table 1. Thirty-six patients had received some form of prior therapy. Thirty-five patients had a SWOG performance status of 0 or 1. All patients were evaluable for toxicity. A total of 123 courses of therapy were given; the median number of courses per patient was two.

Table 1. Patient characteristics

Patients entered	42
Sex	
male	33
female	9
Median age, years (range)	61 (32–81)
Performance status	
0	11
1	24
2	7
Prior therapy	
none	6
chemotherapy	34
radiotherapy	17
Primary tumors	
non-small cell lung	16
colorectal	11
pancreas	2
renal cell	2
ovarian	2
adenocarcinoma-unknown 1°	2
small cell lung	1
prostate	1
head and neck	1
melanoma	1
squamous cell of skin	1
cholangiocarcinoma	1
ampulla of vater	1

Table 2. Hematologic toxicities

	Topotecan dose (mg/m^2)							
	2.5	5.0	8.3	10.4	12.5	15.0	17.5	22.5
No. of patients	3	3	7	6	5	6	7	5
WBC nadir[a] ($\times 10^3$ cells/μl)								
0 = >4.000	2	3	4	3	3	2	2	1
1 = 3.000–3.999	—	—	1	—	1	—	2	1
2 = 2.000–2.999	1	—	—	3	—	3	1	—
3 = 1.000–1.999	—	—	2	—	1	—	1	2
4 = <1.000	—	—	—	—	—	1	1	1
Neutrophil nadir[a]								
0 = >1500	3	3	5	4	4	3	3	1
1 = 1001–1499	—	—	—	1	—	1	—	1
2 = 500–999	—	—	1	—	—	1	2	—
3 = 250–499	—	—	—	1	1	—	—	1
4 = <250	—	—	1	—	—	1	2	2
Platelet nadir[a]								
0 = >100 000	3	3	6	5	5	5	5	3
1 = 75 000–99 999	—	—	—	—	—	1	—	—
2 = 50 000–74 999	—	—	2	1	—	—	1	1
3 = 25 000–49 999	—	—	—	—	—	—	—	—
4 = <25 000	—	—	—	—	—	—	1	1
Hemoglobin nadir[a] (g/dl)								
0 = >10.0	1	2	5	4	3	3	4	3
1 = 9.0–9.9	1	1	—	2	1	1	1	—
2 = 7.0–8.9	1	—	1	—	1	2	1	2
3 = 5.0–6.9	—	—	1	—	—	—	1	—
4 = <5.0	—	—	—	—	—	—	—	—

[a] Nadir value during any course of therapy.

Toxicity

The maximally tolerated dose (MTD) of topotecan given as an i.v. bolus over 30 min every 21 days was 22.5 mg/m^2. Eight dose levels were evaluated to reach this dose. Myelosuppression was the major dose-limiting toxicity. Leukopenia and granulocytopenia were observed in some patients at each dose level above 5 mg/m^2 but was more significant in the final two dose levels (Table 2). WHO grade 4 granulocytopenia (absolute granulocyte count less than 250 cells/μl) occurred in two of seven patients treated with 17.5 mg/m^2 and two of five patients treated with 22.5 mg m^2. An additional patient treated with 22.5 mg m^2 experienced WHO grade 3 granulocytopenia (granulocyte count between 250 and 500 cells μl). The granulocyte nadirs typically occurred between days 10 and 14 of each cycle and were of brief duration (3–5 days). Twelve of the 42 patients received four or more courses of topotecan. In these patients, there was no evidence of cumulative hematologic toxicity during the course of this trial. Six patients required hospitalization and i.v. antibiotics for granulocytopenic fevers. There were two episodes of documented bacteremia during the neutropenic episodes. Both patients were successfully treated with antibiotics and discharged.

In light of the somewhat sporadic nature of granulocytopenia, an attempt was made to assess the possible effect of prior therapy on the incidence of granulocytopenia. Patients were divided into three groups depending on the extent of prior therapy: (i) group 1—no prior anticancer therapy, (ii) group 2—two or fewer chemotherapy regimens, or one chemotherapy regimen plus radiotherapy to a port encompassing more than 20% of the bone marrow, and (iii) group 3—three or more chemotherapy regimens or two regimens plus radiotherapy to more than 20% of the bone marrow. Since granulocytopenia might be dose-related, an analysis of covariance was performed to compare granulocytopenia across the three groups adjusting for the dose administered. Table 3 presents the mean granulocyte nadirs (for any course of topotecan therapy) and their standard errors adjusted for dose. These adjusted means were not significantly

Table 3. Comparison of nadir absolute neutrophil counts (ANCs)

Group	N	Mean nadir ANC ± SE adjusted for dose
1	6	2624 ± 512
2	25	1883 ± 246
3	11	2175 ± 371
		($p = 0.41$)

Group 1: no prior treatment
Group 2: two or fewer prior chemotherapy regimens or one regimen plus radiotherapy to greater than 20% of bone marrow
Group 3: more than two prior chemotherapy regimens or two regimens plus radiotherapy to greater than 20% of bone marrow

different from one another ($p = 0.41$). Thus, the extent of prior treatment did not appear to predict for the severity of myelosuppression resulting from topotecan.

Thrombocytopenia was less frequent and less severe than was neutropenia. One patient at the 17.5 mg/m^2 level and one patient at the 22.5 mg/m^2 level experienced WHO grade 4 thrombocytopenia (platelet count less than 25 000/μl). Ten patients developed WHO grade 2 or greater anemia (hemoglobin less than 9.0 g/dl). A total of three patients required transfusion of packed red blood cells (PRBCs) at some time during their treatment: (i) a 62 year old male with non-small cell lung cancer who was treated with 8.3 mg/m^2 developed grade 3 anemia (hemoglobin 6.8 g/dl) during his fourth cycle of topotecan and received four units of PRBCs; (ii) a 59 year old male with chest wall metastases from non-small cell lung cancer who was treated at the 8.3 mg/m^2 dose level developed bleeding from the ulcerating chest wall mass and required seven units of PRBCs; and (iii) a 65 year old male with non-small cell lung cancer treated with 17.5 mg/m^2 required transfusions of two units of PRBCs during his first cycle and four units during the second cycle. Prior chemotherapy for the lung cancer had consisted of two cycles of cisplatin and etoposide. This patient also developed grade 4 neutropenia and grade 4 thrombocytopenia during each cycle and required platelet transfusions of 10 units of platelets during the nadir period of each course.

Non-myelosuppressive toxicities were mild and generally not dose related. Mild (WHO grade 1 or 2) nausea and vomiting occurred in 23 patients, usually limited to the day of drug administration. Low grade or moderate fevers (99.6–102.0°F) were observed in 21 patients. The onset of fever often followed the drug administration by 6–12 h and the fever usually resolved before the second day of each cycle. Thirty-two patients received two or more courses of topotecan. Only four of these patients experienced a recurrence of fever in any subsequent cycle and none experienced fever with each administered dose of topotecan. In addition to the fevers, six patients developed chills on the day of drug administration. Mild flu-like symptoms (malaise, myalgias, headache and/or fatigue) were noted by six patients. Six patients developed mild diarrhea (WHO grade 1–2) on the day of drug administration. There was no evidence of gastrointestinal bleeding. Alopecia was seen at each dose level of 10.4 mg/m^2 and higher, including four patients receiving 17.5 mg/m^2 and three patients receiving 22.5 mg/m^2. Six patients developed a skin rash during treatment. This usually consisted of a non-generalized, maculopapular, erythematous eruption although some patients had evidence of small pustules giving a more acneiform appearance. One patient who received topotecan at the 22.5 mg/m^2 level developed mucositis, with grade I stomatitis noted during the first week of each of her two cycles. This patient had been extensively treated with multiple agents for metastatic ovarian carcinoma. She also experienced grade 4 granulocytopenia and thrombocytopenia during each course of therapy, despite a dose reduction to 15 mg/m^2 for the second cycle. Other than this instance, there was no evidence of gastrointestinal mucosal toxicity as had been identified in animals during the pre-clinical evaluation of topotecan. Of note, hematuria or urinary bladder complaints were not observed during this trial. One patient experienced a minor infiltration of drug around a forearm i.v. catheter site. She experienced no local symptoms and no soft tissue injury could be detected on subsequent examinations.

Although probably not drug-related, it is interesting to note that two patients receiving topotecan developed Herpes zoster infection, presenting with the usual dermatomal pattern. Neither patient developed systemic illness and in both cases the infection cleared after a typical course. An additional patient presented during course 6 with complaints of chest pain which prompted admission to the hospital. The electrocardiogram revealed changes consistent with an inferior myocardial infarction. At the time of the symptoms the patient's hemoglobin measured 13.6 g/dl. An echocardiogram was normal and a limited graded exercise test produced no symptoms or electrocardiographic

changes. This event was felt to be unrelated to topotecan administration.

Pharmacokinetic results

The pharmacokinetic results are summarized in Table 4. A plasma concentration versus time profile for a representative individual patient at 22.5 mg/m^2 is shown in Figure 2. The average maximum plasma lactone concentration was 581 ng/ml at 22.5 mg/m^2 occurring at the end of infusion. The lactone was the predominant form during the infusion. Fifteen minutes into the infusion and at the end of the infusion, the lactone concentration represented 83% and 69% of the total concentration, respectively. Hydrolysis of the lactone ring occurred rapidly following a 30 min infusion. Within 15 min post-infusion, 50% of the topotecan had been converted to the inactive carboxylate form. There was a linear relationship between peak plasma lactone concentrations versus dose ($r^2 = 0.94$, $p < 0.002$). Likewise, the AUC lactone to AUC total ratio remained relatively constant over the entire dosage range. The plasma lactone concentration declined rapidly with a harmonic mean terminal half-life of 3.4 ($\pm$1.1) h. The mean apparent volume of distribution at steady state was 76.4 ($\pm$18.5) l/m^2 and the mean total body clearance was 25.7 ($\pm$6.7) l/h/m^2. An average of 40% (range 22–60%) of the total dose of topotecan was excreted unchanged in urine over 24 h, with greater than 90% of the excreted amount appearing within the first 12 h of urine collection.

A patient with colon cancer metastatic to the liver had an internal stent in place for common bile duct involvement. Ten days following his first course of topotecan (17.5 mg/m^2) he developed biliary obstruction (total bilirubin 3.8 mg/dl) necessitating that the internal stent be replaced with an externally-draining stent. Upon receiving his second course of topotecan, abbreviated pharmacokinetic studies including concomitant plasma and bile samples were obtained as an outpatient. His total bilirubin and the pH of the bile at the time of sample collection were 1.5 and 7, respectively. Plasma concentrations of total topotecan were similar for his first and second course, C_{pmax} 645.04 and 566.7 ng/ml, respectively. Considerable concentrations of topotecan were detected in the bile compared to the plasma (Figure 3). Not only was there a residual baseline level from the first course, but the peak bile concentration was 1.5 times higher than that of the peak plasma concentration.

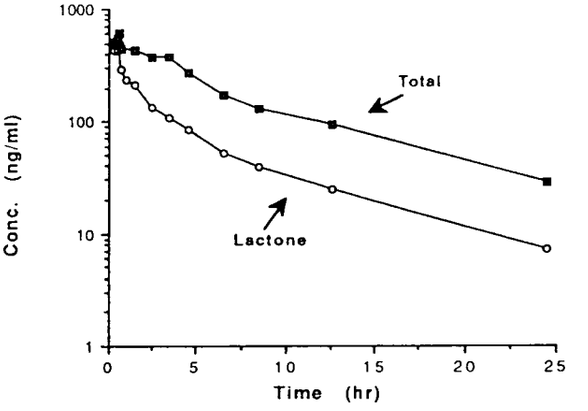

Figure 2. Plasma concentration versus time profile for a representative individual patient receiving 22.5 mg/m^2.

Table 4. Topotecan pharmacokinetic parameters (single dose schedule)

No. of patients	Dose (mg/m^2)	Peak concentration (ng/ml)		Half-lives (h)		Clearance (l/h/m^2)		V_{dss} (l/m^2)		AUC (μg h/ml)		AUC lactone: AUC total[a]
		lactone	total[a]	lactone	total[a]	lactone	total[a]	lactone	total[a]	lactone	total[a]	
$n = 1$	2.5	130	185	2.4	5.5	11.6	3.9	30.2	19.9	0.22	0.64	0.34
$n = 2$	5.0	160	237	4.0	5.1	23.7	6.1	85.9	35.7	0.22	0.82	0.26
$n = 3$	8.3	194	298	2.6	2.7	27.6	8.6	69.2	32.4	0.31	1.00	0.31
$n = 2$	10.4	271	345	3.1	4.5	30.8	10.5	81.5	43.9	0.35	1.02	0.34
$n = 2$	12.5	253	365	3.0	4.9	33.2	11.3	89.3	51.8	0.38	1.11	0.34
$n = 3$	15.0	417	567	3.8	4.5	22.8	8.0	71.2	36.0	0.67	1.91	0.35
$n = 2$	17.5	455	636	3.7	5.3	29.0	10.2	78.8	48.4	0.61	1.74	0.35
$n = 2$	22.5	581	778	5.5	5.7	20.4	6.9	87.8	43.1	1.17	3.38	0.34
Mean (SD) $n = 17$				3.4[b] (1.1)	4.3[b] (1.8)	25.7 (6.7)	8.0 (3.1)	76.4 (18.5)	39.5 (9.8)			0.33 (0.44)

[a] Total = lactone + carboxylate.
[b] Harmonic mean.

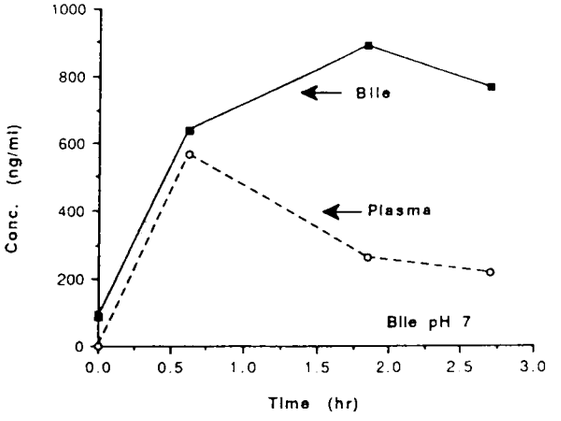

Figure 3. Concentration versus time profiles for bile and plasma in a patient receiving 17.5 mg/m^2.

Responses

Although this was a phase I dose-seeking study and measurable disease was not required as an entry criterion, 16 patients had measurable tumors and received at least two cycles of treatment, making them eligible for evaluation of tumor response. No patients met the standard criteria for partial or complete response. Some suggestion of anticancer activity was observed in three patients. One patient with squamous cell carcinoma of the lung metastatic to a palpable supraclavicular lymph node experienced a decrease in bi-dimensional area of greater than 50% of 3 weeks duration. Another patient with metastatic squamous cell carcinoma of the skin experienced a greater than 50% regression of tumor area of 3 weeks duration. A third patient with metastatic renal cell carcinoma had a dramatic decrease in height of a subcutaneous scalp metastatic nodule, although the cross-sectional measurement as recorded at the initiation of therapy did not allow for the description of the regression as a partial response. Minor responses and periods of disease stabilization were seen in other patients as well. Twelve patients received at least four cycles of therapy, with a maximum duration of treatment of 10 cycles. Of the 12 patients receiving at least four courses of therapy, five patients had manifested clearly progressive disease prior to beginning therapy with topotecan, hinting at some anticancer activity.

Discussion

When given as a 30 min infusion every 21 days, topotecan proved to be a well-tolerated compound. Its major toxicity was myelosuppression, predominantly granulocytopenia, which was of brief duration. Severe anemia and thrombocytopenia were infrequently observed but two patients developed WHO grade 4 thrombocytopenia and required platelet transfusions. A total of 10 patients experienced anemia of WHO grade 2 or greater severity (hemoglobin less than 9.0 g/dl) with three patients receiving transfusion of PRBCs. Non-hematologic toxicities were mild and easily managed. The gastrointestinal and urinary bladder toxicities seen during the initial trials of camptothecin were not observed during this trial with topotecan.

Topotecan undergoes pH-dependent reversible hydrolysis yielding an α,β dihydroxycarboxylic acid functionality (open form). The closed lactone form of the drug is biochemically and pharmacologically active. The lactone was rapidly cleared from the plasma with a total body plasma clearance of 25 l/h/m^2. The mean terminal half-life was 3.4 h with a range from 2.4 to 5.5 h. The only pharmacodynamic relationship noted was the correlation between the grade of leukopenia (WHO grade) and the AUC ($r = 0.6$, $p < 0.05$) for both the lactone and total, as well as peak plasma concentrations ($r = 0.6$, $p < 0.02$). A relationship between concentration (AUC or peak plasma concentration) and neutropenia was not found, perhaps because only four of the 17 patients evaluated with pharmacokinetic analysis experienced a grade 1 or greater neutropenia. Lactone hydrolysis and renal excretion appear to be the major routes of elimination for topotecan. Finding detectable levels of topotecan in bile suggests the possibility of biliary excretion as another route of elimination. No metabolites of topotecan other than the hydrolyzed form were detected in plasma or urine with the analytical conditions described.

Topotecan has been administered in three additional phase I trials, utilizing a different schedule of administration. Recondo *et al.*[25] gave topotecan as a 24 h continuous infusion every 21 days. Hematologic toxicity predominated using this schedule. The MTD in heavily pretreated patients was determined to be 4 mg/m^2. No responses were observed in the 15 patients described in their initial report. Rowinsky *et al.*[26] and Sirott *et al.*[27] both described their experience using topotecan administered as a 30 min infusion on five consecutive days every 21 days. Dose-limiting neutropenia was seen at doses of 1–1.5 mg/m^2. Rowinsky *et al.*[26] accrued 29 patients and described partial responses in three patients with non-small cell lung cancer and one

patient with ovarian cancer. Sirott *et al.*[27] enrolled 16 patients and observed one partial response at the 1.5 mg/m^2 dose level. In each study, non-hematologic toxicity was described as mild to moderate and did not prove to be dose-limiting.

In our phase I trial, topotecan proved to be a well-tolerated drug with somewhat sporadic but generally dose-related myelosuppression. We were unable to demonstrate that prior treatment with chemotherapy was associated with an increased risk of hematologic toxicity. While some tumor regression was observed, objective partial or complete remissions were not seen. In light of the short duration of the active lactone form after a bolus administration of topotecan, the lack of tumor remissions is not unexpected. To capitalize on the short half-life of the active compound, we have initiated a trial utilizing a continuous infusion of topotecan for 72 or 120 h.

References

1. Liu LF. DNA topoisomerase poisons as antitumor drugs. *Annu Rev Biochem* 1989; **58**: 351–5.
2. D'Arpa P, Liu LF. Topoisomerase-targeting antitumor drugs. *Biochem Biophys Acta* 1989; **989**: 163–77.
3. Zijlstra JG, DeJong S, DeVries EGE, *et al.* Topoisomerases, new targets in cancer chemotherapy. *Med Oncol Tumor Pharmacother* 1990; **7**: 11–8.
4. Ross WE, Sullivan DM, Chow K-C. Altered function of DNA topoisomerases as a basis for antineoplastic drug action. In: Devita VT, Hellman S, Rosenberg SA, eds. *Important advances in oncology 1988*. Philadelphia: JB Lippincott 1988: 65–81.
5. Wall ME, Wani MC, Cook CE, *et al.* Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. *J Am Chem Soc* 1966; **88**: 3888–90.
6. Dewys WD, Humphreys SR, Goldin A. Studies on therapeutic effectiveness of drugs with tumor weight and survival time indices of Walker 256 carcinosarcoma. *Cancer Chemother Rep (Pt 1)* 1968; **52**: 229–42.
7. Gottlieb JA, Guarino AM, Call JB, *et al.* Preliminary pharmacologic and clinical evaluation of camptothecin sodium (NSC-100880). *Cancer Chemother Rep (Pt 1)* 1970; **54**: 461–70.
8. Muggia FM, Creaven PJ, Hansen HH, *et al.* Phase I clinical trial of weekly and daily treatment with camptothecin (NSC-100880): correlation with preclinical studies. *Cancer Chemother Rep (Pt 1)* 1972; **56**: 515–21.
9. Moertel CG, Schutt AJ, Reitemeier RJ, *et al.* Phase II study of camptothecin (NSC-100880) in the treatment of advanced gastrointestinal cancer. *Cancer Chemother Rep (Pt 1)* 1972; **56**: 95–101.
10. Gottlieb JA, Luce JK. Treatment of malignant melanoma with camptothecin (NSC-100880). *Cancer Chemother Rep (Pt 1)* 1972; **56**: 103–05.
11. Hsiang Y-H, Hertzberg R, Hecht S, *et al.* Camptothecin induced protein-linked DNA breaks via mammalian DNA topoisomerase I. *J Biol Chem* 1985; **260**: 14873–78.
12. Hsiang Y-H, Liu LF. Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. *Cancer Res* 1988; **48**: 1722–26.
13. Eng W-K, Faucette L, Johnson RK, *et al.* Evidence that DNA topoisomerase I is necessary for the cytotoxic effects of camptothecin. *Mol Pharmacol* 1988; **34**: 755–60.
14. Kingsbury WD, Boehm JC, Jakas DR, *et al.* Synthesis of water-soluble (aminoalkyl) camptothecin analogues: inhibition of topoisomerase I and antitumor activity. *J Med Chem* 1991; **34**: 98–107.
15. Kingsbury WD, Hertzberg RP, Boehm JC, *et al.* Chemical synthesis and structure–activity relationships related to SK&F 104864, a novel water-soluble analog of camptothecin. *Proc Am Ass Cancer Res* 1989; **30**: 622.
16. Creaven PJ, Allen LM, Muggia FM. Plasma camptothecin (NSC-100880) levels during a 5-day course of treatment: relationship to dose and toxicity. *Cancer Chemother Rep (Pt 1)* 1972; **56**: 573–78.
17. *Topotecan (SK&F 104864-A) Investigator Brochure*. Philadelphia: Smith, Kline, and French Laboratories 1990.
18. Hsiang Y-H, Wu H-Y, Liu LF. Proliferation-dependent regulation of DNA topoisomerase II in cultured human cells. *Cancer Res* 1988; **48**: 3230–35.
19. Li LH, Fraser TJ, Olin EJ, *et al.* Action of camptothecin on mammalian cells in culture. *Cancer Res* 1972; **32**: 2643–50.
20. Horwitz SB, Horwitz MS. Effects of camptothecin on the breakage and repair of DNA during the cell cycle. *Cancer Res* 1973; **33**: 2834–36.
21. Giovanella BC, Stehlin JS, Wall ME, *et al.* DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. *Science* 1989; **246**: 1046–48.
22. Johnson RK, McCabe FL, Faucette LF, *et al.* SK&F 104864, a water-soluble analog of camptothecin with broad-spectrum activity in preclinical tumor models. *Proc Am Ass Cancer Res* 1989; **30**: 623.
23. Johnson RK, Hertzberg RP, Kingsbury WD, *et al.* Preclinical profile of SK&F 104864, a water-soluble analog of camptothecin. *Sixth NCI-EORTC Symp on New Drugs in Cancer Therapy* 1989: #301.
24. Gibaldi M. *Biopharmaceutics and clinical pharmacokinetics*, 3rd edn. Philadelphia: Lea & Febiger 1984: 17–28.
25. Recondo G, Abbruzzese J, Newman B, *et al.* A phase I trial of topotecan (TOPO) administered by a 24-hour infusion. *Proc Am Assoc Cancer Res* 1991; **32**: 206.
26. Rowinsky EK, Grochow LB, Hendricks CB. Phase I and pharmacologic study of topotecan: a novel topoisomerase I inhibitor. *J Clin Oncol* 1992; **10**: 647–56.
27. Sirott MN, Saltz L, Young C, *et al.* Phase I and clinical pharmacologic study of intravenous topotecan (T). *Proc Am Soc Clin Oncol* 1991; **10**: 104.

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