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Clinical report

A phase II clinical and pharmacological study of oral 9-nitrocamptothecin in patients with refractory epithelial ovarian, tubal or peritoneal cancer

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9-Nitrocamptothecin (9-NC) is a water-insoluble topoisomerase I inhibitor with a broad antitumor activity in animal models. A phase II study was performed in patients with heavily refractory ovarian, tubal or peritoneal cancer (median number of previous chemotherapy regimens >3) to determine the activity of a daily oral dose of 9-NC. 9-NC dose was 1.5 mg/m^2/day for four consecutive days every week. Increments of 0.25 mg/day were authorized in patients without significant side effects. Of 29 evaluable patients, a 7% remission rate was observed. Thirty-four percent of patients had stable disease. The median survival was 8 months. Toxicity was evaluated in 31 patients. Grade 3 or 4 hematologic toxicity consisted of anemia in 10 patients (32%), neutropenia in eight (26%) and thrombocytopenia in three (10%). Grade ≥2 non-hematologic toxic effects were nausea and vomiting in 26 (84%), diarrhea in 12 (39%), weight loss in seven (22%), chemical cystitis in six (19%) and neutropenic sepsis in six (19%). 9-NC was tolerated for sustained periods of time in some patients (up to 47 weeks). The observed 8-month survival in such a refractory patient population is noteworthy. Further clinical research of prolonged exposure to less toxic analogs of 9-NC is warranted. [© **1999 Lippincott Williams & Wilkins.**]

Key words: Anemia, cystitis, myelosuppression, nausea, neutropenia, thrombocytopenia, weight loss.

Introduction

The water-soluble topoisomerase I (Topo I) inhibitors topotecan (TPT) and irinotecan (CPT-11) have recently been marketed for the treatment of various cancers. Preclinical and clinical trials (phases I and II) have demonstrated activity against ovarian cancer and TPT is FDA approved for the treatment of recurrent ovarian cancer.[1] Many phase II studies of TPT in patients with recurrent ovarian cancer have been published.[2–6] The commonest schedule studied is a dose of 1.5 mg/m^2/day for 5 days, every 21 days. A partial response rate of 10–24% has been observed with a median duration around 10 months. Phase II studies of CPT-11 in patients with ovarian cancers have been performed in Japan and Europe, and are starting in the US. With a schedule of weekly infusion of CPT-11 at 100 mg/m^2, the response rates observed in patients with ovarian cancers are between 21 and 24% with a complete response rate of 9.1%.[7,8] Both TPT and CPT-11 have a modest but definite activity in ovarian carcinoma refractory to platinum therapy. Other analogs are currently in clinical trials.

The camptothecins (CPT), a class of Topo I inhibitors, are indole alkaloids that form a reversible complex with Topo I covalently bound to DNA. This binding prevents DNA replication and transcription.[9–11] The 20*S* stereoisomer of CPT in the lactone form binds the Topo I-DNA complex.[12] The intact lactone form (closed E-ring) binds to the DNA with a 10 times greater affinity than the carboxylate form (open E-ring).[13] Acidic conditions favor the formation of the closed lactone ring, basic conditions favor the formation of the less active open ring.[14–16] Furthermore, CPT has a high affinity for proteins, especially for human serum albumin,[15–17] which binds preferentially to the carboxylate form of CPT. Therefore, in the human plasma, the equilibrium between the lactone form and the carboxylate form

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is shifted in favor of the carboxylate or less active form.

Because of their mechanism of action, the Topo I inhibitors are expected to be most active when exposed for long duration to their target, the cancer cells.[18] This hypothesis has been confirmed in preclinical models.[19] In these models, the water-insoluble compounds 9-aminocamptothecin (9-AC) and 9-nitrocamptothecin (9-NC) were the most active in inhibiting the growth of human xenografts.[20]

We recently reported the results of a phase I study of chronic exposure to 9-NC.[21] In this trial, activity was noted in patients with very refractory ovarian cancers. We now report the results of a phase II study of 9-NC in patients with platinum- and paclitaxel-refractory ovarian cancers.

Patients and methods

Patients

Patients 18 years of age or older, with histologically confirmed epithelial ovarian cancer, fallopian tube cancer or primary peritoneal carcinoma resistant to platinum-based therapy were eligible for this study provided that they met the following criteria: a performance status <3 on ECOG scale and a life expectancy greater than 3 months; measurable tumor with documented progression within 2 months before entry into the study (pleural effusions, ascites and osseous metastases were not considered measurable, and lesions located in previously irradiated areas were excluded from the final evaluation); a neutrophil count above 1.5×10^9/l, a platelet count above 100×10^9/l, a hemoglobin level above 8 g/dl, a serum creatinine level below 2.0 mg/dl, a serum bilirubin level below 2.0 mg/dl and a transaminase level less than 2 times the normal institutional level; any number of prior courses of chemotherapy; no unstable medical condition other than cancer; and signed informed consent. All patients had to have recovered from any toxic effects from previous treatments before entry into the study. Disease that relapsed within 6 months from discontinuation of platinum-based therapy, failure to achieve a complete response after six cycles of cisplatin- or carboplatin-based therapy with no further decrease in measurable disease for at least 2 cycles, or progressed despite platinum-based therapy was considered platinum resistant. Exclusion criteria included prior radiotherapy or chemotherapy within the last 4 weeks (6 weeks for nitrosoureas and/or mitomycin C) before treatment; symptomatic brain metastases; previous or current malignancies (except *in situ* carcinoma of the cervix uteri that underwent cone biopsy, adequately treated basal or squamous cell carcinoma of the skin, or a history of breast cancer greater than 5 years without active disease); significant uropathy; and congestive heart failure or poor medical risk because of non-malignant systemic disease.

Before entry, a complete medical history was recorded and a physical examination done. Performance status was noted and lesions measured. A complete blood cell count and relevant blood chemistries, including urine analysis and CA-125, were analyzed. Chest X-ray and other indicated studies were done as needed, and repeated every 6–12 weeks. Every 3 weeks, a clinical examination and a blood and urine chemistry survey were required.

Treatment plan

Upon registration, each patient was instructed to take 9-NC 1.5 mg/m^2 orally for four consecutive days followed by 3 days off every week. 9-NC was administered on an empty stomach (i.e. 2 h after a meal and no intake of food for 1 h afterwards) with an acidic liquid (orange juice, grapefruit juice, lemon juice or Coca Cola$^{®}$) at the same time every day. Patients were instructed to drink 3 l of water or fluid every day. One course was 3 weeks.

Reductions of dose were allowed for prevention of excessive toxicity. In case of grade 3–4 (<1000 cells/μl) granulocytopenia or a thrombocytopenic episode ($<100\,000$ platelets/μl), that lasted for more than 1 week, and in the case of neutropenic fever, subsequent doses were reduced by 33% and given only upon bone marrow recovery to 1500 granulocytes/μl and to 100 000 platelets/μl. Granulocyte colony stimulating factor (G-CSF), 5 μg/kg/day, was administered s.c. only for development of neutropenia below 500 neutrophils/μl, until recovery to >1500 granulocytes/μl while 9-NC was held. For grade 3 non-hematologic toxicity, the dose was reduced by 33% or the drug discontinued at the discretion of the principal investigator. For delays in chemotherapy administration of 2 weeks or more for any reasons, dose was also reduced by 33%. Dose escalation by steps of 0.25 mg/day was permitted if no side effects had reoccurred.

In case of nausea or vomiting, 10 mg of metoclopromide was prescribed 3 times a day, 30 min before meals as needed. If the nausea and/or vomiting continued despite metoclopromide, promethazine hydrochloride, 25 mg by mouth or suppository, was administered every 8 h as needed. If nausea persisted, odansetron, 8 mg, was administered 30 min before 9-NC, and then every 8 h as needed.

In case of diarrhea, patients were instructed to take loperamide, 4 mg after each liquid stool and to report to the emergency room if the diarrhea could not be controlled in this way.

In case of hematuria, patients were instructed to hold 9-NC and to return to the clinic for evaluation.

Treatment was discontinued if there was evidence of disease progression or side effects became intolerable.

Assessment of response

The response was assessed according to the criteria established by the World Health Organization[22] after a minimum of two courses of treatment had been administered (6 weeks). Development of an effusion was considered progressive disease if substantiated by positive cytologic findings. Occurrence of brain metastases, even in the absence of other signs of progression, was considered to be progressive disease. Death from malignant disease that occurred during the first 4 weeks of treatment was called early death. All documents of the patients were reviewed by the principal investigator and all imaging studies were reviewed by an oncologic radiologist. The duration of remission was measured from the time of documented onset of remission to disease progression. Stable disease, disease-free interval and survival were measured from the start of chemotherapy administration.

Pharmacokinetic evaluation

Blood samples from volunteering patients were obtained to determine the pharmacokinetics of 9-NC after a single dose, two doses or three doses of the drug. The first cohort of patients (n=3) received a single dose of 1.5 mg/m^2 9-NC, the second cohort (n=3) received two doses of 1.5 mg/m^2 24 h apart and the third cohort (n=3) received three doses of 1.5 mg/m^2 every 24 h. Patients were hospitalized for 48–120 h during which blood samples were drawn to analyze the total drug and the lactone concentration in plasma and urine. Blood samples were obtained at predose, and at 0.5, 1, 2, 4, 6, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68 and 72 h in the first cohort. For patients receiving multiple doses of 9-NC, blood samples were obtained similarly for a 24 h period after each dosing and up to 72 h after the last dose. The blood was centrifuged at 6000 g for 30 s to separate the plasma. Total 9-NC concentrations in plasma samples were evaluated using a validated HPLC method, which involved the conversion of 9-NC to 9-AC prior to quantification. Briefly, 400 μl of plasma was run through a Sep-Pak column preconditioned with 20% methanol/water, and eluted with 1 ml acetonitrile:10 mM ammonium (1:1, pH 2.0) to extract the lactone portion that was then immediately frozen at -20°C, and a second 400 μl aliquot of plasma was acidified with 1 ml 10% perchloric acid, processed through a Sep-Pak column as above to extract the total amount of 9-NC and frozen similarly. The samples were analyzed within 1 week of collection by HPLC as described elsewhere.[21]

Urine samples were analyzed every 24 h from pooled urine. Ascites samples were taken in one patient simultaneously with the blood samples, and analyzed for lactone and total drug concentration as described above.

The plasma-concentration time profiles were analyzed by non-compartmental methods. The maximal plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained from evaluation of the plasma concentration versus time data. The area under the plasma concentration versus time curve up to 24 h [AUC(24 h)] was calculated by the trapezoidal rule.[23] The terminal half-life ($T_{1/2}$) was calculated as the ratio of 0.693 and the slope of the terminal phase of the log-linear concentration-time profiles, and were determined only in the first cohort of patients. The accumulation index was determined by dividing the AUC(24 h) after the second and third dose by the AUC(24 h) value obtained after the first dose.

Statistical analysis

The study was designed to test the null hypothesis that the true response rate to 9-NC is 5% versus the alternative hypothesis that the true response rate is 20% or more. The significance level (i.e. the probability of rejecting the null hypothesis when it is true) is 5.1%. The power (i.e. the probability of rejecting the null hypothesis when the alternative hypothesis is true) is 80%.[24] Survival curves were calculated according to the Kaplan-Meier technique.[25]

Results

Patient characteristics

Thirty-five patients were entered in the study. Four patients are not evaluable because they took 9-NC for 1 week or less (four doses or less). Reasons for drug discontinuation are motor vehicle accident with injury (2), early death (1) and refusal to continue treatment

(1). One patient took 9-NC for 2 weeks, then refused to continue treatment. She is included in the analysis. A total of 30 patients received 9-NC for 3 weeks (one course) or more. The characteristics of the evaluable (31) patients are detailed in Table 1.

Response

Twenty-nine patients were evaluable for response. The remission rate was 7%. Two partial responses were observed. The first patient had received four regimens of chemotherapy containing either of the following drugs, carboplatin, cyclophosphamide or paclitaxel, and was refractory to all of them. She also had failed a 2 month trial of immunotherapy with interferon-α, *cis*-retinoic acid and tocopherol. She presented with peritoneal carcinomatosis and a pelvic mass for which a colostomy was performed 2 months before protocol entry. The partial remission occurred 12 weeks after onset of treatment and lasted 17 weeks. She tolerated the 9-NC treatment well. The second patient had received three regimens containing carboplatin, cyclophosphamide or paclitaxel. She also had received an autologous bone marrow transplant after an induction with carboplatin, cyclophosphamide and thiotepa. A partial remission was observed 8 weeks after the onset of 9-NC. However, because of the compromise of her bone marrow, the dose of 9-NC had to be reduced to 1 mg/m^2 and her disease recurred on this lower dose 12 weeks later. Ten patients (34%) had stable disease for a median of 18 weeks (range: 12–47 weeks). Seventeen patients had progressive disease. Table 2 shows the response rate. A serological response (decrease by 50% or more of baseline CA-125) was observed in five patients. The two patients who obtained a partial response had a 92 and 83% decrease, respectively. Three other patients had a 70, 55 and 50% decrease. Of these, the first patient had a transient non-sustained minor response, and the two latter ones stabilized their disease for 9 and 21 weeks, respectively. The third patient eventually refused to continue treatment. Four additional patients had a 30–50%

Table 1. Patient characteristics (*N*=35)

No. of patients evaluated	31
Median age (range)	54 (30–76)
ECOG performance status	
0	6
1	22
2	3
Histology	
papillary serous	22
endometrioid	3
undifferentiated	2
adenocarcinoma	2
clear cell	2
No. of patients who had prior treatments (median no. regimens)	
chemotherapy	31 (>3)
hormonotherapy	12 (1)
immunotherapy	9 (1)
radiotherapy	2
surgery	29

Table 2. Response rate

Response	No. of patients (%)
PR	2 (7%)
NC	10 (34%)
PD	17 (59%)
Total	29 (100%)

PR, partial remission; NC, no change; PD, progressive disease.

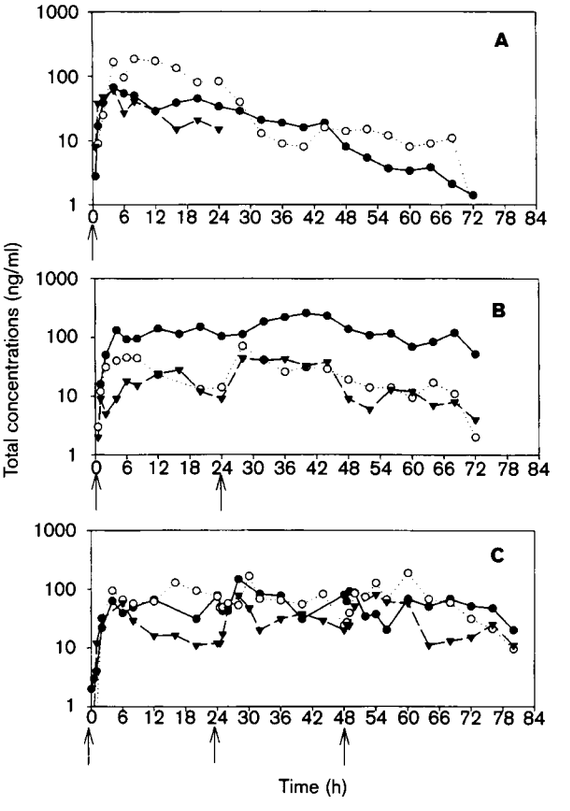

Figure 1. Plasma concentration–time profiles of 9-NC plus 9-AC in patients receiving a single dose (A, *n*=3) two doses (B, *n*=3) or three doses (C, *n*=3) of 9-NC. Arrows represent dosing times.

Table 3. Number of patients presenting with at least one toxic effect (worse grade)

Toxic effects	Grade				Total (%)	Total grade 3+4 (%)
	1	2	3	4		
Anemia	1	12	8	2	23 (74)	10 (32)
Neutropenia	5	5	3	4	18 (58)	7 (23)
Thrombocytopenia	6	4	3		13 (42)	3 (10)
Fever/infection	2	6			8 (26)	–
Nausea	5	25	1		31 (100)	1 (3)
Anorexia	13	12	1		26 (84)	1 (3)
Diarrhea	6	11	1		18 (58)	1 (3)
Vomiting	7	5	4		16 (52)	4 (13)
Weight loss	7	6	1		14 (45)	1 (3)
Constipation	4	7			11 (35)	–
Dehydration	1	6	1		8 (26)	1 (3)
Fatigue	3	22	1		26 (84)	1 (3)
Hematuria	5	5	1		11 (35)	1 (3)
Alopecia	6	1			7 (23)	–
Myalgia	5				5 (16)	–
Skin rash	2	2			4 (13)	–
Stomatitis	1				1 (3)	–
Drug fever	1				1 (3)	–
Nose bleed			1			1 (3)

decrease in CA-125. One refused further treatment after 2 weeks, and the other three stabilized their disease for 16, 28 and 29 weeks. The median survival was 35 weeks (range: 3-101+ weeks).

No correlation was noted between response and histologic characteristics. The median number of weeks on treatment for all patients was 6 (range: 3-47 weeks).

Toxicity

Thirty-one patients could be evaluated for toxicity. A total of 120 courses were administered (median per patient 2; range: 1-13 courses). Toxic effects are shown in Table 3. Four patients had a grade 4 neutropenia for which G-CSF was administered and three a grade 3. For all patients, the median granulocyte count at the nadir was 1650 cells/μl (range: 136 to >5000 cells/μl). A grade 3 thrombocytopenia was seen in three patients. The median platelet nadir was 142 000 cells/μl (range: 41 000-302 000 cells/μl). Two patients developed a grade 4 anemia and required transfusions. The median hemoglobin nadir was 8.5 g/dl (range: 5.3-12.2 g/dl). The median drop in hemoglobin was of 23% (range: 0-59%) from baseline, usually seen at the second course (range: 1-8 courses). Gastrointestinal side effects were severe (above grade 2) in a few patients (Table 3). Grade 2 or higher side effects included nausea (84%), anorexia (42%), diarrhea (39%) and vomiting (29%). A median weight loss of 3.4 kg per patient was seen (range: 0-12.1 kg). This is equivalent to a 4.5% median weight loss from baseline (range: 0-20%). Fatigue was a common complaint. A grade 2 was seen in 71% of patients and one patient had a grade 3 fatigue. Chemical cystitis was observed in 11 patients despite our recommendation to drink a lot of fluids. Most patients who suffered from this condition had a urine density above1022, a witness of poor fluid intake. However, only one patient (3%) presented with clots in the urine. All other side effects were equal or less than a grade 2 in intensity, and included myalgia (16%), skin rash (13%), fever (3%) and stomatitis (3%). Interestingly, one patient suffered recurrent nose bleeds that were clearly related to drug ingestion. Transfusions were needed twice after a bleeding episode.

Pharmacokinetic evaluation

Plasma concentration-time profiles obtained from the three cohorts of patients are shown in Figure 1. It should be noted that since the assay involved reduction of all samples, the concentrations evaluated constitute a summation of the total concentrations of 9-NC and its metabolites, including 9-AC. Following a single dose of 1.5 mg/m^2 9-NC, variability in the systemic exposure of 9-NC and its metabolites was noted (Table 4). In the first cohort of patients (dose 1 only), the C_{max} values ranged from 61 to 167 ng/ml, while the AUC(24 h) values ranged from 624 to

2838 ng.h/ml. The intestinal absorption of 9-NC appeared to be slow with T_{max} values ranging from 4 to 8 h after the first dose. The elimination occurred with a mean terminal $T_{1/2}$ of 10.6 h. Following the administration of multiple doses of 9-NC, variability in the systemic exposure was observed but the ranges of C_{max} and AUC(24 h) values observed in this patients were similar to the first cohort. The variability was fairly consistent between the dosing intervals. For instance, in cohort 2, on day 1, there was a 5.4- and a 6.8-fold range in the C_{max} and the AUC values, respectively. This was matched on day 2 by a 5.9- and a 5.5-fold range in the C_{max} and AUC values, respectively. Similar observations were made in the third cohort of patients. The accumulations observed on days 2 and 3 were similar.

We compared the lactone and total concentrations of 9-NC plus 9-AC formed from 9-NC in the plasma and urine obtained from patients. The lactone concentrations were substantially lower than the total concentrations in the plasma and constituted about 0–20% of the total concentrations. In the urine samples, the ratios of the lactone to total concentrations ranged from 28 to 69%.

We determined the concentrations of lactone and the total concentration of the drug plus its metabolites in ascites obtained from a patient who received a single dose of 9-NC. While the lactone concentrations were undetectable, total concentrations in the fluid ranged from 1.4 to 4.4 ng/ml. Due to the slow elimination rate from the ascites, the ratios of ascites to plasma concentration of the total form increased as a function of time from 3.5% at 10 h after dosing to 126% at 70 h after dosing.

Discussion

The main clinical problem following the administration of CPT is a low therapeutic index. The toxic effects are dose related, reversible, usually non-cumulative, but may be schedule dependent. Duration of exposure is critical in animal models. However, in human trials, the preliminary response rate to continuous infusion is usually not improved over shorter administration time. So far only phase I studies have tested the oral prolonged administration. Toxicity has been defined for oral TPT,[26] oral CPT-11[27] and oral 9-AC.[28] This is the first phase II study of oral prolonged administration.

Despite the low remission rate observed with CPT analogs (10–25% in best conditions), stabilization of ovarian cancer is observed in about half of the treated patients and lasts for about 6 months. The overall

Table 4. Summary of individual pharmacokinetic parameters in patients receiving an oral dose of 1.5mg/m^2 9-NC once, twice or thrice every 24 h

Dose	C_{max} TD (ng/ml)			T_{max} TD (h)			C_{max} LAC (ng/ml)	T_{max} LAC (h)	AUC (0–24 h)(ng.h/ml)			Accumulation index		LAC/TD (%AUC)	$T_{1/2}$ (h)	Response	Hematologic toxicity (worse grade)	GI toxicity (worse grade)
	D1	D2	D3	D1	D2	D3			D1	D2	D3	D2	D3					
1.5 mg/m^2 ×1																		
Pt 1	67			4			4	4	985					4.8	11.6	PD	3	2
Pt 2	186			8			6	4	2838					0.6	14.6	R	0	2
Pt 3	61			4			4	2	694					13.2	16.9	SD	1	2
1.5 mg/m^2 ×2																		
Pt 4	151	260		20	16		52	32	2654	4546		1.71		16.4		PD	4	1
Pt 5	45	71		6	4		5.4	28	603	861		1.43		9.1		SD	2	0
Pt 6	28	44		4	16		0	—	388	832		2.14		0		SD	3	3
1.5 mg/m^2 ×3																		
Pt 7	128	165	187	16	6	12	24	48.5	1818	1638	2075	0.90	1.14	8.5		PD	1	2
Pt 8	78	147	91	24	4	1	13	52	1127	1684	1472	1.49	1.31	9		SD	4	2
Pt 9	58	78	80	6	4	6	6.5	26	546	828	1088	1.52	1.99	5.2		PD	0	2

C_{max}, maximal concentration; TD, total drug; T_{max}, time at maximal concentration; LAC, lactone; AUC, area under the curve; GI, gastrointestinal; D1, day 1; D2, day 2, D3, day 3; Pt, patient; PD, progressive disease; R, reduction in CA-125; SD, stable disease.

median survival of patients with ovarian cancer refractory to platinum treated in second line with TPT is around 10 months, which is similar to the survival obtained with most second-line agents.[6] Myelosuppression is observed in 75% of patients treated with TPT and is the dose-limiting toxicity. The neutropenia seen with TPT correlates with the dose and follows a sigmoidal model, but no correlation could be demonstrated between lactone concentration and neutropenia.[29] Thrombocytopenia is infrequent and a mild depression in the hematocrit level is seen in the majority of patients receiving treatment for five consecutive days.[30] Other side effects include alopecia, rash, vomiting, nausea and diarrhea. The maximum tolerated dose (MTD) varies with the schedule. The MTD of a 21 day continuous infusion repeated every 28 days is 0.53 mg/m^2/day.[31] A phase II study of TPT administered by continuous infusion for 21 days obtained a 37% remission rate in patients with refractory ovarian cancers.[32]

Leukopenia is the major adverse effect seen with CPT-11, with an incidence of 60% of grade 2 and higher. The neutropenia parallels the AUC of CPT-11, but the gastrointestinal adverse effects, especially diarrhea, correlate with the AUC of SN-38.[33] Grades 2–4 gastrointestinal symptoms such as nausea or vomiting (52%), anorexia and diarrhea (39%) are frequently observed. Some patients required fluid resuscitation. SN-38, the active metabolite of CPT-11, could play a role in blocking the reabsorption of sodium and water in the gut. A recently published study by Gupta *et al.*[34] reported that the severity of diarrhea correlates with the patient's ability to metabolize SN-38 to SN-38G and to eliminate SN-38G through the bowels. The severity of the diarrhea correlates with the accumulation of non-glucuronylated SN-38 in the intestines.[34] The other toxic effects of CPT-11 include nausea, vomiting, abdominal pain, mild anemia, alopecia and malaise.[35]

Pantazis *et al.*[36] have demonstrated that Topo I inhibitors such as 9-AC and 9-NC effectively inhibit growth and subsequently induce regression of human ovarian tumors grown in nude mice. 9-NC inhibits the growth of human ovarian carcinoma cells *in vitro* regardless of their ability to induce tumors when xenografted in nude mice and similar morphological changes are seen in both non-tumorigenic and tumorigenic cells.[36] In preclinical studies, both 9-AC and 9-NC demonstrate higher antitumor activity than do the water-soluble CPT.[37,38] 9-NC is a precursor of 9AC. The *in vivo* conversion of 9-NC to 9-AC has been demonstrated in human, dogs and mice.[39] The *in vitro* conversion of 9-NC to 9-AC was shown to be dependent on pH (maximal conversion at pH 6.0) and the presence of human serum albumin.[40] Treatment of various blood cell lines (n=6) with 4 μg/ml 9-NC resulted in the formation of 9-AC with 24 h concentrations ranging from 11.5 to 52 ng/ml.[41] When administered orally in humans (0.1 mg/kg), the C_{max} and AUC of 9-AC was 3 and 12%, respectively, of the C_{max} and AUC of 9-NC.[39] This suggests that in humans, 9-NC is the major component of active drug in the plasma following oral administration of 9-NC, with substantially lower plasma 9AC concentrations. The bioavailability of orally administered 9-AC is 48.6%.[42] In the present investigation, due to poor fluorescent properties of 9-NC, the assay procedure involved the reduction of 9-NC to 9-AC and thus quantified 9-NC plus its metabolite 9-AC. Since both 9-NC and 9-AC possess cytotoxic properties superior to CPT, the combination of 9-NC plus 9-AC would be representative of active drug. In addition, based on low bioavailability of 9-AC and low ratios of 9-AC to 9-NC concentrations following 9-NC administration, the plasma concentrations represented by the present investigation would primarily consist of 9-NC. Furthermore, 9-NC is more stable than 9-AC and is manufactured at a considerably lower cost. Oral administration of 9-NC enclosed in a gelatin capsule circumvents the problems of infusing a non-water-soluble compound. For all these reasons, 9-NC was used in this present study.

Substantial interpatient variability in the disposition of 9-AC was reported following i.v. administration of the drug to children,[43] as well as to adult patients.[44,45] In the present investigation, we observed considerable variability in the systemic exposure to the total concentrations of 9-NC plus 9-AC. The variability could be related to differences in intestinal absorption and first pass metabolism of 9-NC to 9-AC, and could also be due to variable disposition properties of 9-AC and 9-NC.

The observation that the lactone form constituted a small fraction of the total drug concentration in the plasma is similar to the lactone:total ratios reported for 9-AC.[42] Lactone concentrations were undetectable in ascites obtained from one patient. This lactone:total drug ratio is lower than the ratios reported for the more polar camptothecin analogs such as topotecan and irinotecan.[46] With 9-NC, the level of exposure to the lactone form is less than 10% of the total drug exposure. In the patients, there was no correlation between lactone concentrations and response or severity of toxic effects (Table 4). Therefore, the implications of plasma lactone:total ratio in the therapeutic efficacy of 9-NC treatment cannot be conclusively defined. A 7% response rate was observed and three additional patients had a serological remis-

sion (decrease in Ca-125 by more than 50%). Tumor stabilized in 34% of patients for a median of 18 weeks. Median survival was 8 months. In this very refractory patient population, these observations are rather encouraging. Major toxic effects are myelosuppression and gastrointestinal troubles. Grade 3 and 4 granulocytopenia had developed in 26% of patients after 10-20 days of treatment and lasted for an average of 3 days until recovery to 1500 granulocytes/μl. Grade 3 and 4 anemia was also prominent in one-third of the patients. Gastrointestinal side effects included nausea, anorexia, diarrhea, vomiting and weight loss. All patients had at least one of these side effects. Only one hemorrhagic cystitis grade 3 was observed, which is an improvement on our previously reported study.[21] This improvement may be attributable to the recommendation for greater fluid intake.

Conclusion

The main problem in administering CPT analogs to humans is the low levels of lactone forms observed in the plasma, as confirmed in this study. The same is true with the other known analogs available for clinical use. Therefore, the therapeutic index remained low with 9-NC.

Further work needs to be done to improve the therapeutic index. A second generation of compounds is emerging. The compounds of this second generation have a lactone:carboxylate equilibrium in favor of the closed lactone form. The first two of these compounds, DX8951F[47] and 9-NC proprionate,[48] are in phase I trials. Whether these analogs will have a greater clinical antitumor activity remains an open question.

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References

1. ten Bokkel Huinink W, Gore M, Carmichael J, Gordon A, *et al.* Topotecan versus paclitaxel for the treatment of recurrent epithelial ovarian cancer. *J Clin Oncol* 1997; **15**: 2183-93.
2. Gwyther S, Bolis G, Gore M, *et al.* Experience with independent radiological review during a topotecan trial in ovarian cancer. *Ann Oncol* 1997; **8**: 463-8.
3. Creemers GJ, Bolis G, Gore M, *et al.* Topotecan, an active drug in the secondline treatment of epithelial ovarian cancer: results of a large European phase II study. *J Clin Oncol* 1996; **14**: 3056-61.
4. Armstrong D, Rowinsky E, Donehower R, Rosenshein N, Walczak J, Mcguire W. A phase II trial of topotecan as salvage therapy in epithelial ovarian cancer. *Proc Am Soc Clin Oncol* 1995; **14**: A769.
5. Gordon A, Bookman M, Malmstrom H, *et al.* Efficacy of topotecan in advanced epithelial ovarian cancer after failure of platinum and paclitaxel: International Topotecan Study Group Trial. *Proc Am Soc Clin Oncol* 1996; **15**: A763.
6. Kudelka AP, Tresukosol D, Edwards CL, *et al.* Phase II study of intravenous topotecan as a 5-day infusion for refractory epithelial ovarian carcinoma. *J Clin Oncol* 1996; **14**: 1552-7.
7. Takeuchi S, Dobashi K, Fujimoto S, *et al.* A late phase II study of CPT-11 on uterine cervical cancer and ovarian cancer. Research groups of CPT-11 in gynecologic cancers. *Jpn J Cancer Chemother* 1991; **18**: 1681-9.
8. Mori H, Itoh N, Kondoh H, *et al.* Treatment of recurrent gynaecologic malignancies with a new camptothecin derivative. *Eur J Cancer* 1992; **28**: 613-7.
9. Yang L, Liu LF, Li JJ, *et al.* The roles of DNA topoisomerase in SV 40 DNA replication. *UCLA Symp Mol Cell Biol* 1986; **47**: 315-26.
10. Liu LF, Wang JC. Supercoiling of DNA template during transcription. *Proc Natl Acad Sci USA* 1987; **84**: 7024-7.
11. Wang JC, Giaver GN. Action at a distance along a DNA. *Science* 1988; **240**: 300-4.
12. Jaxel C, Kohn KW, Wani MC, *et al.* Structure-activity study of the actions of camptothecin derivatives on mammalian topoisomerase I: evidence for a specific receptor site and a relation to antitumor activity. *Cancer Res* 1989; **49**: 1465-9.
13. Hertzberg RP, Caranfa MJ, Holdern KG, *et al.* Modification of the hydroxy lactone ring of camptothecin: inhibition of mammalian topoisomerase I and biological activity. *J Med Chem* 1982; **32**: 15-9.
14. Wall ME, Wani MC, Cooke, *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.
15. Burke TG, Mi Z. Preferential binding of carboxylate form of camptothecin by human serum albumin. *Ann Biochem* 1993; **212**: 285-7.
16. Mi Z, Burke TG. Marked interspecies variations concerning interactions of camptothecin with serum albumin. *Biochemistry* 1994; **33**: 12540-5.
17. Mi Z, Burke TG. Marked interspecies variations concerning interactions of camptothecin analogues with serum albumin. *Proc Am Ass Cancer Res* 1995; **36**: 444.
18. Takimoto CH, Grem JL, Allegra CJ, Arbuck SG. Current status of the clinical development of 9-aminocamptothecin (9-AC). *Ann Oncol* 1996; **7**(suppl 1): 26.
19. Giovanella BC, Stehlin JS, Wall ME, *et al.* DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. *Science* 1989; **246**: 1046-8.
20. Giovanella BC, Hinz HR, Kozielski AJ, *et al.* Complete growth inhibition of human cancer xenografts in nude mice by treatment with 20(*S*)-camptothecin. *Cancer Res* 1991; **51**: 3052-5.

21. Verschraegen CF, Natelson EA, Giovanella BC, *et al.* A phase I clinical and pharmacological study of oral 9-nitrocamptothecin, a novel water-insoluble topoisomerase I inhibitor. *Anti-Cancer Drugs* 1998; **9**: 36–44.
22. *WHO handbook for reporting results of cancer treatment*. Geneva: WHO, 1979: 48.
23. Gibaldi M. Compartmental and noncompartmental phannacokinetics. In: *Biopharmaceutics and clinical pharmacokinetics*. Lea & Febiger: Philadelphia 1991: 14–23.
24. Simon R. How large should a phase II trial of a new drug be? *Cancer Treat Rep* 1987; **71**: 1079–85.
25. Peto J. The calculation and interpretation of survival curves. In: Buyse M, Staquet M, Sylvester R, eds. *Cancer clinical trials: methods and practice*. Oxford: Oxford University Press 1984: 361–80.
26. Creemers GJ, Gerrits CJ, Eckardt JR, *et al.* Phase I and pharmacologic study of oral topotecan administered twice daily for 21 days to adult patients with solid tumors. *J Clin Oncol* 1997; **15**: 1087–93.
27. Drengler R, Burris H, Dietz A, *et al.* A phase I trial to evaluate orally administered irinotecan HCL (CPT11) given daily ×5 every 3 weeks in patients with refractory malignancies. *Proc Am Soc Clin Oncol* 1996; **15**: A1560.
28. De Jonge M, Punt C, Sparreboom A, *et al.* Phase I and pharmacology study on oral [Peg 1000] 9-amino-camptothecin (9-AC) in patients with advanced solid tumors. In: *Proc 10th NCI-EORTC Symp on New Drugs in Cancer Therapy*, Amsterdam 1998: 64.
29. Grochow LB, Rowinsky EK, Johnson R, *et al.* Pharmacokinetics and pharmacodynamics of topotecan in patients with advanced cancer. *Drug Metab Disp* 1992; **20**: 706–12.
30. Rowinsky EK, Grochow LB, Hendricks CB, *et al.* Phase I and pharmacologic study of topotecan: A novel topoisomerase I inhibitor. *J Clin Oncol* 1992; **10**: 647–56.
31. Creemers GJ, Beijnen JH, Planting AS, *et al.* Phase I trial of low-dose continuous topotecan infusion in patients with cancer: an active and well-tolerated regimen. *J Clin Oncol* 1994; **12**: 553–9.
32. Hochster H, Speyer J, Wadler S, *et al.* Phase II study of topotecan (TPT) 21-day infusion in platinum-treated ovarian cancer: a highly active regimen. *Proc Am Soc Clin Oncol* 1996; **15**: A775.
33. Gay C, Lokiec F, Canal P, *et al.* Pharmacokinetics and pharmacodynamics of the camptothecin analog CPT11 during phase II studies. *Proc Am Ass Cancer Res* 1994; **35**: A1452.
34. Gupta E, Lestingi TM, Mick R, *et al.* Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. *Cancer Res* 1994; **54**: 3723–5.
35. Rothenberg ML. The current status of irinotecan (CPT-11) in the United States. *Ann NY Acad Sci* 1996; **803**: 272–81.
36. Pantazis P, Kozielski AJ, Mendoza JT, *et al.* Camptothecin derivatives induce regression of human ovarian carcinomas grown in nude mice and distinghuish between nontumorigenic and tumorigenic cells *in vitro*. *Int J Cancer* 1993; **53**: 863–71.
37. Pantazis P, Kozielski AJ, Vardeman DM, *et al.* Efficacy of camptothecin congeners in the treatment of human breast carcinoma *Oncol Res* 1993; **5**: 273–1.
38. Pantazis P, Kozielski AJ, Rodriguez R, *et al.* Therapeutic efficacy of camptothecin derivatives against human malignant melanoma xenografts. *Melanoma Res* 1994; **4**: 5–10.
39. Hinz HR, Harris NJ, Natelson EA, Giovanella BC. Pharmacokinetics of the *in vitro* and *in vivo* conversion of 9-nitro-(20*S*)-camptothecin to 9 amino-20(*S*)-camptothecin in humans, dogs, and mice. *Cancer Res* 1994; **54**: 3096–100.
40. Pantazis P, Harris N, Mendoza J, Giovanella B. The role of pH and serum albumin in the metabolic conversion of 9-nitrocamptothecin to aminocamptothecin by human hematopoietic cells. *Eur J Hematol* 1995; **55**: 211–3.
41. Pantazis P, Harris N, Mendoza J, Giovanella B. Conversion of 9-nitrocamptothecin to amino-camptothecin by human blood cells *in vitro*. *Eur J Hematol* 1994; **53**: 246–8.
42. Sparreboom A, Jonge MJA, Punt CJA, *et al.* Pharmacokinetics and bioavailability of oral 9 aminocamptothecin capsules in patients with solid tumors. *Clin Cancer Res* 1998; **4**: 1915–9.
43. Langevin AM, Casto DT, Thomas PJ, *et al.* Phase I trial of 9 aminocamptothecin in children with refractory solid tumors: a Pediatric Oncology Group Study. *J Clin Oncol* 1998; **16**: 2494–9.
44. Rubin E, Wood V, Bharti A, *et al.* A phase I and pharmacokinetic study of a new camptothecin derivative, 9-aminocamptothecin. *Clin Cancer Res* 1995; **1**: 269–76.
45. Dahut W, Harold N, Takimoto C, *et al.* Phase I and pharmacology study of 9-aminocamptothecin given by 72-hour infusion to adult cancer patients. *J Clin Oncol* 1996; **14**: 1236–44.
46. Takimoto CH, Arbuck SG. The camptothecins. In: Chabner SA, Longo DL, eds. *Cancer chemotherapy and biotherapy*. Philadelphia: Lippincott-Raven 1996: 463–8.
47. Hoff PM, Lassere Y, Royce M, *et al.* Phase I study of the new camptothecin analogue DX-8951F administered by 24 hour continuous infusion. In: *Proc 10th NCI-EORTC symp on New Drugs in Cancer Therapy*, Amsterdam 1998: 65.
48. Cao Z, Harris N, Mendoza J, *et al.* Alkyl esters of 9 nitrocamptothecin: synthesis, toxicity and antitumor activity. *Proc Am Ass Cancer Res* 1998; **39**: 420.

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