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Research paper

A phase I clinical and pharmacological study of oral 9-nitrocamptothecin, a novel water-insoluble topoisomerase I inhibitor

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9-nitrocamptothecin (9NC) is a water-insoluble topoisomerase I inhibitor with a broad antitumor activity in animal models. To determine the maximum tolerated oral dose (MTD), a phase I study was performed in patients with advanced cancer refractory to conventional chemotherapy. 9NC was administered orally with escalating doses to cohorts of five patients beginning at 1 mg/m^2/day for five consecutive days every week for 4 weeks. Increments were 0.5 mg/m^2/day for each cohort. Toxicity was evaluated in 28 patients diagnosed with various malignancies. Seven patients received 1 mg/m^2/day for 28 weeks; 10 patients, 1.5 mg/m^2/day for 68 weeks; and 26 patients, 2 mg/m^2/day for 159 weeks. At 1.5 mg/m^2/day or higher, the dose-limiting toxicity was hematologic, with grade 4 anemia in eight (29%); neutropenia in seven (25%) and thrombocytopenia in five (18%). Grade 2 or higher toxic effects occurred at each dose level: nausea and vomiting in 15 (54%), diarrhea in nine (32%), chemical cystitis in seven (25%), neutropenic sepsis in six (21%) and weight loss in five (18%) (*N*=28). Responses were observed after 2–8 weeks of therapy in five patients with pancreatic, breast, ovarian and hematologic tumors. Fourteen patients had a disease stabilization and one patient received treatment up to 18 months. The MTD of 9NC given orally has been estimated at 1.5 mg/m^2/day for five consecutive days weekly. 9NC may be tolerated for sustained periods of time, but has the potential for significant hematologic, gastrointestinal and urinary bladder toxicity. Significant antitumor activity was observed, warranting further clinical investigations. [© 1998 Rapid Science Ltd.]

Key words: Anemia, anorexia, cystitis, myelosuppression, nausea, neutropenia, thrombocytopenia.

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Introduction

The topoisomerase I (topo I) enzyme is a monomeric nuclear protein of 100 kDa, that is necessary for DNA replication and transcription. The binding of topo I to the DNA forms a non-cleavable topo I-DNA complex that induces single-strand breaks that are essential for DNA relaxation, with subsequent religation.[1]

Camptothecin, a natural alkaloid extracted from the leaves and fruits of *Camptotheca acuminata*, is a pentacyclic molecular structure related to the indole alkaloids (Figure 1).[2] Unique features include an α-hydroxylactone system in ring E and a pyridone moiety in ring D. The pentacyclic structure (ring C) is highly unsaturated.[3] Camptothecin has only one asymetric carbon, C20, that is essential for its activity. The naturally existing form, 20*S*, inhibits the topo I-DNA complex in a reversible manner.[4] The 20*R* stereoisomer is inactive.[5–7] Camptothecin stabilizes this topo I-DNA complex. While DNA single-strand breaks are induced, DNA religation is inhibited. This interference in the replication process leads eventually to cell death because nucleases can attack the exposed uncoiled strands of DNA.[8] The antitumor activity is also dependent on tumor cell characteristics. Cells in the S phase are about 1000 times more sensitive to the cytotoxic effect of the topo I inhibitors than are cells in other phases of the cell cycle.[9,10] The cytotoxic effect depends on the activity of polymerase α, which is predominent in the S phase. Experiments with aphidicolin, an inhibitor of polymerases α and γ, have confirmed these observations.[11] The topo I inhibitors induce apoptosis in DNA-replicating cells.[12] However, in the

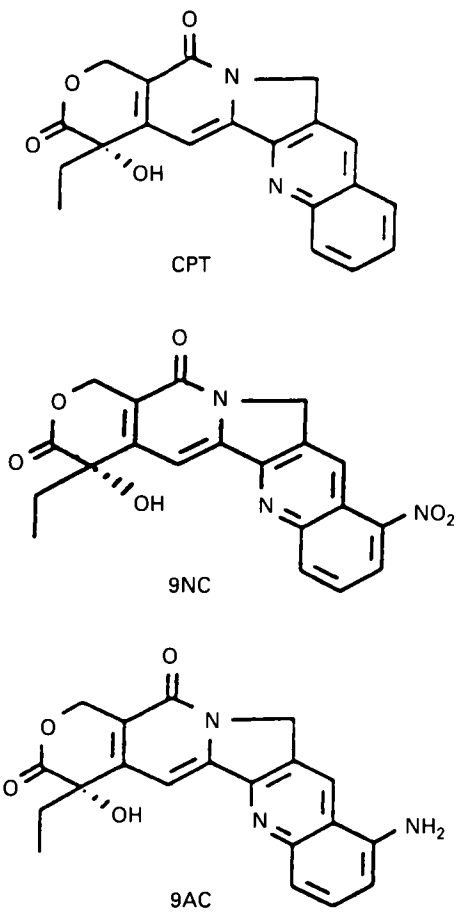

Figure 1. Structure of the camptothecin derivatives.

absence of DNA replication, inhibition of the topo I-DNA complex will be reversible.

The intact lactone form (closed E ring) is probably responsible for the antineoplastic activity. The carboxylate form (open E ring) has little antitumor activity and is toxic to normal cells.[13] The lactone ring is hydrolyzed to the carboxylate form *in vivo* under neutral or basic conditions.[2,14,15] Hydrolysis is accelerated by the presence of serum albumin.[14-16] The carboxylate form of camptothecin binds preferentially to human albumin. This binding shifts the equilibrium from the lactone form to the carboxylate form.

Derivatives of camptothecin include the water-soluble compounds, topotecan and irinotecan, and the water-insoluble compounds, camptothecin and 9-aminocamptothecin (9AC). 9-Nitrocamptothecin (9NC) has a nitro radical in the 9 position and is water insoluble. Pharmacology studies have shown that 9NC is partially metabolized *in vivo* into 9AC.[17] In human tumors xenografted in nude mice: (i) the antitumor activity of 9NC is superior to the activity of the water-soluble compounds;[18,19] and (ii) the daily intragastric administration yields results similar to continuous infusion and is clearly superior to daily i.v. bolus administration. Mouse plasma levels of 9NC showed a good enteric absorption with 60% of the drug in lactone form. Because of this high antitumor activity of 9NC seen in animal models, a phase I study was done in patients with advanced tumors refractory to conventional chemotherapy. Oral administration was chosen because the compound is water insoluble. Based on the previous study of camptothecin,[20] the starting dose was 25% of the maximum tolerated dose (MTD) in dogs.

Patients and methods

Patients

Patients were eligible for this phase I study if they met the following criteria: (i) a histologically confirmed diagnosis of cancer refractory to conventional therapy, (ii) a performance status of 3 or less on the Zubrod scale, (iii) a measurable or evaluable tumor and documented progression within 2 months before entry into the study, (iv) a neutrophil count above 1.5×10^9/l, a platelet count above 100×10^9/l, a serum creatinine level below 2 mg/dl, and a serum bilirubin level below 2 mg/dl, and (v) a signed informed consent. All patients had to have recovered from any toxic effects from previous treatments and could not have a non-neoplastic pre-existing condition of clinical significance. Patients were excluded if they had had prior radiotherapy or chemotherapy within 4 weeks before treatment (6 weeks for nitrosoureas and/or mitomycin), symptomatic brain metastases and other malignancies (except for those who had *in situ* carcinoma of the cervix uteri who underwent cone biopsy, or who had received adequate treatment for basal or squamous cell carcinoma of the skin).

Before entry, a complete medical history was recorded and a physical examination done. Performance status was noted and lesions measured. A complete blood cell (CBC) count, blood chemistries and a urine analysis were performed. Chest X-ray and other studies were done as needed and repeated every 6-12 weeks. Weekly CBC counts were done. Every 4 weeks, a clinical examination, and blood and urine chemistry surveys were required.

9NC formulation

9NC is manufactured at The Stehlin Foundation for Cancer Research according to FDA regulations. It is a

yellow opaque crystalline powder that is stable for 2 months at room temperature (25 C). It is water insoluble. The powder is encapsulated in color-coded gelatin capsules of either 1 or 0.25 mg by a registered pharmacist. The purity is greater than 99% as measured by HPLC.

Treatment plan

9NC was administered orally for five consecutive days every week for 4 weeks. 9NC was ingested on an empty stomach with a glass of citrus fruit juice. One course was defined as 4 weeks of therapy. The starting dose was 1 mg/m^2/day. The daily dose was rounded to the nearest 0.25 mg. A cohort of five patients was treated at each dose level (a total exposure to 9NC of 20 weeks). The dose was escalated by 0.5 mg/m^2/day if the cohort was not affected by grade 2 or higher toxic effects after 4 weeks. Each patient could also be dose escalated if no toxicity was observed during the first 4 weeks of treatment. For any grade 3 or higher hematologic and gastrointestinal (except stomatitis) or grade 2 or higher toxic effects, the drug administration was withheld until complete recovery. Granulocyte-colony stimulating factor (G-CSF) could not be used during the first 4 weeks on treatment. Symptomatologic treatment was used to treat gastrointestinal side effects. After the completion of this phase I, treatment could be continued for a maximum of 2 years or as long as it benefited the patient. Afterwards, if the patient benefited from 9NC administration and was kept on therapy, G-CSF could be used for neutropenic episodes according to the recommendations of the American Society of Clinical Oncology,[21] but not concomitantly with 9NC. Dose modifications were allowed in this part of the study. The drug was held as described above, until full recovery of all side effects. For hematologic side effects, the dose was decreased by one level if recovery occured in less than 2 weeks and by two levels if recovery occured in more than 2 weeks. For gastrointestinal side effects, the days of administration were reduced to 4 days per week instead of 5 days.

Assessment of toxicity and response

All adverse events were recorded and graded according to the NCI Common Toxicity Criteria. Secondarily, the response was assessed according to the World Health Organization rules if a minimum of 8 weeks of treatment had been administered.[22] Development of an effusion was considered as progressive disease if substantiated by positive cytologic findings. Occurrence of brain metastases, even in the absence of other signs of progression, was considered as progressive disease.

Criteria for dose-limiting toxicity and MTD

Dose-limiting toxicity was defined as the dose that caused a grade 4 hematologic toxic effect or grade 3 or higher non-hematologic toxic effects. MTD was defined as the dose level that preceded the dose for which two or less of five patients experienced a dose-limiting toxic effect.

Measurement of 9NC serum levels

Heparinized blood samples were obtained before 9NC administration and at 30 min, and 1, 2, 4 and 6 h after 9NC ingestion. The samples were immediately centrifuged at 6000 *g* for 30 s. Waters C-8 Sep-Pak columns preconditioned by vacuuming with methanol and water were used for 9NC lactone extraction. Two hundred microliters of plasma were passed through the column and the carboxylate form was removed with a water:methanol solvent. The lactone was then eluted with an acetonitrile:methanol solvent. The total amount of 9NC was measured from 100 μl of plasma acidified by ammonium formate (pH 2). The total drug was eluted as described above with an acetonitrile: methanol solvent. 9NC was then converted to 9AC, which is fluorescent. Fluorescence was measured by spectrophotometry and compared with a known 9NC plasma standard.

Results

Patient characteristics

Twenty-eight patients with metastatic cancer refractory to conventional therapy were diagnosed with the following tumors: eight ovarian cancers, five breast cancers, five cervical cancers, three pancreatic cancers and seven other cancers. The median age was 47 years (range 25–69 years). The median Zubrod performance status was 1 (range 0–3). Patient characteristics are summarized in Table 1.

Initial treatments

Five patients received a starting dose of 1 mg/m^2/day for 4 weeks, five patients (two of them had received

1 mg/m^2/day of 9NC for 4 weeks without any toxicity) received a dose of 1.5 mg/m^2/day for 4 weeks and five other patients received a dose of 2 mg/m^2/day for 4 weeks (Table 2). Because of the activity noted in two patients, this phase I study was extended on a compassionate basis and 15 more patients received 2 mg/m^2/day as the initial dose.

All treatments

A dose of 1 mg/m^2/day for 5 days each week was administered for a total of 59 weeks to seven patients (Table 3). None of the five patients who started at 1 mg/m^2/day had adverse events. In three patients (one of them had started at 1 mg/m^2/day, and was then successively increased to 1.5 and then to 2 mg/m^2/day before dose reduction), this dose was the result of a dose reduction because of toxicity at higher doses. These three patients also had their treatment withheld (while on 1 mg/m^2/day) for a total of 7 weeks until side effects resolved.

A dose of 1.5 mg/m^2/day for 5 days each week was given to 10 patients for a total of 68 weeks (median per patient, 4 weeks; range 2-26$^+$ weeks). In three patients, this dose was the result of a dose reduction because of toxicity. In one of these patients, another dose reduction to 1 mg/m^2/day was necessary. 9NC was withheld for a total of 8 weeks (12% of time) in three patients because of the appearance of hematologic side effects (Table 3).

A dose of 2 mg/m^2/day for 5 days each week was given to 26 patients for a total of 159 weeks (median per patient, 5 weeks; range 2-21+ weeks). In 20 patients, this was the initial treatment. In six patients, it was the result of a dose increase. The treatment was withheld for 31 weeks (19% of time) in 12 patients.

Table 1. Patient characteristics (*N*=28)

Median age (range)	47 (25–69)
Zubrod performance status	
0	8
1	14
2	1
3	5
Tumor primary	
epithelial ovarian cancer	8
breast cancer	5
cervical carcinoma	5
pancreatic cancer	3
colon cancer	2
acute myelogenous leukemia	1
prostate cancer	1
endometrial carcinoma	1
non-small cell lung cancer	1
basal cell carcinoma	1
Number of patients who had prior treatments	
chemotherapy	23
hormonotherapy	6
immunotherapy	2
radiotherapy	14
surgery	23

Table 2. Initial treatment

9NC (mg/m^2/day × 5 q week)	No. of patients	No. of weeks on treatment	No. of weeks treatment held
1.0	5	21	0
1.5	5[a]	20	0
2.0	5	20	4 (20%)[b]

[a]Two patients were increased from 1 to 1.5 mg/m^2 and tolerated the dose increase well.
[b]All patients entered at 2 mg/m^2/day had never received 9NC before. Treatment was held for 1 week in two patients and for 2 weeks in another patient.

Table 3. Length of treatment and number of dose modification

Dose [(mg/m^2/day (no. days/week))]	Initial dose (no. of patients)	No. of patients at this dose[a]	Dose increase[b] (no. of patients)	Dose reduction[b] (no. of patients)	Treatment discontinuation (No. of patients)	No. of weeks on treatment	No. of weeks held for toxicity (%)
1 (5)	5	7	6	0	0	28	7 (25)
1.5 (5)	**5**	**10**	**6**	**1**	**0**	**68**	**8 (12)**
1.75 (5)	0	2	0	0	1	11	4
2.0 (5)	**5+15**	**26**	**2**	**11**	**6**	**159**	**31 (19)**
2.25 (5)	0	2	0	0	0	14	0
Any (4)	0	9	1	1	0	13	0

[a]This is the total number of patients who received the indicated dose, at any time during the study, either as the initial dose or as a result of dose modification.
[b]Each patient may have had more than one dose modification.

The dose was raised to 2.25 mg/m^2/day in two patients and reduced because of toxicity in 11 patients. Drug administration was discontinued because of toxicity in six patients. Hence, at 2 mg/m^2/day, a total of 17 patients (65%) had a dose change related to treatment side effects. However, 35% of patients tolerated this dose level (Table 3).

Patients who benefited from the treatment were treated until disease progressed. However, the appearance of toxic effects prompted dose reductions which were achieved in two ways as described above: (i) reduction of the dose per meter squared per day, with treatment given for five consecutive days each week (seven patients) and (ii) maintenance of the daily dose intensity for four consecutive days per week (seven patients). A combination of both reduction methods was used in four patients because an additional dose reduction was necessary. These modifications were required later during the study, and were individualized for each patient to account for tumoral growth stabilization and patient tolerance to the drug.

Toxicity—initial treatments

At doses of 1 and 1.5 mg/m^2/day, 9NC could be administered for 4 weeks without significant side effects. At 2 mg/m^2/day, two patients tolerated 4 weeks of treatment without side effects; after 2 weeks of 9NC administration, one patient developed grade 4 neutropenia and thrombocytopenia which lasted 10 and 15 days, respectively; after 3 weeks of 9NC administration, one patient developed a grade 3 anemia which lasted 2 days, and another patient grade 3 neutropenia and thrombocytopenia which lasted 5 and 2 days, respectively.

Toxicity—all treatments

All patients could be evaluated for toxicity. No dose-limiting toxicity was observed at less than 1.5 mg/m^2/day (Table 4). The main toxicity was hematologic, with 29, 25 and 18% of patients manifesting grade 4 anemia, neutropenia and thrombopenia, respectively. Except for one patient in whom anemia and neutropenia simultaneously developed at 1.5 mg/m^2/day (this patient had previously been treated with extensive pelvic radiotherapy), all these patients had been treated at 2 mg/m^2/day. Six episodes (23%) of neutropenic sepsis were observed in patients treated at 2 mg/m^2/day. Similarly, most grade 3 non-hematologic dose-limiting toxic effects occurred at 2 mg/m^2/day, and included nausea and vomiting (18%), diarrhea (14%), and chemical cystitis with gross hematuria (14%) (Table 4). Two cases each of grade 3 hemorrhagic chemical cystitis occurred at doses of 1.5 and 2 mg/m^2/day at weeks 7, 15, 23 and 30 of treatment, and were preceded by minor urinary complaints (but no hematuria) for 2, 7, 9 and 4 weeks, respectively. A bladder biopsy was performed in one patient. The pathologic examination was consistent with an acute hemorrhagic cystitis of chemical origin.

One patient with a very bulky ovarian cancer died of undetermined cause while still in remission after 8 weeks of treatment. She probably had an internal hemorrhage causing hypovolemic shock and death before any surgical intervention could be attempted. Authorization for autopsy was not granted. A relationship between her death and 9NC is possible, but not probable.

Overall, grade 2 or higher toxic effects were observed at each dose level and are listed in Table 5. Nausea and vomiting, and diarrhea were experienced by 54 and 32% of patients, respectively, and were observed commonly at 1.5 and 2 mg/m^2/day. The nausea was prominent especially on the fifth weekly day of treatment, and accompanied with anorexia and weight loss in 18% of patients (1–11 kg; median 4 kg). Grade 2 or higher chemical cystitis occurred in 25% of patients. Fifteen patients complained of a burning sensation on urination, but only 10 had hematuria (three grade 1, three grade 2 and four grade 3). Alopecia was seen only in patients who experienced

Table 4. Hematologic toxicity

Dose level (mg/m^3/day)	Total no. of patients	Neutropenia (WHO grade)			Thrombocytopenia (WHO grade)			Anemia (WHO grade)		
		2	3	**4**	2	3	**4**	2	3	**4**
1	7	1	0	**0**	0	1	**0**	0	3	**0**
1.5	10	3	0	**1**	0	1	**0**	1	1	**1**
2	26	3	5	**6**	2	5	**5**	4	10	**7**
2.25	2	0	0	**0**	0	0	**0**	0	1	**0**

grade 4 neutropenia. Other observed toxic effects included minor eczematoid skin changes on the hands and hemorrhagic colitis with guaiac positive stools. A colonoscopy showed punctiform ulcerations. No biopsy was done. These ulcerations resolved in 2 days after discontinuation of therapy.

Response

One complete response (CR) was observed by abdominal CT scan in a patient with pancreatic cancer treated at a dose of 1.0 mg/m^2/day. The main lesion was the primary tumor. The onset of partial response (PR) was seen after 8 weeks of therapy and the onset of CR after 21 weeks. The patient has remained on treatment for 12 months. 9NC was then discontinued and the patient has remained free of clinical disease until today, more than 5 months after the end of the treatment. Four PRs were observed in two patients with ovarian carcinoma treated at 2 mg/m^2/day (one patient had a greater than 50% decrease in the size of the abdominal mass by CT scan with a 90% decrease of serum CA-125, and the other patient had a 50% decrease of the abdominal mass by physical examination and a 50% decrease of CA-125), one patient with acute myelogenous leukemia treated at 2 mg/m^2/day (normalization of peripheral blood counts) and one patient with breast cancer treated at 1 then 1.5 mg/m^2/day (disappearance of all but one skin lesion around the mastectomy scar, which was the sole site of recurrence). The onset of response was observed at 2 and 5 weeks for the ovarian cancers, at 3 weeks for the acute myelogenous leukemia, and at 4 weeks for the breast cancer. The patient with acute myelogenous leukemia had a hematologic remission for 2 months but relapsed when the treatment was discontinued because of chemical cystitis. One ovarian cancer patient died of undetermined cause while in remission, and the other ovarian cancer patient was maintained on therapy for 9 months with partial remission and died of a non-neutropenic septic shock with a Gram-negative organism. Her death was felt to be unrelated to 9NC treatment. The breast cancer patient remained on therapy for more than 8 months. Fourteen patients had a minor response or stable disease while on treatment. Of these, seven patients remain on therapy 3-12 months later. Disease progressed in six patients while on therapy. Three patients are inevaluable for response.

Pharmacokinetics

Fourteen patients had plasma levels of total drug and lactone form measured by HPLC on the first day of 9NC administration. Time points at which drug plasma levels were measured allow a very preliminary evaluation of the pharmacology profile. (A long-term pharmacology study is currently ongoing in association with a phase II study.) Four patients had levels remeasured after 8, 18, 34 and 45 days of continuous administration of 9NC and four patients had levels measured for the first time while on treatment at days 8, 21, 28 or 56 (Table 6). On the first day of treatment, the median peak level (C_{MAX}) of the lactone form, occurred at a median of 1 h (range 0.5-6 h) and was 15.5 ng/ml (range 2.8-70 ng/ml). The median C_{MAX} of the total drug occurred at a median of 4 h (range 2-6 h) and was 111 ng/ml (range 6.4-517 ng/ml). The mean percentage of the area under the curve (AUC) (truncated at 6 h) of the lactone form versus the AUC of the total drug was 14.7% (SD=14.3). Plasma levels of 9NC measured in patients on treatment (days 8-56) were similar to those observed in naive patients, with a median peak level of lactone of 10 ng/ml (range 1.8-74 ng/ml). There is at least a second peak around 6-8 h after drug administration that may be related to an enterohepatic cycle. This observation has been confirmed in the long-term pharmacology study currently

Table 5. Number of patients presenting with grade 2 or higher non-hematologic toxicity

Dose (mg/m^2/day)	WHO grade 2			WHO grade 3		
	1.5	2	2.25	1.5	2	2.25
Nausea and vomiting	3	5	2	0	5	0
Diarrhea	1	4	0	1	3	0
Weight loss	2	3	0	0	0	0
Chemical cystitis	0	3	0	2	2	0
Colitis	0	2	0	0	0	0
Dermatitis	0	0	0	0	0	0
Total no. of patients	10	26	2	10	26	2

Table 6. Plasma drug levels

Patient	Day of pharmacology	9NC dose (mg/m^2/day)	$C_{max_{Lactone}}$ (ng/ml) [time (h)]	$C_{max_{Total\ 9NC}}$ (ng/ml) [time (h)]	$AUC_{Lactone}$/ $AUC_{Total\ 9NC}$ (%)
1	1	1	17 [0.5]	131 [4]	5.8
2	1	1	2.8 [6]	58 [6]	3.8
3	1	1	2.8 [1]	37 [2]	3.6
4	1	1	3.3 [1]	22.7 [2]	7.9
5	1	1	3.5 [6]	6.4 [2]	57.8
6	1	2	70 [2]	517 [6]	16.4
7	1	2	16 [1]	301 [4]	2.8
8	1	2	14 [1]	251 [4]	5.6
9	1	2	15.5 [1]	111 [4]	12.1
10	1	2	13 [3]	73 [3]	21
11	1	2	5.5 [2]	55.8 [4]	8.4
12	1	2	15 [0.5]	53 [6]	13
13	1	2	6.3 [0.5]	34.3 [4]	14
14	1	2	8 [2]	26 [4]	33
2	18	1	1.8 [5]	74.6 [4]	2.6
6	34	1	4.9 [2]	106 [6]	4.7
3	45	1.5	13.8 [8]	170 [8]	5.7
6	8	2	74 [4]	209 [4]	33.6
15	8	2	12 [2]	78 [6]	10.6
16	21	2	9 [1]	128 [2]	5.2
17	28	2	10 [1]	160 [4]	3.75
18	56	2.25	8.1 [2]	50 [4]	17

under investigation.[23] The pharmacokinetics of 9NC appear to be non-linear.

Discussion

Phase I trials of chemotherapy agents administered orally are extraordinarily difficult to implement for various reasons, especially if the compound demonstrates anticancer activity. One complete and four partial responses were observed after treatment with 9NC in patients with refractory cancers. More interestingly, half of the patients had either a minor remission or stabilization of their tumor during therapy. Prolonged stabilization of disease has also been observed with other derivatives of camptothecin. Topotecan has been active against various refractory tumors[24,25] and prolonged exposure seems to increase the response rate.[26] An oral form is currently under study. The bioavailability of an oral dose of topotecan is 32%.[27] The equilibrium between carboxylate and lactone forms is maintained after oral administration.[28] The main toxic effect of topotecan is neutropenia.[29] Irinotecan also has a wide range of antitumor activity[30,31] The dose-limiting toxic effects are either neutropenia or diarrhea, which are not directly related to plasma drug concentration.[32,33] Irinotecan is not yet available in an oral form for clinical use. Intravenous formulations of topotecan and irinotecan are commercially available. Camptothecin and 9AC have both been studied in phase I trials.[20,34,35] Camptothecin administered orally induced some remission in refractory tumors.[20] The main side effects observed with both compounds were hematological for 9AC and gastrointestinal for camptothecin (32% diarrhea). Hemorrhagic cystitis occurred in 20% of patients treated with camptothecin. No clinical responses were observed with a 72 h continuous i.v. infusion of 9AC.[34] However, in a phase I study of protracted i.v. administration, antitumor activity was observed.[35] Further studies of 9AC are ongoing.

One-third of patients treated with 2 mg/m^2/day of 9NC experienced a dose-limiting toxicity. Toxic effects of clinical importance included neutropenia, anemia, thrombocytopenia, hemorrhagic cystitis and gastrointestinal toxicity (mainly nausea and anorexia, and less frequently vomiting, diarrhea and weight loss). Weight loss was associated with severe anorexia and nausea secondary to treatment. Except for gastrointestinal intolerance, grade 2 toxic effects progressed in severity if treatment was not interrupted. Anemia was managed by transfusion of blood products. G-CSF was used for neutropenic episodes while treatment was held. The appearance of a grade 2 thrombocytopenia prompted treatment interruption until complete

hematologic recovery. Hemorrhagic cystitis was worrisome and led to treatment discontinuation in two patients. Preliminary urine analyses showed that about half the drug is excreted over the first 24 h and that the lactone form is present as 50% of the total drug amount. Whether the lactone form or a metabolite(s) of 9NC is responsible for these symptoms is not clear. Cystitis may be the result of a cumulative chemical toxicity. We were able to resume 9NC administration in patients who had developed cystitis by increasing their daily fluid intake to a minimum of 3 l/day. The occurrence of toxic effects prompted interruption of the continuous administration, often causing a quick reversal of the observed tumor growth inhibition.

To prove that the drug was absorbed orally, plasma levels were measured in volunteering patients; however, the pharmacokinetic data obtained are very preliminary. There seems to be two or three peaks of drug in plasma after oral ingestion of one dose of 9NC, suggesting the existence of an enterohepatic cycle. Further studies of 9NC pharmacokinetics are ongoing. No direct correlation of pharmacologic parameters could be made with toxicity or response to 9NC and interpatient variability was significant. These findings are similar to those observed in different trials of other camptothecin derivatives.[36,37] In particular, no correlation could be established between a steady-state concentration of CPT-11 or topotecan and response rate. Interpatient variability was observed with 9AC, which is also water insoluble.[34] 9NC is mainly bound to protein in the plasma.[16] Furthermore, because of the lipophilic nature of 9NC, the compound is retained intracellularly, especially in red blood cells (data not shown). This intracellular pool of 9NC constitutes a reserve of drug, that is slowly released into the plasma.[38] This phenomenon may also contribute to the toxicity of 9NC.[39] Therefore, the biological activity of 9NC may depend on factors other than drug plasma concentration.

Conclusion

Because of the antitumor activity observed in this trial, further studies of 9NC are warranted. The hematologic toxicity is predictable and measurable. However, the non-hematologic toxicities, particularly the gastrointestinal toxic effects, need further clarification. More clinical research is needed to understand the pharmacology and pharmacodynamics of this class of drugs. This information may help improve the therapeutic index. Alternative schedules of continuous exposure to 9NC need to be explored further. The data from this trial indicate that 1.5 mg/m^2/day given for five consecutive days each week is the MTD in previously treated patients. We recommend that this dose be used in future phase II trials. A provision for dose increase after 4 weeks of therapy may be considered if no toxic effects are encountered at this starting level.

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References

1. Wang JC. DNA topoisomerases. *Annu Rev Biochem* 1985; **54:** 665-97.
2. 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.
3. Wall ME, Wani MC. Chemistry and antitumor activities of camptothecins. In: Potmesil M, Khon KW, eds. *DNA topoisomerases in cancer*. New York: Oxford University Press 1991: 93-102.
4. 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.
5. Liu LF. DNA topoisomerases—enzymes that catalyze the breaking and rejoining of DNA. *Annu Rev Biochem* 1983; **15:** 19-24.
6. Liu LF, D'Arpa P. Topoisomerase-targeting antitumor drugs: mechanisms of cytotoxicity and resistance. *Import Adv Oncol* 1992; 79-89.
7. Hsiang YH, Liu LF, Wall ME, *et al.* DNA topoisomerase I mediated DNA cleavage and cytotoxicity of camptothecin analogs. *Cancer Res* 1989; **49:** 4385-9.
8. Schneider E, Hsiang YH, Liu L. DNA topoisomerase as anticancer drug targets. *Adv Pharmacol* 1990; **21:** 149-83.
9. Li LH, Fraser TJ, Olin EJ, Bhuyan BK. Action of camptothecin on mammalian cells in culture. *Cancer Res* 1972; **32:** 2643-50.
10. Horwitz SB, Horwitz MS. Effects of camptothecin on the breakage and repair of DNA during the cell cycle. *Cancer Res* 1973; **33:** 2834-6.
11. Hsiang YH, Lihou MG, Liu LF. Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. *Cancer Res* 1989; **49:** 5077-81.
12. Huang R, Tsuda H, Liang J, *et al.* Topoisomerase I inhibitors, irinotecan hydrochloride and SN-38 induce apoptosis in leukemia cell lines. *Proc Am Ass Cancer Res* 1994; **35:** A5.
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. Burke TG, Mi Z. Preferential binding of carboxylate form of camptothecin by human serum albumin. *Anal Biochem* 1993; **212:** 285-7.

15. Mi Z, Burke TG. Marked interspecies variations concerning interactions of camptothecin with serum albumin. *Biochem* 1994; **33:** 12540-5.
16. Mi Z, Burke TG. Marked interspecies variations concerning interactions of camptothecin analogues with serum albumin. *Proc Am Ass Cancer Res* 1995; **36:** 444.
17. Hinz HR, Harris NJ, Natelson EA, *et al.* Pharmacokinetics of the *in vivo* and *in vitro* conversion of 9-nitro-20(*S*)-camptothecin to 9-amino-20(*S*)-camptothecin in humans, dogs, and mice. *Cancer Res* 1994; **52:** 3096-100.
18. Giovanella BC, Stehlin JS, Wall ME, *et al.* DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. *Science* 1989; **246:** 1046-8.
19. 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.
20. Stehlin JS, Natelson EA, Hinz HR, *et al.* Phase I clinical trial and pharmacokinetics results with oral administration of 20(*S*)-camptothecin. In: Potmesil M, Pinedo H, eds. *Camptothecins, new anticancer agents*. Boca Raton, FL: CRC Press 1995: 59-65.
21. American Society of Clinical Oncology recommendations for the use of hematopoietic colony-stimulating factors: evidence-based, clinical practice guidelines. *J Clin Oncol* 1994; **12:** 2471-508.
22. *WHO handbook for reporting results of cancer treatment.* Geneva: WHO 1979: 48.
23. Verschraegen C, Harris N, Steger M, Kavanagh J, Kudelka A, Giovanella B. Pharmacology study of multiple doses of 9-nitrocamptothecin. *Proc Am Ass Cancer Res* 1997; **38:** A709.
24. 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.
25. 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.
26. Hochster H, Speyer J, Wadler S, *et al.* Phase II study of topotecan 21-day infusion in platinum-treated ovarian cancer: a highly active regimen. *Proc Am Soc Clin Oncol* 1996; **15:** 285.
27. Creemers GJ, Schellens JH, Beijnen JH, *et al.* Bioavailability of oral topotecan. *Proc Am Soc Clin Oncol* 1994; **13:** 324.
28. Kuhn J, Rizzo J, Eckardt J, *et al.* Phase I bioavailability study of oral topotecan. *Proc Am Soc Clin Oncol* 1995; **14:** A1538.
29. Kudelka A, Tresukosol D, Edwards C, *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.
30. Kunimito T, Nitta K, Tanaka T, *et al.* Antitumor activity of 7-ethyl-10(4-(1-Piperidino)-1-Piperidino) carbonyloxy-camptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. *Cancer Res* 1987; **47:** 5944-7.
31. Rothenberg ML. CPT-11: an original spectrum of clinical activity. *Semin Oncol* 1996; **1:** 21-6.
32. Chabot G, De Forni M, Abigerges D, *et al.* Clinical trials and pharmacology studies of CPT-11 and its active metabolite SN-38 in France: preliminary pharmacokinetic-pharmacodynamic relationships. In: Potmesil M, Pinedo H, eds. *Camptothecins, new anticancer agents*. Boca Raton, FL: CRC Press 1995: 83-92.
33. 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.
34. 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; **12:** 269-76.
35. Takimoto CH, Dahut W, Harold N, *et al.* A phase I trial of prolonged infusion of 9-aminocamptothecin in adult patients with solid tumors. *Proc Am Soc Clin Oncol* 1996; **15:** A1554.
36. Grochow LB, Rowinsky EK, Johnson R, *et al.* Pharmacokinetics and pharmacodynamics of topotecan in patients with advanced cancer. *Drug Metab Dis* 1992; **20:** 706-12.
37. Kaneda N, Yokokura T. Nonlinear pharmacokinetics of CPT-11 in rats. *Cancer Res* 1990; **50:** 1721-5.
38. Pantazis P, Harris N, Mendoza J, *et al.* Conversion of 9-nitro-camptothecin to 9-amino-camptothecin by human blood cells *in vitro*. *Eur J Haematol* 1994; **53:** 246-8.
39. Chabot G, Gouyette A, Bissery M. Tumor influence on pharmacokinetics of the camptothecin analogue irinotecan and active metabolite SN-38 in mice. *Proc Am Ass Cancer Res* 1994; **35:** A2576.

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