Phase I and Pharmacologic Study of Intermittently Administered 9-Nitrocamptothecin in Patients with Advanced Solid Tumors

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ABSTRACT

Purpose: 9-Nitrocamptothecin (9NC) is an oral camptothecin analogue currently administered at 1.5 mg/m2/day × 5 days/week in Phase III studies for pancreatic cancer. In an effort to increase the dose administered per day and determine whether the daily dose or number of days of treatment influence toxicity, we performed a Phase I study of 9NC using intermittent schedules of administration. Experimental Design: On schedule A, 9NC was administered orally daily × 5 days for 2 weeks every 4 weeks (one cycle). On schedule B, 9NC was administered orally daily × 14 days every 4 weeks (one cycle). Dose levels were determined by adaptive dose finding. Serial blood samples were obtained on day 1 of each schedule for pharmacokinetic studies of 9NC and its 9-aminocamptothecin (9AC) metabolite, and lactone forms were measured by high-performance liquid chromatography.

Results: The recommended Phase II doses for schedules A and B were 2.43 and 1.70 mg/m2/day, respectively, each providing the same dose intensity (i.e., 24 mg/m2/cycle). The primary toxicities on schedules A and B were neutropenia, thrombocytopenia, and diarrhea. On schedule A, two patients with gastric cancer and two patients with pancreatic cancer had stable disease for more than six cycles. On schedule B, one patient with pancreatic cancer had stable disease for more than six cycles, and a patient with pancreatic cancer had a partial response. There was significant interpatient variability in the disposition of 9NC and 9AC. Most of the drug remained in the 9NC form with a ratio of 9NC to 9AC of ~4 to 1.

Conclusions: These studies suggest that 9NC administered on an intermittent schedule is tolerable and may be an active regimen in patients with gastric or pancreatic cancers. Dosing 9NC on a mg/m2 basis does not reduce pharmacokinetic variability.

INTRODUCTION

The camptothecins are DNA topoisomerase I-interactive anticancer agents and have a wide range of antitumor activity (1–3). Currently approved camptothecin analogues (i.e., topotecan and irinotecan) are only available for i.v. administration (4, 5). 9-Nitrocamptothecin (9NC) is administered orally and is partially metabolized to an active metabolite, 9-aminocamptothecin (9AC; Refs. 6, 7). As with other camptothecin analogues, 9NC and 9AC undergo a reversible, pH-dependent hydrolysis between the active-lactone and inactive-hydroxy acid forms (8). In vitro and in vivo preclinical studies suggest that protracted administration of low doses of camptothecin analogues produces better antitumor activity than does less frequent administration of higher doses (9–12). Oral administration of 9NC could mimic the protracted schedule and maximize patient convenience (13). However, oral administration of camptothecin analogues has been characterized by extensive inter- and intrapatient variability in bioavailability (13–16).

In Phase II and III studies, 9NC is administered continuously at 1.25–1.5 mg/m2/day for 5 days/week (17, 18). On this schedule, dose reductions and delays in therapy frequently occur during weeks 3–5 and are due to myelosuppression, diarrhea, and hematuria. In xenograft studies, antitumor activity of camptothecin analogues requires a dose that produces a systemic exposure above a critical threshold (11, 12). It is possible that administration of continuous, low-dose 9NC might not produce a systemic exposure above this critical threshold and, as a result, might fail to produce an antitumor response (19). In contrast, administration of 9NC on an intermittent schedule (e.g., 2 weeks of treatment followed by 2 weeks off) may allow for the administration of a higher dose per day that would produce therapeutic drug concentrations and also avoid toxicities in weeks 3 and 4.

Thus, we evaluated two intermittent schedules of 9NC administration with the goals of increasing the dose administered per day and the dose intensity and determining if the daily dose or number of days of treatment influence toxicity. The objectives of this study were to (a) determine the maximum-
tolerated dose (MTD) and toxicities associated with two intermittent schedules of 9NC, (b) document any antitumor response to 9NC in patients with various solid tumors, and (c) determine the plasma pharmacokinetics of 9NC and 9AC.

PATIENTS AND METHODS

Patients. Patients 18 years of age or older with a histologically or cytologically confirmed malignancy for which no curative or effective therapy was available were eligible for this study. Other eligibility criteria included a Eastern Cooperative Oncology Group performance status of 0–2, adequate bone marrow, hepatic, and renal function as evidenced by the following: absolute neutrophil count ≥ 1500/μl; platelets ≥ 100,000/μl; total bilirubin ≤ 1.5× upper limit of the institutional normal range; aspartate aminotransferase ≤ 1.5× the upper limit of the institutional normal range if liver metastases were not present; and ≤ 4× the upper limit of the institutional normal range if liver metastases were present; and absence of microscopic hematuria. Prior irradiation to brain metastases was allowed if the patient’s neurological status was stable 4 weeks after irradiation. Prior treatment with camptothecin analogues, except 9NC, was permitted. Written informed consent, approved by the Institutional Review Board of the University of Pittsburgh Medical Center, was obtained from all patients before they entered the study.

Dosage and Administration. Two intermittent schedules of 9NC were evaluated. On schedule A, 9NC was administered orally daily for 5 days/week for 2 consecutive weeks and repeated every 4 weeks (one cycle). On schedule B, 9NC was administered orally daily for 14 days and repeated every 4 weeks (one cycle). The daily dose was rounded to the nearest 0.25 mg. On both schedules, 9NC was administered daily on an empty stomach and with an acidic beverage (e.g., orange juice or cola; Refs. 20, 21). Patients were required to increase their oral hydration to at least 2 liters/day during 9NC treatment.

Dose levels for schedules A were determined by adaptive dose finding (22). During stage I of this two-stage method, doses were escalated by a factor of 1.5 until the first dose-limiting toxicity (DLT) occurred. Then, in stage II, escalation was switched to a model-guided mode similar to the continual reassessment method (23). In these methods, the MTD is defined in terms of a fixed probability that patients in the population experience DLT. For our study, this probability was set to 0.3. The DLT experience of all previous cohorts is used to select the dose for a new cohort that will have this probability of DLT. In stage II, the dose-response model is \[ \log [p/(1-p)] = A + B \log (dose) \], where p is the DLT probability, and A and B are constants updated during the study. The two constants are initialized so that \( P = 0.1 \) at half the dose at which the first DLT is observed and \( P = 0.9 \) at 5 times that dose. Six patients were to be entered in the first cohort in this trial; subsequent cohorts were to consist of three patients.

The initial dose level on schedule B was 30% lower than the MTD in schedule A. Because of relatively small dose changes and extensive pharmacokinetic variability in 9NC and 9AC, the adaptive dose finding procedure was not used to calculate dose levels for schedule B.

Response Assessment. After every two cycles of treatment, patients with measurable disease were assessed for response according to the WHO Criteria (24). Toxicity was defined by the National Cancer Institute–Common Toxicity Criteria, version 2.0 (25). DLTs were defined as platelets ≤ 25,000/μl during treatment, absolute neutrophil count ≤ 500/μl for > 1 week; fever accompanied by absolute neutrophil count ≤ 1000/μl; a delay in re-treatment for ≥2 weeks for any reason; and all nonreversible grade 3 and 4 nonhematological toxicities except bone pain. These toxicities were considered DLTs only if they occurred during cycle 1.

Pharmacokinetic Sample Collection and Preparation. On schedules A and B, serial blood samples for pharmacokinetic analysis were obtained on day 1. Blood samples (5 ml) were obtained before administration of 9NC, and at 0.25, 0.5, 1, 2, 3, 6, 8, and 24 h after administration. Blood was placed into heparinized tubes and centrifuged at 7200 × g at 4°C for 5 min. The resulting plasma samples were then processed immediately to measure the lactone forms of 9NC and 9AC.

For measurement of 9AC lactone, plasma was processed by methanolic extraction (13). A total of 600 μl of plasma was added to 1200 μl of cold (−20°C) methanol. The methanol was maintained at −20°C by placing the vials in an insulated cooler containing dry ice. The samples were vortexed and centrifuged at 7200 × g for 5 min. The resulting supernatant was decanted and stored at −80°C until analyzed.

High-Performance Liquid Chromatography Analysis. 9NC and 9AC lactone plasma concentrations were measured using an high-performance liquid chromatography assay with fluorescence detection as described previously (26). Because 9NC is not highly fluorescent, 9NC lactone was measured by chemically reducing 9NC to 9AC. The concentration of 9NC was calculated by subtracting the concentration of 9AC from the concentration of 9NC plus 9AC after the conversion of 9NC to 9AC using iron reduction.

The high-performance liquid chromatography system consisted of a Waters 2695 separation module (Waters, Inc., Milford, MA), a C18 reverse column [Ultrasphere 5-μm absorbance 4.6 × 250-mm; Beckman Coulter, Inc., Fullerton, CA], and a C18 guard column (Brownlee C18 7 μm, 15 × 3.2-mm; Perkin-Elmer Corp., Norwalk, CT). Samples were injected by an autosampler set at 4°C. The isocratic mobile phase consisted of methanol, acetonitrile, and ammonium acetate (10:23:97, v/v/v; pH 5.5) at a flow rate of 1.0 ml/min. Postcolumn acidification (pH 2–3) was performed using 0.3 m trifluoroacetic acid at 0.3 ml/min (26). 9AC was detected by a Waters number 474 fluorescence detector with excitation wavelength of 365 nm and emission wavelength of 440 nm, 18-nm bandwidth, gain 1000, attenuation 16, with RC filter with fast response setting.
MILLENIUM 32 software (Waters, Inc.) was used for data collection and analysis. All of the glassware, including the injection vials, were treated with 3% surfasil in toluene (Fisher Scientific, Inc., Fair Lawn, NJ).

The lower limit of quantitation for 9NC lactone was 0.5 ng/ml, and the assay was linear from 0.5 to 100 ng/ml. The lower limit of quantitation for 9AC lactone was 0.3 ng/ml, and the assay was linear from 0.3 to 100 ng/ml.

Pharmacokinetic Analysis. Compartamental pharmacokinetic analysis of 9NC and 9AC was performed using ADAPT II (27). A linear model with an oral absorption compartment and one-compartment each for 9NC and 9AC was simultaneously fit to 9NC and 9AC concentration versus time profiles. The area under the 9NC and 9AC plasma concentration curves (9NC AUC and 9AC AUC) from 0 to 24 h were calculated using the log trapezoidal method by simulating the concentration to total concentration (27).

Urine Stability Studies. The stability of lactone and total forms of camptothecin (Supergen, Dublin, CA), 9NC, and topotecan (GlaxoSmithKline, Collegeville, PA) in urine at 37°C and at pH values of 5 and 6 was evaluated over 2 h. Camptothecin, 9NC, and topotecan were added to urine to achieve a final concentration of 100 ng/ml. Urinary pH was measured at baseline and after the addition of the drug. Hydrochloric acid and sodium hydroxide were used to adjust the urine to the desired pH. Triplicate urine samples (200 μl) were obtained at baseline and 5, 30, 60, and 120 min. As appropriate, samples were processed immediately via solid-phase extraction for analysis of 9NC lactone and via methanolic extraction for analysis of 9NC total or for camptothecin and topotecan lactone and total as described previously (26). The percent lactone was calculated as (the ratio of lactone concentration to total concentration) × 100.

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Schedule A</th>
<th>Schedule B</th>
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<tbody>
<tr>
<td>Male/Female Enrolled (N)</td>
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<td>5/4</td>
</tr>
<tr>
<td>Male/Female Evaluable (N)</td>
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<td>5/4</td>
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<td>Eastern Cooperative Oncology Group</td>
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<td>1</td>
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<tr>
<td>Prior treatments</td>
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<td>Prior chemotherapy</td>
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<td>4 (0–8)</td>
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<td>Camptothecin analogues</td>
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<td>6</td>
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<tr>
<td>Irinotecan (N)</td>
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</table>

RESULTS

Patient Characteristics. Patient characteristics are summarized in Table 1. Twenty-three patients were enrolled on schedule A, and 9 patients were enrolled on schedule B. Six patients on schedule A were non evaluable; three patients developed elevated serum bilirubins (two due to biliary stent blockage); one patient developed increase in aspartate aminotransferase; one patient had severe nausea and vomiting; and one patient was noncompliant with the oral therapy. All patients enrolled on schedule B were evaluable.

On schedule A, dose levels 1, 2, and 3 were 2.0 mg/m²/day (n = 6 evaluable patients), 2.7 mg/m²/day (n = 4 evaluable patients), and 2.4 mg/m²/day (n = 7 evaluable patients), respectively. The starting dose of schedule B was 30% lower than the MTD (i.e., 2.4 mg/m²/day) on schedule A. On schedule B, dose levels 1 and 2 were 1.7 mg/m²/day (n = 6 evaluable patients) and 2.4 mg/m²/day (n = 3 evaluable patients), respectively.

Toxicity and Tolerability. The toxicity profiles of schedules A and B were similar with diarrhea, nausea, neutropenia, and thrombocytopenia as the primary toxicities (7, 17, 18). A summary of toxicities for evaluable patients on schedule A during cycle 1 is included in Table 2. On schedule A, the most common toxicities for all dose levels were anemia, diarrhea, and vomiting. The majority of adverse effects reported during cycle 1 were grade 1 or 2. There was no DLT at 2.0 mg/m²/day. Two of four evaluable patients at 2.7 mg/m²/day experienced DLT of febrile neutropenia. The dose was then reduced to 2.4 mg/m²/day. One of seven patients at 2.4 mg/m²/day developed DLT of febrile neutropenia, grade 4 thrombocytopenia, grade 4 anemia, and treatment-related death. Therefore, the recommended Phase II dose for schedule A was 2.4 mg/m²/day. Six of 17 evaluable patients developed grade 1 or 2 hematuria during treatment with 9NC, and 4 of these patients developed hematuria during cycle 1. Of note, six patients were found to have grade 1 or 2 hematuria during screening and were considered ineligible.

A summary of toxicities on schedule B during cycle 1 is included in Table 3. On schedule B, the most common toxicities were anemia, diarrhea, thrombocytopenia, nausea, and vomiting. The majority of the adverse effects reported during cycle 1 were grade 1 or 2. No DLTs occurred in patients receiving 1.7 mg/m²/day. As a result, the dose was escalated to 2.4 mg/m²/...
day. During cycle 1, two of three patients treated at 2.4 mg/m²/day developed DLT. One patient developed grade 3 diarrhea and the other patient developed febrile neutropenia with grade 4 thrombocytopenia. Therefore, the recommended Phase II dose for schedule B was 1.7 mg/m²/day. No patients experienced hematuria during treatment on schedule B.

A total of 77 cycles was administered on schedules A and B. The occurrence of selected grade 3 or 4 toxicities on all cycles were as follows: anemia ($n = 8$); neutropenia ($n = 9$); thrombocytopenia ($n = 5$); diarrhea ($n = 1$); nausea ($n = 1$); vomiting ($n = 1$); and hematuria ($n = 1$). Increased neutropenia and thrombocytopenia were seen in patients with higher 9NC and 9AC total and lactone AUC; however, there was no direct relationship between the percentage decrease in neutrophils or platelets and 9NC or 9AC exposure.

**Clinical Activity.** Of the 17 evaluable patients on schedule A, 4 had stable disease. Two of these patients had gastric cancer and two had pancreatic cancer. The two patients with gastric cancer completed eight cycles of treatment at 2.0 mg/m²/day and six cycles of treatment at 2.7 mg/m²/day, respectively. The two patients with pancreatic cancer completed 6 cycles of treatment at 2.0 mg/m²/day and 16 cycles of treatment at 2.4 mg/m²/day. On schedule B, one patient with pancreatic cancer had stable disease, and one patient with pancreatic cancer had partial remission. The patient with stable disease received four cycles of treatment at 2.4 mg/m²/day. The patient with partial remission received eight cycles of treatment at 2.4 mg/m²/day. None of the patients in schedule A or B with stable disease or partial remission had received a camptothecin analogue as prior therapy.

**9NC and 9AC Pharmacokinetics.** Pharmacokinetic studies were performed on day 1 of both schedules A and B. On schedule A, pharmacokinetic data were not available because of lack of i.v. access in two patients and patient refusal in two patients. On schedule B, pharmacokinetic data were available for all patients. 9NC and 9AC lactone AUCs on schedule A and B are presented in Figs. 1 and 2, respectively. There was significant interpatient variability in the exposure of 9NC and 9AC lactone. The 9NC lactone AUCs at individual doses varied from 6- to 16-fold. On schedule A, the mean ± SD 9NC lactone AUCs at 2.0, 2.4, and 2.7 mg/m² were 183 ± 119, 79 ± 54, and 89 ± 91 ng/ml · h, respectively. On schedule B, the mean ± SD 9NC lactone AUCs at 1.7 and 2.4 mg/m² were 156 ± 113 and 278 ± 218 ng/ml · h, respectively.

The 9AC lactone AUCs at individual doses ranged from 6- to 12-fold. On schedule A, the mean ± SD 9AC lactone AUCs at 2.0, 2.4, and 2.7 mg/m² were 34 ± 21, 17 ± 18, and 32 ± 16 ng/ml · h, respectively. On schedule B, the mean ± SD 9AC lactone AUCs at 1.7 and 2.4 mg/m² were 41 ± 17 and 75 ± 70 ng/ml · h, respectively. Most of the drug remains in the 9NC form with ratios of 9NC to 9AC lactone or total of 4 to 1.

The relationships between dose expressed as mg/m² or mg and drug exposure were evaluated (16, 29). The following measures of drug exposure were evaluated: 9NC and 9AC lactone AUC and the sum of 9NC lactone AUC plus 9AC lactone AUC. There was no relationship between 9NC and 9AC AUC and doses based on mg or mg². In addition, there was no relationship between dose and Cmin or Cmax of 9NC or 9AC. Thus, administration of 9NC doses based on mg or mg² were equally poor predictors of exposure.

**Urinary Stability.** 9NC, camptothecin, and topotecan lactone and total concentrations were evaluated in urine at pH 5 and 6. The mean ± SD percentage lactone of 9NC in urine at pH 5 at 5, 30, 60, and 120 min was 93 ± 6%, 99 ± 3, 89 ± 3, and 94 ± 8%, respectively. The mean ± SD percentage lactone of camptothecin in urine at pH 5 at 5, 30, 60, and 120 min was 102 ± 7, 100 ± 9, 98 ± 6, and 99 ± 8%, respectively. The mean ± SD percentage lactone of topotecan in urine at pH 5 at 5, 30, 60, and 120 min was 103 ± 5, 99 ± 8, 101 ± 5, and 101 ± 1%, respectively.

The mean ± SD percentage lactone of 9NC in urine at pH 6 at 5, 30, 60, and 120 min was 108 ± 9, 79 ± 13, 94 ± 7, and 78 ± 14%, respectively. The mean ± SD percentage lactone of camptothecin in urine at pH 6 at 5, 30, 60, and 120 min was 88 ± 4, 90 ± 3, 87 ± 3, and 94 ± 5%, respectively. The mean ± SD percentage lactone of topotecan in urine at pH 6 at 5, 30, 60, and 120 min was 99 ± 6, 98 ± 6, 89 ± 14, and 91 ± 9%, respectively.

**DISCUSSION**

In Phase II and III studies, 9NC was administered orally daily × 5 days/week for 8 weeks (one cycle); however, the optimal schedule of administration of camptothecin analogues, especially 9NC, is unclear (7, 17, 18). As a result, we performed the first study evaluating intermittent schedules of 9NC and have shown that intermittent schedules of 9NC are tolerable and may be active. The MTD on schedules A and B were 2.4 and 1.7 mg/m²/day, respectively; however, the dose intensity (24 mg/m²/cycle) was the same for both schedules. It is currently unclear which of the intermittent schedules of administration is superior. Schedule A may prove to be the optimal intermittent regimen due to administration of a higher daily dose that may be above a critical threshold required for antitumor response (12, 19). However, because the exposures of 9NC and 9AC are similar because of the high interpatient variability in 9NC and 9AC disposition at the MTD of each regimen, administration of 9NC daily for 14 days every 4 weeks (schedule B) may be the most appropriate regimen based on the ability to administer treatment for 14 consecutive days. The dose intensity achieved on either of the intermittent schedules on our study appear less

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**Table 3** Toxicity summary for schedule B during cycle 1

<table>
<thead>
<tr>
<th>Grade</th>
<th>1–2</th>
<th>3–4</th>
<th>1–2</th>
<th>3–4</th>
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</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematuria</td>
<td></td>
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<td>1</td>
<td></td>
</tr>
</tbody>
</table>
than the dose intensity achieved with the continuous schedule at 1.5 mg/m² (30 mg/m²/4 weeks). However, because of the relatively high pharmacokinetic variability, the exposures of 9NC and 9AC achieved with both regimens are similar (17, 18). Moreover, recent studies using the continuous schedule have required dose reductions and delays during weeks 3–5, thus the dose intensity over 4 weeks is less than the intermittent schedules evaluated in our study (17, 18). In our Phase I studies, there were five patients with prolonged (i.e., more than six cycles) stable disease, and one patient with pancreatic cancer achieved partial remission. The DLTs were neutropenia, thrombocytopenia, and diarrhea. There was significant interpatient variability in the disposition of 9NC and 9AC. Most of the drug remained in the 9NC form with an average 9NC to 9AC ratio of 4 to 1. The fact that most of the administered drug remains in the 9NC form is significant because the development of 9AC was stopped because of lack of efficacy (30–32).

As part of our drug development program of camptothecin analogues, we determined the minimum effective dose of 9NC and associated drug exposure in mice bearing human tumor xenografts and compared this to the MTD and associated drug exposure in a Phase I trial using the same regimen (19). In the preclinical studies, the 9NC and 9AC lactone AUCs associated with the minimum dose that produced a response were 43 and 6

![Fig. 1](image1) 9NC lactone AUC after administration of 2.0, 2.4, and 2.7 mg/m²/day on schedule A and 1.7 and 2.4 mg/m²/day on schedule B. Individual 9NC lactone AUCs are represented by ○, and mean 9NC lactone AUCs are represented by ▲.

![Fig. 2](image2) 9AC lactone AUC after administration of 2.0, 2.4, and 2.7 mg/m²/day on schedule A and 1.7 and 2.4 mg/m²/day on schedule B. Individual 9AC lactone AUCs are represented by ○, and mean 9AC lactone AUCs are represented by ▲.
ng/ml h. respectively. Of the six patients with pharmacokinetic studies at 2.4 mg/m$^2$/day, only two (i.e., 27.3 and 37.4 ng/ml h) had 9NC AUC < 43 ng/ml h. In addition, five of the six responders on schedule A or B had 9NC AUC > 43 ng/ml h. In our Phase II study of 9NC administered orally daily for 5 days/week for 8 weeks in patients with refractory colon cancer, three of five patients had a 9NC lactone AUC < 43 ng/ml h (26, 33). Moreover, there were no responders in the Phase II study of 9NC in patients with colon cancer. This lack of response may be associated with the low daily exposures achieved with this regimen. Thus, the intermittent schedule may be more appropriate than the continuous 8-week schedule because it can achieve exposures that are tolerable in humans and above the target threshold defined in xenograft models. This information and study design can be used to make informed decisions regarding the most appropriate dose and schedule of administration of 9NC and other anticancer agents.

Several studies have reported significant interpatient variability in the pharmacokinetics of some orally administered drugs, especially camptothecin analogs (11, 13–16, 34, 35). In this study, at any given dose of 9NC there was 4–16-fold variability in 9NC and 9AC exposure, and there was no relationship between dose and AUC. The method of dose calculation (i.e., mg versus mg/m$^2$) did not reduce the variability in drug exposure. Thus, there is no evidence that dosing 9NC on a mg/m$^2$ basis is warranted to reduce the pharmacokinetic variability. This is an important question for future studies to address because the most appropriate method to use for calculation of doses for oral administration of 9NC and other anticancer agents is unclear (29). It is currently unclear if the pharmacokinetic variability of 9NC and 9AC are due to variable gastrointestinal absorption, hepatic metabolism, and/or biliary elimination.

The minimal hematuria observed in schedule A and lack of hematuria observed in schedule B are in contrast to the 14% of patients that developed grade 3 hemorrhagic cystitis on the prior Phase I study of 9NC, which used an 8-week continuous dosing schedule (7, 17). This decreased hematuria may be associated with the extensive oral hydration and/or the break in therapy during weeks 3 and 4 used in the intermittent study. In addition, six patients had grade 1 or 2 hematuria during screening and were ineligible for this study. Thus, subclinical hematuria may occur patients with cancer, and the occurrence of grade 1 or 2 hematuria may not be clinically relevant or drug induced.

The percentage of the 9NC dose recovered in urine as 9NC or 9AC is relatively low (< 15%; Ref. 26). However, as stated above, 9NC-induced cystitis has required dose reductions and breaks in therapy (7, 17, 18). Currently, patients are instructed to consume 2–3 liters of fluid/day, which becomes problematic in patients with gastrointestinal cancers and in patients with cardiac conditions. Thus, it is critical to determine the mechanism of camptothecin analogue-induced cystitis and develop feasible treatment options. Camptothecin and 9NC produce cystitis; however, the development of cystitis has not been associated with topotecan (7, 17, 18, 34–37). The differential of cystitis induced by camptothecin, 9NC, and topotecan is not associated with the percentage of drug renally eliminated (13, 33, 37). The pH of normal urine ranges from 4.5 to 6.3 (38); however, it is unclear whether the cystitis associated with camptothecin analogues is due to the lactone or hydroxy acid forms. Because of this, we evaluated the stability of total and lactone forms of 9NC, camptothecin, and topotecan in urine at pH 5 and 6. In our study, the percentage lactone and equilibrium between lactone and hydroxy acid forms of 9NC, camptothecin, and topotecan were similar in urine at pH 5 and 6 (39, 40). Thus, the relative exposure of the lactone form of the camptothecin analogues may not be associated with the development of cystitis, and the conversion of lactone to hydroxy acid form via alkalization of the urine may not be a feasible treatment option in the prevention of 9NC-induced cystitis. Alternatively, the lipophilicity and bladder penetration of camptothecin analogues may be related to the development of cystitis (41, 42).

The clinical importance of this study is underscored by the need to evaluate alternate schedules of administration before the initiation of Phase II and III studies (43, 44). Moreover, well-designed translational studies such as those used in the development of 9NC on the intermittent schedule and previously used to develop topotecan and irinotecan in pediatric solid tumors can be highly informative when determining the most appropriate regimen to take forward in development (11, 12, 19). The intermittent regimen of 9NC evaluated in this study has preclinical rationale, acceptable toxicity, and should be evaluated in Phase II trials, especially in patients with pancreatic and gastric cancer.

REFERENCES


Phase I and Pharmacologic Study of Intermittently Administered 9-Nitrocamptothecin in Patients with Advanced Solid Tumors


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