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ORIGINAL ARTICLE

**Laura L. Jung · Ramesh K. Ramanathan
Merrill J. Egorin · Ruzhi Jin · Chandra P. Belani
Douglas M. Potter · Sandra Strychor
Donald L. Trump · Christine Walko
Marwan Fakih · William C. Zamboni**

Pharmacokinetic studies of 9-nitrocamptothecin on intermittent and continuous schedules of administration in patients with solid tumors

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Abstract *Purpose*: Oral administration of 9-nitrocamptothecin (9NC), and the formation of its metabolite 9-aminocamptothecin (9AC), may be associated with high interpatient and intrapatient variability. Therefore, we evaluated the plasma pharmacokinetics and urine recovery of 9NC administered on three different schedules as part of phase I and phase II studies. *Experimental design*: In phase I schedule A, 9NC was administered orally daily for 5 days per week for 2 weeks repeated every 4 weeks. On phase I schedule B, 9NC was administered daily for 14 days repeated every 4 weeks. In Phase II, 9NC was administered daily for 5 days during 8 weeks (one cycle). Serial blood samples were obtained on day 1 and day 10 or 11 for phase I studies, and day 1 and day 50 for the phase II study. Recovery of 9NC and 9AC in urine was evaluated on day 1 and day 10 or 11 in the phase I study. Area under the 9NC and 9AC plasma concentration vs time curves from 0 to 24 h ($AUC_{0-24\ h}$) were calculated using compartmental analysis. *Results*: The mean ± SD 9NC lactone $AUC_{0-24\ h}$ values on day 1 at the maximum tolerated dose of schedules A and B (2.43 and 1.70 mg/m^2, respectively) and the phase II dose (1.5 mg/m^2) were 78.9 ± 54.4, 155.7 ± 112.8, and 48.3 ± 17.5 ng/ml·h, respectively. The mean ± SD 9AC lactone $AUC_{0-24\ h}$ values at these same doses of 9NC were 17.3 ± 17.9, 41.3 ± 16.6, and 31.3 ± 12.8 ng/ml h, respectively. The ratios of 9NC lactone $AUC_{0-24\ h}$ on day 10 or 11 to day 1 on phase I A and B were 1.27 ± 0.68 and 1.73 ± 1.56, respectively, and the ratios 9AC lactone $AUC_{0-24\ h}$ on day 10 or 11 to day 1 on phase I A and B were 2.23 ± 1.02 and 1.65 ± 0.97, respectively. The recovery of 9NC and 9AC in the urine was < 15%. *Conclusions*: There was significant interpatient and intrapatient variability in the disposition of 9NC and 9AC. 9NC and 9AC undergo primarily nonrenal elimination.

Keywords 9-Nitrocamptothecin · 9-Aminocamptothecin · Oral administration · Pharmacokinetics

L. L. Jung · R. K. Ramanathan · M. J. Egorin · R. Jin
C. P. Belani · S. Strychor · D. L. Trump · C. Walko
M. Fakih · W. C. Zamboni
Molecular Therapeutics and Drug Discovery Program,
University of Pittsburgh Cancer Institute,
Pittsburgh, PA 15213, USA

R. K. Ramanathan · M. J. Egorin · C. P. Belani
D. L. Trump · M. Fakih · W. C. Zamboni
Division of Hematology–Oncology,
Department of Medicine, School of Medicine,
University of Pittsburgh, Pittsburgh, PA 15213, USA

M. J. Egorin
Department of Pharmacology,
School of Medicine, University of Pittsburgh,
Pittsburgh, PA 15213, USA

D. M. Potter
Department of Biostatistics,
Graduate School of Public Health,
University of Pittsburgh,
Pittsburgh, PA 15213, USA

D. M. Potter
Biostatistics Facility,
University of Pittsburgh Cancer Institute,
Pittsburgh, PA 15213, USA

W. C. Zamboni
Department of Pharmaceutical Science,
School of Pharmacy,
University of Pittsburgh,
Pittsburgh, PA 15213, USA

W. C. Zamboni (✉)
Hillman Research Pavilion, University of Pittsburgh
Cancer Institute, Room G.27c, 5117 Centre Avenue,
Pittsburgh, PA 15213, USA
E-mail: zamboniwc@msx.upmc.edu
Tel.: +1-412-6231215
Fax: +1-412-6231212

Introduction

The camptothecin analogues are topoisomerase I-interactive anticancer agents [1–3]. 9-Nitrocamptothecin (9NC) is an orally administered camptothecin analogue [4–6]. In vitro and in vivo preclinical studies suggest that protracted administration of low doses of camptothecin analogues produce greater antitumor activity than does less-frequent administration of higher doses [7, 8]. Consistent with the mechanism of action of camptothecin analogues being cell-cycle-specific, prolonged exposures may be more effective than shorter exposures [9–11]. Daily oral administration of 9NC may mimic a protracted parenteral schedule, achieve prolonged exposure, and maximize patient convenience. However, oral administration of camptothecin analogues has been characterized by extensive interpatient and intrapatient variability in bioavailability [12–16].

9NC is partially converted to the metabolite, 9-aminocamptothecin (9AC) [7, 12, 17]. The terminal lactone ring is a key structural feature for the antitumor activity of camptothecin analogues [18–20]. The conversion between lactone and hydroxy acid is reversible and is driven by pH and protein binding [18–20]. Acidic conditions favor the lactone whereas basic conditions favor the hydroxy acid form. Pharmacokinetic data for 9NC and its conversion to 9AC are limited [7, 12, 17, 21, 22]. Therefore, we evaluated the plasma and urinary disposition of 9NC and 9AC as part of phase I and phase II studies investigating three different schedules of administration.

In phase II and III studies, 9NC is administered continuously at 1.25–1.5 mg/m^2 per day for 5 days per week [23, 24]. 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 [7, 8, 11]. 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. In contrast, administration of 9NC on an intermittent schedule (e.g., 2 weeks of treatment followed by 2 weeks off) may allow the administration of a higher daily dose that would produce therapeutic drug concentrations, and also avoid toxicities in weeks 3 and 4. In addition, there is no scientific basis for the administration of 9NC or other camptothecins for 5 days per week followed by a drug holiday for 2 days [7, 8, 11, 23, 24]. Thus, we evaluated 9NC administered daily for 5 days per week for 2 weeks repeated every 4 weeks compared to 9NC administered daily for 14 days repeated every 4 weeks to determine if the 2-day drug holiday affects the deliverable dose or pharmacokinetics. The pharmacokinetics of 9NC and its 9AC metabolite after administration of 9NC on the two intermittent schedules and the 8-week schedule are presented here. The clinical evaluation from these studies has been described previously [25].

Patients and methods

Patients

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. The eligible patients for the phase I study were 18 years of age or older and had a histologically or cytologically confirmed malignancy for which no curative or effective therapy was available. Other eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 and adequate bone marrow, hepatic, and renal function defined as: absolute neutrophil count (ANC) $\geq 1.5\times10^9$ l^{-1}, platelets $\geq 100\times10^9$ l^{-1}, total bilirubin not more than 1.5 times the upper limit of the institutional normal (ULN) range, aspartate aminotransferase (AST) not more than 1.5 times ULN if liver metastases were not present and not more than 4 times ULN if liver metastases were present, and the absence of microscopic hematuria. Prior treatment with camptothecin analogues, except 9NC, was permitted.

Patients eligible for the phase II study had histologically or cytologically confirmed advanced colon carcinoma with measurable disease that had failed to respond or relapsed after receiving at least one prior 5-fluorouracil-based chemotherapy regimen for advanced disease or had evidence of metastatic disease within 6 months of completion of adjuvant therapy. Other eligibility criteria included an ECOG performance status ≤ 2 and adequate bone marrow, hepatic, and renal function defined as: ANC $\geq 1.5\times10^9$ l^{-1}, platelets $\geq 100\times10^9$ l^{-1}, hemoglobin > 10 g/dl, total bilirubin ≤ 2.0 mg/dl, AST and ALT not more than 3 times ULN if liver metastases were not present and not more than 5 times ULN if liver metastases were present, and serum creatinine ≤ 2.0 mg/dl. Patients with hematuria and those that had previously been treated with 9NC or any other camptothecin analogue were excluded from the phase II study.

Dosage and administration

9NC was supplied by the manufacturer, SuperGen (Dublin, Calif.), as a crystalline powder in hard gelatin capsules that contained active drug and lactose excipient. The capsule strengths were 0.5 and 1.25 mg. All doses of 9NC were rounded to the nearest 0.25 mg. 9NC was administered orally on an empty stomach with an acidic beverage (i.e., orange juice, cola) [17, 23]. Patients were also instructed to drink 2–3 l of fluid per day.

In the phase II study, 9NC was administered at 1.5 mg/m^2 per day for 5 days per week. In the phase I study, two intermittent schedules of 9NC were evalu-

ated. The phase I study evaluating schedule A was completed prior to the initiation of schedule B. On schedule A, 9NC was administered orally daily for 5 days per week for two 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). On the phase II study, 9NC was administered for five consecutive days per week for 8 weeks with no dose escalation. Dose levels for schedule A were determined by adaptive dose finding [26]. In this two-stage method, doses are escalated during stage I by a factor of 1.5 until the first dose-limiting toxicity occurs. Then, in stage II, escalation switches to a model-guided mode similar to the continual reassessment method [27]. Based on the number of days of treatment per cycle in schedules A and B, the initial dose level on schedule B was 30% lower than the maximum tolerated dose in schedule A. The adaptive dose-finding procedure was not used to calculate dose levels for schedule B due to high pharmacokinetic variability and small changes in doses calculated by the adaptive dose-finding method. For all studies, patient diaries and interviews were used as documentation of 9NC administration.

Sample collection and preparation

On schedules A and B of the phase I study, serial blood samples for pharmacokinetic analysis were obtained on day 1 and day 10 or 11. Due to scheduling issues, the second pharmacokinetic study was performed on day 10 or 11. On the phase II study, serial blood samples were obtained on days 1 and 50. For each pharmacokinetic study day, blood samples (5 ml) were obtained prior to administration of 9NC, and 0.25, 0.5, 1, 2, 3, 6, 8, and 24 h after administration. Blood was placed into heparinized tubes and immediately centrifuged at $1200 \times g$ at 4°C for 5 min. The resulting plasma sample was then processed immediately in order to separate the lactone and hydroxy acid forms of 9NC and 9AC.

The processing for the measurement of the 9NC lactone in plasma used solid-phase extraction (SPE) with Waters OASIS HLB columns (1 ml, 30 mg). Columns were conditioned with 1 ml methanol and equilibrated with 1 ml water. Plasma (1 ml) was passed through the column, and the column was then washed with 1 ml of 5% methanol in water to remove the hydroxy acid forms of 9NC and 9AC. 9NC lactone was then eluted with 1 ml of methanol and stored at −80°C until analyzed.

For measurement of 9AC lactone, 9NC total (sum of lactone and hydroxy acid) and 9AC total, the plasma was processed by methanolic extraction. A total of 600 μl plasma was added to 1200 μl methanol on dry ice. The samples were vortexed and centrifuged at $10{,}000 \times g$ for 5 min. The supernatant was decanted and stored at −80°C until analyzed.

On day 1 and day 10 or 11 of the phase I studies, 24-h urine collections were performed. Total urine volume was measured and a 600-μl sample was processed by the same methanolic extraction described above for measurement of 9NC and 9AC total.

High-performance liquid chromatography (HPLC) analysis

As 9NC is not highly fluorescent, 9NC lactone and 9NC total were measured by chemically reducing 9NC to 9AC [21, 22, 23, 28]. Reduction of the 9NC to 9AC was accomplished by mixing 500 μl of the 9NC sample solution (methanolic extraction for measurement of 9NC total or the SPE elution for measurement of 9NC lactone) and 25 μl of 12 *N* HCl. Then 50 μl of the Fe-reduction reagent (25 mg reduced pentacarbonyl iron/ml H_2O; Sigma Chemical Co., St Louis, Mo.) was added and the mixture was sonicated for 30 min at 70°C, and then centrifuged at $13{,}400 \times g$ for 5 min at 5°C. For analysis of 9NC lactone, 150 μl of the supernatant was added to 100 μl of 0.5 *M* ammonium acetate (pH 5.5), vortexed, and 100 μl was injected into the HPLC. For analysis of 9NC total in plasma or urine, 150 μl supernatant was added to 75 μl of 0.5 *M* ammonium acetate (pH 5.5), vortexed, and 100 μl was injected into the HPLC.

The concentration of 9NC was calculated by subtracting the concentration of 9AC present before conversion of 9NC to 9AC from the concentration of 9AC after the conversion of 9NC to 9AC. For analysis of 9AC lactone, 150 μl of the methanolic extract was added to 10 μl of 0.5 *M* ammonium acetate. A total volume of 100 μl was then injected into the HPLC. For analysis of 9AC total, 150 μl of the methanolic extract was added to 15 μl of 20% phosphoric acid and vortexed. Then, 10 μl of 0.5 *M* ammonium acetate (pH 5.5) was added and vortexed, and a total volume of 100 μl was injected into the HPLC.

The HPLC system consisted of a Waters 2695 separation module (Waters Corporation, Milford, Mass.), a C18 reverse-phase column (Ultrasphere 5-μm ODS 4.6×250 mm; Beckman Coulter, Fullerton, Calif.), and a C18 guard column (Brownlee C18 7 μm, 15×3.2 mm; PerkinElmer Corporation, Norwalk, Ct.). Samples were injected by an autosampler set at 4°C. The isocratic mobile phase consisted of methanol/acetonitrile/ammonium acetate (10:23:97, v/v/v), pH 5.5, pumped at a flow rate of 1.0 ml/min. Post-column acidification (pH 2–3) was performed using 0.3 *M* trifluoroacetic acid at 0.3 ml/min [28]. 9AC was detected by a Waters 474 fluorescence detector with an excitation wavelength of 365 nm and an emission wavelength of 440 nm. MILLENIUM 32 software (Waters Corporation) was used for data collection and analysis. All glassware, including the injection vials, was treated with 3% Surfasil in toluene (Fisher Scientific, Fair Lawn, N.J.).

Pharmacokinetic analysis

Compartmental pharmacokinetic analysis of 9NC and 9AC was performed using ADAPT II [29]. The estima-

tion procedure and variance model used in the compartmental pharmacokinetic analysis was maximum likelihood estimation and linear models for the variance of the additive errors, respectively. Different pharmacokinetic model structures were considered to characterize the disposition of 9NC and 9AC in plasma. In the model development, one-compartment and two-compartment models were evaluated to describe the systemic disposition of 9NC and 9AC. In addition, we evaluated the use of single and separate apparent volumes of the central compartments for 9NC and 9AC. Akaike's Information Criterion, Schwartz Criterion, estimated error of the model parameters, and residual analysis were used to select the model structure maximizing the fit accuracy while simultaneously minimizing the number of model parameters. The final model structure used for the pharmacokinetic analysis produced identifiable parameters in all patients.

A linear pharmacokinetic model describing oral administration of 9NC was simultaneously fitted to 9NC and 9AC concentration vs time profiles. The model contained one compartment for 9NC systemic disposition, subsequent conversion of 9NC to 9AC, and one compartment for 9AC systemic disposition. Individual parameters estimated were the absorption rate constant (k_a), the lag time prior to absorption (τ), the apparent volume of the central compartment (V_c/F), the rate constant describing conversion of 9NC to 9AC (k_{12}), and the elimination rate constants for 9NC (k_{10}) and 9AC (k_{20}). The apparent clearance of 9NC (9NC CL/F) and 9AC (9AC CL/F) total and lactone were calculated using standard equations [i.e., $V_c/F\times(k_{10}+k_{12})$ and $V_c/F\times k_{20}$, respectively] [29, 30]. The area under the 9NC and 9AC plasma concentration vs time curves (9NC $AUC_{0-24\ h}$ and 9AC $AUC_{0-24\ h}$) from zero to 24 h were calculated using the log trapezoidal method by simulating the concentration vs time data from each patient using patient-specific parameters [29]. Intrapatient variability of 9NC and 9AC on the phase I study was estimated as the ratio of $AUC_{0-24\ h}$ on day 10 or 11 to $AUC_{0-24\ h}$ on day 1. Intrapatient variability of 9NC and 9AC on the phase II study was estimated as the ratio of $AUC_{0-24\ h}$ on day 50 to the $AUC_{0-24\ h}$ on day 1.

In another attempt to evaluate the intrapatient variability in the disposition of 9NC and 9AC, patient-specific pharmacokinetic parameters describing the disposition of 9NC and 9AC on day 1 were used to simulate the 9NC and 9AC $AUC_{0-24\ h}$ expected in each patient on day 10, and the simulated $AUC_{0-24\ h}$ were compared with the 9NC and 9AC $AUC_{0-24\ h}$ actually measured in each patient on day 10.

Statistical analysis

Statistical analysis was performed using the two-sided Wilcoxon signed ranks test. The level of significance was set at $P<0.05$.

Results

High-performance liquid chromatography (HPLC) analysis

As stated in the "Patients and methods" section, 9NC was measured indirectly as 9AC. There were no endogenous materials in plasma or urine that interfered with measurement of 9AC. With the chromatography conditions described, 9AC eluted at 7.1 min. The sample preparation described resulted in >90% recovery of 9AC when compared to the direct injection of an equivalent amount of 9AC in mobile phase. The conversion of 9NC to 9AC via the reduction method described was $54\pm4.6\%$. When stored at −80°C the percentage change in measured concentration of 9NC and 9AC lactone and total from baseline was <10% at 2 months. The stability of 9NC and 9AC lactone and total on the autosampler at 24 h was >90%.

The lower limits of quantitation (LLQ) for 9NC lactone in plasma and 9NC total in plasma and urine were 0.5 ng/ml, and the assay was linear from 0.5 to 100 ng/ml. The correlation coefficients for three successive 9NC lactone triplicate standard curves in plasma and 9NC total triplicate standard curves in plasma or urine were >0.99. When expressed as a percentage coefficient of variation, the within-day and between day variations in 9NC lactone in plasma and total in plasma and urine triplicate standards were always <15%.

The LLQ for 9AC lactone and 9AC total was 0.3 ng/ml, and the assay was linear from 0.3 to 30 ng/ml. The average (±SD) correlation coefficients for three successive 9AC lactone triplicate standard curves in plasma and 9AC total triplicate standard in plasma or urine curves were >0.99. When expressed as percentage coefficients of variation, the within-day and between day variations in 9AC lactone triplicate standards in plasma and total triplicate standards in plasma or urine were always <15%.

Phase I study 9NC and 9AC lactone pharmacokinetics

A summary of 9NC and 9AC lactone pharmacokinetic parameters from the phase I study is presented in Table 1 and $AUC_{0-24\ h}$ values are presented in Tables 2 and 3. There were no difference ($P>0.05$) in the pharmacokinetic parameters after administration of different doses of 9NC; thus data from all doses were pooled on day 1 or day 10 and 11. Representative concentration vs time profiles of 9NC and 9AC total and lactone after administration of 9NC 2.43 mg/m^2 per day on day 1 and day 11 of schedule A of the phase I study within the same patient are presented in Figs. 1 and 2, respectively. The 9NC and 9AC lactone $AUC_{0-24\ h}$ values on day 1 and day 10 or 11 of schedules A and B are presented in Figs. 3 and 4, respectively.

Table 1 9NC and 9AC lactone pharmacokinetic parameters for phase I schedules A and B and phase II. Values are means ± SD

Parameter	Units	Schedules A, B, and phase II Day 1	Schedule A Day 10 or 11	Schedule B Day 10 or 11	Phase II Week 8, day 1
Lactone		$n=34$	$n=11$	$n=9$	$n=4$
9NC CL/F	l/h/m^2	27.0 ± 33.7	9.5 ± 16.2	16.5 ± 32.5	59.8 ± 45.8
9AC CL/F	l/h/m^2	4.0 ± 8.8	0.4 ± 0.4	2.8 ± 5.5	1.2 ± 0.9
k_a	h^{-1}	0.3 ± 0.4	0.04 ± 0.04	0.1 ± 0.1*	0.3 ± 0.2
τ	h	0.5 ± 0.3	0.8 ± 1.3	0.2 ± 0.2	0.4 ± 0.1
k_{12}	h^{-1}	0.04 ± 0.03	0.09 ± 0.08	0.3 ± 0.3*	0.1 ± 0.02
V_c/F	l/m^2	37.5 ± 46.2	10.0 ± 0.1	4.9 ± 7.0	99.9 ± 98.4
Total		$n=39$	$n=13$	$n=9$	$n=4$
9NC CL/*F*	l/h/m^2	6.3 ± 6.2	2.0 ± 3.1*	1.4 ± 1.1	4.6 ± 6.6
9AC CL/*F*	l/h/m^2	2.2 ± 3.6	0.3 ± 0.3*	0.2 ± 0.1	1.0 ± 1.5
k_a	h^{-1}	0.4 ± 0.7	0.02 ± 0.02*	0.03 ± 0.04	0.1 ± 0.1
τ	h	0.4 ± 0.2	0.7 ± 0.6	0.2 ± 0.1	0.3 ± 0.1
k_{12}	h^{-1}	0.04 ± 0.03	0.1 ± 0.1*	0.1 ± 0.1	0.1 ± 0.1
V_c/F	l/m^2	27.5 ± 35.4	11.0 ± 10.0	10.2 ± 1.2	8.1 ± 12.4

*$P < 0.05$, two-sided Wilcoxon signed ranks test

On schedule A, the ratio of 9NC lactone $AUC_{0-24\ h}$ on day 10 or 11 to day 1 was 1.27 ± 0.68 (mean ± SD). On schedule A, the ratio of 9AC lactone $AUC_{0-24\ h}$ on day 10 or 11 to day 1 was 2.23 ± 1.02. On schedule B, the ratio of 9NC lactone $AUC_{0-24\ h}$ on day 10 to day 1 was 1.73 ± 1.56. In addition, on schedule B the ratio of 9NC lactone $AUC_{0-24\ h}$ simulated on day 10 to the $AUC_{0-24\ h}$ measured on day 10 was 1.6 ± 1.1. On schedule B, the ratio of 9AC lactone $AUC_{0-24\ h}$ on day 10 to day 1 was 1.65 ± 0.97. In addition, on schedule B the ratio of 9AC lactone $AUC_{0-24\ h}$ simulated on day 10 to the $AUC_{0-24\ h}$ measured on day 10 was 3.7 ± 4.2.

Phase I study 9NC and 9AC total pharmacokinetics

A summary of 9NC and 9AC total pharmacokinetic parameters is presented in Table 1 and $AUC_{0-24\ h}$ are presented in Tables 2 and 3. There was no difference ($P > 0.05$) in the pharmacokinetic parameters after administration of different doses of 9NC; thus data from all doses were pooled on day 1 or day 10 and 11. On schedule A, the ratio of 9NC total $AUC_{0-24\ h}$ from day 10 or 11 to day 1 was 1.62 ± 0.57 (mean ± SD). On schedule A, the ratio of 9AC total $AUC_{0-24\ h}$ from day 10 or 11 to day 1 was 2.08 ± 0.75. On schedule B, the ratio of 9NC total $AUC_{0-24\ h}$ from day 10 to day 1 was 1.40 ± 0.61. In addition, on schedule B the ratio of 9NC total $AUC_{0-24\ h}$ simulated on day 10 to the $AUC_{0-24\ h}$ measured on day 10 was 1.7 ± 1.0. On schedule B, the ratio of 9AC total $AUC_{0-24\ h}$ from day 10 to day 1 was 1.50 ± 0.45. In addition, on schedule B the ratio of 9AC total $AUC_{0-24\ h}$ simulated on day 10 to the $AUC_{0-24\ h}$ measured on day 10 was 6.5 ± 4.4.

Phase II 9NC and 9AC lactone and total pharmacokinetics

Summaries of 9NC and 9AC pharmacokinetic parameters from the phase II study are presented in Tables 1 and 4. The ratio of 9NC lactone $AUC_{0-24\ h}$ on day 50 to day 1 was 1.16 ± 0.89 (mean ± SD). The ratio of 9AC lactone $AUC_{0-24\ h}$ on day 50 to day 1 was 1.74 ± 0.13. The ratio of 9NC total $AUC_{0-24\ h}$ on day 50 to day 1

Table 2 9NC and 9AC lactone and total $AUC_{0-24\ h}$ (ng/ml·h) values and 9AC to 9NC AUC ratios on phase I schedule A, presented as means ± SD (range)

	Day 1			Day 10 or 11		
	2.00 mg/m^2	2.43 mg/m^2	2.68 mg/m^2	2.00 mg/m^2	2.43 mg/m^2	2.68 mg/m^2
Lactone	$n=6$	$n=7$	$n=4$	$n=6$	$n=7$	$n=3$
9NC AUC	183.1 ± 119.0 (78.4–412.7)	78.9 ± 54.4 (27.0–187.2)	89.2 ± 90.9 (45.8–287.9)	254.7 ± 190.0 (110.4–671.9)	102.8 ± 121.0 (11.0–360.7)	229.6 ± 66.8 (154.6–316.8)
9AC AUC	33.6 ± 20.7 (10.81–66.14)	17.3 ± 17.9 (5.2–60.6)	32.2 ± 16.1 (7.3–51.5)	45.1 ± 23.4 (20.1–80.4)	43.7 ± 42.5 (8.3–136.1)	88.8 ± 34.6 (42.0–124.5)
9AC to 9NC AUC ratio	0.19 ± 0.07 (0.11–0.29)	0.23 ± 0.13 (0.06–0.41)	0.18 ± 0.04 (0.11–0.21)	0.21 ± 0.13 (0.11–0.48)	0.70 ± 0.66 (0.14–2.06)	0.41 ± 0.19 (0.19–0.65)
Total	$n=6$	$n=7$	$n=4$	$n=6$	$n=7$	$n=3$
9NC AUC	647.4 ± 294.5 (136.3–1037.0)	482.1 ± 597.6 (109.0–1,936.0)	942.0 ± 443.6 (292.0–1,474.0)	1075.3 ± 811.3 (408.4–2,646.0)	651.6 ± 774.7 (62.8–2,487.0)	1,663.3 ± 556.8 (1,115.0–2,427.0)
9AC AUC	196.4 ± 134.1 (54.5–441.2)	83.5 ± 78.9 (31.1–274.0)	245.5 ± 122.4 (49.7–382.0)	304.9 ± 197.1 (112.9–668.0)	178.3 ± 179.8 (23.4–582.0)	537.4 ± 238.9 (201.9–739.4)
9AC to 9NC AUC ratio	0.30 ± 0.10 (0.14–0.43)	0.21 ± 0.05 (0.14–0.29)	0.25 ± 0.05 (0.17–0.31)	0.23 ± 0.05 (0.15–0.29)	0.33 ± 0.12 (0.14–0.50)	0.32 ± 0.12 (0.18–0.46)

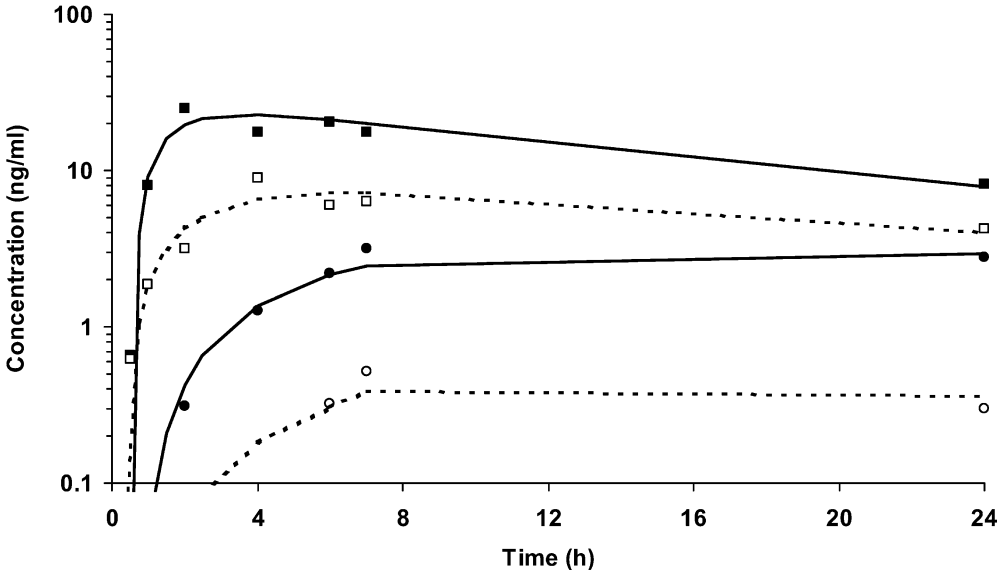

Fig. 1 Representative concentration vs time profiles of 9NC and 9AC total and lactone profiles in a single patient after oral administration of 9NC 2.43 mg/m^2 per day on day 1 of schedule A of the phase I study. The concentration vs time profiles in this figure and Fig. 2 are from the same patient. Individual data and best-fit line for 9NC total (*closed squares*, —) and lactone (*open squares*, ---) concentration vs time profiles are presented. Individual data and best-fit line for 9AC total (*closed circles*, —) and lactone (*open circles*, ---) concentration vs time profiles are also presented

was 1.68 ± 0.61. The ratio of 9AC total $AUC_{0-24\,h}$ on day 50 to day 1 was 1.50 ± 0.76.

Renal elimination

On schedule A of the phase I study, the percentages of the doses renally eliminated on day 1 as 9NC total and 9AC total were 5.9 ± 4.3 and 3.5 ± 1.7%, respectively. On schedule A of the phase I study, the percentages of the doses renally eliminated as 9NC total and 9AC total on day 10 or 11 were 5.6 ± 3.8 and 4.2 ± 2.2%, respectively. On schedule B of the phase I study, the percentages of the doses renally eliminated on day 1 as 9NC total and 9AC total were 11.3 ± 3.8 and 5.0 ± 2.6%, respectively. On schedule B of the phase I study, the percentages of the doses renally eliminated as 9NC total and 9AC total on day 10 or 11 were 9.0 ± 6.2 and 5.5 ± 1.9%, respectively.

Discussion

Although phase III trials of 9NC have been completed, there are limited pharmacokinetic data on 9NC and its conversion to 9AC. The pharmacokinetics of 9NC after oral and inhaled administration have been investigated in previous studies [21–24, 31–33]. However, this is the first pharmacokinetic study in which the interpatient and intrapatient variabilities of 9NC and its 9AC metabolite on continuous and intermittent schedules of administration have been evaluated. There is high interpatient and intrapatient pharmacokinetic variability of 9NC and 9AC regardless of the schedule of administration

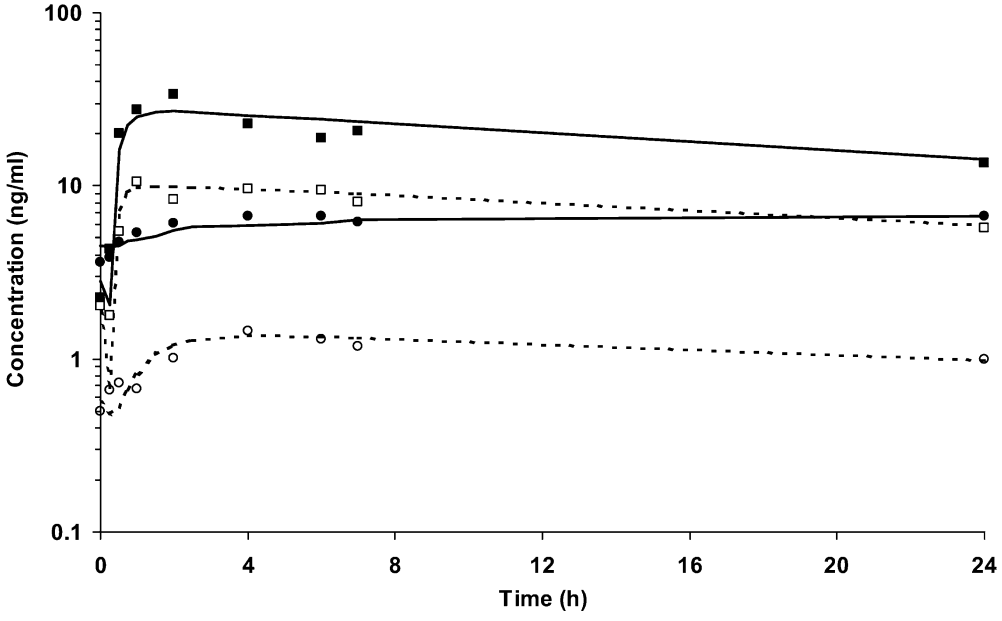

Fig. 2 Representative concentration vs time profiles of 9NC and 9AC total and lactone profiles in a single patient after oral administration of 9NC 2.43 mg/m^2 per day on day 10 of schedule A of the phase I study. The concentration vs time profiles in Fig. 1 and this figure are from the same patient. Individual data and best-fit line for 9NC total (*closed squares*, —) and lactone (*open squares*, ---) concentration vs time profiles are presented. Individual data and best fit line for 9AC total (*closed circles*, —) and lactone (*open circles*, ---) concentration vs time profiles are also presented

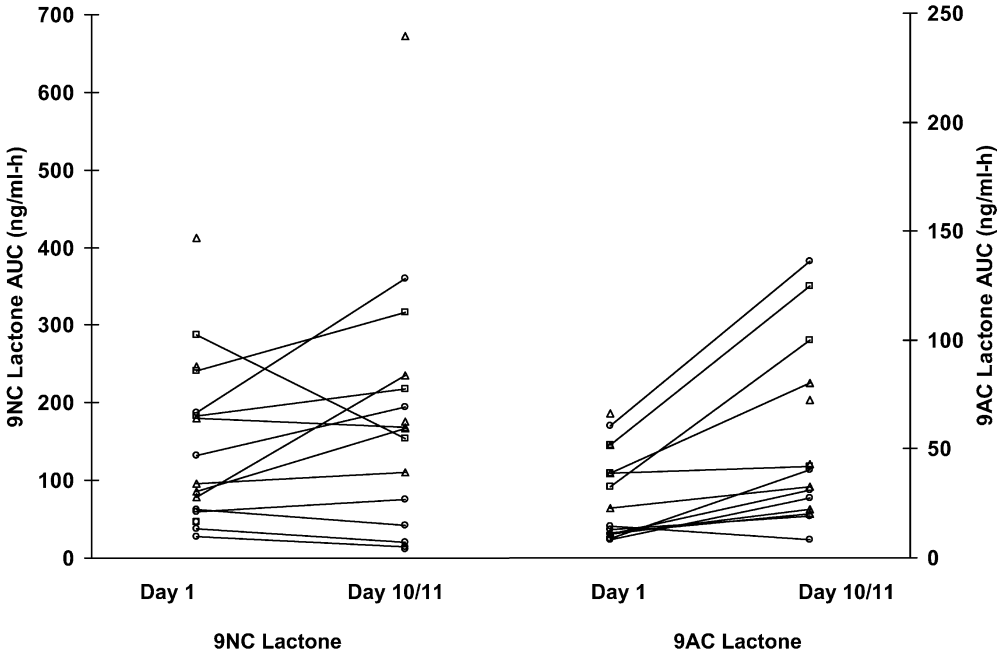

Fig. 3 interpatient and intrapatient variability in 9NC and 9AC lactone AUCs on schedule A of the phase I study. Individual 9NC and 9AC lactone AUC values for patients administered 2.00 mg/m^2 (*triangles*), 2.43 mg/m^2 (*circles*), and 2.68 mg/m^2 (*squares*) are presented. Data from the same patient from day 1 to day 10 or 11 are connected by *solid lines*

[12–16]. The clinical importance of this study can be underscored by the need to evaluate the pharmacokinetics of anticancer agents that are orally administered and have a steep relationship between exposure and response (i.e., antitumor activity or toxicity).

Preliminary evidence suggests that 9NC is metabolized extensively in the liver by an NADPH-dependent system, probably involving a cytochrome P450 isotype [33]. Previous in vitro studies have demonstrated that the conversion of 9NC to 9AC is relatively minor (i.e., 12–25%), but highly variable [33–35]. In our current study, most of the drug remained in the 9NC form with a mean ratio of 9NC to 9AC conversion of 4 to 1, which is consistent with the previous studies. The fact that most of the administered drug remains in the 9NC form is significant because the development of 9AC was stopped due to lack efficacy [4–6]. However, our studies also demonstrated highly variable conversion of 9NC to 9AC, as two patients had undetectable 9AC concentrations and four patients had higher exposures of 9AC compared to 9NC. In addition, from day 1 to day 10 in the phase I study or day 1 to day 50 in the phase II study some patients had greater accumulation of 9NC than of 9AC, whereas others had greater accumulation of 9AC.

Although the complete metabolic pathway of 9NC and 9AC is unclear, our study suggests that the clear-

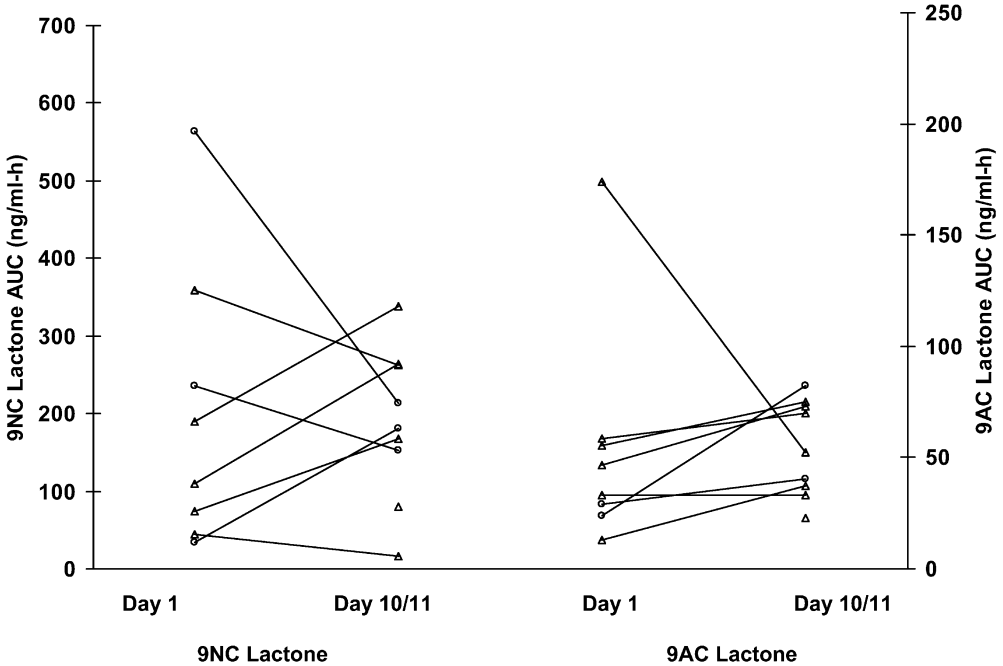

Fig. 4 interpatient and intrapatient variability in 9NC and 9AC lactone AUCs on Schedule B of the Phase I study. Individual 9NC and 9AC lactone AUC values for patients administered 1.70 mg/m^2 (*triangles*) and 2.40 mg/m^2 (*circles*) are presented. Data within the same patient from day 1 to day 10 or 11 are connected by *solid lines*

Table 3 9NC and 9AC lactone and total $AUC_{0-24\ h}$ (ng/ml h) values and 9AC to 9NC AUC ratios on phase I schedule B, presented as means ± SD (range)

	Day 1		Day 10 or 11	
	1.70 mg/m^2	2.40 mg/m^2	1.70 mg/m^2	2.40 mg/m^2
Lactone	$n=5$	$n=3$	$n=6$	$n=3$
9NC AUC	155.7 ± 112.8 (45.2–359.2)	277.9 ± 218.4 (34.1–563.9)	188.1 ± 112.3 (15.8–338.5)	182.7 ± 25.1 (152.8–241.3)
9AC AUC	41.3 ± 16.6 (13.1–58.3)	75.4 ± 69.6 (23.6–173.8)	51.8 ± 21.2 (22.9–74.9)	58.4 ± 17.7 (40.3–82.5)
9AC to 9NC AUC ratio	0.38 ± 0.24 (0.13–0.79)	0.37 ± 0.24 (0.12–0.69)	0.62 ± 0.78 (0.10–2.36)	0.32 ± 0.10 (0.25–0.46)
Total	$n=5$	$n=3$	$n=6$	$n=3$
9NC AUC	431.0 ± 213.7 (91.0–737.4)	289.5 ± 92.5 (161.8–377.7)	534.3 ± 339.7 (83.6–1,065.0)	337.4 ± 22.2 (321.7–368.8)
9AC AUC	183.8 ± 109.8 (31.6–345.2)	281.7 ± 198.3 (114.1–560.2)	233.6 ± 155.8 (73.6–446.9)	306.6 ± 123.3 (218.7–481.0)
9AC to 9NC AUC ratio	0.46 ± 0.25 (0.15–0.90)	0.95 ± 0.54 (0.45–1.70)	0.51 ± 0.23 (0.23–0.88)	0.92 ± 0.41 (0.59–1.49)

* Area under the plasma concentration vs time curves were calculated from 0 to 24 h ($AUC_{0-24\ h}$)

ance of 9NC appears to be non-renal with possible biliary elimination. The percentage of the dose recovered in the urine was low (i.e., <15%). However, if the oral bioavailability, which is currently unknown because no intravenous formulation exists, is only 30% then the percentage of the dose recovered in the urine would be 50%. Patients with who had elevated bilirubin levels related to biliary stent blocks or disease had the highest 9NC and 9AC exposures. A plateau of 9NC and 9AC concentrations from 6 to 24 h, and T_{max} which ranged from 0.5 to 24 h, suggests that 9NC and 9AC may undergo enterohepatic circulation [31]. Similar results have been reported for 9NC and with other camptothecin analogues. However, the long duration of 9NC exposure was much greater than reported for other orally administered camptothecin analogues [12–16].

Several studies have shown significant interpatient variability in the pharmacokinetics of orally administered camptothecin analogues [11, 13–16, 34, 35]. Our study also demonstrated relatively high interpatient and intrapatient pharmacokinetic variability of 9NC and 9AC. At individual doses there was a 4- to 16-fold variability in 9NC and 9AC exposure among different patients and there was no relationship between dose and $AUC_{0-24\ h}$. The ratio of 9NC or 9AC $AUC_{0-24\ h}$ from day 1 to day 10 in the phase I study or day 50 in the phase II study ranged from 0.6 to 4. Some accumulation of 9NC was seen on both the intermittent and continuous schedules of administration. However, the accumulation of 9NC or 9AC from day 1 to day 10 or 11 in the phase I studies or from day 1 to day 50 on the phase II study was not as great as predicted by the pharmacokinetics on day 1. This also suggests that the disposition of 9NC and 9AC is not consistent over time.

Table 4 9NC and 9AC lactone and total $AUC_{0-24\ h}$ (ng/ml h) values and 9AC to 9NC AUC ratios in phase II, presented as means ± SD (range)

	Day 1 1.50 mg/m^2	Day 50 1.50 mg/m^2
Lactone	$n=5$	$n=3$
9NC AUC	48.3 ± 17.5 (23.4–69.3)	51.9 ± 31.1 (11.9–87.7)
9AC AUC	31.3 ± 12.8 (10.2–44.8)	38.8 ± 19.7 (19.1–58.5)
9AC to 9NC AUC ratio	0.81 ± 0.65 (0.28–1.92)	0.63 ± 0.41 (0.22–1.04)
Total	$n=9$	$n=3$
9NC AUC	377.0 ± 221.2 (90.7–798.1)	310.7 ± 178.2 (89.8–526.1)
9AC AUC	88.5 ± 41.32 (27.0–141.8)	97.8 ± 79.5 (16.1–205.5)
9AC to 9NC AUC ratio	0.31 ± 0.21 (0.10–0.83)	0.27 ± 0.09 (0.18–0.39)

The high interpatient and intrapatient variability in 9NC and 9AC disposition could be due to variability in oral absorption, the affect of food, pH of the gastric tract, and hepatic and extrahepatic conversion of 9NC to 9AC [7, 12–17, 23]. The presence of a nitro group at the nine-position makes 9NC water-insoluble and an intravenous formulation has not been developed. Thus, the absolute oral bioavailability of 9NC cannot be determined in patients and the influence of oral absorption and systemic metabolism on the overall variability in 9NC and 9AC disposition cannot be determined. Schoffski and colleagues demonstrated that a meal consisting of 58% fat, 15% protein, and 27% carbohydrate consumed 30 min prior to drug administration led to a 50% reduction in the oral absorption of 9NC [23]. In our study, 9NC was administered on an empty stomach with an acidic beverage; however, it is still unclear if coadministration with an acidic beverage improves oral absorption and reduces variability [17, 23]. Patients in this study were allowed to continue any routine medications, including histamine-2 blockers and proton pump inhibitors. There was no relationship between the administration of these agents and exposure of TNC and 9AC. Similar results have been seen with topotecan after administration of ranitidine [17]. In

addition, there was no relationship between the level of compliance as documented by patient diaries and interviews and the exposure of 9NC and 9AC. Thus, the factors associated with the extensive variability in the disposition of 9NC and 9AC are unknown.

The results of our study are similar to those of the studies by Schoemaker et al. and Raymond et al. [21, 22]. For example, the values of k_a in our study and the study by Schoemaker et al. were $0.4 \pm 0.7\ h^{-1}$ and $0.8\ h^{-1}$, respectively. In addition, the values of CL/F of 9NC in our study and the study by Schoemaker et al. were 6.3 ± 6.2 l/h/m^2 and 1.7 l/h, respectively. The slight difference in CL/F may be explained by administration of different doses and a slightly different model framework used between the two studies, and additional samples and evaluation of more patients in our study. In the study by Raymond et al., following administration of a single dose of 9NC at 1.5 mg/m^2, the 9NC and 9AC $AUC_{0-24\ h}$ values were 231 ± 137 ng/ml h and 36.9 ± 28.5 ng/ml h, respectively, which are similar to the $AUC_{0-24\ h}$ values in our studies after administration of 9NC at 2.0 mg/m^2. In addition, the concentration vs time profiles of 9NC and 9AC, delayed absorption, increases in 9AC concentrations over the 24-h period, and no drug accumulation over the 21 days of treatment in the study by Raymond et al. are very similar to the findings of our study.

We used an HPLC assay with fluorescence detection to measure 9NC and 9AC. As stated previously, 9NC was measured indirectly by chemically reducing 9NC to 9AC using an Fe-reduction reagent [27]. The need to convert 9NC to 9AC via Fe reduction is highly complicated and logistically difficulty. Moreover, the conversion of 9NC to 9AC is only approximately 54% which requires the indirect estimation of 9NC concentrations by subtracting the concentration of 9AC present before conversion of 9NC to 9AC from the concentration of 9AC after the conversion of 9NC to 9AC. The use of an LC/MS or LC/MS/MS assay for 9NC would not require the conversion of 9NC to 9AC and may allow the simultaneous determination of lactone and hydroxy acid forms of 9NC and 9AC.

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