Cancer Chemother Pharmacol (2006) 57: 631–639
DOI 10.1007/s00280-005-0084-6

ORIGINAL ARTICLE

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Clinical and pharmacokinetic study evaluating the effect of food on the disposition of 9-nitrocamptothecin and its 9-aminocamptothecin metabolite in patients with solid tumors

Received: 13 April 2005 / Accepted: 25 July 2005 / Published online: 5 October 2005

Abstract *Background*: 9-Nitrocamptothecin (9NC) is an orally administered camptothecin analogue that has completed phase III trials for pancreatic cancer. In biological matrices, camptothecin analogues exist in equilibrium between the active-lactone (LAC) and inactive-hydroxy acid (HA) forms. 9NC has been administered on an empty stomach; however, it is unclear if food alters the absorption and disposition of 9NC and its 9-aminocamptothecin (9AC) active-metabolite. Thus, we evaluated the disposition of 9NC and 9AC after administration of 9NC under fasting conditions and after a standard meal. *Methods*: Patients were randomized to receive 9NC as a single oral dose at 1.5 mg/m^2 with 8 ounces (oz) of an acidic beverage under fasting conditions, or after a meal consisting of two eggs, 8 oz of orange juice, buttered toast, 8 oz of milk, and 4 oz of hash brown potatoes. Following a 72 h washout period, 9NC was administered with the alternative condition (i.e., with food or fasting). 9NC was then continued for 5 days of every week. Serial blood samples were obtained prior to and from 0.25 to 24 h after administration of 9NC. The total (sum of LAC + HA) of 9NC and 9ACwere measured by an LC-MS/MS assay. Area under the plasma concentration versus time curve (AUC) for 9NC and 9AC total were calculated. After the pharmacokinetic section of the study, patients received 9NC 1.5 mg/m^2 orally under fasting conditions daily for 5 days per week for 8 weeks. *Results*: Sixteen patients with median (range) age 62 (47–83) years, diagnoses of colorectal (six patients), lung (two patients), and other (eight patients) malignancies, received 83 [median (range) 4 (2–9)] weeks of therapy. Patients with toxicities greater than grade 2: were diarrhea (1), nausea (2), vomiting (2), fatigue (2), anemia (3), neutropenia (3), and febrile neutropenia (2). Three patients (lung, unknown primary, and colon) had stable disease for eight weeks. The mean ± SD of 9NC AUC_{food} and 9NC AUC_{fast} ($n = 9$) were 330 ± 182 and 558 ± 379 ng/ml·h, respectively ($P < 0.05$). The mean ± SD of 9AC AUC_{food} and 9AC AUC_{fast} ($n = 9$) were 244 ± 60 and 256 ± 101 ng/ml·h, respectively ($P > 0.05$). The mean ± SD ratio of 9NC AUC_{food} to $AUC_{fasting}$ in individual patients ($n = 9$) was 0.67 ± 0.22. The mean ± SD ratio of 9AC AUC_{food} to $AUC_{fasting}$ in individual patients ($n = 9$) was 1.14 ± 0.61. *Conclusions*: Co-administration of 9NC with food reduces the oral absorption of 9NC; however, there was no difference in

Supported, in part, by grant NCI 2P30 CA47904, grant NIH / NCRR / GCRC /#5M01 RR00056, and a grant from Supergen Inc., Dublin, California

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the exposure of 9AC. The is high interpatient variability in the effect of food on the absorption of 9NC and the interpatient variability in the effect of food on the disposition of 9AC is even greater when compared to 9NC.

Keywords Nitrocamptothecin · Aminocamptothecin · Metabolite · Solid tumors

Introduction

The camptothecin analogues are topoisomerase I-interactive anticancer agents [5, 16, 19]. 9-Nitrocamptothecin (9NC, RSF2000, rubitacan) is an orally administered camptothecin analogue [21, 35, 48]. In vitro and in vivo preclinical studies suggest that protracted administration of low doses of camptothecin analogues produce greater anti-tumor activity than less frequent administration of higher doses [9, 49]. Consistent with the mechanism of action of camptothecin analogues being cell-cycle-specific, prolonged exposures may be more effective than shorter exposures [7, 15, 17]. 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 inter-and intra-patient variability in bioavailability and may be clinically significant [8, 12, 23, 24, 51].

9-Nitrocamptothecin is partially converted to a 9-aminocamptothecin (9AC) active-metabolite [1, 9, 51]. 9NC and 9AC both occur in equilibrium between the active-lactone and inactive-hydroxy acid forms [28, 29, 44]. In our previous pharmacokinetic studies of 9NC and its 9AC metabolite, we found that for any given dose of 9NC, there was 4–16-fold variability in 9NC and 9AC exposure [33]. The method of dose calculation (i.e., mg vs. mg/m^2) did not reduce the variability in drug exposure. It is currently unclear whether the pharmacokinetic variability of 9NC and 9AC is due to variable gastrointestinal absorption, hepatic metabolism, and/or biliary elimination.

As is standard for orally administered drugs, the effect of food on the oral absorption and bioavailability of drugs should be evaluated [8, 12, 23, 24, 51]. Schoffski et al. [43] evaluated the effect of food intake on the bioavailability of orally administered 9NC in a randomized crossover study in which patients were administered 9NC with and without food. Because administration of 9NC with food resulted in a 50% reduction in the exposure of 9NC, the authors concluded that 9NC should be administered under fasting conditions. However, the study did not evaluate the disposition of 9AC which has ranged from 20 to 200% of the 9NC exposure in patients. Thus, we evaluated the disposition of 9NC and 9AC after administration of 9NC with and without a standard meal in a randomized crossover study.

Methods

Patient selection

Eligible patients were required to have an advanced solid malignancy confirmed on histology and refractory to standard treatment; age greater than or equal to 18 years; measurable or evaluable disease; ECOG performance status (PS) ≤ 2; and an adequate organ function as defined by absolute neutrophil count (ANC) $\geq 1{,}500/mm^3$, platelet count $\geq 100{,}000/mm^3$, hemoglobin >9 gm/dl; creatinine clearance ≥ 50 ml/min; serum bilirubin within 1.5× institutional normal limits (INL); and ALT and AST ≤ 3 times INL. In addition, patients were required to be able to swallow oral medication, and have adequate venous access to permit a repeat blood sampling. Patients with malabsorption syndrome or gastric or small bowel resection were excluded, as were pregnant or nursing women. Patients of childbearing age were required to practice contraception. Patients were required to have at least a 4-week interval from any previous surgery, chemotherapy, and radiotherapy prior to initiation of the study. All patients gave written informed consent approved by the Institutional Review Boards at the Albert Einstein College of Medicine (AECOM) and the Montefiore Medical Center (MMC).

Study design

This trial was a two-period crossover design with patients randomized to receive the study medication either in the ''fasting'' or the ''fed'' state (period 1). They received one dose of the drug on the first day. After a 72-h washout, the patients were crossed over to the other arm (period 2). After a repeat washout period of at least 72 h, patients were entered on to period 3 of the study. This entailed ingestion of 9NC for 5 days on a weekly basis for a period of 8 weeks. It was an open-label, single-arm trial conducted at the AECOM and the MMC from December 2002 to May 2004.

Study drugs and administration

9-Nitrocamptothecin was supplied by Supergen, Inc (Dublin, CA, USA) as 1.25 and 0.5 mg tablets. The drug was stored in the outpatient pharmacy in a secure, limited access area at 15–25°C (59–77°F) in a dry place protected from light. The dose was calculated as 1.5 mg/m^2 and rounded to the closest multiple of 0.25 mg. 9NC was administered by the study staff under supervision during periods 1 and 2. The standard meal consisted of two eggs, 8 ounces (oz) of orange juice, buttered toast, 8 oz of milk, and 4 oz of hash brown potatoes. During period 3, the patient was given a 5-day supply each week and the drug was self-administered at home. Each dose was administered with 6–8 oz of an acidic beverage, such

as an orange juice [51]. During the ''fasting'' period and in period 3, the dose of 9NC was preceded and followed by a 2 h fasting period. During the ''fed'' period, the 9NC was swallowed within 5 min of ingestion of the standard meal. The study drug administration was repeated every 7 days, until disease progression or development of intolerable toxicities. Objective disease evaluations were performed at the end of 8 weeks of period 3.

Patient evaluation and follow-up

All patients underwent a complete medical history, physical examination, and performance status evaluation within 2 weeks of entry into the study, prior to drug administration for periods 1 and 2, and at the start of every week of therapy during period 3. Complete blood count with differential (CBC), a complete chemistry profile, and a urinalysis were obtained with each physical examination. Imaging studies of chest, abdomen, and pelvis were performed as indicated for tumor measurements within 4 weeks of initiation of study drugs. Toxicity assessments were performed weekly. Tumor assessment was performed after every 8 weeks of weekly therapy using the World Health Organization criteria.

A complete response (CR) was defined as the disappearance of all measurable lesions at two examinations at least 4 weeks apart. Partial response (PR), stable disease (SD), and progressive disease (PD) were defined as ≥50% decrease, <25% decrease or <25% increase, and ≥25% increase in the sum of bi-dimensional measurements of tumor burden, respectively. Appearance of any new lesion constituted disease progression. Response duration was the time from documentation of first response to the first date of objective progression of disease. After removal from study, all patients underwent follow-up examinations and toxicity assessments every 2 months until their deaths.

Study endpoints and dose modifications

The primary and secondary endpoints of the study were to determine the effect of food on the oral absorption of 9NC and 9AC and to characterize toxicities, respectively. The starting dose was 1.5 mg/m^2 for all patients and for all periods 1–3. If a patient tolerated the first 4 weeks of study drug without any grade 2 toxicities, the dose could be escalated to 2 mg/m^2 for all subsequent administrations. Appearance of grade 2 thrombocytopenia, grade 3 neutropenia, grade 3 non-hematologic toxicity, or grades1–2 chemical cystitis, necessitated dose reduction by one level, i.e. 1.5 mg/m^2 administered for 4 days every week. Appearance of grade 3 thrombocytopenia, grade 4 neutropenia, grade 4 non-hematologic toxicity, or grade 3 chemical cystitis, necessitated dose reduction by two levels, i.e., 1 mg/m^2 administered for 4 days every week. Grade 4 chemical cystitis required discontinuation of protocol therapy. All toxicities were graded based on the expanded NCI CTC version 2.0.

Sample collection and preparation

Blood samples (5 ml) were obtained after administration of 9NC, on an empty stomach or with a standard meal, prior to administration and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after administration. Blood was placed into heparinized tubes and immediately centrifuged at 1,200×*g* at 4°C for 5 min. The resulting plasma was processed via methanolic extraction to measure 9NC and 9AC total as previously described [8, 33, 51].

Mass spectrometric assay

In previous studies, 9NC was measured indirectly by conversion to 9AC via a logistically difficult iron-reduction process [33, 47]. To overcome these issues, we developed a highly sensitive and specific HPLC-tandem mass spectrometric assay to simultaneously and directly quantitate 9NC and 9AC total concentrations in human plasma. Our assay was modified from the assay developed by Xenobiotics Laboratories, Inc. (Plainsboro, NJ, USA) [32].

Chromatography was performed on a Waters Alliance 2695 system (Milford, MA, USA) and a Phenomenex Luna C_{18} (5 μm, 150×2 mm) analytical column (Torrance, CA, USA). The isocratic mobile phase, consisting of 0.1% formic acid in acetonitrile: 50 μM ammonium acetate buffer (40:60, v/v), was pumped at 0.2 ml/min, and the run time was 7 min. Mass detection was carried out using a Waters Quattro micro triple-stage, bench top quadrupole mass spectrometer with electrospray ionization in positive-ion, multiple reaction mode (MRM). The settings of the mass spectrometer were as follows: capillary voltage 4 kV; cone voltage 40 V; source temperature 120°C; and desolvation temperature 450°C. The cone and desolvation gas flows were 110 and 550 l/h, respectively. The collision voltage was 30 V. 9NC, 9AC, and camptothecin ions monitored in MRM scans were m/z 394 > 350, 364 > 320, and 349 > 304, respectively. The LC system and mass spectrometer were controlled by Waters Mass Lynx software (version 4.0), and data were collected with the same software. 9NC, 9AC, and camptothecin eluted at 5.04, 2.70, and 3.83 min, respectively.

Pharmacokinetic analysis

Compartmental pharmacokinetic analysis of 9NC and 9AC was performed using ADAPT II [6]. The estimation 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- 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 Criteria (AIC), 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 as described below.

A linear PK model describing oral administration of 9NC was simultaneously fit to 9NC and 9AC concentration versus 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 were calculated using standard equations (i.e. $V_c/F\times(k_{10} + k_{12})$ and $V_c/F\times k_{20}$, respectively) [6, 40]. The area under the 9NC and 9AC plasma concentration versus time curves (9NC $AUC_{0-24\ h}$ and 9AC $AUC_{0-24\ h}$) from 0 to 24 h were calculated using the log trapezoidal method by simulating the concentration versus time data from each patient using patient-specific parameters [6]. Effect of food on the disposition of 9NC and 9AC was evaluated as the ratio of AUC with food (AUC_{food}) to AUC without food ($AUC_{fasting}$).

Statistical analysis

Statistical analysis was performed by comparing all parameters for 9NC and 9AC after administration with and without food on paired data only using the Wilcoxon signed ranked test. All analysis was performed using the SPSS version 10.0 (Chicago, IL, USA).

Results

Patient characteristics

Sixteen patients with a median (range) age of 62 (29–85) years and ECOG PS of 1 (15 patients) or 2 (1 patient) were enrolled onto this trial (Table 1). All patients had received prior treatment with 3(1–4) [median (range)] chemotherapy regimens. The primary sites included patients with colorectal ($n = 6$), lung ($n = 2$), and others ($n = 8$).

Pharmacokinetic studies were performed in 16 patients. Samples from the first five patients were inadequate for analysis due to a storage malfunction. One patient had pharmacokinetic studies performed after administration of 9NC with food and then withdrew from the study prior to the crossover to administration of 9NC without food. One patient only had blood samples obtained from 0 to 6 h after administration of 9NC with and without food and thus an AUC from 0 to 24 h could not be determined. In two patients administered 9NC with food, AUC from 0 to 24 h could not be estimated due to a limited number of samples from 1 to 6 h after administration. Thus, paired pharmacokinetic data after administration of 9NC with food and under fasting conditions were available in nine patients with only two additional patients only having data after administration of 9NC under fasting conditions.

Table 1 Patient characteristics

Patient characteristics	Number of patients
Patients enrolled	16
Males	5
Females	11
Age: median (range), years	62 (29–85)
ECOG performance status	
1	15
2	1
# Prior chemotherapy regimens	
1	2
2	3
3	8
4	3
Primary site	
Colorectal	6
NSCLC	2
Others*	8
Evaluable for PK	12

*Other sites (8): pancreas (1), esophageal (1), ovarian (1), cervix (1), endometrial (1), adenocarcinoma of unknown primary (1), thyroid (1) and head and neck (1)

ECOG Eastern Cooperative Oncology Group; # number; *PK* pharmacokinetics

Mass spectrometric assay

The sample preparation described resulted in >90% recovery of 9NC and 9AC when compared to the direct injection of an equivalent amount of 9NC or 9AC in mobile phase. When stored at −80°C the percentage change in measured concentration of 9NC and 9AC total from baseline was < 10% at 2 months. The stability of 9NC and 9AC total on the autosampler at 24 h was >90%. The assay for 9NC and 9AC total in plasma was linear from 0.5 to 1,000 ng/ml. The correlation coefficients for three successive 9NC and 9AC triplicate standard curves in plasma were >0.99. When expressed as a percentage coefficient of variation, the within-day and between-day variation in 9NC and 9AC triplicate standards was always <15%.

Table 2 9-Nitrocamptothecin and 9AC pharmacokinetic parameters after administration of 9NC with food and under fasting conditions

Parameters	Units	Food mean ± SD ($n=9$)[b]	Fasting mean ± SD ($n=9$)[b]
9NC			
k_a	h^{-1}	0.12 ± 0.11[a]	1.26 ± 1.18[a]
τ	h	0.26 ± 0.31	0.16 ± 0.28
k_{12}	h^{-1}	0.21 ± 0.17	0.12 ± 0.18
k_{10}	h^{-1}	0.15 ± 0.19	0.29 ± 0.55
Vd	l	17.0 ± 12.0	27.4 ± 25.2
CL/F	$l/h/m^2$	1.4 ± 1.8	1.7 ± 1.4
C_{max}	ng/ml	26.1 ± 13.5[a]	52.9 ± 27.7[a]
T_{max}	h	5.3 ± 1.5[a]	3.8 ± 3.1[a]
$AUC_{0\text{-}24}$	ng/ml h	330 ± 182[a]	557 ± 379[a]
9AC			
k_{20}	h^{-1}	0.18 ± 0.22	0.11 ± 0.11
CL/F	$l/h/m^2$	2.2 ± 1.7	2.7 ± 2.7
C_{max}	ng/mL	15.9 ± 4.4	17.5 ± 11.5
T_{max}	h	12.0 ± 5.2	12.7 ± 7.0
$AUC_{0\text{-}24}$	ng/ml h	244 ± 60	256 ± 102

[a]Values for 9NC administered under fasting conditions are significantly different ($P < 0.05$) than for 9NC administered with food

[b]This table only includes paired pharmacokinetic data from nine patients after administration of 9NC with food and under fasting conditions

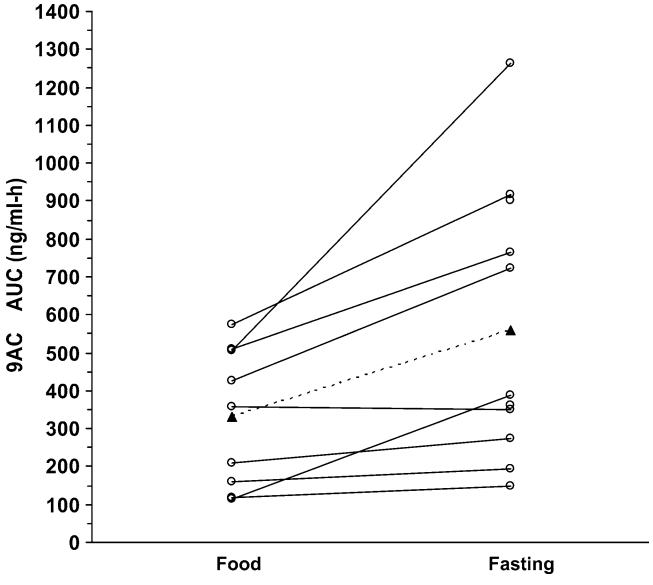

Fig. 1 9-Nitrocamptothecin AUCs in individual patients when 9NC was administered with food ($n=9$) and under fasting conditions ($n=11$). Individual data points are presented by (*open circle*) and data within a single patient are connected by a *solid line*. The mean value presented for each group is for the paired data only is represented by (*filled triangle*) and connected by a *dashed line*

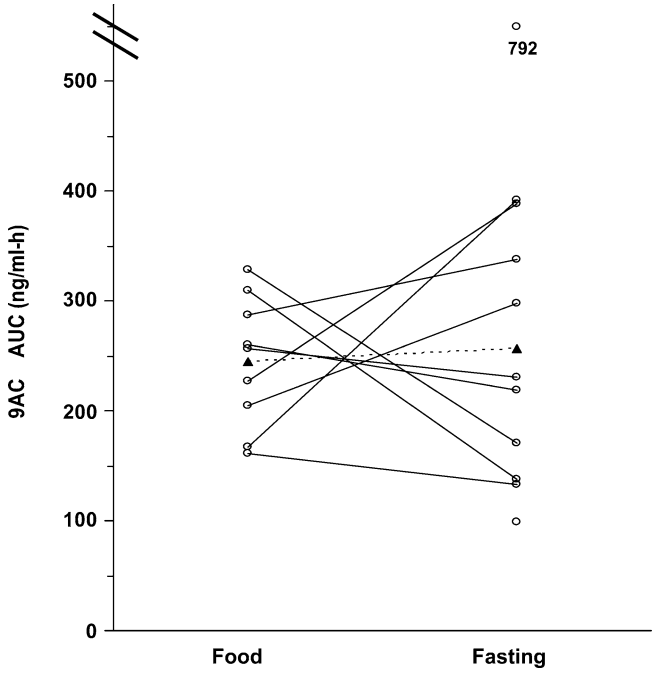

Fig. 2 9-Aminocamptothecin AUCs in individual patients when 9NC was administered with food ($n=9$) and under fasting conditions ($n=11$). Individual data points are presented by (*open circle*) and data within a single patient are connected by a *solid line*. The mean value presented for each group is for the paired data only is represented by (*filled triangle*) and connected by a *dashed line*

Pharmacokinetics

Paired pharmacokinetic data after administration of 9NC with food and under fasting conditions were available in nine patients. Pharmacokinetic data was available on two additional patients, only after administration of 9NC under fasting conditions. The 9NC and 9AC AUC in the first patient was 900 and 792 ng/ml h, respectively. The 9NC and 9AC AUC in the second patient was 346 and 99 ng/ml h, respectively. All further pharmacokinetic summaries will only include data for the nine patients with paired pharmacokinetic studies of 9NC with food and under fasting conditions. 9NC and 9AC pharmacokinetic parameters after administration of 9NC with a standard meal and under fasting conditions are presented in Table 2. 9NC and 9AC AUCs after administration of 9NC with food and under fasting conditions are presented in Figs. 1, 2 respectively. The ratio of 9AC AUC to 9NC AUC after administration of 9NC with food and under fasting conditions is presented in Fig. 3.

The mean ± SD of the ratio of 9NC AUC_{food} to 9NC $AUC_{fasting}$ in individual patients was 0.67 ± 0.22. The mean ± SD of the ratio of 9AC AUC_{food} to 9AC $AUC_{fasting}$ in individual patients was 1.14 ± 0.61. The mean ± SD of the ratio of 9AC $AUC_{food}/AUC_{fasting}$ to 9NC $AUC_{food}/AUC_{fasting}$ in individual patients was 1.80 ± 0.99.

Table 3 Non-hematologic toxicities (by number of patients)

Toxicity (CTC)	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	5	1	2	
Vomiting	2		2	
Diarrhea	1	3	2	
Fatigue	5	2	2	
Anorexia	3		1	
Mucositis		1		
Dysuria		2		
Proteinuria	4			
Hematuria	4	3		
UTI		2	1	
Skin rash		2		

UTI Urinary tract infection; *CTC* common toxicity criteria version 2.0

Toxicities

Overall, non-hematologic toxicity was more common than hematologic toxicity (Table 3). The most common non-hematologic toxicity observed was nausea, which occurred in eight patients. Other grade 2 toxicities included vomiting, diarrhea, fatigue, urinary infection (UTI), and anorexia. Skin rash was observed in two patients and resulted in an interruption of therapy for a week until the spontaneous resolution of the rash. This was a diffuse, macular–papular rash, more prominent on the trunk and the back, associated with itching, and resolved within 1–2 weeks, and did not reappear with the reintroduction of the drug at a lower dose. Also observed were renal changes as evidenced by hematuria, proteinurea, dysuria and UTI. One patient, a 64-year woman with metastatic colon cancer, had grade 3 diabetic nephropathy-induced proteinurea at baseline, which did not appear to worsen during administration of 9NC.

Grades 3–4 neutropenia was observed in three patients (Table 4). Two patients experienced febrile neutropenia. Both patients recovered with appropriate antibiotics and without any sequelae. The median (interquartile range) of the nadir neutrophil count was 2,400/mm^3 (1,200–3,600/mm^3). One patient experienced grade 2 thrombocytopenia. Grade 3 anemia, requiring transfusion, occurred in three patients. One of these patients was a patient with metastatic colon cancer that developed episodes of gastrointestinal bleeding which contributed to the anemia.

Table 4 Hematologic toxicities (by number of patients)

Toxicity (CTC)	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia	2	1	1	2
Febrile neutropenia			2	
Anemia	3	6	3	
Thrombocytopenia	3	1		

CTC Common toxicity criteria version 2.0

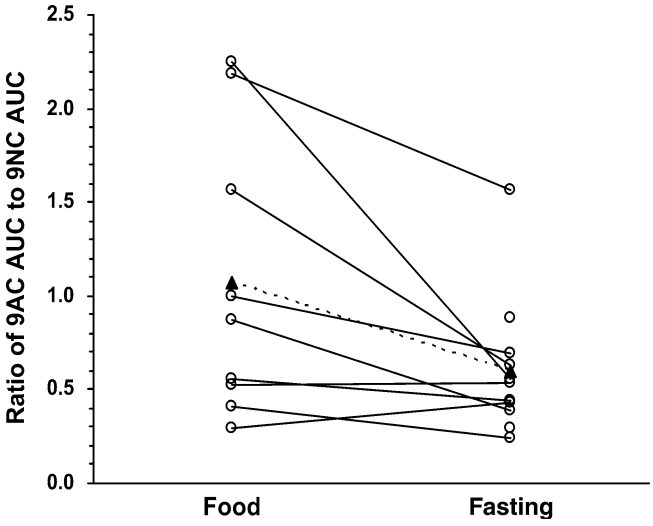

Fig. 3 The ratio of 9AC AUC to 9NC AUC patients when 9NC was administered with food ($n=9$) and under fasting conditions ($n=11$). Individual data points are presented by (*open circle*) and data within a single patient are connected by a *solid line*. The mean value presented for each group is for the paired data only is represented by (*filled triangle*) and connected by a *dashed line*

Antitumor response

Although response evaluation was not the primary end point of this study, 13 patients were evaluable for objective response. Of the three patients not evaluable for response, one was withdrawn from study after only 4 weeks of therapy because of hematuria, and two patients withdrew consent and did not complete the planned 8 weeks of therapy. There was no objective tumor response observed. Three patients (lung, unknown primary, and colon) had stable disease for a period of 8 weeks.

Discussion

Previous studies evaluated the oral bioavailability of camptothecin analogues administered with and without food [13, 22–24, 31]. Schoffski et al. [43] reported that the oral bioavailability of 9NC was found to be strongly dependent on the timing of food intake. The plasma exposure of 9NC was approximately half when administered with food as compared to fasting conditions. However, that study did not evaluate the disposition of 9AC, the major metabolite of 9NC, which ranged from 20 to 200% of the 9NC exposure in patients [10, 13, 33, 34]. Thus, our study is the first report evaluating the disposition of 9NC and 9AC when administered with and without food. In our study, food inhibited the oral absorption of 9NC with a mean ± SD ratio of 9NC AUC_{food} to 9NC $AUC_{fasting}$ of 0.67 ± 0.22. Administration of 9NC with food did not alter the exposure of 9AC with a mean ± SD ratio of 9AC AUC_{food} to 9AC $AUC_{fasting}$ of 1.14 ± 0.61. However, the effect of food on the absorption of 9NC is highly variable and is less

consistent for 9AC compared to 9NC. The mechanism associated with differential effects of food on the exposure of 9NC and 9AC are currently unclear. One potential explanation is that 9NC is converted to 9AC in the gastrointestinal tract in addition to the liver and food does not alter the absorption of 9AC [10, 11, 34]. The differential effects of food on 9NC and 9AC is significant because the development of 9AC was stopped due to lack of efficacy and significant toxicity in clinical trials [21, 36, 50]. Thus, to maximize the exposure of 9NC relative to 9AC, 9NC should be administered orally without food [38].

The results from our study are similar to previous studies evaluating the pharmacokinetics of 9NC when administered under fasting conditions [10, 14, 34, 38, 42, 43]. For example, the k_a in our study and the study by Schoemaker et al. were 1.26 ± 1.18 h^{-1} and 0.8 h^{-1}, respectively [42]. In the study by Raymond et al. [38] in which 9NC was administered at 1.5 mg/m^2 ×1, the 9NC and 9AC $AUC_{0-24\ h}$ were 231 ± 137 and 36.9 ± 28.5 ng/ml h, respectively, which are similar to the 9NC and 9AC $AUC_{0-24\ h}$ in this current study and our prior phase I study after administration of 9NC at 2.0 mg/m^2. In addition, the concentration versus time profiles of 9NC and 9AC were representative of delayed absorption, and the 9AC concentrations increase over the 24 h period.

9-Nitrocamptothecin is an orally administered camptothecin analogue with significant interpatient variability in drug disposition [14, 33, 34, 38, 42]. The interpatient variability in 9NC was similar when administered with (8.0-fold) and without food (6.2-fold). In addition, the interpatient variability in 9AC was similar when administered with (3.9-fold) and without (6.4-fold) food. Thus, there is still a need to identify factors responsible for the variability and develop techniques to reduce the interpatient variability in the exposure of 9NC and 9AC [20, 23, 33, 43]. The interpatient variability in the exposure of 9NC and 9AC may be explained by variability in the expression and function of the ATP-binding cassette (ABC) transporters [3, 18, 23, 26, 30, 37, 46]. The ABC transporters are membrane proteins that modulate a wide variety of substrates, including metabolic products, lipids and sterols, and drugs, across extra-and intracellular membranes, such as the gastrointestinal tract [25, 45]. ABC transporters have been reported to modulate camptothecin analogues and are associated with camptothecin resistance in cancer cell lines [3, 18, 26, 30, 37, 46]. Fumitremorgin C, a potent and specific inhibitor of ABCG2, has been evaluated as a way to increase the absolute oral bioavailability and reduce interpatient variability in oral absorption of camptothecins analogues [2]. Further studies evaluating the effects of fumitremorgin C on 9NC should be performed.

Acknowledgments This study is supported by a grant from Supergen, Inc. SM is supported by a clinical investigator award from the Damon Runyon Cancer Research Foundation (CI: 15–02), and Institutional Research Grant from the American Cancer Society (ACS IRG 9824701).

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