Relationship between Plasma Exposure of 9-Nitrocamptothecin and Its 9-Aminocamptothecin Metabolite and Antitumor Response in Mice Bearing Human Colon Carcinoma Xenografts

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9-Nitrocamptothecin has completed phase III studies in patients with newly diagnosed and refractory pancreatic cancer; however, the optimal 9-nitrocamptothecin treatment regimen is unclear. We used an intermittent schedule of 9-nitrocamptothecin to evaluate the relationship between plasma exposure of 9-nitrocamptothecin and its 9-aminocamptothecin metabolite and antitumor response in mice bearing human colon carcinoma xenografts. 9-Nitrocamptothecin was given orally at 0.44, 0.67, or 1.0 mg/kg qd × 5d × 2 weeks repeated q 4 weeks for two cycles to female C.B-17 SCID mice bearing HT29 or ELC2 human colon xenografts. Pharmacokinetic studies were done after oral administration of 0.67 mg/kg × 1. Serial samples were obtained and 9-nitrocamptothecin and 9-aminocamptothecin lactone concentrations in plasma were determined by high-performance liquid chromatography analysis with fluorescence detection. The areas under plasma concentration versus time curve (AUC) from 0 to infinity for 9-nitrocamptothecin and 9-aminocamptothecin were calculated. The antitumor activity of 9-nitrocamptothecin was dose-dependent in both colon xenografts. At all doses, 9-nitrocamptothecin treatment resulted in significant antitumor activity in both xenografts compared with vehicle-treated and control groups and achieved levels of tumor regression that met criteria (minimum %T/C ≤ 40%) for antitumor activity. In mice bearing HT29 xenografts, the 9-nitrocamptothecin and 9-aminocamptothecin lactone AUCs after administration of 9-nitrocamptothecin at 0.67 mg/kg were 41.3 and 5.7 ng/mL h, respectively. The responses seen in these xenograft models occurred at systemic exposures that are tolerable in adult patients. These results suggest that the intermittent schedule of 9-nitrocamptothecin may be an active regimen in patients with colorectal carcinoma.

Abstract

9-Nitrocamptothecin has completed phase III studies in patients with newly diagnosed and refractory pancreatic cancer; however, the optimal 9-nitrocamptothecin treatment regimen is unclear. We used an intermittent schedule of 9-nitrocamptothecin to evaluate the relationship between plasma exposure of 9-nitrocamptothecin and its 9-aminocamptothecin metabolite and antitumor response in mice bearing human colon carcinoma xenografts. 9-Nitrocamptothecin was given orally at 0.44, 0.67, or 1.0 mg/kg qd × 5d × 2 weeks repeated q 4 weeks for two cycles to female C.B-17 SCID mice bearing HT29 or ELC2 human colon xenografts. Pharmacokinetic studies were done after oral administration of 0.67 mg/kg × 1. Serial samples were obtained and 9-nitrocamptothecin and 9-aminocamptothecin lactone concentrations in plasma were determined by high-performance liquid chromatography analysis with fluorescence detection. The areas under plasma concentration versus time curve (AUC) from 0 to infinity for 9-nitrocamptothecin and 9-aminocamptothecin were calculated. The antitumor activity of 9-nitrocamptothecin was dose-dependent in both colon xenografts. At all doses, 9-nitrocamptothecin treatment resulted in significant antitumor activity in both xenografts compared with vehicle-treated and control groups and achieved levels of tumor regression that met criteria (minimum %T/C ≤ 40%) for antitumor activity. In mice bearing HT29 xenografts, the 9-nitrocamptothecin and 9-aminocamptothecin lactone AUCs after administration of 9-nitrocamptothecin at 0.67 mg/kg were 41.3 and 5.7 ng/mL h, respectively. The responses seen in these xenograft models occurred at systemic exposures that are tolerable in adult patients. These results suggest that the intermittent schedule of 9-nitrocamptothecin may be an active regimen in patients with colorectal carcinoma.

9-Nitrocamptothecin (rubitecan, RFS2000) has completed phase III studies in patients with newly diagnosed and refractory pancreatic cancer. However, the optimal dose, schedule, and route of administration of 9-nitrocamptothecin and other camptothecin analogues, such as topotecan and irinotecan, are currently unclear (1–5). In vitro and in vivo preclinical studies suggest that protracted administration of low doses of camptothecin analogues achieve better antitumor activity than does less frequent administration of higher doses (6–9). Repeated oral administration of 9-nitrocamptothecin could mimic the protracted schedule, maximize patient convenience, and minimize the use of health care resources. However, oral administration of camptothecin analogues has been associated with extensive interpatient and intrapatient variability in bioavailability (10–13). In addition, the antitumor activity associated with camptothecin analogues, such as topotecan and irinotecan, may require a dose that achieves a systemic exposure above an exposure threshold (8, 9, 14, 15). Thus, continuous and prolonged administration of low doses of camptothecins, which achieve systemic exposures below this threshold, may not produce an antitumor response. Thus, the highly schedule-dependent antitumor activity, steep relationship between systemic exposure and antitumor activity, and pharmacokinetic variability associated with oral absorption of camptothecin analogues, require studies evaluating the relationship between drug exposure and antitumor effect in preclinical and clinical studies. This process has been used in the development of topotecan, irinotecan, and 9-aminocamptothecin (6, 9, 16).

9-Nitrocamptothecin is given orally and is partially metabolized to an active metabolite, 9-aminocamptothecin (5, 13, 17, 18). As with other camptothecin analogues, 9-nitrocamptothecin and 9-aminocamptothecin undergo a reversible, pH-dependent hydrolysis between the active lactone and inactive hydroxyl acid forms (19). At acidic pH, the lactone form predominates, and at physiologic pH, the hydroxyl acid form predominates (19–21). However, changes in plasma pH, serum-albumin concentration, and route of administration

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may affect the conversion between the lactone and hydroxyl acid forms (20, 21). Thus, measurement of the active lactone form of the drug may be basic to understanding the clinical pharmacology of 9-nitrocamptothecin and other camptothecin analogues (19–23). Due to the differences in oral absorption, elimination, and percentage of 9-nitrocamptothecin and 9-amino camptothecin lactone in mice and humans, comparing the dose or total (sum of lactone plus hydroxyl acid) exposure that produces antitumor response in mice and humans would not be appropriate. We evaluated the relationship between plasma exposure of 9-nitrocamptothecin and 9-amino camptothecin lactone and antitumor response in mice bearing human colon carcinoma xenografts. In addition, we compared the 9-nitrocamptothecin and 9-amino camptothecin systemic exposures associated with antitumor response in the xenograft models to those reported in a phase I study of 9-nitrocamptothecin that used the same intermittent schedule as in the xenograft studies and used allometric scaling analysis to normalize the data (1, 24).

Materials and Methods

Mice. This study was approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh, and all animals were handled in accordance with the Guide to the Care and Use of Laboratory Animals (National Research Council, 1996). Mice (female C.B-17 SCID, 4-6 weeks of age, specific pathogen free), were obtained from the National Cancer Institute Animal Production Program (Frederick, MD) and were allowed to acclimate to the University of Pittsburgh Central Animal Facility for 1 week before initiation of study. Mice were housed in autoclaved micro isolator cages and were allowed Prolab ISOPRO RMH 3000 Irradiated Lab Diet (PMI Nutrition International, Brentwood, MO) and water ad libitum. Animal rooms were maintained at 22 ± 2°C on a 12-hour light and dark cycle with at least 12 air changes per hour. Tri-monthly analysis of sentinel mice (Assessment Plus, Charles River, Boston, MA) housed in 1 of 5 dirty bedding confirmed that the study mice remained murine antibody profile negative throughout the study.

Tumor lines, implantation, and measurements. HT29 and EL2 human colon xenografts were obtained from the National Cancer Institute Tumor Repository (Frederick, MD) and Dr. Janet Houghton at St. Jude Children’s Research Hospital (Memphis, TN), respectively, and were murine antibody profile test negative. HT29 and EL2 cells were passaged in C.B-17 SCID female mice. When the tumors reached 500 to 1,000 mm³ (500-1,000 mg), the tumors were harvested and ~25 mg fragments were implanted s.c. on the right flank of study mice using aseptic techniques. Mice were observed twice daily. Body weights and tumor measurements were recorded twice weekly. Tumor volumes were calculated from the formula: length × (width)² / 2, where length is the longest diameter and width is the shortest diameter perpendicular to the length (24, 25). Median days to one and two doublings of tumor volume and median optimal %T/C {ratio of median tumor volume for treatment group (T) to median tumor volume for control group (C)} × 100] were calculated (25).

Efficacy studies. Efficacy studies were done in female C.B-17 SCID mice bearing HT29 or EL2 human colon carcinoma xenografts. Mice bearing HT29 xenografts were stratified on day 19 post-implantation when the tumor volumes were 26 to 71 mm³. Mice bearing EL2 xenografts were stratified on day 17 post-implantation when the tumor volumes were 68 to 195 mm³. Mice were stratified into groups of 10 such that the mean body weight and tumor volumes for the groups were not statistically different. The stratification was done 3 days before treatment (day –3). 9-Nitrocamptothecin, obtained from Supergen (Dublin, CA), was prepared in 1 mmol/L phosphoric acid/polyethylene glycol 400/29% N,N-dimethylacetamide (48:50:2, v/v/v). 9-Nitrocamptothecin concentrations of 0.1, 0.067, or 0.044 mg/mL were prepared for the 1, 0.67, and 0.44 mg/kg/d doses, respectively. 9-Nitrocamptothecin dosing solutions or vehicle were given at 0.01 mL/g body weight using a curved, 20-gauge, oral gavage needle attached to a 1-mL syringe. Mice (n = 10 per group) bearing HT29 xenografts were treated with 9-nitrocamptothecin daily for 5 days per week for two consecutive weeks repeated every 4 weeks for a total of two cycles. To serve as a positive control, 5-fluorouracil was prepared at 2 mg/mL in 0.9% NaCl and given at 0.01 mL/g body weight (26). Mice (n = 10 per group) bearing HT29 tumors were treated with 5-fluorouracil i.p. at 20 mg/kg/d for 5 days per week for two consecutive weeks repeated every 4 weeks for a total of two cycles (26). For mice bearing HT29 xenografts, treatment days during cycle 1 were days 0 to 4 and days 7 to 11, and treatment days during cycle 2 were days 28 to 32 and days 35 to 39. At the completion of the study, mice were euthanized by CO₂ inhalation, and complete necropsies were done.

Mice (n = 10 per group) bearing EL2 xenografts were treated with 9-nitrocamptothecin at the same doses and on the same schedules as mice bearing HT29 xenografts; however, the mice bearing EL2 tumors received only the first 5 days of the treatment of the second cycle. The study was terminated early because the tumor volumes in the control and vehicle-treated groups reached 2,000 m³, and the mice in these groups had significant (>10%) loss of body weight. Treatment days during cycle 1 were days 0 to 4 and days 7 to 11, and treatment days during cycle 2 were days 28 to 32. At the completion of the study, mice were euthanized by CO₂ inhalation, and complete necropsies were done.

Pharmacokinetic studies. Plasma pharmacokinetic studies were done in C.B-17 SCID mice bearing HT29 xenografts at 27 days post-tumor implantation and in non-tumor-bearing mice. Mice were stratified into three mice per group such that the mean body weight and tumor volumes were not statistically different (26).

Animals were fasted overnight before dosing and 9-nitrocamptothecin was given at 0.67 mg/kg as a single oral dose. After dosing, mice (n = 3 per time point) were euthanized by CO₂ inhalation, and blood (–1 mL) was collected, by cardiac puncture using heparinized syringes at 0.08, 0.25, 0.5, 1, 1.5, 2, 4, 6, 7, 18, 24, and 48 hours after 9-nitrocamptothecin administration. In addition, vehicle-treated mice were euthanized at 5 minutes after administration using the same procedure as stated above. Blood was transferred to microcentrifuge tubes and immediately placed on ice until centrifuged.

Sample preparation. Plasma was prepared by centrifuging blood samples at 13,000 × g at room temperature for 4 minutes. For analysis of 9-nitrocamptothecin lactone, solid-phase extraction was used to separate the lactone and hydroxyl acid forms of 9-nitrocamptothecin and 9-amino camptothecin (13). Waters OASIS HLB columns (1 mL, 30 mg; Milford, MA) were used for the solid-phase extraction. The columns were conditioned with 1 mL of methanol and equilibrated with 1 mL of water before loading plasma samples (0.2 mL) on the columns. The columns were then washed with 1 mL of methanol/water (5:95, v/v) to remove the hydroxyl acid forms of 9-nitrocamptothecin and 9-amino camptothecin. 9-Nitrocamptothecin lactone was then eluted with 0.5 mL of methanol and stored at −80°C until analyzed. For analysis of 9-amino camptothecin lactone, plasma was processed using methanol extraction as previously described and stored at −80°C until analyzed (13).

High-performance liquid chromatography analysis. The concentrations of 9-nitrocamptothecin and 9-amino camptothecin lactone in plasma were quantitated using a high-performance liquid chromatography assay with fluorescence detection modified from that described in our previous pharmacokinetic studies in humans (13). Because 9-nitrocamptothecin is not highly fluorescent and 9-amino camptothecin is, 9-nitrocamptothecin lactone was measured by chemically reducing 9-nitrocamptothecin to 9-aminocamptothecin (13, 28, 29). The concentration of 9-nitrocamptothecin was calculated by subtracting the concentration of 9-amino camptothecin from the
concentration of 9-nitrocamptothecin plus 9-aminocamptothecin after the conversion of 9-nitrocamptothecin to 9-aminocamptothecin (13, 29). The lower limit of quantitation for 9-nitrocamptothecin lactone was 0.5 ng/mL, and the assay was linear from 0.5 to 100 ng/mL. The lower limit of quantitation for 9-aminocamptothecin lactone was 0.3 ng/mL, and the assay was linear from 0.3 to 100 ng/mL. When expressed as a percentage coefficient of variation, the within-day and between-day variation in 9-nitrocamptothecin and 9-aminocamptothecin lactone triplicate standards in plasma were <15%.

Pharmacokinetic analysis. Compartmental pharmacokinetic methods were used to analyze the 9-nitrocamptothecin and 9-aminocamptothecin lactone plasma concentration versus time data from mice bearing HT29 human colon tumors and non–tumor-bearing mice. Compartmental modeling was done with the ADAPT II computer program (30). 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 9-nitrocamptothecin and 9-aminocamptothecin in plasma. In the model development, one- and two-compartment models were evaluated to describe the systemic disposition of 9-nitrocamptothecin and 9-aminocamptothecin. Akaike’s Information Criteria, Schwartz Criteria, estimated error of the model variables, and residual analysis were used to select the model structure that maximized the fit accuracy and simultaneously minimized the number of model variables. The final model structure used for the pharmacokinetic analysis produced identifiable variables in both groups.

A four-compartment model with an oral absorption compartment was simultaneously fit to the mean plasma concentration versus time profiles of 9-nitrocamptothecin and 9-aminocamptothecin (30). Individual variables estimated by the model included the rate constant describing oral absorption ($k_a$), intercompartmental rate constants for 9-nitrocamptothecin ($k_{12}$ and $k_{23}$) and 9-aminocamptothecin ($k_{14}$ and $k_{34}$), the rate constant describing conversion of 9-nitrocamptothecin to 9-aminocamptothecin ($k_{13}$), the elimination rate constants for 9-nitrocamptothecin ($k_{20}$) and 9-aminocamptothecin ($k_{30}$), and the apparent volume of the central compartments for 9-nitrocamptothecin ($Vc1/F$) and 9-aminocamptothecin ($Vc3/F$). The apparent clearance ($CL/F$) and half-life ($t_{1/2}$) of 9-nitrocamptothecin and 9-aminocamptothecin lactone were calculated using standard equations (31). The areas under the 9-nitrocamptothecin and 9-aminocamptothecin lactone plasma concentration versus time curves (AUC) from 0 to infinity were calculated using the log trapezoidal method by simulating the concentration versus time data based upon model-specific variables (30).

Allometric scaling. Allometric scaling was used to compare the disposition of 9-nitrocamptothecin and 9-aminocamptothecin lactone in patients with refractory solid tumors and in mice bearing HT29 human colon xenografts (1, 24). The 9-nitrocamptothecin and 9-aminocamptothecin lactone concentration versus time data in patients was from a phase I study of orally given 9-nitrocamptothecin daily for five consecutive days per week for 2 weeks repeated every 4 weeks (1). Pharmacokinetic studies were done on day 1 after a single dose of 9-nitrocamptothecin at 2.4 mg/m$^2$ ($n$ = 9), which was the maximum tolerated dose. The mean 9-nitrocamptothecin and 9-aminocamptothecin lactone concentration at each time point was used in the allometric calculations.

Allometric scaling is based on the power-law relationship between physiologic and pharmacokinetic processes and body weight among mammals (24). Complex Dedrick Plots were used to normalize the concentration versus time profiles in patients and mice (24). Interspecies concentrations and time were normalized by dividing the plasma concentration (ng/mL) by the dose (mg/kg) and by dividing the time (hours) by body weight$^{0.25}$ (kg). The 9-nitrocamptothecin and 9-aminocamptothecin lactone AUCs from 0 to last measured concentration-time point for the Complex Dedrick Plots were calculated using the log trapezoidal method (31).

Statistics. Body weights and tumor volumes from both of the efficacy studies were analyzed for statistical significance using the statistical software package Minitab (Minitab, Inc., State College, PA; ref. 27). Values were expressed as the mean ± SD and median. Mean data were analyzed by one-way ANOVA followed by pairwise comparisons using Dunnett’s test. Median data were analyzed using Kruskal-Wallis followed by pairwise comparisons by Mann-Whitney test (27). The a priori level of significance was set at $P = 0.05$.

Results

Efficacy and toxicity. Tumor growth curves for HT29 and ELC2 xenografts are presented in Figs. 1 and 2, respectively.
Days to 1 and 2 doublings of tumor volume and median optimal %T/C for HT29 and ELC2 xenografts are presented in Tables 1 and 2, respectively. 9-Nitrocamptothecin treatment resulted in significant antitumor activity in both human colon xenografts as compared with the vehicle-treated and control groups (*P* < 0.05; ref. 25). All doses of 9-nitrocamptothecin in both xenograft lines achieved levels of activity that met criteria for antitumor activity (minimum %T/C ≥ 40%; ref. 25).

The antitumor activity of 9-nitrocamptothecin in both xenografts was dose dependent (Figs. 1 and 2). In mice bearing HT29 xenografts, the days to one and two tumor doublings were significantly longer after administration of 1 mg/kg compared with 0.67 and 0.44 mg/kg (*P* < 0.05). For HT29 xenografts, the median optimal % T/C after administration of 1, 0.67, and 0.44 mg/kg were 9.8%, 23.0%, and 34.7%, respectively, as measured on day 43. In mice bearing ELC2 xenografts, the days to one and two tumor doublings were significantly longer after administration of 1 and 0.67 mg/kg compared with 0.44 mg/kg (*P* < 0.05). For ELC2 xenografts, the median optimal % T/C after administration of 1, 0.67, and 0.44 mg/kg were 18.4%, 21.8%, and 31.1%, respectively, as measured on day 35.

The primary toxicity associated with 9-nitrocamptothecin in mice bearing HT29 and ELC2 human colon xenografts was soft stools and diarrhea. After administration of 9-nitrocamptothecin at 1 mg/kg in mice bearing HT29 and ELC2 xenografts, all mice had soft stools by day 3 of treatment and developed diarrhea by day 7 of treatment. After administration of 9-nitrocamptothecin at 0.67 mg/kg in mice bearing HT29 and ELC2 xenografts, mice had soft stools by day 7 and developed

### Table 1. Doubling times for tumor volume and median optimal % T/C for HT29 human colon xenografts.

<table>
<thead>
<tr>
<th>Treatment groups (n = 10)</th>
<th>Days to 1 doubling of tumor volume, mean ± SD (median)</th>
<th>Days to 2 doublings of tumor volume, mean ± SD (median)</th>
<th>Median optimal % T/C (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.3 ± 2.0 (5.0)</td>
<td>22.5 ± 7.3 (21.0)</td>
<td>—</td>
</tr>
<tr>
<td>5-FU (20 mg/kg)</td>
<td>8.8 ± 2.9 (10.0)</td>
<td>33.3 ± 14.0 (34.0)</td>
<td>44.7 (43)</td>
</tr>
<tr>
<td>9NC (1 mg/kg)</td>
<td>48.7 ± 12.0* (52.5)*</td>
<td>58.7 ± 6.1* (59.7)*</td>
<td>9.8 (43)</td>
</tr>
<tr>
<td>9NC (0.67 mg/kg)</td>
<td>28.9 ± 8.7* (29.0)*</td>
<td>46.9 ± 5.6* (47.9)*</td>
<td>23.0 (43)</td>
</tr>
<tr>
<td>9NC (0.44 mg/kg)</td>
<td>25.5 ± 10.3* (26.6)*</td>
<td>42.0 ± 6.9* (41.5)*</td>
<td>34.7 (43)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6.9 ± 2.3 (6.5)</td>
<td>30.9 ± 6.7 (30.1)</td>
<td>73.1 (43)</td>
</tr>
</tbody>
</table>

Abbreviations: 5-FU, 5-fluorouracil; 9NC, 9-nitrocamptothecin; T, median tumor volume in treated group; C, median tumor volume in control group.

*Significantly different compared with control and vehicle at *P* ≤ 0.05 as determined by Dunnett’s Test.
†Significantly different compared with 0.67 and 0.44 mg/kg at *P* ≤ 0.05 as determined by Dunnett’s Test.
‡Significantly different compared with control and vehicle at *P* ≤ 0.05 as determined by Mann-Whitney Test.
§Significantly different compared with 0.67 and 0.44 mg/kg at *P* ≤ 0.05 as determined by Mann-Whitney Test.
diarrhea by days 9 to 10. After administration of 9-nitrocamptothecin at 0.44 mg/kg and vehicle in mice bearing both tumor xenografts, soft stools developed during the second week of treatment. During the 2-week break in treatment (days 13-28), stools became more solid. The pattern of soft stools and diarrhea was similar during cycles 1 and 2.

Pharmacokinetic studies. Pharmacokinetic studies of 9-nitrocamptothecin and 9-aminocamptothecin lactone were done in non–tumor-bearing mice and mice bearing HT29 xenografts after administration of a single dose of 9-nitrocamptothecin at 0.67 mg/kg. The 9-nitrocamptothecin and 9-aminocamptothecin lactone concentration versus time profiles in mice bearing HT29 xenografts are presented in Fig. 3. In mice bearing HT29 xenografts, peak plasma concentrations of 9-nitrocamptothecin and 9-aminocamptothecin were detected at 15 minutes after administration. The mean ± SD peak plasma concentrations of 9-nitrocamptothecin and 9-aminocamptothecin lactone were 45.4 ± 8.1 and 4.2 ± 1.4 ng/mL, respectively. Pharmacokinetic variables of 9-nitrocamptothecin and 9-aminocamptothecin in mice bearing HT29 xenografts are summarized in Table 3. The 9-nitrocamptothecin and 9-aminocamptothecin lactone AUCs were 41.3 and 5.7 ng/mL/h, respectively.

The 9-nitrocamptothecin and 9-aminocamptothecin lactone concentration versus time profiles in non–tumor-bearing mice were similar to those in mice bearing HT29 colon xenografts and are presented in Fig. 3. In non–tumor-bearing mice, peak plasma concentrations of 9-nitrocamptothecin and 9-aminocamptothecin lactone were detected at 15 minutes after administration. The mean ± SD peak plasma concentrations of 9-nitrocamptothecin and 9-aminocamptothecin lactone were 46.8 ± 27.6 and 3.3 ± 1.8 ng/mL, respectively. Pharmacokinetic variables of 9-nitrocamptothecin and 9-aminocamptothecin in non–tumor-bearing mice are summarized in Table 3. The 9-nitrocamptothecin and 9-aminocamptothecin lactone AUCs were 40.7 and 5.2 ng/mL/h, respectively.

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Table 2. Doubling times for tumor volume and median optimal % T/C for ELC2 human colon xenografts.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Days to 1 doubling of tumor volume, mean ± SD (median)</th>
<th>Days to 2 doublings of tumor volume, mean ± SD (median)</th>
<th>Median optimal % T/C (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.6 ± 3.7 (10.5)</td>
<td>24.1 ± 6.6 (26.0)</td>
<td>—</td>
</tr>
<tr>
<td>9NC (1 mg/kg)</td>
<td>35.0 ± 1.3 (35.0)</td>
<td>35.0 ± 1.3 (35.0)</td>
<td>18.4 (35)</td>
</tr>
<tr>
<td>9NC (0.67 mg/kg)</td>
<td>34.2 ± 2.5 (35.0)</td>
<td>35.0 ± 2.1 (35.0)</td>
<td>21.8 (35)</td>
</tr>
<tr>
<td>9NC (0.44 mg/kg)</td>
<td>25.7 ± 8.9 (26.9)</td>
<td>34.8 ± 4.2 (35.0)</td>
<td>31.1 (35)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>12.2 ± 5.6 (13.3)</td>
<td>24.2 ± 5.7 (25.1)</td>
<td>111.8 (35)</td>
</tr>
</tbody>
</table>

Abbreviations: 9NC, 9-nitrocamptothecin; T, median tumor volume in treated group; C, median tumor volume in control group.

*Significantly different compared with control and vehicle at P = 0.05 as determined by Dunnett’s Test.

†Significantly different compared with 0.44 mg/kg at P = 0.05 as determined by Dunnett’s Test.

‡Significantly different compared with control and vehicle at P = 0.05 as determined by Mann-Whitney Test.

§Significantly different compared with 0.44 mg/kg at P = 0.05 as determined by Mann-Whitney Test.

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Fig. 3. 9-Nitrocamptothecin (9NC) and 9-aminocamptothecin (9AC) lactone concentration versus time profiles in mice bearing HT29 human colon xenografts and non–tumor-bearing mice after administration of 9-nitrocamptothecin at 0.67 mg/kg × 1 via oral gavage. For mice bearing HT29 tumors, individual data points and best fit line of the data for 9-nitrocamptothecin lactone (●, —) and 9-aminocamptothecin lactone (▲, —). For non–tumor-bearing mice, individual data points and best fit line of the data for 9-nitrocamptothecin lactone (○, —) and 9-aminocamptothecin lactone (△, —) are presented. Individual concentration versus time points represents the average concentration of 9-nitrocamptothecin or 9-aminocamptothecin from three mice at each time point.
Allometric scaling studies. Representative plasma concentration versus time profiles of 9-nitrocamptothecin and 9-amino-camptothecin lactone on day 1 in a patient after oral administration of 9-nitrocamptothecin at 2.4 mg/m² is presented in Fig. 4. Complex Dedrick Plots of 9-nitrocamptothecin and 9-aminocamptothecin lactone in patients after administration of 9-nitrocamptothecin at 2.4 mg/m² (n = 9) and in mice bearing HT29 human colon xenografts after administration of 9-nitrocamptothecin at 0.67 mg/kg are presented in Fig. 5. The 9-nitrocamptothecin and 9-amino-camptothecin lactone AUCs for the Complex Dedrick Plots in the patients treated at 2.4 mg/m² were 393 and 60 (ng/mL per mg/kg)·h/kg⁰.²⁵, respectively. The 9-nitrocamptothecin and 9-aminocamptothecin lactone AUCs for the Complex Dedrick Plots in mice bearing HT29 human colon xenografts were 193 and 30 (ng/mL per mg/kg)·h/kg⁰.²⁵, respectively.

Discussion

It takes a considerable length of time for a drug to be approved for the treatment of cancer (32–35). Thus, there is a need to expedite the preclinical and clinical studies of anticancer agents and determine which agents should continue, or be stopped, in development (32, 36, 37). During the past 25 years, there has been a progressive shift from syngeneic transplantable tumors to the use of human tumor xenografts for the identification and development of new anticancer agents (6–9, 32). Support for these preclinical studies is based on the proposed improved ability of xenograft models to identify agents with clinical utility. The factors associated with correctly predicting the activity of anticancer agents in patients using xenograft models are currently unclear (7, 9, 16, 19–21). Camptothecin analogues are some of the most highly active agents evaluated in xenograft models. However, mice are able to tolerate 10- to 20-fold greater doses and systemic exposures of camptothecin analogues (i.e., topotecan, irinotecan, 9-aminocamptothecin) compared with humans (6, 7, 9, 16). Therefore, the activity of camptothecins in xenograft models may not translate to clinical activity in humans. To address this issue as related to 9-nitrocamptothecin, we evaluated the relationship between plasma exposure of 9-nitrocamptothecin and 9-aminocamptothecin lactone and antitumor response in mice bearing human colon xenografts and compared this with clinically relevant exposures in patients (1). Comparing the lactone forms of 9-nitrocamptothecin and 9-aminocamptothecin overcomes potential differences in the lactone-to-hydroxyl acid ratio associated with protein binding and other factors between mice and humans (6). This process has previously

### Table 3. Pharmacokinetic variables of 9NC and 9AC in mice bearing HT29 human colon xenografts and non–tumor-bearing mice

<table>
<thead>
<tr>
<th>Variables</th>
<th>Units</th>
<th>HT29-bearing mice</th>
<th>Non–tumor-bearing mice</th>
</tr>
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<tbody>
<tr>
<td>9NC AUC</td>
<td>ng/mL·h</td>
<td>41.3</td>
<td>39.1</td>
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<tr>
<td>9NC CL/F</td>
<td>L/h/m²</td>
<td>61.6</td>
<td>52.6</td>
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<td>9NC, t₁/₂β</td>
<td>h</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td>kₐ</td>
<td>h⁻¹</td>
<td>1.2</td>
<td>3.3</td>
</tr>
<tr>
<td>Vc1/F</td>
<td>L/m²</td>
<td>4.5</td>
<td>4.0</td>
</tr>
<tr>
<td>k₁₀</td>
<td>h⁻¹</td>
<td>10.8</td>
<td>12.9</td>
</tr>
<tr>
<td>k₁₂</td>
<td>h⁻¹</td>
<td>3.4</td>
<td>3.0</td>
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<tr>
<td>k₂₁</td>
<td>h⁻¹</td>
<td>0.8</td>
<td>1.2</td>
</tr>
<tr>
<td>k₃₁</td>
<td>h⁻¹</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>9AC AUC</td>
<td>ng/mL·h</td>
<td>5.7</td>
<td>5.2</td>
</tr>
<tr>
<td>9AC CL/F</td>
<td>L/h/m²</td>
<td>26.3</td>
<td>9.6</td>
</tr>
<tr>
<td>9AC, t₁/₂β</td>
<td>h</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Vc₃/F</td>
<td>L/m²</td>
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<td>1.1</td>
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<tr>
<td>k₄₃</td>
<td>h⁻¹</td>
<td>5.3</td>
<td>1.3</td>
</tr>
<tr>
<td>k₃₀</td>
<td>h⁻¹</td>
<td>21.8</td>
<td>8.8</td>
</tr>
</tbody>
</table>

Abbreviations: 9NC, 9-nitrocamptothecin; 9AC, 9-aminocamptothecin.
been used in the development of topotecan, irinotecan, and 9-aminocamptothecin (6, 9, 16).

9-Nitrocamptothecin produced significant antitumor activity at all doses evaluated in both human colon xenografts. The 9-nitrocamptothecin and 9-aminocamptothecin lactone AUCs after administration of 9-nitrocamptothecin at 0.67 mg/kg were 41.3 and 5.7 ng/mL-h, respectively. The dose of 0.67 mg/kg evaluated in the pharmacokinetic study was selected based on the greater overall antitumor activity after administration of 0.67 mg/kg compared with 0.44 mg/kg and less diarrhea after administration of 0.67 mg/kg compared with 1 mg/kg. In the phase I study using the same regimen as in the xenograft studies, four of six patients treated at the maximum tolerated dose (2.4 mg/m²/d) had a 9-nitrocamptothecin AUC > 41 ng/mL-h (13). In addition, five of the six patients who responded had a 9-nitrocamptothecin AUC > 41 ng/mL-h. In our phase II study of 9-nitrocamptothecin given orally daily for 5 days per week for 8 weeks in patients with refractory colon cancer, three of eight patients had a 9-nitrocamptothecin lactone AUC < 41 ng/mL-h (13). Moreover, there were no responders in phase II studies of 9-nitrocamptothecin in patients with colon cancer (2). The overall lack of 9-nitrocamptothecin response in patients with refractory colon carcinoma raises concerns about the ability of mice bearing human colon xenografts to predict 9-nitrocamptothecin response in humans (19–21). However, this lack of response in the phase II studies may be associated with the low daily exposures achieved with this regimen. The intermittent schedule of 9-nitrocamptothecin may be an active regimen because it achieves exposures that are tolerable in humans and above the exposure associated with antitumor response in the colon xenograft models.

Using the approach comparing the drug exposure associated with response in preclinical xenografts models and in patients may also explain the variable clinical response of camptothecin analogues (6, 9, 16). The topotecan systemic exposure associated with antitumor response in the xenograft models of neuroblastoma was tolerable and was associated with clinical responses in children with neuroblastoma (9). Kirstein et al. evaluated the relationship between 9-aminocamptothecin systemic exposure and tumor response in human solid tumor xenografts (29). The systemic exposure of 9-aminocamptothecin required for antitumor effect in the xenograft models was in excess of that achievable in patients, possibly explaining why 9-aminocamptothecin has not produced significant antitumor activity in clinical trials (16, 38, 39). In our studies, the conversion of 9-nitrocamptothecin to 9-aminocamptothecin in mice and patients was similar, with most of the drug remaining in the 9-nitrocamptothecin form. Thus, the antitumor activity associated with administration of 9-nitrocamptothecin is most likely due to the parent compound and not the 9-aminocamptothecin metabolite.

Although the exposures of 9-nitrocamptothecin associated with response in the xenograft models are tolerable in clinical trials, there are potential differences in the disposition of 9-nitrocamptothecin between mice and humans and tumor-specific issues that must be addressed (6, 9, 13, 16, 20). The concentration versus time profiles of 9-nitrocamptothecin and 9-aminocamptothecin in mice and humans are different. Thus, there may be differences in the duration of exposure that 9-nitrocamptothecin and 9-aminocamptothecin are maintained above a potential threshold required for activity (13, 17, 18, 40–42). However, the normalized concentration versus time profiles in the Complex Dedrick Plots were greater in patients compared with mice. The relationship between 9-nitrocamptothecin and 9-aminocamptothecin exposure and response may be different for specific tumors; thus, these types of studies may need to be done for each tumor type (13, 40–42). The tumor exposures of anticancer agents in xenografts located on the flank of mice may be different than the exposure in tumors of patients and thus evaluating plasma exposures in mice and man may not be an accurate comparison (43, 44).

The use of preclinical translational studies may be fundamental to the design and interpretation of clinical trials in humans. The direct comparison of drug exposures will remove
variability associated with differences in metabolism or elimination and allometric scaling can also be used to normalize the concentration versus time profiles in mice and patients (6, 9, 16, 25, 32–35). This information can then be used to make informed decisions in the development of new anticancer agents for the treatment of solid tumors and other malignancies (6, 32). Based on these results presented in our study, we recommend performing phase II studies of 9-nitrocamptothecin using the intermittent schedule of administration.

References

Relationship between Plasma Exposure of 9-Nitrocamptothecin and Its 9-Aminocamptothecin Metabolite and Antitumor Response in Mice Bearing Human Colon Carcinoma Xenografts


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