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ORIGINAL ARTICLE

Tumor, tissue, and plasma pharmacokinetic studies and antitumor response studies of docetaxel in combination with 9-nitrocamptothecin in mice bearing SKOV-3 human ovarian xenografts

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Abstract
Purpose We evaluated the antitumor activity of two different schedules of docetaxel and 9-nitrocamptothecin (9NC) in mice bearing human SKOV-3 ovarian carcinoma xenografts and evaluated the plasma, tissue, and tumor disposition of each agent alone and in combination.
Experimental design The following treatment groups were evaluated: (1) docetaxel 10 mg/kg IV on days 0 and 7; (2) 9NC 0.67 mg/kg PO qdx5dx2wk; (3) 9NC 0.67 mg/kg PO qdx5dx2wk in combination with docetaxel 10 mg/kg IV on days 0 and 7; and (4) 9NC 0.67 mg/kg PO qdx5dx2wk in combination with docetaxel 10 mg/kg IV on days 4 and 11; (5) vehicle controls for each agent; and (6) no treatment controls.
Results All treatment regimens produced significant antitumor activity as compared with control groups ($P < 0.05$). Docetaxel administered on days 0 and 7 or on days 4 and 11 in combination with 9NC resulted in similar antitumor activity ($P > 0.05$). High docetaxel concentrations in tumor were maintained at late time points as compared with plasma and tissues with the retention of docetaxel at 24 h being 132-fold and 15-fold higher in tumor than in plasma and liver, respectively. After administration of 9NC alone, the ratio of the 9-aminocamptothecin (9AC) area under the concentration versus time curve (AUC) to 9NC AUC in plasma and tumor was 0.15 and 1.34, respectively.
Conclusions The combination of docetaxel and 9NC was effective against SKOV-3 xenografts. The lack of a difference in sequence-dependent antitumor activity may reflect the sensitivity of the SKOV-3 xenograft to 9NC. The factors associated with tumor-specific retention of docetaxel and the ratio of 9NC to 9AC in tumors is unknown.

Keywords Docetaxel · 9-Nitrocamptothecin · Ovarian xenografts · Tumor retention

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Introduction

While most patients with ovarian cancer initially respond to therapy, the majority will relapse [1]. Therefore, there is an urgent need to develop new therapies and improve existing treatments for ovarian cancer [1]. Antitumor activity can often be enhanced by combining agents [2–6]. However, the sequence of administration and schedule of those agents

can alter the pharmacokinetics, toxicity, and antitumor response of the combination [2, 7–9]. Therefore, attention to drug interactions, which may be sequence- or schedule-dependent, are necessary. Taxanes and camptothecin analogues have a broad spectrum of antitumor activity in preclinical models and in patients with solid tumors, including ovarian cancer [10, 11]. Taxane and camptothecin analogues have overlapping toxicities (e.g., myelosuppression) but are non-cross resistant and their combination may be synergistic [3]. The combination of docetaxel and topotecan has been evaluated in clinical trials, but the optimal schedule of the combination may not have been used [3, 8, 9, 12–14].

Docetaxel is approved for IV administration every 21 days as treatment for locally advanced or metastatic breast cancer, non-small lung cancer, androgen-independent prostate cancer, and squamous cell carcinoma of the head and neck, and is also used in the treatment of newly diagnosed and relapsed ovarian cancer [15, 16]. Randomized trials comparing docetaxel administered every 3 weeks with docetaxel administered weekly have reported similar efficacy for the two regimens, but a significantly lower incidence of severe neutropenia with the weekly regimen [17–21].

The optimal dose, schedule, and route of administration of camptothecin analogues are unclear [22–26]. In vitro and in vivo preclinical studies suggest protracted administration of low doses of camptothecin analogues produce better antitumor activity than does less frequent administration of higher doses [27–30]. Repeated oral administration of camptothecin analogues could mimic the protracted schedule and maximize patient convenience. However, oral administration of camptothecin analogues has been associated with extensive inter- and intra-patient variability in bioavailability [31–34]. 9-Nitrocamptothecin (9NC, rubitecan, RFS2000) is an orally administered camptothecin analogue that is partially metabolized to an active-metabolite, 9-aminocamptothecin (9AC) [26, 31, 35–37]. We have previously reported that 9NC administered orally daily for 5 days per week for two consecutive weeks repeated every 4 weeks may be an active regimen in patients with colorectal carcinoma [22, 31, 38].

Docetaxel undergoes primarily hepatic metabolism via cytochrome P450 (CYP) 3A4 and 3A5 [9, 15, 39–42]. We have previously reported that topotecan inhibits docetaxel clearance, and this interaction was associated with increased neutropenia [14]. 9NC and 9AC also undergo hepatic metabolism; however, identification of the specific CYP isoenzymes involved in their metabolism and potential drug interactions have not been evaluated [30]. The dose-limiting toxicities associated with 9NC are neutropenia and thrombocytopenia [22–25]. Therefore, there is potential for docetaxel and 9NC to have pharmacokinetic and pharmacodynamic drug interactions based on metabolism by CYP3A and bone marrow suppression, respectively.

Information obtained from human tumor xenograft models can be used to make informed decisions with regard to the most appropriate regimen for the combination of a taxane and a camptothecin analogue [27, 29, 38]. Xenograft models can also be used to evaluate potential pharmacokinetic and pharmacodynamic interactions of drugs in blood and tumor [28, 30, 37, 43]. The objectives of this study were: (1) To evaluate the effect of the sequence of administration on antitumor response to docetaxel and 9NC in combination by mice bearing a human ovarian carcinoma xenograft; and (2) to evaluate the plasma, tumor, and tissue pharmacokinetics of docetaxel when administered alone, 9NC when administered alone, and docetaxel when administered in combination with single or multiple doses of 9NC.

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 Taconic Farms (Germantown, NY), and were allowed to acclimate to the University of Pittsburgh Cancer Institute Animal Facility for 1 week prior to initiation of study. Mice were housed in autoclaved, microisolator caging and had access to 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-h light and dark cycle with at least 12 air changes per hour. Analysis every 3 months of sentinel mice (Assessment Plus, Charles River, Boston, MA) indicated that the study mice remained murine antibody profile (MAP)-negative throughout the study.

Tumor lines, implantation, and measurements

SKOV-3 human ovarian xenografts were obtained from the National Cancer Institute Tumor Repository (Frederick, MD) and were MAP test-negative. SKOV-3 cells were passaged in C.B-17 SCID female mice. Tumors were harvested from passage mice when they reached 500–1,000 mm^3 (500–1,000 mg) and approximately 25-mg fragments were implanted subcutaneously, using aseptic techniques, on the right flank of study mice [44]. Mice were observed twice daily. Body weights and tumor measure-

ments 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 longest diameter perpendicular to the length. Median days to 1 and 2 doublings of tumor volume and median maximum *%T/C* [(ratio of median tumor volume for treatment group (*T*) to median tumor volume for control group (*C*)) × 100] were calculated [45].

Efficacy studies

On day 39 post-implantation, when the tumor volumes were 23–246 mm^3, mice were stratified into groups of ten such that the mean body weight and tumor volumes for the treatment groups were not statistically different. Stratification was performed 3 days prior to treatment (day 3). 9NC (SuperGen Inc., Dublin, CA) was dissolved at 0.067 mg/ml in 1 mM phosphoric acid:polyethylene glycol 400:*N*,*N*-dimethylacetamide (48:50:2, *v*/*v*/*v*). 9NC dosing solution or vehicle was administered using a curved, 20 gauge, oral gavage needle at 0.01 ml/g body weight. The docetaxel clinical formulation (Aventis Pharmaceuticals, Bridgewater, NJ) was diluted to 1 mg/ml in 0.9% NaCl and was administered IV at 0.01 ml/g body weight. To serve as a positive control, the paclitaxel clinical formulation (UDL Laboratories Inc., Rockford, IL) was diluted to 2 mg/ml in 0.9% NaCl and was administered IV at 0.01 ml/g body weight.

The doses and regimens of docetaxel, 9NC, and paclitaxel were based on previous studies [38, 46, 47]. The first day of treatment was defined as day 0. The following treatment groups were evaluated: (1) control group that received no drug or vehicle; (2) 9NC vehicle PO daily for 5 days per week for two consecutive weeks (qdx5dx2wk); (3) docetaxel vehicle IV on days 0 and 7; (4) paclitaxel 20 mg/kg IV on days 0 and 7; (5) docetaxel 10 mg/kg IV on days 0 and 7; (6) 9NC 0.67 mg/kg PO qdx5dx2wk; (7) 9NC 0.67 mg/kg PO qdx5dx2wk in combination with docetaxel 10 mg/kg IV on days 0 and 7 administered 30 min prior to 9NC; and (8) 9NC 0.67 mg/kg PO qdx5dx2wk in combination with docetaxel 10 mg/kg IV on days 4 and 11 administered 30 min after 9NC. At the completion of each study, mice were euthanized by CO_2 inhalation, and complete necropsies were performed.

Statistics

Body weights and tumor volumes from the efficacy study were analyzed for statistical significance using the Minitab statistical software package (Minitab Inc., State College, PA). Values were expressed as the mean ± SD and median. Mean data were analyzed by one-way ANOVA followed by pair-wise comparisons using Dunnett's test. Median data were analyzed using Kruskal–Wallis followed by pair-wise comparisons by Mann–Whitney test. The a priori level of significance was set at $P = 0.05$ [44,45].

Pharmacokinetic studies

Mice were stratified into groups of three such that the mean body weights and tumor volumes among the groups were not statistically different. Pharmacokinetic studies were performed after administration of docetaxel alone at 10 mg/kg IV × 1, 9NC alone at 0.67 mg/kg PO × 1, docetaxel at 10 mg/kg IV × 1 administered 30 min prior to 9NC at 0.67 mg/kg PO × 1 on day 0, and docetaxel at 10 mg/kg IV × 1 administered 30 min after 9NC on day 4 following administration of 9NC on days 0–4.

Mice were fasted overnight prior to all pharmacokinetic studies. After dosing with docetaxel or 9NC alone, mice ($n = 3$ per time point) were euthanized at 0.08, 0.25, 0.5, 1, 1.5, 2, 4, 6, 7, 18, and 24 h. After administration of docetaxel 30 min before 9NC on day 0, mice ($n = 3$ per time point) were euthanized at 0.08, 0.25, 0.5 h after docetaxel administration, and 0.08, 0.25, 0.5, 1, 1.5, 2, 4, 6, 7, 18, 24, and 48 h after 9NC administration. After administration of 9NC 30 min before 9NC on day 4, mice ($n = 3$ per time point) were euthanized at 0.08, 0.25, 0.5 h after 9NC administration, and 0.08, 0.25, 0.5, 1, 1.5, 2, 4, 6, 7, 18, 24, and 48 h after docetaxel administration. Three additional mice were euthanized 5 min after administration of vehicle in each study. Blood was collected by cardiac puncture using heparinized syringes and placed on ice until centrifuged at 12,000*g* for 4 min. The resulting plasma was collected, immediately frozen on dry ice, and stored at −80°C. Tumor, liver, kidneys, spleen, and brain were also obtained, weighed, frozen in liquid nitrogen, and stored at −80°C until analyzed [38, 44].

Docetaxel concentrations were measured in plasma, tumor, liver, kidney, and spleen after administration of docetaxel alone. In studies of docetaxel in combination with 9NC on day 0 or 4, docetaxel concentrations were measured only in plasma and tumor samples. Plasma, tumor, liver, kidney, and brain samples were assayed for 9NC and 9AC after administration of 9NC alone and in combination with docetaxel on day 0 and 4.

Sample preparation

Docetaxel plasma samples collected after the administration of docetaxel alone were prepared by combining up to 100 µl of sample plasma with control plasma (Lampire Biological Lab., Pipersville, PA) to achieve a final volume of 500, and 10 µl of 1 µM paclitaxel was added as the internal standard (IS). Plasma samples were then processed by solid phase extraction (SPE) as described previously [48]. Ten

microliters of sample was injected into the liquid chromatography/electrospray-mass spectrometer (LC–MS).

Because of matrix interferences, plasma collected after the administration of docetaxel in combination with 9NC and all tumor and tissue samples for docetaxel analysis were processed by protein precipitation followed by SPE. Protein precipitation of the plasma sample was performed by mixing 500 µl of plasma with 1 ml of acetonitrile. Tumor and tissue samples were homogenized in phosphate-buffered saline (PBS), pH 7.4 (1:3, *w*/*v*). A 200 µl aliquot of tumor or tissue homogenate sample was mixed with 1 ml of acetonitrile. Plasma, tumor, and tissue samples in acetonitrile were vortexed and centrifuged at 21,000*g* for 6 min at 4°C. The supernatant was decanted and evaporated to dryness under nitrogen at 37°C. The dried residues were re-suspended in 1 ml of 0.01 M ammonium acetate buffer, pH 5.0: methanol (80:20, *v*/*v*), which was then processed by SPE as described previously, and 10 µl of sample was injected into the LC–MS [48].

Plasma samples for 9NC and 9AC analysis were processed by mixing 100 µl of plasma with 300 µl of methanol that contained 10 µl of 10 µg/ml camptothecin as the IS. Tumor and tissue samples of 9NC and 9AC were homogenized in PBS, pH 7.4 (1:3, *w*/*v*). A 200 µl aliquot of homogenate was mixed with 10 µl of IS and 1 ml of acetonitrile. The samples were centrifuged at 21,000*g* for 6 min at 4°C, and the resulting supernatant was decanted and evaporated to dryness under nitrogen at 37°C. Each dried residue was re-suspended in 100 µl of mobile phase, and 25 µl was injected into a liquid chromatography-electrospray tandem mass spectrometer (LC–MS/MS) [49].

Liquid chromatography–mass spectrometry

Docetaxel concentrations in plasma, tumor, and all tissues were determined by an LC–MS assay that was modified from an assay originally developed for the detection of docetaxel in human plasma [48]. The docetaxel assay was linear for from 0.001 to 0.6 µM in plasma and from 0.003 to 0.6 µM in tumor and tissues. 9NC and 9AC concentrations in plasma, tumor, and tissues were determined by an LC–MS/MS assay that was originally developed for the detection of 9NC and 9AC in human plasma [49]. The assay was linear for 9NC and 9AC from 1 to 1,000 ng/ml.

Pharmacokinetic analysis

The compartmental pharmacokinetic analysis of docetaxel concentrations in plasma was performed using a three-compartment model in ADAPT II [50]. The estimation procedure and variance model used in the compartmental pharmacokinetic analysis were maximum likelihood estimation and linear models for the variance of the additive errors, respectively. Estimated model parameters included: the volume of the central compartment (V_c); intercompartmental rate constants, (k_{12}, k_{21}, k_{13}, k_{31}); and the elimination rate constant from the central compartment (k_{10}), and were identifiable in all studies [50]. Using standard equations, systemic clearance (CL) and elimination half-life ($t_{1/2}$) of docetaxel were calculated from parameter estimates [51]. The area under the docetaxel plasma concentration versus time curve from 0 to infinity ($AUC_{0-\infty}$), or 0 to the last time point, were calculated using the log trapezoidal method by simulating the concentration versus time data based upon model-specific parameters [50].

After administration of docetaxel alone, the area under the docetaxel concentration versus time curves in tumors and tissues were calculated from 0 to 24 h ($AUC_{0-24\,h}$) using the log trapezoidal method [51]. After administration of docetaxel in combination with 9NC on days 0 and 4, the area under the docetaxel concentration versus time curves in tumor and tissues were calculated from 0 to 48 h ($AUC_{0-48\,h}$) and from 0 to 48.5 h ($AUC_{0-48.5\,h}$), respectively. After administration of 9NC alone and in combination with docetaxel, the area under the 9NC and 9AC concentration versus time curves in plasma and tumor were calculated from 0 to infinity ($AUC_{0-\infty}$) [51]. The $AUC_{0-\infty}$ was reported unless the extrapolated AUC was > 15%. If extrapolated AUC was >15%, the AUC from 0 to the last time point was reported. The maximum concentration (C_{max}), time of C_{max} (T_{max}), concentration at 24 h ($C_{24\,h}$), and concentration at 48 h ($C_{48\,h}$) were determined by visual inspection for all studies [51].

Results

Efficacy and toxicity

Tumor growth curves for SKOV-3 human ovarian xenografts are presented in Fig. 1. Days to 1 and 2 doublings of tumor volume and median optimal *%T/C* for SKOV-3 xenografts are presented in Table 1. All treatments regimens resulted in significant antitumor activity as compared with the vehicle-treated and control groups ($P < 0.05$) (Table 1). Administration of paclitaxel as the positive control produced antitumor activity consistent with previous studies [46]. Single-agent docetaxel and single-agent 9NC produced antitumor activity similar to that produced by single-agent paclitaxel ($P < 0.05$). Treatment with docetaxel on days 0 and 7 in combination with 9NC and treatment with docetaxel on days 4 and 11 in combination with 9NC produced greater antitumor activity than did treatment with single-agent docetaxel or paclitaxel ($P < 0.05$). The antitumor activity resulting from docetaxel administered on days 0 and 7 and days 4 and 11 in combination with 9NC was

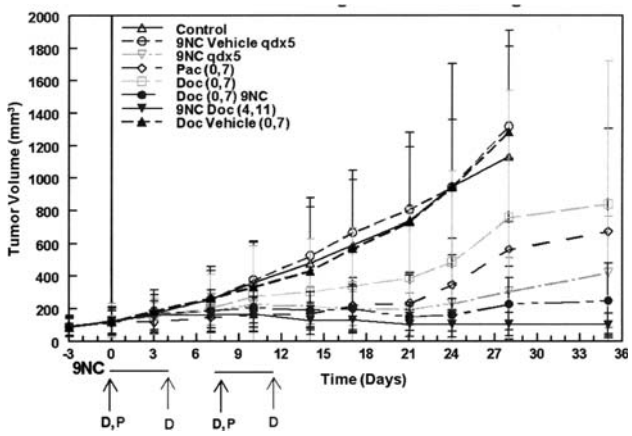

Fig. 1 Tumor growth curves for SKOV-3 human ovarian xenografts. Mice were treated with (1) control group receiving no drug or vehicle; (2) 9NC vehicle PO daily for 5 days per week for two consecutive weeks (qdx5dx2); (3) docetaxel vehicle IV on days 0 and 7; (4) paclitaxel 20 mg/kg IV on days 0 and 7; (5) docetaxel 10 mg/kg IV on days 0 and 7; (6) 9NC 0.67 mg/kg PO qdx5dx2; (7) 9NC 0.67 mg/kg PO qdx5dx2 in combination with docetaxel 10 mg/kg IV on days 0 and 7 administered 30 min prior to 9NC; and (8) 9NC 0.67 mg/kg PO qdx5dx2 in combination with docetaxel 10 mg/kg IV on days 4 and 11 administered 30 min after 9NC. The *vertical line* within the figure at day 0 represents the start of treatment. The *horizontal line* under the *x*-axis represents the oral administration of 9NC. The *vertical arrows* under the *x*-axis represent the IV administration of docetaxel (*D*) or paclitaxel (*P*). The data *points* and *bars* represent the mean tumor volumes (mm^3) and SD, respectively

similar ($P > 0.05$). Administration of docetaxel on days 4 and 11 in combination with 9NC resulted in significantly greater activity as compared with 9NC alone ($P < 0.05$). However, administration of docetaxel on days 0 and 7 in combination with 9NC resulted in similar antitumor activity as compared with 9NC alone ($P > 0.05$). Throughout the study, the body weight of mice corrected for tumor weight was similar in all groups ($P > 0.05$). The maximum decrease in body weight was <20% in all groups.

Pharmacokinetic studies

The concentration versus time profiles of docetaxel in plasma, tumor, liver, kidney, and spleen after administration of docetaxel alone are presented in Fig. 2a and b. The concentration versus time profiles of docetaxel in plasma and tumor after administration of docetaxel alone and in combination with 9NC on days 0 and 4 are presented in Fig. 3a and b. Pharmacokinetic parameters of docetaxel alone and in combination with 9NC on days 0 and 4 are presented in Tables 2 and 3.

The peak docetaxel concentrations in plasma and tissues were approximately 7-fold and 40-fold greater, respectively, compared with those in SKOV-3 tumor. The disposition of docetaxel in plasma and tissues was representative of a bi- or tri-phasic elimination, whereas the docetaxel concentrations in tumor remained relatively constant from 6 to 48 h. Docetaxel concentrations at late time points were much greater than those in other tissues with the mean docetaxel concentration being 132-fold and 15-fold higher in tumor as compared with plasma and liver, respectively. In addition, the concentration of docetaxel in tumor at 48 h after administration of docetaxel in combination with 9NC was relatively stable at approximately 2 μM, where as the

Table 1 Tumor doubling times and median optimal *%T/C* in C.B-17 SCID mice bearing SKOV-3 xenografts

Treatment groups ($n = 10$ per group)	Days to 1 tumor doubling (median days to reach doubling)	Days to 2 tumor doublings (median days to reach doubling)	Median maximum *%T/C* (day 28)
Control	6.7 ± 1.6 (6.8)	15.5 ± 5.4 (15.3)	100.0
9NC Vehicle PO qdx5x2	5.8 ± 1.7 (5.3)	12.3 ± 2.4 (11.3)	124.0
Docetaxel Vehicle IV day 0,7	7.7 ± 3.6 (6.5)	15.6 ± 4.2 (15.3)	84.6
Paclitaxel 20 mg/kg IV day 0,7	22.2 ± 10.3[a,b] (22.0)[2]	28.9 ± 8.4[a,b] (31.5)[2]	28.6
Docetaxel 10 mg/kg IV day 0,7	18.0 ± 9.8[a,b] (16.8)[2]	28.4 ± 8.0[a,b] (29.5)[2]	37.5
9NC 0.67 mg/kg PO qdx5x2	26.5 ± 10.4[a,b] (27.3)[2]	34.0 ± 4.6[a,b] (36.0)[2]	33.1
Docetaxel 10 mg/kg IV day 0,7 9NC 0.67 mg/kg PO qdx5x2	27.6 ± 11.4[a,b,c] (36.0)	34.7 ± 2.9[a,b,c] (36.0)	19.7
9NC 0.67 mg/kg PO qdx5x2 Docetaxel 10 mg/kg IV day 4,11	>36.0[a,c,d] (>36.0)	>36.0[a,d] (>36.0)	7.9

Tumors were implanted in C.B-17 SCID female mice as 25-mg fragments, and mice were stratified on day 39 when tumors had reached 23–246 mm^3. Treatment began 3 days later. Results of time to one doubling and time to two doublings are presented as the mean ± SD (median)

[a] Value significantly different than control and vehicle, $P \leq 0.05$ by Dunnett's test

[b] Value significantly different than control and vehicle, $P \leq 0.05$ by Mann–Whitney test

[c] Value significantly different than Docetaxel, $P \leq 0.05$ by Dunnett's test

[d] Value significantly different than 9NC, $P \leq 0.05$ by Dunnett's test

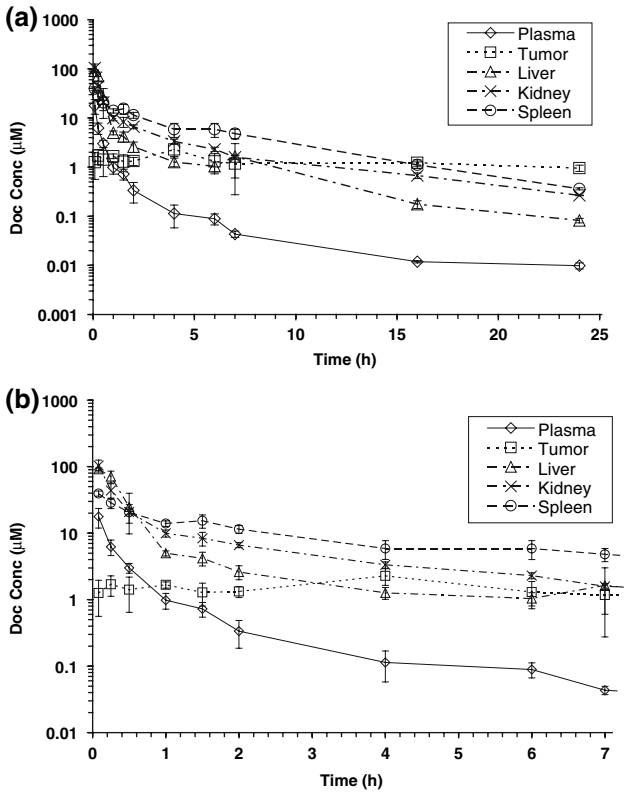

Fig. 2 Concentration versus time profiles of docetaxel in plasma, tumor, liver, kidney, and spleen after administration of docetaxel alone. The plasma, tissue, and tumor concentrations from 0 to 24 h and 0 to 8 h are presented in **a**, **b**, respectively. Individual concentration versus time points represent the mean ± SD concentration obtained from three mice at each time point from 5 min to 24 h after administration of docetaxel

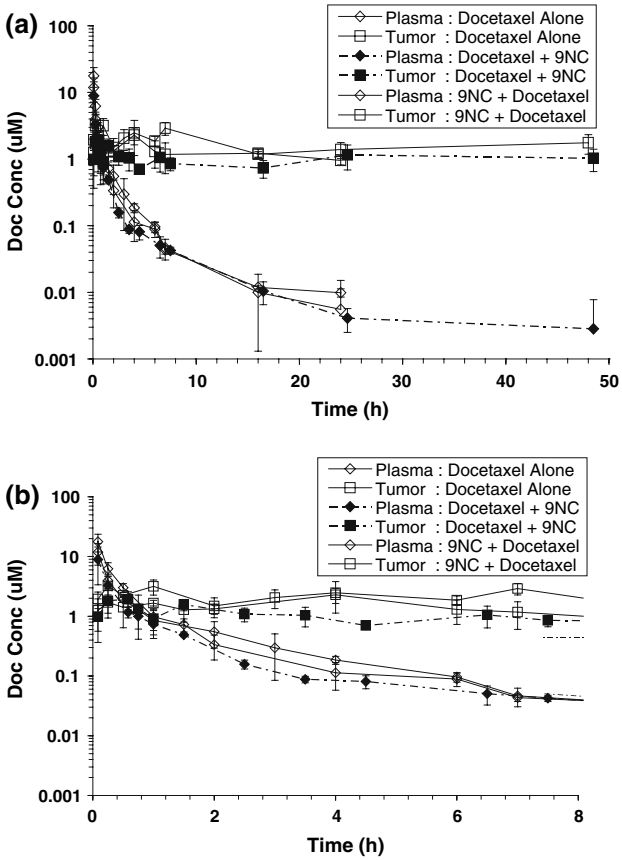

Fig. 3 Concentration versus time profiles of docetaxel in plasma and tumor after administration of docetaxel alone (*Docetaxel Alone*) and in combination with 9NC on days 0 (*Docetaxel + 9NC*) and 4 (*9NC + Docetaxel*). The plasma and tumor concentrations from 0 to 50 h and 0 to 8 h are presented in **a**, **b**, respectively. Individual concentration versus time points represents the mean ± SD concentrations from 3 mice at each time point. For docetaxel administered alone (*Docetaxel Alone*), samples were obtained 5 min to 24 h after administration. For docetaxel administered in combination with 9NC on day 0 (*Docetaxel + 9NC*), docetaxel was administered 30 min prior to 9NC and samples were obtained from 5 min to 48.5 h after administration of docetaxel. For docetaxel administered in combination with 9NC on day 4 (*9NC + Docetaxel*), docetaxel was administered 30 min after 9NC and samples were obtained from 5 min to 48 h after administration of docetaxel

concentration of docetaxel in plasma was below the lower limit of quantitation (BLQ).

Pharmacokinetic parameters of 9NC and 9AC after administration of 9NC alone and in combination with docetaxel are presented in Table 4. The disposition of 9NC and 9AC were similar after administration of 9NC alone or in combination with docetaxel on day 0. However, exposure of 9NC was approximately two-fold lower on day 4 in combination with docetaxel as compared to day 0 alone or in combination with docetaxel suggesting a reduction in exposure of 9NC with repeated dosing. The exposure of 9NC in plasma was 5 to 18-fold higher than 9AC.

After administration of 9NC alone or in combination with docetaxel, the exposure of 9NC and 9AC in tumor was more variable than in plasma. The exposure of 9AC in tumor after administration of 9NC alone and with docetaxel on day 0 was 1.3 and 6.6-fold greater, respectively, than 9NC. Consistent with the metabolism of 9NC to 9AC in the liver, the exposure of 9AC in the liver was greater than 9NC.

Discussion

The combination of taxanes and camptothecin analogues has been evaluated in preclinical and clinical studies; however, the optimal sequence of administration has not been determined [3, 8, 9, 12–14, 51–54]. As such, we performed the first study evaluating the effect of the sequence of administration of docetaxel and 9NC in mice bearing human tumor xenografts. In addition, we performed studies evaluating the tissue and tumor disposition of docetaxel and 9NC.

Prior to the use of an LC–MS assay and the development of acetonitrile precipitation followed by solid phase

Table 2 Compartmental pharmacokinetic parameters for the plasma disposition of docetaxel after administration of docetaxel alone and in combination with 9NC on days 0 and 4

Parameter	Units	Docetaxel alone	Docetaxel + 9NC on day 0	Docetaxel + 9NC on day 4
k_{10}	h^{-1}	3.8	3.5	4.5
k_{12}	h^{-1}	2.1	3.8	3.5
k_{21}	h^{-1}	2.0	1.3	2.5
k_{13}	h^{-1}	0.7	0.3	1.0
k_{31}	h^{-1}	0.2	0.1	0.2
V_C	l/m^2	1.2	1.7	1.7
CL	$l/h/m^2$	4.6	5.8	7.9
$t_{1/2}$[a]	h	4.8	5.8	4.7

The CV% for each parameter in each study was <15%

[a] $t_{1/2}$ is the elimination half life

extraction performed in our study, interfering substances made the detection of docetaxel in tumor and tissues difficult. Previous studies have evaluated the plasma, tumor, and tissue disposition of taxanes; however, our study is the first to report retention of docetaxel in an ovarian tumor as compared to plasma and normal tissues [47, 55–58]. This retention of docetaxel in tumors has not been reported for most anticancer agents, with exceptions being geldanamycin-analogues and carrier-mediated anticancer agents (e.g., liposomes, nanoparticles, and conjugates) [44, 59–62]. After administration of radio-labeled paclitaxel to non-tumor bearing CD_2F_1 mice, the highest peak concentrations were observed in the liver, followed by the kidneys, lungs, and spleen [55]. In mice bearing a human ovarian carcinoma xenograft, the peak concentrations of paclitaxel were considerably lower in tumor compared with plasma; however, the tumor concentrations were >2 μg/g at 48 h [55]. Paclitaxel tumor retention was also observed in ICR mice bearing S.180 and M109 tumors [55]. A prolonged tumor exposure of docetaxel has been reported in mice bearing colon adenocarcinoma 38 [47]. The concentration of docetaxel in plasma was not detectable after 6 h; whereas, the concentration in tumor was approximately 2 μg/ml at 24 h. The extensive tissue distribution of docetaxel in our study was similar to previous studies of paclitaxel in mice [56, 57].

The sequence of administration of taxane and camptothecin analogues may greatly enhance the antitumor activity of these two agents in combination [2, 7–9, 63]. Administration of docetaxel or paclitaxel prior to topotecan or 9AC significantly reduced the IC_{50} and was associated with an increase in topoisomerase I for MDAH 231B human breast cancer cells [63]. We previously evaluated the pharmacokinetics and pharmacodynamics of docetaxel and topotecan when co-administered on different sequences of administration as part of phase I trials [8, 9]. The results of those clinical studies suggested that to avoid pharmacokinetic interactions between docetaxel and 9NC and improve antitumor activity, docetaxel should be administered on days 0 and 7 in combination with 9NC.

In the current preclinical study, the antitumor response was similar after administration of docetaxel on days 0 and 7 or days 4 and 11 in combination with 9NC. The lack of a difference in sequence-dependent antitumor activity may be associated with the sensitivity of the SKOV-3 to 9NC treatment as depicted by the significant antitumor response of 9NC alone. Thus, administration of 9NC at a lower dose in

Table 3 Non-compartmental pharmacokinetic parameters of docetaxel after administration of docetaxel alone and In combination with 9NC on days 0 and 4

Parameter	Units	Plasma	Tumor	Liver	Kidney	Spleen
Docetaxel alone						
AUC	μM h	5.9[a]	30.5[b]	50.3[a]	68.2[a]	98.9[a]
T_{max}	h	0.083	4	0.083	0.083	0.083
C_{max}	μM	17.7 ± 5.8	2.3 ± 0.6	91.5 ± 3.8	104.2 ± 21.0	39.5 ± 3.4
$C_{24\,h}$	μM	0.009 ± 0.001	1.0 ± 0.1	0.08 ± 0.01	0.3 ± 0.01	0.4 ± 0.02
Docetaxel + 9NC on day 0						
AUC	μM h	3.4[a]	48.7[c]	–	–	–
T_{max}	h	0.083	0.58	–	–	–
C_{max}	μM	8.9 ± 5.5	1.9 ± 0.7	–	–	–
$C_{24.5\,h}$	μM	0.004 ± 0.002	1.2 ± 0.5	–	–	–
$C_{48.5\,h}$	μM	BLQ	1.0 ± 0.4	–	–	–
9NC + docetaxel on Day 4						
AUC	μM h	4.7[a]	80.5[c]	–	–	–
T_{max}	h	0.083	0.25	–	–	–
C_{max}	μM	11.8 ± 4.7	3.2 ± 0.3	–	–	–
$C_{24\,h}$	μM	BLQ	1.4 ± 0.4	–	–	–
$C_{48\,h}$	μM	BLQ	1.8 ± 0.6	–	–	–

BLQ below lower limit of quantitation

[a] $AUC_{0-\infty}$

[b] $AUC_{0-24\,h}$

[c] $AUC_{0-48\,h}$

Table 4 Non-compartmental pharmacokinetic parameters of 9NC and 9AC after administration of 9NC Alone, 9NC in combination with docetaxel on day 0, and 9NC in combination with docetaxel on day 4

Parameter	Units	Plasma	Tumor	Liver	Kidney
9NC alone					
9NC					
$AUC_{0-\infty}$	ng/ml h	66.3	46.8	N/A	51.6
T_{max}	h	0.25	0.5	0.5	0.25
C_{max}	ng/ml	68.4 ± 18.4	30.8 ± 22.3	2.7 ± 4.7	91.8 ± 33.5
9AC					
$AUC_{0-\infty}$	ng/ml h	10.1	63.0	615.7	259.5
T_{max}	h	0.5	1.0	0.083	0.25
C_{max}	ng/ml	6.3 ± 2.2	45.4 ± 34.1	680.9 ± 220.4	195.0 ± 48.1
Docetaxel + 9NC on day 0					
9NC					
$AUC_{0-\infty}$	ng/ml h	64.0	8.1	24.8	35.8
T_{max}	h	0.083	0.25	0.5	0.5
C_{max}	ng/ml	72.4 ± 13.0	17.0 ± 18.1	21.0 ± 1.8	26.3 ± 12.4
9AC					
$AUC_{0-\infty}$	ng/ml h	3.5	53.2	1131.2	472.5
T_{max}	h	0.083	0.5	0.5	0.25
C_{max}	ng/ml	9.3 ± 6.4	31.7 ± 16.7	1093.6 ± 514.0	213.1 ± 72.5
9NC + docetaxel on day 4					
9NC					
AUC	ng/ml h	29.2	15.9	N/A	17.6
T_{max}	h	0.25	0.58	0.083	0.25
C_{max}	ng/ml	52.2 ± 43.0	11.7 ± 10.8	30.6 ± 49.8	40.2 ± 36.6
9AC					
AUC	ng/ml h	5.5	N/A	190.2	133.1
T_{max}	h	0.58	0.25	0.083	0.25
C_{max}	ng/ml	4.8 ± 0.85	5.6 ± 6.6	394.0 ± 246.6	191.8 ± 146.2

N/A = unable to estimate AUC due to limited number of detectable sample concentrations. In the 9NC alone study, there was one detectable 9NC concentration at 0.5 and at 1.0 h

combination with docetaxel or administration of docetaxel in combination with 9NC in a less sensitive tumor line may produce sequence-dependent effects. There was no pharmacokinetic interaction between docetaxel and 9NC in this study. This finding is inconsistent with previous results of docetaxel in combination with topotecan in phase I studies [8, 9]. The difference in pharmacokinetic interactions between docetaxel and topotecan compared with 9NC may be due to differences in elimination between topotecan and 9NC [8, 9, 31, 35]. The exposure of 9NC and 9AC in the kidney is consistent with the renal elimination of these forms. After administration of 9NC alone, the ratio of 9AC AUC to 9NC AUC in plasma and tumor was 0.15 and 1.34, respectively. These results highlight the importance of evaluating the tumor disposition of anticancer agents because the tumor disposition is not always consistent with the plasma disposition. The factors associated with the conversion of 9NC to 9AC and altered distribution of 9NC and 9AC in tumor and tissue are unknown.

Future studies need to determine if the tumor retention of taxanes is related to the sensitivity of the xenograft tumor, tumor matrix factors, cellular efflux, or the interaction between taxane analogues and tubulin [40, 59], and if the tumor retention occurs in xenografts other than ovarian and colon cancer. In addition, future studies also need to determine if the tumor retention of taxanes occurs in patients with solid tumors and how the tumor disposition of taxanes impacts antitumor response in patients with solid tumors.

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