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ORIGINAL ARTICLE

Phase 1 and pharmacokinetic study of weekly docosahexaenoic acid-paclitaxel, Taxoprexin®, in resistant solid tumor malignancies

Paula M. Fracasso · Joel Picus · Jonathan D. Wildi · Sherry A. Goodner · Allison N. Creekmore · Feng Gao · Ramaswamy Govindan · Matthew J. Ellis · Benjamin R. Tan · Gerald P. Linette · ChauHwei J. Fu · Helen S. Pentikis · Stephen C. Zumbrun · Merrill J. Egorin · Robert E. Bellet

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Abstract
Purpose To determine the maximum tolerated dose, dose-limiting toxicity (DLT), and pharmacokinetics of weekly docosahexaenoic acid-paclitaxel (DHA-paclitaxel), a taxane fatty acid conjugate.
Experimental design Docosahexaenoic acid-paclitaxel was administered by 2-hour i.v. infusion weekly for three out of four weeks. DHA-paclitaxel 200 mg/m^2 was dose escalated by 100 mg/m^2 per cohort to 600 mg/m^2. Blood samples for pharmacokinetics of DHA-paclitaxel and paclitaxel derived from DHA-paclitaxel were collected.
Results Twenty-one patients received 42 cycles of treatment over five dose levels. Grade 3/4 neutropenia occurred in five patients but was not dose limiting. Grade 3 hyperbilirubinemia, a DLT, and grade 1 sensory neuropathy occurred at the highest dose level. PK analyses demonstrated dose proportional C_{max} and AUC_{0-24}. Limited accumulation of DHA-paclitaxel or paclitaxel occurred with weekly treatment. Increased DHA-paclitaxel and paclitaxel AUC_{0-24} were associated with increased neutropenia. Of the 19 patients evaluable for response, three patients with esophageal, melanoma and colon carcinoma had stable disease for 11, 16, and 17 weeks, respectively.
Conclusion Docosahexaenoic acid-paclitaxel administered weekly to a maximum dose of 600 mg/m^2 was well-tolerated. The slow release of paclitaxel from DHA-paclitaxel and the weekly schedule approximates continuous infusion paclitaxel which may be more active than every 3 week or weekly taxanes.

Keywords Taxoprexin® · DHA-paclitaxel · Phase 1

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P. M. Fracasso · J. Picus · J. D. Wildi · S. A. Goodner · A. N. Creekmore · F. Gao · R. Govindan · M. J. Ellis · B. R. Tan · G. P. Linette
Alvin J. Siteman Cancer Center,
Washington University School of Medicine, St Louis, MO, USA

C. J. Fu
Applied Analytical Industries International, Shawnee, KS, USA

H. S. Pentikis
ICON Development Solutions, Ellicott City, MD, USA

S. C. Zumbrun · M. J. Egorin
Molecular Therapeutic/Drug Discovery Program,
University of Pittsburgh Cancer Center, Pittsburgh, PA, USA

R. E. Bellet
Luitpold Pharmaceuticals, Inc., Norristown, PA, USA

P. M. Fracasso (✉)
Department of Medicine, Division of Hematology/Oncology,
University of Virginia Health System, P·O. Box 800716,
Charlottesville, VA 22908, USA
e-mail: fracasso@virginia.edu

Introduction

Paclitaxel, a taxane diterpenoid, is one of the most active anticancer agents in the treatment of several solid tumor malignancies including breast, lung, and ovarian carcinomas [5, 19, 20]. Exerting its cytotoxic effect by binding to tubulin, promoting polymerization and subsequent assembly of microtubules resistant to disassembly, paclitaxel interferes with cell division eventually causing cell death [18, 25]. The toxicities of paclitaxel include myelosuppression, peripheral neuropathy, and hypersensitivity reactions which may limit its use [5, 19, 20]. With its mechanism of action and significant activity of this agent in solid tumor

malignancies, there has been considerable effort expended in developing taxane analogues with improved activity and less toxicity [7, 18].

Docosahexanenoic acid-paclitaxel (DHA-paclitaxel) is a covalently linked conjugate of paclitaxel and DHA. DHA is an omega-3, C22 fatty acid that is found in human milk and certain dietary fats [15, 16]. It is added to infant formula and is a constituent of the GRAS (Generally Recognized As Safe) list by the FDA in the United States [15, 16]. In preclinical animal studies, DHA has been found to be preferentially taken up by tumor cells from the arterial blood supply, presumably for use as a precursor for metabolic and biochemical pathways [12, 21–23]. DHA is covalently linked to paclitaxel at the 2′-hydroxyl position of paclitaxel via acylation to produce DHA-paclitaxel [2, 3]. Acylation at the 2′ position has been shown to eliminate the in vitro microtubule assembly activity of paclitaxel until it is cleaved by hydrolysis [3, 13]. Preclinical cell culture data indicate that DHA-paclitaxel is 1,000-fold less cytotoxic than unconjugated paclitaxel [3]. However, in animal xenograft models, DHA-paclitaxel has been shown to have significantly more antitumor activity than paclitaxel at equimolar and equitoxic doses [3]. Further work demonstrated that the areas under the curve (AUCs) of DHA-paclitaxel and the paclitaxel derived from it were higher in tumor than the AUCs produced by i.v. paclitaxel, supporting the concept that DHA conjugation to paclitaxel targets the drug to tumor [3]. At sublethal doses, DHA-paclitaxel shared the same toxicities as paclitaxel, and both drugs had myelosuppresion as the dose-limiting toxicity [3].

Clinical trials have further delineated the differing and more favorable properties of DHA-paclitaxel when compared to paclitaxel [29, 31]. A phase 1 study administered DHA-paclitaxel once every 3 weeks and found the maximum tolerated dose (MTD) to be 1,100 mg/m^2 [31]. Neutropenia was the dose limiting toxicity (DLT), and there was only one incident of febrile neutropenia at the 1,100 mg/m^2 dose level. No patient developed alopecia, and no peripheral neuropathy >grade 1 was observed. One patient with breast cancer had a partial response. Pharmacokinetic analyses showed a prolonged half-life and sustained levels of DHA-paclitaxel within the plasma [29, 31]. Results of eight phase 2 trials administering single-agent DHA-paclitaxel every 3 weeks revealed a favorable toxicity profile as well as modest activity in a variety of solid tumors [1, 8–10, 14, 17, 24] .

Metronomic chemotherapy has been shown to block angiogenesis by targeting the endothelial cells associated with tumor and upregulating expression of thrombospondin, a natural inhibitor of angiogenesis [4, 11]. Given the relatively long half-life of DHA-paclitaxel and its prolonged presence in plasma, weekly scheduling of this agent may further enhance its efficacy and potentially lessen toxicity. We performed a dose-escalation phase 1 clinical trial of weekly DHA-paclitaxel to determine MTD, DLT, and to investigate the effect of the new dosing schema on the pharmacokinetics of DHA-paclitaxel and its active metabolite, paclitaxel.

Patients and methods

Patient eligibility

Patients ≥18 years of age were eligible for enrollment onto the study if they had a histologically or cytologically confirmed malignant solid tumor, failed all conventional chemotherapy options available, and had an Eastern Cooperative Oncology Group (ECOG) performance status of 0−2. Laboratory criteria for eligibility included absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$ cells/L, platelet count $\geq 100 \times 10^9$ cells/L, bilirubin ≤ upper limit of normal, creatinine ≤ upper limit of normal, and alanine transaminase and aspartate transaminase ≤ 1.5 times the upper limit of normal. No chemotherapy or radiation therapy was permitted within 4 weeks of study entry, and no major surgery was permitted within 2 weeks of study entry. Patients with the following conditions were ineligible: active central nervous system metastases, baseline peripheral neuropathy ≥ grade 2 (National Cancer Institute Common Toxicity Criteria v.2), serious concurrent medical illness, or a known hypersensitivity to Cremophor®. Women who were either pregnant or breast feeding were ineligible. All patients gave written informed consent before study entry in compliance with institutional and federal regulations.

Treatment plan

Patients received DHA-paclitaxel (Taxoprexin®, Luitpold Pharmaceuticals, Inc., Norristown, PA) as a 2-hour i.v. infusion at one of six dose levels: 100, 200, 300, 400, 500, or 600 mg/m^2 on days 1, 8 and 15 every 28 days. The initial dose level of DHA-paclitaxel was designed to be <1/3 of the total dose of the phase 1 study by Wolff et al., which administered DHA-paclitaxel every three weeks [31]. Prophylactic medications were administered 0.5–1 h prior to each therapy and included dexamethasone 10 mg i.v., diphenhydramine 25 mg i.v., and cimetidine 300 mg i.v. Dose escalation was permitted after all patients at the prior level had successfully completed cycle 1.

Evaluation of toxicity, dose modifications, and assessment of response

Patients were eligible to continue treatment unless there was evidence of unacceptable toxicity or progressive

disease. Three to six patients were enrolled at each dose level. Toxicities were graded in accordance with the NCI Common Toxicity Criteria (NCI CTC) version 2.0. DLT was determined by toxicity observed during cycle 1. DLT was defined as grade 4 neutropenia (ANC $<0.5 \times 10^9$cells/L) lasting $\geq$5 days, neutropenia complicated by fever (>38.5°C) or infection, grade 4 thrombocytopenia, grade 3 thrombocytopenia lasting $\geq$5 days or associated with bleeding, grade 2 hemorrhage, $\geq$grade 3 non-hematological toxicity (excluding nausea, vomiting, diarrhea, and hypersensitivity reactions), grade 2 hemorrhage or neurologic toxicities, or any toxicity causing a delay of >14 days in cycle 1. If a DLT occurred in one of the three patients treated at a given dose level, up to three additional patients would be treated at that dose level. If two patients at this dose level experienced a DLT, enrollment would be terminated. If only one of six patients experienced a DLT, dose escalation proceeded. The MTD of DHA-paclitaxel was defined as one dose level lower than that of which at least two patients experienced a DLT.

Dose modifications for DHA-paclitaxel included dose delays and reductions. DHA-paclitaxel dosing was delayed if any of the following toxicities was present on the day of treatment: ANC $< 1.5 \times 10^9$ cells/L, platelet count $< 100 \times 10^9$ cells/L, increase of >1 grade from baseline bilirubin, alanine transaminase, and/or aspartate transaminase, and any grade 3 non-hematologic toxicities excluding nausea and vomiting. Treatment was resumed at a one level dose reduction if toxicities recovered to the following values: ANC $\geq 1.5 \times 10^9$ cells/L, platelet count $\geq 100 \times 10^9$ cells/L, LFT(s) within one grade of the baseline value(s), and resolution of grade 3 nonhematologic toxicities to $\leq$grade 1. Treatment delays of up to 2 weeks were allowed for recovery of toxicities, and patients were removed from study for delays greater than 2 weeks. If there was a dose reduction within a treatment cycle, then dose escalation to the original dose at the start of the following cycle was allowed providing the patient tolerated the reduced dose. If neutropenia resulted in hospitalization for neutropenic fever/infection, or if thrombocytopenia resulted in a bleeding event, then all subsequent DHA-paclitaxel was resumed at a one level dose reduction and was not escalated.

Antitumor response was evaluated by physical examination and/or imaging prestudy and every two cycles. Responses were defined by Response Evaluation Criteria In Solid Tumors [30].

Bioanalytical methods

Pharmacokinetic sampling for total DHA-paclitaxel and its metabolite, paclitaxel, was performed during cycle 1 only. Blood samples were collected at 0 (prior to the start of the infusion), 2 (prior to the end of the infusion), 2.5–3.0, 3.5–4.0, 8.5–9.0, and 26 h after the start of the infusion, and just prior to the next weekly treatment on days 8, 15, and 29.

Analysis of both total DHA-paclitaxel and unconjugated paclitaxel were determined under Good Laboratory Practice at the Kansas City Facility of Applied Analytical Industries International (AAII-KC, Shawnee, KS) by analytical assays developed and validated by AAII-KC. The plasma samples collected from patients were analyzed by two validated LC/MS/MS methods, one method for total DHA-paclitaxel and another method for free paclitaxel.

DHA-paclitaxel

DHA-paclitaxel and the internal standard (IS), paclitaxel–2′-EPA, were extracted from potassium EDTA-anticoagulated plasma (50 μL) by a protein precipitation cleanup procedure using 750 μL of acetonitrile. After centrifugation, the supernatant (250 μL) was combined with 4.0 mL of loading mobile phase A. This combined extract was then subjected to reverse phase high performance liquid chromatography on a 5 μm, 20 × 2.0 mm Aquasil DASH-18 column. Two sets of mobile phase solutions were used to enhance the formation of Na^+ adducts and elute the analytes with a valve switching system involved. Loading mobile phase A consisted of 35/54/1 acetonitrile/10 mM ammonium acetate (pH 5.5)/100 mM sodium chloride (v/v/v). Elution mobile phase A consisted of 25% methanol in 10 mM ammonium acetate (pH 5.5). Mobile phase B was 100% acetonitrile. The analytes were detected by a PE/Sciex (Foster City, CA) API 3000 LC/MS/MS system equipped with Turbo IonSpray interface. Quantitation was achieved by monitoring the product ions (*m/z* 618.5 and 592.4 for DHA-paclitaxel and IS, respectively) of precursor ions of *m/z* 1,186.5 and 1,160.6, respectively. The lower limit of quantitation (LLOQ) was 400 ng/mL. The analytical range was 400–10,000 ng/mL. No significant matrix effect was observed. For samples with DHA-paclitaxel concentrations >10,000 ng/mL, a validated dilution technique was applied to obtain quantitative results. The overall accuracy (relative recovery) was 94.9–101.9%. The overall precision (%CV) was 4.4–19.1%. For matrix stability evaluation, no significant degradation was observed for DHA-paclitaxel in human plasma at room temperature (6.8 h) and at −70°C (1,155 days), or when subjected to freeze and thaw procedures (4 cycles).

Paclitaxel

Paclitaxel and the IS, cephalomannine, were extracted from potassium EDTA-anticoagulated plasma (100 μL) by a liquid−liquid extraction procedure using 3.0 mL of ethyl acetate as the extraction solvent. The extract was evaporated to

dry and then reconstituted with 100 µL of 10/90 acetonitrile/10 mM ammonium acetate (pH 4.2). This reconstituted extract was then subjected to reverse phase high performance liquid chromatography on a 5 µm, 10 × 2.0 mm Hypersil ODS-C18 column. Mobile phase A consisted of 25% methanol in 10 mM ammonium acetate (v/v, pH 5.5). Mobile phase B was 100% acetonitrile. The analytes were detected by a PE/Sciex API III + LC/MS/MS system equipped with Turbo IonSpray interface. Quantitation was achieved by monitoring the product ions (*m/z* 568.8 and 263.8 for paclitaxel and IS, respectively) of precursor ions of *m/z* 871.4 and 832.2, respectively. The lower limit of quantitation (LLOQ) was 10.0 ng/mL. The analytical range was 10.0–500 ng/mL. No significant matrix effect was observed. The overall accuracy (relative recovery) was 93.2–106.7%. The overall precision (%CV) was 5.6–14.0%. For matrix stability evaluation, no significant degradation was observed for paclitaxel in human plasma at room temperature (5.0 h) and at −70°C (1,283 days), or when subjected to freeze and thaw procedures (3 cycles).

Pharmacokinetic analysis

A noncompartmental analysis was performed on the plasma concentration-time data for DHA-paclitaxel and paclitaxel using WinNonlin v 4.0.1. (Pharsight Corporation, Mountain View, CA). Standard noncompartmental pharmacokinetic parameters were calculated (C_{max}, T_{max}, and AUC_{0-24}). Only C_{max}, T_{max} and AUC_{0-24} were calculated because samples at times between 24 h from the first infusion and predose the second infusion were not collected and therefore, the estimation of the terminal disposition rate, λ_z, and the half-life of the terminal disposition phase, $T_{1/2}$, was not possible.

All the data analyses were descriptive in nature. Due to the variability in pharmacokinetic parameters, the relationships between pharmacokinetic parameters (AUC, C_{max}, and trough levels) and patient/treatment characteristics (age, gender, dose level, and BSA) were explored based on relative ranks using Kendall's correlation coefficients and Mann−Whitney's rank test as appropriate. Relationships between dose or pharmacokinetic parameters and myelosuppression were explored using linear and sigmoid Emax models.

Results

Patient characteristics

Twenty-one eligible patients were enrolled from April 2003 to November 2004. Characteristics of those enrolled are summarized in Table 1. All patients had received prior chemotherapy, and nine patients had received a taxane.

Table 1 Patient characteristics

Characteristics	No. patients (n = 21)
Sex	
Female/male	7:14
Age (year)	
Mean (range)	58 (18–78)
ECOG performance status	
0	4
1	16
2	1
Diagnosis	
Colorectal	5
Lung	4
Breast	2
Other (cervical, esophageal, melanoma, mesothelioma, osteosarcoma, ovarian, pancreatic, prostate, renal cell, unknown primary carcinomas)	10
Prior therapy	
Chemotherapy	21
Mean no. regimens (range)	4 (2–6)
Previous taxane chemotherapy	9
Radiotherapy	10

ECOG, Eastern Cooperative Oncology Group

Treatment

Twenty-one patients received 42 cycles (median, 2 cycles) of treatment. All were evaluable for toxicity, and 19 were evaluable for response. Both of the patients not evaluable for response received DHA-paclitaxel 600 mg/m^2 (dose level 5). One of these patients experienced grade 3 hyperbilirubinemia, a DLT, in cycle 1 and was subsequently taken off from study. The other patient withdrew consent because of transportation issues. Of the 19 patients evaluable for response, one did not complete cycle 1 because of early progressive disease.

Toxicity

While neutropenia was the most frequent hematologic toxicity observed in this study, no patients experienced febrile neutropenia (Table 2). Grade 3 or 4 neutropenia in cycle 1 was noted in five patients, four of whom received DHA-paclitaxel 600 mg/m^2. Seven patients (one each at DHA-paclitaxel 300, 400, and 500 mg/m^2, and 4 at DHA-paclitaxel 600 mg/m^2) had cycle 1 doses held and reduced due to neutropenia. Neutropenia first occurred on day 15 in six of the seven patients, and only two of the seven patients

Table 2 Hematologic toxicities during cycle 1

DHA-paclitaxel (mg/m^2)	No. patients	ANC nadir (cells $\times 10^9$ L^{-1})		Neutropenia grade		Hemoglobin nadir (g/dL)		Platelet nadir (cells $\times 10^9$ L^{-1})	
		Mean	Range	1/2	3/4	Mean	Range	Mean	Range
200	3	4.600	3.629–5.695	0/0	0/0	11.3	9.1–13.5	240	202–315
300	4	2.123	0.726–3.082	0/0	1[a]/0	11.3	8.9–13.0	136	86–185
400	3	2.791	1.260–5.072	0/1[a]	0/0	11.6	8.6–13.9	301	228–413
500	3	2.355	1.120–4.236	1/1[a]	0/0	12.3	10.9–13.3	221	205–233
600	8	1.693	0.442–6.494	0/2[c]	2[a]/2[a,b]	10.6	8.5–12.6	188	87–280

[a] Six patients required a dose delay and reduction at day 15

[b] One patient required a dose delay and reduction at day 8

[c] Neither of these patients had a dose reduction or delay as one patient had a normal ANC on day of treatment and the other patient went off from study due to grade 3 hyperbilirubinemia, a DLT after only one week of treatment

had delays >1 week. When cycle 1 resumed, six out of the seven patients had their doses reduced by one level.

The nonhematologic toxicities for all cycles of therapy worst grade by patient are summarized in Table 3. The most common toxicities were grades 1 and 2 fatigue, anorexia, and nausea/vomiting. One patient, with pancreatic cancer, and extensive liver metastases, suffered grade 3 hyperbilirubinemia after a single 600 mg/m^2 dose of DHA-paclitaxel. The patient's prestudy bilirubin was 1.5 mg/dL. Within a week after this infusion, the bilirubin increased to 5.0 mg/dL, but returned to baseline within ~3 weeks after the infusion. Radiographic evaluation ruled out post-obstructive jaundice, and no obvious cause was detected for the hyperbilirubinemia. As this event was considered a DLT, the patient was taken off study. The only episode of neuropathy was a grade 1 sensory neuropathy at the highest dose level. Overall, the treatment regimen was well-tolerated.

Table 3 Non-hematologic toxicities for all cycles

Adverse event	DHA-paclitaxel (mg/m^2)										
Grade of adverse event	200		300		400		500		600		
	1	2	1	2	1	2	1	2	1	2	3
Constitutional symptoms											
Fatigue/malaise				1				1		3	1
Gastrointestinal											
Anorexia			2				1			1	
Diarrhea				1							
Hyperbilirubinemia											1[a]
Nausea/vomiting	1		1	1	1	1			1		1
Stomatitis									1		
Neurologic											
Neuropathy-Sensory									1		

[a] Cycle 1-DLT

Pharmacokinetics

Plasma concentration versus time profiles for both DHA-paclitaxel and unconjugated-paclitaxel are presented in Fig. 1a, b, respectively. Fig. 1a shows the DHA-paclitaxel plasma concentrations during the infusion and for the first 24-hour period after the end of the infusion. Plasma concentrations of DHA-paclitaxel were substantial and measurable at all time points. With the administration of weekly DHA-paclitaxel 500 and 600 mg/m^2, DHA-paclitaxel trough concentrations remained over 1 μg/mL throughout the first cycle and trough concentrations remained detectable on day 29, which was 2 weeks after the last infusion (data not shown). As expected, plasma concentrations of unconjugated-paclitaxel were much lower when compared to concentrations of DHA-paclitaxel (Fig. 1b). With the administration of DHA-paclitaxel doses of 500 and 600 mg/m^2, unconjugated-paclitaxel trough concentrations were >10 ng/mL in one patient and two patients, respectively. Both of the patients who received DHA-paclitaxel 600 mg/m^2 had treatment held on day 15 due to neutropenia. All other patients had undetectable unconjugated-paclitaxel trough concentrations (<10 ng/mL) on day 8 and 15. Pharmacokinetic analyses and comparison of trough samples on days 8, 15, and 29 demonstrated that there was not a substantial accumulation of DHA-paclitaxel or unconjugated-paclitaxel with weekly treatment (data not shown).

Plasma pharmacokinetic parameters for DHA-paclitaxel and unconjugated-paclitaxel are separated by dose level and summarized in Table 4. The parameter analysis was limited to the first 2-hour DHA-paclitaxel infusion through the 24 h after the end of that infusion. No patient had his or her dosing or infusion time modified during the first administration. DHA-paclitaxel $C_{max,}$ C_{24} and AUC_{0-24} increased with dose when considered on a mg/m^2 or total dose basis. DHA-paclitaxel AUC_{0-24} correlated closely with

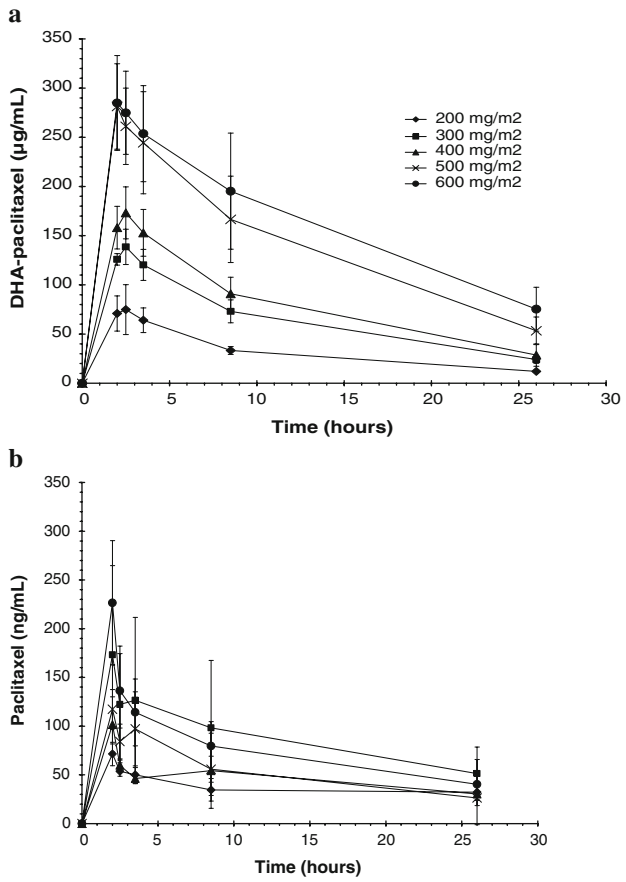

Fig. 1 **a** DHA-paclitaxel plasma concentration versus time profiles by dose level. **b** Unconjugated-paclitaxel plasma concentration versus time profiles by dose level

DHA-paclitaxel $C_{max,}$ and $C_{24,}$ with the relationships being described by the equations $AUC_{0–24} = 12.19\ C_{max}$ ($R^2 = 0.945$) and $AUC_{0–24} = 50.1\ C_{max}$ ($R^2 = 0.805$). Paclitaxel C_{max}, C_{24}, and $AUC_{0–24}$ were more variable, though the data suggested an increase in plasma concentration with increasing dose (Table 4). As with DHA-paclitaxel, paclitaxel $AUC_{0–24}$ correlated closely with paclitaxel $C_{max,}$ and $C_{24,}$ with the relationships being described by the equations $AUC_{0–24} = 9.06\ C_{max}$ ($R^2 = 0.512$) and $AUC_{0–24} = 39.58\ C_{24}$ ($R^2 = 0.0.416$). Paclitaxel $AUC_{0–24}$ correlated closely with DHA-paclitaxel $AUC_{0–24}$ and paclitaxel $C_{max,}$correlated closely with DHA-paclitaxel $C_{max,}$, with the relationships being described by the equations paclitaxel $AUC_{0–24} = 0.0005$ DHA-paclitaxel $AUC_{0–24}$ ($R^2 = 0.708$) and paclitaxel $C_{max,} = 0.0007$ DHA-paclitaxel $C_{max,}$ ($R^2 = 0.246$). In contrast, the paclitaxel C_{24} did not correlate with the DHA-paclitaxel C_{24}.

The percentage decrease in ANC increased with dose, but there was substantial variability at each dose (Fig. 2a). When the relationship of percentage decrease in ANC to PK parameters was explored, there were obvious relationships between exposure to DHA-paclitaxel and paclitaxel, as reflected by $AUC_{0–24}$, although substantial interpatient variability remained (Fig. 2b, c).

Response

Nineteen patients with measurable disease were evaluable for response; three had stable disease and sixteen had progressive disease. Of the patients with stable disease, none had had a previous taxane. Two of the patients, one each with colon cancer and melanoma, were treated with DHA-paclitaxel 400 mg/m^2 and had stable disease for 16 weeks and 17 weeks, respectively. One patient with esophageal cancer was treated with DHA-paclitaxel 600 mg/m^2, and experienced stable disease for 11 weeks.

Table 4 Paclitaxel and DHA-paclitaxel C_{max}, T_{max}, and $AUC_{0–24}$ as a function of dose

Dose (mg/m^2)	Paclitaxel PK Parameters			DHA-Paclitaxel PK Parameters		
	C_{max} (ng/mL) (%CV)	T_{max} (h) (%CV)	$AUC_{0–24}$ (ng h/mL) (%CV)	C_{max} (µg/mL) (%CV)	T_{max} (h) (%CV)	$AUC_{0–24}$ (µg h/mL) (%CV)
200 ($n = 3$)	71.4 ± 12.0 16.8%	1.9 ± 0.2 8.8%	864.7 ± 275.2 31.8%	76.5 ± 22.6 29.6%	2.5 ± 0.3 12.0%	758.3 ± 112.4 14.8%
300 ($n = 4$)	178.1 ± 94.0 52.8%	2.4 ± 0.9 36.0%	2384.2 ± 1239.1 52.0%	141.1 ± 13.2 9.3%	2.5 ± 0.4 18.1%	1477.1 ± 178.0[a] 12.1%
400 ($n = 3$)	101.7 ± 28.3 27.8%	2.0 ± 0.0 2.8%	1096.1 ± 410.7 37.5%	173.2 ± 26.5 15.3%	2.8 ± 0.1 4.6%	1885.0 ± 486.1 25.8%
500 ($n = 3$)	117.4 ± 20.1 17.1%	2.0 ± 0.9 1.0%	1253.0 ± 250.7 20.0%	281.3 ± 43.4 15.4%	2.0 ± 0.0 0.9%	3296.7 ± 793.3 24.1%
600 ($n = 8$)	226.5 ± 63.7 28.1%	2.1 ± 0.1 6.7%	1822.7 ± 308.4 16.9%	290.4 ± 44.3 15.3%	2.2 ± 0.3 13.9%	3605.3 ± 726.2[a] 20.1%

Data are expressed as mean ± standard deviation

C_{max} maximum concentration, T_{max} time of maximum concentration, *AUCO-24* area-under the concentration–time curve, *CV%* coefficient of variation

[a] $n = 3$ for 300 mg/m^2 dose level and $n = 7$ for 600 mg/m^2 dose level due to incomplete PK profile

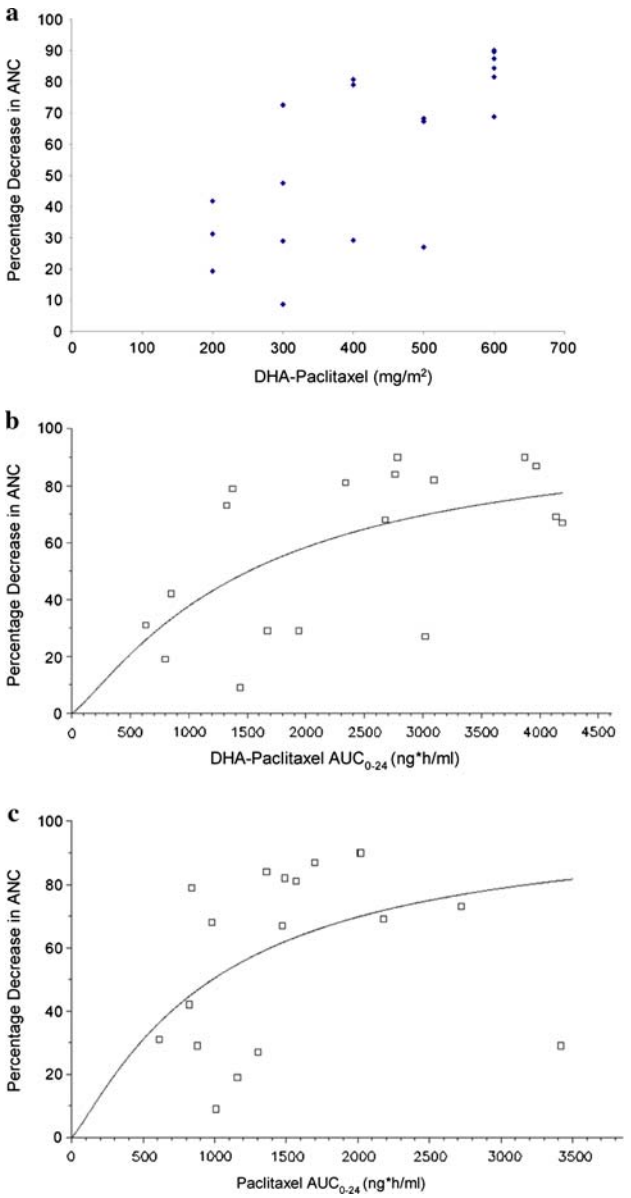

Fig. 2 **a** Relationship of DHA-paclitaxel dose to percentage decrease in ANC. **b** Relationship of DHA-paclitaxel AUC_{0-24} to percentage decrease in ANC. **c** Relationship of paclitaxel AUC_{0-24} to percentage decrease in ANC

Discussion

DHA-paclitaxel is a promising taxane derivative that has been shown to have significant antitumor activity in preclinical models and a favorable toxicity profile in a previous phase I study administering DHA-paclitaxel every 3 weeks [29, 31]. We performed a phase I study administering DHA-paclitaxel weekly three out of every four weeks and obtained limited pharmacokinetics on DHA-paclitaxel and its active metabolite, paclitaxel.

In this phase 1 study, we determined that a regimen of DHA-paclitaxel 600 mg/m^2 administered weekly for 3 weeks as part of a 28-day cycle was safe and well tolerated. No MTD was determined. As with the every 3-week schedule of DHA-paclitaxel reported by Wolff et al. [31], the most common toxicity was neutropenia. No patient experienced febrile neutropenia or prolonged neutropenia. Only one incidence of grade 1 sensory neuropathy occurred at the highest dose level (DHA-paclitaxel 600 mg/m^2) which is consistent with the every 3-week schedule of DHA-paclitaxel [31].

Pharmacokinetic analyses of DHA-paclitaxel and paclitaxel in plasma demonstrated a dose-dependent increase in C_{max}, C_{24}, and AUC_{0-24} for DHA-paclitaxel and paclitaxel. The pharmacokinetic parameters described for DHA-paclitaxel and paclitaxel in the current study, are very consistent with those previously reported by Wolff et al. [31]. As expected, there was increased neutropenia associated with increasing DHA-paclitaxel dose as well as several DHA-paclitaxel and paclitaxel pharmacokinetic parameters; however, more precise definition of relationships between pharmacokinetic parameters and neutropenia as well as comparison to previously a published relationship of that nature [6] was precluded by the inability to define precisely the time that plasma paclitaxel concentrations remained above 0.05 μM as well as the lack of good pharmacokinetic-neutropenia models for paclitaxel when administered on a weekly basis. The weekly schedule of DHA-paclitaxel used in the current study also made it impossible to compare directly the pharmacokinetic-neutropenia relationships described with those previously described by Sparreboom et al. in their study that used an every 3-week schedule of DHA-paclitaxel administration [29]. Our phase 1 study demonstrated that weekly DHA-paclitaxel administration for 3 weeks as part of 28-day cycle is well-tolerated. Weekly DHA-paclitaxel administration allowed for constant exposure of the prodrug and prolonged exposure to its metabolite, paclitaxel, while minimizing peripheral neuropathy, the dose limiting toxicity of weekly paclitaxel. The slow release of paclitaxel from DHA-paclitaxel with its documented half-life of 85 h [31] coupled with the weekly schedule administered in this study should approximate continous infusion paclitaxel. Given that paclitaxel has been shown to be more active in metastatic breast cancer when given as a longer infusion [26–28], DHA-paclitaxel may be more active than every 3 week or weekly taxanes. We await the results of clinical trials using this weekly schedule as a single agent in metastatic melanoma and hepatobiliary carcinoma and in combination with carboplatin in non-small cell lung carcinoma.

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