Phase I Trial of Combretastatin A-4 Phosphate with Carboplatin

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ABSTRACT

Purpose: **Preclinical evidence of synergy led to a phase I trial employing combretastatin A-4 phosphate (CA4P), a novel tubulin-binding antivascular drug, in combination with carboplatin.**

Experimental Design: **Based on preclinical scheduling studies, patients were treated on day 1 of a 21-day cycle. Carboplatin was given as a 30-minute i.v. infusion and CA4P was given 60 minutes later as a 10-minute infusion.**

Results: **Sixteen patients with solid tumors received 40 cycles of therapy at CA4P doses of 27 and 36 mg/m^2 together with carboplatin at area under the concentration-time curve (AUC) values of 4 and 5 mg min/mL. The dose-limiting toxicity of thrombocytopenia halted the dose escalation phase of the study. Four patients were treated at an amended dose level of CA4P of 36 mg/m^2 and carboplatin AUC of 4 mg min/mL, although grade 3 neutropenia and thrombocytopenia were still observed. Three lines of evidence are adduced to suggest that a pharmacokinetic interaction between the drugs results in greater thrombocytopenia than anticipated: the carboplatin exposure (as AUC) was greater than predicted; the platelet nadirs were lower than predicted; and the deviation of the carboplatin exposure from predicted was proportional to the AUC of CA4, the active metabolite of CA4P. Patient benefit included six patients with stable disease lasting at least four cycles.**

Conclusion: **This study of CA4P and carboplatin given in combination showed dose-limiting thrombocytopenia. Pharmacokinetic/pharmacodynamic modeling permitted the inference that altered carboplatin pharmacokinetics caused the increment in platelet toxicity.**

INTRODUCTION

Combretastatin A-4 Phosphate (CA4P) is a synthetic, phosphorylated prodrug of the natural product combretastatin A-4 (CA4). The combretastatins are heterocyclic plant alkaloids derived originally from the bark of the African bush willow, *Combretum caffrum*, and have been investigated as anticancer agents because of their activity as inhibitors of tumor blood flow (1, 2). *In vitro*, CA4 is a strong tubulin-binding agent that prevents tubulin polymerization and is toxic to actively proliferating vascular endothelial cells at concentrations in the low micromolar range (3–6). CA4 is also cytotoxic to a variety of human cancer cell lines. In some studies, activity was observed at concentrations higher than those toxic to endothelial cells (3 orders of magnitude), although in others, significant toxicity was also observed at low concentrations (7, 8).

The antivascular effects of CA4 have been shown in several *in vitro* and *in vivo* models and seem to be mediated through endothelial cell damage (3, 9–11). CA4P is rapidly converted to the active hydrophobic form CA4 by membrane-bound phosphatases, which are widely expressed on endothelial cells. CA4P and CA4 have been shown to induce apoptosis in human umbilical vein endothelial cells (HUVEC), impair HUVEC migration, and disrupt the endothelial cytoskeleton (7, 9). Alterations in cell shape and in microtubule stability are reported to occur at CA4 concentrations in the nanomolar range (12).

In rodent tumor models, CA4P increases vascular resistance, reduces tumor blood flow, and induces central tumor necrosis (10, 11). CA4P has been shown to cause measurable reductions in tumor blood flow to human tumors (13–15). In animal models, CA4P caused ischemic necrosis at the center of tumor xenografts, leaving a viable rim behind (11). These observations prompted preclinical combination studies with various cytotoxic drugs and radiation in the hope that tumor cells less affected by the ischemic insult could also be eliminated. CA4P has been shown to act synergistically with 5-fluorouracil, cisplatin, and carboplatin in rodent models (3, 16–18). In a murine reticulosarcoma tumor model, carboplatin was administered alone at its maximum tolerated dose of 90 mg/kg, given i.v. every 4 days at three doses, resulting in a log cell kill of 1.4 but no tumor regression (3). CA4P given alone on the same schedule had no activity in this tumor model. However, combining CA4P and carboplatin resulted in a log cell kill of 2.0, suggesting synergistic antitumor activity.

Three phase I single-agent studies of CA4P in humans have been reported (13, 19, 20). Tumor pain, cardiopulmonary toxicity, and assorted neuropathies are dose limiting. This side-effect profile, in conjunction with available preclinical data, suggested that CA4P given in combination with carboplatin would be well tolerated in humans.

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PATIENTS AND METHODS

Study Design. This study was a dose-escalating phase I combination trial of i.v. CA4P, coadministered with i.v. carboplatin, in adults with refractory solid tumors. It was designed to assess the dose-limiting toxicity and maximum tolerated dose of this combination, with the secondary end of describing antitumor activity. This study was approved by the Institutional Review Board of the University of Pennsylvania and all enrolled patients provided written informed consent.

Patient Selection. Patients were required to have a histologically or cytologically confirmed solid malignant tumor refractory to treatment or for which no effective treatment existed. Patients were also required to have measurable disease, a life expectancy of ≥12 weeks, WHO performance status ≤2, age ≥18 years, and the ability to provide written informed consent (21). Patients were excluded if they had inadequate bone marrow reserve (neutrophils $<1.5 \times 10^9$/L, platelets $<100 \times 10^9$/L), inadequate liver function, or inadequate renal function (serum creatinine >2.0 mg/dL or creatinine clearance of ≤60 mL/min). Patients were also excluded if they had had extensive prior radiation or a prior myocardial infarction.

Treatment and Dose Escalation. CA4P was supplied as a sterile, freeze-dried disodium salt in glass vials containing 90 mg CA4P. The drug was reconstituted with 11.0 mL of sterile water for injection and further diluted with ~100 to 150 mL of normal saline. The carboplatin infusion was prepared from commercially available vials containing sterile aqueous solution, which were diluted with 250 mL of 5% dextrose injection.

After antiemetic prophylaxis with ondansetron, carboplatin was given as a 30-minute i.v. infusion on day 1. Sixty minutes after the start of the carboplatin infusion, CA4P was administered as a 10-minute infusion on day 1. Patients were treated every 21 days as outpatients.

Table 1 summarizes the dose escalation schedule. Dose escalations were based on the occurrence of dose-limiting toxicity, defined as drug-related adverse events, and graded according to the National Cancer Institute Common Toxicity Criteria 2.0. Dose-limiting toxicities were defined as any of the following occurring in the first cycle: QTc prolongation ≥500 ms; grade ≥2 ventricular arrhythmia; grade 3/4 nonhematologic toxicity (except fatigue, asthenia, nausea, or vomiting); toxicity resulting in treatment delay >14 days; absolute neutrophil count < 500 cells/mm^3 ≥5 consecutive days or febrile neutropenia with ANC <1,000 cells/mm^3; thrombocytopenia <10,000 cells/mm^3 or bleeding episode requiring platelet transfusion; any grade toxicity requiring patient removal from the study in the judgment of investigators.

If a dose-limiting toxicity was observed in none of three patients at a dose level, then escalation to the next dose level was permitted. If a dose-limiting toxicity was observed in one of the first three subjects at a dose level, then three additional patients were treated at that level. If two or more subjects experienced a dose-limiting toxicity at a dose level, that dose level was deemed the maximum dose level to be tested. If a dose-limiting toxicity occurred, retreatment could be delayed for up to 14 days.

Table 1 Dose escalation schedule

Dose level	CA4P (mg/m^2)	Carboplatin (AUC, mg×min/mL)	No. patients
1	27	5	6
2	36	5	6
3	36	4	4

Treatment Assessment. Upon enrollment in the study, patients underwent a complete medical history, physical, and laboratory assessment. Thereafter, laboratory studies were obtained once to twice weekly. Tumor evaluations were done at screening and then after every two cycles for the first two assessments and then every three cycles. Criteria for response were based on the Response Evaluation Criteria in Solid Tumors (22). Patients were removed from the study upon disease progression.

Pharmacokinetic Sampling and Analysis. The pharmacokinetics of CA4P, CA4, combretastatin A-4 glucuronide (CA4G), and carboplatin were evaluated during cycle 1 in 16 patients. Blood samples were collected immediately before carboplatin administration and after the start of the infusion at 15, 28, 58, 69 minutes, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, and 24 hours. Carboplatin was administered over 30 minutes. CA4P was infused over 10 minutes starting at 60 minutes after the start of the carboplatin infusion. Urine collections were obtained at the following intervals: 0 to 3, 3 to 6, and 6 to 24 hours following the initiation of the carboplatin infusion.

Plasma concentration data for CA4P, CA4, CA4G, and carboplatin plasma ultrafiltrate concentrations were analyzed noncompartmentally using WinNonlin (Version 4.0, Pharsight Corporation, Mountain View, CA). Peak concentrations (C_{max}) were determined by visual inspection. The terminal elimination rate constants (λ_z) were determined by linear regression analysis of the terminal log-linear part of the concentration-time curve. The total area under the observed plasma concentration-time curve (AUC) and the area under the first moment curve values were calculated for each analyte from time zero to the last measured concentration, using the linear-log trapezoidal rule. AUC values were extrapolated from the last observed time point to infinity by dividing the last measured concentration by λ_z. Clearance was calculated by Dose/AUC and for carboplatin was normalized for bovine serum albumin. Steady-state volume of distribution was determined by the following equation:

$$\mathrm{Vd_{ss}} = (\text{infuse dose})(\mathrm{AUMC}) / (\mathrm{AUC})^2 - (\text{infused dose})(\mathrm{T}) / 2(\mathrm{AUC})$$

where $\mathrm{Vd_{ss}}$ is the steady-state volume of distribution, AUMC is the area under the first moment curve value, and T represents the infusion time.

The ratios of AUC values of CA4P to CA4 and CA4G were calculated for each patient. One-way ANOVA was done to test for evidence of linearity between AUC and C_{max} values for CA4P and CA4 at each dose level.

Statistical Analysis. Descriptive statistical analyses were done retrospectively using STATA (Version 7.0, Stata Corporation, College Station, TX). The carboplatin AUC was calculated using the Cockroft-Gault equation as an approximation of creatinine clearance ($\mathrm{CL_{CR\text{-}CG}}$; ref. 23):

$$\mathrm{CL_{CR-CG}(mL/min)} = \frac{\{[140 - \text{age(y)}] \times \text{weight(kg)}\} \times \{0.85 \text{ if female}\}}{[0.813 \times \text{serum creatinine concentration } (\mu\text{mol/L})]}$$

resulting in the following dosing formula:

$$\text{Dose} = \text{AUC}_{\text{CG}}\,(\text{mg min/mL}) \times (\text{CL}_{\text{CR-CG}} + 25)$$

Platelet trends were analyzed in the context of a previously published formula relating carboplatin dosage and changes in platelet count (24):

$$\text{Dosage}\,(\text{mg/m}^2) = (0.091)(\text{creatinine clearance}) / (\textit{body surface area})[(\text{pretreatment platelet count} - \textit{platelet nadir desired}) / (\text{pretreatment } \textit{platelet count})(100) - 17] + 86.$$

where the Cockroft-Gault estimation was employed for creatinine clearance.

RESULTS

Patient Characteristics. The demographic characteristics of the patients entered are shown in Table 2. Sixteen patients with a median age of 54 years were enrolled, of whom 15 had been treated previously with chemotherapy (median number of regimens, 2; range, 0-8). A total of 40 courses of CA4P and carboplatin were given for a median of 2 per patient (range 1-7).

Dose-Limiting Toxicity. Patients were enrolled at three dose levels (Table 1). At the first dose level of 27/5 (denotes CA4P mg/m^2/carboplatin AUC mg×min/mL), one of six patients experienced grade 3 neutropenia, which resolved after 6 weeks. This treatment delay was considered a dose-limiting toxicity. At the second dose level of 36/5, two of six patients experienced dose-limiting toxicity of grade 4 thrombocytopenia. One patient with head and neck cancer and prior radiation therapy and chemotherapy with two platinum-containing regimens experienced a platelet nadir of 7,000 cells/mm^3, required a transfusion, but had no bleeding complications. The second patient had advanced gastroesophageal junction adenocarcinoma and five prior chemotherapy regimens. His platelet nadir measured 2,000 cells/mm^3, which was accompanied by bleeding and required transfusion. These dose-limiting toxicities halted the dose escalation phase of the study and subsequent patients were enrolled at an amended dose level of 36/4, in which the carboplatin dose was reduced. Grade 2 and 3 thrombocytopenia occurred at the modified dose level. Further enrollment onto this study was halted based on the conclusion that a drug interaction on this schedule was responsible for the unexpectedly severe platelet toxicity, and that alternative schedules should be explored.

Table 2 Patient characteristics

Entered/evaluable	16/16
Male/female	11/5
Median age, y (range)	54 (26-75)
<65 y	11
≥65 y	5
Performance status	
0	5
1	10
2	1
Primary tumor site	
Anaplastic thyroid	2
Breast	2
Esophagus	2
Pancreas	2
Renal/renal pelvis	2
Other	6
Prior chemotherapy regimens	
0-1	4
2-4	9
≥5	3
Prior radiotherapy, yes/no	8/8

Table 3 Hematologic toxicity

(A) Hematologic toxicity during cycle 1

		Neutropenia (grade)					Thrombocytopenia (grade)				
Dose level	No. patients	0	1	2	3	4	0	1	2	3	4
1	6	3	—	2	1	—	1	2	—	3	—
2	6	3	—	—	1	2	2	1	—	1	2
3	4	2	1	—	1	—	1	1	1	1	—

(B) Worst hematologic toxicity (all cycles)

		Neutropenia (grade)					Thrombocytopenia (grade)				
Dose level	No. patients	0	1	2	3	4	0	1	2	3	4
1	6	2	—	3	1	—	—	2	—	3	1
2	6	3	—	—	1	2	2	—	—	2	2
3	4	2	1	—	1	—	—	2	1	1	—

Hematologic Toxicity. Hematologic toxicity occurred in patients at all dose levels and cycles of treatment. At the first dose level, three of six patients exhibited grade 2 or grade 3 neutropenia in cycle one (Table 3*A*). Three of six patients exhibited grade 3 thrombocytopenia. At the second and third dose levels, grade 3 and 4 neutropenia and thrombocytopenia were also common, as previously described, and summarized in Table 3*A*. Cumulative hematologic toxicity was moderate although the median number of cycles given per patient was small (Table 3*B*). An exception was one patient treated at the first dose level who had advanced breast cancer and eight prior chemotherapy regimens. In her fourth cycle, this patient experienced grade 4 thrombocytopenia which necessitated hospitalization and platelet transfusion.

Nonhematologic Toxicity. There was no grade 3 or 4 nonhematologic toxicity in cycle one. Nausea and vomiting were common but manageable (Table 4). Grade 2 neuropathy occurred in one patient at dose level one and a second patient at dose level two. Both patients had had prior platinum exposure and experienced a sensory neuropathy in the distal extremities. Of interest, grade 1/2 tumor pain was reported by five patients in cycle one at sites of known disease. It was transient, beginning within 1 hour of the CA4P infusion and resolved within 6 to 8 hours. These symptoms were similar in distribution, severity, and time course to those in the previously reported single agent CA4P study (13).

In subsequent cycles of therapy, various mild nonhematologic toxicities were observed (Table 5). Mild sensory neuropathy was seen in 6 patients in a total of 10 treatment cycles. Three patients experienced grade 1 perirectal itching at the time of the CA4P infusion, which resolved within 6 to 8 hours, as previously observed (13). One patient with renal cell carcinoma and abnormal renal function at baseline showed a grade 3 elevation in his serum

Table 4 Selected nonhematologic toxicity (cycle 1)

Dose level	No. patients	Infusion-related tumor pain (grade)				Neurosensory (grade)				Nausea (grade)				Vomiting (grade)			
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
1	6	4	2	—	—	3	2	1	—	2	3	1	—	3	3	—	—
2	6	4	2	—	—	5	—	1	—	3	2	1	—	3	3	—	—
3	4	3	—	1	—	3	1	—	—	2	1	1		3	1	—	—

creatinine. He improved with hydration and continued on study for an additional cycle after a dose reduction in carboplatin. Of note, no cardiopulmonary toxicities such as hypotension, electrocardiogram changes, dyspnea, or chest pain were observed.

CA4P/CA4 Pharmacokinetics. Complete plasma data were available for all 16 patients to determine pharmacokinetic variables of CA4P and its metabolites (Tables 6*A* and 6*B*). Twenty-four-hour urine collections were evaluable for measurement of carboplatin and CA4G in 14 of 16 patients.

The mean terminal elimination half-life of CA4P was 22 [range, 11-38; coefficient of variation (CV), 32%] minutes. The mean steady-state volume of distribution was 8.6 L (range, 4.3-16.2; CV, 40%). The mean systemic CA4P clearance was 43.8 (range, 19.62-117.6; CV, 52%) L/h.

Appearance of CA4 and CA4G in the plasma was detected within 9 minutes of beginning of parent drug infusion. Peak plasma levels of both metabolites were present within 5 minutes after the infusion of CA4P was complete. A mean of 66% of the administered dose of CA4P was excreted in the urine within the first 24 hours as glucuronide (CA4G). These kinetic characteristics are consistent with single-agent data (13).

Carboplatin Pharmacokinetics. Complete plasma data were available for 15 of 16 patients for estimation of pharmacokinetic variables for carboplatin. Table 7 summarizes pharmacokinetic data for carboplatin. The mean terminal elimination half-life of ultrafilterable carboplatin was 175 minutes and ranged from 81 to 306 minutes with a CV of 46%. The mean normalized steady-state volume of distribution was 10.6 L/m^2 (range, 6.2-13.8; CV, 29%). The mean normalized systemic carboplatin clearance was 58 mL/min/m^2 and ranged from 31 to 79 mL/min/m^2 with a CV of 29%. The mean ratio of observed and predicted carboplatin AUC was 1.16 (range, 0.888-1.675; CV, 18%). A mean of 63% of the administered dose of carboplatin was excreted in the urine within the first 24 hours. The percentage dose excreted ranged from 40% to 89%.

Patient Benefit. Six of the 16 patients showed stable disease as their best response. Four of these patients were on study for four cycles, reflecting the following tumor types: breast (2), head and neck, and mesothelioma. One patient with ovarian cancer remained on study for five cycles. A patient with cancer of the renal pelvis showed stable disease for 10 cycles. An extension protocol was written and Institutional Review Board–approved for this patient that reduced the carboplatin dose to an AUC of 4 mg min/mL and increased the CA4P to 54 mg/m^2. The patient was treated for an additional three cycles before progressing. Ten patients experienced progressive disease with two receiving only one treatment cycle.

Evidence for a Carboplatin/Combretastatin A-4 Phosphate Drug-Drug Interaction. Upon observation of dose-limiting thrombocytopenia, we conducted an unplanned analysis to elucidate a putative drug-drug interaction. Owing to thrombocytopenia being the dose-limiting toxicity of carboplatin, we compared carboplatin AUCs predicted by a modified Calvert formula (glomerular filtration rate approximated by the Cockroft-Gault equation) with measured AUCs. As seen in Table 8, the mean ratio of observed AUC to predicted AUC was 1.16, indicating a general trend of modest overestimation.

The relationship between the carboplatin dose and the platelet nadir has been previously described and prospectively validated (24). Accordingly, we calculated the predicted platelet nadirs in a retrospective fashion and compared these results to those observed for each patient in the study. As shown in Table 8,

Table 5 Worst nonhematologic toxicity (per course)

Grade	No. courses (%)				
	0	1	2	3	4
Fatigue	21 (52)	5 (12)	13 (32)	1 (2)	—
Nausea	27 (68)	11 (28)	2 (5)	—	—
Sensory neuropathy	30 (75)	6 (15)	4 (10)	—	—
Vomiting	30 (75)	10 (25)	—	—	—
Infusion pain	33 (82)	6 (15)	1 (2)	—	—
Creatinine elevation	36 (90)	—	3 (8)	1 (2)	—
Constipation	37 (92)	—	3 (8)	—	—
Fever, flu-like symptoms	38 (95)	1 (2)	1 (2)	—	—
Hemagglutinin	38 (95)	2 (5)	—	—	—
Anorexia	38 (95)	2 (5)	—	—	—
Aspartate aminotransferase/alanine aminotransferase elevation	38 (95)	2 (5)	—	—	—
Abdominal pain	39 (98)	1 (2)	—	—	—
Diarrhea	39 (98)	1 (2)	—	—	—
Rash	39 (98)	1 (2)	—	—	—

Table 6 Pharmacokinetic variables

(A) Pharmacokinetic variables of CA4P in patients during cycle 1

Dose group (mg/m^2)	No. patients	C_{max} (ng/mL)	$AUC_{0-\infty}$ (h×ng/mL)	CL (L/min)	$t_{1/2}$ (min)	Vd_{ss} (L)
27	6	15.24 ± 6.3	182 ± 50.7	0.72 ± 0.2	21.17 ± 3.3	7.67 ± 3.7
36	6	14.62 ± 4.4	226.9 ± 92.5	0.83 ± 0.6	22.00 ± 7.7	8.77 ± 2.7
36	4	21.74 ± 7.6	273.9 ± 135	0.61 ± 0.2	22.00 ± 11.2	9.75 ± 4.6

(B) Pharmacokinetic variables of CA4 in patients during cycle 1

Dose group (mg/m^2)	No. patients	C_{max} (μmol/L)	$AUC_{0-\infty}$ (h×ng/mL)	CL (L/min)	$t_{1/2}$ (min)	Vd_{ss} (L)
27	6	1.5 ± 0.6	49.1 ± 8.0		90.33 ± 19.1	
36	6	1.4 ± 0.5	63.4 ± 20.1		78.33 ± 26.3	
36	4	3.8 ± 4.2	78.27 ± 40.5		74.0 ± 29.2	

NOTE. Mean ± SD.

the predicted mean platelet nadir was 127 whereas the actual mean platelet nadir was 117, although there was a greater SD of actual platelet nadir counts with lower than predicted platelet counts having clinical significance for two patients.

Finally, we investigated whether the greater than predicted carboplatin exposure could be explained in a dose-dependent fashion on CA4 exposure, the active metabolite of CA4P. We constructed a two-way simple linear regression model in which the observed to predicted ratio of carboplatin AUC was set as the dependent variable and CA4 AUC was defined as the independent variable. The two-sided t test for the model was 3.13, translating into a statistically significant P value of 0.008. A scatter plot of the observed to predicted ratio of carboplatin and CA4 AUC (mg min/mL) is shown in Fig. 1 with the accompanying fitted regression line.

DISCUSSION

Myelosuppression, an expected side effect of carboplatin, was not observed in the reported single-agent studies for CA4P. Dowlati et al. (19) treated 25 patients with advanced cancer with CA4P at escalating doses, administering a 10- to 60-minute infusion every 3 weeks. Tumor pain occurred in 10% of cycles. Four dose-limiting toxicities were observed at doses ≥60 mg/m^2, including two episodes of acute coronary syndrome. Rustin et al. (25) treated 34 patients with 10-minute weekly infusions for 3 weeks followed by a 1-week rest. The only toxicity attributed to doses up to 40 mg/m^2 was tumor pain. At higher doses, dose-limiting toxicities included reversible ataxia, motor neuropathy, vasovagal syncope, and bowel ischemia. In a previous study at the University of Pennsylvania, we treated patients with CA4P as a 10-minute infusion daily for 5 consecutive days, repeated every 3 weeks (13). Thirty-seven patients received 133 treatment cycles at dose levels ranging from 6 to 75 mg/m^2 daily. Severe pain at sites of known tumor was dose limiting at 75 mg/m^2. Dose-limiting cardiopulmonary toxicity (syncope, dyspnea/hypoxia) was noted in two patients. Other toxicities included hypotension, ataxia, dyspnea, nausea/vomiting, headache, and transient sensory

Table 7 Pharmacokinetic variables of carboplatin during cycle 1, by patient

Patient no.	C_{max} (ng/mL)	K_{elim} (min^{-1})	$t_{1/2}$ (min)	Vd_{ss} (L/m^2)	CL (mL/min/m^2)	$AUC_{0-\infty}$ (mg×min/mL)	AUC	AUC_{obs}/AUC_{pred}	% Urine excretion
1	39,200	0.005	135	13	72.9	5.8	5	1.16	73.4
2	56,700	0.0058	118	8.3	59.56	6.34	5	1.268	53.3
3	46,900	0.0024	283	13.8	48.88	5.07	5	1.014	61.6
4	20,800	0.0033	211	17	65.64	5.11	5	1.022	48.8
5	62,500	0.0045	156	7.1	45.1	6.02	5	1.204	74.9
6	45,600	0.0066	105	8.5	73.34	4.76	5	0.952	81.9
7	20,900	0.0023	306	12.5	30.91	6.28	5	1.256	
8	48,500	0.0086	81	9.4	91.22	4.44	5	0.888	67.1
9	30,600	0.0026	263	11.8	37.11	6.46	5	1.292	49.3
10	47,300	0.0059	118	8.9	63.16	5.06	5	1.012	51.7
11	55,200	0.0063	111	6.2	57.03	5.35	5	1.07	51.8
12	27,600	0.0028	248	13.8	41.56	7.26	5	1.452	89.3
13	72,800	0.0057	122	7.1	52.49	6.7	4	1.675	40.4
14	36,600	0.0025	277	11	46.18	4.92	4	1.23	
15	47,200	NE	NE	NE	NE	NE	NE	NE	72.9
16	35,200	0.0076	91	9.9	78.77	3.78	4	0.945	67.3
Mean	43,350	0.0048	175	10.55	57.59	5.56	4.80	1.16	63.13
Median	46,250	0.0050	135	9.90	57.03	5.35	5.00	1.16	64.36
SD	14,559	0.0021	80	3.06	16.77	0.95	0.41	0.21	14.36
SE	3,639	0.0005	21	0.79	4.33	0.98	0.43	0.05	0.05
CV%	33.6	42.9	45.8	28.96	29.12	17.07		18.24	18.24

neuropathy. Taken together, these studies suggest that single-agent CA4P is well tolerated without myelosuppression at doses less than 40 mg/m^2, a level that was not exceeded in our study.

The thrombocytopenia we observed is consistent with the dose-limiting toxicity of single-agent carboplatin. However, the degree of toxicity was more than expected and required dose modifications to levels much lower than conventionally used. Carboplatin-induced thrombocytopenia is directly related to drug exposure as measured by AUC (24, 26). We approached the analysis of this toxicity in three ways to determine whether a drug-drug interaction was responsible. We analyzed the pharmacokinetics of carboplatin in relation to expected values as defined by Calvert et al. (27); we analyzed the pharmacodynamic effect on platelet nadirs in relation to expected profiles as presented by Egorin et al. (28); and we determined the relationship of the pharmacokinetic deviation to exposure to the active metabolite of CA4P, CA4.

Whereas the mean observed to predicted ratio of carboplatin AUC of 1.16 may not seem dramatic at first inspection, it is notable when considered in the context of the clinical literature. It has been shown that when the Cockroft-Gault estimate of glomerular filtration rate is employed, the dose of carboplatin may be underestimated by as much as 20% (29). Other investigators have reported similar underestimates (30). By these precedents, it would have been reasonable to expect an observed to predicted ratio of 0.8, considerably less than the 1.16 we observed.

As illustrated in Table 8, a formula validated by Egorin et al. (24) for heavily pretreated populations illustrates that pretreatment platelet counts and the carboplatin dose are reasonable predictors of platelet nadir, even when Cockroft-Gault approximation of creatinine clearance is employed. Our analysis showed that for a given dose of carboplatin, platelet toxicity was greater than expected. This resulted in some patients with marginal (100-150,000) baseline platelet counts having nadirs in the grade 4 range. The patient cohort was not especially heavily pretreated, but pretreatment is a factor that should be considered in an analysis such as this. The results are, however, consistent with those from the pharmacokinetic analysis.

Table 8 Predicted and actual platelet nadirs in cycle 1

Patient	Observed platelet nadir	Predicted platelet nadir
1	43	118
2	32	69
3	130	133
4	30	59
5	140	101
6	206	195
7	363	256
8	409	240
9	22	127
10	7	55
11	109	70
12	2	37
13	48	213
14	50	120
15	91	100
16	194	142
Mean	117	127
Median	70	119
SD	122	67

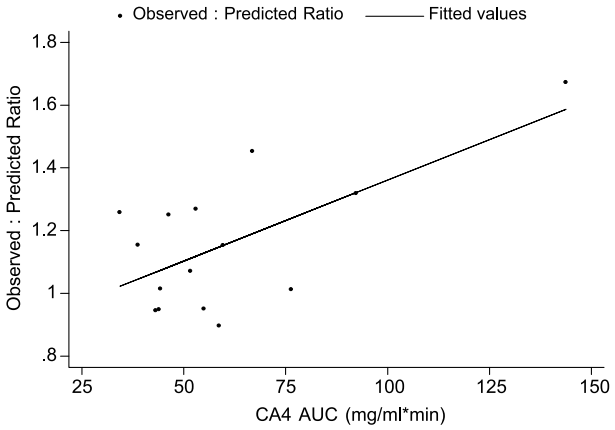

Fig. 1 Plot of observed/predicted ratio of carboplatin AUC by CA4 AUC (mg×min/mL) with fitted regression line (P = 0.008).

Our analysis of the observed-to-predicted carboplatin AUC ratio to CA4 exposure was highly suggestive of a pharmacokinetic interaction (Fig. 1). The data indicate that CA4 modulates carboplatin pharmacokinetics, increasing the AUC. This observation favors the hypothesis that the thrombocytopenia we observed in this study resulted from direct carboplatin toxicity. We cannot exclude the possibility of a drug-drug interaction at the level of the bone marrow. It has been shown that another tubulin-binding agent, paclitaxel, antagonizes the cytotoxicity of carboplatin in the megakaryoblast cell line MEG-01, perhaps accounting for the "platelet-sparing effect" described for this drug combination (31, 32). By extension, it is possible that CA4P and carboplatin similarly interact, although without a platelet-sparing effect. The observed changes in the pharmacokinetics would seem to offer a sufficient explanation without invoking a direct pharmacodynamic interaction.

In conclusion, we explored the combination of CA4P and carboplatin, given on day 1 of a 21-day cycle, in 16 patients with advanced cancer. Three dose levels were studied, although thrombocytopenia halted the dose escalation and ultimately led to the early termination of this study. The basis for this decision was the observation that to give these drugs at tolerable doses in combination, each drug would need to be dosed at levels substantially lower than standard, with concomitant potential for increased variability in pharmacodynamic effects. Past clinical experience with CA4P suggests that doses less than 50 mg/m^2 have relatively small effects on tumor perfusion (14). Our data do, however, suggest that these doses are sufficient to alter carboplatin disposition. Similarly, a carboplatin dose of 4 is commonly regarded as subtherapeutic, although in this combination, the actual exposure was equivalent to that of a higher dose. Rather than trying to modulate the interaction and its variability (Fig. 1), it seems wiser to modify the schedule of administration of these agents in future studies.

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