A Phase I Pharmacokinetic and Translational Study of the Novel Vascular Targeting Agent Combretastatin A-4 Phosphate on a Single-Dose Intravenous Schedule in Patients with Advanced Cancer


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ABSTRACT

Combretastatin A-4 phosphate (CA4P) is a novel antitumor vascular targeting agent, the first agent of this class of compounds to enter the clinic. We performed a Phase I trial to determine the maximum-tolerated dose, safety, and pharmacokinetic profile of CA4P on a single-dose i.v. schedule. We also obtained preliminary data on its effect on tumor blood flow using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) techniques and cell adhesion molecules at the higher-dose levels. Twenty-five assessable patients with advanced cancer received a total of 107 cycles over the following dose escalation schema: 18, 36, 60, 90 mg/m² as a 10-min infusion and 60 mg/m² as a 60-min infusion at 3-week intervals. There was no significant myelotoxicity, stomatitis, or alopecia. Tumor pain was a unique side effect, which occurred in 10% of cycles, and there were four episodes of dose-limiting toxicity at dosages ≥60 mg/m², including two episodes of acute coronary syndrome. Pharmacokinetics revealed rapid dephosphorylation of the parent compound (CA4P) to combretastatin A4 (CA4), with a short plasma half-life (~30 min). A significant (P < 0.03) decline in gradient peak tumor blood flow by DCE-MRI in six of seven patients treated at 60 mg/m² was observed. A patient with anaplastic thyroid cancer had a complete response and is alive 30 months after treatment. The toxicity profile is consistent with a drug that is “vascularly active” and devoid of traditional “cytotoxic” side effects. Dosages ≤60 mg/m² as a 10-min infusion define the upper boundary of the maximum-tolerated dose.

INTRODUCTION

CA4P (disodium combretastatin-A-4–3-0-phosphate) is a novel antitumor vascular targeting agent that is the first of a series of combretastatin analogs to enter the clinic (1–7). CA4P is a prodrug and is rapidly dephosphorylated to the active compound CA4. Combretastatin has a broad range of cytotoxicity against several tumor cell lines and is effective in mouse xenograft models (8–12). The precise mechanism of action of combretastatin is unknown. The drug is structurally similar to colchicine (Fig. 1), binds the colchicine-binding site on tubulin, and inhibits tubulin polymerization (13–15). Histological studies have shown that several tubulin-binding agents (colchicine, podophyllotoxin, vincristine, and vinblastine) as well as other antineoplastic agents (tumor necrosis factor α, flavone acetic acid, and related compounds) can induce vascular damage within tumors but only at doses approximating the MTD, which has limited their applicability (6, 16–25). In contrast, CA4P induces vascular shutdown within tumors at doses less than one-tenth of the MTD in murine models (7).

In vitro studies indicate that a short exposure of quiescent endothelial cells to CA4 results in profound long-term cytotoxic effects that become evident when the cells are stimulated to proliferate (7). In animal studies, CA4 induces immediate and selective shutdown of the tumor vasculature through the induction of endothelial cell apoptosis (7, 26–28). The peak effect on tumor blood flow is demonstrable within 6 h after administration and, depending on dose, is sustained for 24 h (7). Vascular shutdown within the tumor induces central necrosis (7, 29). In rats, the dose that caused severe toxicity or death in 10% (STD10) of the animals was 360 mg/m². Single-dose studies in the dog did not cause lethality or serious irreversible toxicity; however, reversible bradycardia was observed at 25 mg/m².

On the basis of the above preclinical rationale, we embarked on a Phase I trial of CA4P administered to patients with advanced solid tumors as a single i.v. dose at 3-week intervals. The primary objective of our trial was to determine the MTD, safety, and pharmacokinetic profile of CA4P on this schedule. We also set out to obtain preliminary data on antitumor activity, its effects on tumor blood flow using DCE-MRI techniques, and measurement of circulating levels of cell adhesion molecules at the higher dose levels. A conservative starting dose of 18 mg/m² was selected and initially given as a single 10-min infusion; a second cohort of patients received the drug as a 60-min infusion. Observations from our study helped define the boundaries of the MTD level across other studies (30, 31).

PATIENTS AND METHODS

Selection Criteria

Patients with histologically documented advanced solid tumors for whom no standard effective therapy was available were entered into the protocol. Signed informed consent was obtained from all of the patients in keeping with United States Food and Drug Administration and institutional guidelines. All of the patients were at least 18 years of age, had Eastern Cooperative Oncology Group PS values ≤2, a life expectancy of at least 12 weeks, and had received no prior chemotherapy, radiation, or other investigational agent for 4 weeks before entry (32). Adequate bone marrow function (absolute neutrophil count, ≥1500/µl; platelets, ≥100,000/µl; hemoglobin, ≥9.0 g/dl), clotting parame-

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Drug Administration and Dose Escalation

CA4P was protected from light exposure and administered in escalating doses initially as a 10-min, and subsequently as a 60-min infusion in a dimly lit room every 21 days. A minimum of three new patients were entered at each dose level. Dose escalation was allowed to proceed if no grade 3 or higher nonhematological toxicity (except vomiting, suboptimally treated diarrhea, fatigue, alopecia, or treatment-induced tumor pain), grade 4 hematological toxicity, QTc interval prolongation of ≥500 ms or increase of 25% over baseline, or treatment delay of more than 2 weeks because of unresolved toxicity in patients who experienced at least grade 3 or greater hematological toxicity, or any grade 2 nonhematological toxicity. Any of these toxicities were considered to be a DLT. For purposes of this trial, “DLT events” were defined during the first cycle of therapy. In the event that one of three patients encountered DLT, three more patients were treated at the same dose level. Toxicity was reported using National Cancer Institute common toxicity criteria, including the category for tumor pain.10

Drug Formulation

Lyophilized CA4P was supplied by ILEX Oncology, Inc. (San Antonio, TX) in aseptically filled 10-ml vials containing 100 mg of CA4P. The drug was stored at 2–8°C. The formulation was reconstituted with 10 ml of water for injection to make a concentration of 10 mg/ml, and was further diluted in sodium chloride 0.9% (minimum volume, 100 ml; maximum, 150 ml).

Patient Follow-Up and Assessment

Documentation of disease extent was done within 4 weeks, history and physical examination within 2 weeks; and complete laboratory evaluation (complete blood counts with differential, prothrombin time/plasma thromboplastin time, serum chemistries, and urinalysis) within 1 week of study enrollment. EKGS were performed hourly through the first 4 h and repeated 24 h after dosing. Hourly 12-lead EKGS beyond 4 h and continuous cardiac monitoring was performed for patients with QTc, interval prolongation of ≥480 ms and/or a QTc interval increase of ≥25% from baseline demonstrated within the first 4 h after dosing. Patients were transferred to a telemetry unit for QTc prolongation ≥500 ms. QTc intervals were manually measured by one author of this study (B.S.) and recalculated using Bazett’s formula [QTc = QT / (RR)1/2]. Laboratory assessments were performed at weekly intervals during all of the cycles, and clinical restaging evaluation was performed every 2 cycles. Standard solid tumor response criteria were used (32). A protocol amendment-incorporated DCE-MRI assessment of tumor blood flow at baseline and 4–6 h after infusion on the first day of cycle number one, starting at dose level 3 (60 mg/m²). Not all of the patients were consecutively evaluated, because they had to have tumors amenable to MRI. A final protocol amendment enhanced cardiac monitoring for the last 3 patients entered, which included 3 EKGS on the day of treatment to obtain an average baseline QTc interval before dosing, continuous bedside and Holter monitoring, and transmission of real-time electrophysiological data to a core laboratory.

Pharmacokinetic Sampling and Analytic Method

The pharmacokinetics of CA4P and its primary metabolic products were evaluated on the first dose (cycle 1). Thirteen serial blood samples were collected at the following time points: immediately before drug administration, 1 min prior to the end of infusion (10- or 60-min infusions), and at 5, 15, 30, 45, 60, and 90 min, and at 2, 4, 8, 12, and 24 h after infusion. Simultaneously timed urine collections were obtained at: 0–4, 4–8, 8–12, and 12–24 h. Plasma and urine samples were analyzed by high-performance liquid chromatography for parent CA4P and for CA4, and CA4G according to published methods (33).

Pharmacokinetic Methods

All patient CA4P, CA4 and CA4G plasma concentration-time data sets were individually evaluated by a noncompartmental method. The absolute dose (nonnormalized) in micromoles was used as the input variable for all analyses. Because the molecular weight of CA4P is 440.3, doses given in mg were multiplied by 2.2712 to convert the values to micromoles. AUC, data provided in noncompartmental analyses, were estimated by the linear trapezoidal rule with extrapolation using the 1/observed concentration² weight scheme for determination of the terminal elimination rate constant. One way ANOVA was performed to test whether the normalized AUC and the Cmax, values for the active metabolite (CA4) increased with dose and whether there was any evidence of linearity using a two-sided test.

DCE-MRI Assessment of Tumor Blood Flow

Data Acquisition. Patients were studied using a 1.5T scanner (Siemens Magnetom Vision). Perfusion studies were obtained with a single-slice FLASH sequence (TR = 10, TE = 4, FA = 30°, slice thickness = 10 mm, matrix = 128 × 256, FOV = 16 × 25 cm-35 × 35 cm) repeated 128 times, once every 2.6 s. The first 10 images were obtained without contrast enhancement, after which, an ~10-s bolus of gadolinium-DTPA contrast agent (Magnevist, Berlex Laboratories) was administered i.v. Gadolinium is a paramagnetic agent that, proportional to its concentration, decreases both the longitudinal magnetization recovery time (T1), and the transverse magnetization decay time (T2*) in tissue. In MRI, shorter T1 increases signal intensity, whereas shorter T2* decreases it. However, for the FLASH sequence described above, the signal decrease caused by T2* shortening is negligible (less than 2% signal change over the range of obtainable Gd-DTPA tissue concentrations at the study dosage). It can also be shown that, over the same range of tissue concentrations, the relative signal increase (postcontrast/baseline) has an essentially linear dependence on tissue concentration. This allows us to interpret proportional change in signal intensity over time as equivalent to proportional change in contrast agent tissue concentration (34). DCE-MRI was performed twice on each patient; once to obtain a pretreatment baseline, and once 4–6 h after the infusion of CA4P. The slice position and orientation between days within each patient was carefully matched to obtain data from the same region of interest in the tumor.

Post-processing and Analysis. Image processing was performed off-line using a custom software package that permitted a trained rater to view all 128 images in a study and to interactively identify the tumor region of interest. Mean tumor signal intensity versus time curve for each subject was generated.
These curves were then converted to proportional enhancement versus time curves by dividing by the precontrast mean signal intensity. Four measures were obtained from each set of curves: Peak rate of change [Gradient peak ($G_{peak}$)]; time to gradient peak ($t_{gp}$), measured from the first arrival of contrast agent; time until gradient drops to 10% of $G_{peak}$ ($t_{gp10}$), also measured from the arrival of the contrast agent bolus; and value of the proportional enhancement ($E$) measured at $t_{gp10}$ (Enhancement at 10% of Gradient Peak).

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![Graph showing tumor perfusion curves and measures](image)

**Fig. 2. Measures from tumor perfusion curves.**
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**Cell Adhesion Molecules**

Plasma samples collected from patients prior to and at 1, 2, 4, 8, 12, and 24 h after CA4P infusion were analyzed for levels of sICAM-1, sVCAM-1, and soluble (s)E-selectin using commercially available ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. All of the samples were assayed in triplicate. A two-tailed paired $t$ test was performed on intragroup comparisons.

**Pharmacodynamic Analyses**

The relationship between CA4P and CA4 systemic exposure and toxicity (QTc changes on EKG) were explored. Maximum concentrations ($C_{max}$) and area under the concentration-versus-time curve (AUC) obtained were used as indices of systemic exposure. The only relevant demonstrable parameter of toxicity assessed was QTc changes. The relevant parameter of changes in blood flow assessed by MRI flow images that was evaluated included the percentage decrease in $G_{peak}$, which was calculated as:

$$\text{Decrease in } G_{peak} = \frac{100\% \times (\text{pretreatment } G_{peak} - \text{posttreatment } G_{peak})}{\text{Pretreatment } G_{peak}}$$

Relationships between CA4P and CA4 exposure and pharmacodynamic effect were assessed by univariate correlation analysis.

**RESULTS**

**Patient Characteristics.** Twenty-five patients with a variety of solid tumors (six colorectal and five thyroid cancer) were treated (Table 1). The majority of patients had excellent PS and had prior treatment.

<table>
<thead>
<tr>
<th>Table 1 Patient characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients (male/female)</td>
</tr>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>Performance status (ECOG* scale)</td>
</tr>
<tr>
<td>Primary tumor</td>
</tr>
<tr>
<td>Prior therapy</td>
</tr>
</tbody>
</table>

*ECOG, Eastern Cooperative Oncology Group; NSCLC, non-small cell lung cancer.
Dose Escalation. A total of 107 cycles of therapy were administered and were fully evaluated for toxicity (Table 2). The dose escalation proceeded swiftly until the 10th patient was treated at dose level 4 (90 mg/m²). This patient had dose-limiting pulmonary toxicity. The second patient treated at this dose level developed coronary vasospasm during cycle 2, which, although reversible, was considered dose limiting. The protocol was amended to analyze a cohort of patients in which the CA4P was administered as a 60-min infusion (60 mg/m², dose level 3A) to determine whether the infusion duration would have an effect on the acute toxicity profile observed with the 10-min infusion schedule.

Toxicity. The toxicity encountered over the CA4P dose-escalation was limited to short-term, acute side effects that resolved by 24 h. There were minimal cumulative side effects, except mild and, rarely, moderate fatigue. Most striking was the absence of traditionally cytotoxic side effects (e.g., myelosuppression, stomatitis, and alopecia). There were only three episodes of clinically meaningful myelosuppression (grade 3 each: anemia, neutropenia, and thrombocytopenia) over 107 courses of therapy. There was no evidence of cumulative myelosuppression in the three patients who received 10, 15, and 24 cycles of treatment over 10, 12.5, and 18.5 months of therapy, respectively.

The clinical toxicity observed during the first cycle of therapy is summarized in Table 3 and consisted of a variable symptom complex, across all of the dose levels, that included flushing, hot flashes, pruritus, headache, diarrhea, cramping abdominal pain, and dose-related nausea and vomiting. Transient, clinically insignificant, changes in blood pressure and heart rate were also seen. Four episodes of atypical chest pain (≤ grade 2) were encountered, and all were without ischemic changes on EKG.

There were seven episodes of grade 1 QTc interval prolongation seen on the hourly 12-lead EKG tracings (all at 60 mg/m², with four on the 10-min and three on the 60-min infusion schedules). When these QTc intervals were manually determined and recalculated using Bazett’s formula, only a single QTc interval prolongation was greater than 500 ms on subsequent 5 cycles of therapy for safety reasons for a total course of 24 cycles of therapy.

### Table 2. Dose escalation (see “Dose Escalation” section for details)

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Combretastatin A4 Phosphate (mg/m²) (i.v. 10-min infusion)</th>
<th>No. of patients</th>
<th>No. of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>9</td>
<td>40</td>
</tr>
<tr>
<td>3A</td>
<td>60 (1-h infusion)</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>25</td>
<td>107</td>
</tr>
</tbody>
</table>

* DLT encountered in first patient (during cycle 1) and second patient (during cycle 2) treated at this level.

** Third patient dose reduced to 60 mg/m² for cycle 2 over next 18 cycles and subsequently to 50 mg/m² for last 5 cycles of therapy for safety reasons for a total course of 24 cycles of therapy.

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>No. of patients</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 18</td>
<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>2) 36</td>
<td>3</td>
<td>Hot flash</td>
</tr>
<tr>
<td>3) 60</td>
<td>9</td>
<td>Flush</td>
</tr>
<tr>
<td>3A) 60 (60 min)</td>
<td>7</td>
<td>Hot flash</td>
</tr>
<tr>
<td>4) 90</td>
<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>5) 120</td>
<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>6) 180</td>
<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>7) 240</td>
<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>8) 300</td>
<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>9) 360</td>
<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>10) 420</td>
<td>3</td>
<td>Flush</td>
</tr>
</tbody>
</table>

** DLT encountered: acute reversible myocardial infarction without chest pain.

### Table 3. Overview of clinical toxicity by Common Toxicity Criteria34 encountered over dose escalation for cycle 1 (see “Toxicity” section for details)

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>No. of patients</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 18</td>
<td>3</td>
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<td>2) 36</td>
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<td>3A) 60 (60 min)</td>
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<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>5) 120</td>
<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>6) 180</td>
<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>7) 240</td>
<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>8) 300</td>
<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>9) 360</td>
<td>3</td>
<td>Flush</td>
</tr>
<tr>
<td>10) 420</td>
<td>3</td>
<td>Flush</td>
</tr>
</tbody>
</table>

* Abd. cramps, abdominal cramps; prolong., QTc prolongation.

A unique toxicity encountered in this study was self-limited tumor pain associated with the infusion of CA4P. In all instances, this occurred at the 60-mg/m² dose level both on the 10- and 60-min schedules occurring within 2–5 h after dosing. The pain was responsive to narcotic analgesia and completely resolved 24 h after drug administration. Of the 11 episodes of tumor pain at grade 2 or higher, 4 occurred with the 10-min infusion schedule (10% of cycles) and 7 occurred with the 60-min infusion schedule (47% of cycles). The two episodes of grade 4 tumor pain in two patients occurred during the first cycle of therapy, in each case starting within 10–15 min of completion of the infusion and lasting for several h. One patient had extensive intra-abdominal and pelvic tumor from metastatic colon...
carcinoma, and the other had metastatic tumor involvement of the right hip and pelvis from an osteosarcoma.

Four episodes of DLT were encountered during the course of this study. The first two occurred at dose level 4 (90 mg/m²). A 66-year-old woman with advanced ovarian cancer and pleural involvement developed grade 3 shortness of breath, several h after CA4P infusion. There were no significant changes on EKG, oxygen saturation remained stable, and there was no evidence of pulmonary embolism. The episode resolved within 24 h. The second patient, a 57-year-old man with pancreatic cancer and no cardiac risk factors, developed crushing substernal chest pain 80 min after infusion of CA4P during cycle 2. The EKG was consistent with an acute myocardial infarction. Within 22 min of the episode the patient underwent cardiac catheterization, which demonstrated minimal occlusion of the distal left anterior descending coronary artery that was not amenable to further intervention. He was managed medically and recovered without further incident. The EKG normalized, and there was no elevation of creatine phosphokinase-MB fraction (CPK-MB) bands or troponin levels. An echocardiogram performed later the same day showed normal myocardial wall motion. The patient was thought to have acute coronary vasospasm related to the CA4P infusion, which was considered grade 4, dose-limiting cardiac ischemia.

Two patients treated at dose level 3 (60 mg/m², 10-min. infusion) had DLT. A 53-year-old man with anaplastic thyroid cancer and bulky neck mass developed grade 2 tumor pain within 30 min after the infusion of CA4P, which required i.v. narcotic analgesia. He became acutely ill, with respiratory stridor, hypoxia, and stupor (grade 3 pulmonary toxicity), which were reversible with naloxone. Direct laryngoscopy confirmed a patent airway. At 24 h after dosing, the patient was found to have a prolonged QTc interval on 12-lead EKG. The patient was discharged from the hospital on day 4 after CA4P treatment with resolution of QTc interval prolongation. Eleven days after CA4P infusion the patient was found unresponsive at home and died. Autopsy examination confirmed progressive metastatic anaplastic thyroid carcinoma. Examination of the heart was without evidence of clinically significant coronary artery disease. The last patient enrolled, a 77-year-old man with metastatic anaplastic thyroid carcinoma, developed asymptomatic acute ST-T wave changes consistent with myocardial ischemia 21 h after CA4P infusion. This patient had DLT. A 53-year-old man with anaplastic thyroid cancer and bulky right hip and pelvis from an osteosarcoma.

Seven of 10 patients studied had paired DCE-MRI assessments of tumor blood flow suitable for analysis. A summary table of all variables, pre- and posttreatment, and the change observed for each patient is shown in Table 5. Time to gradient peak, tgp, was not statistically significantly different between pre- and posttreatment session, indirectly supporting the conclusion that there were no systematic differences in contrast agent administration (Wilcoxon Z = 0.730; P = 0.47). Gradient peak (Gpeak), the flow-dependent parameter least sensitive to variations in EES volume fraction, was statistically significantly reduced after treatment (Wilcoxon Z = 2.20; P = 0.028), as was enhancement E, (Z = -2.20; P = 0.028). This was expected because both parameters increased monotonically with blood flow in simulations in the two-compartment model simulations. The last parameter, tgp10, was not statistically different between pre- and posttreatment scans, although this could well be attributable to the nonlinear relationship with flow and the sensitivity of tgp10 to possible changes in both the EES volume fraction and the arterial input function.

Cell Adhesion Molecules. Twelve patients had measurements of sICAM, sVCAM, and sE-selectin obtained immediately preinfusion of CA4P and at 1 and 24 h postinfusion. Paired comparisons (Table 6) of pretreatment levels of sICAM (451 ± 358) to posttreatment levels at 1 h (477 ± 373) and 24 h (499 ± 392) showed a significant increase (P = 0.02 for pretreatment levels versus both 1-h and 24-h levels). No statistical difference was seen between 1-h and 24-h postinfusion levels. Similarly no significant change was seen at the three measured time points for sVCAM or sE-selectin.

Pharmacokinetic Correlations. No correlation was observed between percentage of change in QTc, and dose level (r = -0.1788; P = 0.4). An inverse correlation was seen between both CA4P and CA4 Cmax concentrations and percentage decrement in Gpeak (Fig. 4; P = 0.05; r = 0.543). However no correlation was seen between AUC of CA4P or CA4 and changes in Gpeak.

Antitumor Activity. A 55-year-old man with refractory metastatic anaplastic thyroid carcinoma, treated at dose level 3 (60 mg/m², 10-min. infusion), had a complete response. At the time of study enrollment, there was persistent abnormality in the left lobe of the thyroid extending into the mediastinum on computed tomography scan, and palpable left neck nodal involvement (3.0 cm). By the end of cycle 2, there was complete resolution of the palpable left neck node, and after eight cycles of therapy, there was complete resolution of abnormalities in the neck on computed tomography scan consistent with a clinical complete response. The patient subsequently underwent exploratory neck dissection to excise micrometastatic disease. At the time of surgery, there was no evidence of residual disease in the bed of the thyroid, confirming a pathological complete remission. The patient then received two additional cycles of therapy beyond com-

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complete remission and has remained disease free for more than 30 months since completing therapy.

Two other patients experienced prolonged freedom from progression of their disease while receiving CA4P on the 10-min infusion schedule. Patient 12 with metastatic colon carcinoma received a total of 24 cycles of therapy over 19 months. She received her first dose at 90 mg/m², which was decreased to 60 mg/m² for 18 cycles and, subsequently, to 50 mg/m² for the next 5 cycles. Patient 23 with metastatic medullary thyroid cancer received a total of 15 cycles of therapy with freedom from progression of her disease for over 12 months. This patient was initially treated at 60 mg/m² for the first cycle and her dose was reduced to 50 mg/m² for the remainder of her course. Dose reduction in these patients was thought prudent for safety reasons because of cardiac side effects that were encountered during the study in the 11th and 25th patients enrolled.

A patient with metastatic renal cancer received eight cycles of therapy at 18 mg/m² and had stable disease for 6 months. Another patient with non-small cell lung cancer had a 34% reduction in measurable disease after two cycles of therapy, but developed progressive disease after four cycles at 36 mg/m².

**DISCUSSION**

In 1991, Denekamp and Hill (36) framed the concept of vascular targeting. The target is either the endothelial cells lining tumor vessels or a protein product elaborated by tumor endothelium (37, 38). Antitumor effects in this instance are mediated either by induction of endothelial and/or subsequent tumor cell apoptosis or via vascular shutdown with resultant hemorrhagic necrosis (39, 40). There are important biological differences between normal and tumor blood vessels, which provides a scientific rationale for vascular targeting and therapeutic exploitation. These differences include the constant remodeling of tumor vessels as opposed to the quiescent endothelium of normal vasculature, a lack of associated pericytes, and the greater vascular permeability of tumor vessels (39–43).

The precise mechanism of combretastatin induction of vascular shutdown is not clear. It is postulated that cytoskeletal changes in endothelial cells, as a result of tubulin-binding, may contribute to endothelial conformational changes leading to increased tumor-vessel permeability and the disruption of blood flow (39). Preclinical studies identified marked diminution in tumor blood flow, on the order of two logs, with only modest decreases in systemic blood flow in normal tissues, in which the greatest reduction occurred in the spleen (27). We confirmed a significant diminution in the gradient peak tumor blood flow in 6 of 7 patients treated at the 60 mg/m² dose level. This preliminary observation confirms the potential to perturb tumor blood flow with the systemic administration of an antivascular targeting agent and is consistent with other observations with this agent (44).

There is also an apparent inverse correlation of CA4 plasma concentration and percentage of decrement in gradient peak, which may be attributable to the patient with the highest C_{max} (Fig. 4). This paradox is consistent with preclinical antiangiogenesis model systems, in
which the greatest antiangiogenic effects are, in fact, seen at the lowest drug concentrations (45, 46).

DCE-MRI blood flow studies are based on the rate of uptake of contrast into the EES from plasma across the endothelium. In the general case, this is governed by two factors: the permeability-surface area product (PS) of the endothelium with respect to the contrast agent, and tissue perfusion (F) (35). However in the limiting case in which PS is very high with respect to perfusion F, the rate of uptake is almost exclusively dependent on changes in blood flow. Similarly when PS is low compared with flow, the effect of blood flow becomes negligible, and differences in the rate of uptake are dominated by differences in permeability. The permeability of the endothelium to current clinically approved paramagnetic contrast agents, such as Gd-DTPA, is not low enough to make DCE-MRI an index of flow only (35). Therefore, the changes in the rate of uptake observed in this study can be attributable to reductions in either F or PS, or both. In fact, if combretastatin acts to cutoff portions of the capillary network from the flow of blood, the surface area of those portions would be deducted from the PS product, whereas their flow component would be deducted at least in part from the total perfusion F.

Despite demonstrable cytotoxicity in vitro, the clinical toxicity profile of CA4P is highly suggestive of an agent that is “vascularly active” and devoid of traditional “cytotoxic” side effects. There was negligible myelosuppression, no stomatitis, and only a single episode of alopecia, which is remarkable because 92% of patients had received prior systemic therapy. On the other hand, a variable symptom complex across all of the dose levels, comprised of cutaneous flushing and hot flashes, diffuse abdominal cramping pain (perhaps attributable to vascular changes in small vessels in the gastrointestinal tract), and changes in hemodynamic parameters, was observed in the peri-infusion period. In addition, the intensity of induced tumor pain shortly after the infusion of CA4P further substantiates perturbation in tumor blood flow. Tumor pain was thought to be potentially a desirable effect of treatment. In two patients tumor pain was severe but, nonetheless, manageable. Given the symptom complex described above, and especially the emergence of two episodes of acute coronary syndrome with EKG evidence of cardiac ischemia, it is possible that CA4P, in addition to disrupting tumor vasculature, may cause transient changes in other vascular beds. In one patient, the EKG changes were attributable to coronary vasospasm without elevation in troponin. In the second patient, the ischemic episode is difficult to sort out, because this patient had significant coronary risk factors and underlying, asymptomatic, severe coronary artery disease.

We confirmed a consistent and significant prolongation of the QTc interval at 3–4 hours after infusion of CA4P; this was not associated with clinically meaningful prolongation. Only one patient had a confirmed QTc interval prolongation of more than 500 ms, and no ventricular arrhythmia was observed. These electrophysiological changes are consistent with an agent that blocks the K+ ion channel. It is of interest that combretastatin B1 (CB1; also referred to as the “hICCup nut” toxin), a combretastatin B analogue, is a K+ ion channel blocker and prolongs the action potential duration in excitable tissues (47).

The pharmacokinetic observations from our study replicate those predicted from preclinical models. The parent compound is rapidly dephosphorylated on entry into the systemic circulation with a very short plasma half-life, with CA4 and CA4G displaying longer disposition profiles on the order of 4 h. Over the dose range studied, AUC and Cmax values increased with dose, and there was no evidence of nonlinearity in parent drug clearance. The degree of variability in systemic clearance that we observed with CA4P (CV, 53%) would not be considered extraordinary compared with other parenteral anticancer drugs such as irinotecan, doxorubicin, and cytarabine (48–50). Given the acute toxicity profile encountered within 5 h of drug infusion we felt that it was not appropriate to escalate the dose beyond the 7.5 mg/m2 level that we ultimately reached.

Table 5: Summary of pre- and posttreatment MR tumor blood flow estimation in seven patients treated with combretastatin (CA4P)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose (mg/m²)</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>tgp (s)</td>
<td>Gpeak</td>
<td>tpeak (s)</td>
</tr>
<tr>
<td>16</td>
<td>60 (1 h)</td>
<td>5.24</td>
<td>3.63</td>
<td>36.60</td>
</tr>
<tr>
<td>17</td>
<td>60 (1 h)</td>
<td>15.70</td>
<td>3.23</td>
<td>31.40</td>
</tr>
<tr>
<td>18</td>
<td>60 (1 h)</td>
<td>7.86</td>
<td>8.69</td>
<td>18.30</td>
</tr>
<tr>
<td>19</td>
<td>60 (1 h)</td>
<td>13.10</td>
<td>4.62</td>
<td>39.30</td>
</tr>
<tr>
<td>20</td>
<td>60 (1 h)</td>
<td>7.86</td>
<td>5.00</td>
<td>34.00</td>
</tr>
<tr>
<td>21</td>
<td>60 (10 min)</td>
<td>11.50</td>
<td>7.30</td>
<td>39.10</td>
</tr>
</tbody>
</table>

* MR, magnetic resonance; tgp, time to gradient peak; Gpeak, gradient peak; tpeak (s) time until gradient drops to 10% of Gpeak; E, enhancement.

Table 6: Summary of serial sICAM determinations (ng/ml) by ELISA at baseline and 1 and 24 h post-CA4P-infusion, and change from baseline

<table>
<thead>
<tr>
<th>Patient nos.</th>
<th>Dose (mg/m²)</th>
<th>Pre-Rx Mean</th>
<th>1 h Post</th>
<th>Change vs. Pre-Rx</th>
<th>P</th>
<th>24 h Post</th>
<th>Change vs. Post</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>90</td>
<td>645.58</td>
<td>609.15</td>
<td>−36.43</td>
<td>0.10</td>
<td>684.18</td>
<td>38.59</td>
<td>0.05</td>
</tr>
<tr>
<td>11</td>
<td>90</td>
<td>194.32</td>
<td>210.72</td>
<td>16.4</td>
<td>0.18</td>
<td>232.57</td>
<td>38.25</td>
<td>0.54</td>
</tr>
<tr>
<td>12</td>
<td>90</td>
<td>325.23</td>
<td>354.49</td>
<td>29.26</td>
<td>&lt;0.05</td>
<td>413.80</td>
<td>88.57</td>
<td>0.02</td>
</tr>
<tr>
<td>13</td>
<td>60 (10 min)</td>
<td>335.07</td>
<td>406.31</td>
<td>73.24</td>
<td>0.18</td>
<td>443.32</td>
<td>110.25</td>
<td>0.23</td>
</tr>
<tr>
<td>14</td>
<td>60 (1 h)</td>
<td>512.88</td>
<td>579.49</td>
<td>66.61</td>
<td>0.11</td>
<td>619.38</td>
<td>106.05</td>
<td>0.01</td>
</tr>
<tr>
<td>15</td>
<td>60 (1 h)</td>
<td>423.71</td>
<td>480.24</td>
<td>56.53</td>
<td>0.06</td>
<td>527.32</td>
<td>24.62</td>
<td>0.12</td>
</tr>
<tr>
<td>16</td>
<td>60 (1 h)</td>
<td>462.09</td>
<td>470.41</td>
<td>8.32</td>
<td>0.54</td>
<td>565.64</td>
<td>103.54</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>17</td>
<td>60 (1 h)</td>
<td>1509.64</td>
<td>1594.82</td>
<td>84.44</td>
<td>&lt;0.01</td>
<td>1644.07</td>
<td>134.44</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>18</td>
<td>60 (1 h)</td>
<td>345.39</td>
<td>367.90</td>
<td>22.51</td>
<td>0.10</td>
<td>390.42</td>
<td>54.97</td>
<td>0.07</td>
</tr>
<tr>
<td>19</td>
<td>60 (10 min)</td>
<td>225.98</td>
<td>262.30</td>
<td>36.32</td>
<td>0.19</td>
<td>250.00</td>
<td>24.02</td>
<td>0.06</td>
</tr>
<tr>
<td>20</td>
<td>60 (10 min)</td>
<td>354.63</td>
<td>349.86</td>
<td>−4.77</td>
<td>0.64</td>
<td>338.70</td>
<td>−15.93</td>
<td>0.62</td>
</tr>
<tr>
<td>21</td>
<td>60 (10 min)</td>
<td>270.09</td>
<td>289.17</td>
<td>19.07</td>
<td>0.10</td>
<td>251.77</td>
<td>−18.33</td>
<td>0.16</td>
</tr>
</tbody>
</table>

* Pre Rx, pretreatment; Post, posttreatment.
* a Patient 19, absorbance (maximum value) at 20-fold dilution.
* b Insufficient replicates to determine P for the mean.
administration, it was hoped that this could be a manifestation of drug (CA4) peak plasma concentration. Lengthening the infusion schedule of CA4P did not result in appreciably different CA4 C_{\text{max}}. There was no demonstrable difference in the toxicity profile of either the 10- or the 60-min infusion schedules. Most importantly, achievable CA4 plasma concentrations obtained even at the lowest dose level (range, 1.21–3.83 µM, over the dose range studied), exceeded that required for in vitro cytotoxicity of proliferating human umbilical-vein endothelial cells (1 µM; Ref. 7).

It will be of further interest to discern whether the tolerable doses of this agent will vary despite alternative administration schedules. The MTD was not precisely defined in our study but is likely between 50 and 60 mg/m², which is within the boundary framed by other Phase I trials of this agent on a weekly-for-3-weeks schedule at 28-day intervals, and a daily-for-5-days schedule at 21-day intervals (30, 31). This dosing observation would be akin to that of another class of tubulin-binding agents, the taxanes, in which the dosing schedule does not necessarily alter the range of total tolerable dose (51, 52).

Because the primary target of CA4P is the endothelial cell, we hypothesized that CA4-induced damage and/or apoptosis of vascular endothelial cells would be associated with the release of endothelial cell-specific markers (sICAM, sVCAM, and sE-selectin) into the plasma (53–55). The rationale for this hypothesis is supported by detection of elevated levels of thrombomodulin and plasminogen activator type I in the plasma of patients with thrombotic thrombocytopathic purpura, a disorder characterized by endothelial apoptosis (56). Similarly, elevated levels of sVCAM and sE-selectin are indicative of endothelial dysfunction in patients with essential hypertension and glucose intolerance, diseases characterized by small vessel pathology (57). Moreover, elevated levels of sVCAM have been observed in patients receiving the angiogenesis inhibitor CM-101 and have also been proposed as a surrogate marker of tumor angiogenesis (58, 59). These results imply that serial measurement of endothelial cell-specific marker(s) may provide insight into the effects of antiangiogenic drugs and/or vascular targeting agents on the putative endothelial cell target.

This study is the first clinical trial conducted with the novel vascular targeting agent CA4P. The drug appears to have demonstrable clinical activity early in its clinical development. Important clinical observations with CA4P include: a lack of traditional cytotoxic effects; and, while targeting tumor vasculature, the induction of transient changes in other vascular beds. Previous studies in rats have reported transient increases in vascular resistance in various normal tissues and a resultant increase in blood pressure (27). These effects could be responsible for some of the adverse events observed in the present study and, if so, will require further study to elucidate the underlying mechanism. It will be critically important to determine whether the therapeutic index of this agent can be enhanced and whether preferential effects can be directed toward tumor vasculature.

In light of this, our data on tumor blood flow using DCE-MRI and the correlation with achievable plasma levels of CA4 are suggestive that lower dosages of this agent may be more suitable for further clinical development and not simply a empirically derived MTD dose from this and other ongoing trials (30, 31). Continued assessment of tumor blood flow will be important at dosages below those (<60 mg/m²) that we explored, especially as imaging techniques evolve (60, 61). Furthermore, combining this compound (with its limited myelotoxicity) with other, cytotoxic, antiangiogenesis agents and with other modalities, especially radiation, will likely be an attractive prospect.

ACKNOWLEDGMENTS

We are most appreciative of the patients who volunteered to participate on this study and of the support of Dr. G. Robert Pettit and colleagues at the Cancer Research Institute at the Arizona State University in Tempe. We acknowledge the support of the nursing and research personnel of the University Hospitals of Cleveland General Clinical Research Center for their dedicated care and monitoring of the patients enrolled on this study. Meri Armour provided exceptional administrative support during the conduct of this trial. Drs. Cindy Connell and Nathan Levitan followed patients on protocol and participated in the conduct of the study. Dr. Carol Buchter provided clinical cardiology support for this trial. Lisa Folkes at the Gray Laboratory provided pharmacokinetic analytic support. ILEX Oncology, Inc. (San Antonio, TX) was the study monitor for the project. Lastly, we would like to acknowledge OXiGENE, Inc. (Boston, MA) and, in particular, the efforts of Scott Young and Dr. David Sherris for their support and for providing us the opportunity to evaluate this interesting compound.

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A Phase I Pharmacokinetic and Translational Study of the Novel Vascular Targeting Agent Combretastatin A-4 Phosphate on a Single-Dose Intravenous Schedule in Patients with Advanced Cancer

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