Cancer Chemother Pharmacol (1995) 36: 513–523

© Springer-Verlag 1995

ORIGINAL ARTICLE

Pedro M. Politi · Fuming Xie · William Dahut
Harry Ford, Jr. · James A. Kelley · Anne Bastian
Ann Setser · Carmen J. Allegra · Alice P. Chen
J. Michael Hamilton · Susan F. Arbuck
Peter Linz · Harry Brammer · Jean L. Grem

Phase I clinical trial of continuous infusion cyclopentenyl cytosine

Received: 19 May 1994/Accepted: 25 October 1994

Abstract Cyclopentenyl cytosine (CPE-C) is an investigational drug that is active against human solid tumor xenografts. The 5′-triphosphate of CPE-C inhibits CTP synthase, and depletes CTP and dCTP pools. We conducted a phase I clinical trial of CPE-C given as a 24-h continuous i.v. infusion every 3 weeks in 26 adults with solid tumors. The starting dose rate, 1 mg/m^2 per h, was selected on the basis of both preclinical studies and pharmacokinetic data from two patients obtained after a test dose of 24 mg/m^2 CPE-C as an i.v. bolus. Dose escalation was guided by clinical toxicity. A total of 87 cycles were given, and ten patients received four or more cycles. The mean CPE-C steady-state plasma levels (Cpss) increased linearly from 0.4 μM to 3.1 μM at dose levels ranging from 1 to 5.9 mg/m^2 per h (actual body weight); the mean total body clearance was 146 ± 38 ml/min per m^2. CPE-C was eliminated by both renal excretion of intact drug and deamination to cyclopentenyl uracil in an apparent 2:1 ratio. CTP synthase activity in intact bone marrow mononuclear cells was inhibited by 58% to 100% at 22 h compared to matched pretreatment samples at all CPE-C dose levels. When all data were combined, flux through CTP synthase was decreased by 89.6% ± 3.1% at 22 h (mean ± SE, $n = 16$), and remained inhibited by 67.6% ± 7.7% ($n = 10$) for at least 24 h post-CPE-C infusion. Granulocyte and platelet toxicities were dose-dependent, and dose-limiting myelosuppression occurred during the initial cycle in two of three patients treated with 5.9 mg/m^2 per h. Four of 11 patients (4 of 20 cycles) who received 4.7 mg/m^2 per h CPE-C experienced hypotension 24–48 h after completion of the CPE-C infusion during their first ($n = 2$), third ($n = 1$) and sixth cycles ($n = 1$), respectively. Two of these patients died with refractory hypotension despite aggressive hydration and cardiopulmonary resuscitation. One of 12 patients (28 total cycles) treated with 3.5 mg/m^2 per h CPE-C experienced orthostatic hypotension during cycle 1, and this patient had a second episode of orthostatic hypotension at a lower dose (3.0 mg/m^{2}per h). Hypotension was not seen in patients receiving ≤ 2.5 mg/m^2 per h CPE-C. The occurrence of hypotension did not directly correlate with either CPE-C Cpss, CPE-U plasma levels, pretreatment cytidine plasma levels, baseline CTP synthase activity, or with the degree of enzyme inhibition during treatment. While the hypotension appeared to be dose-related, its unpredictable occurrence and the uncertainty concerning the mechanism preclude a recommendation of a tolerable dose for future studies.

Key words: Cyclopentenyl cytosine · Phase I trials · CTP synthase · Pharmacokinetics · Hypotension

P.M. Politi[1] · W. Dahut · A. Bastian · A. Setser · C.J. Allegra · A.P. Chen · J.M. Hamilton · J.L. Grem ✉
NCI-Navy Medical Oncology Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD 20889, USA

F. Xie[2] · H. Ford · J.A. Kelley
Laboratory of Medicinal Chemistry, Developmental Therapeutics program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, USA

S.F. Arbuck
Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, USA

P. Linz
Department of Internal Medicine, Division of Cardiology, National Naval Medical Center, Bethesda, MD, USA

H. Brammer
Department of Radiology, National Naval Medical Center, Bethesda, MD, USA

Present addresses:
[1] Department of Pharmacology, School of Medicine, University of Buenos Aires, Paraguay 2155, 15th floor, 1214 Buenos Aires, Argentina
[2] Bioanalytical Systems, Inc., 2701 Kent Ave., West Lafayette, IN 47906, USA

Abbrevations *CPE-C* Cyclopentenyl cytosine · Cyd cytidine · *CPE-CTP* cyclopentenyl cytidine triphosphate · *CPE-U* cyclopentenyl uracil · *Cp* plasma concentration · *Cpss* plasma concentration at steady-state · *Urd* uridine · *HPLC* high-performance liquid chromatography · *DCT* Division of Cancer Treatment · *NCI* National Cancer Institute · *EKG* electrocardiogram

Introduction

CPE-C (NSC 375575) is an investigational Cyd analogue in which a carbocyclic cyclopentenyl moiety replaces the furan ring of the ribose sugar [1, 2]. CPE-C is metabolized intracellularly to its 5'-monophosphate by Urd/Cyd kinase (E.C. 2.7.1.48), and readily forms the corresponding di- and triphosphate derivatives [3–5]. CPE-CTP potently inhibits CTP synthase (E.C. 6.3.4.2), leading to depletion of CTP and dCTP pools, inhibition of RNA and DNA synthesis, and S-phase accumulation [2–7]. CPE-C is active against murine and human leukemias and carcinomas in both in vitro and in vivo models [1, 4–8]. CPE-CTP has an apparent intracellular half-life of 12–24 h [4, 5]. [^{3}H]CPE-C is incorporated into RNA at low levels, predominantly in low molecular mass (4–8 S) species [5].

In a human colon cancer cell line, a 24-h exposure to 0.5 μM CPE-C results in $> 90\%$ lethality, and is accompanied by persistent CTP depletion for at least 96 h [5]. Continuous exposure to Cyd provides dose-dependent protection: 0.5 μM Cyd, a physiological concentration, cannot rescue cells against ≥ 1 μM CPEC for 24 h, while 10 μM Cyd is protective [5, 9]. Saturation of phosphorylation to the monophosphate form occurs at extracellular concentrations of 5–10 μM, suggesting that the target Cpss in a clinical trial should not exceed this level.

Preclinical studies with CPE-C suggested species differences in both drug disposition and toxicity [10–13]. CPE-C is markedly more toxic to CD2F1 mice than to Fischer-344 rats, presumably because of the relatively high plasma Cyd levels in rats [10]. Continuous infusion of CPE-C in dogs leads to weight loss, pancytopenia, and abnormalities in both bone marrow and lymph nodes, and the maximally tolerated doses are 5 mg/m^2 per h over 24 h and 1.6 mg/m^2 per h over 72 h [11]. Rodents and dogs eliminate the drug primarily through renal excretion, with a clearance of 90–102 ml/min per m^2 [10–12].

Reversible granulocytopenia has been shown to occur in primates following a 24-h continuous i.v. infusion of 12.5 mg/m^2 per h CPE-C, with a Cpss of 2.1 μM [13]. In primates, CPE-C is rapidly deaminated to its Urd analogue, CPE-U. Although CPE-U is an inhibitor of Urd/Cyd kinase, it is not cytotoxic. CPE-U interferes with CPE-C phosphorylation if present concurrently at a tenfold or more excess with respect to CPE-C, whereas delayed administration of CPE-U does not reverse CPE-C toxicity [14]. After i.v. bolus administration, CPE-C is rapidly eliminated from the plasma in a biphasic manner with a total body clearance of 662 ml/min per m^2, about fivefold higher than reported for rodents and dogs [12].

Based upon its preclinical activity, CPE-C appeared to merit clinical evaluation as an antineoplastic agent. The rapid deamination of CPE-C in non-human primates, its saturable metabolism, and the desire to avoid potentially antagonistic concentration ratios of CPE-U to CPE-C provided the rationale for continuous i.v. infusion. In addition, the Cpss of CPE-C tolerated by primates are in the range associated with in vitro cytotoxicity to cancer cells. The objectives of this trial were to establish the toxicities of CPE-C given as a 24-h continuous i.v. infusion every 21 days, to determine the pharmacokinetic profile of CPE-C, and to measure the effects of CPE-C on target enzyme activity (CTP synthase) in mononuclear cells isolated from bone marrow. The interspecies differences in pharmacokinetics suggested that selection of a starting dose based on murine and canine toxicology might underestimate the dose that would be associated with toxicity in humans. The starting dose rate, 1 mg/m^2 per h for 24 h, was therefore selected not only on the basis of preclinical studies in subhuman primates, but also by pharmacokinetic data from the first two patients obtained after a test dose of 24 mg/m^2 CPE-C as an i.v. bolus. The initial dose level was 8% of the dose that was tolerated in subhuman primates, and 20% of the maximally tolerated dose in dogs.

Patients, materials and methods

Materials

CPE-C and CPE-U (NSC 375574) of pharmaceutical purity were obtained from the Drug Synthesis and Chemistry Branch, and [5-^{3}H]CPE-C (15 Ci/mmol) and tetrahydrouridine (NSC 112907) from the Pharmaceutical Resources Branch, Developmental Therapeutics Program, DCT, NCI. [5-^{3}H]Urd (20 Ci/mmol) was purchased from Moravek Biochemicals (Brea, Calif.). Phosphate-buffered isotonic saline (PBS), pH 7.2 and RPMI-1640 medium were obtained from Biofluids, Inc. (Rockville, Md.). Fetal bovine serum was from Gibco (Grand Island, N.Y.). Lymphocyte Separation Medium (LSM) was purchased from Organon Teknika (Durham, N.C.). Cyd was purchased from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals and solvents were reagent grade and were used without further purification.

Patients

Eligibility requirements included age ≥ 18 years, refractory solid tumors, a Zubrod (Eastern Cooperative Oncology Group) performance status of 2 or better, an absolute granulocyte count $\geq 2000/\mu l$, a platelet count $\geq 100\,000/\mu l$, a serum bilirubin ≤ 1.6 mg/dl, serum glutamate oxaloacetate transferase up to four times

above the upper limits of the institutional norm, and a serum creatinine ≤ 1.6 mg/dl. Objectively measurable disease was not required. At least 4 weeks must have elapsed since completing any previous chemotherapy, and the patient had to be fully recovered from the toxicities of previous therapies. The protocol, which included the rationale for obtaining biochemical studies, was approved by the institutional review boards of the NCI and the National Naval Medical Center, and all patients gave written informed consent.

Treatment plan

Drug treatment was administered on an inpatient basis. CPE-C was provided as lyophilized powder (10 mg) with 50 mg mannitol in a 10-ml vial. Reconstitution with 2 ml sterile water for injection (USP) provided a 5 mg/ml solution of CPE-C and 25 mg/ml of mannitol at a pH of 6–7. The appropriate total daily dose of CPE-C was diluted in 500 ml 0.9% sodium chloride injection (USP) and was administered via a fixed-rate infusion pump over 24 h (CADD-1 Ambulatory Infusion Pump, Pharmacia-Deltec, Inc., St. Paul, Minn).

The body surface area for determining individual CPE-C dose was calculated on the basis of actual body weight in patients who were either underweight or of appropriate weight and on the basis of ideal body weight in overweight individuals. To avoid the confounding effect of any possible circadian variation in CPE-C pharmacokinetics, CTP synthase activity, and/or drug metabolism, infusions were started between 10:00 a.m. and noon. Patients remained hospitalized for an additional 24–48 h following completion of therapy for observation and pharmacologic monitoring. Chemotherapy was delivered every 21 days provided that clinical toxicity had resolved.

The initial two patients enrolled into the study received the same total dose of CPE-C as specified for the initial dose level, 24 mg/m^2, as a 5-min i.v. infusion to permit determination of CPE-C pharmacokinetics. These patients then received CPE-C 2 weeks later, administered as a 24-h infusion at 1 mg/m^2 per h. Individual cohorts of at least three patients were treated at each dose level. Because the mean achieved CPE-C Cpss at dose level 1 was in the concentration range associated with in vitro cytotoxicity, subsequent dose escalation was guided by clinical toxicity as follows: 1.67-fold, 1.5-fold, 1.4-fold, and 1.33-fold. Once grade 2 toxicity (excluding nausea and vomiting) occurred in two or more patients, dose-escalation proceeded in 25% increments. If grade 3 or 4 toxicity occurred in one patient, additional patients were entered at the same dose level. Dose-escalations ceased once two patients at a given dose level experienced non-hematologic toxicity at grade 3 or above or hematologic toxicity at grade 4; that dose level would be considered the maximally tolerated dose. Individual patients were allowed to escalate to the next dose level every third cycle if clinical toxicity was grade 1 or less in severity during the preceding two cycles. The dose of CPE-C was decreased one dose level if the patient experienced toxicity at grade 3 or above. For control of vomiting, 0.15 mg/kg ondansetron was administered i.v. every 4 h for 24 h starting immediately before CPE-C and continuing for up to 24 h post-CPE-C.

Sample collection for pharmacokinetics

Blood was drawn from a peripheral vein in the non-CPE-C infused arm. A 20-ml pretreatment blood sample was obtained from all patients. For the two patients given a 5-min infusion of CPE-C, 5-ml blood samples were obtained at 2, 5, 10, 20, 30, 45, 60 and 90 min as well as at 2, 3, 4, 6 and 24 h after drug administration. For patients treated with a 24-h infusion of CPE-C, a 5-ml blood sample was obtained at 6 h and three "steady-state" samples were obtained during the 20–24 h period of the infusion. Once the CPE-C Cpss was consistently > 1 μM, blood samples were also obtained during the post-infusion period at 5, 15, 30 and 60 min and at 2, 4, 6 and 24 h for the determination of elimination kinetics[1]. Blood samples were collected in 10-ml heparinized tubes containing sufficient tetrahydrouridine, a Cyd deaminase inhibitor, so that the concentration in whole blood was 100 μM. The blood was immediately placed on ice and plasma was separated by centrifugation at 800 *g* for 10 min at 4°C. The plasma was stored at –70°C until analysis.

A pretreatment urine sample, and urine in 8-h increments for the infusion period and for the 24 h post-infusion period were collected. The total urine volume for each time period was measured. An aliquot was removed for creatinine analysis, and another aliquot was immediately frozen on dry ice and stored at – 70°C for subsequent HPLC analysis.

HPLC assay for CPE-C pharmacokinetic studies

An HPLC method was used to measure levels of CPE-C, CPE-U and pretreatment Cyd [12, 13]. Isocarbodine (1 nmol) was added to 0.5 ml plasma or to 1.0 ml urine (diluted by 1:20 or 1:100) as an internal standard [15]. Plasma was ultrafiltered in an Amicon Centrifree Micropartition System (Beverly, Mass.) by centrifugation at 2000 *g* for 50 min, while urine was filtered through a Millipore (Bedford, Mass.) Millex-GS 0.22-μm filter unit. A phenylboronic acid (PBA) solid-phase extraction cartridge (Analytichem, Varian Sample Preparation Products, Harbor City, Calif.) was activated by washing with 2 ml methanol followed by 2 ml 0.01 M sodium phosphate buffer (pH 10.0). Exactly 0.30 ml plasma ultrafiltrate or 0.80 ml urine filtrate was added to the activated PBA cartridge and washed with an additional 2 ml pH-10 buffer. The cartridge was then dried with a positive pressure of nitrogen. The nucleosides were eluted with 0.25 ml 2 M formic acid, and neutralized with approximately 50 mg solid ammonium bicarbonate. A 10-μl aliquot was then analyzed on a multidimensional HPLC system.

Two narrow-bore C-18 reversed phase columns were connected in tandem and eluted isocratically with 0.1 M ammonium formate buffer (pH 5.0) at a flow rate of 0.15 ml/min. The precolumn consisted of two Brownlee 2.0 × 30 mm ODS cartridges (Applied Biosystems, Santa Clara, Calif.) in a 60-mm column holder, while the analytical column was a 2.1 × 250 mm Supleco LC-18-S column (Bellefonte, Pa.). When the nucleosides of interest had eluted from the precolumn (3.5–5 min), a six-position Valco (Houston, Tx.) EC6U electronic switching valve was automatically activated so that the precolumn was backflushed and analytical column elution remained constant at 0.15 ml/min. When the final component of interest had eluted from the analytical column (25–28 min), the valve was switched again so that precolumn and analytical column were in series and the system could equilibrate for 5 min before the next injection. CPE-C had a retention time of 12.3 min, while Cyd, CPE-U and isocarbodine eluted at 15.4, 17.2 and 21.6 min, respectively. A Gilson (Middleton, Wis.) Model 116 variable UV detector was used to measure CPE-C, Cyd and isocarbodine peak areas at 278 nm, and CPE-U and isocarbodine peak areas at 265 nm.

Standard curves of CPE-C and CPE-U in plasma and diluted urine were prepared for each patient by addition of known amounts of CPE-C and CPE-U to the corresponding pretreatment samples. Selected pretreatment plasma samples were also spiked with known amounts of Cyd. All samples were then processed and analyzed in the same manner as the unknown samples. The ratios of the area of

[1] For patients in whom elimination kinetics were determined, the i.v. bag contained the appropriate daily dose plus 10% overfill to insure that CPE-C would still be infusing when the pump was discontinued at 24 h. In these patients, CPE-C was diluted in 264 ml 0.9% sodium chloride injection (USP) and 240 ml was given at a constant rate over 24 h

each analyte to that of internal standard were calculated, and these values were then used to define a standard curve by linear least-squares regression analysis. All standard curves consisted of a minimum of five standard points plus a blank, and typically had correlation coefficients > 0.997. For samples from patients receiving ≥ 2.5 mg/m^2 per h and for whom post-infusion samples were analyzed, spiked standards covered the range 0.1–4 μM for CPE-C and CPE-U in plasma and 0.1–8 μM in diluted urine. Cyd plasma standards covered the range 0–2 μM and the standard curve was constructed by a standard addition method where the ratio of the area of endogenous Cyd to internal standard was subtracted from each point. This assay allowed a limit of quantitation (signal-to-noise ratio greater than five and above or at the lowest point on the standard curve) in biological samples of 0.1 μM (25 ng/ml) for CPE-C, 0.2 μM (50 ng/ml) for Cyd, and 0.3 μM (70 ng/ml) for CPE-U.

Pharmacokinetic analysis

Data from the CPE-C bolus doses of 24 mg/m^2 and from the post-infusion period of CPE-C continuous infusion whose rates were high enough (≥ 2.5 mg/m^2 per h) to observe plasma elimination were fitted to both biexponential and triexponential equations of the form

$$\mathrm{Cp}(t) = \sum_{i=1}^{n} A_i e^{-k_i t}$$

using GraphPad Software for DOS (San Diego, Calif.), where Cp(t) is the plasma concentration at time t, A_i is the exponential coefficient and k_i is the rate constant. For the bolus dose data, areas-under-the-curve were also estimated using the trapezoid rule and equations derived from statistical moment theory. The resulting pharmacokinetic parameters were then calculated using standard equations [16]. For samples obtained during continuous infusion of CPE-C, Cpss was defined as the mean of measured Cp for three samples obtained during the 20–24 h period of the infusion. The total body clearance for infusion doses was then defined as the rate of infusion divided by the Cpss.

CTP synthase assay

Bone marrow was aspirated from consenting patients under local anesthesia with lidocaine to obtain 1–2 ml samples from either the sternum or posterior iliac crest. The samples were collected in heparinized tubes prior to the infusion, at 22 h during the infusion, and 24 and 48 h after completion of the infusion, and were immediately placed on ice. The samples were diluted with an equal volume of PBS, carefully layered onto LSM and centrifuged at 20°C (400 × g for 20 min). The cell layer at the plasma/LSM interface was gently collected and washed twice with 50 ml RPMI-1640 medium. The mononuclear cell pellet was then resuspended in 1–2 ml complete medium (RPMI-1640 with 1.8 mM glutamine and 10% fetal bovine serum) and transferred to an 80-cm^2 tissue culture flask. An aliquot of the mononuclear cell suspension was counted using a hemacytometer. Nucleated cell recovery tended to decrease with sequential aspirations (mean number of cells × 10^6 range): pretreatment 64, 18–445 ($n = 16$); 22 h 48.5, 3–439 ($n = 16$); 48 h 27, 5.8–184 ($n = 10$); 72 h 10.4, 5.4–15.3 ($n = 2$).

The mononulcear cells were then incubated for 4 h with [^{3}H]Urd (1 μM, 1 μCi/nmol) in 10 ml complete medium at 37°C in a humidified atmosphere containing 5% CO_2 [17]. The non-adherent cells were then collected, washed once with ice-cold PBS, and centrifuged (400 g) at 4°C for 10 min. The pellet was extracted with 3 ml ice-cold 0.5 N perchloric acid. The acid-soluble fraction was isolated and neutralized as previously described [5]. The sample was then lyophilized, and stored at − 70°C. Immediately prior to HPLC analysis, the samples were dissolved in 600 μl distilled water and clarified by centrifugation (8000 g) at 4°C for 10 min.

A Waters analytical HPLC system (Millipore Corp., Bedford, Mass.) with an in-line Flo-One Beta liquid scintillation flow detector (Radiomatic, Tampa, Fl.) was employed [5]. A Waters SAX Radial-Pak column (0.8 × 10 cm) was developed using two buffers: 0.007 M ammonium phosphate, pH 4.5 (buffer A), and 0.75 M ammonium phosphate, pH 4.5 (buffer B). A linear gradient was run over 5 min from 100% A to 75% A/25% B, followed by isocratic conditions for the next 50 min. A linear gradient to 20% A/80% B was run from 55 to 65 min, followed by a linear gradient to 100% B at 70 min. The column was washed with 100% B for 5 min and then allowed to equilibrate with 100% A for 15 min prior to the next run. [^{3}H]UTP and [^{3}H]CTP eluted at 48 and 57 min, respectively. Statistical and graphic analysis of the pharmacodynamic data was performed using SigmaStat Statistical Analysis Software v. 1.0 and SigmaPlot Scientific Graphing Software for DOS v. 5.0 (both from Jandel Scientific, San Rafael, Calif.).

Results

Patients

Of 26 patients entered into this trial, 23 were either asymptomatic or minimally symptomatic (Table 1). The majority of patients had colorectal cancer; all had received prior chemotherapy, and half had received prior radiotherapy. The starting dose was 1 mg/m^2 per h for 24 h. The measured CPE-C clearance in the initial two patients who received a 5-min i.v. infusion dose of 24 mg/m^2 CPE-C predicted that this starting dose-rate was appropriate and would yield CPE-C Cpss in the submicromolar range.

After dose-limiting granulocyte toxicity occurred in two of three patients entered at 5.9 mg/m^2 per h, the protocol was amended to permit further study of the tolerability of the 3.5 and 4.7 mg/m^2 per h dose levels and better delineation of the duration of the biochemical effects. A total of 87 cycles were administered (median, 2 cycles; range, 1–10 cycles).

Table 1 Patient characteristics

Age (years; median, range)	54 (26–71)
Males/females	17/9 (65%/35%)
Zubrod Performance Status	
0	19 (73%)
1	4 (15%)
2	3 (12%)
Primary histology	
Colorectal	22 (84%)
Breast	1 (4%)
Pancreas	1 (4%)
Head and neck	1 (4%)
Soft tissue sarcoma	1 (4%)
Prior therapy	
Radiotherapy	13 (50%)
[^{131}I]Col-1 monoclonal antibody	3 (12%)
Chemotherapy	26 (100%)
Median number of prior regimens	2 (range 1–5)

Clinical toxicity

CPE-C was associated with dose-dependent, reversible decreases in the granulocyte and platelet counts (Fig. 1). The median day of the granulocyte and platelet nadirs during cycle 1 were 17 days (range 7–27 days) and 11 days (range 3–19 days), respectively. The median granulocyte nadirs during cycle 1 were 1500/μl, 1000/μl and 450/μl, at dose levels 3.5, 4.7 and 5.9 mg/m^2 per h, respectively (Fig. 1). No patient experienced grade 4 hematologic toxicity during their initial cycle at dose levels $\leq$ 4.7 mg/m^2 per h, but two of three patients treated with $\leq$ 5.9 mg/m^2 per h experienced a granulocyte count $\leq$ 500/μl during cycle 1. The median granulocyte and platelet nadirs at each dose level for all cycles were similar to those occurring during cycle 1 (data not shown), suggesting that retreatment with CPE-C was not associated with greater myelosuppression. The incidence of hematologic toxicity at grade 3 or above for all cycles of therapy is presented in Table 2. A granulocyte nadir below 1000/μl occurred in 25% and 50% of the cycles with 4.7 and 5.9 mg/m^2 per h CPE-C, respectively. However, a platelet nadir below 50 000/μl only occurred during one cycle (at 5.9 mg/m^2 per h). Mild to moderate anemia occurred frequently at most dose levels, but the hemoglobin nadir was generally $\geq$ 8.0 g/dl.

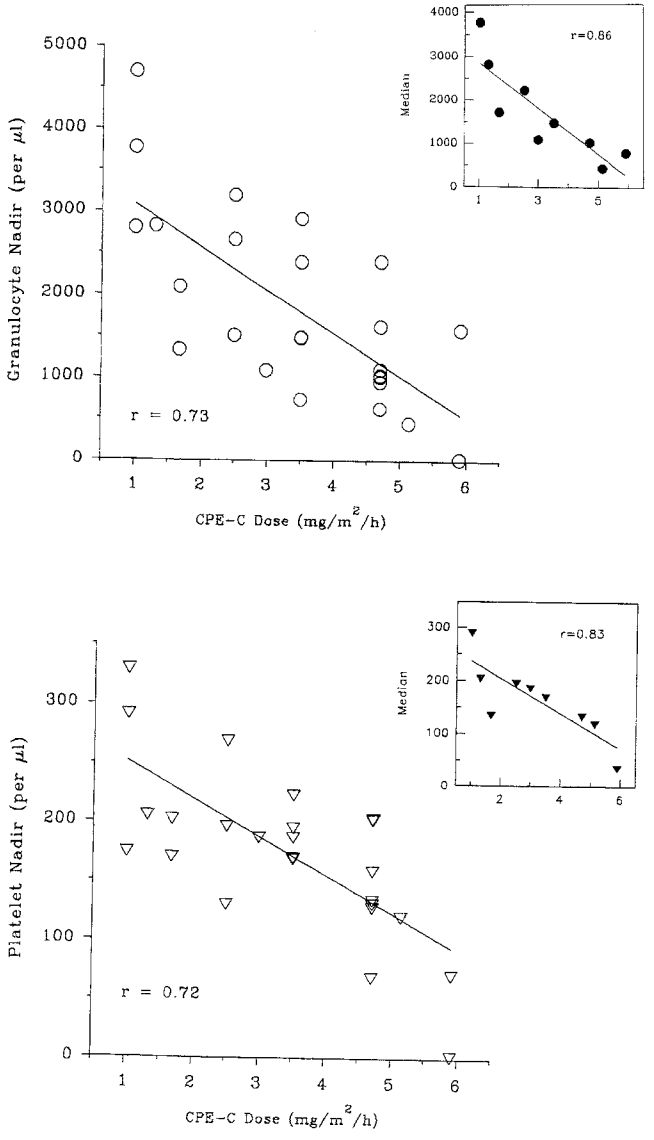

Fig. 1a,b Relationship between the granulocyte (**a**) and platelet (**b**) nadirs and CPE-C dose (based on actual body weight) during the first cycle of therapy. The insets show the median nadirs according to CPE-C dose. The *solid lines* represent a straight line fit of the data by linear regression

Patients began to experience mild nausea and vomiting during the CPE-C infusion at the 3.5 mg/m^2 per h dose level. A prophylactic antiemetic (ondansetron) was subsequently employed, which ameliorated this toxicity. Grade 2 diarrhea occurred in one of six patients (17%) and two of eight patients (25%) during cycle 1 at 3.5 and 4.7 mg/m^2 per h, respectively. Only one patient (treated at 5.9 mg/m^2 per h CPE-C) experienced grade 2 mucositis during cycle 1.

The most serious non-hematologic toxicity was hypotension (Table 3). Six episodes of hypotension occurred in five patients, and varied from orthostatic hypotension that resolved in hours to a few days, to life-threatening or fatal hypotension. None of the hypotensive patients had clinical evidence of dehydration. The onset of hypotension was between hours 48 and 72 (24–48 h after completion of the CPE-C infusion). The first patient to experience hypotension (PB) had transient orthostatic hypotension during her third cycle of therapy when her dose was escalated to 4.7 mg/m^2 per h. A life-threatening episode of hypotension occurred in the sixth patient (AT) entered at 4.7 mg/m^2 per h CPE-C. Systolic hypotension developed on day 3 of her initial cycle, which did not resolve with initial i.v. crystalloid hydration. The patient eventually made

Table 2 Hematologic toxicity for all cycles

CPE-C dose (mg/m^2/h)	No. of cycles[a] (no. of patients	Number of cycles complicated by grade 3–4 toxicity (number of patients experiencing grade 3–4 toxicity at each level)		
		Granulocyte	Platelet	Hemoglobin
1.0	6(3)	0	0	0
1.67	9(4)	0	0	1(1)
2.5	15(6)	0	0	3(1)
3.0	3(1)	1(1)	0	2(1)
3.5	28(12)	3(3)	0	0
4.7	20(10)	5(5)	0	1(1)
5.9	4(3)	2(2)	1(1)	1(1)

[a]Hematologic toxicity could not be analyzed for the two cycles associated with fatal hypotension; thus, hematologic toxicity was evaluable in 85 cycles and in 10 of 11 patients treated with 4.7 mg/m^2 per h CPE-C

Table 3 Hypotensive episodes during CPE-C treatment (*ND* not done)

Patient (ID/sex)	Age (years)	CPE-C dose ($mg/m^2/h$)	Cycle	CPE-C Cpss(μM)		CPE-U Cpmax (μM)	Cyd Cp pretreatment (μM)	Symptoms/blood pressure (mmHg)
				During treatment	24 h post treatment			
MB/F	37	3.5	1	1.8	ND	0.8	0.59	Day 3[c], orthostatic hypotension
		3	4	ND	ND	ND	ND	Day 4, orthostatic hypotension
PB/F	45	4.7	3	2.5	0.24	1.0	ND	Day 3, orthostatic hypotension
AT/F	67	4.7	1	2.3	0.52	1.5	0.95	Day 3, systolic BP 70–80 supine
BG/M	67	4.7	1	2.9	0.1	2.0	0.81	Day 3, systolic BP 60; refractory
DM/M	49	4.7	6	2.75[a]	0.13	2.0	0.32	Day 3, systolic BP 40; refractory
				1.73[b]	ND	0.6	0.73	

[a]Cycle 1 (pharmacokinetic data not available for cycle 6) 1[a] and Cycle 4[b], respectively.
[b]Cycle 4 (pharmacokinetic data not availabe for cycle 6)
[c]Day 3 refers to the period 24–48 h after the end of the 24-h CPE-C infusion

a complete recovery. A question about a possible drug interaction between prazosin, an adrenergic blocker she was taking for hypertension, ondansetron, and CPE-C was raised. Neither patient received additional CPE-C therapy.

The treatment plan was then modified to include 0.9% sodium chloride 100 ml/min (given through a separate i.v. line) starting the evening before CPE-C administration. Hydration continued at the same rate during and for 24 h following the CPE-C infusion. Vital signs, including orthostatic blood pressure measurements, were taken every 4 h. Three additional patients were then entered at the dose level below, 3.5 mg/m^2 per h. One of these patients (MB) had orthostatic blood pressure changes on day 3 of her first cycle, but she was minimally symptomatic. Two additional patients were then entered at 4.7 mg/m^2 per h. One of these patients experienced a transient near-syncopal episode while walking on day 4 of her first cycle, but her vital signs were normal seconds later. Cardiac monitoring over the next 24 h was unremarkable. A 67-year-old male (BG) with no prior cardiac history experienced orthostatic hypotension on day 3 of his initial cycle, which progressed over several hours to frank systolic hypotension. Despite aggressive hydration, he remained hypotensive. He subsequently developed bradycardia followed by cardiac arrest; resuscitation efforts were unsuccessful. There was no clinical evidence of acute myocardial infarction, adrenal insufficiency, or infection; permission for post-mortem examination was denied. After this episode of fatal hypotension, no additional patients were entered into the study.

Laboratory studies in the hypotensive patients revealed abnormalities consistent with hypoperfusion, including metabolic acidosis, hypoxemia, elevation of LDH, and increased BUN and creatinine. Decreased voltage was noted on EKGs; echocardiograms showed vigorous left ventricular contraction and no evidence of pericardial effusion.

The three patients undergoing treatment on the protocol were advised of the hypotensive death, and offered the options of either discontinuing protocol therapy or receiving their next cycle of therapy in the coronary care unit with hemodynamic monitoring. All three patients elected to remain in the study. Two of these patients received a dose reduction from 3.5 and 4.7 mg/m^2 per h to 3.0[2] and 3.5 mg/m^2 per h, respectively, for their second cycle. The third patient had tolerated three prior cycles at 4.7 mg/m^2 per h without toxicity, and remained at this dose for the monitored cycle. A decrease in the central venous pressure was noted in two patients, but each had normal cardiac output and low normal to normal systemic vascular resistance. Serial echocardiograms in all three patients revealed a hyperdynamic picture, and EKGs showed decreased voltage. Patient DM had minimal hemodynamic abnormalities during the monitored (fourth) cycle; unfortunately, he experienced fatal treatment-associated hypotension during his sixth cycle at 4.7 mg/m^2 per h. This patient felt weak on day 3, then developed vomiting and chest discomfort. Upon presentation at a local emergency room, he was found to be hypotensive, and was treated with fluids and dopamine. An EKG showed low voltage, and Swan Ganz catheterization revealed a pulmonary artery pressure of 43/15 mmHg (mean pressure 26). Before

[2]The dose of CPE-C was decreased by one-half dose level rather than a full dose level for patient MB because she experienced few symptoms during the period of orthostatic blood pressure changes at 3.5 mg/m^2 per h

further measurements could be taken, he experienced a cardiac arrest with electrocardiographic changes consistent with electromechanical dissociation. Cardiopulmonary resuscitation was unsuccessful. Post-mortem examination revealed global subendcardial necrosis, a minimal pericardial effusion, and non-atherosclerotic coronary arteries; other organs were unremarkable.

CPE-C pharmacokinetics and plasma Cyd levels

CPE-C was rapidly eliminated from the plasma of two patients after an i.v. bolus test dose of 24 mg/m^2. Parent drug Cp decreased from an initial maximum of approximately 10 μM to less than 1 μM by 2 h. Both patients exhibited similar plasma elimination kinetics with two phases of fairly rapid elimination (average half-lives 8.0 and 101 min, respectively). A third, more prolonged, elimination phase was suggested by the fact that CPE-C could still be detected in plasma 24 h following drug administration, but the Cp data were insufficient to define this phase since the values (≤ 0.1 μM) were below the limit of quantitation of the HPLC assay. Based on the average total body clearance of 199 ml/min per m^2, a continuous infusion of 1 mg/m^2 per h CPE-C was predicted to yield a Cpss of 0.3–0.4 μM. This was indeed the case for these two patients whose individual total body clearances during continuous infusion were within 12% of the corresponding bolus value.

During a 24-h infusion, CPE-C Cpss was proportional to dose and increased in a linear manner with escalating doses (Fig. 2). The measured Cpss of three overweight patients dosed on the basis of ideal body weight were consistently lower than other patients treated at the same dose levels. Therefore, Cpss according to CPE-C dosage based on actual body weight are presented in Fig. 2. The plasma clearance (mean ± SD) of CPE-C was 146 ± 34 ml/min per m^2 ($n = 36$). The CPE-C Cp versus time profile for a representative patient treated at 4.7 mg/m^2 per h is shown in Fig. 3. Steady-state was achieved around hour 12 of infusion. The Cp of CPE-C exceeded that of CPE-U during the 24-h infusion, and the average ratio of CPE-C Cpss to the maximum CPE-U level was 1.65 ± 0.44 (mean ± SD, $n = 31$); CPE-U levels increased in proportion to CPE-C Cpss ($r = 0.90$, data not shown).

Post-infusion samples were obtained in patients treated at ≥ 3.5 mg/m^2 per h. CPE-C Cp decreased rapidly to 23 ± 10% (mean ± SD, $n = 9$) of the Cpss values by 4–6 h after the completion of the infusion (0.63 ± 0.17 μM). However, CPE-C was still detected in plasma 24 h post-infusion at 10% ± 5% of the Cpss (0.21 ± 0.12 μM, $n = 11$), indicating a slow terminal phase of elimination. Urinary excretion of CPE-C and CPE-U was 32% ± 14% and 17% ± 8%, respectively ($n = 22$), of the administered dose for the first 48 h of therapy. The Cpss of CPE-C and the ratio of CPE-C

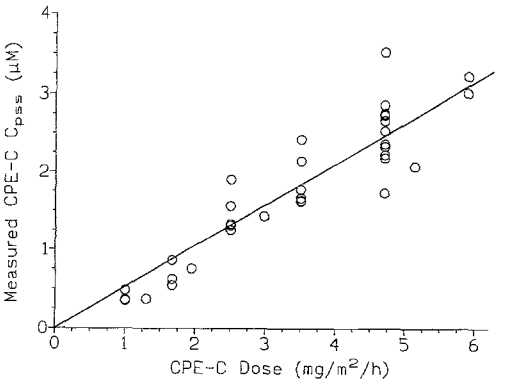

Fig. 2 Relationship between CPE-C steady-state plasma concentration (Cp) and dose. Individual values for CPE-C Cpss are the mean of three plasma level measurements made during 20–24 h of the infusion period. Measurements are from all 26 patients and represent 35 cycles of therapy. The CPE-C dose for three overweight patients (dosed on the basis of ideal body weight) has been recalculated on the basis of their actual weight for this figure. The *solid line* represents the best linear least-squares fit for the data with a forced zero intercept: Cpss = 0.5253(dose), $r = 0.92$

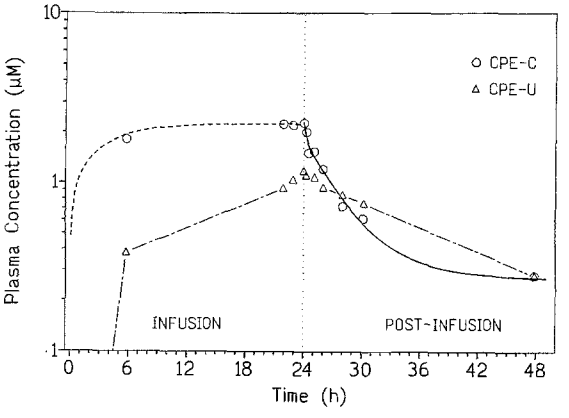

Fig. 3 Plasma concentration versus time profile of CPE-C (○) and CPE-U (△) for a patient receiving a 24-h continuous infusion of CPE-C at a rate of 4.7 mg/m^2 per h. The *solid curve* through the post-infusion CPE-C points represents the best fit of the data to a three-compartment open model. The calculated half-lives are 10.3 min, 2.6 h, and 63 h, respectively, for the phases defined by this model. The *dashed curve* connecting the infusion time CPE-C points is the curve calculated from the parameters defined by the experimentally fitted plasma elimination curve. CPE-U points are connected by the *long-short dashed line*

to CPE-U in patients who experienced hypotension were not higher than those achieved in other patients at the same dose level (Table 3 and Fig. 2). Pretreatment plasma Cyd levels were 0.62 ± 0.24 μM (mean ± SD, range 0.3–1.2 μM, $n = 24$), and were not lower in patients experiencing hypotension. The ratio of CPss CPE-C to pretreatment Cyd increased with higher CPE-C doses ($r = 0.71$, data not shown), and ranged from 0.6 to 9.7.

The pharmacodynamic relationship between achieved CPE-C Cpss and hematologic toxicity was explored. The absolute granulocyte nadirs in 12 cycles

in which the CPE-C Cpss was ≤ 1.5 μM (median 2807/μl, range 1092–6408) were significantly higher than the nadirs in 22 cycles with a Cpss ≥ 1.6 μM (median 1484/μl, range 20–5184; $P = 0.003$, Mann-Whitney rank sum test). The percentage change in granulocyte and platelet counts during the initial cycle tended to increase as the Cpss of CPE-C increased ($r = 0.66$ and $r = 0.68$, respectively, data not shown).

Biochemical studies

In preliminary studies, [^{3}H]CPE-C metabolism was studied in mononuclear cells isolated from peripheral blood and bone marrow from normal volunteers. Following a 4-h in vitro exposure to 0.1 μM and 1 μM [^{3}H]CPE-C, CPE-CTP formation was 174 and 992 fmol/10^6 cells in bone marrow mononuclear cells. CPE-CTP formation in peripheral blood mononuclear cells, however, was four- and sixfold lower. Therefore, bone marrow was selected as the surrogate tissue for biochemical monitoring.

Bone marrow aspirates were obtained immediately prior to treatment and again at 22 h during the initial CPE-C infusion. The mononuclear cells were isolated and incubated with 1 μM [^{3}H]Urd (10 μCi total, 1 μCi/nmol). [^{3}H]CTP formation in pretreatment samples was compared with that in samples obtained during or following CPE-C treatment. Based on the relatively long intracellular half-life, CPE-CTP levels were not expected to appreciably decrease during the isolation and incubation of bone marrow. CTP synthase activity was expressed in two ways: as the absolute amount of CTP formed over time per million cells, and as the ratio of [^{3}H]CTP to [^{3}H]UTP. Preclinical studies indicated that UTP pools did not increase noticeably despite inhibition of CTP synthase, which may be explained by the much smaller endogenous CTP pools compared to UTP pools [3, 5]. For these experiments, a 4-h incubation period rather than a brief pulse was selected to permit greater time for equilibration of precursor and flux through the enzyme pathway.

Paired baseline and 22-h bone marrow samples were obtained in 16 patients. Only one patient was studied at each of the first three dose levels, whereas three or more patients were studied at dose levels 4–6. The duration of CPE-C-mediated biochemical effects was studied during the initial cycle in ten patients receiving ≥ 3.5 mg/m^2 per h CPE-C.

Baseline CTP synthase activity was 172 ± 67 fmol/h per 10^6 cells (mean ± SE; median, 99.5; range 13–1111; $n = 16$). There was evidence of a biochemical effect at 22 h even at the lowest CPE-C dose levels; flux through the CTP synthase pathway was inhibited by more than 58% in all samples (data not shown).

Therefore, data from all dose levels were combined. CTP synthase activity at 22 h during CPE-C infusion had decreased to 9.2 ± 3.0 fmol/h per 10^6 cells (median, 7; range 0–42; $n = 16$; $P = 0.023$, paired t-test). Although some recovery was evident at 48 h (24 h post-CPE-C infusion), CTP synthase activity remained depressed: 27.0 ± 8.3 fmol/h per 10^6 cells (median, 18; range, 2–83, $n = 9$).

Estimation of CTP synthase activity as the ratio of [^{3}H]CTP to [^{3}H]UTP allows for any possible differences in [^{3}H]Urd transport and phosphorylation, permits internal normalization of each sample, and is relatively independent of cell number [17]. Consequently, this estimate may be more reliable and have less variability than the expression of activity as absolute amounts of product formed over time per million cells. As shown in Fig. 4, the ratio of [^{3}H]CTP to [^{3}H]UTP in all pretreatment samples combined was 0.096 ($n = 16$). At 22 h during the CPE-C infusion, the ratio was dramatically decreased to 0.010 (10.5 ± 3.1% of the paired baseline samples; $P < 0.001$, paired t-test). By 48 h, although partial recovery was noted, the ratio remained significantly depressed (32.5 ± 7.7%) compared to the paired baseline samples ($P < 0.001$), and was decreased by $\geq 50\%$ in eight of ten patients. The degree of enzyme inhibition at 48 h appeared to be greater with higher CPE-C doses (Table 4). Diminished flux through CTP synthase persisted at 72 h in two patients receiving 3.5 and 4.7 mg/m^2 per h, although greater recovery was noted in the patient receiving the lower dose. These results suggest that although the

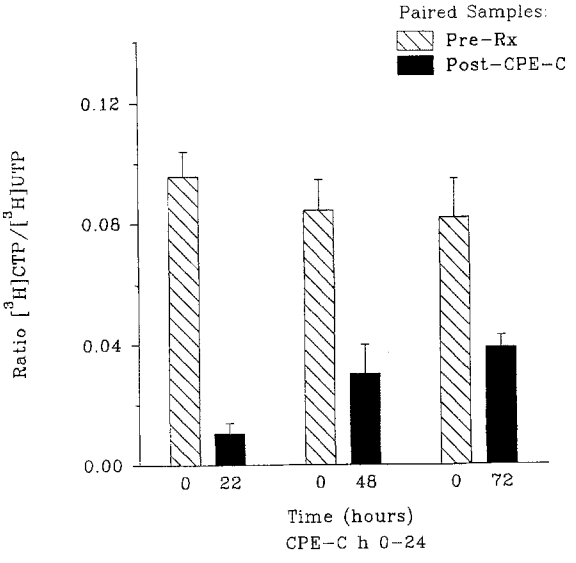

Fig. 4 Effect of CPE-C treatment on CTP synthase in intact bone marrow mononuclear cells. The impact of CPE-C infusion of CTP synthase activity was estimated by comparing the ratio of [^{3}H]CTP to [^{3}H]UTP in paired pretreatment samples with those obtained at 22 h ($n = 16$), 48 h ($n = 10$) and 72 h ($n = 2$) as described in the Methods. Since profound biochemical effects were observed at all CPE-C doses, the data were combined, and are presented as the mean ± SE (22 h and 48 h) or as the mean ± range (72 h). The decreases in the [^{3}H]CTP to [^{3}H]UTP ratio compared with the matched baseline samples were highly significant at 22 h and 48 h ($P < 0.001$, paired t-test)

Table 4 Effect of CPE-C on CTP synthase activity in bone marrow mononuclear cells (*NA* no sample available)

CPE-C dose ($mg/m^2/h$)	No. of patients	CTP Synthase Inhibition (%, mean ± SD)		
		22 h	48 h	72 h
1–2.5	3	92.9 ± 9.5	NA	NA
3.5	4	98.2 ± 3.6	56.7(36.4–60.6; $n = 2$)	34.8 ($n = 1$)
4.7	6	84.4 ± 16.7	60.6 ± 26.8 ($n = 5$)	63.2 ($n = 1$)
5.9	3	85.0 ± 6.3	86.6 ± 4.1 ($n = 3$)	NA

CTP synthase activity was estimated as the ratio of [^{3}H]CTP to [^{3}H]UTP in bone marrow samples obtained during or following the 24-h infusion of CPE-C. The percentage inhibition was calculated from the following expression: [1-(post-treatment/pretreatment enzyme activity)] × 100

degree of CTP synthase inhibition during the CPE-C infusion (22-h sample) was comparable at all doses studied, the duration of enzyme inhibition may be longer at higher doses. However, impaired flux through CTP synthase at either 22 h or 48 h did not closely correlate with the severity of granulocytopenia (data not shown).

Biochemical studies were obtained in four of the five patients who ultimately experienced hypotension, but only two patients were studied during the cycle in which hypotension occurred. CTP synthase activity using either definition indicated that the spectrum of values overlapped in patients who did and did not experience hypotension. For example, the ratio of [^{3}H]CTP to [^{3}H]UTP was decreased by over 90% at 22 h (mean ± SD, 95 ± 4%) during the CPE-C infusion compared to their pretreatment values in all four of the patients who experienced hypotension. The degree of enzyme inhibition at 22 h, however, was not appreciably different in patients who did not experience hypotension (mean ± SD, 86 ± 17%, $n = 12$). One patient experiencing fatal hypotension had evidence of persistent enzyme inhibition by 68% at 48 h, the day that he died. CTP synthase was also inhibited by 77% at 48 h in patient MB treated with 3.5 mg/m^2 per h, although partial recovery (38% inhibition) was noted at 72 h, the day she experienced orthostatic hypotension.

Clinical response

All but one of the 26 patients had documented disease progression in the 3 months prior to entering into this trial. The median time to treatment failure after starting CPE-C therapy was 7.9 weeks (range 0.3–34.7 weeks), and was > 3 months in 11 patients (42%). No partial or complete responses were seen.

Discussion

We evaluated the clinical toxicities, pharmacokinetics and biochemical effects of the investigational antimetabolite CPE-C given as a 24-h continuous i.v. infusion in this phase I trial. A 24-h infusion was selected for several reasons. An infusion of 12.5 mg/m^2 per h for 24 h in subhuman primates has been shown to be tolerated with minimal toxicity, and achieves a Cpss in the concentration range associated with in vitro cytotoxicity and prolonged biochemical effects. The projected CPE-C plasma levels would be lower than those associated with saturation of intracellular phosphorylation. The ratio of CPE-U to CPE-C during the 24-h infusion in primates was favorable and would be predicted not to cause biochemical antagonism. In addition, the physiological plasma levels of Cyd would not be expected to protect cancer cells from CPE-C toxicity. With this schedule, the median granulocyte and platelet nadirs decreased with increasing doses of CPE-C, and dose-limiting hematologic toxicity occurred in two of three patients treated at 5.9 mg/m^2 per h. Five patients ultimately experienced hypotension on six occasions. Four hypotensive episodes occurred in 20 cycles (in 4 of 11 patients) at CPE-C 4.7 mg/m^2 per h, including two fatal events despite attempted cardiopulmonary resuscitation. One episode of hypotension occurred in 28 cycles (1 of 12 patients) at CPE-C 3.5 mg/m^2 per h; this patient subsequently experienced another episode of orthostatic hypotension at a lower dose, 3.0 mg/m^2 per h. Hypotension was not observed in 30 cycles administered at CPE-C doses ≤ 2.5 mg/m^2 per h. Hypotension occurred 48–72 h after the start of CPE-C treatment (24–48 h after discontinuation of CPE-C). Invasive hemodynamic monitoring showed decreased right-sided filling pressures, suggesting that venous dilatation preceded arterial vasodilation. The occurrence of hypotension was unpredictable in that one fatal episode occurred in a patient during his sixth consecutive cycle at 4.7 mg/m^2 per h. This patient not only had a normal cardiovascular status prior to entry into the study, but had no appreciable hemodynamic abnormalities detected by invasive monitoring during cycle 4. In addition, another patient with minimally symptomatic orthostatic hypotension at 3.5 mg/m^2 per h tolerated two consecutive cycles at 3.0 mg/m^2 per h before experiencing symptomatic orthostatic hypotension during the next cycle.

Hypotension was not suspected after completion of the initial toxicology studies; however, blood pressure and pulse rate monitoring are not routinely included in such studies. Acute fatalities within the first 3 days after CPE-C administration have been reported to occur only in dogs receiving very high single i.v. bolus doses; the cause of death in these animals was unclear. Two other antimetabolites that also inhibit CTP synthase have previously been clinically evaluated: 3-deazauridine, a Urd analogue, and acivicin, a glutamine antagonist. Hypotension was not reported with either of these agents on several different schedules, including a 72-h continuous i.v. infusion schedule for acivicin [18–20]. A number of Cyd and deoxycytidine analogues have been studied in clinical trials as antineoplastic agents. One of these agents, cyclocytidine, an analogue of cytosine arabinoside with a longer plasma half-life, is associated with postural hypotension, but the mechanism has not been identified [21]. Other chemotherapeutic agents associated with hypotension include teniposide, paclitaxel, flavone acetic acid (a flavonoid), and homoharringtonine (a cephalotaxine ester) [22–24]. Teniposide- and paclitaxel-associated hypotension occurs acutely during the infusion, and may be accompanied by other anaphylactoid symptoms. This toxicity has been attributed to the presence of Cremaphor in the formulation, which may induce histamine release. The incidence of hypotension and anaphylactoid reactions is reduced by lengthening the infusion, and, in the case of paclitaxel, prophylactic use of antihistamines and corticosteroids. The underlying mechanisms for the clinical hypotension associated with flavone acetic acid and homoharringtonine are not known, but this toxicity is also ameliorated by lengthening the duration of infusion. Unlike teniposide and paclitaxel, CPE-C is water soluble, and the reconstituted clinical formulation has a neutral pH. All patients in this trial received the same lot of CPE-C, and re-analysis of vials from this lot after the episodes of hypotension indicated that the clinical formulation was 99.95% pure.

Mean CPE-C Cpss increased in a linear manner from 0.4 μM to 3.1 μM over the dose rate used in this study, 1–5.9 mg/m^2 per h. CPE-C Cp initially declined rapidly upon discontinuation of the infusion. CPE-C levels were detectable 24 h after completion of the infusion, however, indicating a more prolonged third phase of elimination; this phenomenon may reflect slow release of CPE-C from tissues. In vitro, CPE-C concentrations in the 0.1–0.5 μM range for 24 h are associated with cytotoxicity in several cancer cell lines. There was a trend for decreasing granulocyte and platelet nadirs with increasing dose and CPE-C Cpss levels. CPE-U Cp levels also increased in a linear manner from 0.2–2.3 μM in proportion to CPE-C Cpss, and there was no evidence for saturation of elimination. Deamination of CPE-C was not as extensive as in subhuman primates, and the clearance in humans was only 22% of that obtained in monkeys. Urinary excretion of CPE-C accounted for 32% of the clearance, whereas deamination to CPE-U accounted for 17%. Prolonged exposure to 1 mM CPE-U is not cytotoxic to human leukemia cells in vitro [14], and very large doses of CPE-U have been given to mice without toxicity [25]. CPE-U levels were neither unusually high nor low in patients experiencing hypotension.

CTP synthase activity in intact bone marrow mononuclear cells at 22 h of the infusion was significantly inhibited at all CPE-C dose levels compared to the paired baseline sample; $\geq$ 80% inhibition was noted in 14 of 16 samples. With an in situ assay, expression of CTP synthase activity as the absolute amount of CTP formed per hour per million cells might be inaccurate if differences in [^{3}H]Urd transport and/or conversion to [^{3}H]UTP occurred on the different study days, particularly if the incubation period was brief and recovery of mononuclear cells varied from baseline to the final day of evaluation. We used a relatively long incubation (4 h) to permit more time for equilibration. The mononuclear cell numbers isolated from the bone marrow aspirates at baseline and 22 h were similar, but mononuclear cell recovery at 48 and 72 h was much lower than baseline (and presumably reflected acute drug effects). For these reasons, CTP synthase activity was also calculated as the ratio of [^{3}H]CTP to [^{3}H]UTP, since any possible differences in [^{3}H]UTP formation are taken into account for each sample [17]. The effect of CPE-C on flux through CTP synthase was similar using either definition, but there was less variability using the ratio of [^{3}H]CTP to [^{3}H]UTP. As there was no evidence for dose-dependent inhibition of enzyme activity at 22 h, there also was no correlation between enzyme inhibition during CPE-C infusion and hematologic or other clinical toxicities. Inhibition of CTP synthase persisted for at least 24 h after completion of the CPE-C infusion, and the duration of enzyme inhibition appeared to be longer at higher doses. Other variables which might affect toxicity include the circulating concentrations of Cyd, since Cyd rescues cancer cells from CPE-C toxicity in a dose-dependent manner [4–6, 9, 26]. Pretreatment Cyd Cp ranged from 0.3 to 1.2 μM in this study. Although the ratio of CPE-C Cpss to Cyd increased with increasing CPE-C dose, there was no apparent correlation between Cyd levels and clinical toxicity.

In summary, a 24-h continuous infusion of CPE-C was associated with predictable, dose-dependent myelosuppression. While hypotension also appeared to be dose-related, its unpredictable occurrence and the uncertainty concerning the mechanism preclude a recommendation of a tolerable dose for future studies. Additional preclinical studies are in progress to elucidate the mechanism of CPE-C-associated hypotension, and to identify any therapeutic interventions which might counter the vascular effects. We have previously reported that a brief (3-h) pre-exposure to a non-toxic,

but biochemically active, concentration of CPE-C (0.5 μM) enhances the metabolism and cytotoxicity of cytosine arabinoside in a human colon cancer cell line [27]. If the basis of the hypotension can be ascertained, CPE-C administered at low doses may have potential clinical use as a biochemical modulator of cytosine arabinoside.

Acknowledgements We wish to express our gratitude to the Medical Oncology clinical associates and nursing staff of the Medicine and Navy Medical Oncology Branches, NCI, for their excellent care and management of the patients.

References

1. Marquez VE, Lim M-I, Treanor SP, Plowman J, Priest MA, Markovac A, Khan MS, Kaskar B, Driscoll JS (1988) Cyclopentenylcytosine. A carbocyclic nucleoside with antitumor and antiviral properties. J Med Chem 31: 1687
2. Lim M-I, Moyer JD, Cysyk RL, Marquez VE (1984) Cyclopentenyluridine and cyclopentenylcytidine analogues as inhibitors of uridine-cytidine kinase. J Med Chem 27: 1536.
3. Kang GJ, Cooney DA, Moyer JD, Kelley JA, Kim H-Y, Marquez VE, Johns DG (1989) Cyclopentenylcytosine triphosphate. Formation and inhibition of CTP synthetase. J Biol Chem 264: 713
4. Ford H Jr, Cooney DA, Ahluwalia GS, Hao Z, Rommel ME, Hicks L, Dobyns KA, Tomaszewski JE, Johns DG (1991) Cellular pharmacology of cyclopentenyl cytosine in Molt-4 lymphoblasts. Cancer Res 51: 3733
5. Yee LK, Allegra CJ, Trepel JB, Grem JL (1992) Metabolism and RNA incorporation of cyclopentenyl cytosine in human colorectal cancer cells. Biochem Pharmacol 43: 1587
6. Glazer RI, Knode MC, Lim M-I, Marquez VE (1985) Cyclopentenyl cytidine analogue. An inhibitor of cytidine triphosphate synthesis in human colon carcinoma cells. Biochem Pharmacol 34: 2535
7. Moyer JD, Malinowski NM, Treanor SP, Marquez VE (1986) Antitumor activity biochemical effects of cyclopentenyl cytosine in mice. Cancer Res 46: 3325
8. Glazer RI, Cohen MB, Hartman KD, Knode MC, Lim M-I, Marquez VE (1986) Induction of differentiation in the human promyelocytic leukemia cell line HL-60 by the cyclopentenyl analogue of cytidine. Biochem Pharmacol 35: 1841
9. Dahut WL, Allegra CJ, Grem JL (1993) Modulation of cyclopentenyl cytosine toxicity by cytidine and dipyridamole in human colon cancer cells. Proc Am Assoc Cancer Res 34: 284
10. Tomaszewski JE, Heath JE, Grieshaber CK, Page JG (1990) Toxicity of cyclopentenyl cytosine (NSC-375575) in CD2F1 mice and Fischer-344 rats. Proc Am Assoc Cancer Res 31: 441
11. Page JG, Heath JE, Tomaszewski JE, Grieshaber CK (1990) Toxicity and pharmacokinetics of cyclopentenyl cytosine (NSC 375575) in beagle dogs. Proc Am Assoc Cancer Res 31: 442
12. Zaharko DS, Kelley JA, Tomaszewski JE, Hegedus L, Hartman NR (1991) Cyclopentenyl cytosine: interspecies predictions based on rodent plasma and urine kinetics. Invest New Drugs 9: 9
13. Blaney SM, Balis FM, Hegedus L, Heideman RL, McCully C, Murphy RF, Kelley JA, Poplack DG (1990) Pharmacokinetics and metabolism of cyclopentenyl cytosine (CPE-C) in nonhuman primates. Cancer Res 50: 7915
14. Blaney S, Balis FM, Grem J, Cole DE, Adamson PC, Poplack DG (1992) Modulation of the cytotoxic effect of cyclopentenylcytosine by its primary metabolite, cyclopentenyl uridine. Cancer Res 52: 3503
15. Russ PL, Hegedus L, Kelley JA, Barchi JJ, Marquez VE (1992) The controlled stereospecific reduction of cyclopentenyl cytosine to carbodine and isocarbodine. Nucleosides Nucleotides 11: 353
16. Garibaldi M, Perrier D (1982) In: Swarbrik J (ed) Pharmacokinetics, 2nd edn, vol 15, Marcel Dekker, New York, pp
17. Fischer PH, Willson JKV, Risueno C, Tutsch K, Bruggink J, Ranhosky A, Trump DL (1988) Biochemical assessment of the effects of acivicin and dipyridamole given as a continuous 72-hour intravenous infusion. Cancer Res 48: 5591
18. Moriconi WJ, Slavik M, Taylor S (1986) 3-deazauridine (NSC 126849), an interesting modulator of biochemical response. Invest New Drugs 4: 67
19. Ahluwalia GS, Grem JL, Hao Z, Cooney DA (1990) Metabolism and action of amino acid analog anticancer agents. Pharmacol Ther 46: 243.
20. O'Dwyer PJ, Alonso MT Leyland-Jones B (1984) Acivicin: a new glutamine antagnosit in clinical trials. J Clin Oncol 2: 1064
21. Lokich JJ, Chawla PL, Jaffe N, Frei E III (1975) Phase I evaluation of cyclocytidine (NSC-145668). Cancer Chemother Rep 59: 389
22. Bender RA, Hamel E, Hande KR (1990) Plant alkaloids. In: Chabner BA, Collins JM (eds) Pharmacologic principles of cancer treatment, 2nd edn. W.B. Saunders, Philadelphia, p 180
23. Bibby MC, Double JA (1993) Flavone acetic acid-from laboratory to clinic and back. Anticancer Drugs 4: 3
24. Grem JL, Cheson BD, King SA, Leyland-Jones B, Suffness M (1988) Cephalotaxine esters: antileukemic advance or therapeutic failure? J Natl Cancer Inst 80: 1095
25. Cysyk RL, Malinowski N, Marquez V, Zaharevitz D, August EM, Moyer JD (1994) Cyclopentenyl uracil: an effective inhibitor of uridine salvage in vivo. Biochem Pharmacol 49: 203
26. Ford H Jr, Driscoll JS, Hao Z, Dobyns KA, Rommel ME, Stowe E, Anderson JO, Waud WR, Plowman J, Johns DG, Cooney DA (1994) Reversal by cytidine of cyclopentenyl cytosine-induced toxicity in mice without compromise of antitumor activity. Biochem Pharmacol 49: 173
27. Grem JL, Allegra CJ (1990) Enhancement of the toxicity and DNA incorporation of arabinosyl-5-azacytosine and cytosine arabinoside by cyclopentenyl cytosine. Cancer Res 50: 7279