Cancer Chemother Pharmacol (1994) 34: 103–108

Cancer Chemotherapy and Pharmacology

In vitro characterization of the myelotoxicity of cyclopentenyl cytosine

Donna A. Volpe[1,*], De-lin Du[1,], Charles K. Grieshaber[2,*], Martin J. Murphy, Jr.[1]**

[1] Hipple Cancer Research Center, Dayton, OH, USA
[2] National Cancer Institute, Bethesda, MD, USA

Received: 29 September 1993/Accepted: 3 January 1994

Abstract. We studied the toxicity of a new experimental anticancer drug, cyclopentenyl cytosine (CPE-C), to human and murine hematopoietic progenitor cells in vitro. Due to CPE-C's in vivo myelotoxicity, it was important to characterize its potential adverse effects on human marrow cells during preclinical development of the drug. Marrow cells were exposed to CPE-C for either 1 h prior to addition in clonal assays or continuously during their culture period. The inhibitory effects of CPE-C on myeloid (CFU-gm) and erythroid (CFU-e, BFU-e) colony formation were concentration- and time-dependent, with continuous CPE-C exposure being significantly more inhibitory than 1-h exposure. The results of both exposure experiments were combined to investigate colony inhibition as a function of overall drug exposure (concentration × time, AUC) and data analyzed by the nonlinear Emax equation. Human and murine CFU-gm had similar AUC-response curves and $IAUC_{70}$ values (i.e., AUC at 70% colony inhibition) of 40.8 and 41.9 μ*M*h, respectively. In contrast, murine CFU-e and BFU-e were more sensitive to CPE-C, having lower $IAUC_{70}$ values (both, 21.1 μ*M*h) than human CFU-e and BFU-e (107.8 and 33.0 μ*M*h, respectively). This difference was most prominent with the late erythroid progenitor, CFU-e, in that the human cells were 5 times more resistant to inhibition by CPE-C. CPE-C was myelotoxic in vitro to human and murine marrow cells and toxicity correlated with overall drug exposure.

Introduction

Cyclopentenyl cytosine (CPE-C; NSC-375575) is a carbocyclic analog of cytidine, having significant antitumor activity in experimental systems. CPE-C demonstrates in vitro cytotoxic effects against several human and murine cancer cell lines [10, 15], increases the life span of mice bearing leukemia and melanoma cells [18], and suppresses the growth of human xenografts in nude mice [16]. CPE-C also induces myeloid differentiation in the human promyelocytic leukemia cell line HL-60 [11] and is active against several DNA and RNA viruses in vitro [15]. Phosphorylation of CPE-C at the 5′-position by uridine-cytidine kinase results in its active metabolite, CPE-C triphosphate (CPE-CTP) [14]. CPE-CTP inhibits cytidine triphosphate (CTP) synthetase, thus diminishing intracellular CTP pools and halting cell growth [5, 10]. Cytidine can avert the cytotoxicity of CPE-C in vitro by decreasing CPE-CTP formation and elevating CTP pools, and it can reduce CPE-C's in vivo toxicity [8].

In preclinical toxicology studies using rhesus monkeys, CPE-C has caused a schedule-dependent neutropenia [2]. The target organs of CPE-C toxicity in beagle dogs have been the gastrointestinal tract and bone marrow, with anemia being a major toxicity [21]. Due to CPE-C's in vivo myelotoxicity, it is important to characterize its potential adverse hematopoietic effects on human marrow cells and to understand the relationships between data obtained in preclinical animal studies and those seen in clinical trials. We report herein the in vitro myelotoxicity of CPE-C, whose inhibitory activity against normal human and murine granulocyte-macrophage colony-forming units (CFU-gm), erythroid colony-forming units (CFU-e), and erythroid burst-forming units (BFU-e) is compared using clonal assays. Colony inhibition was examined in relation to overall drug exposure, i.e., the product of drug concentration and time, or AUC (area under the concentration × time curve).

Materials and methods

Bone-marrow cell collection. Human bone marrow cells were obtained from discarded femoral canal reamings or epiphyseal and diaphyseal fragments from orthopedic surgery patients with informed consent [28]. Mononuclear cells (MNC) were collected after centrifugation on a Ficoll-Paque gradient (Pharmacia, Inc., Piscataway, N.J.), washed,

* *Present address:* Food and Drug Administration, Center for Drug Evaluation and Research, Rockville, MD, USA

** *Present address:* Institute of Basic Medical Sciences, Beijing, PRC

Correspondence to: Martin J. Murphy, Hipple Cancer Research Center, 4100 South Kettering Boulevard, Dayton, OH 45439-2092, USA

and resuspended in Iscove's modified Dulbecco's medium (IMDM; Sigma Chemical Co., St. Louis, Mo.). The cell suspension was diluted to a concentration of 1.0–2.5 × 10^6 MNC/ml for drug exposure. BD_2F_1 ($C57BL \times DBA_2F_1$) female mice aged 6–14 weeks (National Cancer Institute, Bethesda, Md.) were killed by cervical dislocation. Femoral and tibial marrow cells were flushed out with IMDM, and the cell suspension was adjusted to 1.0–2.5 × 10^6 nucleated cells/ml for drug exposure.

Drug exposure. CPE-C and 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) were provided by the National Cancer Institue (NCI). CPE-C was diluted in phosphate-buffered saline (PBS; Gibco, Grand Island, N.Y.) prior to each experiment. BCNU was initially dissolved in ethanol, stored at –70° C, and diluted with PBS for each experiment. For 1 h exposure, human and murine marrow cells were incubated with CPE-C at final concentrations of 0.01, 0.1, 1, 10, and 100 μ*M*. Cell suspensions containing 23, 47, and 94 μ*M* BCNU served as positive controls, and negative control cultures were treated with equal volumes of PBS. Following 1 h incubation at 37° C in a humidified incubator containing 5% CO_2, the cells were centrifuged, washed, and resuspended in IMDM. The cell count was determined prior to addition in hematopoietic clonogenic assays. For continuous-exposure experiments, drug solutions were directly added to hematopoietic culture mixtures to yield the desired final concentrations.

Human hematopoietic progenitor assays. A microcapillary clonogenic assay system was used to culture human precursor cells [6]. For CFU-gm, a culture mixture was prepared with 2.5 × 10^5 MNC/ml, 20% heat-inactivated fetal calf serum (FCS; HyClone Laboratories, Inc., Logan, Utah), 20% human placenta-conditioned medium [3], and 0.2% agarose (SeaPlaque; FMC BioProducts, Rockland, Me.). CFU-e and BFU-e cultures consisted of 30% heat-inactivated fetal bovine serum (FBS; Whittaker M.A. BioProducts, Walkersville, Md.), 1% deionized bovine serum albumin (BSA; Armour Pharmaceutical Co., Kankakee, Ill.), 0.1 m*M* 2-mercaptoethanol (2-ME, Sigma), 2 (CFU-e) or 4 U (BFU-e) human urinary erythropoietin/ml (Epo; Terry Fox Laboratories, Vancouver, Canada), 2.5 × 10^5 MNC/ml, and 0.2% agarose. A pipette was used to draw 75 μl of the agarose mixture into a capillary tube, with six replicates comprising each group. The cultures were allowed to gel at 4° C and then incubated at 37° C in a fully humidified atmosphere of 5% CO_2 in air for 7 (CFU-e), 10 (CFU-gm), or 14 (BFU-e) days. CFU-gm colonies were identified as cell clones of at least 40 cells. CFU-e cultures were stained with dianisidine [8] and aggregates of eight or more positive cells were counted as erythroid colonies. BFU-e were defined as aggregates of more than 60 hemoglobin-containing cells.

Murine hematopoietic progenitor assays. Murine clonogenic cell assays were conducted in triplicate 35-mm plastic petri dishes (Nunc, Inc., Naperville, Ill.). All cultures were incubated at 37° C in a humidified atmosphere of 5% CO_2 in air. The culture mixture for CFU-gm contained 20% FCS, 20% mouse lung-conditioned medium [25], 2 × 10^5 marrow cells/ml, and 0.3% agarose. Aliquots of 1 ml were plated, gelled at 4° C, and incubated for 7 days. CFU-gm colonies were counted as containing 40 or more cells.

The methylcellulose assay [19] for murine BFU-e contained 25% FBS, 1% BSA, 10% pokeweed mitogen-stimulated spleen cell-conditioned medium [13], 330 μg iron-saturated human transferrin/ml (Sigma), 0.1 m*M* 2-ME, 2 U Epo/ml, 2 m*M* glutamine (Gibco), 2 × 10^5 marrow cells/ml, and 0.84% methylcellulose (Fisher Scientific Co., Fair Lawn, N.J.). Of this mixture, 1 ml was plated per petri dish and incubated for 9 days. BFU-e colonies were identified as having at least 60 dianisidine-positive cells after staining [9]. Murine CFU-e were assayed in plasma clot cultures consisting of 25% FBS, 1% BSA, 0.5 m*M* 2-ME, 10% bovine embryo extract (Gibco), 2.5 × 10^5 marrow cells/ml, 2 U Epo/ml, 0.5 m*M* $CaCl_2$, and 10% citrated bovine plasma (Gibco) [17]. Aliquots of 0.4 ml were allowed to clot in petri dishes and then 0.6 ml IMDM was added around each clot. Following 2 days of incubation, the cultures were dried and fixed with 5% glutaraldehyde. The clots were stained with 1% dianisidine and counterstained with hematoxylin. Colonies with eight or more dianisidine-positive cells were scored as CFU-e.

Data analysis. Colony counts were expressed in terms of plating efficiency (PE), i.e., the number of colonies per 10^5 nucleated marrow cells. Drug exposure (AUC, area under the curve) is the product of the drug concentration (C, μ*M*) multiplied by the incubation time (T, h). For example, a 48-h exposure at 0.1 μ*M* yields an AUC (C × T) of 4.8 μ*M*h. These calculations enable comparisons across species and among progenitors as incubation periods are varied. The percentage of colony inhibition was determined by comparing the PE of drug-treated groups (A) with that of negative (untreated) controls (B):

$$\% \text{ Inhibition} = \frac{B - A}{B} \times 100.$$

Analysis of the AUC-response curve was performed with the sigmoid Emax equation [12] utilizing SigmaPlot 4.1 software (Jandel Scientific, Corte Madera, Calif.). This equation best describes the data in a temporal causal-effect relationship, correlating effect (colony inhibition) to drug exposure (AUC):

$$E = \frac{E_{max} \times AUC^n}{IC_{50}^n + AUC^n},$$

where *E* is the percentage of inhibition, *AUC* is the drug exposure (expressed as micromolar values per hour), *Emax* is the maximal effect, IC_{50} is the AUC at the half-maximal effect, and *n* is the sigmoidicity factor (measure of the curve's steepness). From this equation, an $IAUC_{70}$ value was calculated for each progenitor, i.e., the AUC (C × T) at which 70% colony inhibition occurs. We have found a good correlation between plasma AUCs resulting in grade 3–4 neutropenia and in vitro AUCs that inhibit human CFU-gm by 75% or more [22]. The $IAUC_{70}$ value may be more appropriate to represent maximal AUCs from which hematopoiesis can recover [22]. Even though the IC_{50}/$IAUC_{50}$ value is the normal standard for comparisons across cell lines, the $IAUC_{70}$ may be a better value for toxicity comparisons.

Results

The plating efficiencies (number of colonies per 10^5 cells) of normal marrow progenitor cells (mean value ± SE) were 114 ± 9 (*n* = 7), 106 ± 10 (*n* = 6), and 178 ± 25 (*n* = 7) for human CFUgm, BFU-e, and CFU-e, respectively, and 87 ± 6 (*n* = 5), 26 ± 1 (*n* = 6), and 215 ± 27 (*n* = 6) for murine CFU-gm, BFU-e, and CFU-e, respectively. BCNU, the positive control, inhibited human and murine colony formation by 88%–100% following both 1 h and continuous exposure at 94 μ*M* (data not shown). Drug treatment for 1 h was somewhat less toxic to the progenitor cells than was continuous BCNU exposure, and murine cells were suppressed to a greater extent than were their human counterparts (data not shown). Therefore, BCNU toxicity to progenitor cells is manifested within the 1st h of drug exposure. The lack of a significant difference in BCNU myelotoxicity between 1 h and continuous exposures is not surprising, since this drug decomposes in aqueous solution with a half-life of approximately 7 h at 37° C [26].

The effect of CPE-C exposure on human and murine hematopoietic progenitors was both concentration- and time-dependent. Continuous drug exposure induced significantly (more than 1 log) greater colony inhibition than did 1 h exposure. For example, 100 μ*M* CPE-C inhibited human progenitors by 69%–74% after 1 h preincubation, whereas 10 μ*M* continuous drug exposure resulted in 91%–98% colony inhibition for the same marrow precursors. For both human and murine cells, BFU-e were the most resistant to 1 h CPE-C exposure and CFU-e were the least resistant. However, BFU-e were the most sensitive when

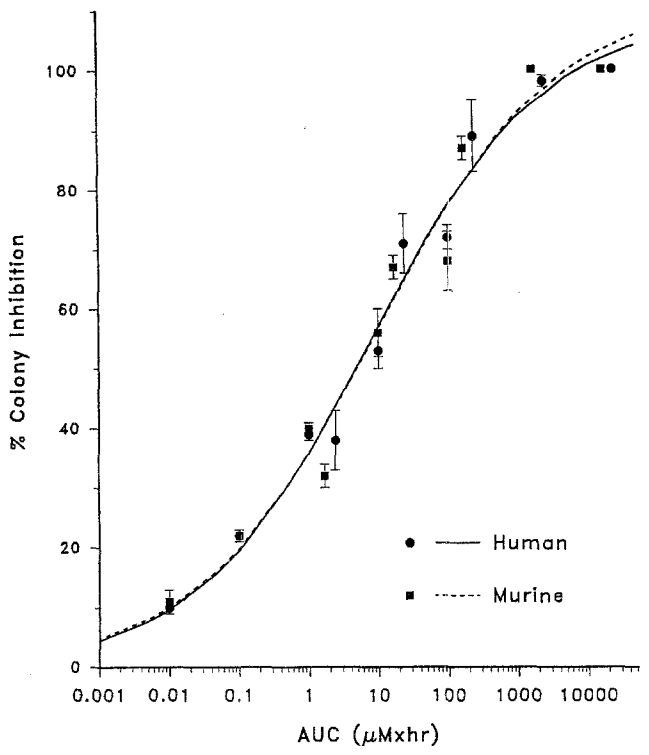

Fig. 1. Effects of CPE-C exposure on human (●——●) and murine (■- - - -■) CFU-gm. *Symbols* represent mean values ±SE for 3 individual experiments; *lines* were generated from the Emax equation. Each experiment consisted of 6 capillaries (human) or 3 dishes (murine)

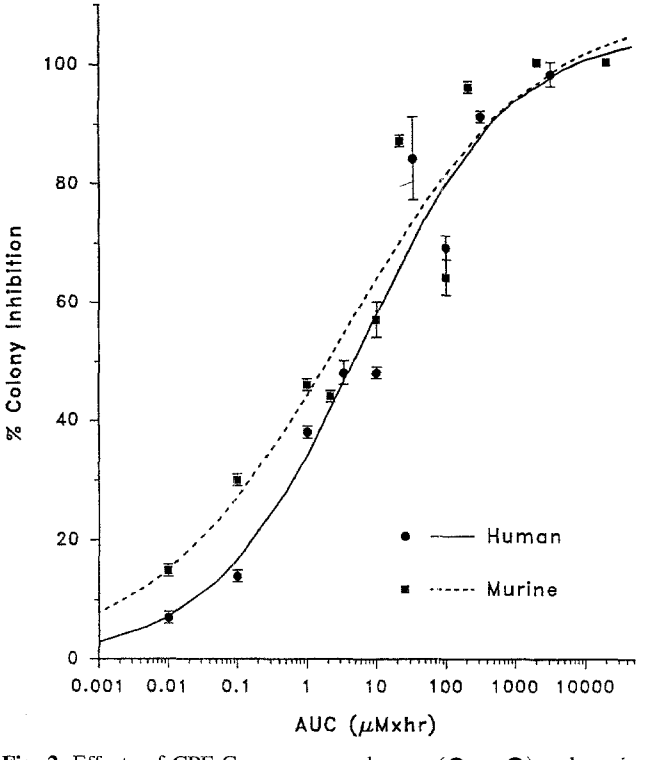

Fig. 2. Effects of CPE-C exposure on human (●——●) and murine (■- - - -■) BFU-e. *Symbols* represent mean values ±SE for 3 individual experiments; *lines* were generated from the Emax equation. Each experiment consisted of 6 capillaries (human) or 3 dishes (murine)

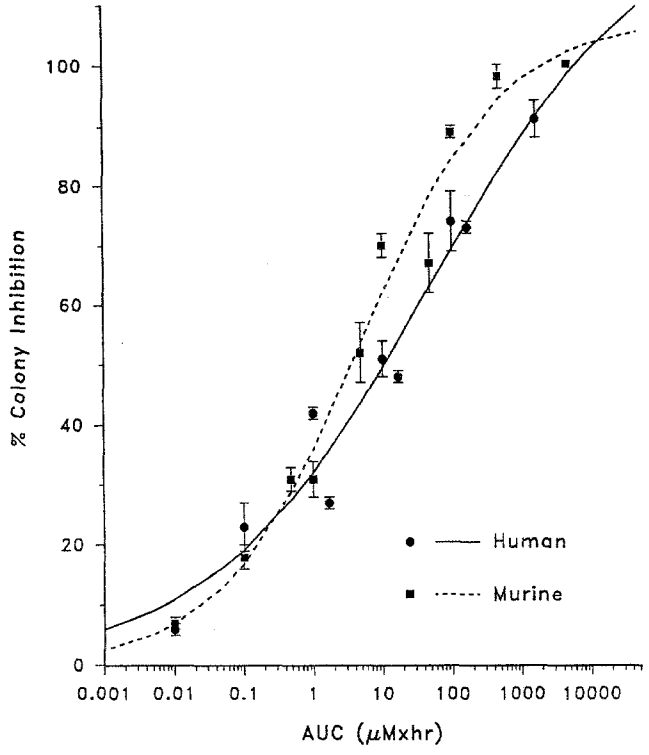

Fig. 3. Effects of CPE-C exposure on human (●——●) and murine (■- - - -■) CFU-gm. *Symbols* represent mean values ±SE for 3 individual experiments; *lines* were generated from the Emax equation. Each experiment consisted of 6 capillaries (human) or 3 dishes (murine)

human marrow was continuously incubated with CPE-C. The results of colony formation at both time levels were combined to examine suppression as a function of drug exposure (AUC, concentration × time). These data combinations were done to compare toxicity more precisely among the hematopoietic precursors as culture periods for continuous drug exposure were varied from 48 h (murine CFU-e) to 2 weeks (human BFU-e).

Figure 1 shows that the effects of CPE-C on human and murine CFU-gm are nearly identical with superimposable curves. Table 1 mathematically demonstrates that human and murine myeloid progenitors have essentially similar sigmoidicity numbers (n, 0.34/0.35) and $IAUC_{70}$ values (40.8/41.9 μ*M*h), respectively. The sigmoidicity number is a shape factor in the Emax equation; when $n > 1$, the curve has a steep slope in the central portion, and when $n < 1$, the curve is shallow in the central portion [11]. CPE-C-induced colony inhibition of human and murine BFU-e (Fig. 2) resulted in curves with similar sigmoidicity numbers but a slightly lower $IAUC_{70}$ for murine BFU-e than for human BFU-e (Table 1). CPE-C was more toxic to murine CFU-e than to human CFU-e at drug exposures ranging between 5 and 1000 μ*M*h, resulting in a steeper curve for the murine erythroid cells (Fig. 3). The $IAUC_{70}$ for murine CFU-e was 5 times lower than that for human CFU-e (Table 1).

Human CFU-e were approximately 3 times more resistant to the toxicity of CPE-C than were human BFU-e and CFU-gm as noted by the higher $IAUC_{70}$ value (Table 1). The curves generated for human CFU-gm (Fig. 1) and BFU-e (Fig. 2) were not significantly different, resulting in

Table 1. $IAUC_{70}$ values for CPE-C exposure

Progenitor	Human		Murine	
	$IAUC_{70}$ (μM)	n	$IAUC_{70}$ (μM)	n
CFU-gm	40.8	0.35	41.9	0.34
BFU-e	33.0	0.41	21.1	0.32
CFU-e	107.8	0.28	21.1	0.43

$IAUC_{70}$, Exposure (AUC) at 70% colony inhibition as calculated from the Emax equation from combined data of three individual experiments; n = , sigmoidicity factor

only a slightly lower $IAUC_{70}$ for the erythroid cells. In contrast, murine CFU-e and BFU-e were more sensitive to CPE-C than were murine CFU-gm. Greater colony suppression was observed for murine BFU-e (Fig. 2) at drug exposures of less than 5 μMh as compared with murine CFU-e (Fig. 3).

Discussion

The results of this study demonstrate that in vitro exposure of human and murine marrow to CPE-C suppresses progenitor cell proliferation in a concentration-dependent manner. Inhibition of myeloid and erythroid colony formation correlated with overal drug exposure ($C \times T$, or AUC). The significance of these data in fitting the Emax model is that a toxic effect of a drug (i.e., myelosuppression) can be predicted on the basis of the plasma AUC [4]. Continuous exposure to CPE-C during the culture period was highly toxic to hematopoietic precursors, resulting in colony inhibition significantly greater than that produced by 1 h preincubation. This was not unexpected, as CPE-C is more suppressive of cytosine triphosphate (CTP) formation, DNA and RNA synthesis, and cell growth following longer drug exposures in colon carcinoma and promyelocytic leukemia cell lines [10, 11]. In addition, CPE-C is a cell-cycle-phase-specific inhibitor [29] and there is a substantial difference in the number of cycling marrow cells subjected to CPE-C between 1 h preincubation and continuous exposure. CPE-C can affect more cycling cells under continuous-exposure conditions than during 1 h preincubations, resulting in higher colony inhibition.

Yee et al. [29] examined the effect of CPE-C on human colorectal cancer cell lines in tumor clonal assays and noted that the IC_{50} (concentration at 50% cytotoxicity) decreased as the duration of CPE-C exposure was increased from 3 (0.4–0.8 μM) to 7 h (0.01–0.06 μM). They also surveyed toxicity in relation to drug exposure, calculating $IAUC_{50}$ and $IAUC_{90}$ values (AUC at 50% and 90% cytotoxicity, respectively) for each cell line. Total exposure (AUC) was more critical than CPE-C concentration in inhibiting cell-line growth and clonogenic capacity. A narrow AUC range was associated with a given toxicity level; thus, as the duration of CPE-C exposure was increased from 3 to 24 h, a disproportionately lower total AUC was required to yield 50% and 90% cytotoxicity [29]. For example, the $IAUC_{50}$ values ranged from 15 to 30 μM h for 3 h CPE-C exposure and from 4.6 to 10.1 μM h for 24 h exposure of colon carcinoma cell lines. Following continuous CPE-C exposure (7 days), $IAUC_{50}$ values varied from 4.6 to 10.1 μM h and $IAUC_{90}$ values ranged from 22.8 to 85.9 μM h [29]. The $IAUC_{50}$ values obtained for continuous CPE-C exposure (7–14 days) of human marrow progenitors in the present study ranged from 23.7 to 129.0 μM h (data not shown), which results in a favorable in vitro therapeutic index. Total AUC values that would result in 50% (4–10 μM h) and 90% (20–100 μM h) colon cancer cell lethality [29] inhibited human and murine hematopoietic colony formation by approximately 50% and 70%, respectively (Figs. 1–3), suggesting a narrow but positive in vitro therapeutic index.

Preclinical studies in vivo have determined the plasma AUC to be 80 μM h in mice given four doses of the maximum tolerated CPE-C dose of 6 mg/kg (~18 mg/m^2) [30]. At this level, murine myeloid and erythroid marrow progenitors are inhibited in vitro by over 70% by CPE-C (Figs. 1–3). Mice treated with a single CPE-C i.v. bolus of 20–140 mg/kg (60–420 mg/m^2) exhibited few toxic signs and no death [27]. However, mice given five daily i.v. CPE-C doses of 15 mg/kg (total dose, 225 mg/m^2) died by day 7 [27]. This finding compares with our observations that a short-term exposure (1 h) to CPE-C was less toxic than continuous drug exposure. Unfortunately, the murine studies dealing with the toxic effects of CPE-C did not report plasma concentrations or AUCs in the same animals. Rhesus monkeys given a single nontoxic i.v. bolus of 100 mg/m^2 (~8 mg/kg) CPE-C had a plasma AUC of 11 μM h, and no adverse effect was reported [2]. CPE-C concentrations in monkey plasma reached 2.23 μM at steady state during a 24-h infusion of 12.5 mg/m^2 (~1 mg/kg), with reversible neutropenia being noted as the only toxicity [2]. Our $IAUC_{70}$ values for human progenitors ranged from 33.0 to 107.8 μM h (Table 1), being above the plasma AUC levels seen in monkeys, thus providing at least circumstantial evidence that there is a positive in vivo therapeutic index in primates. A phase I clinical trial has been initiated at the NCI, with CPE-C being given as a 24-h continuous infusion (1.0–5.9 mg m^{-2} h^{-1}) to patients with solid tumors [23]. Dose-limiting neutropenia and thrombocytopenia increased with dose and steady-state CPE-C plasma levels. Grade 4 myelotoxicity was noted in two of three patients given CPE-C at 5.9 mg m^{-2} h^{-1}, with steady-state plasma levels being 2.8 ± 0.6 μM [23]. Anemia and vomiting were also common toxicities [23].

Muus et al. [20] found that cytosine arabinoside (Ara-C), another anticancer cytidine analog with cell-cycle specificity, was more myelotoxic to human CFU-gm, CFU-e, and BFU-e following continuous exposure as compared with 1 h preincubation. They, too, noted that the toxicity of Ara-C to human marrow cells was more dependent on the exposure time than on the drug concentration [20]. Raijmakers et al. [24] made similar observations in that continuous exposure (10 days) to Ara-C resulted in significantly greater inhibition of human CFU-gm than did 1 h exposure.

Studies on the molecular and biochemical pharmacology of CPE-C indicate that it is a potent inhibitor of CTP synthetase [10]. CPE-C reduces intracellular concentrations of CTP, correlating with its cytotoxic activity [10, 18]. As would be expected, tumor cells are protected in vitro from

the growth-inhibitory effect of CPE-C by co-incubation with cytidine or deoxycytidine [8, 10], and even more appropriately, exogenous cytidine can reduce the in vivo toxic effects in mice after CPE-C administration [8]. Cytidine given up to 8 h after CPE-C administration can eliminate CPE-C-induced toxicity in normal and L1210 leukemia-bearing mice without producing a concomitant decrease in antitumor activity [8]. Interestingly, deoxycytidine can reduce the in vitro myelotoxicity of Ara-C without reducing its cytotoxicity to leukemic cell lines [1]. Significant inhibition (70%–80%) of CTP synthetase activity occurred in marrow MNC of patients given 4.7–5.9 mg m^{-2} h^{-1} CPE-C after 22 h of continuous infusion [23]. This inhibition of CTP synthetase activity lasted for at least 24 h postinfusion (50%–85%) [23].

In our current studies, human and murine CFU-gm were comparably sensitive to CPE-C, resulting in similar $IAUC_{70}$ values. In contrast, there was some difference among human and murine erythroid progenitors (Table 1). The differences in progenitor response to CPE-C may reflect the ability of these cells to phosphorylate the drug to its active species CPE-CTP. Alternatively, CPE-C can be deaminated to cyclopentenyl uridine (CPE-U), a nontoxic metabolite [2]. Blaney et al. [2] have found CPE-C to be converted to CPE-U by cytidine deaminase in the plasma of rhesus monkeys, but the catabolite is not seen in plasma of mice or dogs. Molt-4 human lymphoblast cells can convert CPE-C to CPE-U [7], which suggests that bone marrow cells may also be capable of converting CPE-C. Raijmakers et al. [24] found that Ara-C was deaminated to arabinoside uracil (Ara-U) by human marrow cells similarly in liquid and agar assays. Ara-U concentrations increased with time as levels of Ara-C decreased [24]. The ability of human and murine hematopoietic precursors to metabolize CPE-C to active (CPE-CTP) and inactive (CPE-U) metabolites may relate to CPE-C-induced colony suppression.

In summary, as anticipated, CPE-C was myelotoxic in vitro to human and murine hematopoietic progenitor cells. Toxicity was correlated to overall drug exposure, measured herein as concentration × time (AUC). Our data, along with the results of preclinical animal studies [2, 21] and an initial clinical trial [23], show that myelosuppression will occur in patients given CPE-C at high doses over short periods and at low doses over long infusion periods.

Acknowledgements. This study was supported by a contract with the National Cancer Institute (N44-CM-77831). We thank Dr. Kenneth P. Pohl for providing patient bone-marrow samples, Dr. William J. Bachman for programming the Emax equation in SigmaPlot, Dr. Jerry Collins for constructive comments, and Patricia Klopfenstein for assistance in the preparation of this manuscript.

References

1. Bhalla K, MacLaughlin W, Cole J, Arlin Z, Baker M, Graham G, Grant S (1987) Deoxycytidine preferentially protects normal versus leukemic myeloid progenitor cells from cytosine arabinoside-mediated cytotoxicity. Blood 70: 568–571
2. Blaney SM, Balis FM, Hegedus L, Heideman RL, McCully C, Murphy RF, Kelley JA, Poplack DG (1990) Pharmacokinetics and metabolism of cyclopentenyl cytosine in nonhuman primates. Cancer Res 50: 7915–7919
3. Burgess AW, Wilson EM, Metcalf D (1977) Stimulation by human placental conditioned medium of hematopoietic colony formation by human marrow cells. Blood 49: 573–583
4. Collins JM, Grieshaber CK, Chabner BA (1990) Pharmacologically guided phase I clinical trials based upon preclinical drug development. J Natl Cancer Inst 82: 1321–1326
5. Cooney DA, Rommel ME, Kang G, Wilson Y, Tseng J, Johns DG (1990) Cellular pharmacology in human cells of cyclopentenyl cytosine, a new antineoplastic nucleoside (abstract). Proc Am Assoc Cancer Res 31: 421
6. Du DL, Volpe DA, Murphy MJ (1989) Microcapillary clonogenic assays for human marrow hematopoietic progenitor cells. Int J Cell Cloning 7: 303–313
7. Ford H, Cooney DA, Ahluwalia GS, Hao Z, Rommel ME, Hicks L, Dobyn KA, Tomaszewski JE, Johns DG (1991) Cellular pharmacology of cyclopentenyl cytosine in Molt-4 lymphoblasts. Cancer Res 51: 3733–3740
8. Ford H, Cooney DA, Dobyns KA, Rommel ME, Johns DG (1991) Time-dependence of cytidine rescue from the cytotoxicity of cyclopentenyl cytosine (abstract). Proc Am Assoc Cancer Res 32: 414
9. Gallicchio VS, Murphy MJ (1979) In vitro erythropoiesis. II. Cytochemical enumeration of erythroid stem cells (CFU-e and BFU-e) from normal mouse and human hematopoietic tissues. Exp Hematol 7: 219–224
10. Glazer RI, Knode MC, Lim MI, Marquez VE (1985) Cyclopentenyl cytidine analogue. An inhibitor of cytidine triphosphate synthesis in human colon carcinoma cells. Biochem Pharmacol 34: 2535–2539
11. Glazer RI, Cohen MB, Hartman KD, Knode MC, Lim MI, Marquez VE (1986) Induction of differentiation in the human promyelocytic leukemia cell line HL-60 by the cyclopentenyl analogue of cytidine. Biochem Pharmacol 35: 1841–1848
12. Holford NHG, Sheiner LB (1981) Understanding the dose-effect relationship: clinical application of pharmacokinetic-pharmacodynamic models. Clin Pharmacokinet 6: 429–453
13. Johnson GR, Metcalf D (1977) Pure and mixed erythroid colony formation in vitro stimulated by spleen-conditioned medium with no detectable erythropoietin. Proc Natl Acad Sci USA 74: 3879–3882
14. Kang GJ, Cooney DA, Moyer JD, Kelley JA, Kim HY, Marquez VE, Johns DG (1989) Cyclopentenylcytosine triphosphate: formation and inhibition of CTP synthetase. J Biochem Chem 264: 713–718
15. Lim MI, Moyer JD, Cysyk RL, Marquez VE (1984) Cyclopentenyluridine and cyclopentenylcytidine analogues as inhibitors of uridine-cytidine kinase. J Med Chem 27: 1536–1538
16. Marquez VE, Lim MI, 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: 1687–1694
17. McLeod DL, Shreeve MM, Axelrad AA (1974) Improved plasma culture system for production of erythrocytic colonies in vitro: quantitative assay method for CFU-E. Blood 44: 517–534
18. Moyer JD, Malinowski NM, Treanor SP, Marquez VE (1986) Antitumor activity and biochemical effects of cyclopentenyl cytosine in mice. Cancer Res 46: 3325–3329
19. Murphy MJ, Sullivan ME (1978) Culture of erythroid stem cells from murine and human marrow and blood. In: Murphy MJ (ed) In vitro aspects of erythropoiesis. Springer, New York Berlin Heidelberg, pp 262–265
20. Muus P, Haanen C, Raijmakers R, deWitte T, Salden M, Wessels J (1987) Influence of dose and duration of exposure on the cytotoxic effect of cytarabine toward human hematopoietic clonogenic cells. Semin Oncol 14 [Suppl 2]: 238–244
21. Page JG, Heath JE, Sparks RM, Kelley JA, Tomaszewski JE (1991) Preclinical toxicology studies of cyclopentenylcytosine (CPEC; NSC-375575) given by continuous intravenous infusion to beagle dogs (abstract). Proc Am Assoc Cancer Res 32: 423

22. Parchment RE, Volpe DA, LoRusso PM, Erickson-Miller CL, Murphy MJ, Grieshaber CK (1994) An in vivo-in vitro correlation of the myelotoxicity of 9-methoxypyrazoloacridine (PZA, NSC-366140, PD115934) to myeloid and erythroid hematopoietic progenitors from human, murine and canine marrow. J Natl Cancer Inst 86: 273–280
23. Politi P, Setser A, Bastian A, Ford H, Xie F, Kelley J, Jordan E, Dahut B, Allegra C, Hamilton JM, Arbuck S, Chen A, Agbaria R, Grem J (1993) Pharmacologically guided phase I trial of cyclopentenyl cytosine (CPE-C) with biochemical monitoring (abstract). Proc Am Soc Clin Oncol 12: 142
24. Raijmakers R, deWitte T, Linssen P, Wessels T, Haanen C (1986) The relation of exposure time and drug concentration in their effect on cloning efficiency after incubation of human bone marrow with cytosine arabinoside. Br J Haematol 62: 447–453
25. Sheridan JW, Metcalf D (1972) A low molecular weight factor in lung-conditioned medium stimulating granulocyte and monocyte colony formation in vitro. J Cell Physiol 81: 11–24
26. Tepe P, Hassenbusch SJ, Benoit R, Anderson JH (1991) BCNU stability as a function of ethanol concentration and temperature. J Neurooncol 10: 121–127
27. Tomaszewski JE, Heath JE, Grieshaber CK, Page JG (1990) Toxicity of cyclopentenylcytosine (CPEC, NSC-375575) in CD2F1 mice and Fischer-344 rats (abstract). Proc Am Assoc Cancer Res 31: 441
28. Volpe DA, Du DL, Pohl KP, Campbell KP, Murphy MJ (1991) Utility of human bone marrow obtained incidental to orthopedic surgery for hematopoietic clonal assays. Pathobiology 59: 53–56
29. Yee LK, Allegra CJ, Trepel JB, Grem JL (1992) Metabolism and RNA incorporation of cyclopentenyl cytosine in human colorectal cancer cells. Biochem Pharmacol 43: 1587–1599
30. Zaharko DS, Kelley JA, Tomaszewski JE, Hegedus L, Hartman NR (1990) Cyclopentenyl cytosine: interspecies predictions based on rodent plasma and urine kinetics. Invest New Drugs 9: 9–18