Pharmacokinetics and Metabolism of Cyclopentenyl Cytosine in Nonhuman Primates

Susan M. Blaney, Frank M. Balis, Lajos Hegedus, Richard L. Heideman, Cynthia McCully, Robert F. Murphy, James A. Kelley, and David G. Poplack

Walter Reed Army Medical Center, Washington, DC 20307 [S. M. B.], and The Pediatric Branch [F. M. B., R. L. H., C. M., R. F. M., D. G. P.] and the Laboratory of Medicinal Chemistry [L. H., J. A. K.], National Cancer Institute, Bethesda, Maryland 20892

ABSTRACT

The plasma and cerebrospinal fluid pharmacokinetics of cyclopentenyl cytosine (CPE-C) were studied following i.v. bolus and continuous i.v. infusion in male rhesus monkeys. Following an i.v. bolus dose of 100 mg/m² plasma elimination of CPE-C was biexponential with a mean t₁/₂ of 8.4 min, a mean t₁/₂β of 36 min, and a total clearance (CL₉₀) of 662 ml/min/m², which is 5- to 10-fold higher than clearance rates in rodents and dogs. Less than 20% of the total dose of CPE-C was excreted unchanged in the urine. The remainder was excreted as the inactive deamination product cyclopentenyl uridine (CPE-U). The ratio of the areas under the plasma concentration versus time curves of CPE-U to CPE-C was 7.6 ± 2.4 following i.v. bolus CPE-C. The cerebrospinal fluid:plasma ratios of CPE-C and CPE-U were 0.08 and 0.30, respectively. Continuous i.v. infusion of CPE-C was compared to continuous infusion of 1-β-D-arabinofuranosylcytosine in two monkeys. Steady state plasma concentrations, normalized to a dose of 12.5 mg/m²/h of CPE-C and an equimolar dose of 1-β-D-arabinofuranosylcytosine, were 2.1 and 0.53 μM, respectively. The steady state concentrations of their corresponding uridine metabolites (CPE-U and 1-β-D-arabinofuranosyluridine) were 8.2 and 15.5 μM. The rapid elimination of CPE-C by deamination in the primate resulted in a much higher CL₉₀ and considerably lower total drug exposure than in rodents and dogs that clear CPE-C at a much lower rate by renal excretion. These significant interspecies differences in the disposition of CPE-C should be considered in the selection of a starting dose and schedule for human trials and suggest that a pharmacologically directed dose escalation scheme should be used in the planned phase I studies.

INTRODUCTION

CPE-C (NSC 375575; Fig. 1), a synthetic cytidine analogue in which a cyclopentenyl moiety replaces the furan ring of the ribose sugar (1), is currently undergoing extensive preclinical testing and has been demonstrated to have both antiviral and antitumor activity (2). This compound is active in vivo against the P388 and L1210 murine leukemias and against human lung, melanoma, and breast cancer xenografts grown in athymic mice (2, 3). In addition, 1-β-D-arabinofuranosylcytosine resistant murine leukemia cells are collaterally sensitive to CPE-C (3, 4).

CPE-C, like ara-C, is phosphorylated intracellularly to its mono-, di-, and triphosphorylated nucleotide forms with predominance of the triphosphate (5–7). Intracellular concentrations of CPE-CTP exceed 200 μM in L1210 cells exposed to 1 μM CPE-C (7). Exposure to CPE-C results in rapid depletion of intracellular endogenous CTP pools (5, 7), which is apparently the result of inhibition of CTP synthetase by CPE-CTP (5, 7). Other possible mechanisms of action are currently being investigated. In contrast to ara-C, CPE-C is a poor substrate for mouse kidney cytidine deaminase (3).

Studies of the pharmacokinetic behavior of CPE-C in mice, rats, and dogs have revealed that CPE-C is eliminated primarily through renal excretion (8). There is no evidence of enzymatic deamination of CPE-C in these species. Clearance values are 50 to 100 ml/min/m² and plasma disappearance is triexponential with a long terminal half-life of 20 to 40 h (8).

In the present study the pharmacokinetics of CPE-C was examined in a nonhuman primate model and contrasted to the pharmacokinetics of CPE-C in rodents and dogs. Significant differences in catabolism and rate of clearance were observed which most likely relate to the known differences in the levels of cytidine deaminase in these species (9). The disposition of CPE-C was also directly compared to that of ara-C in the primate model. Additionally, in an attempt to predict the human pharmacokinetic parameters for CPE-C, the interspecies and interspecies differences in disposition of other cytidine analogues were compared to data that have been accumulated with these agents in both primates and humans. The implications of these findings for the planned clinical trials of this agent in humans are discussed.

MATERIALS AND METHODS

Drugs. CPE-C, tetrahydrouridine, and cyclopentenyl uridine were obtained from the Pharmaceutical Resources Branch, National Cancer Institute, Bethesda, MD. The purity of the parent compound was greater than 99% as confirmed by standard methods. Both compounds were reconstituted in sterile water to a final concentration of 10 mg/ml for the i.v. bolus doses. For the continuous i.v. infusion experiments, CPE-C was reconstituted to 5 mg/ml. [3H]CPE-C (specific activity, 15.0 Ci/mmol), labeled at position 5 of cytosine, was obtained from Research Triangle Institute (Research Triangle Park, NC). [3H]CPE-C was mixed with cold CPE-C to a specific activity of 3.0 μCi/mg prior to administration to a single monkey. Isocarbodine (1R,2S,3R,4S)-1-[2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]cytosine, which was used as an internal standard in the HPLC assay for CPE-C and CPE-U, was obtained from the Laboratory of Medicinal Chemistry, National Cancer Institute. ara-C was obtained from the Upjohn Company, Kalamazoo, MI, in 500-mg vials. This was reconstituted in sterile water to a final concentration of 5.3 mg/ml for one animal and 10.6 mg/ml for the second animal. All drugs were filtered through a 0.22-μm filter prior to administration.

Monkeys. Five adult male rhesus monkeys (Macaca mulatta) ranging in weight from 6.4 to 15.0 kg were used in these experiments. The animals were fed NIH Open Formula Extruded Non-Human Primate Diet twice daily and individually housed in accordance with published procedures (10). Blood samples were drawn through a catheter placed in either the femoral or the saphenous vein opposite from the site of drug administration. CSF samples were obtained from a chronically indwelling Pudenz catheter attached to a s.c.-implanted Ommaya res-
The preclinical pharmacology of cyclopentenyl cytosine

The HPLC system used consisted of a Waters model 680 automated gradient controller with two model 510 pumps, a U6K injector, a WISP 710B autoinjector, and a 490 programmable variable wavelength UV detector (Waters Associates, Milford, MA). UV spectral scans were obtained on peaks of interest using a Waters 990 diode array detector (Waters Associates). The precolumn consisted of two 30- x 4.6-mm Brownlee ODS-GU Spheri 5 cartridges (Applied Biosystems, San Jose, CA) and the analytical column was a 250- x 4.6-mm Beckman Ultrasphere ODS (UE3531) with a 5-μm particle size (Beckman Instruments, San Ramon, CA). A Valco electric valve actuator (Supelco, Inc., Bellefonte, PA) was incorporated into the HPLC system after the injector to backwash the precolumn automatically after the last peak of interest had reached the analytical column. At 3.5 min after injection the valve actuator automatically switched the flow of mobile phase to bypass the precolumn and flow directly through the analytical column. CPE-C was monitored at 278 nm and CPE-U was monitored at 266 nm. A mobile phase of 0.1 M ammonium formate buffer, pH 5.0, at a flow rate of 1.0 ml/min provided good separation of CPE-C, CPE-U, and ICN with retention times of 8.0, 11.3, and 14.0 min, respectively (Fig. 2). The limit of quantitation was 0.2 μM for CPE-C and 0.5 μM for CPE-U. Standard curves in monkey plasma and saline were prepared for each experiment by addition of known amounts of CPE-C and CPE-U to both plasma and saline. Standard curves were linear (r² ≥ 0.995) over a range of 0.2 to 50 μM for CPE-C and a range of 0.5 to 50 μM for CPE-U.

Plasma, CSF, and urine samples from the animal receiving [3H]CPE-C were prepared and assayed under similar conditions. One-min fractions of the analytical column eluent were collected and counted in a Beckman LS 8100 scintillation counter (Beckman Instruments).

ara-C and ara-U in plasma were measured with a reverse-phase HPLC assay modified from a previously described method (12). Forty μl of plasma ultrafiltrate were injected onto the analytical column, a 4-μm C18 Nova-pak column (Waters Associates) and eluted with 0.2 M KH2PO4 buffer at a flow rate of 1.5 ml/min. ara-C and ara-U were monitored at 272 nm. Retention times for ara-C and ara-U were 5.7 and 9.7 min, respectively. The limit of quantitation was 0.5 μM for both ara-C and ara-U. Standard curves in monkey plasma and saline were prepared for each experiment by addition of known amounts of ara-C and ara-U to both plasma and saline. Standard curves were linear (r² ≥ 0.995) over a range of 0.2 to 50 μM for ara-C and 0.5 to 50 μM for ara-U.
Continuous infusion, CLTB was determined by dividing the infusion rate over a range of 0.5 to 20 \( \mu M \). The fraction of drug penetrating into the CSF was derived from the ratio of the AUCs in CSF and plasma after the bolus dose administration.

RESULTS

Plasma Pharmacokinetics. The plasma disappearance of CPE-C following an i.v. bolus dose (Fig. 3) was best fitted by a biexponential equation. CPE-C was rapidly eliminated from plasma with a mean \( t_{1/2,\alpha} \) of 8.4 min (range 8.1 to 10.8 min) and a mean \( t_{1/2,\beta} \) of 36 min (range, 20 to 67 min). The mean total body clearance was 662 ml/min/m² (range, 563 to 803 ml/min/m²). The pharmacokinetic parameters for the i.v. bolus doses are listed in Table 1.

<table>
<thead>
<tr>
<th>Monkey</th>
<th>CLTB (ml/min/m²)</th>
<th>( V_d(P) ) (liters/m²)</th>
<th>AUC* (µM·min)</th>
<th>AUC** (µM·min)</th>
<th>CSF:P</th>
<th>( T_a ) (min)</th>
<th>AUC* (µM·min)</th>
<th>AUC** (µM·min)</th>
<th>CSF:P</th>
<th>( T_a ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>440</td>
<td>803</td>
<td>23.6</td>
<td>482</td>
<td>31.8</td>
<td>0.066</td>
<td>9.6</td>
<td>66.6</td>
<td>4480</td>
<td>1220</td>
<td>0.268</td>
</tr>
<tr>
<td>340U</td>
<td>563</td>
<td>10.8</td>
<td>732</td>
<td>87.3</td>
<td>0.119</td>
<td>10.8</td>
<td>21.9</td>
<td>5310</td>
<td>2700</td>
<td>0.468</td>
</tr>
<tr>
<td>385</td>
<td>620</td>
<td>14.0</td>
<td>688</td>
<td>31.0</td>
<td>0.045</td>
<td>8.1</td>
<td>20.3</td>
<td>3040</td>
<td>548</td>
<td>0.178</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>662±125</td>
<td>16.1±6.7</td>
<td>634±133</td>
<td>50.0±32.3</td>
<td>0.077±0.038</td>
<td>8.4±1.1</td>
<td>36.3±26.3</td>
<td>4270±1150</td>
<td>1490±1100</td>
<td>0.305±0.148</td>
</tr>
</tbody>
</table>

\* P, plasma; CSFP, ratio of the AUC** to AUC*.

DISCUSSION

The preclinical pharmacokinetics of CPE-C was studied in a nonhuman primate model that has previously been predictive of anticancer drug disposition in humans (17, 18). In monkeys, CPE-C was rapidly eliminated with a terminal half-life of 20 to 67 min. The mean steady state plasma concentrations normalized to a dose of 52.3 µmol/m²/h (equivalent to 12.5 mg/m²/h of CPE-C and 12.7 mg/m²/h of ara-C) were 2.1 µM for CPE-C and 0.53 µM for ara-C, and 8.2 and 15.5 µM, for CPE-U and ara-U, respectively. The mean total body clearance for CPE-C derived from the steady state plasma concentration was 417 ml/min/m² compared to a mean clearance of 1770 ml/min/m² for ara-C. The pharmacokinetic parameters from these experiments are summarized in Table 2.
60 min and a total clearance exceeding 600 ml/min/m². The primary route of elimination was by deamination to the inactive uridine metabolite, CPE-U (1). Less than 20% of the parent drug was excreted unchanged in the urine. This pharmacokinetic profile and route of elimination of CPE-C in primates is strikingly different than that observed in rodents and dogs, the classical preclinical models used to define the toxicology and pharmacokinetics of new anticancer agents prior to human studies (19).

In contrast to primates the pharmacokinetics of CPE-C in rodents and dogs are characterized by triexponential plasma disappearance with a long terminal half-life and renal excretion of unchanged CPE-C as the primary route of elimination. No evidence exists for the in vivo deamination of CPE-C to CPE-U in these species. As shown in Table 3, this interspecies difference in CPE-C elimination was responsible for a 5- to 10-fold lower total drug exposure (AUC) at an equivalent CPE-C dose in monkeys compared to rodents and dogs. This marked differences in the disposition of CPE-C between primate and rodents have important implications for the planned phase I clinical trials of CPE-C. If the drug is eliminated primarily by deamination in humans, as it is in the rhesus monkey, then a starting dose for phase I trials based on toxicology studies in rodents and dogs (19) is likely to be too low. This would mean that a large number of dose escalations would be required to reach the maximum tolerated dose in humans and that most of the patients treated would receive a suboptimal dose. In an attempt to predict the disposition of CPE-C in humans and determine a more rational starting dose for phase I trials, the pharmacokinetic behavior of other cytidine analogues (ara-C and dideoxycytidine) that have been studied in both humans and our nonhuman primate model were compared (Table 4). ara-C is rapidly deaminated in both monkeys and humans (20), although the higher clearance value and larger ara-U:ara-C ratio indicate that the monkey more efficiently deaminates this drug. In contrast, deamination of 2',3'-dideoxycytidine is not significant in either species and the total clearance under these circumstances approaches renal clearance (21, 22). In the nonhuman primate model, deamination and total clearance of CPE-C are intermediate between ara-C and 2',3'-dideoxycytidine, suggesting that the total clearance of CPE-C in humans will be approximately 300 to 500 ml/min/m².

A continuous i.v. infusion schedule is being considered for human phase I trials. Toxicology studies to correlate the toxicity and the plasma Cₜₐ₀ of CPE-C in dogs treated on a similar schedule are under way. With this information, a safe starting infusion rate for humans that yields a plasma Cₜₐ₀ below the toxic range could be calculated from the estimated total body clearance above.

The proposed pharmacologically directed dose escalation scheme for phase I trials, which can minimize the number of dose escalations required to reach the maximum tolerated dose (23), may be useful for the initial trials with CPE-C. This pharmacokinetically guided dosage escalation strategy relates the initial human plasma drug concentrations to the plasma drug concentrations that produced toxicity in the toxicology studies in rodents. The rate of subsequent dosage escalations is guided by how close the plasma drug concentrations in humans is to the toxic drug concentrations in rodents. The primary advantage of this pharmacologically directed dose escalation method for a drug like CPE-C is that it accounts for interspecies differences in drug disposition as was seen between rodents and primates.

Adjusting the starting dose or dose escalation scheme based solely on pharmacokinetic considerations is valid only if the rate and extent of intracellular activation (phosphorylation) of CPE-C are comparable in the different species. For example, if human cells take up and phosphorylate CPE-C more efficiently than rodents or dogs, then the same plasma Cₜₐ₀ or AUC will be more toxic in humans.

The relatively poor penetration of CPE-C into the CSF compared to that of CPE-U is consistent with results from a
previous study which demonstrated that the nucleobase is a major determinant of the CSF penetration of some nucleosides (24). In that study, the penetration of the 2',3'-dideoxy and 3'-azido analogue of cytidine gained access to the CSF.

In vitro cytotoxicity studies of CPE-C have demonstrated that the effect of CPE-C, like other antimetabolites, is schedule dependent with longer exposures resulting in greater cytotoxicity associated with these infusions was reversible neutropenia, suggesting that this schedule will be feasible in humans.

In summary, interspecies differences in the catabolism of CPE-C markedly influence the rate of the elimination of this drug and therefore the total systemic drug exposure at a given dose. These significant interspecies differences in the disposition of CPE-C should be considered in the selection of a starting dose and schedule for human trials and suggest that a pharmacologically directed dose escalation scheme should be used in the planned phase I studies.

REFERENCES


Pharmacokinetics and Metabolism of Cyclopentenyl Cytosine in Nonhuman Primates

Susan M. Blaney, Frank M. Balis, Lajos Hegedus, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/50/24/7915

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.