Pharmacokinetics and Metabolism of Cyclopentenyl Cytosine in Nonhuman Primates

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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/β of 36 min, and a total clearance (CL₁β) 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 7.0 ± 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 constant 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-arabinofuranoosylcytosine, were 2.1 and 0.53 μM, respectively. The steady state concentrations of their corresponding uridine metabolites (CPE-U and 1-β-D-arabinofuranoosyluridaine) were 8.2 and 15.5 μM. The rapid elimination of CPE-C by deamination in the primate resulted in a much higher CL₁β 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 lines 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.¹³¹CPE-C (specific activity, 150 Ci/mmol), labeled at position 5 of cytosine, was obtained from Research Triangle Institute (Research Triangle Park, NC).²³¹CPE-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,4R)-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 blood administration. CSF samples were obtained from a chronically indwelling Pudenz catheter attached to a s.c.-implanted Ommaya reservoir.
eriovor (11). Lumbar CSF samples were also obtained from several animals from an indwelling temporary lumbar catheter.

Experiments. The pharmacokinetics of CPE-C was studied in three animals following an i.v. bolus dose of 100 mg/m² (one animal received [3H]CPE-C). Blood was collected in heparinized tubes containing 50 µl of 1 mM tetrahydrouridine prior to the dose and at 5, 10, 20, and 30 min and 1, 1.5, 2, 3, 4, 6, 8, 10, and 24 h after the dose. Plasma was immediately separated by centrifugation. CSF samples were collected from an Ommaya reservoir or lumbar catheter prior to the dose and at 5, 15, and 30 min and 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h. The reservoir was pumped four times before and after each sample collection to ensure adequate mixing with ventricular CSF. Urine was collected in 4-hour fractions for 8 to 24 h. All samples were frozen at −70°C until assayed.

Two animals received a continuous i.v. infusion of CPE-C over 24 h. The planned dose of 12.5 mg/m²/h was calculated to achieve a steady state plasma level of 1.25 µM based on i.v. bolus kinetics. The actual dose of CPE-C administered as determined by HPLC analysis was 12.5 mg/m²/h for the first animal and 10 mg/m²/h for the second animal. Blood samples were collected prior to the infusion and during the infusion at 18, 19, 20, 22, and 24 h. The same two animals also received a continuous i.v. infusion of ara-C at doses of 12.5 and 25.0 mg/m²/h, respectively. Blood was collected in tubes containing tetrahydrouridine to ensure adequate mixing with ventricular CSF. Urine was collected in 4-hour fractions for 8 to 24 h. All samples were frozen at −70°C until assayed.

Sample Analysis. CPE-C and CPE-U concentrations in plasma, urine, and CSF were measured with a reverse-phase HPLC assay modified slightly from a recently developed method. From 0.5 to 1.2 µg of ICN, an analogue of CPE-C in which the double bond of the cyclopentenyl ring has been reduced, was added to 0.5 to 1.0 ml of plasma samples and spiked plasma standards as an internal standard. An ultrafiltrate was prepared by centrifuging the samples and standards through a Centrifree Micropartition System (Amicon Corp., Harbor City, CA). Ultrafiltrates (250–500 µl) were loaded onto an activated phenylboronic acid solid-phase extraction column (PBA cartridge; Analytichem International, Danvers, MA) and washed with 2.0 ml of 0.1 M Na₂HPO₄, pH 10.0. The cartridge was aspirated dry, and CPE-C, CPE-U, and ICN were eluted with 250 µl of 2.0 M formic acid. Eluants from a direct injection of plasma ultrafiltrate. However, it was subsequently determined that increasing the volume of the 2.0 M formic acid eluent to 500 µl resulted in more consistent CPE-U recovery (68 ± 20%). CSF and urine samples were prepared under similar conditions with the omission of the ultrafiltration step. Urine samples were diluted 10 to 100 times before solid phase extraction.

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-GS 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. 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).

**Fig. 2. Chromatograms of extracted plasma samples from monkey 8485.** Assay conditions are described in "Material and Methods." Eluent was monitored with an UV detector at a wavelength of 278 nm. A, prior to a dose of CPE-C; B, during a continuous infusion of 12.5 mg/m²/h of CPE-C. Peak 1, CPE-C; Peak 2, CPE-U; Peak 3, ICN.
0.996) over a range of 0.5 to 20 \( \mu M \).

Pharmacokinetic Analysis. Plasma concentration versus time data from the CPE-C i.v. bolus experiments were fitted to both biexponential \((n = 2)\) and triexponential \((n = 3)\) equations

\[
C(t) = \sum_{i=1}^{n} A_i e^{-\lambda_i t}
\]

using MLAB, a nonlinear curve fitting program, where \(C\) is the plasma concentration of CPE-C at time \(t\), \(A_i\) the coefficient and \(\lambda_i\) is the rate constant \((13)\). Akaike’s information criterion \((14)\) was used to determine which equation best fit the data. The half-life for each phase of elimination was calculated by dividing 0.693 by the rate constant \((\lambda_i)\) for that phase. Model independent methods were used to calculate other pharmacokinetic parameters. The AUC was derived by the linear trapezoidal method \((15)\) and extrapolated to infinity by adding the quotient of the final plasma concentration divided by the terminal rate constant \((\lambda_a)\). Total body clearance \((CL_B)\) was determined by dividing the infusion rate constant \((X)\). Total body clearance \((CL_B)\) was determined by dividing the quotient of the final plasma concentration divided by the terminal rate constant \((\lambda_a)\). Total body clearance \((CL_B)\) was determined by dividing the dose by the AUC. In the monkeys that received CPE-C and ara-C continuous infusion, \(CL_{TB}\) was determined by dividing the infusion rate by the plasma steady state concentration. The volume of distribution at steady state \((Vd_a)\) was calculated using the area under the moment curve \((16)\).

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 \(t_{\alpha}\) of 8.4 min (range 8.1 to 10.8 min) and a mean \(t_{\beta}\) of 36 min (range, 20 to 67 min). The mean total body clearance was 662 ml/min/m\(^2\) (range, 563 to 803 ml/min/m\(^2\)). The pharmacokinetic parameters for the i.v. bolus doses are listed in Table 1.

![Fig. 3. Plasma concentration-time profiles of CPE-C and CPE-U following i.v. bolus administration of 100 mg/m\(^3\) of CPE-C. Points, geometric mean from 3 animals; bars, SD.](Image)

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

<table>
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<th>Monkey</th>
<th>CL(_{TB}) (ml/min/m²)</th>
<th>V(_d) (liters/m²)</th>
<th>AUC(_{\alpha}) (µM min)</th>
<th>AUC(_{\beta}) (µM min)</th>
<th>CSF:P</th>
<th>(T_a) (min)</th>
<th>AUC(_{\alpha}) (µM min)</th>
<th>AUC(_{\beta}) (µM min)</th>
<th>CSF:P</th>
<th>(T_a) (min)</th>
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<td>440</td>
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<td>732</td>
<td>87.3</td>
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<td>10.8</td>
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<td>0.045</td>
<td>8.1</td>
<td>20.3</td>
<td>3040</td>
<td>0.178</td>
<td>183</td>
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<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>0.305 ± 0.148</td>
<td>273 ± 165</td>
</tr>
</tbody>
</table>

\(\alpha\), plasma; CSF:P, ratio of the AUC\(_{\alpha}\) to AUC\(_{\beta}\).
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 interspecies difference is directly related to the significant differences in cytidine deaminase levels in these animals. The rat, which has virtually no detectable cytidine deaminase in any body tissue (9), has the highest total drug exposure and lowest CL_T of CPE-C. In contrast, the relatively low AUC and rapid CL_T of CPE-C in rhesus monkeys is consistent with the high levels and broad tissue distribution of cytidine deaminase in this species (9).

These 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 Cl 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 Cl 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 Cl of 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


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