Plasma and Cerebrospinal Fluid Pharmacokinetics of Clofarabine in Nonhuman Primates

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Abstract

Introduction: Clofarabine (2-chloro-2′fluoro-2′-deoxy-9-β-D-arabinofuranosyladenine) is a purine nucleoside analogue that is active in the treatment of acute leukemia. We studied the pharmacokinetics and cerebrospinal fluid penetration of clofarabine in a nonhuman primate model.

Methods: A dose of 2.3 mg/kg of clofarabine was given i.v. over 2 hours to each of four animals. Plasma and cerebrospinal fluid (CSF) samples were obtained at specified intervals and the clofarabine concentration determined by reverse-phase high-pressure liquid chromatography with mass spectroscopy.

Results: The median clofarabine clearance was 17 mL/min/kg (range, 15-20), the median plasma area under the concentration-time curve was 452 µmol/L minutes (range, 380-487), and the median terminal half-life was 105 minutes (range, 78-138). Concentrations of clofarabine in CSF could not be modeled reliably because the terminal rate constant was not well defined. The median CSF penetration was 5% (range, 3-26%).

Conclusion: Clofarabine penetrates into the CSF only modestly, but the concentrations obtained may approach those that are cytotoxic in vitro. Evaluation of the contribution of clofarabine to central nervous system preventive therapy should be considered in future studies.

Nucleoside analogues are among the most commonly used anticancer agents. These agents usually include modification of either the sugar moiety, the base, or both, to render the pharmaceutical characteristics of the compound more favorable, especially with regard to protection from natural pathways of degradation. Purine nucleoside analogues in widespread use include fludarabine, cladribine (2-chlorodeoxyadenosine), and clofarabine (2-chloro-2′fluoro-2′-deoxy-9-β-D-arabinofuranosyladenine). The chlorine substitution on the adenine base in clofarabine deamination renders the drug resistant to deamination (1), whereas the fluorine on the sugar moiety confers resistance to degradation by purine nucleoside phosphorylase (2).

The use of antimetabolites given either systemically or by the intrathecal route has been a mainstay of both prevention and treatment of central nervous system leukemia (3, 4). Some nucleoside analogues penetrate relatively well into the cerebrospinal fluid (CSF) after systemic administration. The CSF penetration of cytarabine is ~20%, although the CSF/plasma ratio may be dose dependent (5, 6). The penetration of cladribine is ~20% during a continuous i.v. infusion (7). We studied the CSF penetration of clofarabine in a nonhuman primate model that has been highly predictive of anticancer drug distribution in humans (8).

Materials and Methods

Drug. Clofarabine was supplied by Genzyme Oncology, San Antonio, TX in 20-mL vials containing 1 mg/mL of clofarabine. The appropriate dose of drug was diluted in 0.9% sodium chloride to a final total volume of 150 mL.

Animals. Four adult male rhesus monkeys (Macaca mulatta) weighing 12.7 to 15.2 kg were used in these experiments. The animals were fed Open Formula Extruded Non-Human Primate Diet twice daily and group housed in accordance with Guide for the Care and Use of Laboratory Animals (9). Drug was given through a surgically implanted central venous catheter. Blood samples were drawn through a catheter placed in the contralateral femoral or saphenous vein. Ventricular CSF samples were obtained from a chronically indwelling fourth ventricular catheter attached to a s.c. implanted Ommaya reservoir (8). The reservoir was pumped four times before and after each CSF sample collection to ensure adequate mixing with ventricular CSF.

Experiments. Four animals received 2.3 mg/kg (~46 mg/m²) of clofarabine given i.v. over 2 hours. Blood samples and ventricular CSF were collected immediately before the dose, at 1 hour during the infusion, at the end of the infusion, and at 5, 15, 30 minutes, and 1, 2, 4, 6, 8, and 24 hours after the infusion. Plasma was separated immediately by centrifugation at 1,500 rpm for 10 minutes. Plasma and CSF were frozen immediately after collection. Clinical laboratory studies including complete blood counts, electrolytes, liver function tests, and renal function tests were obtained on a weekly basis for a minimum of 3 weeks after the clofarabine infusion. Animals were also

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observed on a daily basis for a minimum of 3 weeks after infusion for any evidence of clinical toxicity.

**Sample analysis.** Plasma samples containing clofarabine, cladribine (as the internal standard), and EDTA as the anticoagulant were precipitated with acetonitrile. The supernatant was evaporated and reconstituted in mobile phase consisting of water/acetonitrile/formic acid (90:10:0.1, v/v/v). CSF samples were prepared by dilution with 0.1% formic acid. Both sample types were analyzed by reverse-phase high-pressure liquid chromatography (Agilent Technologies 1100 series, Wilmington, DE) using a Synergy Polar RP column (150 x 2.0 mm, 4 μm, Phenomenex, Torrance, CA) maintained at 45°C. The mobile phase at a flow rate of 0.3 mL/min was nebulized using heated nitrogen in a Z-spray source/interface and the ionized compounds were detected using a Quattro LC tandem quadrapole mass spectrometer (Waters, Milford, MA) with ion transitions monitored at 304.05 > 170.02 for clofarabine and 286.05 > 169.98 for the internal standard. The linear range of the method was 10 to 5,000 ng/mL. The limit of quantitation was 10 ng/mL. All samples were run on the same day. The intraday variability of the assay was <7%.

**Pharmacokinetic analysis.** Plasma concentration-time data were modeled in ADAPT II (10). One-, two-, and three-compartment models were fit to each data set and the best fit was determined using Akaike's information criterion (11). Clearance was calculated as the product of the central volume and the elimination rate constant and half-lives were derived from the estimates of model variables by using standard techniques (12). For both plasma and CSF, the areas under the concentration-time curve (AUC) were determined by the linear trapezoidal method to the last measured concentration (AUClast) and extrapolated to infinity (AUCinf) using the terminal rate constant (12). CSF penetration was calculated as CSF AUC/plasma AUC. The ratio was calculated two ways, once using the AUC of both plasma and CSF extrapolated to infinity, and once using the AUCs calculated only to the last measured time point.

**Results**

Pharmacokinetic variables after i.v. administration of clofarabine over 2 hours are presented in Table 1. The two-compartment model fit the plasma concentration-time curve (Fig. 1) better than the one-compartment model for all animals and better than the three-compartment model for three of four animals (data not shown). The median clearance was 17 mL/min/kg (range, 15-20), the median AUCinf was 452 μmol/L minutes (range, 380-487), and the median terminal half-life was 105 minutes (range, 78-138). The median central compartment volume was 1,000 mL/kg (range, 260-1,530). Concentrations of clofarabine in CSF could not be modeled reliably because the terminal rate constant was not well constrained.

![Fig. 1. Plasma (▲, ■, ◆, and ○) and CSF (○, □, ◆, and △) concentrations of clofarabine after a 2-hour i.v. infusion of a 2.3 mg/kg dose. Symbols represent measured concentrations; lines represent model-predicted concentrations; and ○, animal 1; ■, animal 2; ◆, animal 3; △, animal 4.)]("cid:image/png"

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**Table 1. Pharmacokinetic variables for clofarabine after 2-hour i.v. infusion in nonhuman primates**

<table>
<thead>
<tr>
<th>Animal</th>
<th>$k_{10}$ (min$^{-1}$)</th>
<th>$k_{12}$ (min$^{-1}$)</th>
<th>$k_{21}$ (min$^{-1}$)</th>
<th>Clearance (mL/kg/min)</th>
<th>$t_12$ α (min)</th>
<th>$t_12$ β (min)</th>
<th>Plasma AUC$_{\text{last}}$ (μmol/L min)</th>
<th>CSF AUC$_{\text{last}}$ (μmol/L min)</th>
<th>AUC CSF/AUC plasma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0165</td>
<td>0.0057</td>
<td>0.0075</td>
<td>17.6</td>
<td>28.1</td>
<td>138.2</td>
<td>426</td>
<td>109</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>0.0108</td>
<td>0.0029</td>
<td>0.0117</td>
<td>16.5</td>
<td>37.2</td>
<td>102.7</td>
<td>445</td>
<td>26</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>0.0208</td>
<td>0.0186</td>
<td>0.0230</td>
<td>19.7</td>
<td>13.0</td>
<td>77.6</td>
<td>373</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>0.0581</td>
<td>0.1562</td>
<td>0.0263</td>
<td>14.9</td>
<td>3.0</td>
<td>106.2</td>
<td>473</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td>Median</td>
<td>0.0186</td>
<td>0.0121</td>
<td>0.0173</td>
<td>17.1</td>
<td>20.6</td>
<td>104.5</td>
<td>436</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>SD</td>
<td>0.0214</td>
<td>0.0739</td>
<td>0.0090</td>
<td>2.0</td>
<td>15.3</td>
<td>24.9</td>
<td>42</td>
<td>45</td>
<td>11</td>
</tr>
</tbody>
</table>

* $k_{10}$, $k_{12}$, and $k_{21}$ are the rate constants for transfer from the central compartment out, from the central compartment to the peripheral compartment, and from the peripheral compartment to the central compartment.
defined. The median CSF AUC$_{last}$ was 25 μmol/L minutes (range, 10-109). The median CSF penetration (CSF AUC$_{last}$/plasma AUC$_{last}$) was 5% (range, 3-26%).

The animals tolerated the drug without significant clinical or laboratory toxicity.

**Discussion**

Although the plasma pharmacokinetic behavior of clofarabine in the nonhuman primate was relatively consistent, the CSF concentrations were somewhat variable. The median CSF penetration calculation by CSF AUC$_{last}$/plasma AUC$_{last}$ is 9%. However, because of the variability in CSF clofarabine concentrations, which prevents accurate estimation of the terminal rate constant, the extrapolation of the AUC of clofarabine in CSF to infinity results in >75% of the AUCs being extrapolated (data not shown). Thus, this result is unreliable. Therefore, we recalculated the CSF penetration by comparing the ratio of CSF AUC$_{last}$/plasma AUC$_{last}$, a calculation that does not require any extrapolation beyond the measured drug concentrations. By this method the median CSF penetration is 5% (range, 3-26%). Because very little of the plasma AUC$_{inf}$ was extrapolated (only an average of 3%; range, 2-4%; data not shown), this calculation is essentially the same as the ratio of CSF AUC$_{last}$/plasma AUC$_{inf}$. Thus, the CSF penetration of clofarabine could be underestimated in this study by omitting the extrapolated portion of the CSF AUC but is not overestimated.

Clofarabine plasma pharmacokinetics has been noted to be variable in humans, and the plasma half-life has not been reported in phase 1 and 2 studies (13, 14). In a population pharmacokinetic model, the systemic clearance of clofarabine was 17 to 30 L/h/m$^2$ (15), a result very similar to the 17 mL/min/kg or ~20 L/h/m$^2$ clearance observed in the nonhuman primates. Studies of clofarabine CSF penetration in humans have not been reported. However, the CSF penetration of clofarabine that we observed after a 2-hour infusion in the nonhuman primates is only slightly lower than that reported for cladribine in children during a continuous infusion (7). The concentration of clofarabine required to inhibit 50% of cell growth (IC$_{50}$) in human lymphoblast cell lines after a 3-hour exposure is 0.095 μmol/L (16); after a 24- to 48-hour exposure, the IC$_{50}$ ranges from 0.003 to 0.23 μmol/L in various cell lines (17). Clofarabine approached this concentration in the CSF (Fig. 1) after a dose of 2.3 mg/kg (~46 mg/m$^2$). The recommended phase 2 dose in patients with leukemia is 40 mg/m$^2$. Thus, cytotoxic concentrations of clofarabine may be approached in the CSF after systemic administration.

We have shown that clofarabine is present in the CSF of nonhuman primates at potentially cytotoxic concentrations after systemic administration of a dose similar to that recommended for use in patients with leukemia. Because the blood-CSF and blood-brain barriers create a pharmacologic sanctuary for leukemic cells, use of intrathecal chemotherapy and other central nervous system–preventive strategies is a critical part of leukemia treatment. Systemic administration of drugs that penetrate into the CSF may form part of these strategies. Evaluation of the contribution of clofarabine to central nervous system preventive therapy should be considered in future studies.

**References**

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