Clinical Pharmacology of Low-Dose Cytosine Arabinoside

By D. Spriggs, J. Griffin, J. Wisch, and D. Kufe

Low doses of cytosine arabinoside (ara-C) have recently been administered by intravenous (IV) infusion and intermittent subcutaneous (SC) injection to patients with preleukemia and acute leukemia. Our studies have demonstrated that the continuous IV infusion of low-dose (20 mg/m²/d) ara-C produces hematologic improvement in patients with preleukemic syndromes. The present work has monitored plasma ara-C levels in five of these patients. The results demonstrate mean steady-state plasma levels ranging from 1.8 to 6.9 \( \times 10^{-6} \) mol/L. The range for total drug exposure (area under the curve) for the 14-day course was 6.6 to 15.9 \( \times 10^{-6} \) mol/L x hour. These findings have been compared to the pharmacokinetics of ara-C (10 mg/m²/d) given by bolus SC injection. This dose schedule resulted in peak ara-C levels 15 minutes after injection that were tenfold to 30-fold higher than the mean plasma level achieved during continuous IV infusion in the same patient. Furthermore, there was no detectable plasma ara-C at six hours after bolus injection. The differences in ara-C pharmacology for the continuous IV infusion and bolus SC injection dose schedules may contribute to the variability in response and toxicity achieved with these regimens.

CYTOSINE arabinoside (ara-C) is one of the most effective agents in the treatment of acute myelogenous leukemia (AML). This agent incorporates into DNA and not RNA of human myeloblasts. Furthermore, there is a highly significant relationship between formation of (ara-C)DNA and decreases in clonogenic survival of leukemic cells. The incorporated ara-C residue behaves as a relative chain terminator, and the extent of ara-C incorporation into DNA correlates significantly with inhibition of DNA synthesis.

In vitro studies using low doses of ara-C have demonstrated that slowing of DNA synthesis can induce differentiation of human leukemic cells. These findings have prompted the evaluation of low-dose ara-C in the treatment of preleukemia and acute leukemia. One study has demonstrated that administration of ara-C at 10 mg/m² by intermittent subcutaneous (SC) injection can produce responses in patients with both preleukemia and leukemia. Another study using a similar dose schedule has shown that the response rate may be as low as 10%. Our studies have used continuous intravenous (IV) infusion ara-C at 20 mg/m²/d for 14 to 21 days. Using this dose schedule, 11 of 16 patients with preleukemic syndromes have had significant hematologic improvement with increased platelets, neutrophils, and absence of transfusion requirements for two to 24+ months.

Although each of these studies has administered low doses of ara-C, the results have indicated discrepancies in therapeutic response. In the present study, we have attempted to characterize the differences in plasma pharmacokinetics of ara-C administered by continuous IV infusion and by bolus SC injection.

MATERIALS AND METHODS

Patients

Five patients with preleukemic syndromes in varying degrees of leukemic transformation were treated with a continuous IV infusion of ara-C at a dose of 20 mg/m²/d. Nine courses of therapy were monitored for plasma ara-C levels. Each course was 14 days in duration except for the second course administered to patient 4, which was terminated after seven days. Patients 1 and 5 each received a single SC bolus injection of 10 mg/m² prior to beginning the 14-day infusion. All patients had normal hepatic and renal function.

Sample Collection

Five-milliliter blood samples were collected in chilled vacutainers containing 50 units of heparin and 0.1 mmol/L of tetrahydrounidine (Calbiochem, San Diego) to inhibit plasma cytidine deaminase. The samples were promptly centrifuged at 200 g for five minutes, and the plasma was frozen at −20°C until analysis.

Assay

The plasma concentration of ara-C was determined by radioimmunoassay. The preparation of [1H]-ara-C (16 Ci/mmol, Moravek Biochemicals, Brea, Calif) was purified by high-pressure liquid chromatography prior to use in the assay. Sheep anti-ara-C serum (batch G/S/747/X11A) was purchased from Guildhay Antisera, Guilford, Surrey, England. All dilutions and assays were performed in 0.05 mol/L of K2PO4 (pH 7.6), 0.6% NaCl, and 0.1% gelatin. The unbound ara-C was separated by a dextran-charcoal suspension (Wien Laboratories, Succasunna, NJ) for each assay. All sample analyses were performed at least three times. Patient plasma samples without ara-C treatment were also analyzed to exclude false-positive results. No blank plasma sample was available in one patient (No. 3).

RESULTS

Plasma ara-C levels were monitored in five preleukemic patients receiving ara-C by continuous IV infusion.
sion at a dose of 20 mg/m²/d. Representative results obtained for three patients are illustrated in Fig 1. Plasma samples were obtained at varying intervals throughout the 14-day period of infusion. All samples for each treatment course were assayed at the same time. The plasma ara-C levels ranged from \(1.5 \times 10^{-8}\) mol/L to nearly \(10^{-7}\) mol/L. The area under the curve (AUC) was calculated by the trapezoidal rule\(^4\) as a measure of total drug exposure. The values obtained for these patients ranged from 6.5 to \(21.0 \times 10^{-6}\) mol/L x hour. Plasma 1-β-D-arabinofuranosyluracil (ara-U) levels are not detected in this radioimmunoassay.\(^3\) Furthermore, plasma ara-U levels were not detectable by reverse-phase high-pressure liquid chromatography.\(^2\)

The variability in plasma ara-C levels within individual patients is listed in Table 1. The data includes nine courses administered to the five patients. The differences in mean plasma ara-C levels obtained between the first and second week of therapy were not significantly different, thus suggesting that neither drug accumulation nor enhanced degradation occurred during the 14-day study period. In contrast, there were significant differences in mean plasma ara-C levels from patient to patient. Paired \(t\) test comparisons\(^1\) of mean plasma levels demonstrated that patients 1, 3, and 4 had significantly different ara-C levels \((P < .002)\). Patient 2 had a significantly higher level than patient 3 \((P < .001)\), but the differences in mean levels from patient 1 and 4 did not reach statistical significance.

Two patients received single SC bolus injections of ara-C at 10 mg/m² to provide a comparison with the levels achieved by continuous IV infusion. The results obtained are illustrated in Fig 2. The peak ara-C concentration was observed 15 minutes after injection in each of these patients. The peak plasma ara-C level was ten to 30 times higher than the mean plasma ara-C level achieved during the continuous infusion schedule in the same patient. At three hours after bolus injection, the plasma ara-C level had fallen below the mean plasma ara-C level achieved by continuous infusion. There was no detectable ara-C present in plasma at six hours after bolus injection. The AUC as determined by the trapezoidal rule was \(532\) nmol/L x hour for patient 1 (Fig 2A) and \(1,044\) nmol/L x hour for patient 5 (Fig 2B). The AUC for 12-hour continuous IV infusions calculated from the mean plasma ara-C level was \(771\) nmol/L x hour for patient 1 and \(630\) nmol/L x hour for patient 5. Thus, the total drug exposure was similar when the bolus SC or continuous IV infusion regimen was used.

**DISCUSSION**

Low-dose ara-C regimens have had varying success in the treatment of preleukemia and acute leukemia. Several studies using 10 to 20 mg/m² ara-C as a bolus injection every 12 hours have had generally disappointing results.\(^{10,16-18}\) These studies utilized a twice-daily

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<th>Table 1. Plasma Ara-C Levels in Patients Receiving 20 mg/m²/d by Continuous IV Infusion</th>
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<tr>
<td><strong>Ara-C Levels (nmol/L)</strong></td>
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Results are expressed as the mean ± SE. Ara-C, cytosine arabinoside.
schedule for seven to 21 days. A similar study using 10 mg/m² every 12 hours resulted in improvement in 71% of the 21 patients treated. Our experience in 16 patients, all with preleukemia, treated with ara-C at 20 mg/m²/d by continuous IV infusion for 14 days has resulted in 11 patients achieving beneficial hematological response. The basis for the discrepancies in response rates has not been identified. The pharmacokinetic behavior of ara-C in these regimens has not been previously defined. We have measured plasma levels in two patients receiving ara-C by the same bolus SC dose schedule. The data obtained demonstrate peak plasma levels of approximately 10⁻⁶ mol/L. Furthermore, plasma ara-C levels were undetectable six hours after bolus injection. Thus, this dose schedule results in a six-hour interval with no detectable drug before the next injection. This varies substantially from the pharmacokinetic behavior of ara-C as a continuous infusion.

The administration of ara-C by continuous IV infusion results in plasma ara-C levels ranging between 10⁻⁶ and 10⁻⁷ mol/L. We have previously demonstrated that ara-C concentrations between 10⁻⁶ and 10⁻⁴ mol/L partially inhibit HL-60 DNA replication and induce maturation along the monocyte lineage. Similarly, we have recently demonstrated that slowing of K562 human erythroleukemia cell proliferation by >10⁻⁸ mol/L of ara-C induces hemoglobin synthesis and results in loss of self-renewal capacity. In these studies, 10⁻⁶ mol/L of ara-C was cytotoxic and decreased the appearance of a differentiated phenotype. Our in vitro findings would suggest that the continuous exposure of leukemic cells to concentrations up to 10⁻⁷ mol/L of ara-C could provide an effective approach for inducing differentiation.

We have also monitored AUC for three patients who received ara-C by continuous IV infusion. There were significant differences between the total amount of drug exposure for each of these patients. Thus, drug metabolism may account for some variability in terms of response and emphasizes the value of pharmacokinetic measurements in patients under study. The number of patients studied thus far is too small to draw any conclusions about the relationship between dose schedule, plasma level, and response. The AUC for a bolus SC injection is similar to that achieved during a 12-hour interval of continuous IV infusion. The cytotoxic effects of these dose schedules should therefore be similar. The major difference, however, is that the bolus regimen does not provide continuous exposure to ara-C. Because the available clinical data does not distinguish between cytotoxicity and induction of differentiation, additional pharmacokinetic and comparative studies of the various dose schedules will now both needed to determine more efficacious low-dose ara-C regimens.

REFERENCES


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