Clinical pharmacology of N^4^-palmitoyl-1-β-D-arabinofuranosylcytosine in patients with hematologic malignancies

Takanori Ueda^1, Toru Nakamura^1, Daizaburo Kagawa^1, Michihiko Uchida^1, Naohica Domae^1, Masatoshi Sugiyama^2, Yasunori Ueda^3, Masataka Sasada^3, and Haruto Uchino^3

1 First Department of Medicine, 2 Department of Pharmacy, Fukui Medical School, Matsuoka, Fukui, 910-11 and 3 First Department of Medicine, Faculty of Medicine, Kyoto University, Sakyo, Kyoto, 606, Japan

Summary. The pharmacokinetics of oral N^4^-palmitoyl-1-β-D-arabinofuranosylcytosine (PLAC), a lipophilic and deaminase-resistant derivative of 1-β-D-arabinofuranosylcytosine (ara-C), were determined in patients with hematologic malignancies. The concentration of ara-C and 1-β-D-arabinofuranosyluracil (ara-U), metabolites of PLAC, were measured by radioimmunoassay and gas chromatography-mass spectrometry-mass fragmentography, respectively. The concentration of PLAC was determined by measuring ara-C, which was derived from PLAC by hydrolyzation. In six patients given an oral bolus of PLAC (300 mg/m²), the plasma disappearance curve of PLAC corresponded to a one-compartment open model, including first-order absorption. The peak plasma level was 22.9±6.4 ng/ml, and the predicted time to reach the peak level was 2.5±1.0 h. The elimination half-life was 3.8±2.7 h. The plasma ara-C level increased slowly to 6.9 ng/ml during the 1st 2–3 h after administration and remained over 1.0 ng/ml for 12 h. Plasma ara-U was detectable for at least 24 h, with a peak concentration of 376 ng/ml at 6 h. Urinary PLAC excretion was below the limit of detection (5 ng/ml) in all cases. Prolonged urinary ara-C and ara-U excretion was detected, but the total recovery rate was low (6.7% in 24 h) and varied between patients. In spite of the lipophilic nature of the drug, the PLAC concentration in the cerebrospinal fluid, measured at 3 or 6 h, was below the limit of detection in all four patients with no meningeal involvement. This study showed low but persistent levels of PLAC in plasma and tissues, with a continuous release of small amounts of ara-C, which demonstrated antitumor activity in patients with hematologic malignancies.

Introduction

1-β-D-Arabinofuranosylcytosine (ara-C) is one of the most useful drugs for the treatment of patients with acute leukemia or lymphoma [5]. However, in humans this drug has a short half-life as a result of its conversion by cytidine deaminase to 1-β-D-arabinofuranosyluracil (ara-U), an inactive metabolite of ara-C [4, 9, 17]. Several attempts have been made to overcome this limitation [3, 11, 16]. N^4^-acyl-ara-C is a derivative of ara-C with a long-chain fatty acid introduced at the N^4^-position. It is deaminase-resistant and also has high lipophilicity and a long half-life compared with ara-C [1, 2]. Among the N^4^-acyl-ara-C derivatives, N^4^-behenoyl-1-β-D-arabinofuranosylcytosine (BHAC) has been used clinically by intravenous infusion and has shown a therapeutic effect equivalent to that of ara-C [15], with fewer side effects. We have reported the results of a precise pharmacological study of BHAC [18, 24].

N^4^-palmitoyl-1-β-D-arabinofuranosylcytosine (PLAC) is another acyl-ara-C derivative. Besides the unique therapeutic advantages of N^4^-acyl-ara-C derivatives vs ara-C, this drug offers patients another benefit: it is given orally. According to a preclinical study [10], PLAC is absorbed from the gastrointestinal tract and small amounts of ara-C are released slowly. Phase I and II clinical studies carried out in Japan have shown promising results with tolerable side effects in patients with hematologic malignancies, including acute leukemia, myelodysplastic syndrome, and myeloproliferative disorder [14, 19]. A comprehensive pharmacokinetic study of oral PLAC was carried out in patients with hematologic malignancies to determine an appropriate schedule for administration.

Materials and methods

PLAC was kindly supplied by Asahi Chemical Industry Co., Ltd. (Tokyo, Japan), as 50-, 100-, and 200-mg capsules containing dioctyl sodium sulfosuccinate (Nikkol OTP-100S; Nihon Surfactant Kogyo K. K., Tokyo, Japan) and polyoxyethylene 40 stearate (Nikkol MYS-40; Nihon Surfactant Kogyo K. K., Tokyo, Japan) as a detergent and stabilizer.

Patients. All patients eligible for this study were diagnosed as having hematologic malignancies by the usual hematologic and clinical criteria (Table 1). Informed consent for this investigation was obtained from each patient or his family prior to drug treatment and pharmacokinetic evaluation. None of the patients had hepatic or renal dysfunction, as determined by a serum bilirubin value of ≥2.0 mg/dl and a serum creatinine level of ≥1.5 mg/dl. No other drugs were given until sample collection was completed.

* This study was supported in part by Grants-in-Aid from the Ministry of Health and Welfare (62-18 and 63-3), Japan

Offprint requests to: T. Ueda, First Department of Medicine, Fukui Medical School, Matsuoka, Fukui 910-11, Japan
PLAC administration and sample collection. PLAC was given orally as a bolus with 200 ml H2O after an overnight fast. Patients were allowed to resume normal p.o. intake 2 h after drug administration. Blood samples were collected at designated intervals for 24 h in heparinized tubes containing tetrahydrofuran, an inhibitor of cytidine deaminase, at a final concentration of 0.1 mM [9]; they were immediately put on ice. Plasma samples were then separated by centrifugation at 4 °C (700 g, 10 min). Urine samples were also collected at designated intervals for 24 h. Samples of plasma and urine were stored at -20 °C until the assays for PLAC, ara-C, and ara-U were carried out. For the assay of drug concentration in the cerebrospinal fluid (CSF), spinal taps were carried out in four patients who had acute leukemia with no meningeal involvement at 3 or 6 h after PLAC administration; 2 ml liquid was collected in tubes containing tetrahydrofuran (0.1 mM).

Determination of concentrations of PLAC, ara-C, and ara-U. PLAC concentration in the samples was determined by a method described in detail elsewhere [19]. Briefly, PLAC was hydrolyzed by sodium hydroxide to ara-C, and the latter was measured by radioimmunoassay. A 1-ml plasma samples was diluted with an equal volume of 0.9% sodium chloride and 10 µl ethanol and was mixed with 2 ml methanol and 4 ml chloroform. After sonication and centrifugation, the chloroform layer was collected and 3 ml was mixed with 2 ml methanol and 0.2 ml 0.2 N sodium hydroxide, kept overnight at 37 °C, and dried in vacuo. Then, PLAC was hydrolyzed and recovered as ara-C. The residue was mixed with 1 ml 0.01 M phosphate-buffered saline (pH 7.4) containing 0.5% bovine serum albumin, then sonicated and centrifuged. The ara-C concentration in the supernatant was determined by the radioimmunoassay method described elsewhere [24]; the limit of detection was 1 ng/ml for ara-C and 5 ng/ml for PLAC.

The ara-C concentration in the samples was determined directly by the radioimmunoassay method described above; the limit of detection was 1 ng/ml. The ara-U concentration in the samples was determined by the method of Mizuno et al., using a gas chromatography-mass spectrometry-mass fragmentography procedure described elsewhere [24]; the limit of detection was 10 ng/ml.

Analysis of the plasma PLAC concentration. Plasma concentrations (Ct) of PLAC after a single oral dose were fitted to a one-compartment open model, including first-order absorption, as described by the following equation [6]:

\[ Ct = \frac{F \cdot D \cdot Ka}{Vd (Ka - Kel)} \left[ e^{-kel(t-t_0)} - e^{-ka(t-t_0)} \right], \]

where \( K_a \) is the apparent first-order absorption rate constant, \( K_a \) is the apparent first-order elimination rate constant, \( V_d \) is the apparent volume of distribution, \( F \) is the apparent fraction of the available dose (which could not be determined in this case), \( D \) is the dose given, and \( t_0 \) is the lag time preceding the initiation of absorption.

The predicted time of the peak plasma level (\( C_{max} \)) after administration (\( T_{max} \)) was calculated as the time when \( dCt/dt = 0 \). The elimination half-life was calculated as 0.693/Kel. The AUC from time zero to infinity (AUCinf) was computed using the trapezoidal rule. The total plasma clearance (Cltot/F) was estimated from D/AUCinf. The plasma PLAC concentration data was analyzed by nonlinear least-squares regression analysis, MULTI [28], including Akaike’s information criterion [27].

Results

Plasma PLAC levels

The pharmacokinetic parameters obtained by computer analysis of the plasma PLAC concentration-vs-time curve (Fig. 1) are summarized in Table 2.

The curves corresponded to a one-compartment open model with first-order absorption. \( C_{max} \) of PLAC varied from 16.2 to 33.7 µg/l, with a mean ± SD of 22.9 ± 6.4 µg/l. \( T_{max} \) was 2.5 ± 1.0 h. The plasma \( T_{1/2} \) for PLAC varied from patient to patient (range, 1.4–6.6 h), with an average of 3.8 ± 2.7 h. The mean ± SD of \( V_d/F \) was 262 ± 148 l/kg (range, 178–556 l/kg), and the mean AUC was 174 µg·h/l. The total clearance (Cltot/F) was 63.4 ± 33.6 l/h per kg.

Urinary excretion of PLAC, ara-C, and ara-U

Unchanged PLAC was not excreted in the urine in any patient. However, in 24 h, 0.05% of the dose appeared as ara-C and 6.67%, as ara-U; thus, 6.72% of PLAC was eliminated in the urine in 24 h.

Plasma levels of ara-C and ara-U

The plasma concentration of ara-C, the active metabolite of PLAC, was measured in the same samples as were used for calculating plasma PLAC levels (Fig. 1). The concentration of ara-C increased slowly to 6.9 ± 4.8 ng/ml during the first 2–3 h after administration and was maintained for 12 h after drug administration. The apparent mean half-life for ara-C, calculated in four cases (patients 2, 4, 5, and 6), was 2.49 h. Plasma ara-U was detectable at least