Monitoring of Intracellular 1-β-D-Arabinofuranosylcytosine 5′-Triphosphate in 1-β-D-Arabinofuranosylcytosine Therapy at Low and Conventional Doses

Takahiro Yamauchi,[1, 3] Yasukazu Kawai,[1] Shinji Kishi,[1] Nobuyuki Goto,[2] Yoshimasa Urasaki,[1] Shin Imamura,[1] Toshihiro Fukushima,[1] Akira Yoshida,[1] Hiromichi Iwasaki,[1] Hiroshi Tsutani,[1] Mikio Masada[2] and Takanori Ueda[1]

[1]First Department of Internal Medicine and [2]Department of Hospital Pharmacy, Fukui Medical University, 23 Shimoaizuki, Matsuoka, Fukui 910-1193

1-β-D-Arabinofuranosylcytosine (ara-C) is used empirically at a low, conventional, or high dose. Ara-C therapy may be optimal if it is directed by the clinical pharmacokinetics of the intracellular active metabolite of ara-C, 1-β-D-arabinofuranosylcytosine 5′-triphosphate (ara-CTP). However, ara-CTP has seldom been monitored during low- and conventional-dose ara-C therapies because detection methods were insufficiently sensitive. Here, with the use of our newly established method (*Cancer Res.*, 56, 1800–1804 (1996)), ara-CTP was monitored in leukemic cells from acute myelogenous leukemia patients receiving low- or conventional-dose ara-C [subcutaneous ara-C administration (10 mg/m^2) (3 patients), continuous ara-C infusion (20 or 70 mg/m^2/24 h) (7 patients), 2-h ara-C infusion (70 mg/m^2) (4 patients), and 2-h infusion of N^4-behenoyl-1-β-D-arabinofuranosylcytosine, a deaminase-resistant ara-C derivative (70 mg/m^2) (6 patients)]. Ara-CTP could be determined at levels under 1 μM. There was a close correlation between the elimination half-life values of the plasma ara-C and the intracellular ara-CTP. The presence of ara-C in the plasma was important to maintain ara-CTP. The continuous ara-C and the 2-h N^4-behenoyl-1-β-D-arabinofuranosylcytosine infusions maintained ara-CTP and the plasma ara-C longer than the subcutaneous ara-C or the 2-h ara-C infusion. They also afforded relatively higher ara-CTP concentrations, and consequently produced ara-CTP more efficiently than the 2-h ara-C infusion. Different administration methods produced different quantities of ara-CTP even at the same dose.

Key words: Ara-C — Ara-CTP — Clinical pharmacokinetics — Low and conventional dose

1-β-D-Arabinofuranosylcytosine (ara-C), a pyrimidine nucleoside analog, is one of the most effective anticancer agents for the treatment of patients with leukemia.[1–3] There are several methods of administering ara-C and its derivatives. Conventional-dose ara-C (60–100 mg/m^2) is given by intravenous infusion for remission induction therapy of patients with newly diagnosed acute myelogenous leukemia.[2, 3] N^4-Behenoyl-1-β-D-arabinofuranosylcytosine (BHAC), one of the N^4-acyl derivatives of ara-C, is frequently used instead of conventional-dose ara-C in Japan.[4] While ara-C is rapidly inactivated by cytidine deaminase in the plasma, BHAC is resistant to deamination[5] and its plasma level is well maintained, resulting in continuous release of ara-C.[6] Low-dose ara-C (3–10 mg/m^2) is administered subcutaneously or by continuous infusion to elderly patients with leukemia or myelodysplastic syndrome in the expectation of a mild cytotoxic effect.[7–9] Patients with refractory leukemia receive ara-C at high doses (1000–3000 mg/m^2) to overcome the drug resistance.[10, 11] Thus, ara-C is used at doses over a 1000-fold range. However, the doses and the schedules are empiri-

cally decided from the clinical diagnosis and the hematological findings.

As regards the mechanism of action, after being transported into leukemic cells, ara-C is phosphorylated to 1-β-D-arabinofuranosylcytosine 5′-monophosphate and then to its active metabolite, 1-β-D-arabinofuranosylcytosine 5′-triphosphate (ara-CTP).[12] The cytotoxic effect of ara-C is thought to be due to the inhibition of DNA polymerases by ara-CTP in competition with deoxyribonucleoside 5′-triphosphate.[13–15] A small portion of ara-CTP is incorporated into the DNA strand and terminates the DNA elongation.[16] Thus, intracellular ara-CTP is the key metabolite in the mechanism of action.[13–15]

The clinical pharmacokinetics of ara-CTP has been investigated mainly in high-dose ara-C therapy.[17–22] It was reported that the therapeutic efficacy was more closely related to the amount of ara-CTP in leukemic cells than to the plasma ara-C concentration.[19–22] It was further recommended that ara-C therapy should be constructed so as to afford the largest amount of ara-CTP. Thus, ara-C therapy may be effective and reasonable if it is directed on the basis of the pharmacokinetics of ara-CTP in each individual patient. However, ara-CTP has seldom been measured during low- and conventional-dose ara-C therapies because

detection methods for ara-CTP have been insufficiently sensitive.[17]

We established a new method for the determination of intracellular ara-CTP,[23] using a combination of high-performance liquid chromatography and radioimmunoassay. In brief, acid-soluble metabolites were extracted from leukemic cells of patients receiving ara-C. Ara-CTP was fractionated from other nucleotides by high-performance liquid chromatography, and dephosphorylated to ara-C by alkaline phosphatase. The ara-C was detected by radioimmunoassay using anti-ara-C serum. This method was 50-fold more sensitive than previous methods and suitable for clinical use.

In the present study, using this method, we monitored ara-CTP in leukemic cells from patients receiving ara-C or BHAC, the prodrug of ara-C, at a low or a conventional dose. We further evaluated the pharmacokinetics of ara-CTP to find the most efficient method of ara-C administration.

MATERIALS AND METHODS

Chemicals and enzymes Ara-C, ara-CTP and bacterial alkaline phosphatase (EC 3.1.3.1) were purchased from Sigma Chemical Co. (St. Louis, MO). Na_2HPO_4, acetonitrile, NaCl, Tris, perchloric acid, KOH, and dextran were purchased from Nacalai Tesque Inc. (Kyoto). Tetrahydrouridine was obtained from Calbiochem-Novabiochem Corp. (La Jolla, CA). Charcoal was purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). [5-^{3}H]Ara-C (30 Ci/mmol) was purchased from Amersham International (Buckinghamshire, UK). Anti-ara-C serum was kindly supplied by Asahi Chemical Industry Co., Ltd. (Tokyo).[24]

Patients and treatments Twenty patients entered into this study had been diagnosed as having acute myelogenous leukemia. Informed consent for this study was obtained in advance. The patients' characteristics are shown in Table I. Low-dose ara-C was administered in 2 ways. One was the subcutaneous administration of 10 mg/m^2 ara-C (SC ara-C (10)) twice daily (3 patients), and the other was the continuous intravenous infusion of 20 mg/m^2 ara-C for 24 h (Cont IV ara-C (20)) (4 patients). Conventional-dose ara-C including the equivalent dose of BHAC was administered in 3 ways, i.e., 2-h intravenous infusion of 70 mg/m^2 ara-C (2-h IV ara-C (70)) once daily (4 patients), continuous intravenous infusion of 70 mg/m^2 ara-C for 24 h (Cont IV ara-C (70)) (3 patients), and 2-h intravenous infusion of 70 mg/m^2 BHAC (2-h IV BHAC (70)) twice daily (6 patients).

Blood sampling Blood samples were drawn into heparinized tubes containing tetrahydrouridine, an inhibitor of cytidine deaminase, at a final concentration of 0.1 mM.[25] The plasma and the leukemic cells were fractionated using a Ficoll-Paque density centrifugation procedure.[26] Both

Table I. Patients' Characteristics

No. of patients entered	20
Sex (n)	
Male	13
Female	7
Age (y)	
Median	60
Range	24–82
Diagnosis by FAB classification (n)	
M0	2
M1	4
M2	13
M3	0
M4	1
M5	0
M6	0
M7	0

n, number of patients; FAB, French-American-British.

the plasma ara-C concentration and the ara-CTP concentration in leukemic cells were measured.

Determination of plasma ara-C concentration The plasma ara-C concentration was measured using the radioimmunoassay described by Shimada *et al*.[24] In brief, 50 μl of the plasma was mixed with 200 μl of 0.01 M phosphate buffer (containing 0.5% bovine serum albumin and 0.9% NaCl) (pH 7.4), 50 μl of anti-ara-C serum, and 50 μl of [5-^{3}H]ara-C. The mixture was incubated for 16 h at 4°C. Five hundred microliters of dextran-coated charcoal, which was composed of 100 mg of dextran and 1000 mg of charcoal per 100 ml of the above phosphate buffer, was added to the mixture. This mixture was allowed to stand for 30 min at 4°C, and centrifuged (1000g, 15 min, 4°C). The radioactivity of the supernatant was determined. The detection limit was 0.4 nM.

Determination of intracellular ara-CTP concentration The intracellular ara-CTP concentration was determined by the newly established method.[23] The nucleotide pool including ara-CTP was extracted from leukemic cells (2×10^7 cells) using cold perchloric acid (final concentration 0.3 M), and then neutralized with KOH. Ara-CTP was separated from the nucleotide pool by high-performance liquid chromatography with an ion-exchange column, TSK gel DEAE-2 SW (250×4.6 mm inside diameter: particle size 5 μl: TOSOH Corp., Tokyo). Elution was done with 0.05 M Na_2HPO_4 (pH 6.9)-20% acetonitrile at a constant flow rate of 0.7 ml/min. Ara-CTP was monitored at 269 nm and fractionated. The ara-CTP fraction was freeze-dried, dissolved again in 300 μl of 0.1 M Tris buffer (pH 10.1), and mixed with alkaline phosphatase (10 units). The mixture was incubated for 12 h at 55°C, during which time, ara-CTP was dephosphorylated to ara-C. The ara-C

derived from ara-CTP was measured using the radioimmunoassay described above. The obtained value of ara-CTP was divided by the packed cell volume (2×10^7 cells) to determine the intracellular concentration. The detection limit was 20 nM.

Pharmacokinetic analysis The area under the concentration-time curve from 0 to 24 h (AUC) and the pharmacokinetic parameters were obtained by moment analysis.[27] To further evaluate the intracellular pharmacokinetics, three parameters were added to the usual pharmacokinetic parameters. They were C_{ratio}, AUC_{ratio}, and $AUC_{/dose}$. The C_{ratio} is the ratio of the concentration (C_{max} or C_{ss}) of intracellular ara-CTP to that of plasma ara-C. The AUC_{ratio} is the ratio of the AUC of intracellular ara-CTP to that of plasma ara-C. Both of them were used for direct comparison of the absolute values of plasma ara-C and intracellular ara-CTP concentrations. The $AUC_{/dose}$ was defined as the AUC of ara-CTP ($\mu M \cdot$h) produced by 1 mg of ara-C, which is the ratio of the AUC to the dose of ara-C administered. $AUC_{/dose}$ ($\mu M \cdot$h/mg) was used to find the most efficient method of drug administration that would yield the greatest AUC of ara-CTP. The dose of BHAC was converted into that of ara-C, according to the ratio of their molecular weights.

Statistical analysis Statistical analysis was performed with STATVIEW 5.0 software (Abacus Concepts, Berkeley, CA). The Mann-Whitney test was used for the evaluation of differences in C_{ratio}, AUC_{ratio}, and $AUC_{/dose}$. The level of significance (P) was set at 0.05.

RESULTS

Our new method enabled measurement of extremely low ara-CTP levels (under 1 μM). The ara-CTP pharmacokinetics could be determined even in patients receiving low-dose ara-C. The pharmacokinetic parameters (mean±

SD values) of both the plasma ara-C and the intracellular ara-CTP are summarized in Table II. They varied among patients, doses, and administration methods.

Low-dose ara-C After SC ara-C (10), the plasma ara-C reached the maximal concentration (C_{max}) within 1 h and decreased rapidly thereafter. The intracellular ara-CTP also reached the C_{max} and decreased rapidly thereafter (Fig. 1A). During Cont IV ara-C (20), the plasma ara-C reached a steady-state concentration (C_{ss}). The intracellular ara-CTP also reached the C_{ss} (Fig. 1B). Thus, Cont IV ara-C (20) maintained the concentrations of not only the plasma ara-C but also ara-CTP constant during the infusion, while SC ara-C (10) showed decreases in both.

Conventional-dose ara-C After 2-h IV ara-C (70), the plasma ara-C reached the C_{max} at the end of the infusion and then decreased thereafter. The intracellular ara-CTP reached the C_{max} at almost the same time and thereafter decreased (Fig. 2A). During Cont IV ara-C (70), both the plasma ara-C and the intracellular ara-CTP reached the C_{ss} (Fig. 2B). Thus, Cont IV ara-C (70) maintained the concentrations of both ara-CTP and the plasma ara-C constant during the infusion, while 2-h IV ara-C (70) showed decreases in both after the end of the infusion.

BHAC, the prodrug of ara-C, is resistant to deamination and releases ara-C in the plasma continuously.[6] As shown in Fig. 2C, after 2-h IV BHAC (70), the plasma ara-C reached the C_{max} at the end of the infusion and decreased slowly thereafter. The elimination half-life ($t_{1/2}$) of the plasma ara-C concentration yielded by 2-h IV BHAC was significantly longer than that by 2-h IV ara-C (70) ($P=0.03$, the Mann-Whitney test). The intracellular ara-CTP also reached the C_{max} and decreased slowly thereafter. The $t_{1/2}$ of the intracellular ara-CTP concentration yielded by 2-h IV BHAC was longer than that by 2-h IV ara-C (70), although the difference was not significant ($P=0.39$, the Mann-Whitney test). Thus, 2-h IV BHAC (70) main-

Table II.　Pharmacokinetic Parameters (Mean±SD) of Each Administration Method

Agent (mg/m^2)	n	Ara-C			Ara-CTP		
		C_{max} or C_{ss} (μM)	AUC ($\mu M \cdot$h)	$t_{1/2}$ (h)	C_{max} or C_{ss} (μM)	AUC ($\mu M \cdot$h)	$t_{1/2}$ (h)
[Low dose]							
SC ara-C (10)	3	0.25±0.14	0.2±0.1	0.8±0.2	1.3±1.3	5.5±5.3	2.1±0.4
Cont IV ara-C (20)	4	0.05±0.04	1.1±0.7		1.0±0.7	24.0±16.6	
[Conventional dose]							
2-h IV ara-C (70)	4	0.83±0.56	1.4±0.4	2.1±1.5	3.1±1.4	16.6±4.1	4.5±2.1
Cont IV ara-C (70)	3	0.14±0.55	3.2±1.3		3.8±2.2	92.2±53.9	
2-h IV BHAC (70)	6	0.11±0.02	0.8±0.4	8.1±7.5	3.1±2.1	28.1±8.7	8.7±6.0

n, number of patients; ara-C, 1-β-D-arabinofuranosylcytosine; ara-CTP, 1-β-D-arabinofuranosylcytosine 5′-triphosphate; BHAC, N^4-behenoyl-1-β-D-arabinofuranosylcytosine; SC, subcutaneous administration; 2-h IV, intravenous infusion for 2 h; Cont IV, continuous intravenous infusion; C_{max}, maximal concentration; C_{ss}, steady-state concentration; AUC, area under the concentration-time curve; $t_{1/2}$, elimination half-life.

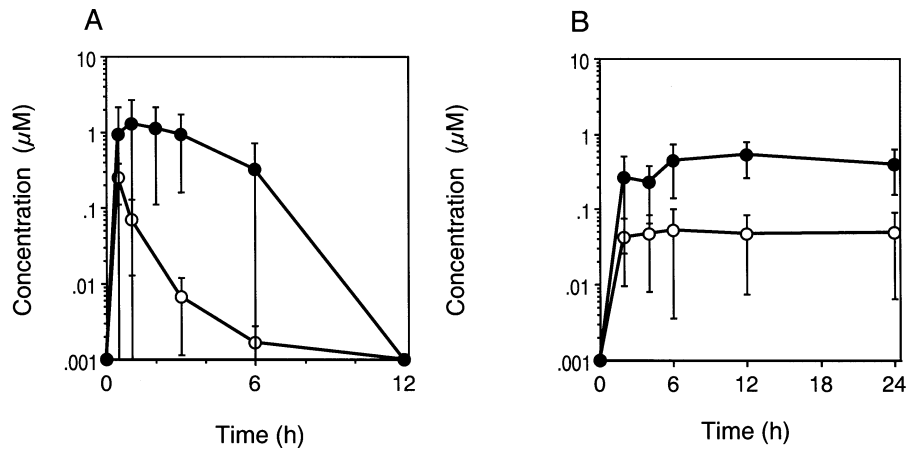Fig. 1. Mean±SD concentration-time curves of plasma ara-C (open circles) and intracellular ara-CTP (solid circles) in acute myelogenous leukemia patients receiving low-dose ara-C. (A) The subcutaneous administration of 10 mg/m² ara-C (3 patients). (B) The continuous intravenous infusion of ara-C (20 mg/m²/24 h) (4 patients). Bars represent SD.
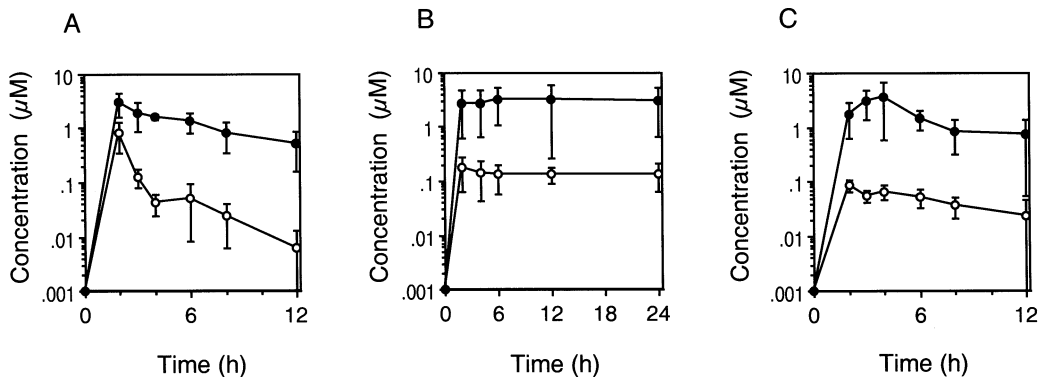

Fig. 2. Mean±SD concentration-time curves of plasma ara-C (open circles) and intracellular ara-CTP (solid circles) in acute myelogenous leukemia patients receiving conventional-dose ara-C or BHAC. (A) The 2-h intravenous infusion of 70 mg/m² ara-C (4 patients). (B) The continuous intravenous infusion of ara-C (70 mg/m²/24 h) (3 patients). (C) The 2-h intravenous infusion of 70 mg/m² BHAC (6 patients). Bars represent SD.

tained the concentrations of both ara-CTP and the plasma ara-C for longer than 2-h IV ara-C (70).

The plasma ara-C concentration at the end of the 2-h infusion of ara-C and BHAC could not be the C_{max} in the strictest sense because there were no data during the infusion. However, concerning ara-C, the plasma ara-C concentration was reported to be maximal at the end of the infusion in a 1-h infusion of an intermediate dose (1 g/m²)[28] and in a 3-h infusion of a high dose (3 g/m²).[18] Concerning BHAC, a pharmacokinetic study of conventional-dose BHAC infusion, which included the time points during the infusion, revealed that the C_{max} occurred at the end of the infusion.[6] Therefore, we regarded the highest concentration arising at the end of the infusion as the C_{max}, although we had no time points during the infusion.

Relationship between the plasma ara-C and the intracellular ara-CTP pharmacokinetics Pharmacokinetic parameters were compared between the plasma ara-C and the intracellular ara-CTP. There was a close correlation only between the $t_{1/2}$ values of ara-C and ara-CTP ($P=0.02$, the Spearman's rank correlation test). The longer ara-C was present in the plasma, the longer ara-CTP remained in leukemic cells. This suggests that the presence of ara-C in the plasma would be crucial to the maintenance of the intracellular ara-CTP. Except for $t_{1/2}$, there was no indication of a close relationship between the plasma and the intracellular pharmacokinetics.

Comparison of the C_{ratio} values The C_{ratio} value was calculated to determine the relative value of the intracellular ara-CTP concentration (C_{max} or C_{ss}) to the corresponding

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plasma ara-C concentration (C_{max} or C_{ss}) in each administration method (Fig. 3A). The C_{max} of intracellular ara-CTP was 4-fold greater than that of plasma ara-C in 2-h IV ara-C (70) (C_{ratio}; 4.3±2.3, mean±SD). The C_{ss} of the intracellular ara-CTP was 20-fold greater than that of the plasma ara-C in Cont IV ara-C (20, 70) (C_{ratio}; 24.2±11.8, mean±SD). The C_{ratio} for Cont IV ara-C (20, 70) was significantly greater than that for 2-h IV ara-C (70) ($P=0.01$). The C_{ratio} for 2-h BHAC (70) (C_{ratio}; 31.5±22.4, mean±SD) was also greater than that for 2-h IV ara-C (70) ($P=0.01$). No significant difference was obtained between the C_{ratio} values for Cont IV ara-C and 2-h IV BHAC ($P=0.8$). Thus, Cont IV ara-C (20, 70) and 2-h IV BHAC (70) afforded relatively higher ara-CTP concentrations than 2-h IV ara-C (70).

Comparison of the AUC_{ratio} values The AUC_{ratio} value was calculated to determine the relative value of the AUC of ara-CTP to the corresponding AUC of the plasma ara-C (Fig. 3B). The AUC of intracellular ara-CTP was 12-fold greater than that of plasma ara-C in 2-h IV ara-C (70) (AUC_{ratio}; 12.4±4.9, mean±SD). The AUC of intracellular ara-CTP was 26-fold greater than that of plasma ara-C in Cont IV ara-C (20, 70) (AUC_{ratio}; 26.1±11.4, mean±SD). The AUC_{ratio} for 2-h BHAC (70) was 40.8±19.8 (mean±SD). Thus, the AUC_{ratio} was significantly greater in Cont IV ara-C (20, 70) ($P=0.02$) and 2-h IV BHAC (70) ($P=0.02$) than in 2-h IV ara-C (70). No significant difference was obtained between the AUC_{ratio} values for Cont IV ara-C and 2-h IV BHAC ($P=0.2$). This suggests that if leukemic cells are loaded with the same AUC of plasma ara-C by different administration methods, the corresponding AUC of ara-CTP may be greater in Cont IV ara-C and 2-h IV BHAC, than in 2-h IV ara-C.

Comparison of the $AUC_{/dose}$ values $AUC_{/dose}$ was examined to find the most efficient method of administration that could provide the greatest amount of intracellular ara-CTP (Fig. 3C). The $AUC_{/dose}$ values for Cont IV ara-C (20, 70) (1.26±0.72 $\mu M \cdot h/mg$, mean±SD) and 2-h IV BHAC (70) (0.95±0.29 $\mu M \cdot h/mg$, mean±SD) were significantly greater than the value for 2-h IV ara-C (70) (0.23±0.07 $\mu M \cdot h/mg$, mean±SD) ($P=0.01$ for both). There was no significant difference of the $AUC_{/dose}$ value between Cont IV ara-C (20, 70) and 2-h IV BHAC (70) ($P=0.62$). Thus, the comparison of the $AUC_{/dose}$ suggests that Cont IV ara-C (20, 70) and 2-h IV BHAC (70) yielded ara-CTP more efficiently than 2-h IV ara-C.

DISCUSSION

The clinical pharmacology of ara-CTP has been examined mainly in high-dose ara-C therapy, because a sensitive monitoring method was lacking.[17–22] In conventional-dose ara-C therapy, only the C_{ss} during continuous infusion could be measured.[29] No information was available on the intracellular pharmacokinetics of low-dose ara-C therapy. In the present study, we successfully monitored extremely low levels of ara-CTP and evaluated the intracellular pharmacokinetics even in low-dose ara-C therapy. The pharmacokinetic behavior of ara-CTP varied widely among patients, doses, and administration methods of ara-C. The presence of ara-C in the plasma was important to the maintenance of ara-CTP in leukemic cells. Cont IV ara-C maintained both the plasma ara-C and the intracellular ara-CTP concentrations constant, while SC ara-C and 2-h IV ara-C showed decreases in both. In addition, Cont IV ara-C afforded a relatively higher ara-CTP concentra-
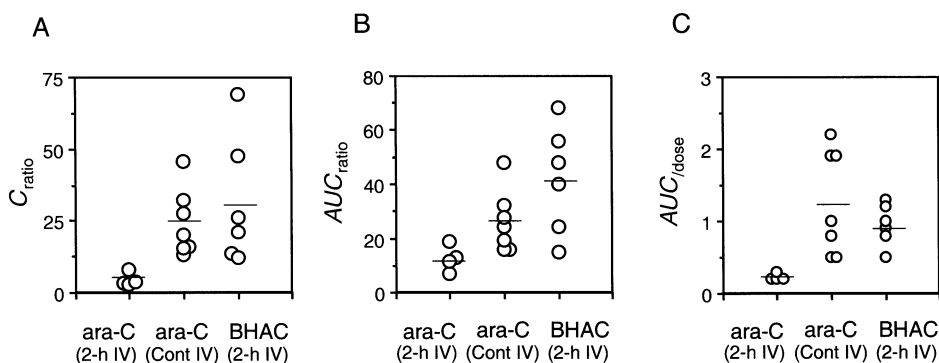

Fig. 3. (A) Comparison of the C_{ratio} values among 3 methods: the 2-h intravenous infusion of 70 mg/m² ara-C (4 patients), the continuous intravenous infusion of ara-C (20 or 70 mg/m²/24 h) (7 patients), and the 2-h intravenous infusion of 70 mg/m² BHAC (6 patients). C_{ratio} is the ratio of the concentration (C_{max} or C_{ss}) of intracellular ara-CTP to that of plasma ara-C. (B) Comparison of the AUC_{ratio} values among the above 3 methods. AUC_{ratio} is the ratio of the AUC of intracellular ara-CTP to that of plasma ara-C. (C) Comparison of the $AUC_{/dose}$ values among the above 3 methods. $AUC_{/dose}$ ($\mu M \cdot h/mg$) is the AUC of ara-CTP produced by 1 mg of ara-C, which is the ratio of the AUC to the dose administered. The dose of BHAC was converted into that of ara-C according to the ratio of their molecular weights. Each bar represents the mean value of each administration method.

tion, and consequently yielded ara-CTP more efficiently than 2-h IV ara-C. Similarly, 2-h IV BHAC also produced ara-CTP efficiently.

In our previous *in vitro* study,[30]) when HL-60 leukemic cells were incubated with 2 μM ara-C, ara-C was rapidly taken up into the cells, and metabolized in 2 ways. The intracellular ara-C was mainly deaminated to 1-β-D-arabinofuranosyluracil, and continuously transported outside of the cell. But, in part, the intracellular ara-C was phosphorylated to ara-CTP, which accumulated in the cell. The total concentration of the intracellular ara-C metabolites (including ara-C and metabolites of ara-C) surpassed the ara-C concentration in the medium within only 7 min from the start of the incubation, and became 4-fold greater than the medium ara-C concentration at 1-h incubation. Similarly, the intracellular ara-CTP concentration exceeded the medium ara-C concentration at 10 min from the start of the incubation, and became 3-fold greater than the medium ara-C concentration at 1-h incubation. Thus, the increase in the total concentration of intracellular ara-C metabolites was mainly attributed to the ara-CTP fraction (70–80% of total ara-C metabolites). We did not measure the intracellular ara-C concentration in the present study. However, these *in vitro* findings would accord with the present observation that the intracellular ara-CTP concentration was higher than the plasma ara-C concentration even very early after the drug administration, although the exact mechanism is unclear. Liliemark *et al.* have also demonstrated similar pharmacokinetic behavior in a 3-h infusion of high-dose ara-C.[18]) On the other hand, during the incubation with ara-C, the intracellular ara-C concentration itself did not exceed the medium ara-C concentration because of its rapid conversion into 1-β-D-arabinofuranosyluracil or ara-CTP in the cell. Moreover, while the intracellular ara-CTP concentration was reduced to half at 3 h after the drug washout, the presence of ara-C even at a low concentration in the medium maintained ara-CTP in the cell. Our pharmacokinetic study demonstrated that, even clinically, the presence of ara-C in the plasma maintained the level of intracellular ara-CTP. Moreover, only the continuous infusion of ara-C, which provided a steady concentration of plasma ara-C, maintained the intracellular ara-CTP constant. Therefore, we speculated that plasma ara-C would be incorporated into the cell continuously, and ara-CTP would be maintained as long as ara-C was present in the plasma.

The C_{ratio} for Cont IV ara-C (20, 70) was higher than that for 2-h IV ara-C (70). Liliemark *et al.* reported that the C_{max} of the intracellular ara-CTP was 4.4-fold greater than that of the plasma ara-C in a 2-h infusion of high-dose ara-C (3000 mg/m^2).[18]) They also reported that the C_{ss} of ara-CTP was 50-fold greater than that of ara-C in a continuous infusion of 3000 mg/m^2 ara-C. These values were similar to our data in the low- and conventional-dose

therapies. A continuous infusion might yield a relatively higher ara-CTP concentration than a 2-h infusion, regardless of the dose. This suggests that the momentary C_{max} of plasma ara-C yielded by the 2-h infusion would not provide sufficient intracellular ara-C retention to lead to the efficient formation of ara-CTP.

From the comparison of the $AUC_{/dose}$ values, Cont IV ara-C (20, 70) produced ara-CTP more efficiently than 2-h IV ara-C (70). Iacoboni *et al.* reported that the AUC of ara-CTP by 2-h infusion of 3000 mg/m^2 ara-C was 1270 μmol·h/liter (median value).[20]) Although the $AUC_{/dose}$ (0.42 μmol·h/liter·mg), which was calculated from the ratio of 1270/3000, was close to that of 2-h IV ara-C (70), it was only half the value of Cont IV ara-C (20, 70). At a given dose of ara-C, continuous infusion might produce ara-CTP more efficiently than a 2-h infusion.[21]) The comparison of the AUC_{ratio} values suggests that the difference of this efficiency could not be attributed to the quantitative difference of the AUC of plasma ara-C. As described above, the continuous infusion maintained ara-CTP constant. Moreover, it afforded a relatively higher ara-CTP concentration than the 2-h infusion. These two factors seem to be responsible for the efficient production of ara-CTP by continuous infusion.

BHAC is resistant to deamination, unlike ara-C, because it has a long-chain fatty acyl group at the 4-amino position of ara-C.[5]) BHAC is well maintained in the plasma and is converted slowly into ara-C.[6]) In the present study, both the intracellular ara-CTP and the plasma ara-C were maintained longer by 2-h IV BHAC (70) than by 2-h IV ara-C (70). Low but long-lasting plasma ara-C would maintain ara-CTP longer in leukemic cells. 2-h IV BHAC (70) also gave a high C_{ratio} value, and produced ara-CTP more efficiently than 2-h IV ara-C (70). The $AUC_{/dose}$ value for 2-h IV BHAC (70) was similar to that for Cont IV ara-C (20, 70). On the other hand, the C_{ratio} and AUC_{ratio} values for BHAC were higher than the respective values for Cont IV ara-C, although no significant difference was found. This may be partly because the plasma BHAC would be directly transported into leukemic cells to some extent and converted into ara-C, which was phosphorylated to ara-CTP thereafter.[6])

When ara-C or BHAC was administered at the same dose of 70 mg/m^2 by a 2-h infusion, the C_{max} of the plasma ara-C was different, but the C_{max} of the intracellular ara-CTP was almost the same. As the molecular weight of ara-C (Mw=243) is nearly half the value of BHAC (Mw=565), 70 mg of BHAC is substantially half the dose of 70 mg of ara-C. In addition, BHAC is converted slowly into ara-C in the plasma. Thus, because of the use of a substantially smaller dose and the slow conversion into ara-C, 2-h IV BHAC (70) yielded a lower C_{max} of plasma ara-C, compared with 2-h IV ara-C (70). On the other hand, to form intracellular ara-CTP, ara-C should be accu-

mulated in the cell. 2-h IV ara-C (70) did produce a higher C_{max} of plasma ara-C than 2-h IV BHAC (70), but this peak ara-C concentration was not maintained. As was discussed in the comparison between Cont IV ara-C and 2-h IV ara-C, high but transient plasma ara-C would not induce sufficient accumulation of intracellular ara-C to produce a high ara-CTP concentration. In contrast, 2-h IV BHAC (70) yielded a low but long-lasting level of plasma ara-C. We speculated that this prolonged ara-C retention in the plasma would lead to sufficient ara-C accumulation in the cell, although we have no data for intracellular ara-C. Consequently, the increased accumulation of intracellular ara-C would generate a relatively higher ara-CTP concentration, compared with the case of 2-h IV ara-C (70).

The cytotoxic effect of ara-C has been thought to be S-phase-specific and time-dependent.[31] Intermittently proliferating cells have a greater chance of being exposed to ara-C if the drug is given for a longer time. To obtain a maximal cytotoxic effect, ara-C is usually infused continuously,[3] because the activity of ara-C is time-dependent[30, 31] and, moreover, ara-C is rapidly inactivated by cytidine deaminase in the plasma.[5] Our present findings confirm

the superiority of the continuous infusion of ara-C and the use of the long-acting ara-C derivative, BHAC, from the viewpoint of the intracellular pharmacokinetics.

In summary, our study is the first to describe the ara-CTP pharmacokinetics after low-dose ara-C administration. We demonstrated that different administration methods produce different levels of ara-CTP even at the same dose. Further studies are under way to investigate the correlation between the ara-CTP pharmacokinetics and the therapeutic effect in low- and conventional-dose ara-C therapies. A population pharmacokinetic model for ara-CTP will be developed to reduce the necessary number of blood samplings and the frequency of measurements.[32]

REFERENCES

1) Berman, E. Chemotherapy in acute myelogenous leukemia: high dose, higher expectations? *J. Clin. Oncol.*, **13**, 1–4 (1995).

2) Rowe, J. M. What is the best induction regimen for acute myelogenous leukemia? *Leukemia*, **12** (Suppl. 1), S16–S19 (1998).

3) Fukushima, T. and Ueda, T. Current situation and perspective for treatment of acute myelogenous leukemia in adults. *Jpn. J. Cancer Chemother.*, **25**, 295–302 (1998) (in Japanese).

4) Kimura, K., Ohno, R., Amaki, I., Hattori, K., Hirota, Y., Hoshino, A., Ichimaru, M., Ito, M., Kimura, I., Maekawa, T., Masaoka, T., Nakamura, T., Ogawa, M., Oguro, M., Ohta, K., Osamura, S., Shimoyama, M., Takaku, F., Uzuka, Y. and Yamada, K. Treatment of acute myelogenous leukemia in adults with N[4]-behenoyl-1-β-D-arabinofuranosylcytosine. *Cancer*, **56**, 1913–1917 (1985).

5) Aoshima, M., Tsukagoshi, S., Sakurai, Y., Oh-ishi, J., Ishida, T. and Kobayashi, H. N[4]-behenoyl-1-β-D-arabinofuranosylcytosine as a potential new antitumor agent. *Cancer Res.*, **37**, 2481–2486 (1977).

6) Ueda, T., Nakamura, T., Ando, S., Kagawa, D., Sasada, M., Uchino, H., Johno, I. and Akiyama, Y. Pharmacokinetics of N[4]-behenoyl-1-β-D-arabinofuranosylcytosine in patients with acute leukemia. *Cancer Res.*, **43**, 3412–3416 (1983).

7) Cheson, B. D. Standard and low-dose chemotherapy for the treatment of myelodysplastic syndromes. *Leuk. Res.*, **22**, S17–S21 (1998).

8) Ossenkoppele, G. J., Wijermans, P. W., Nauta, J. J., Huijgens, P. C. and Langenhuijsen, M. M. Low-dose cyt-

arabine for acute myeloid leukaemia and myelodysplastic syndromes: *in vivo* and *in vitro* cytotoxicity. *Eur. J. Cancer*, **27**, 842–845 (1991).

9) Miller, K. B., Kyungmann, K., Morrison, F. S., Winter, J. N., Bennett, J. M., Neiman, R. S., Head, D. R., Cassileth, P. A. and O'Connell, M. J. The evaluation of low-dose cytarabine in the treatment of myelodysplastic syndromes: a phase-III intergroup study. *Ann. Hematol.*, **65**, 162–168 (1992).

10) Herzig, R. H., Wolff, S. N., Lazarus, H. M., Phillips, G. L., Karanes, C. and Herzig, G. P. High-dose cytosine arabinoside therapy for refractory leukemia. *Blood*, **62**, 361–369 (1983).

11) Miyawaki, S., Mizoguchi, H., Tomonaga, M., Kanamaru, A., Tsukagoshi, S., Nakamura, T., Ohashi, Y., Yoshida, S. and Ohno, R. A phase II study with high-dose cytarabine (NS-075) in adult patients with relapsed and refractory acute leukemia. *Jpn. J. Cancer Chemother.*, **25**, 2229–2242 (1998) (in Japanese).

12) Chou, T.-C., Hutchinson, D. J., Schmid, F. A. and Philips, F. S. Metabolism and selective effects of 1-β-D-arabinofuranosylcytosine in L1210 and host tissues *in vivo*. *Cancer Res.*, **35**, 225–236 (1975).

13) Graham, F. L. and Whitmore, G. F. The effect of 1-β-D-arabinofuranosylcytosine on growth, viability, and DNA synthesis of mouse L-cells. *Cancer Res.*, **30**, 2627–2635 (1970).

14) Graham, F. L. and Whitmore, G. F. Studies in mouse L-cells on the incorporation of 1-β-D-arabinofuranosylcytosine into DNA and on inhibition of DNA polymerase by

1-β-D-arabinofuranosylcytosine 5′-triphosphate. *Cancer Res.*, **30**, 2636–2644 (1970).

15) Chou, T.-C., Arlin, Z., Clarkson, B. D. and Philips, F. S. Metabolism of 1-β-D-arabinofuranosylcytosine in human leukemic cells. *Cancer Res.*, **37**, 3561–3570 (1977).

16) Spriggs, D., Robbins, G., Ohno, Y. and Kufe, D. Detection of 1-β-D-arabinofuranosylcytosine incorporation into DNA *in vivo*. *Cancer Res.*, **47**, 6532–6536 (1987).

17) Plunkett, W., Hug, V., Keating, M. J. and Chubb, S. Quantitation of 1-β-D-arabinofuranosylcytosine 5′-triphosphate in the leukemic cells from bone marrow and peripheral blood of patients receiving 1-β-D-arabinofuranosylcytosine therapy. *Cancer Res.*, **40**, 588–591 (1980).

18) Liliemach, J. O., Plunkett, W. and Dixon, D. O. Relationship of 1-β-D-arabinofuranosylcytosine in plasma to 1-β-D-arabinofuranosylcytosine 5′-triphosphate levels in leukemic cells during treatment with high-dose 1-β-D-arabinofuranosylcytosine. *Cancer Res.*, **45**, 5952–5957 (1985).

19) Plunkett, W., Iacoboni, S., Estey, E., Danhauser, L., Liliemark, J. O. and Keating, M. J. Pharmacologically directed ara-C therapy for refractory leukemia. *Semin. Oncol.*, **12** (Suppl. 3), 20–30 (1985).

20) Iacoboni, S. J., Plunkett, W., Kantarjian, H. M., Estey, E., Keating, M. J., McCredie, K. B. and Freireich, E. J. High-dose cytosine arabinoside: treatment and cellular pharmacology of chronic myelogenous leukemia blast crisis. *J. Clin. Oncol.*, **4**, 1079–1088 (1986).

21) Plunkett, W., Liliemark, J. O., Adams, T. M., Nowak, B., Estey, E., Kantarjian, H. and Keating, M. J. Saturation of 1-β-D-arabinofuranosylcytosine 5′-triphosphate accumulation in leukemia cells during high-dose 1-β-D-arabinofuranosylcytosine therapy. *Cancer Res.*, **47**, 3005–3011 (1987).

22) Heinemann, V., Estey, E., Keating, M. J. and Plunkett, W. Patient-specific dose rate for continuous infusion high-dose cytarabine in relapsed acute myelogenous leukemia. *J. Clin. Oncol.*, **7**, 622–628 (1989).

23) Yamauchi, T., Ueda, T. and Nakamura, T. A new sensitive method for determination of intracellular 1-β-D-arabinofuranosylcytosine 5′-triphosphate content in human materials *in vivo*. *Cancer Res.*, **56**, 1800–1804 (1996).

24) Shimada, N., Ueda, T., Yokoshima, T., Oh-ishi, J. and Ohoka, T. A sensitive and specific radioimmunoassay for 1-β-D-arabino-furanosylcytosine. *Cancer Lett.*, **24**, 173–178 (1984).

25) Ho, D. H. W. and Frei, E., III Clinical pharmacology of 1-β-D-arabinofuranosylcytosine. *Clin. Pharmacol. Ther.*, **12**, 944–954 (1971).

26) Fallon, H. J., Frei, E., III, Davidson, J. D., Trier, J. S. and Burk, D. Leukocyte preparations from human blood: evaluation of their morphologic and metabolic state. *J. Lab. Clin. Med.*, **59**, 779–791 (1962).

27) Yamaoka, K., Nakagawa, T. and Uno, T. Statistical moments in pharmacokinetics. *J. Pharmacokinet. Biopharm.*, **6**, 547–558 (1978).

28) Ueda, T., Matsuyama, S., Yamauchi, T., Kishi, S., Fukushima, T., Tsutani, H., Nakamura, T., Goto, N. and Masada, M. Clinical pharmacology of intermediate and low-dose cytosine arabinoside (ara-C) therapy in patients with hematologic malignancies. *Adv. Exp. Med. Biol.*, **431**, 647–651 (1998).

29) Boos, J., Hohenlöchter, B., Schulze-Westhoff, P., Schiller, M., Zimmermann, M., Creutzig, U., Ritter, J. and Jürgens, H. Intracellular retention of cytosine arabinoside triphosphate in blast cells from children with acute myelogenous and lymphoblastic leukemia. *Med. Pediatr. Oncol.*, **26**, 397–404 (1996).

30) Kamiya, K., Uchida, M., Ueda, T. and Nakamura, T. Studies on intracellular kinetics of ara-C triphosphate in HL-60, human leukemia cells in relation to reasonable administration of ara-C. *J. Jpn. Soc. Cancer Ther.*, **25**, 1419–1427 (1990) (in Japanese).

31) Ozawa, S., Sugiyama, Y., Mitsuhashi, J. and Inaba, M. Kinetic analysis of cell killing effect induced by cytosine arabinoside and cisplatin in relation to cell cycle phase specificity in human colon cancer and Chinese hamster cells. *Cancer Res.*, **49**, 3823–3828 (1989).

32) Thomson, A. H. and Whiting, B. Bayesian parameter estimation and population pharmacokinetics. *Clin. Pharmacokinet.*, **22**, 447–467 (1992).