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Cellular pharmacology of 1-β-D-arabinofuranosylcytosine in human myeloid, B-lymphoid and T-lymphoid leukemic cells*

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Summary. The in vitro inhibitory action and metabolism of 1-β-D-arabinofuranosylcytosine (ara-C) on human myeloid (HL-60), B-lymphoid (RPMI-8392), and T-lymphoid (Molt-3) leukemic cells was compared. Ara-C produced greater inhibitory effects in Molt-3 cells than in either HL-60 or RPMI-8392 cells. At a 48 h exposure, ara-C was 7 and 10 times more cytotoxic to Molt-3 cells than to HL-60 and RPMI-8392 cells, respectively. The total ara-C uptake to nucleotides and the formation of 1-β-D-arabinofuranosylcytosine 5′-triphosphate (ara-CTP) was about 5 times greater in Molt-3 cells than in either HL-60 or RPMI-8392 cells. The incorporation of ara-C into DNA was also higher in Molt-3 cells than in either HL-60 or RPMI-8392 cells. The mean intracellular half-life of ara-CTP was 31.7, 59.4, and 155 min for RPMI-8392, HL-60, and Molt-3 leukemic cells, respectively. The Km and V_{max} values of ara-C for deoxycytidine kinase and the feedback inhibition of this enzyme by ara-CTP in the different leukemic cell lines could not explain the differences in metabolism of this analogue in these cells. These data indicate that the increased sensitivity of T-lymphoid leukemic cells to ara-C than as compared with B-lymphoid and myeloid leukemic cells was due to an inreased rate of formation and a longer half-life of ara-CTP in the T-cells.

Introduction

1-β-D-Arabinofuranosylcytosine (ara-C) is one of the most effective agents for the treatment of acute myeloid leukemia [7, 21]. To be an active inhibitor, ara-C must first be phosphorylated by the enzyme deoxycytidine kinase [14] and then be converted to its triphosphate form, ara-CTP [17]. Leukemic cells deficient in deoxycytidine kinase are resistant to ara-C [2, 8]. The active inhibitor in the cell is ara-CTP, which, after its incorporation into DNA by replicative DNA polymerase, produces a pseudo chain-terminating effect [12, 13] that results in a potent inhibition of DNA synthesis [3]. The repair DNA polymerase can also incorporate ara-CTP into DNA [10]. The incorporation of ara-C into DNA correlates with the lethal action of this analogue [11]. Cell death is probably due to the damage to DNA produced by the incorporation of ara-C into DNA and the subsequent inhibition of DNA synthesis.

Although ara-C is a primary chemotherapeutic agent for the treatment of acute myeloid leukemia, it plays a secondary role in the treatment of acute lymphoid leukemia [4, 6]. In vitro data obtained by several investigators have shown that human T-lymphoid leukemic cells may be a good target for chemotherapy with ara-C. Ohnuma et al. [16] demonstrated that human T-lymphoid leukemic cells (Molt-3) were much more sensitive to the growth-inhibitory effects of ara-C than were several human B-lymphoid leukemic cells. This observation was confirmed by Abe et al. [1], who also found that ara-CTP formation as well as the half-life of ara-CTP were much greater in the T-lymphoid line than in the B-lymphoid leukemic cells. These investigators postulated that dephosphorylation of ara-CTP plays an important role in the cytotoxic action of this analogue. Verhoef and Fridland [20] also observed that the half-life of ara-CTP was greater in T- than in B-lymphoid leukemic cells.

In studies on leukemic cells from patients, Wiley et al. [22] observed that upon incubation with ara-C, the formation of ara-CTP was greatest in the T-cells, intermediate in myeloid cells, and lowest in the B-cells. These investigators also showed that the number of nucleoside transport sites showed a strong correlation with the amount of ara-CTP formed in these cell types. In a similar study, Muus et al. [15] reported that the half-life of ara-CTP was longer in lymphoid than in myeloid leukemic cells. The present study compared the in vitro cytotoxic action of ara-C on

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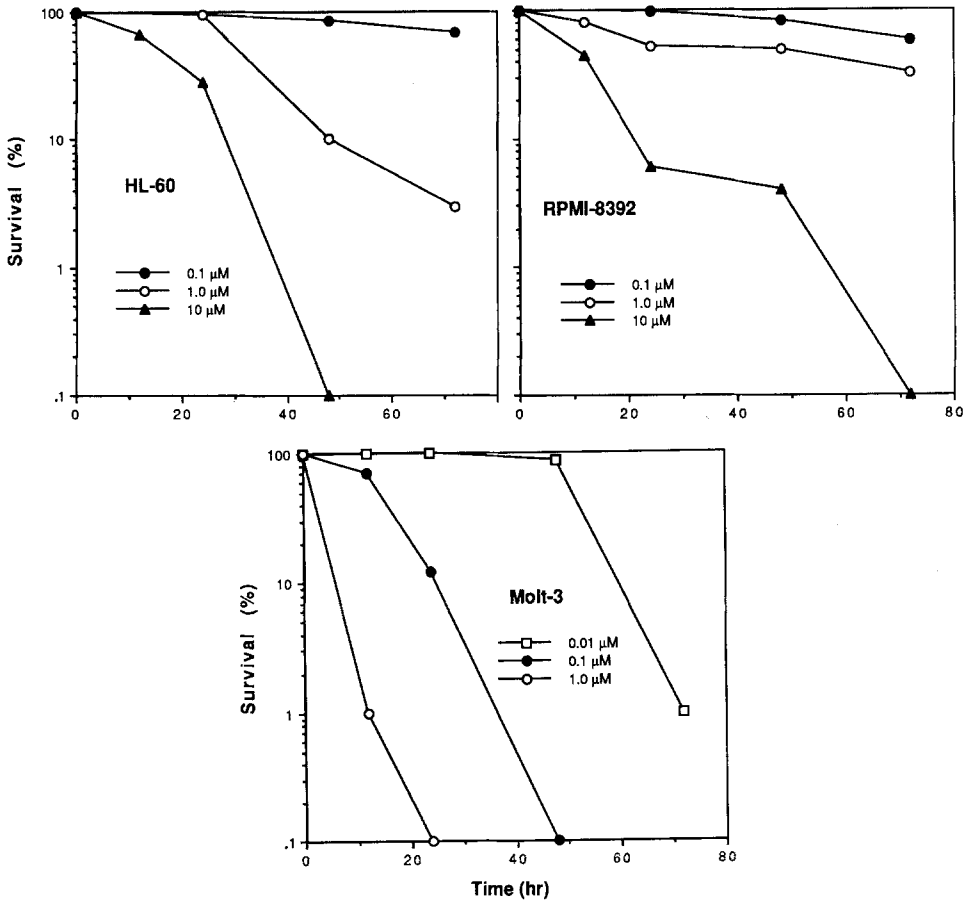

Fig. 1. Cytotoxicity of different concentrations of ara-C for different exposure times to HL-60 myeloid, RPMI-8392 B-lymphoid, and Molt-3 T-lymphoid leukemic cells. Cells were treated with the indicated concentrations of ara-C. At the time indicated aliquots of cells were removed and placed in soft agar for determination of colony-forming activity as described in Materials and methods. Ara-C concentrations: 0.01 μM (□); 0.1 μM (●); 1 μM (○); 10 μM (△). *Points* represent the means of 2–3 experiments carried out in duplicate (SE, <10%)

human myeloid and T- and B-lymphoid leukemic cells and correlated it to the metabolism of this analogue to ara-CTP. In addition, the various mechanisms that regulate the pool size of ara-CTP were investigated so as to elucidate the biochemical events responsible for the differences in sensitivity to ara-C between the leukemic cell lines.

Materials and methods

Materials. Ara-C was obtained from the Upjohn Company of Canada. [5-^{3}H]-Ara-C was obtained from Moravek Biochemicals Inc. (Brea, Calif.) and Amersham (Oakvile, Ontario). The [5-^{3}H]-ara-C was purified by HPLC on a column of Spherisorb ODS C18 (5 μm, 0.4×25 cm; Chromatography Sciences, Montreal) using 1 mM potassium phosphate (pH 6.8) as the mobile phase. [5-^{3}H]-Deoxycytidine and [^{3}H-methyl]-thymidine were obtained from Du Pont Canada Inc. (Mississauga, Ontario).

Cell culture. Human HL-60 myeloid leukemic cells were obtained from Dr. R. Gallo, National Cancer Institute (Bethesda, Md). Human RPMI-8392 B-lymphoid leukemic cells and human Molt-3 T-lymphoid leukemic cells were obtained from the Institute for Medical Research (Canden, N.J.) and American Type Tissue Culture Collection (Rockville, Md.). The cell lines were maintained in suspension culture in minimal essential medium containing non-essential amino acids (Gibco, Grand Island, N.Y.) and 10% heat-inactivated fetal calf serum (Flow Laboratories, Mississauga, Ontario). The doubling time for the cell lines was 20–24 h.

Growth and colony assay. For growth inhibition experiments, 5 ml leukemic cells (10^5/ml) were placed in a tissue-culture flask with the indicated concentration of ara-C. Cell counts were made at the indicated times with a Model Z Coulter counter. The proliferative viability of the leukemic cells after exposure to ara-C was determined by cloning. At the termination of drug exposure, the cells were centrifuged and suspended in drug-free medium. An aliquot of 150–200 cells was placed in 2 ml 0.3% soft-agar medium containing 15% serum. After incubation for 14–16 days at 37° C in an incubator containing 5% CO_2 the number of colonies (>500 cells) were counted. The cloning efficiency of the control cells was in the range of 40%–60%.

Metabolism of ara-C. The leukemic cells (10^6/ml) were incubated at 37° C in a shaker bath with 1 μM [5-^{3}H]-ara-C (2–4 μCi/ml) in medium containing 5% dialyzed fetal calf serum (Gibco). For measurement of the total uptake of ara-C, at the indicated times $1.25–2.5\times10^5$ cells were removed, diluted with 2 ml cold medium, and placed on 2.4-cm Whatman GF/C glass-fiber filters. The filters were washed with 0.9% NaCl, dried, placed in scintillation fluid (Omnifluor, Du Pont), and assayed for radioactivity. For measurement of the incorporation of [5-^{3}H]-ara-C into DNA, 10^6 cells were placed on the GF/C filter. The filters were washed with 0.9% NaCl, cold 5% TCA, and ethanol; after drying, they were placed in scintillation fluid and assayed for radioactivity.

HPLC analysis for ara-CTP. Using the incubation conditions described for the metabolism of ara-C, at the indicated times aliquots containing 2.5×10^6 cells were removed from the flask and centrifuged at 200 *g* for 5 min. The cell pellet was washed twice with 0.9% NaCl containing 1 mg/ml glucose, then suspended in 75 μl cold 5% TCA. The supernatant was obtained after centrifugation at 12,000 *g* for 15 min; it was neutralized with 75 μl 0.35 M TRIS base, and 1 μmol each of dCMP, dUMP,

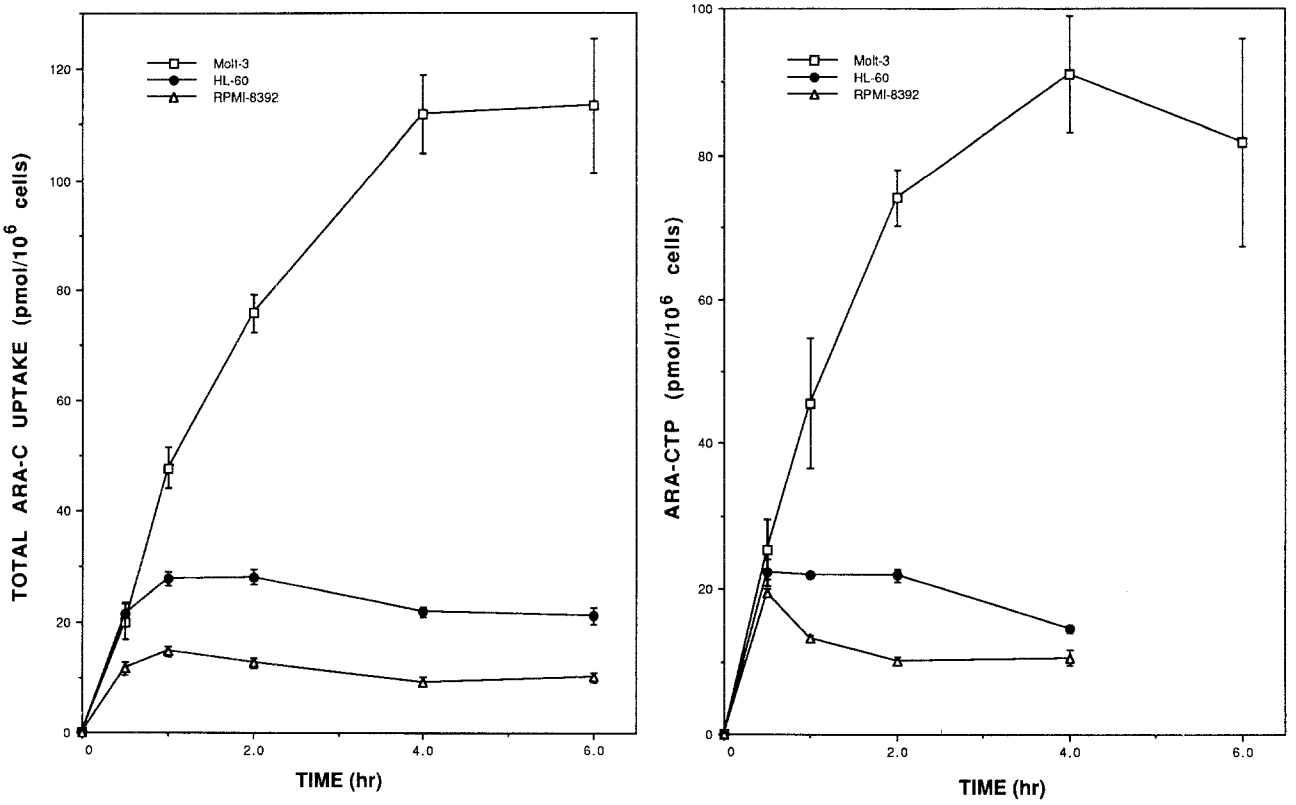

Fig. 2. Total ara-C uptake and formation of ara-CTP in HL-60 myeloid, RPMI-8392 B-lymphoid, and Molt-3 T-lymphoid leukemic cells as a function of time. Cells were incubated with 1 μM [5-^{3}H]-ara-C (2–4 μCi/ml) and at the time indicated, aliquots of cells were removed for determination of total uptake of ara-C and ara-CTP as described in Materials and methods. Molt-3 (□); HL-60 (●); RPMI-8392 (△). *Points* represent the means ± SE of 6–8 experiments (total ara-C) or of 2–3 experiments carried out in duplicate (ara-CTP)

dCDP, and dCTP was added as a cold carrier. The sample was stored at –70° C until assay. HPLC was performed on a column of Partisil SAX (10 μm, 0.4 × 25 cm; Whatman, Clifton, N.Y.) that was equilibrated with 30 mM ammonium phosphate buffer (pH 4.0). The flow rate of the mobile phase was 1.5 ml/min. After the elution of radioactive ara-CMP as detected by absorbance at 254 and 280 nm, the mobile phase was changed to 400 mM ammonium phosphate (pH 4.0). The peak of ATP from each cell extract was used as an internal standard; peaks corresponding to radioactive ara-CMP, ara-CDP, and ara-CTP were collected in separate tubes and assayed for radioactivity in scintillation fluid (Aquasol II, Du Pont).

Deoxycytidine kinase assay. The cell extract was prepared by centrifugation of leukemic cells in log growth phase at 200 *g* for 5 min and washing of the cell pellet in 0.9% NaCl. The cell pellet (3×10^7 cells) was suspended in 100 μl 5 mM TRIS-Cl (pH 7.4) and the cells were lysed by freezethawing three times. The supernatant was recovered after centrifugation at 12,000 *g* for 15 min. The concentration of KCl was adjusted to 0.1 M and the supernatant was stored at –70° C. The reaction mixture (100 μl) for the enzyme assay contained 50 mM TRIS-Cl (pH 7.4), 2.5 mM ATP, 5 mM $MgCl_2$, and 0.25 μCi or the indicated concentration of either [5-^{3}H]-ara-C or [5-^{3}H]-deoxycytidine. After addition of the cell extract (5–10 μl), the mixture was incubated for 30 min at 37° C, diluted with 3 ml cold H_2O, and placed on a 2.4-cm diameter DEAE-cellulose filter (Whatman). Before use the filters were washed with H_2O, 1 ml 0.05 *N* HCl, and H_2O. The mixture flowed through the filter by gravity to enable complete binding of the phosphorylated nucleosides. The filter was washed with 10 ml H_2O and 3 ml ethanol, then dried and assayed for radioactivity in scintillation fluid (Omnifluor). For studies on the feedback inhibition using ara-CTP or dCTP, the enzyme was purified by removal of nucleic acids with 5.9 mg/ml streptomycin sulfate and centrifugation at 12,000 *g* for 15 min. Saturated ammonium sulfate solution was added to the supernatant to a final saturation of 56%, and the protein precipitate obtained after centrifugation was dissolved in 50 mM TRIS-Cl (pH 7.4) containing 20% glycerol. The purified enzyme was stored at –20° C.

Deoxynucleotidase assay. The reaction mixture (100 μl) contained 50 mM TRIS-Cl (pH 7.4), 5 mM $MgCl_2$, 200 μM [^{3}H]-dCMP or [^{3}H]-ara-CMP (0.5 μCi), and 5 μl enzyme prepared as described above. After incubation for 30 min at 37° C, the amount of radioactive nucleotide bound to DEAE-cellulose filters was determined as described for the deoxycytidine kinase assay; 1 unit enzyme activity was defined as the amount of enzyme that dephosphorylates 1 nmol nucleotide/min under these experimental conditions. [^{3}H]-Ara-CMP was synthesized enzymatically from [^{3}H]-ara-C using deoxycytidine kinase in the reaction mixture described above. After incubation for 40 min at 37° C, the reaction mixture was spotted on 20- × 20-cm DEAE-cellulose plates (type KPNS, 250 μm thick; Analtech, Newark, Del.) and thin-layer chromatography was performed in 0.03 *N* HCl. The band containing [^{3}H]-ara-CMP was cut out, eluted with 0.1 *N* HCl, neutralized, and adjusted to a final concentration of 2 mM with cold ara-CMP.

Results

The cytotoxicity exerted by different concentrations of ara-C at different exposure times on the three leukemic cell lines are shown in Fig. 1. The RPMI-8392 leukemic cells were the least sensitive to the cytotoxic effects of ara-C, whereas HL-60 cells were of intermediate sensitivity and Molt-3 cells were the most sensitive. The concentrations of ara-C that produced 50% cell kill (LC_{50}) over a 48-h exposure in HL-60, RPMI-8392, and Molt-3 cells were in the range of 0.3, 2, and 0.02 μM, respectively. The Molt-3

Table 1. Intracellular pharmacokinetics of total ara-C and ara-CTP in human myeloid and B- and T-lymphoid leukemic cell lines

Leukemic cell line	Intracellular half-life	
	Total ara-C (min)	ara-CTP (min)
HL-60 (myeloid)	66.6 ± 8.9	59.4 ± 4.9
RPMI-8392 (B-lymphoid)	28.5 ± 6.5	31.7 ± 5.1
Molt-3 (T-lymphoid)	253 ± 28	155 ± 3

Data represent the mean ± SE of 2–3 experiments carried out in duplicate. Cells were incubated with 1 μM [5-^{3}H]-ara-C (2–4 μCi/ml) for 120 min and then washed free of drug. The incubation was continued and aliquots of cells were removed at intervals of 0, 20, 45, 90, and 180 min (Molt-3) for determinaton of the total uptake of ara-C and ara-CTP as described in Materials and methods

Table 2. Incorporation of ara-C into DNA in human myeloid and B- and T-lymphoid leukemic cell lines

Leukemic cell line	Incorporation of ara-C into DNA	
	2-h incubation (pmol/10^6 cells)	4-h incubation (pmol/10^6 cells)
HL-60 (myeloid)	0.89 ± 0.10	2.01 ± 0.28
RPMI-8392 (B-lymphoid)	0.83 ± 0.08	1.70 ± 0.34
Molt-3 (T-lymphoid)	1.25 ± 0.12	3.94 ± 0.40

Data represent the mean ± SE of 6–10 experiments. Cells were incubated with 1 μM [5-^{3}H]-ara-C (2–4 μCi/ml) for 2 and 4 h and the amount of ara-C incorporated into DNA was determined as described in Materials and methods

leukemic cells were also much more sensitive to the growth-inhibitory effects of ara-C than were the other leukemic cells lines. The ara-C concentrations that inhibited growth by 50% (IC_{50}) over a 48-h exposure in HL-60, RPMI-8392, and Molt-3 leukemic cells were in the range of 0.8, 0.5, and 0.01 μM, respectively.

To elucidate the differences observed in sensitivity to ara-C, the uptake of total ara-C and the formation of ara-CTP were measured in each of the leukemic cell lines (Fig. 2). The total uptake of ara-C appeared to reach a plateau after 1 h in HL-60 and RPMI-8392 leukemic cells; however, in Molt-3 leukemic cells it increased up to 4 h, reaching a plateau that was about 4-fold that of HL-60 and RPMI-8392 cells. The measurement of ara-CTP formation gave a pattern similar to that for total ara-C uptake. In Molt-3 leukemic cells, the formation of ara-CTP obtained a plateau at about 4 h and was about 4-fold that of HL-60 and RPMI-8392 leukemic cells. In latter, ara-CTP reached a plateau after only 30 min.

The intracellular half-life of total ara-C and ara-CTP was determined by placing the cells in drug-free medium after incubation with the radioactive analogue for 120 min and then measuring the concentration of the ara-C nucleotides at different intervals (Table 1). The intracellular half-life of total ara-C in HL-60 and RPMI-8392 leukemic cells was 66.6 and 28.5 min, respectively, whereas that in Molt-3 leukemic cells was much longer (253 min). The half-life of ara-CTP in HL-60 and RPMI-8392 cells was 59.4 and 31.7 min, respectively, a value similar to that obtained for total ara-C. In Molt-3 leukemic cells the half-life of ara-CTP was estimated to be 155 min, which was much longer than that determined for the other cell lines but considerably less than the total ara-C half-life in the former cells.

The incorporation of radioactive ara-C into DNA after 2 and 4 h incubation was determined for each cell line (Table 2). For both time points, the incorporation of ara-C into the DNA of Molt-3 leukemic cells was significantly greater than that observed in either HL-60 or RPMI-8392 cells.

Table 3. Kinetic data on the phosphorylation of ara-C by deoxycytidine kinase in human myeloid and B- and T-lymphoid leukemic cell lines

Leukemic cell line	Enzyme kinetic parameters	
	Km (μM)	V_{max} (nmol h^{-1} mg^{-1})
HL-60 (myeloid)	5.8 ± 0.4	6.3 ± 0.2
RPMI-8392 (B-lymphoid)	4.1 ± 0.1	2.6 ± 0.3
Molt-3 (T-lymphoid)	3.7 ± 0.4	6.5 ± 0.2

Data represent the mean ± SE of 3–5 experiments. Cell extracts prepared from leukemic cell lines during exponential growth were incubated at 37° C for 30 min in the presence of different concentrations (5–15 μM) of [5-^{3}H]-ara-C (0.25 μCi) and then assayed for deoxycytidine kinase activity as described in Materials and methods

The phosphorylation of ara-C by deoxycytidine kinase was investigated in extracts of the leukemic cell lines (Table 3). The Km values for ara-C in RPMI-8392 and Molt-3 cells were similar and were slightly lower than the value obtained for HL-60 leukemic cells. In contrast, the V_{max} values for HL-60 and Molt-3 leukemic cells were similar and were significantly higher than that determined for RPMI-8392 cells. The effect of ara-CTP and dCTP on the feedback inhibition of deoxycytidine kinase from the different leukemic cell lines, using ara-C or deoxycytidine as the substrate, is shown in Table 4. In these studies, the enzyme was partially purified to remove the nucleotides present in the cell extracts. For each of the leukemic cell lines, dCTP was a more potent feedback inhibitor than was ara-CTP when either ara-C or deoxycytidine was used as the substrate. With ara-C as the substrate, each of the cell lines showed similar sensitivity to feedback inhibition produced by either ara-CTP or dCTP. On the other hand, with deoxycytidine as the substrate, ara-CTP appeared to be a more potent feedback inhibitor of deoxycytidine kinase from Molt-3 leukemic cells than of that from HL-60 cells; the inhibition produced by the combination of ara-CTP (100 μM) and dCTP (10 μM) was subadditive as compared with that produced by these nucleotides alone.

The cytoplasmic deoxynucleotidase activity found in the different leukemic cell lines using ara-CMP or dCMP as the substrate is shown in Table 5. With ara-CMP as the substrate, there did not appear to be a significant difference in the rate of dephosphorylation in the three leukemic cell lines. When dCMP was used as the substrate, the rate of dephosphorylation was slightly higher in Molt-3 leukemic cells than in HL-60 cells.

Table 4. Feedback inhibition of deoxycytidine kinase from leukemic cells by ara-CTP and dCTP using either deoxycytidine or ara-C as the substrate

Enzyme source	Ara-C substrate: % Inhibition produced by			Deoxycytidine substrate: % Inhibition produced by		
	Ara-CTP 100 μM	dCTP 10 μM	Ara-CTP+dCTP 100 μM+10 μM	Ara-CTP 100 μM	dCTP 10 μM	Ara-CTP+dCTP 100 μM+10 μM
HL-60	73±6	94±2	98±2	15±15	60± 3	70± 2
RPMI-8392	79±6	94±3	99±1	26± 5	50± 6	66± 7
Molt-3	75±3	96±2	99±2	36± 7	37±15	60±13

Data represent the mean ± SD of 4 experiments. The reaction mixture (100 μl) contained 10 μM [^{3}H]-ara-C or [^{3}H]-deoxycytidine (0.5 μCi), 100 μM ara-CTP and/or 10 μM dCTP, and 5 μl enzyme. The mixture was incubated for 30 min at 37° C and then assayed as described in Materials and methods

Table 5. Deoxynucleotidase activity in HL-60 myeloid, RPMI-8392 B-lymphoid, and Molt-3 T-lymphoid leukemic cell lines

Leukemic cell line	Deoxynucleotidase activity	
	Ara-CMP substrate (units/mg)	dCMP substrate (units/mg)
HL-60	3.6±0.4[a]	3.2±0.3[b]
RPMI-8392	3.8±0.2	3.6±0.3
Molt-3	3.9±0.4	4.1±0.2

[a] Mean ± SD (n = 6)
[b] Mean ± SD (n = 9)
The reaction mixture (100 μl) contained 5 μl enzyme, 50 mM TRIS-Cl (pH 7.4), 5 mM $MgCl_2$, and 200 μM [^{3}H]-ara-CMP or [^{3}H]-dCMP (0.5 μCi). The mixture was incubated at 37° C for 30 min and then assayed as described in Materials and methods

Discussion

Since ara-C is a very effective agent for the treatment of acute myeloid leukemia [7, 22], it is of interest to evaluate the potential effectiveness of this analogue in the treatment of acute lymphoid leukemia. Ohnuma et al. [16] reported that ara-C produces much stronger growth inhibition in T-lymphoid leukemic cells (Molt-4) than in B-lymphoid cells, suggesting that this analogue may be used for the treatment of T-cell leukemia. Abe et al. [1] also observed that T-lymphoid cells (Molt-4) were more sensitive to the growth-inhibitory effects of ara-C than were B-lymphoid leukemic cells. We also observed that ara-C was a much more potent inhibitor of growth in T-lymphoid cells (Molt-3) than in either B-lymphoid or myeloid leukemic cells.

One problem with the use of cell growth in evaluating the chemotherapeutic potential of an experimental drug is that some agents may inhibit growth with only a minimal loss of clonogenicity. The loss of the proliferative potential of the malignant cells should be the major parameter for the assessment of antineoplastic activity. Since Onhuma et al. [16] and Abe et al. [1] did not present any data on the cytotoxicity produced by ara-C, full assessment of the importance of their work is difficult. For this reason, we used a colony assay to evaluate the cytotoxic selectivity of ara-C with respect to the phenotype of the leukemic cells. We observed that ara-C was much more cytotoxic to T-lymphoid (Molt-3) than to B-lymphoid or myeloid leukemic cells (Table 2).

The biochemical basis for this selectivity of ara-C is probably attributable to differences in the metabolism of this analogue in the different types of leukemic cells. Abe et al. [1] reported that the formation of ara-CTP was higher in T-lymphoid leukemic cells than in B-lymphoid cells. Using leukemic cells from patients, Wiley et al. [22] observed that the formation of ara-CTP was greater in T-cells than in either B-cells or myeloid cells. Our experimental data are in agreement with these reports. We found that the total ara-C uptake and the formation of ara-CTP were greater in the T-lymphoid cell line than in the B-lymphoid or myeloid leukemic cells (Fig. 2). In addition, we observed that the incorporation of ara-C into the DNA of T-cells was higher than that in B-cells or myeloid cells (Table 2). Major et al. [11] demonstrated that the lethal action of ara-C correlates with the amount that is incorporated into DNA.

One possible explanation for the higher levels of ara-CTP found in T-lymphoid leukemic cells is that the rate of degradation of this nucleotide analogue is slower in this cell type than in leukemic cells of a different phenotype. In accordance with this hypothesis, Abe et al. [1] have reported that the half-life of ara-CTP is 204 min in T-cells (Molt-4) as compared with 26–31 min in (Raji and Daudi) B-cells. Verhoef and Fridland [20] also observed that the half-life of ara-CTP was longer in human T-cells (CEM) than in B-cells (PF-23). In leukemic cells from patients, Muus et al. [15] showed that the half-life of ara-CTP in lymphoblasts was longer than that in myeloblasts. Our data are in agreement with these investigators. We found that the half-life of ara-CTP in T-lymphoid leukemic cells (Molt-3) was 155 min as compared with 31.7 and 59.4 min in B-cells (RPMI-8392) and myeloid cells (HL-60), respectively (Table 1). The half-life we found for ara-CTP in Molt-3 cells (155 min) is considerably shorter than those reported by Abe et al. [1] and Townsend et al. [19] in Molt-4 cells (204 and 240 min, respectively). This difference is probably related to slight differences in the metabolism of ara-CTP in these two clones of the same leukemic cell line.

The half-life of ara-CTP in leukemic cells is an important parameter with respect to the cytotoxic action of this analogue and to predictions of the response of patients with acute leukemia to chemotherapy. Kufe et al. [9] demonstrated that for HL-60 myeloid leukemic cells, there is a correlation between the duration of the pool size of ara-CTP and the amount that is incorporated into DNA. Rustum and Preisler [18] reported that the duration of complete remission in patients with acute leukemia in whom ara-CTP showed a long half-life (retention time)

was longer than that in patients in whom the half-life of this nucleotide analogue was short. Plunkett et al. [17] observed that leukemic patients with resistant disease exhibited a shorter half-life for ara-CTP than did those who actieved complete remission.

The molecular mechanisms that regulate the half-life of ara-CTP in leukemic cells are not yet completely understood. Abe et al. [1] proposed that the maintenance of the ara-CTP pool is dependent on both anabolic (kinases) and catabolic (dephosphorylation) events in the cell. In an attempt to elucidate those mechanisms, we performed enzyme kinetic studies with deoxycytidine kinase using ara-C as the substrate (Table 3). Although there were slight differences in the Km and V_{max} value for ara-C in the three leukemic cell lines, we felt that these differences were not sufficient to account for the large discrepancy in the half-life of ara-CTP in T-cells as compared with B-cells and myeloid cells. In addition, we investigated the feedback inhibition of deoxycytidine kinase by ara-CTP and dCTP (Table 4). dCTP was a more potent feedback inhibitor of this enzyme that was ara-CTP in each of the cell lines. With ara-C as the substrate, ara-CTP at a concentration of 100 μM produced about the same extent of inhibition of deoxycytidine kinase in each of the leukemic cell lines, suggesting that this phenomenon did not account for the differences in the half-life of ara-CTP in these cells.

Another possible explanation for the longer half-life of ara-CTP in leukemic T-cells as compared with non-T-cells is that the latter cells contain greater deoxynucleotidase activity, which results in a higher rate of dephosphorylation of nucleotides. In support of this hypothesis, Iizasa and Carson [5] have reported that B-lymphoid cells, but not T-lymphoid cells, secrete substantial amounts of deoxycytidine into the medium; these investigators suggested that the B-cells have higher deoxynucleotidase activity than do the T-cells. In the assay that we used, we did not observe any significant differences in deoxynucleotidase activity when ara-CMP was used as the substrate in the three leukemic cell lines (Table 5), suggesting that dephosphorylation at the nucleoside 5′-monophosphate level does not explain the difference in the half-life of ara-CTP in these cells. The biochemical mechanisms that regulate the pool size of ara-CTP in T-lymphoid leukemic cells remain unclear. It is possible that compartmentalization of anabolic and catabolic enzymes may play a crucial role in this process.

The present study shows that T-lymphoid leukemic cells are very sensitive to the cytotoxic action of ara-C and that this appears to be due to the long half-life of ara-CTP in these cells. These results support clinical investigations recommending ara-C for the treatment of T-cell leukemia and lymphoma.

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