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Prediction of Sensitivity to 1-β-D-Arabinofuranosylcytosine by the Plateau Level of its 5′-Triphosphate in Human Lymphoblastic Cell Lines *in Vitro*

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Abstract—*Parameters for metabolism of 1-β-D-arabinofuranosylcytosine (ara-C) were examined to know whether the prediction of ara-C sensitivity is possible or not using 9 human lymphoblastic cell lines, 3 T cell lines and 6 B cell lines* in vitro. *Neither the capacity for synthesis, degradation of 1-β-D-arabinofuranosylcytosine 5′-triphosphate (ara-CTP), generally lower in the T than B cell lines, nor deamination of ara-C, negligible in the B cell lines, could be correlated to the drug sensitivity. On the other hand, significant correlation was obtained between the sensitivity and the plateau level of ara-CTP. We consider that ara-C sensitivity could be predicted by measuring the plateau ara-CTP level before commencement of chemotherapy.*

INTRODUCTION

THE STUDY of the metabolism of ara-C has been pursued with the goal of finding a way to predict the effectiveness of this anticancer drug prior to chemotherapy in order to cure the cancer patients without unfavorable side-effects. Kessel *et al.* [1] stated that the sensitivity to ara-C was correlative to the capacity for ara-C phosphorylation in mouse leukemia cell lines. Chou *et al.* [2] stated that DNA synthesis in the host tissue and L1210 leukemia cells was equally inhibited by similar levels of ara-CTP. Rustum and Preisler [3] showed the importance of retention of ara-CTP in ara-C sensitivity of human leukemic myeloblasts. Our previous reports [4, 5] raised the possibility that the plateau level of ara-CTP would be correlative to, thus predictive of, the sensitivity to ara-C in human lymphoblastic cell lines. In this paper predictability of ara-C sensitivity was investigated and the relevance of the metabolic parameters to it was analyzed in nine human lymphoblastic cell lines *in vitro*.

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MATERIALS AND METHODS

Radiochemicals

[^{3}H]-Ara-C (26 Ci/mmol) and [^{3}H]-thymidine (42 Ci/mmol) were purchased from Amersham International, Amersham, U.K.

Cell culture

Nine human lymphoblastic cell lines were used. The three T cell lines (all acute lymphocytic leukemia) were MOLT-4F [6], HPB-ALL [7] and CCRF-CEM [8]. Among the six B cell lines, Raji [9], Daudi [10] and P3HR-1 [11] are Burkitt's lymphoma; BALL-1 [12] is an acute lymphocytic leukemia; and C-6 (clone of NC-37 [13]) and RITC1000 [Yamane and Uno; unpublished] are normal lymphatic cell lines. Population doubling time of these cell lines was between 1 and 1.5 days under the present culture conditions, details of which were described previously [4]. In brief, the cell suspensions (3×10^5/ml) in Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal calf serum (GIBCO, Grand Island, NY, U.S.A.), 30 mM HEPES buffer, pH 7.4, and antibiotics were cultured in 5% CO_2 and air and subcultured every 2 or 3 days. Cells in the exponential phase of growth were used in the following experiments.

Inhibition of DNA synthesis

The cell suspension (5×10^5/ml) was pre-incubated with 0.1 μM ara-C (gift of Nippon Shin-yaku Co., Kyoto, Japan) for 4 hr. A 300-μl portion of the suspension was poured into the well of a microplate containing 20 μl of [^{3}H]-thymidine (50 μCi/ml) and incubated for 1 hr. Portions (300 μl) of the suspension were processed for estimation of the radioactivity incorporated into the acid-insoluble fraction as described before [4].

Metabolism of ara-C in the intact cells

Accumulation of [^{3}H]-ara-CTP was followed for up to 8 hr by incubation of the cell suspension (5×10^5/ml) with 0.1 μM [^{3}H]-ara-C (2.6 μCi/ml final volume). For estimating the degradation of [^{3}H]-ara-CTP unlabeled ara-C (0.8 mM final volume) was added to interrupt further phosphorylation of [^{3}H]-ara-C after 4 hr incubation. Degradation of [^{3}H]-ara-CTP was evident immediately upon its addition. Aliquots (200 μl) were sampled at appropriate intervals during accumulation and degradation of [^{3}H]-ara-CTP and centrifuged. The resultant pellet was treated with chilled (–20°C) 60% methanol to extract ara-C nucleotides. The extract was then applied to a PEI-cellulose-precoated plastic sheet and developed with 0.66 N acetic acid containing 0.66 M LiCl at 4°C for 30 min for separation of ara-CTP. The thin-layer plate was washed with distilled water and dried before use. Since the ara-CTP level almost reached the plateau after 4 hr of incubation, the 4-hr value was tentatively designated as the plateau level.

Deamination of ara-C to 1-β-D-arabinofuranosyluracil (ara-U) was estimated in 10-μl aliquots of the cell suspension incubated as above. Aliquots applied to the PEI-cellulose-precoated plastic sheet were developed with ethylacetate–isopropanol–water (22°C). Further details have been described previously [4].

RESULTS

When the inhibition of DNA synthesis (expressed as a percentage of the controls) after 4 hr exposure to ara-C was plotted against the plateau (4 hr) ara-CTP level (Fig. 1), a significant correlation ($r = -0.81$, $P < 0.01$) was obtained between the two. The initial level (30 min) of ara-CTP could not be associated to the sensitivity.

The plateau level of ara-CTP observed was coincident with the value calculated in the B cell lines (Fig. 2). The calculated value was obtained according to Price *et al.* [14] using the initial (30 min) level and the half-life of the nucleotide under the assumption that the synthesis and degradation of ara-CTP are the only determinants of the ara-CTP level. In the T cell lines, however, the observed value was somewhat lower than the predicted value.

Among the metabolic parameters for ara-C in the intact cells (Table 1), the calculated capacity

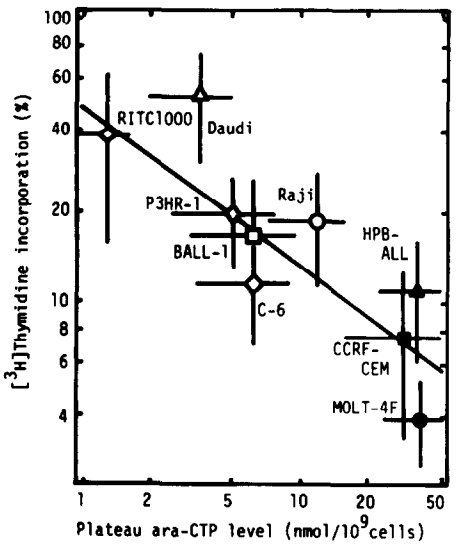

Fig. 1. Correlation between the plateau ara-CTP level (x) *and the inhibition of DNA synthesis* (y). *Cell suspension was incubated with* [^{3}H]*-ara-C for* 4 hr, *when the accumulation of* [^{3}H]*-ara-CTP reached the plateau. Open symbols, B cell lines; closed symbols, T cell lines. Bars, S.D. Correlation coefficient,* $r = -0.81$ ($P < 0.01$). *Regression line,* $\log y = -0.57 \log x + 1.72$.

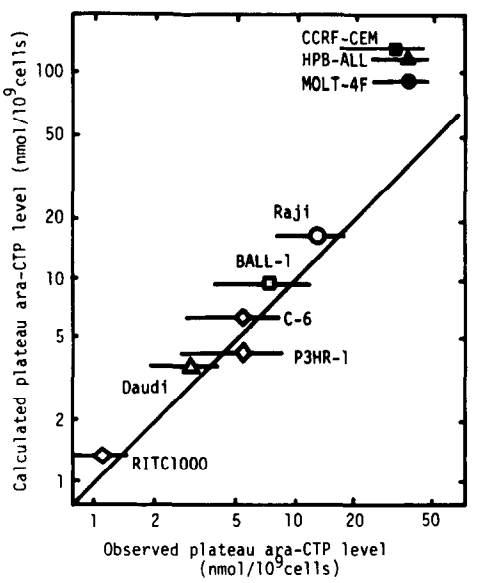

Fig. 2. Correlation between the plateau ara-CTP level observed and that calculated. The observed value was obtained by sampling aliquots of cell suspension incubated with 0.1 μM [^{3}H]*-ara-C for* 4 hr. *The calculated plateau ara-CTP level* (C_n) *was obtained according to Price* et al. [14] *as follows:*

$$C_n = K_s/K_d = T_{1/2} \cdot K_s/0.693,$$

where K_s *and* $T_{1/2}$ *are given in Table* 1. *Open symbols, B cell lines; closed symbols, T cell lines. Horizontal bars, S.D.*

Table 1. Metabolic parameters for ara-C in the intact lymphoblastic cell lines

	Rate of ara-CTP synthesis (K_s)*	Deamination of ara-C	Halflife of ara-CTP ($T_{1/2}$)
	(nmol/10^9 cells/min)		(min)
T cell lines			
MOLT-4F	0.24	0.03 ± 0.02†	195 ± 66†
HPB-ALL	0.22	0.05 ± 0.03	408 ± 68
CCRF-CEM	0.19	0.03‡	536 ± 126
B cell lines			
Raji	0.41	<0.01	32 ± 13
Daudi	0.11	<0.01	33 ± 12
P3HR-1	0.06	<0.01	39 ± 4
BALL-1	0.09	<0.01	64 ± 22
C-6	0.09	<0.01	49 ± 20
RITC1000	0.02	<0.01	55 ± 39

*Rate of ara-CTP synthesis (K_s) was calculated according to Price *et al.* [14] as follows:

$$K_s = Ct_i \cdot K_d/(1\text{-}e^{-K_d \cdot t_i}),$$

where Ct_i is the intracellular level of ara-CTP at time t_i and K_d, equal to $0.693/T_{1/2}$, is the rate constant of ara-CTP degradation. Values at 30 min (t_i) were used to minimize the influence of ara-C deamination, which grew with time. Thence the present K_s value of MOLT-4F should be more accurate than the previous one [4]. Cell density, 5×10^5/ml; [^{3}H]-ara-C, 0.1 μM.
Mean ± S.D. or mean of †3 or ‡2 experiments respectively.

for ara-C phosphorylation was higher in the T than B cell lines except for Raji, which showed the highest value. Degradation of ara-CTP was marked in the B cell lines. In contrast, ara-C deamination was obvious in the T but negligible in the B cell lines.

DISCUSSION

In the nine human lymphoblastic cell lines tested, significant ($P<0.01$) correlation was observed between the plateau, not initial, level of ara-CTP accumulated within the cells and inhibition of DNA synthesis (Fig. 1). The T cell lines were more sensitive to ara-C than the B cell lines ($P<0.01$, Student's t test) in terms of inhibition of DNA synthesis, discordant from our previous results [5], as discussed later. The finding that the order of the inhibition was not strictly the same as that of the plateau ara-CTP level may imply that additional factors such as DNA polymerase levels play a role in drug sensitivity [15]. The view of Chou *et al.* [16] that a high initial rate of ara-C phosphorylation was not a sufficient condition for drug sensitivity is supported by our data. Kessel *et al.* [1] showed the clear correlation between the initial level of ara-CTP and the ara-C sensitivity in mouse leukemia lines. It may be that the capacity for ara-CTP degradation or ara-C deamination was not much different among those animal cell lines in contrast to the present human cell lines. The fact that the calculated plateau ara-CTP levels were coincident with those observed in the B cell lines examined (Fig. 2) gives new evidence that the ara-CTP level is determined not only by the synthesis but also the degradation of this nucleotide [4]. The finding that nucleotide degradation was more marked in the B than T cell lines may be useful in differentiating these two categories of lymphocytes (Table 1).

Although ara-C deamination was very low in the B cell lines tested, this observation may not be generalized to other B cell lines. Certain B cell lines have been reported to be more susceptible to ara-C in the presence of the cytidine deaminase inhibitor, tetrahydrouridine [17, 18]. That the observed ara-CTP level in the T cell lines was lower than the calculated one (Fig. 2) may be attributable, at least in part, to a decrease in ara-C by deamination. In this connection it should be noted that our previous stocks of the two T cell lines, CCRF-CEM and RPMI8402, were slightly sensitive to ara-C [5] due to vigorous deamination, contrary to our results (Fig. 1) and earlier reports [17, 18]. Recently, those stocks were revealed to have been heavily infected with *Mycoplasma* [Komatsu and Miyaji, personal communication], which had the capacity for ara-C deamination [Abe *et al.*, unpublished data]. The present cell lines were judged to be *Mycoplasma*-free.

There have been several attempts to relate ara-C sensitivity to deoxycytidine kinase or cytidine deaminase or to the ratio of these two activities in

cell-free extracts. However, correlation between these parameters and drug sensitivity was not so tight (data not shown) that it was suspected that the prediction of the sensitivity would be difficult from one of these parameters alone.

In conclusion, the present study showed the plateau ara-CTP level to be a predictor of sensitivity to ara-C. It may be of value to re-examine the correlation between the ara-C sensitivity and the plateau level of ara-CTP in intact cells of human myeloid leukemia.

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