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Cell cycle related change of Ara-C transport in HL-60 cells after differentiation induction

Tetsuya Takimoto, Masaru Kubota, Satoru Tsuruta, Toshiyuki Kitoh, Akihiko Tanizawa, Yuichi Akiyama and Haruki Mikawa

Department of Pediatrics, Kyoto University, 54-Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606, Japan

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Using a promyelocytic leukemia cell line, HL-60, we studied the membrane transport of Ara-C before and after differentiation induced by retinoic acid (RA). In RA-treated cells, Ara-C transport was reduced and there was a concomitant increase of the ID_{50} values of Ara-C in comparison with the controls. By three different procedures to synchronize untreated cells, i.e. density arrest G_1 phase enrichment, aphidicolin-induced S phase accumulation and the double isoleucine block method, we found that Ara-C transport was 30–50% higher in the S phase than in the G_1 phase. Therefore, the observed decrease in Ara-C transport is, in part, due to the retarded growth accompanied by an accumulation of cells in the G_1 phase after differentiation induction.

Arabinofuranosylcytosine transport; Differentiation; Cell cycle

1. INTRODUCTION

The efficacy of chemotherapy for leukemia is usually related to the degree of maturation arrest of the neoplastic clones. That is, leukemia cells with a mature phenotype are more resistant to anticancer agents. From this point of view, the relationship between cell differentiation and drug sensitivity is a clinically important issue. Recently, we demonstrated that human T-lymphoblastoid cell line MOLT-4 cells became resistant to 1-β-D-arabinofuranosylcytosine after differentiation induced by TPA, and this acquired resistance was attributed in part to a decrease in the membrane transport of Ara-C [1]. The doubling time of TPA-treated MOLT-4 cells was 2.6-fold that of the controls, and in accordance with the retarded growth, an accumulation of cells in the G_1 phase was observed following differentiation.

Therefore, to determine whether such reduction of Ara-C transport in differentiated cells is due to the altered cell cycle distribution, we evaluated cell cycle-related changes in nucleoside transport.

Correspondence address: M. Kubota, Department of Pediatrics, Kyoto University, 54-Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606, Japan

Abbreviations: Ara-C, 1-β-D-arabinofuranosylcytosine; Ara-A, 1-β-D-arabinofuranosyladenine; CdA, 2-chlorodeoxyadenosine; dCK, deoxycytidine kinase; RA, retinoic acid

2. MATERIALS AND METHODS

2.1. *Cell culture*

The human promyelocytic cell line HL-60 was cultivated in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cultures were kept at 37°C in a humidified incubator in a 95% air/5% CO_2 atmosphere. The cells were checked regularly for mycoplasma contamination. For differentiation induction, cells (1×10^5/ml) were exposed to 1 μM RA for four days. Then, the ability of cells to reduce nitroblue tetrazolium dye was evaluated as an indicator of differentiation.

2.2. *Drug sensitivity*

Cells (2×10^5/ml) were cultured in media containing serial dilutions of several nucleosides: Ara-C, Ara-A and CdA. After 48 h of incubation, the concentration of the nucleosides required to inhibit growth by 50% (ID_{50}) was determined by trypan blue dye exclusion.

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2.3. *Determination of Ara-C transport*

The membrane transport of Ara-C was evaluated by the silicon-oil technique described in our earlier report [1].

2.4. *Cell synchronization and cell cycle analysis*

Cell synchronization was achieved by three different procedures. The percentage of cells in each phase of the cell cycle was determined with a FACS 440 (Becton-Dickinson) [1]. First, the proliferation of HL-60 cells was arrested by making the cell density approx. 2×10^6/ml. One day later, when more than 75% of the cells had accumulated in the G_1 phase, termed the 'confluent phase', the transport assay was performed.

Second, the double isoleucine block method, described in an earlier report [2] was used. Following release from the second isoleucine starvation, cultures were pulse-labeled with [*methyl*-^{3}H]thymidine at 1 h intervals up to 24 h and the incorporation of thymidine into DNA was measured.

Finally, the cells were synchronized in the S phase by 24 h treatment with 0.2 μg/ml aphidicolin, an inhibitor of DNA polymerase α [3]. Ara-C transport was evaluated 3 h after the removal of aphidicolin, when almost 100% of the cells were in the S phase.

3. RESULTS

3.1. *Ara-C transport before and after differentiation*

After four days of treatment with RA, HL-60 cells stopped proliferating and more than 90% showed a positive reaction in the nitroblue tetrazolium dye reduction test. Concomitantly, the transport of Ara-C in RA-treated HL-60 cells fell to about 50–60% of the control value irrespective of the extracellular concentration of Ara-C (fig.1). The affinity constant (K_m; the concentration of substrate required to achieve half-maximal transport) determined graphically from Lineweaver-Burk plots did not show significant change between control and RA-treated cells (3.4 ± 1.1 μM and 4.4 ± 1.6 μM, respectively).

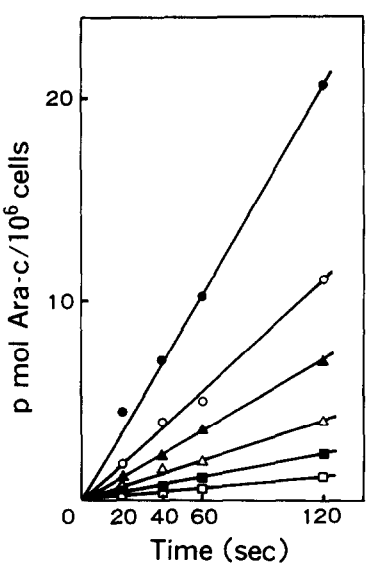

Fig.1. A representative time course of the transport of [^{3}H]Ara-C in RA-treated (open symbols) and untreated (closed symbols) HL-60 cells. The extracellular Ara-C concentrations are 5 μM (●,○), 1 μM (▲, △) and 0.2 μM (■, □).

Table 1

Sensitivity of HL-60 cells to several nucleoside analogs before and after differentiation induction

Treatment	ID_{50} (M)		
	Ara-C	Ara-A	CdA
None	3.8×10^{-8}	2.7×10^{-6}	4.5×10^{-8}
1 μM retinoic acid	1.7×10^{-6}	3.4×10^{-5}	3.5×10^{-6}

3.2. *Drug sensitivity*

The ID_{50} values of three synthetic nucleosides

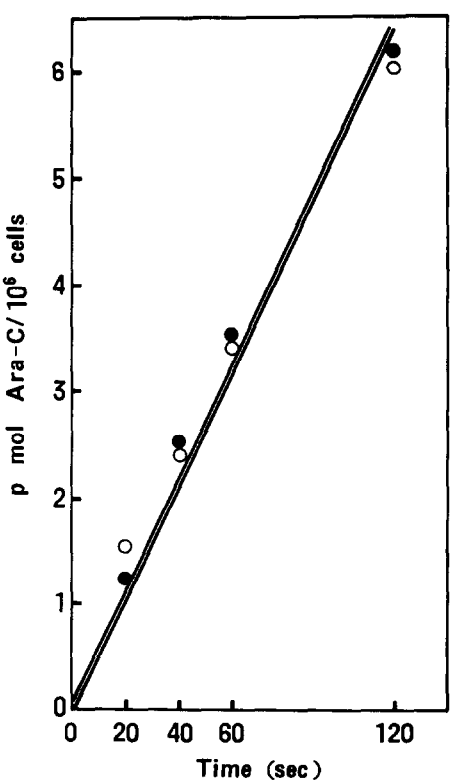

Fig.2. Transport of 1 μM [^{3}H]Ara-C by wild type cells (●) and mutant cells deficient in deoxycytidine kinase (○).

(Ara-C, Ara-A, CdA) are shown in table 1. Cells became approximately 10–220-fold more resistant to the drugs after RA-induced differentiation.

3.3. *Ara-C transport in mutant cells deficient in deoxycytidine kinase*

After entry into cells, Ara-C is rapidly converted to Ara-CTP. Deoxycytidine kinase (dCK) is the first and the rate-limiting step of this process. Therefore, we evaluated Ara-C transport in HL-60 mutant cells deficient in dCK to examine the possibility that the intracellular metabolism of Ara-C may affect the transport rates. The mutant was selected in our laboratory on the basis of being resistant to Ara-C, and it contained less than 1% of the dCK activity of wild type cells [4]. As shown in fig.2, Ara-C transport in mutant cells was almost equivalent to that in the parent cells. This was also true even after RA-induced differentiation (data not shown).

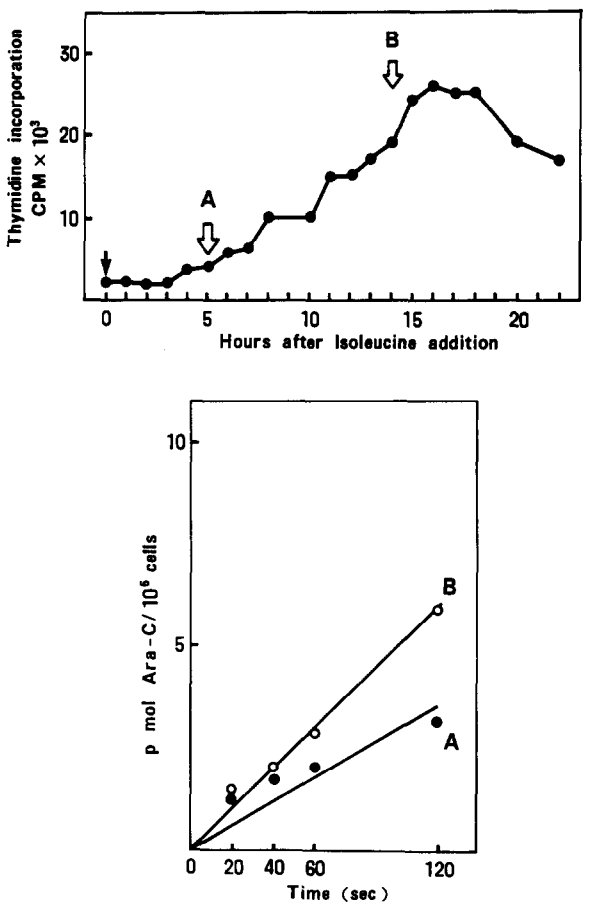

Fig.3. [^{3}H]Thymidine incorporation into DNA after release from isoleucine starvation (↓) in HL-60 cells synchronized by the double isoleucine block method (upper panel). At the indicated times (A,B), Ara-C transport was examined (lower panel).

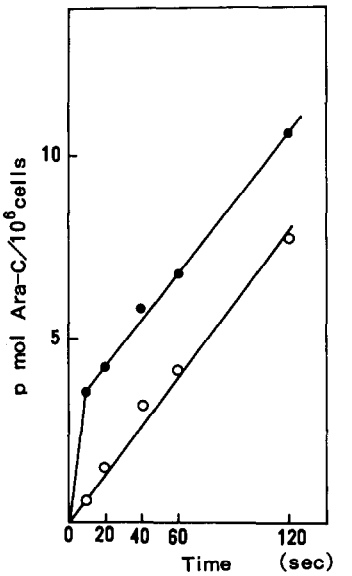

Fig.4. Ara-C transport of aphidicolin (0.2 μg/ml for 24 h)-treated (●) and untreated (○) HL-60 cells. The assay was performed 3 h after the removal of aphidicolin.

3.4. *Changes of Ara-C transport during the cell cycle*

First, we evaluated Ara-C transport in density-arrested HL-60 cells. A decrease of about 50% in Ara-C transport was observed in a confluent phase compared to that in a logarithmic phase. Next, cells were initially synchronized in the G_1 phase by the double isoleucine block method. Ara-C transport increased for cells to traverse the cell cycle after the replacement of isoleucine. Indeed, when the phases of the cell cycle were defined by [^{3}H]thymidine incorporation into DNA, Ara-C transport was about 1.5-times greater in the S phase than in the G_1 phase. Third, synchronization in the S phase was performed by treatment with 0.2 μg/ml aphidicolin. Ara-C transport was also significantly greater in the synchronized S phase-rich cells (fig.4).

4. DISCUSSION

In the present study, we demonstrated that the

transport of Ara-C in HL-60 leukemia cells decreased after RA-induced differentiation, which was accompanied by an increase of the ID_{50} value of Ara-C and the other nucleosides. Mutant cells deficient in dCK followed the same time course of Ara-C transport as that of wild type cells. This fact suggests that the change in dCK activity, if any, does not explain the altered Ara-C transport after differentiation.

There have been several studies showing that nucleoside transport is reduced during cellular differentiation [5,6]. Chen et al. reported that a decrease in the number of specific binding sites per cell for a given nucleoside was related to the reduction of transport in differentiation-induced HL-60 cells [6]. In general, cell differentiation and arrest of proliferation is a coupled process [7]. Actually, HL-60 cells stopped growing four days after RA treatment. Such alteration in the growth characteristics is inevitably accompanied by a change in cell cycle distribution; namely, an accumulation of cells in the G_1 phase with a concomitant decrease in the S and G_2+M phases [1]. Therefore, our finding that Ara-C transport in HL-60 cells decreases in the G_1 phase and increases in the S phase suggests that the decrease in nucleoside transport following differentiation is, in part, a function of altered cell cycle distribution. This is further supported by the observation that the number of nitrobenzylthioinosine-binding sites per cell increased as cells progressed from the G_1 through the S phase in HeLa cells [8].

Finally, our present results suggest one possible biochemical basis for the reduced sensitivity to various nucleoside analogs in more differentiated leukemia.

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