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Modulation of the Effect of 1-β-D-Arabinofuranosylcytosine by 6-Mercaptopurine in L1210 Cells

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In L1210 cells incubated with 1-β-D-arabinofuranosylcytosine (ara-C), 6-mercaptopurine (6-MP) significantly potentiated 1-β-D-arabinofuranosylcytosine 5′-triphosphate (ara-CTP) accumulation and ara-C incorporation into DNA (ara-C/DNA). The cytotoxicity of these two drugs was assessed to be at least additive by clonogenic assay. 1-β-D-Arabinofuranosyluracil (ara-U) level in a cell suspension was suppressed by 6-MP in a concentration-dependent fashion, though intracellular cytidine deaminase (CDD) activity was not affected by 6-MP. In addition, extracted CDD activity was not directly inhibited by 6-MP or by its intracellular metabolites *in vitro*. After preincubation in the presence or absence of 6-MP, the cell suspension was fractionated to obtain the spent medium and cell pellet. Then, each fraction was incubated with ara-C. Ara-U formation in the spent medium was found to increase conspicuously in relation to the time of preincubation in the control and it was suppressed by 6-MP pretreatment. Ara-U formation in the cell compartment increased slightly in relation to the time of preincubation in the control and substantially no suppression of ara-U formation was observed in spite of 6-MP pretreatment. In conclusion, intracellularly synthesized CDD was thought to be rapidly shed into the medium and the released CDD could play an important role in ara-C inactivation. 6-MP interrupted some step between synthesis and shedding of CDD, resulting in a decrease of the ara-C deamination in the medium and enhancement of its antileukemic effect.

Key words: 1-β-D-Arabinofuranosylcytosine — 6-Mercaptopurine — Cytidine deaminase

Ara-C[1] and its derivatives are among the main drugs used in the treatment of acute leukemia and their action mechanism has been studied in a number of laboratories, including ours.[1-4] Most administered ara-C is converted to an inactive metabolite, ara-U, by a process catalyzed by CDD *in vivo*, because the level of CDD activity in human plasma and liver is exceptionally high.[5] The remainder is transported into leukemic cells across the cell membrane by a facilitated diffusion mechanism,[6] converted to its nucleotide, ara-CMP, by DCK (ultimately to ara-CTP)[7] and also incorporated into DNA. The cytotoxic activity of ara-C is dependent on intracellular ara-CTP accumulation and/or ara-C incorporation into DNA.[8,9] Because of its complicated metabolism, the cellular pharmacology of ara-C could be modulated by the concomitant use of other antineoplastic agents. Among conventional anticancer agents, methotrexate,[10] hydroxyurea[11] and VP-16[12] were reported to modulate intracellular ara-C metabolism.

The simultaneous administration of ara-C and oral thiopurine has an important position in standard regimens of chemotherapy of leukemia.[13,14] In spite of the long history of usage of these drugs, details of intracellular ara-C pharmacology in the presence of thiopurine remain unclear. The present work was undertaken to clarify the effect of 6-MP on the cellular pharmacology of ara-C in the mouse leukemic cell line L1210 *in vitro* in order to investigate the usefulness of this combination.

[1] Abbreviations : ara-C, 1-β-D-arabinofuranosylcytosine; ara-U, 1-β-D-arabinofuranosyluracil; CDD, cytidine deaminase (EC 3.5.4.5); ara-CMP, 1-β-D-arabinofuranosylcytosine 5′-monophosphate; DCK, deoxycytidine kinase (EC 2.7.1.74); ara-CTP, 1-β-D-arabinofuranosylcytosine 5′-triphosphate; 6-MP, 6-mercaptopurine; 6-TG, 6-thioguanine; THU, tetrahydrouridine; PEI cellulose, polyethyleneimine-impregnated cellulose; PBS, phosphate-buffered saline; TCA, trichloroacetic acid; 2-ME, 2-mercaptoethanol; EDTA, ethylenediaminetetraacetic acid; 6-TX, 6-thioxanthine; methyl-6-MP, 6-methylmercaptopurine; 6-MPR, 6-mercaptopurine riboside; 6-TGR, 6-thioguanine riboside; TIMP, thioinosinic acid; LDH, L-lactate NAD oxidoreductase; 6-thio-ITP, 6-mercaptopurine ribonucleoside triphosphate; ANLL, acute non lymphocytic leukemia; ara-C/DNA, ara-C incorporation into DNA.

MATERIALS AND METHODS

Chemicals [^{3}H]Ara-C (30 Ci/mmol) and NCS were purchased from Amersham Japan (Tokyo). 6-MP, 6-TG, ara-C, THU and silicone oil were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Clear-sol and other chemicals were purchased from Nacalai Tesque (Kyoto). Polyethyleneimine-impregnated cellulose and cellulose thin-layer chromatography plates were obtained from Macherey-Nagel (Postlach, Germany). AG 50W-X8 resin was purchased from Japan Bio-Rad (Tokyo).

Cell culture and clonogenic assay L1210 cells were maintained in suspension culture in RPMI 1640 medium

supplemented with 10% fetal calf serum at 37°C in a humidified atmosphere containing 5% CO_2. All studies were carried out with cells at the exponential stage of growth. In order to maintain the cell culture under constant conditions, the cells were re-suspended in fresh medium at 5.0×10^5/ml and incubated for 16 h. Then drugs were added. Clonogenic assay was performed by a slight modification of the method described by Kufe *et al.*[8] After drug exposure, L1210 cells were washed twice with PBS, and harvested on 35 mm^2 dishes (Falcon #3001, Becton Dickinson, Lincoln Park, NJ) in triplicate. Each dish contained 1.0 ml of RPMI 1640 medium, 10% fetal calf serum, 0.88% methylcellulose and 200 cells. After incubation for 6 days, colonies consisting of groups of 50 or more cells were counted with an inverted microscope.

Determination of ara-C metabolites Cells (1.0×10^6/ml) were incubated at 37°C with 2.0 μCi of [^{3}H]ara-C. After the incubation period, cells and medium were rapidly separated from 500 μl of cell suspension by means of the silicone oil method previously described.[15] The metabolites of ara-C were extracted from cells by adding 100 μl of ice-cold 6% TCA to the cell pellet for 15 min.[16] Metabolites of ara-C in the medium were also assayed after deproteinization with an equal volume of 10% TCA. After neutralization with two volumes of cold Freon containing 0.5 *M* tri-*n*-octylamine as described by Tanaka *et al.*,[17] the sample was subjected to thin-layer chromatography. Samples from the cell pellets were spotted on PEI cellulose plates and developed with 0.45 *M* LiCl to separate ara-C nucleosides and nucleotides. Samples of the medium were spotted on cellulose plates and developed with ethyl acetate:isopropanol:water (2:2:1, v/v) to separate ara-C, ara-U and its nucleotides. After development, plates were cut into 1.0 cm strips and the radioactivity was counted with a liquid scintillation counter (Aloka LSC-3500, Tokyo). The quantity of ara-C metabolites was calculated by multiplying the percentage of the product by total radioactivity of cells or medium. Ara-CTP production was expressed as pmol of ara-CTP per 10^6 cells and ara-U production was expressed as pmol of ara-U per 1.0 ml of cell suspension or medium.

Ara-C/DNA After incubation with [^{3}H]ara-C, nuclei of L1210 cells were collected to obtain highly pure DNA at the first step. Cells were washed twice with ice-cold PBS, stirred in SKTM buffer (0.25 *M* sucrose, 25 m*M* KCl, 50 m*M* Tris-HCl pH 7.5, 5 m*M* $MgCl_2$) containing 0.2% Triton X-100 and placed on ice for 10 min. Nuclei were collected by centrifugation at 800*g* for 5 min, and then subjected to proteinase K and RNAase A digestion, followed by phenol extraction and ethanol precipitation to obtain the DNA fraction, as described elsewhere.[18] The amount of DNA was estimated spectrophotometrically, and ara-C incorporation into DNA was calculated from the radioactivity of obtained DNA. The quantity of ara-C incorporation was expressed as fmol of ara-C per microgram of DNA.

Half life of ara-CTP retention Ara-CTP dephosphorylation in L1210 cells was assayed using the method of Grant *et al.* but with slight modifications.[19] After 4.0 h exposure to 400 μCi/μmol [^{3}H]ara-C with or without 6-MP, the cells were washed twice with warmed RPMI 1640 medium and incubated again with or without 6-MP in fresh medium. At various intervals, aliquots of cell pellets were collected by centrifugation and ara-CTP pool size was determined as described above. Values for the half life of ara-CTP retention were obtained from regression lines of the natural logarithm of the ara-CTP pool size plotted against time.

Initial velocity of ara-C influx After pretreatment with 20 μM 6-MP for 4.5 h, 150 μl of cell suspension was taken and overlaid on a 750 μl silicon oil layer in a 2 ml microtube as described by Wang *et al.*[20] Then, 0.48 μCi of [^{3}H]ara-C was added to the cell suspension. After 45 s, ara-C uptake was terminated by centrifugation at 12×10^3g for 6 s. The medium and oil layers were aspirated, and the bottoms of microtubes, containing the cell pellet, were cut into glass vials. Cell pellets were dissolved in NCS and the radioactivity was counted after addition of 5.0 ml of Clear-sol.

CDD assay The CDD assay was performed by the method of Steuart and Burke, with slight modifications.[21] Crude enzyme solution was obtained by sonication of cells suspended in 50 m*M* Tris-HCl (pH 8.0) containing 10 m*M* 2-ME and 1 m*M* EDTA, then clarified by centrifugation at 100×10^3g for 60 min. The enzyme was incubated in a reaction buffer containing 50 m*M* Tris-HCl (pH 8.0), 200 μM [^{3}H]ara-C (specific activity 25 μCi/μmol), 2 m*M* 2-ME, and 1 m*M* EDTA at 37°C for 30 min. The reaction was terminated by the addition of an equal volume of 10% TCA. After removal of the precipitate by centrifugation, the supernatant of the mixture was loaded on a 0.5×5 cm AG 50W-X8 resin column and subsequently eluted with 9.0 ml of distilled water. The radioactivity of eluted [^{3}H]ara-U was directly counted with a liquid scintillation counter after adding Clear-sol. The enzyme activity was expressed as pmol of ara-U formed/mg protein/h.

DCK assay DCK was assayed by the method of Ives and Durham.[22] Crude enzyme was extracted as described above. A buffer (60 μl) containing 40 m*M* Tris-HCl (pH 8.0), 20 μM [^{3}H]ara-C (specific activity 250 μCi/μmol), 10 m*M* $MgCl_2$, 10 m*M* ATP, 10 m*M* 2-ME and 1 m*M* THU was used as the reaction buffer. Ara-CMP production was controlled within 10% of total substrate. After adding 10% TCA, the acid soluble fraction was subjected to thin-layer chromatography developed with 0.4 *M* LiCl. The plates were cut into 1.0 cm strips, and radio-

activity was counted as described above. Enzyme activity was expressed as pmol of ara-CMP formed/mg protein/h. Protein was quantified by the method of Bradford.[23]

Statistics The significance of differences between experimental groups was calculated by ANOVA one way analysis, followed by the unpaired Student's *t* test.

RESULTS

Clonogenic assay Cytotoxicity of ara-C and 6-MP in simultaneous exposure was estimated by clonogenic assay (Table I). To quantify the interaction of ara-C and 6-MP, the method of Valeriote and Lin was employed.[24] The observed survival fraction of 1.0 μM ara-C and 20 μM 6-MP in combination exposure (46%) was equivalent to the calculated product of those of the two agents (51%) and the observed survival fraction of 5.0 μM ara-C and 20 μM 6-MP in simultaneous exposure (13%) was significantly smaller than the expected figure (22%). Hence the drug interaction in this condition was assessed to be at least additive.

Ara-CTP accumulation and ara-C incorporation into DNA in the cells treated with 6-MP In order to investigate the effect of 6-MP on ara-C, we measured the ara-CTP accumulation (Fig. 1) and ara-C incorporation into DNA (Fig. 2) in cells exposed to ara-C in the presence of 6-MP. When the cells were incubated in the presence to 5.0 μM ara-C alone, ara-CTP pool size was increased linearly up to 21.6 pmol/10^6 cells at 3.0 h incubation then gradually decreased. Simultaneous exposure of 6-MP in addition to ara-C led to a significant increment of ara-CTP accumulation in a time- and concentration-dependent fashion. At 4.5 h incubation, 1.5 and 2.2 fold increment of ara-CTP was observed in cells exposed to 5.0 μM ara-C and 5.0 or 20 μM 6-MP, respectively. The extent of enhancement of ara-CTP pool size by 20 μM 6-MP was equivalent to that of 400 μM THU, so that under this condition 90% of ara-C deamination was suppressed in the cell suspension (data not

Table I. Effect of Simultaneous Exposure to Ara-C and 6-MP on Colony Formation of L1210[a]

	Conc. (μM)	% of survival fraction[b]		
		alone	with 6-MP	estimated[c]
6-MP	20	59±3.9	—	—
Ara-C	1.0	86±18	46±17	51±6.7
Ara-C	5.0	38±2.5	13±2.5[d]	22±2.8

a) Cells were incubated with drugs for 4.5 h then clonogenic assay was performed as described in "Materials and Methods."
b) Percent survival fraction was calculated by dividing the number of colonies exposed to the drug by that of the control ×100. Values are the means±1SD of three separate experiments in triplicate.
c) Estimated value was calculated by multiplying the survival fraction with ara-C alone by that with 6-MP alone.
d) Significantly smaller than the estimated figure ($P<0.05$).

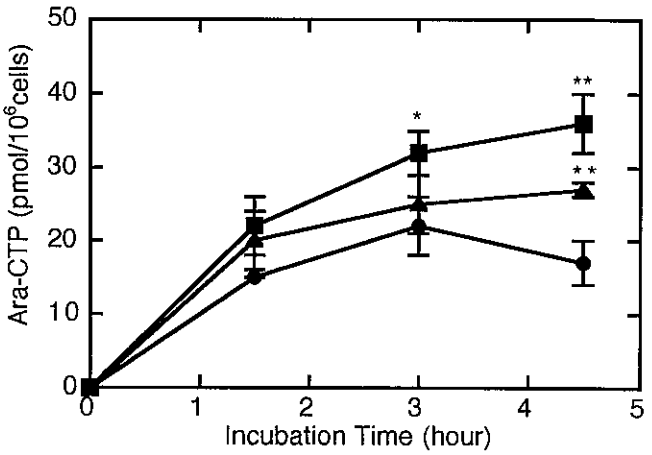

Fig. 1. Effect of 6-MP on the ara-CTP accumulation in L1210 cells exposed to 5.0 μM [^{3}H]ara-C. Cells were incubated in the presence of 6-MP (●, 0 μM; ▲, 5.0 μM; ■, 20 μM) for the indicated time. After incubation, the ara-CTP pool was determined as described in "Materials and Methods." Values are the means±1SD of three separate experiments in duplicate. Significantly greater than the control (* $P<0.05$, ** $P<0.01$).

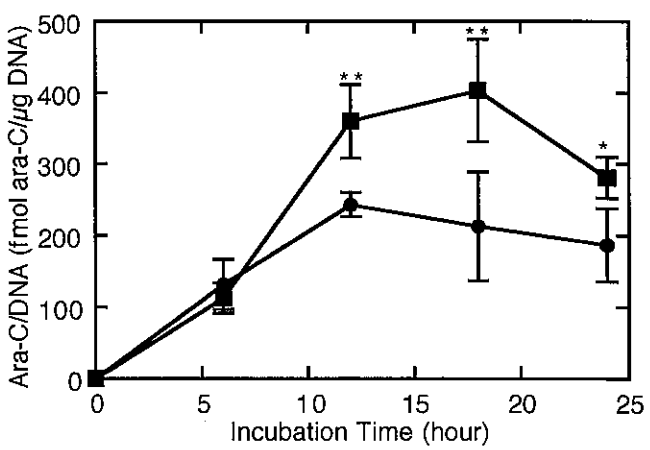

Fig. 2. Effect of 6-MP on the ara-C incorporation into DNA in L1210 cells. Cells were incubated with 5.0 μM [^{3}H]ara-C in the presence of 6-MP (●, 0 μM; ■, 20 μM) for the indicated time. After incubation, DNA was extracted as described in "Materials and Methods" and ara-C/DNA was calculated from the radioactivity of DNA. Values are the means±1SD of three separate experiments. Significantly greater than the control (* $P<0.05$, ** $P<0.01$).

Table II. Ara-CTP Accumulation in L1210 Cells Exposed to Various Concentrations of Ara-C in the Presence of 6-MP

Ara-C conc. (μM)	Ara-CTP[a]		P value
	without 6-MP	with 6-MP[b]	
0.2	0.2±0.1	0.3±0.1	0.025
1	0.6±0.2	1.5±0.5	0.017
5	11±1.8	24±3.6	0.003
25	43±6.7	57±7.0	0.032
125	105±23	135±42	0.202

a) Ara-CTP accumulation pmol/10^6 cells/3.0 h. Values are the means±1SD of three separate experiments in duplicate. *b*) Concentration of 6-MP: 20 μM.

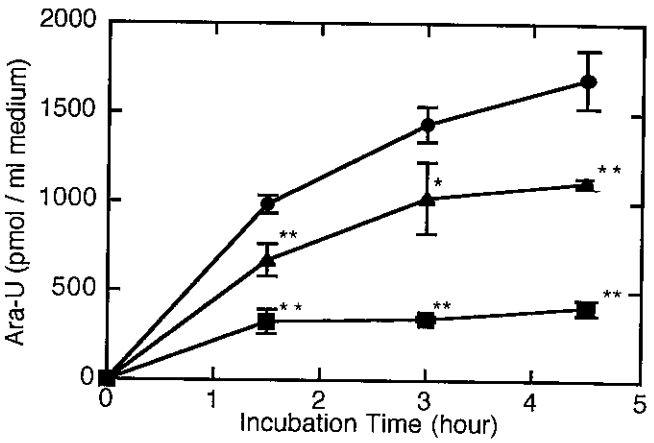

Fig. 3. Effect of 6-MP on ara-U formation in a cell suspension. L1210 cells (1.0×10^6/ml) were incubated with 5.0 μM ara-C in the presence of 6-MP (●, 0 μM; ▲, 5.0 μM; ■, 20 μM) for the indicated time. After incubation, metabolites of ara-C in the medium were determined as described in the text. Values are the mean±1SD of three separate experiments in duplicate. Significantly smaller than the control (* $P<0.05$, ** $P<0.01$).

shown). A significant increase of ara-CTP accumulation by 20 μM 6-MP was observed over a wide range of ara-C concentration from 0.2 to 25 μM (Table II). Increment of ara-CTP formation was also observed by 6-TG treatment, to the same extent as by 6-MP, but not by other physiological purine bases such as guanine, hypoxanthine and inosine (data not shown).

Ara-C/DNA also increased linearly for 12 h before reaching a plateau level in the control culture (Fig. 2). The increment of ara-C/DNA in the presence of 20 μM 6-MP was significant at 12 and 18 h incubations ($P<0.01$). Ara-C/DNA enhancement in the presence of 6-MP was interpreted as being a result of ara-CTP increment in the cells, because the extent of ara-C/DNA can be predicted from the product of ara-CTP concentration and time.[8] Ara-C/DNA in the presence of 20 μM 6-MP and ara-C for 24 h incubation was less than that for 12 or 18 h incubation. It was suspected that cells lethally damaged by increased ara-C/DNA began to disintegrate at 24 h of incubation. Thus DNA obtained from the remaining cells contained less ara-C.

Effect of 6-MP on cellular ara-C pharmacology In order to investigate the mechanism of increased accumulation of ara-CTP in the cells treated with 6-MP, we measured ara-C metabolites and ara-C transport across the cell membrane. Ara-U level in the cell suspension was found to be significantly suppressed in the presence of 6-MP in a concentration-dependent fashion (Fig. 3). However, the half life of ara-CTP retention was not different, being 266 min in control cells and 302 min in the cells treated with 20 μM 6-MP. Inital ara-C influx was also unchanged by 6-MP pretreatment. It was 19±1.9 pmol/10^7 cells/s in control cells and 18±2.9 pmol/10^7 cells/s after 4.5 h treatment with 20 μM 6-MP.

Effect of 6-MP on enzyme activities catalyzing ara-C metabolism CDD and DCK activities in L1210 cells were assayed to determine whether 6-MP affects the enzyme activity responsible for ara-C metabolism. Intracellular CDD activity was 1.0±0.4 pmol ara-U/mg/h in control cells and 1.0±0.1 pmol ara-U/mg/h after 4.5 h treatment with 20 μM 6-MP. Intracellular DCK activity was also unchanged, being 51±18 pmol ara-CMP/mg/h in control cells and 56±19 pmol ara-CMP/mg/h after 4.5 h treatment with 20 μM 6-MP. Next, we examined whether various known metabolites of 6-MP (6-TG, methyl-6-MP, 6-MPR, 6-TGR, TIMP)[25,26] directly inhibit CDD activity *in vitro*. However, 800 μM 6-MP and the above metabolites had no inhibitory effect on CDD activity (Table IIIA). Furthermore, the supernatant of cell culture exposed to 6-MP for 4.5 h was also studied to ascertain whether some unknown metabolite(s) of 6-MP inhibits CDD activity. However, CDD activity was not directly inhibited by the supernatant (Table IIIB). Consequently, it was suggested that CDD was not inhibited by 6-MP or its metabolites.

CDD shedding into the medium and its suppression by 6-MP It was necessary to investigate the distribution of CDD, because L1210 cells have a weaker CDD activity than DCK activity, in apparent conflict with the fact that the amount of ara-U produced in the cell suspension was approximately 80 fold higher than that of ara-CTP (Figs. 1 and 3). Thus, cell suspension cultured in the presence of 6-MP prior to ara-C administration was separated into the cells and the spent medium compartment. The ara-U formation in these two fractions was measured after a 3.0 h additional incubation with 5.0 μM ara-C. In the cell suspension maintained without 6-MP, ara-U formation in the spent medium was found to increase in a time-

Table III. Effect of 6-MP or Its Metabolites (A) and Supernatant of Culture Exposed to 6-MP (B) on CDD Activity

(A)	Metabolites[a)]	CDD activity[b)]
	6-MP	123±12
	6-TG	97±15
	6-TX	99±21
	methyl-6-MP	92±6.0
	6-MPR	93±0.6
	6-TGR	92±25
	TIMP	108±15
	THU	11±12
(B)	Chemicals[c)]	CDD activity[b)]
	6-MP	96±6.9
	THU	11±1.3
	—	104±7.2

a) CDD activity was measured in the presence of 800 μM 6-MP or its known intracellular metabolites.
b) CDD activity in the presence of various substrates is displayed as % of control. Control = 1.0 pmol ara-U/mg/h. Values are the means±1SE of two separate experiments in duplicate.
c) CDD activity was measured in the presence of 30 μl of supernatant of the culture in which cells were exposed to 20 μM 6-MP or THU.

dependent fashion after an appropriate lag time. Ara-U produced in the cell fractions tended to increase with increment of viable cell number, but the rate of increase was not as marked as that of medium (Fig 4A). CDD shed into the spent medium was derived from L1210 cells, because the increment of ara-U formation in the spent medium was not seen in cell-free culture under the same experimental conditions (data not shown). LDH (EC 1.1.1.27) activity in the medium was also evaluated as an internal standard, but was not increased in the medium in spite of cell culture (data not shown). Consequently, we suspected that a major part of CDD synthesized in L1210 cells is rapidly shed into the medium. In the presence of 6-MP, the amount of ara-U produced in the medium fractions was found to be suppressed in a concentration-dependent fashion. In contrast, suppression of ara-U production in the cell fractions was not observed in the presence of 6-MP (Fig. 4B, C). Viable cell number in the medium was equivalent to that of the control in spite of 6-MP treatment.

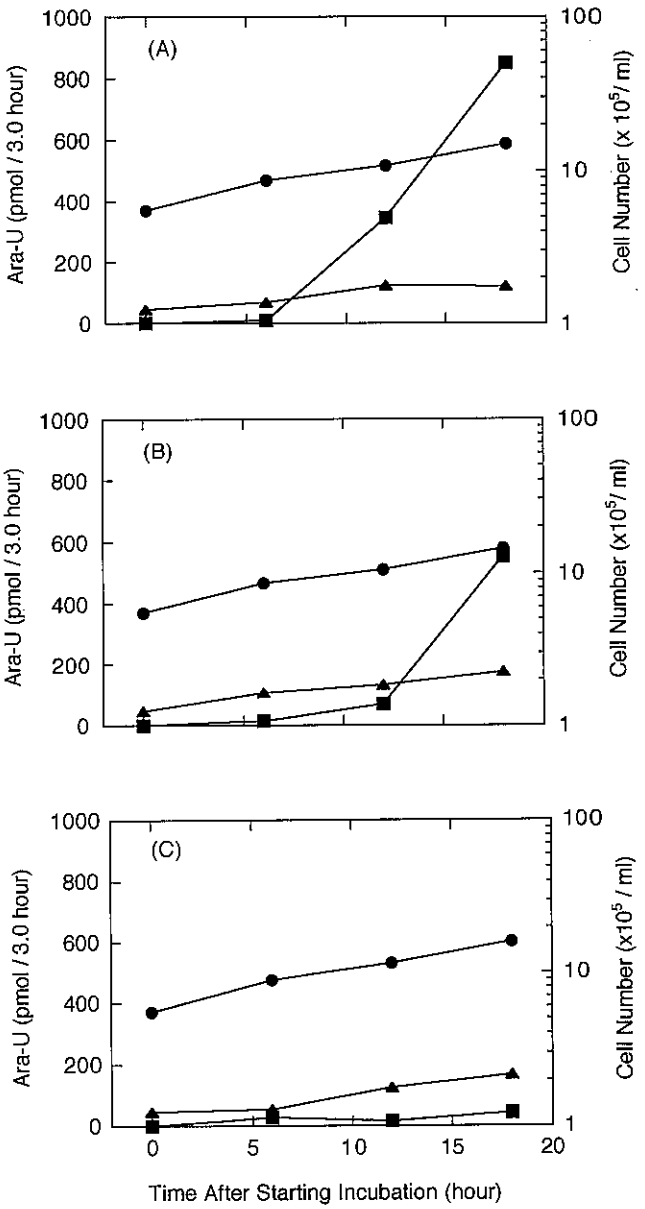

Fig. 4. CDD shedding in the medium and its suppression by 6-MP treatment. L1210 cells (5.0×10^5/ml) were suspended in fresh RPMI 1640 medium supplemented with 10% FCS and placed in a CO_2 incubator in the presence of various concentrations of 6-MP (A, 0 μM; B, 5.0 μM; C, 20 μM) but in the absence of ara-C. After 0, 6, 12 and 18 h, cell suspension was separated into the spent medium and cell pellet by centrifugation. The cell pellets were resuspended in the same volume of fresh RPMI 1640 medium supplemented with 10% FCS. Spent medium and resuspended cells were separately incubated with 5.0 μM ara-C for an additional 3.0 h at 37°C in a water bath. After deprotenization with 10% TCA, ara-U formed in the spent medium (■) and cell fraction (▲) was measured, as described in the text. Values are the means of two separate experiments in duplicate. Closed circles represent mean viable cell number.

DISCUSSION

The present study indicated that 6-MP enhanced the anti-leukemic effect of ara-C by decreasing ara-U formation, resulting in the persistence of high extracellular ara-C concentration and thus increasing the intracellular ara-CTP pool size and ara-C incorporation into DNA.

The maintenance of ara-C level was suggested to be effective for increasing ara-CTP pool size in leukemic cells, because ara-C deamination was the main pathway of its elimination.[27] Indeed, we have reported that if ara-C is present in the extracellular fluid even at a low concentration, it will be taken up into cells against the concentration gradient, leading to maintenance or increase of ara-CTP pool size.[28]

It should be emphasized that the distribution of CDD in the cell suspension was characteristic. CDD was found to be shed into the medium during incubation, while intracellular CDD activity was unchanged. The shedding of CDD was observed not only in L1210 cells *in vitro*, but also in an animal model. Ho demonstrated a conspicuous rise of CDD activity in mouse ascites fluid containing L1210 leukemic cells.[29] Furthermore, we previously reported that CDD activity was increased in the medium during primary culture of fresh leukemic cells directly obtained from patients.[1] Although the mechanism of shedding of CDD remains unknown at present, CDD could be shed by a specific mechanism and not just as a result of cell degradation, because LDH, a representative enzyme in leukemic cells, was not shed into the medium.

6-MP suppressed the ara-C deamination to a similar extent but in a different manner from THU, a typical competitive inhibitor of CDD.[30] In the presence of 6-MP, ara-U formation in the spent medium was suppressed. In contrast, the suppression of ara-U formation in cell fractions was not observed, perhaps because the changes were below the limit of detection. We suspected that 6-MP did not suppress CDD shedding, but suppressed the overall synthesis of CDD, because ara-U in the cell suspension was suppressed in the presence of 6-MP (Fig. 3). It is likely that the transcription of CDD might be suppressed non-specifically, reflecting the disturbance of RNA metabolism as a result of 6-MP incorporation into RNA[31] or RNA polymerase inhibition by 6-thio-ITP.[32] The transcription of cytidine deaminase is thought to be more susceptible than that of deoxycytidine kinase to 6-MP treatment, because deaminase activity was far weaker than that of the kinase in L1210 cells, as indicated in our study and supported by Ho.[29]

The suppressive effect of 6-MP on ara-C deamination (Fig. 3) required at least 12 to 18 h preincubation of cell suspensions with 6-MP before the simultaneous treatment with ara-C and 6-MP. Because CDD activity in the medium fraction was markedly increased in this period, as shown in Fig. 4A, 6-MP treatment at this point was thought to be effective for suppression of CDD shedding, resulting in the suppression of ara-C deamination.

The combination of ara-C and oral 6-MP has been used as a standard treatment of ANLL for 25 years.[13, 14] At this conventional dose of oral 6-MP (75 mg/m^2), the average peak concentration of 6-MP was 0.89 μM *in vivo*, which was 5 to 20 times lower than that used in this study *in vitro*.[33]

Clinical trials of prolonged intravenous infusion of 6-MP are in progress. Zimm *et al.* reported that the average steady-state plasma concentration was 6.9 μM when 6-MP was administered at a dose of 50 mg/m^2/h.[34] Pinkel *et al.* have described the administration of high-dose intravenous 6-MP (1,000 to 1,250 mg/m^2) by 24 h continuous infusion followed by ara-C 500 mg/m^2/day 24 h countinuous infusion for 4 days.[35, 36] Of 7 children with ANLL in relapse after conventional chemotherapy, 6 experienced complete remission. The median steady-state level for 6-MP was 4.3 μM and that for ara-C was 1.6 μM, these levels being similar to those used in the present study. Although it is difficult to estimate the clinical significance of high-dose intravenous 6-MP on ara-C pharmacology *in vivo* from our limited data, we speculate as follows. As well as in leukemic cells, ara-C deamination might be suppressed by 6-MP in the liver, kidney and mucous membrane of the gastrointestinal tract to some extent, which might result in a change of plasma pharmacokinetics of ara-C without severe adverse effects in these critical organs, because CDD activity in these organs are exceptionally high, permitting the deamination of ara-C even at high concentration, corresponding to the high-dose ara-C administration regimen. Consequently, part of the favorable clinical effect without severe adverse effects, as reported by Lockhart *et al.*, might be due to the inhibition of ara-C deamination in major organs and plasma with high CDD activity, followed by an enhancement of ara-C anabolism in leukemic cells with relatively low CDD activity. Indeed, ara-C deamination could be responsible for ara-C resistance in some patients, and a tendency for positive response to ara-C was found in slow ara-C deaminators *in vivo*.[37] Thus, high-dose intravenous administration of 6-MP might provide a new mode of chemical modulation of ara-C *in vivo*. Further clinical studies on the pharmacokinetics of ara-C and CDD activity in plasma are needed in order to elucidate the effect of high concentrations of 6-MP on ara-C metabolism *in vivo*.

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