Increased Deoxycytidine Kinase Activity by Etoposide in L1210 Murine Leukemic Cells

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Deoxycytidine kinase (dCK) is a rate-limiting enzyme for the activation of ara-C. It is responsible for the phosphorylation of ara-C which is widely used in the treatment of leukemia. We examined the effect of etoposide on dCK in L1210 cells and found that incubation with 10 μ M etoposide for 1 h increased dCK activity to 170% of the control. This effect of etoposide on dCK activity was concentration-dependent up to at least 100 μ M of the substance.

Key words ara-C; etoposide; deoxycytidine kinase; L1210 leukemic cell

Deoxycytidine kinase (dCK) is an enzyme in the salvage pathway used for pyrimidine synthesis. It is important for the phosphorylation of deoxycytidine and several deoxycytidine analogues.^{1,2)} ara-C is an analogue of deoxycytidine and is used as a standard drug in the chemotherapy of leukemia.³⁾ To be cytotoxic, ara-C must be phosphorylated to ara-CTP, and the rate-limiting enzyme in this process is dCK. The cytotoxic effect of ara-C is dependent on its phosphorylation by dCK. One of the main mechanisms of resistance to ara-C is a decrease in dCK activity.⁴⁾ Combination chemotherapy of etoposide and ara-C has been shown to be very effective in the treatment of human leukemia.^{5,6)} Etoposide produces topoisomerase II-mediated strand breaks in DNA.^{7,8)} We previously demonstrated that pretreatment with etoposide increased ara-C incorporation and that this effect was compatible with the sequence-dependent antitumor effect of etoposide and ara-C. It was of note that 3 and 6 h pretreatment of etoposide increased ara-C incorporation up to 250% of that of ara-C alone.⁹⁾ The mechanism of increased ara-C incorporation with this pretreatment was investigated here by examining the effect of etoposide on dCK activity.

MATERIALS AND METHODS

Experimental Leukemic Cells Murine L1210 leukemic cells were obtained from Shionogi Pharmacological Laboratory (Osaka, Japan). Cells were routinely maintained under conditions of logarithmic growth in RPMI-1640 medium supplemented with 10% fetal calf serum, 1% glutamine and 20 μ M 2-mercaptoethanol.

Anticancer Drug and Chemicals Etoposide was purchased from Nippon Kayaku Co., Ltd. (Tokyo, Japan), and [³H]deoxycytidine (dC) from Amersham International, Ltd. (Tokyo, Japan). All other chemicals were purchased from Sigma Chemical Co., Ltd. (Tokyo, Japan).

Measurement of dCK Activity in Cell Extract dCK activity was measured by the modified method of Cheng *et al.*¹⁰⁾ Briefly, leukemic cells were washed once with saline and collected by centrifugation at 1500 $\times g$ for 5 min. 2×10^7 cells were resuspended in extract buffer A

containing 50 mM Tris-HCl (pH 7.5), 2 mM dithiothreitol (DTT), 20% glycerol, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF) in a final volume of 200 μ l. The solution was prepared by repeated freezing and thawing three times and centrifugation at 14000 $\times g$ for 5 min. The supernatant was transferred to a microcentrifuge tube and stored at -80°C until measurement. The assay reaction mixture for dCK contained 10 μ M [³H]dC, 10 mM ATP, 10 mM MgCl₂, 50 mM Tris-HCl (pH 7.5), 15 mM NaF, 10 mM DTT and 1 mM cytidine (buffer B). Reactions were started by the addition of 20 μ l of cell lysate supernatant to 30 μ l of buffer B at 37 $^\circ\text{C}$. After 30 min of reaction time, 50 μ l samples were pipetted onto Whatman DE-81 filter discs. The filters were washed three times with a large quantity of water. Radioactivity associated with the filter was measured by scintillation counting. Each data point is the average of triplicate measurements. The results were expressed as pmol of dCMP/min per milligram of protein. Protein concentrations were determined using 25% human serum albumin as a standard.

RESULTS

L1210 cells were incubated with etoposide at 37 $^\circ\text{C}$ for 1 h at concentrations ranging from 1 to 100 μ M. Ten and 100 μ M etoposide increased dCK activity to 171% and 216% of the control (Fig. 1). This increasing effect was observed even at as low a concentration as 1 μ M. Next, we measured the time course of dCK activity after continuous incubation with 1 and 10 μ M etoposide. After 20 min of incubation, 10 μ M etoposide increased dCK activity to 125% of the control, but no increase was observed at 1 μ M etoposide. After a dramatic increase in dCK activity at 1 h, the activity continued to increase gradually for at least 2 h more up to 3 h (Fig. 2).

DISCUSSION

In our previous study, we demonstrated that the antitumor effect of etoposide and ara-C was significantly sequence-dependent, and that pretreatment with etoposide produced the best therapeutic results. We further dem-

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Fig. 1. Effect of Etoposide on dCK Activity in L1210 Cells

L1210 cells were incubated with etoposide for 1 h. All values show the means $\pm$ S.D. * $p < 0.001$ as compared with the control by Student's *t*-test.

Fig. 2. Effect of Continuous Incubation with Etoposide on dCK Activity in L1210 Cells

L1210 cells were incubated with etoposide for 20, 60 or 180 min. All values show the means $\pm$ S.D. * $p < 0.001$ as compared with the control by Student's *t*-test.

onstrated that 3 and 6 h pretreatment with etoposide increased ara-C incorporation into DNA to more than 200% that of ara-C alone.⁹⁾ In the present study, we demonstrated that dCK activity was increased within 1 h after exposure to etoposide, and that this effect was concentration-dependent but significant, even at a low concentration of 1 μM . dCK activity increased rapidly within 1 h and continued to increase gradually for at least the next 2 h. This increased dCK activity might be the main mechanism of increased ara-C incorporation following pretreatment with etoposide; however, the mechanism of the increase in dCK activity by etoposide is not yet known. MTX also increased dCK activity to 178% of the control at 3 h after exposure (data not shown). In our previous study, MTX reduced the dCTP pool,¹¹⁾ and the feedback control due to this decrease might be the main mechanism involved in increasing dCK activity by MTX. Etoposide, however, expanded the dCTP pool, so that¹²⁾ this feedback control did not seem to be the mechanism of increase in dCK activity by this substance.

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