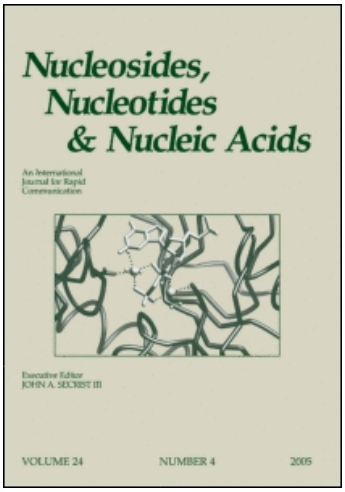

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Inhibition of the ERK Pathway Promotes Apoptosis Induced by 2-Chloro-2'-Deoxyadenosine in the B-Cell Leukemia Cell Line Eheb

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INHIBITION OF THE ERK PATHWAY PROMOTES APOPTOSIS INDUCED BY 2-CHLORO-2′-DEOXYADENOSINE IN THE B-CELL LEUKEMIA CELL LINE EHEB

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□ *2-Chloro-2′-deoxyadenosine (CdA) is a nucleoside analogue active in B-cell chronic lymphocytic leukemia (B-CLL). Although the mechanism of action of CdA has been extensively investigated in leukemic cells, the possibility that this nucleoside analogue interacts with the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway has never been explored. In this study, we show that CdA, at concentrations close to the IC_{50}, activated the ERK pathway in the B-cell line EHEB. Because activation of this pathway is assumed to exert anti-apoptotic effect, we combined CdA with inhibitors of the ERK pathway. The latter were found to enhance CdA-induced apoptosis. These results suggest that the efficacy of CdA could be strengthened by combination with inhibitors of the ERK pathway.*

Keywords Cladribine; MAPK/ERK; B-CLL; Lymphocytes

C. Smal and S. Lisart have equally contributed to this work.

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INTRODUCTION

CdA, an analogue of 2′-deoxyadenosine, is used for the treatment of lymphoid malignancies, including B-CLL. CdA is a prodrug and has to be converted to CdATP by the successive action of deoxycytidine kinase and nucleoside monophosphate and diphosphate kinases to exert its cytotoxicity. Intracellular accumulation of CdATP results in inhibition of DNA synthesis (replication or repair), leading to DNA lesions and eventually to apoptosis by p53-dependent pathways. CdATP also can directly induce mitochondrial depolarization and co-operate with cytochrome c and Apaf-1 to activate caspase-3.[1,2] Whether CdA interacts with the mitogen-activated protein kinases (MAPKs), which play key roles in survival, proliferation and apoptosis,[3] had never been studied. Here, we investigated the influence of CdA on the prosurvival MAPK/extracellular signal-regulated kinase (ERK) pathway because activation of the latter could compromise the therapeutic efficacy of CdA.

MATERIALS AND METHODS

EHEB cells, used as an *in vitro* model of B-CLL lymphocytes,[4] were cultured in RPMI-1640 with Glutamax, supplemented with 10% heat-inactivated FCS at 37°C in an atmosphere of 5% CO_2 in air. Phospho-ERK or total ERK were measured by Western blot after SDS-Page on a 10% polyacrylamide gel, using specific rabbit antibodies (Promega). Caspase-3 activity was determined by its ability to cleave the synthetic peptide ac-DEVD-AMC. The release of AMC was monitored by a spectrofluorometer with an excitation wavenlength of 380 nm and an emission wavenlength of 460 nm.

RESULTS AND DISCUSSION

EHEB cells were incubated for increasing times with 5 μM CdA, a concentration close to the IC_{50} in this cellular model.[5] Cell extracts were subjected to immunoblot to examine ERK phosphorylation and expression after CdA treatment (Figure 1). ERK phosphorylation was nearly undetectable in basal conditions and was not modified at short times after addition of CdA. However, an increase of ERK phosphorylation was clearly observed after a 24 hour-incubation in the presence of CdA, whereas ERK level remained fairly constant under these conditions.

The dose response of ERK to CdA was also investigated. A maximal effect, although larger after 48 hours than 24 hours, was already observed with 2 μM CdA (results not shown). Taken together, these results suggest that CdA activate the MAPK/ERK pathway in EHEB cells at cytotoxic concentrations.

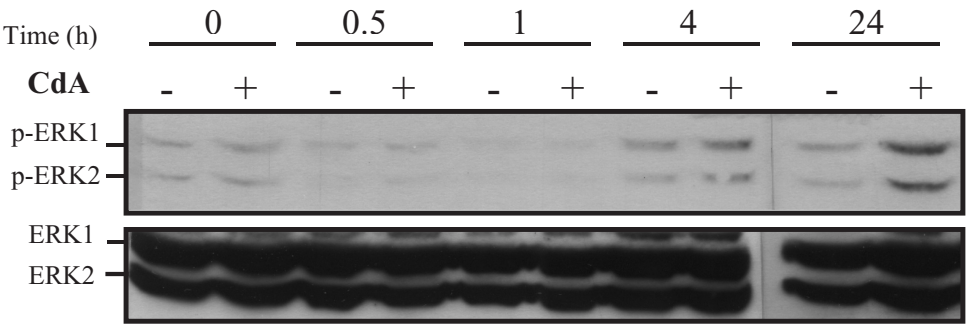

FIGURE 1 Time-course effect of CdA on ERK phosphorylation. EHEB cells were incubated with 5 μM CdA for 30 min to 24 h, and cell lysates were subjected to immunoblot analysis for phosphorylated ERK (pERK1/2) and total ERK (ERK1/2).

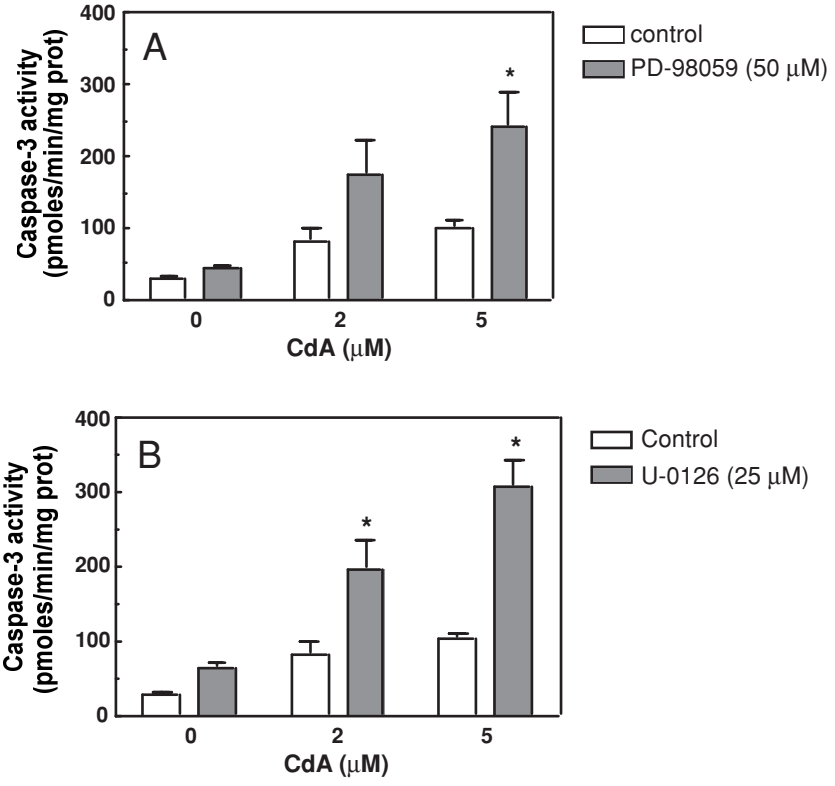

FIGURE 2 Effect of MAPK/ERK pathway inhibitors on the activation of caspase-3 by CdA. EHEB cells were incubated for 24 h in the absence or in the presence of 2 or 5 μM CdA, and with or without an inhibitor of the ERK pathway, PD-98059 (A) or U-0126 (B). * = $P < 0.05$.

Since activation of the MAPK/ERK pathway is considered as an anti-apoptotic signal that can antagonize chemotherapy, we examined the influence of two structurally unrelated inhibitors of this pathway, PD-98059 and U-0126,[6] on apoptosis induced by CdA. We firstly verified that both inhibitors indeed inhibited the phosphorylation of ERK at recommended concentrations (not shown). We then measured activity of caspase-3, a key executioner of apoptosis, 24 h after addition of CdA to cells preincubated with or without the ERK pathway inhibitors (Figure 2).

Both inhibitors enhanced the activation of caspase-3 induced by 2 or 5 μM CdA. Taken together, these results indicate that CdA, like other anti-cancer drugs,[7] may activate the MAPK/ERK pathway, and suggest that combination of CdA with inhibitors of this pathway might improve the clinical efficacy of CdA. Further studies are planned to examine whether ERK activation is observed in B-CLL lymphocytes at clinically relevant concentrations of CdA.

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