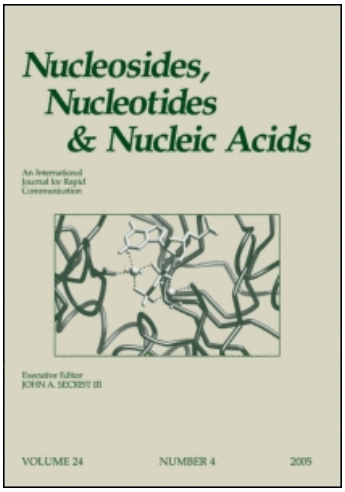

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Stimulation of Deoxycytidine Kinase Results in Prolonged Maintenance of the Enzyme Activity

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ABSTRACT

A number of genotoxic and antiproliferative agents such as 2-chlorodeoxyadenosine (Cladribine; CdA) and aphidicolin (APC) have been shown to stimulate the activity of deoxycytidine kinase, the main deoxynucleoside salvage enzyme in lymphocytes. Here we show that enzyme activation could be prevented by treating cells with the membrane-permeant calcium chelator BAPTA-AM. Long-term incubations demonstrated that CdA and APC not only stimulated but also sustained deoxycytidine kinase activity in the cellular context, as compared to the control and BAPTA-AM treated enzyme activities.

Key Words: Deoxycytidine kinase; 2-Chlorodeoxyadenosine; Enzyme activation; BAPTA-AM; Calcium chelation.

INTRODUCTION

Deoxycytidine kinase (dCK; EC 2.7.1.74.) plays a pivotal role in the mammalian nucleoside salvage pathways by recycling deoxynucleosides either released from the

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intracellular DNA catabolism or provided via nucleoside-specific membrane transporters from the extracellular environment.[1] This enzyme is responsible for the first and rate-limiting 5′-phosphotransfer from ATP or UTP[2] to 2′-deoxycytidine and to the two purine deoxynucleosides.[3] Moreover, dCK efficiently phosphorylates and thereby activates a wide range of nucleoside analogues, fundamental components of anti-leukemic and antiviral therapeutic regimens.[4]

Previously we have found that upon short-term treatments of human primary lymphocyte cultures with nucleoside analogues, with a variety of non-nucleoside genotoxic agents such as aphidicolin, etoposide,[5,6] and even with the G-protein modulator sodium fluoride, dCK activity has elevated several fold.[7,8] Opposite results were obtained with λ protein phosphatase treatment, pointing to a presumable regulatory role of reversible protein phosphorylation.[6] Gamma-irradiation also augments dCK function,[9] and some recent papers also corroborate our findings.[10] The increase of dCK activity has been suggested as a compensatory mechanism of cells, induced by different ''inhibitors'' of DNA synthesis resulting in damaged DNA. The increased dCK activity is able to supply the repair of DNA with dNTPs in quiescent cells, which seems to be supported by the counteracting effect of extracellular dCyd too.[11]

Most of the drugs eliciting dCK activation in lymphoid cells have also been shown to induce apoptotic cell death too.[12] Transient Ca^{2+} spikes were shown within minutes after CdA addition,[12] and cytochrome c and deoxyguanosine kinase were released into the cytosol, which promote the formation of the apoptosome complex.[12,13]

In this study, the long-term effects of deoxycytidine kinase stimulation by CdA and aphidicolin were analyzed. BAPTA-AM treatment prevented the activation of the enzyme and resulted in a gradually decreasing enzyme activity in lymphocytes.

MATERIALS AND METHODS

CdA was synthesized by Z. Kazimierczuk.[14] Aphidicolin and BAPTA-AM (1, 2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester) were obtained from Sigma, the isotopes from Amersham, the rest of the chemicals were from Reanal.

Human tonsillar lymphocytes were freshly isolated from surgically removed tonsils of 3–6-year-old children. Cells (10^7/ml) were resuspended in serum-free Eagle's MEM and incubated at 37°C in the presence or absence of the different drugs for the indicated time periods.

Crude cell extracts were prepared by three consecutive freeze-thaw cycles (liquid nitrogen–ice) in extraction buffer containing 50 mM Tris-HCl pH 7.6, 2 mM dithiothreitol, 0.5 mM phenyl-methyl-sulphonyl-fluoride (PMSF), 20 v/v% glycerol and 0.5% Nonidet P-40 non-ionic detergent. After centrifugation for 30 min at 14,000 rpm at 4°C, supernatants with an average protein concentration of 5 mg/ml were subjected to dCK and TK isoenzyme activity determinations.

dCK and TK activities were measured using ^{3}H-dCyd and ^{3}H-dThd as substrates (both 10 μM; specific activities: 500–1000 cpm/pmol), respectively, in a kinase assay containing 50 mM Tris-HCl pH 7.6, 5 mM $MgCl_2$, 5 mM ATP, 2 mM dithiothreitol (DTT), 10 mM NaF and 25 μg cell extract essentially as described.[8,9,11] TK2

isoenzyme was measured in the presence of 1 mM dCyd in the TK reaction mixture. TK2 activity was less than 1% of total TK activity in our cells and did not change during treatments.[8,9,11]

RESULTS AND CONCLUSIONS

Long-term effects of CdA and APC alone or in combination with BAPTA-AM, a cell-permeable calcium chelator, on the activation of deoxycytidine kinase were investigated in human tonsillar lymphocytes. Enzyme activity data are depicted in Fig. 1, as percentages of the control activities in each time group.

Both CdA and APC stimulated dCK activity (2.41 fold and 2.92 fold after 2 hours, respectively), while thymidine kinase levels were unaffected (data not shown). Potentiation of dCK activity was maintained and even slightly augmented during prolonged incubations with the drugs (4, 6 and 16 hours), expressed as relative values over control activities (Table 1, first and third rows).

These results indicate that stimulated dCK activity is sustained in the cells as compared to the activity of the non-stimulated enzyme. The difference is much more conspicuous in case of the BAPTA-treated cells. Intracellular calcium chelation completely prevented dCK activation induced by either CdA or APC; moreover, it gradually decreased enzyme activity as compared to the untreated control (compare columns 1 to columns 3 and 5). However, BAPTA-AM did not inhibit dCK activity when it was added directly to the in vitro kinase reaction (data not shown). After 16 hour treatments, the dCK activity of CdA-treated cells was 3.26 fold over the activity of the control and 5.98 fold over the activity of the CdA + BAPTA-AM treated cells. These quotients were 3.44 fold versus 14.04 fold, respectively, in APC-treated cells, suggesting that calcium deprivation gradually decreases the catalytic activity of deoxycytidine kinase via inactivation of the enzyme.

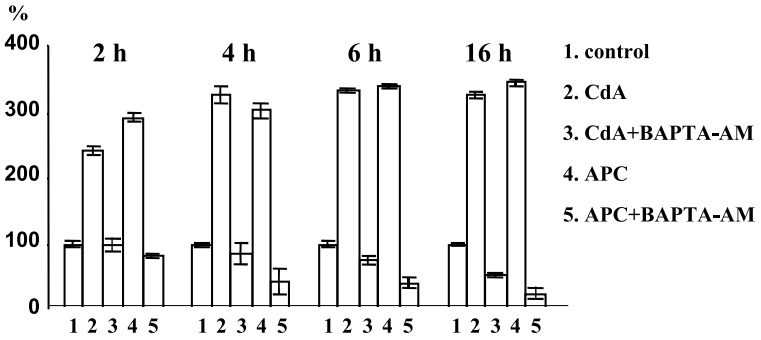

Figure 1. Long-term effects of Cladribine (CdA), aphidicholin (APC) and BAPTA-AM treatments on dCK activity in lymphocytes. Freshly isolated human tonsillar lymphocytes were incubated in Eagle's MEM for the indicated time periods in the absence or presence of the enlisted drugs and dCK activities were subsequently determined as described. Columns represent dCK activity as percentage of the untreated controls. **1.** control cells **2.** CdA 2 μM **3.** CdA 2 μM + BAPTA-AM 50 μM **4.** APC 2 μg/ml **5.** APC 2 μg/ml + BAPTA-AM 50 μM.

Table 1. Activation quotients (dCK fold activity of treated cells over that of control or BAPTA-AM-coincubated cells) from treatments shown in Fig. 1.

	Incubation time			
Activation quotients	2 hrs	4 hrs	6 hrs	16 hrs
Cladribine (CdA)/control	2.41	3.26	3.31	3.26
CdA/CdA + BAPTA-AM	2.46	3.88	4.40	5.98
Aphidicolin (APC)/control	2.92	3.02	3.38	3.44
APC/APC + BAPTA-AM	3.65	6.69	8.15	14.04

Our recent model of dCK activation suggested that the enzyme exists in an inactive and active form in cells and the transition between them might be regulated by reversible phosphorylation.[9] Results presented here further support this theory by showing that CdA and APC keep up dCK activity for a long time probably by continuous stimulation of a putative 'dCK kinase.' On the other hand, Ca^{2+} deprivation might cripple this kinase, therefore dCK activity gradually decreases due to the action of calyculin A-sensitive protein phosphatases.[9]

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