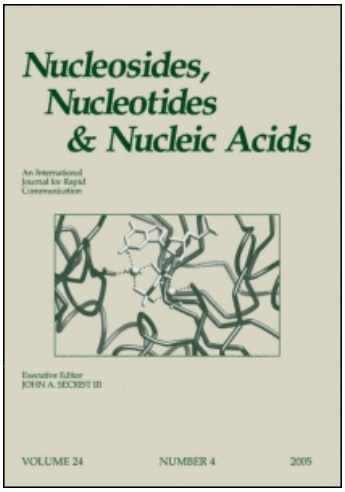

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Cell Cycle Dependent Regulation of Deoxycytidine Kinase, Deoxyguanosine Kinase, and Cytosolic 5'-Nucleotidase I Activity in MOLT-4 Cells

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To cite this Article Fyrberg, A. , Mirzaee, S. and Lotfi, K.(2006) 'Cell Cycle Dependent Regulation of Deoxycytidine Kinase, Deoxyguanosine Kinase, and Cytosolic 5'-Nucleotidase I Activity in MOLT-4 Cells', Nucleosides, Nucleotides and Nucleic Acids, 25: 9, 1201 — 1204
To link to this Article: DOI: 10.1080/15257770600894386
URL: http://dx.doi.org/10.1080/15257770600894386

Nucleosides, Nucleotides, and Nucleic Acids, 25:1201–1204, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600894386

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CELL CYCLE DEPENDENT REGULATION OF DEOXYCYTIDINE KINASE, DEOXYGUANOSINE KINASE, AND CYTOSOLIC 5′-NUCLEOTIDASE I ACTIVITY IN MOLT-4 CELLS

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□ *Activation of nucleoside analogues is dependent on kinases and 5′-nucleotidases and the balance between the activity of these enzymes. The purpose of this study was to analyze deoxycytidine kinase, deoxyguanosine kinase, and 4 different 5′-nucleotidases during cell cycle progression in MOLT-4 cells. The activity of both kinases was cell cycle dependent and increased during proliferation while the activity of cytosolic 5′-nucleotidase I decreased. We could show that the kinase activity was higher than the total nucleotidase activity, which was unchanged or decreased during cell cycle progression. These data may be important in designing modern combination therapy with nucleoside analogues.*

Keywords Deoxycytidine kinase; Deoxyguanosine kinase; 5′-Nucleotidase; Nucleoside analogues

INTRODUCTION

Nucleoside analogues are cytotoxic drugs used in the treatment of several haematological malignancies. Their cytotoxic effects are dependent on their phosphorylation to active drug by the cytosolic/nuclear enzyme deoxycytidine kinase (dCK) and to some extent by the mitochondrial deoxyguanosine kinase (dGK). The activation of nucleoside analogues is reversed by 5′-nucleotidases (5′-NTs). Resistance to nucleoside analogues

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is a common problem and is often due to decreased activity of dCK[1,2] and/or altered expression of 5′-nucleotidases.[3] The aim of this study was to investigate the activities of dCK, dGK and 4 5′-nucleotidases during cell cycle progression in the human leukemic cell line MOLT-4, and to correlate these data with cytotoxicity measurements.

MATERIALS AND METHODS

MOLT-4 cells were starved without serum for 24 hours to arrest in G0/G1 phase of the cell cycle, then 10% serum was added and cells were harvested every 12 hours. The activity of dCK and dGK was measured as previously described[2] using [8-^{3}H]-2′-deoxycytidine and [6-^{3}H]-2′-deoxyguanosine as substrates. The 4 nucleotidases; extracellular NT (ecto-NT), the cytosolic NTs, cN-I, and cN-II and 5′-(3′)-deoxyribonucleotidase (dNT-I) was measured also as previously described.[4] Substrates used were [^{3}H]-AMP, [^{3}H]-IMP, [^{3}H]-dUMP, and [^{3}H]-CMP for cN-I, cN-II, dNT-I, and ecto-NT, respectively. Cell cycle distribution was determined using flow cytometry. The cytotoxicity towards nucleoside analogues was examined using the MTT assay after incubating the cells with drugs for 72 hours as described earlier.[2]

RESULTS AND DISCUSSION

We could show that as more cells entered S-phase, the activities of dCK and dGK enzymes increased. In the case of dCK, there was an almost 2-fold increase in activity at 36 hours after addition of serum compared to resting cells (0 hours, Figure 1a). At this time-point approximately 60% of the cells were in S-phase compared to 0 hours when about 30–35% of the cells were in S-phase, indicating a relationship between the increase in activity and the percentage of cells in S-phase. For dGK there was a 4-fold increase in activity at 36 hours compared to resting cells (Figure 1b). The activity of dCK has previously been suggested to be cell cycle regulated[5–8] but the activity of dGK has been proposed not to be influenced by the cell cycle since there is no synchronization between mitochondrial replication and the cell cycle.[8] The cell cycle dependent activation of dCK is rather modest but may be of significance since dCK is regarded as the rate-limiting enzyme for the activation of several nucleoside analogues. Rodgrigues et al.[8] have shown that the formation of 9-β-D-arabinofuranosylguanine (AraG) monophosphate was greater when cells were in S-phase and that there were a strong linear relationship between the number of S-phase cells and AraG triphosphate-mediated DNA synthesis inhibition.

The activity of cN-I started to decrease after serum starvation and were kept at the same level and did not return to baseline level as cells started to

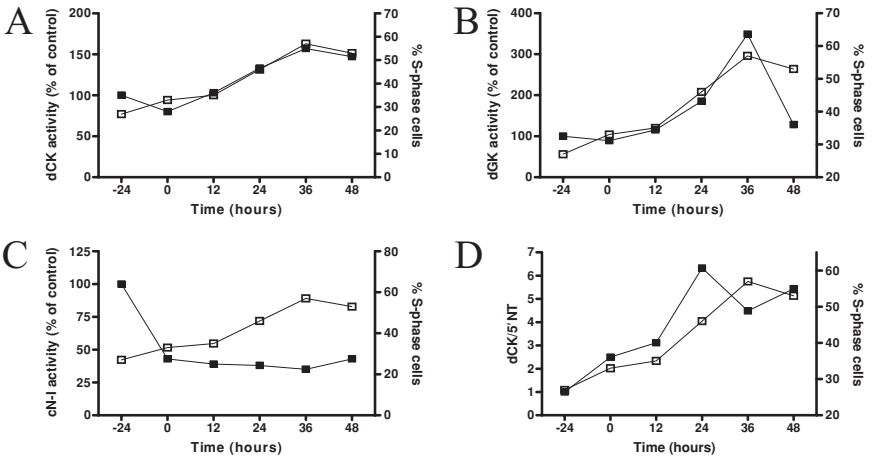

FIGURE 1 MOLT-4 cells were starved without serum for 24 hours, then 10% serum was added (0 hours) and cells were harvested every 12 hours. A-D, enzymatic activity (■) compared to percentage of cells in S-phase (□). Representative data from one out of 3 experiments.

proliferate (Figure 1c). cN-II and dNT-I decreased with 50% or more after addition of serum while the activity of ecto-NT was rather constant at all time points (data not shown). When looking at a quotient of dCK to 5′-NT activity during cell cycle progression the increase in dCK activity was greater than the total change in nucleotidase activity up to 24 hours after addition of serum (Figure 1d). This suggests that it may be beneficial to stimulate cells to proliferation before treating them with cytotoxic drugs like nucleoside analogues.

The MOLT-4 cells were most sensitive to Cytarabine (AraC), Clofarabine (CAFdA), and Cladribine (CdA) with IC_{50} values of 0.06 ± 0.02, 0.14 + 0.01, and 0.51 + 0.08 μM, respectively. These cells were more insensitive to 2-fluoro-9-β-arabinofuranosyladenine (FaraA) and AraG with IC_{50} values of 4.8 ± 0.6 and 9.5 ± 0.2 μM. CdA and CAFdA are much better substrates for dCK than FaraA,[9] which may be one explanation for the higher IC_{50} value of FaraA. The phospohorylation of AraG is dependent of both dCK and dGK but to what extent is unclear. AraG is predominatly incorporated into mitochondrial DNA but resistance to AraG has been shown to be due to both dCK and dGK down regulation.[10] In the case of MOLT-4 cells the activity of dGK represented 8–16% of the total kinase activity which may be one explanation for the higher IC_{50} value of AraG.

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