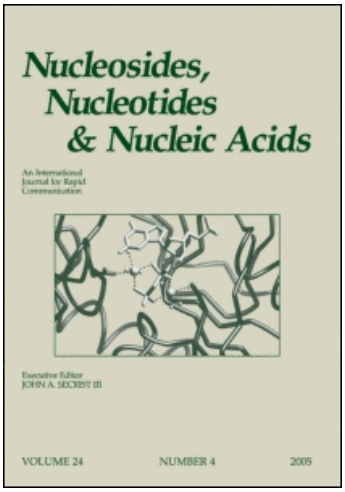

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T. Spasokoukotskaja[a]; Z. Csapó[a]; S. Virga[a]; M. Sasvári-Székely[a]; M. Staub[a]; G. Keszler[a]
[a] Institute of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary

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EFFECTS OF INTRACELLULAR CALCIUM CHELATION AND PIFITHRIN-α ON DEOXYNUCLEOTIDE METABOLISM IN HUMAN LYMPHOCYTES

T. Spasokoukotskaja, Z. Csapó, S. Virga, M. Sasvári-Székely, M. Staub, and G. Keszler □ *Institute of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary*

□ *Previously, we have found that activation of deoxycytidine kinase elicited by various DNA-damaging chemical agents could be prevented by BAPTA-AM, a cell-permeable calcium chelator or by pifithrin-α, a pharmacological inhibitor of p53. Here, we show that stimulation of deoxycytidine kinase by UV-light also is calcium-dependent and pifithrin-α-sensitive in tonsillar lymphocytes, while thymidine kinase 1 activity is stabilised in the presence of BAPTA-AM. Importantly, both UV-irradiation and calcium chelation decreased the incorporation of labelled deoxycytidine and thymidine into DNA. Pifithrin-alpha dramatically reduced the labelling of both the nucleotide and DNA fractions, possibly due to inhibition of transmembrane nucleoside transport.*

Keywords Deoxycytidine kinase; Metabolic labelling; Enzyme activation; BAPTA-AM; Pifithrin-α

INTRODUCTION

In contrast to thymidine kinase 1 (TK1), the expression and activity of deoxycytidine kinase (dCK) is reportedly constant throughout the cell cycle. However, it turned out that the activity of dCK could be enhanced several fold upon chemical[1] or physical[2,3] genotoxic stress at the post-translational level.[2,3] We found that activation of dCK by 2-chloro-2′-deoxyadenosine or by aphidicolin could be prevented by pretreating normal human lymphocytes either with BAPTA-AM, a hydrophobic calcium chelator,[4] or with pifithrin-alpha, a pharmacological inhibitor of p53.[5] Here, we extend the above studies by showing that UV-induced dCK

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Address correspondence to T. Spasokoukotskaja, Institute of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, POB 260, H-1444 Budapest, Hungary. E-mail: spas@puskin.sote.hu

activation also is counteracted by calcium chelation and pifithrin-α, and a novel inhibitory effect of pifithrin-α on the cellular uptake of nucleosides is proposed.

MATERIALS AND METHODS

Isolation of human tonsillar lymphocytes, maintenance of primary cell cultures, preparation of whole-cell extracts, and determination of dCK and TK1 activities, as well as metabolic labelling of primary cultures were performed essentially as described in Csapó et al.[2] Primary cultures were irradiated with 100 J/m^2 in 12-well plates by means of a Bio-Rad UV-lamp (Hercules, CA, USA) delivering its bulk at 260 nm. BAPTA-AM and pifithrin-α were purchased from Sigma-Aldrich (St. Louis, MO, USA).

RESULTS AND DISCUSSION

Van den Neste et al. showed that the activity of dCK elevated 3-fold upon UV-irradiation in B-CLL cells.[3] As depicted in Figure 1, similar results were obtained in UV-irradiated normal human lymphocytes exhibiting 2.6-fold greater dCK levels. BAPTA-AM and pifithrin-α proved to be equally potent inhibitors of UV-induced activation of dCK, suggesting that stimulation of the enzyme by UV-light might be calcium- and p53-dependent in human tonsillar lymphocytes. As far as TK1 activities are concerned, it seems

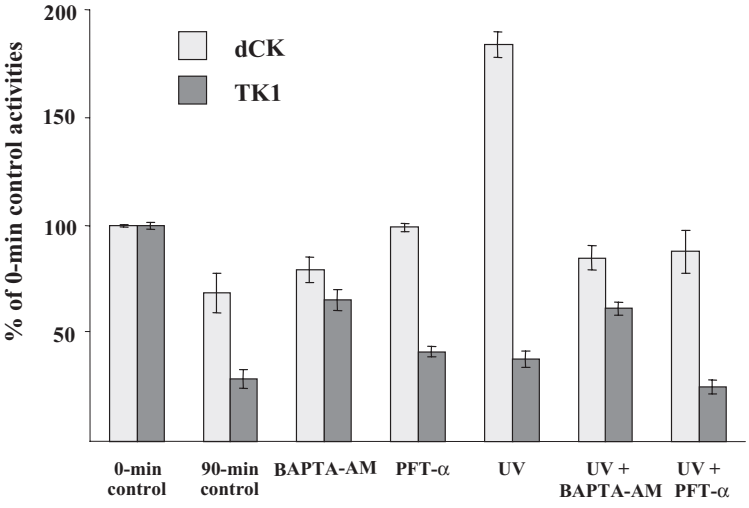

FIGURE 1 Effect of BAPTA-AM and pifithrin-α on dCK and TK1 activities in UV-irradiated lymphocytes. Cells were incubated for 0 or 90 minutes in the absence or in the presence of 50 μM BAPTA-AM or 50 μM pifithrin-α (PFT-α), respectively. Cells were irradiated with 100 J/m^2 UV-dose at 60 minutes of incubation if indicated. After harvesting at 90 minutes, cell extracts were prepared and enzyme activities were measured.[2] Assays were performed in triplicate; SEM are indicated.

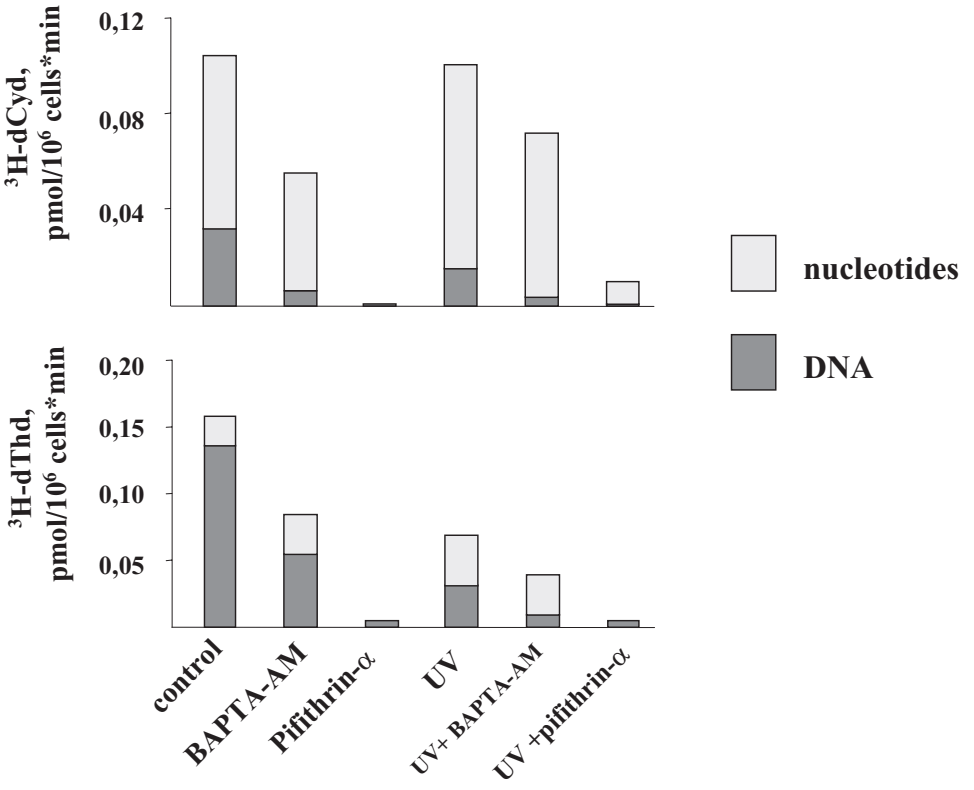

FIGURE 2 Effect of BAPTA-AM and pifithrin-α on deoxynucleoside metabolism in UV-irradiated lymphocytes. Cells were exposed to 100 J/m^2 UV-irradiation and subsequently labelled with 2 μCi/ml (0.08 μmol/l final concentration) tritiated dCyd or dThd for 30 minutes. Radioactivity was measured in ethanol-soluble (nucleotide) and ethanol-insoluble (DNA) fractions as desribed in Csapó et al.[2]

that BAPTA-AM but not pifithrin-alpha partially rescues the spontaneous inactivation of TK1, raising the possibility that reduced cytosolic calcium levels might stabilize the enzyme. This observation is in sharp contrast with the suggestion of O'Day and coworkers[6] who assumed that human TK1—based on sequence analogy—might be a Ca/calmodulin-activated enzyme.

In contrast to data obtained in CLL lymphocytes,[3] where replicative DNA synthesis does not occur, UV-irradiation of tonsillar lymphocytes significantly reduced the incorporation of labelled dCyd and dThd into DNA, while labelling of the nucleotide pool slightly increased (Figure 2). The rate of DNA synthesis was reduced further in the presence of BAPTA-AM that might be explained by the calcium dependence of some factors of DNA synthesis.[7]

Practically no labelling was detected either in the nucleotide or in the macromolecular fraction in the presence of pifithrin-α. Since pifithrin-α does not inhibit dCK or TK1 activities in vitro,[5] the possibility arises

that this non-specific p53 inhibitor might interfere with transmembrane nucleoside transport.

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