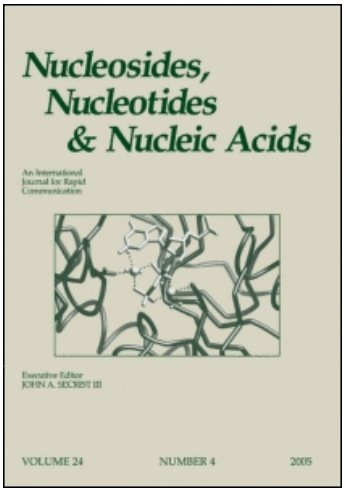

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Effects of 2-Chloro-2'-Deoxyadenosine on the Cell Cycle in the Human Leukemia EHEB Cell Line

S. Cardoen[a]; E. Van Den Neste[ab]; C. Smal[a]; J. F. Rosier[c]; A. Ferrant[b]; G. Van den Berghe[a]; F. Bontemps[a]
[a] Laboratory of Physiological Chemistry, Christian de Duve Institute of Cellular Pathology, Université Catholique de Louvain, Brussels, Belgium [b] Department of Hematology, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium [c] Tumoral Metabolism Research Unit, Centre Hospitalier de Jolimont-Lobbes, La Louvière, Belgium

Online publication date: 27 October 2004

To cite this Article Cardoen, S. , Neste, E. Van Den , Smal, C. , Rosier, J. F. , Ferrant, A. , Berghe, G. Van den and Bontemps, F.(2004) 'Effects of 2-Chloro-2'-Deoxyadenosine on the Cell Cycle in the Human Leukemia EHEB Cell Line', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1425 — 1429
To link to this Article: DOI: 10.1081/NCN-200027653
URL: http://dx.doi.org/10.1081/NCN-200027653

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1425–1429, 2004

Effects of 2-Chloro-2′-Deoxyadenosine on the Cell Cycle in the Human Leukemia EHEB Cell Line

S. Cardoen,[1,*] E. Van Den Neste,[1,2] C. Smal,[1] J. F. Rosier,[3] A. Ferrant,[2] G. Van den Berghe,[1] and F. Bontemps[1]

[1]Laboratory of Physiological Chemistry, Christian de Duve Institute of Cellular Pathology and [2]Department of Hematology, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium
[3]Tumoral Metabolism Research Unit, Centre Hospitalier de Jolimont-Lobbes, La Louvière, Belgium

ABSTRACT

To explain why 2-chloro-2′-deoxyadenosine (CdA) is unable to block DNA synthesis and cell cycle progression, and paradoxically enhances progression from G1 into S phase in the CdA-resistant leukemia EHEB cell line, we studied its metabolism and effects on proteins regulating the transition from G1 to S phase. A low deoxycytidine kinase activity and CdATP accumulation, and a lack of p21 induction despite p53 phosphorylation and accumulation may account for the inability of CdA to block the cell cycle. An alternative pathway involving pRb phosphorylation seems implicated in the CdA-induced increase in G1 to S phase progression.

Key Words: 2-Chloro-2′-deoxyadenosine; CdA resistance; G1/S transition; Cell cycle; pRb; B-CLL.

*Correspondence: S. Cardoen, Laboratory of Physiological Chemistry, Christian de Duve Institute of Cellular Pathology, Université Catholique de Louvain, B-1200 Brussels, Belgium.

DOI: 10.1081/NCN-200027653
1525-7770 (Print); 1532-2335 (Online)

INTRODUCTION

CdA, an analogue of the nucleoside 2′-deoxyadenosine, is used for the treatment of lymphoid malignancies, including chronic lymphocytic leukemia (CLL). To exert its antileukemic effects, CdA has to be phosphorylated by deoxycytidine kinase (dCK) into CdAMP and subsequently converted into CdADP and CdATP. CdATP can be incorporated into newly synthesised DNA in place of its natural homolog dATP, causing chain termination, inhibition of DNA synthesis and arrest of the cell cycle in S phase, which leads progressively to accumulation of cells at the G1/S border.[1] In previous work,[2] we observed that the EHEB cell line (CLL) was much less sensitive to CdA (IC_{50} at 96 h = 5 μM) than other leukemic cell lines like the CCRF-CEM cell line (IC_{50} at 24 h = 0.2 μM). We also observed that 10 μM CdA induced in EHEB cells a dose- and time-dependent increase in DNA synthesis, which was the consequence of a paradoxical increase in the number of cells in S phase, synthesizing DNA. The aims of this study were, firstly, to explain why CdA is unable to inhibit DNA synthesis and to arrest cell cycle progression in EHEB cells and, secondly, to investigate the reasons of the peculiar increase in cells in S phase.

MATERIALS AND METHODS

Activity of dCK was measured by incubating cell extracts with 50 μM [8-^{3}H] CdA as substrate, and by measuring its conversion into CdA nucleotides.[2] The intracellular accumulation of CdATP was measured after incubation of the cells with [8-^{3}H] CdA, followed by separation of the CdA nucleotides by HPLC.[2] Cell cycle analysis was performed by flow cytometry after the cells had been incubated without or with 10 μM CdA, pulse-labeled for 1 h with 10 μM BrdUrd allowing measurement of DNA synthesis, and DNA-marked with propidium iodide allowing measurement of DNA content. Effets of CdA on p53, p21 and pRb were measured by Western blot.

RESULTS AND DISCUSSION

Metabolism of CdA

Since cell lines deficient in dCK are resistant to the toxic effects of CdA,[3] we compared dCK activity in the EHEB and in the CCRF-CEM cell lines. At 50 μM CdA, activities were 90 ± 3 pmol/min/mg protein and 365 ± 27 pmol/min/mg protein, respectively. Western blot analysis revealed a lower amount of dCK protein in the EHEB than in the CCRF-CEM cells. EHEB cells incubated during 24 h with 10 μM CdA accumulated less CdATP (0.6 ± 0.07 μM) than CCRF-CEM cells (13 ± 2 μM). CdATP was also less incorporated into the DNA of EHEB than of CCRF-CEM cells, which could account for the inability of CdA to inhibit DNA synthesis and cell cycle progression in this cell line, since it has been shown that the cellular response is determined by the amount of drug incorporated into DNA.[4,5]

Effect of CdA on Progression in the Cell Cycle

In order to elucidate if the CdA-induced increase in the proportion of EHEB cells in S phase was due to an enhancement of entry into S phase or to a delay in progression therein, we performed kinetic and synchronisation experiments. Kinetic experiments revealed that the EHEB cells incubated with 10 μM CdA accumulated in early S phase after 4 h and in mid-late S phase after 16 h. Synchronisation experiments with serum deprivation, blocking the cells in G1 phase, revealed that cells incubated with CdA enter S-phase earlier than control cells. These results indicate that the accumulation of EHEB cells in S phase is due to a CdA-induced increase in the progression of the cells from G1 to S phase. These findings contrast with those observed in the CCRF-CEM cells in which 0.5 μM CdA inhibits DNA synthesis and blocks cell cycle progression with an accumulation of cells in S phase already after 4 h.

Effects of CdA on Proteins Regulating the G1/S Transition

To explain the effect of CdA on the progression of the EHEB cells from G1 to S phase, we investigated its effect on different proteins regulating the G1/S checkpoint (Fig. 1). CdA has been shown to induce p53 and p21 in chronic lymphocytic leukemia cells[6] and expression of some of these cell cycle regulating proteins has been shown to influence sensitivity of cancer cells to this drug.[7] Gemcitabine, another nucleoside analogue, misincorporated into DNA, has also been reported to be recognised in acute myeloid leukemia cells as a DNA damaging agent triggering a biochemical cascade leading to p53 phosphorylation on serine 15, accumulation and activation.[4]

We observed a time- and dose-dependent increase in the amount of p53 phosphorylation on serine 15 (Fig. 2a) as well as an increase in its total amount (Fig. 2b). This indicates that CdATP, despite its low misincorporation into DNA in

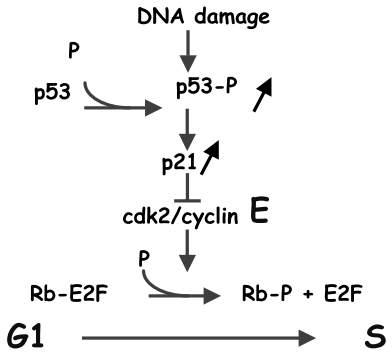

Figure 1. Control of the G1/S checkpoint. During the G1/S transition, the retinoblastoma (Rb) protein is phosphorylated on serine 795 by the cyclin-dependent kinase cdk2, which is associated with cyclin E. This allows its dissociation from the transcription factor E2F, which can then increase the transcription of genes necessary for S phase. When DNA is damaged, sensor kinases phosphorylate protein p53 on serine 15, which stabilises it and allows its accumulation. p53 increases the transcription of protein p21, which is an inhibitor of cdk2 able to block the cell cycle at the G1/S checkpoint. This block allows the cell to repair its DNA to avoid cancerisation.

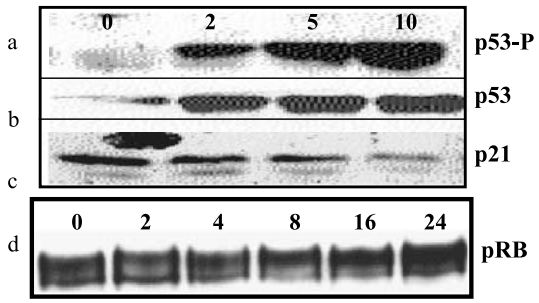

Figure 2. EHEB cells were incubated with CdA 0, 2, 5 and 10 μM during 24 h (a, b, c) or during increasing time intervals (0, 2, 4, 8, 16, 24 h) with 10 μM CdA (d).

EHEB cells, is recognised as a DNA damaging agent triggering p53 phosphorylation and accumulation. Contrary to expectation, the accumulated p53 protein failed to increase the amount of p21 (Fig. 2c), suggesting an abnormality in p21 induction. This defect of p21 induction could also account for the inability of CdA to block cell cycle progression.

CdA dose- and time-dependently increased the amount of hyperphosphorylated Rb (Fig. 2d) as well as the phosphorylation of Rb on serine 795 (not shown). This suggests that the CdA-induced increase in G1 to S phase progression involves phosphorylation of the Rb protein.

On the contrary, in CCRF-CEM cells, CdA time- and dose-dependently decreased Rb phosphorylation (not shown), in accordance with the inhibition of cell cycle progression observed by flow cytometry. Since the CCRF-CEM cells are p53-mutated, contrary to EHEB cells, and since we could not detect any p21 protein expression, we suggest that in the latter cell line, CdA blocks the cell cycle progression by a p53-independent pathway, possibly an intra-S phase checkpoint, as described for fludarabine.[8]

In conclusion, we show in EHEB cells a new mode of cellular response to CdA, involving modification of the cell cycle regulation leading to enhanced DNA synthesis. We propose that this peculiar effect might be implicated in some types of as yet unexplained resistance of leukemic cells to CdA.

REFERENCES

1. Huang, M.C.; Ashmum, R.A.; Avery, T.L.; Kuehl, M.; Blackley, R.L. Effects of cytotoxicity of 2-chloro-2′-deoxyadenosine and 2-bromo-2′-deoxyadenosine on cell growth, clonogenicity, DNA synthesis, and cell cycle kinetics. Cancer Res. **1986**, *46*, 2362–2368.
2. Cardoen, S.; Van Den Neste, E.; Smal, C.; Rosier, J.F.; Delacauw, A.; Ferrant, A.; Van den Berghe, G.; Bontemps, F. Resistance to 2-chloro-2′-deoxyadenosine of the human B-cell leukemia cell line EHEB. Clin. Cancer Res. **2001**, *7*, 3559–3566.
3. Carson, D.A.; Wasson, D.B.; Kaye, J.; Ullman, B.; Martin, D.W.; Robins, R.K.; Montgomery, J.A. Deoxycytidine kinase-mediated toxicity of deoxyadenosine

analogs toward malignant human lymphoblasts in vitro and toward murine L1210 leukemia in vivo. Proc. Natl. Acad. Sci. U. S. A. **1980**, *77*, 6865–6869.
4. Achanta, G.; Pelicano, H.; Feng, L.; Plunkett, W.; Huang, P. Interaction of p53 and DNA-PK in response to nucleoside analogues: potential role as a sensor complex for DNA damage. Cancer Res. **2001**, *16*, 8723–8729.
5. Shi, Z.; Azuma, A.; Sampath, D.; Li, Y.X.; Huang, P.; Plunkett, W. S-phase arrest by nucleoside analogues and abrogation of survival without cell cycle progression by 7-hydroxystaurosporine. Cancer Res. **2001**, *61*, 1065–1072.
6. Gartenhaus, R.B.; Wang, P.; Hoffman, M.; Janson, D.; Rai, K.R. The induction of p53 and WAF/CIP1 in chronic lymphocytic leukemia cells treated with 2-chlorodeoxyadenosine. J. Mol. Med. **1996**, *74*, 143–147.
7. Galmarini, C.M.; Voorzanger, N.; Falette, N.; Jordheim, L.; Cros, E.; Puisieux, A.; Dumontet, C. Influence of p53 and $p21^{WAF1}$ expression on sensitivity of cancer cells to cladribine. Biochem. Pharmacol. **2003**, *65*, 121–129.
8. Sampath, D.; Shi, Z.; Plunkett, W. Inhibition of cyclin-dependant kinase 2 by the Chk1-Cdc25A pathway during the S-phase checkpoint activated by fludarabine: dysregulation by 7-hydroxystaurosporine. Mol. Pharmacol. **2002**, *62*, 680–688.