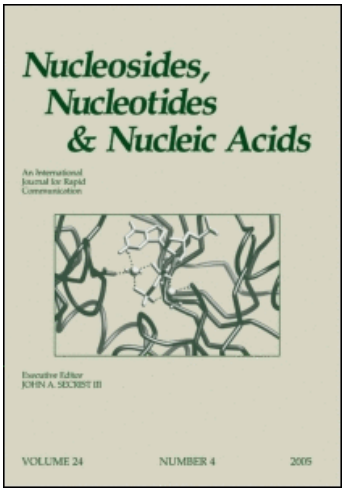

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STUDY OF THE EFFICACY OF A PRONUCLEOTIDE OF 2-CHLORO-2′-DEOXYADENOSINE IN DEOXYCYTIDINE KINASE-DEFICIENT LYMPHOMA CELLS

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□ *2-Chloro-2′-deoxyadenosine (CdA, cladribine) is a nucleoside analogue (NA) used for the treatment of lymphoproliferative disorders. Phosphorylation of the drug to CdAMP by deoxycytidine kinase (dCK) and its subsequent conversion to CdATP is essential for its efficacy. DCK deficiency is a common mechanism of resistance to NA, which could be overcome by the pronucleotide approach. The latter consists of using the nucleoside monophosphate conjugated to a lipophilic group enabling CdAMP to enter the cells by passive diffusion. In this study, we show that* cyclo*Saligenyl-2-chloro-2′-deoxyadenosine monophosphate (*cyclo*Sal-CdAMP) is 10-fold more potent that CdA in a dCK-deficient lymphoma cell line. These results suggest that the use of* cyclo*Sal-nucleotides could be a strategy to counteract resistance caused by dCK deficiency.*

Keywords 2-Chloro-2′-deoxyadenosine; NA resistance; *cyclo*Sal-pronucleotide.

INTRODUCTION

CdA, an analogue of 2′-deoxyadenosine, is used for the treatment of lymphoid malignancies, including hairy cell leukemia and chronic

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lymphocytic leukemia (CLL). To exert its antileukemic effects, CdA has to be phosphorylated by deoxycytidine kinase (dCK) into CdAMP and is subsequently converted into CdADP and CdATP, the latter being the active metabolite of CdA.[1] The step catalyzed by dCK is rate-limiting and critical for nucleoside analogue (NA) efficiency. Indeed, dCK activity has been shown to be decreased or absent in different cell lines resistant to NA. (For a review see Galamarini et al.[2]). One of the potential strategies for overcoming this common mechanism of resistance to NA is the prodrug approach, designed in attempts to release the nucleoside 5′-monophosphate directly into the cell. In this strategy, the polar nucleoside monophosphate is converted to a neutral lipophilic phosphate ester by linking protecting groups to the phosphate moiety.[3] In this study, we investigated the effectiveness of a pronucleotide of CdA, the *cyclo*Saligenyl(*cyclo*Sal)-2-chloro-2′-deoxyadenosine monophosphate (*cyclo*Sal-CdAMP) that incorporates unique phosphate ester bonds into saligenol, in a dCK-deficient lymphoma cell line. The cycloSal concept is one of several pronucleotide systems reported so far, but is the only approach in which a pronucleotide is cleaved successfully by a simple but selective chemical hydrolysis.[4]

MATERIALS AND METHODS

The RL-7 cell line was derived from a human follicular lymphoma. The resistant variant RL-G was produced by continuous exposure of the parental RL-7 cells to increasing concentrations of gemcitabine. Both cell lines were cultured in RPMI 1640 with Glutamax, containing 10% FCS and 2% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. *Cyclo*Sal-CdAMP (Figure 1) was synthesized in the Institute of Organic Chemistry (University of Hamburg).[4] Drug cytotoxicity was analysed by the MTT assay after 5 days of incubation. *Cyclo*Sal-CdAMP and its metabolites were measured by HPLC. DCK activity was measured with 10 μM [^{3}H]-deoxycytidine as the substrate.

RESULTS AND DISCUSSION

As reported by Galmarini et al.,[5] the RL-G cell line was resistant to the cytotoxic effect of deoxynucleoside analogues gemcitabine and cytarabine. These results were correlated to a very low dCK expression. In accordance, we measured a dCK activity of 0.53 pmol/min/mg protein in the RL-G cells versus an activity of 174.3 pmol/min/mg protein in the parental RL-7 cells. Low dCK activity in RL-G cells could predict cross-resistance to CdA since its activation is dCK dependent. Indeed, we observed that RL-G cells were approximately 1,100-fold more resistant to the cytotoxic effect of CdA

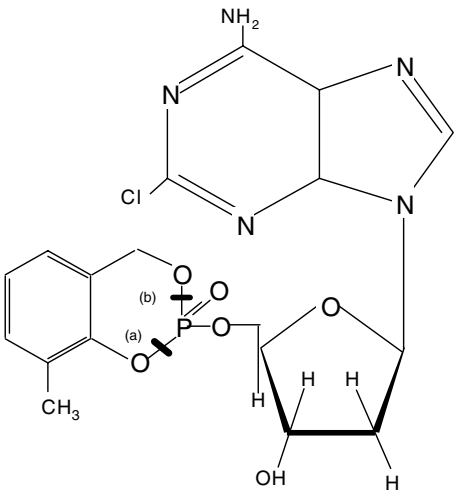

FIGURE 1 Structure of *Cyclo*Sal-CdAMP. The generation of the nucleoside-5′-monophosphate is initiated by selective hydrolysis of the phenolic ester bound (a). Subsequently, spontaneous cleavage of the benzylic phosphate ester bond (b) releases the nucleoside 5′-monophosphate and salicyl alcohol.

than the parental RL-7 cells (IC_{50} value of 87 versus 0.08 μM, respectively) (Figure 2).

Comparison of CdA with *cyclo*Sal-CdAMP showed that the parental RL-7 cells were slightly less sensitive to cycloSal-CdAMP than to CdA (0.20 versus 0.08 μM, respectively) (Figure 2A). This result, also observed in CCRF-CEM

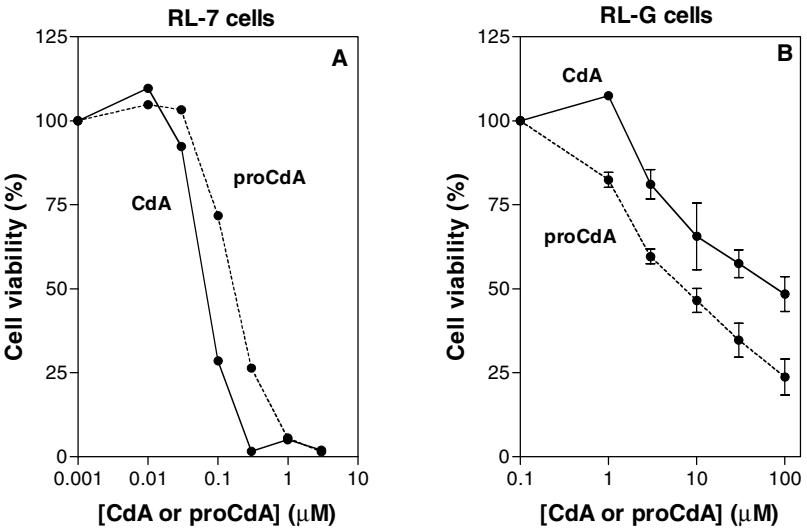

FIGURE 2 Comparative cytotoxicity of CdA and *cyclo*Sal-CdAMP (proCdA) in RL-7 (A) and RL-G cells (B). Cell viability was measured by the MTT assay after 5 days of incubation. Results shown for RL-G cells are means ± SEM of 3 separate experiments.

cells and in B-CLL lymphocytes (data not shown), was expected because cycloSal-CdAMP enters cells by passive diffusion whereas CdA rapidly enters cells via nucleoside transporters. In contrast, *cyclo*Sal-CdAMP was 10-fold more potent than CdA in dCK-deficient cells (8.2 versus 87 μM, respectively) (Figure 2B), which indicates that the pronucleotide is able to cross the plasma membrane and to deliver CdAMP intracellularly. Nevertheless, the efficacy of *cyclo*Sal-CdAMP in dCK-deficient cells is much lower than expected. This observation could be explained by the presence of other mechanisms contributing to the CdA resistance and/or by conversion of *cyclo*Sal-CdAMP into CdA in the incubation medium that cannot be activated in dCK-deficient cells. In accordance with the latter hypothesis, we observed that the pronucleotide of CdA became nearly undetectable in the medium after 48 h of incubation. Metabolite analysis in the medium demonstrated that the pronucleotide was converted spontaneously into CdAMP that subsequently was dephosphorylated into CdA by phosphatase/5′-nucleotidase present in FCS (data not shown). In conclusion, our data indicate that use of *cyclo*Sal-CdAMP could be a strategy to counteract resistance caused by dCK deficiency, but pronucleotide efficiency and/or stability still has to be improved.

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