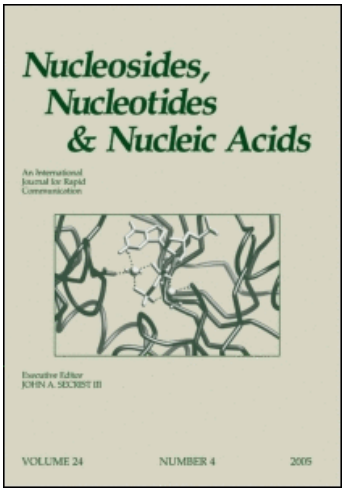

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Bjerke, M. , Franco, M. , Johansson, M. , Balzarini, J. and Karlsson, A.(2006) 'Alterations of Mitochondrial DNA in CEM Cells Selected for Resistance Toward DDC Toxicity', Nucleosides, Nucleotides and Nucleic Acids, 25: 9, 987 — 990
To link to this Article: DOI: 10.1080/15257770600889055
URL: http://dx.doi.org/10.1080/15257770600889055

Nucleosides, Nucleotides, and Nucleic Acids, 25:987–990, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600889055

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ALTERATIONS OF MITOCHONDRIAL DNA IN CEM CELLS SELECTED FOR RESISTANCE TOWARD DDC TOXICITY

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□ *2′,3′-dideoxycytidine (ddC) is a nucleoside analog that has been shown to produce a delayed toxicity which may be due to the depletion of mitochondrial DNA (mtDNA). In order to gain further understanding of the events involved in mitochondrial toxicity, two different CEM cell lines were selected for resistance to the delayed ddC toxicity.*

Keywords Nucleoside analog; 2′,3′-Dideoxycytidine; Mitochondrial DNA; Delayed toxicity

INTRODUCTION

2′,3′-dideoxycytidine (ddC) is an analog of the natural nucleoside 2′-deoxycytidine. It is initially phosphorylated by cytosolic deoxycytidine kinase (dCK) to the 5′-monophosphate derivative. ddC exerts its biological effects through its metabolite dideoxycytidine triphosphate (ddCTP) and is currently used in HIV-therapy.[1] The principal mode of action of ddC is through inhibition of DNA synthesis after incorporation of ddCTP into the replicating DNA strand. Treatment with ddC has been shown to produce a delayed toxicity which may be due to the depletion of mitochondrial DNA (mtDNA). This depletion is believed to be caused by incorporation of ddCTP into mtDNA and the inhibition of DNA polymerase γ, which is believed to be responsible for mtDNA synthesis.[2] In order to gain further

This work was supported by grants from the Swedish Cancer Society, the Swedish Research Council and the Medical Faculty of the Karolinska Institute.

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understanding of the events involved in mitochondrial toxicity, two different CEM cell lines were selected for resistance to the delayed ddC toxicity. The cells were then analyzed with regard to growth rate, levels of mtDNA, and growth response to ddC and other nucleoside analogs.

MATERIAL AND METHODS

Cell Culture

The CEM/wt and the two ddC resistant cell lines were cultured in RPMI 1640 medium supplemented with heat-inactivated 10% fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin in a humidified atmosphere with 5% CO_2 at 37°C. Cell culture media were renewed every 3–4 days.

Selection of ddC Resistant Cell

The CEM/wt cells were grown in RPMI medium for 50 days in the presence of 0.25 μM ddC. After the delayed toxicity the cell growth returned to normal and the resistant cells were selected. These cells (CEM/ddC1) were then grown in the presence of 1 μM ddC for 125 days and selected for the higher resistance (CEM/ddC2).

We used real time PCR to explore if mtDNA was altered in the resistant cells.[3]

RESULTS

CEM cells were grown at different subtoxic concentrations of ddC and were then selected for resistance to the delayed ddC toxicity. After 10–20 days, toxicity was apparent at certain ddC concentrations but, after further incubation, the cell growth returned to normal in cells grown at

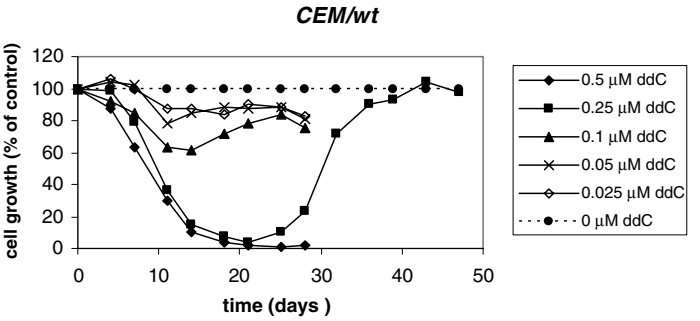

FIGURE 1 The selection of cells resistant to 0.25 μM ddC (CEM/ddC1) were made after 50 days of incubation.

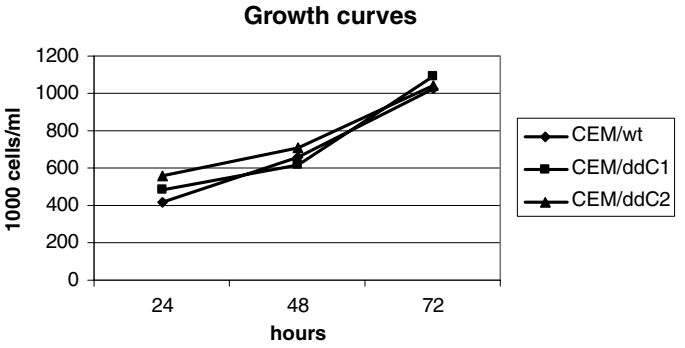

FIGURE 2 The growth curves of CEM/wt, CEM/ddC1, and CEM/ddC2 are all similar

0.25 μM ddC or less. The first cells to be characterized were the CEM cells selected for resistance towards 0.25 μM ddC (CEM/ddC1) (Figure 1). In a second experiment the CEM/ddC1 cells incubated were further at increasing concentrations of ddC resulting in a second cell line with resistance toward delayed toxicity of 1 μM ddC (CEM/ddC2) (data not shown).

The growth rates of the resistant cells were similar to the wt control (Figure 2).

When the cells were grown without the presence of any toxic compound, there was an increase in the level of mtDNA in the CEM/ddC1 compared to the wild type cells. An increase was also shown in the CEM/ddC2 cells although not as high as in the CEM/ddC1 (Figure 3).

The cytostatic activity of ddC and two other deoxycytidine analogs (araC and dFdC) were measured against the wt and ddC resistant CEM cells (Table 1). The sensitivity against araC and dFdC were similar for all the three cell lines. However, there was a 3-fold increase in the resistance toward the

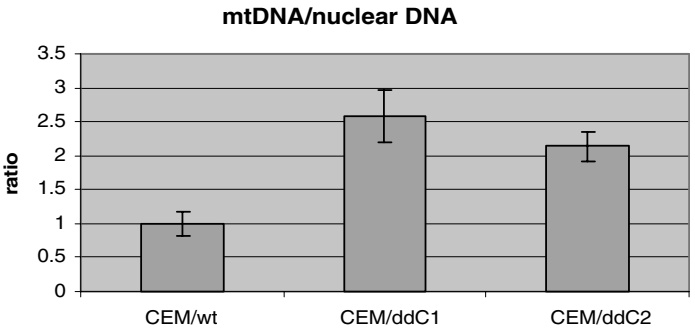

FIGURE 3 Relative mtDNA content in the wt and ddC resistant cell lines. The data represent the ratio of the mean mtDNA value to the mean nuclear DNA value for a given extract. The ratios are expressed relative to the value obtained for the wt.

TABLE 1 Cytostatic Activity of Test Compounds Against Mutant CEM Cell Lines (AraC = 1-β-D-Arabinofuranosylcytosine; dFdC = 2′, 2′-Difluorodeoxycytidine)

	IC_{50} (μM)		
	CEM/0	CEM/ddC–0.25 μM	CEM/ddC–1.0 μM
ddCyd	3.2±0.6	9.1±0.9	12±7
araC	0.035±0.006	0.036±0.008	0.042±0.019
dFdC	0.14	0.07 ± 0.07	0.084±0.009

ddC in the CEM/ddC1 cells compared to the wt cells. Also the CEM/ddC2 cells showed an increase in comparison to the wt.

DISCUSSION

In the present study CEM cells have been selected for resistance toward the delayed toxicity caused by ddC. The cells kept their sensitivity to the anti-HIV activity of ddC indicating that the activation of ddC to its triphosphate derivative is unchanged in the resistant cells. Accordingly, the cells kept their sensitivity toward different cytotoxic dCyd analogs phosphorylated by dCK. Thus, the mechanism of resistance did not involve any of the enzymes phosphorylating ddC to its active triphosphate form. The resistant cells did not show any alterations in growth rate and they were able to infect HIV in a similar way as the wild type control cells. Among the different possibilities to obtain resistance toward mitochondrial toxicity are compensatory changes in mitochondrial DNA levels and alterations in mitochondrial transport of ddC or its phosphorylated derivatives. The ddC resistant cells in this study were found to have an increased level of mitochondrial DNA, and we conclude that this alteration in the ratio between mitochondrial and nuclear DNA is at least one of the mechanisms for the decreased sensitivity of the toxic effects of ddC on the mitochondrial DNA.

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