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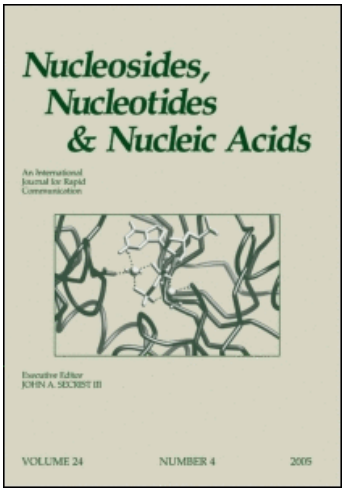

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Reduced Levels of Mitochondrial DNA Increases the Toxicity of 9-ß-D-Arabinofuranosylguanine (araG)

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REDUCED LEVELS OF MITOCHONDRIAL DNA INCREASES THE TOXICITY OF 9-β-D-ARABINOFURANOSYLGUANINE (araG)

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□ *Incubation of cells with thymidine (dThd) is known to cause dNTP pool imbalance as well as deletions and depletion of the mtDNA. In order to gain further understanding in the events involved in dThd toxicity over time, H9 cells were cultured for 20 months in the presence or absence of 1μM dThd. The level of mtDNA was reduced by 90% in the cells grown in dThd as compared to the control cells. The H9/dThd cells also showed a 100-fold increased sensitivity towards the cytotoxicity of the antileukemic compound 9-β-D-arabinofuranolsylguanine (araG).*

Keywords Mitochondrial DNA; thymidine; araG; H9 cells

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INTRODUCTION

Thymidine (dThd) is a deoxyribonucleoside needed for DNA synthesis. It is a natural substrate of the cytosolic thymidine kinase 1 (TK1) and the mitochondrial thymidine kinase 2 (TK2). dThd is known to be toxic to mammalian cells by accumulating intracellularly as dTTP.[1] High levels of dThd can lead to depletion, deletion, and point mutations in mitochondrial DNA (mtDNA).[2] Incubation of cells in the presence of dThd causes a dNTP pool imbalance that can affect both DNA replication and repair. AraG is an antileukemic compound that is a substrate of mitochondrial deoxyguanosine kinase and previous studies have shown incorporation of araG into mtDNA.[3] In order to further study the contribution of mitochondria and mitochondrial enzymes to the activation of nucleoside analogs, the T-lymphoblastoid cell line H9 was grown for 20 months in the presence of 1 μM dThd. The cells were analyzed with regard to levels of mtDNA and cytostatic activity of araG and other nucleoside analogs.

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MATERIALS AND METHODS

Cell Culture

The H9 cells were cultured in RPMI-1640 medium supplemented with heat-inactivated 10% fetal bovine serum(Gibco, UK), 100 U/ml penicillin and 0.1 mg/ml streptomycin(Gibco, UK) in a humidified atmosphere with 5% CO_2 at 37°C. Cell culture media were renewed every three to four days. Every two days 1 μM dThd was added to the medium (H9/dThd).

Quantification of mtDNA

4×10^6 logarithmically growing cells from each cell line were harvested. Genomic DNA was extracted using the Easy DNA extraction kit (Invitrogen). The samples were then quantified by real-time quantitative PCR, as described previously.[4]

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Cell Proliferation Assay

Approximately 2.5×10^5 – cells $\times$ mL^{-1} were seeded in 200 μL-wells of 96-well microtiter plates in the presence of serial 5-fold dilutions of the test compounds starting at 500 μM. The cells were then allowed to proliferate at 37°C for 72 hours. After this time period, control cells (in the absence of test compounds) were almost at the end of the exponential growth phase. The cell number was determined by use of a Coulter counter type ZM (Coulter Electronics, USA). Each sample was measured in triplicates.

RESULTS

The H9 cells were grown in the presence of 1 μM dThd for 20 months. The growth rate of the wildtype H9/wt and H9/dThd cells were similar (data not shown). When the cells were grown in the presence of dThd there was a 10-fold decrease in the level of mtDNA in the H9/dThd over time compared to the wildtype cells (Figure 1).

The cytostatic activity of dThd, 2′-3′-dideoxycytidine (ddC), 9-β-D arabinofuranosyladenine (araA) and araG was measured in the H9/wt and H9/dThd cells (Table 1). A 5- to 10-fold increase in cytotoxicity was found in the H9/dThd cells for all the tested nucleoside analogs as compared to the control cells. The most pronounced difference between the cell lines was the 100-fold increase in araG sensitivity in the H9/dThd cells compared to the wildtype cells.

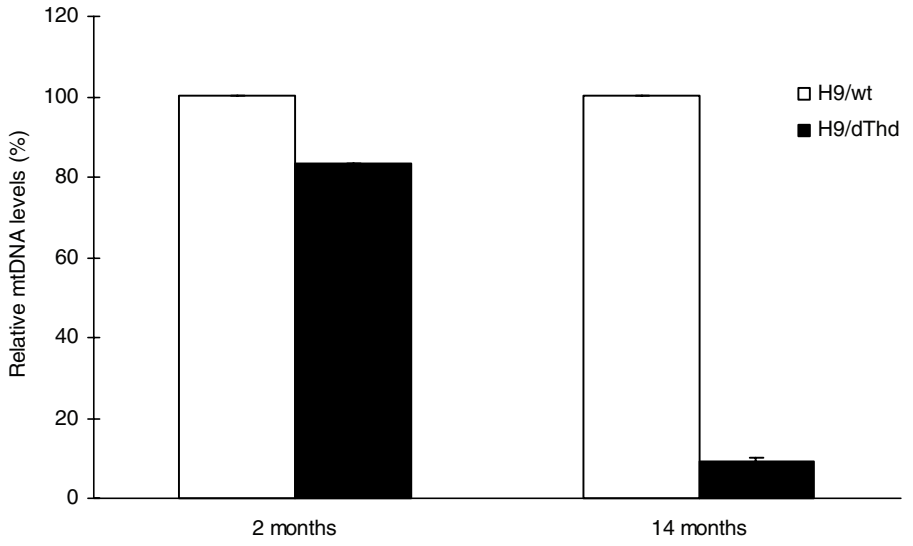

FIGURE 1 The relative mtDNA content in H9/wt and H9/dThd cell lines. The mtDNA quantification was made by TaqMan Real-time PCR. The data represent the percentage of the mean mtDNA value to the mean nuclear DNA value for a given extract. The percentages are expressed relative to the value obtained for the H9/wt control cells (designated 100%). Each sample was measured four times in two different experiments and the mean and sd are shown.

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DISCUSSION

In the present study, H9 cells were cultured in a low concentration of dThd for almost 2 years. The H9/dThd cells did not show any alterations in growth rate or apparent morphological changes as compared to the wildtype cells although the mtDNA levels were dramatically reduced. The function of the mitochondria clearly was sufficient to support the major cellular processes as judged by the unaltered viability of the H9/dThd cells. The most pronounced difference found in the H9/dThd cells was a hundred fold

TABLE 1 Cytostatic activity

	IC_{50} (μM)	
	H9/wt	H9/dThd
dThd	>500	45 ± 6
ddC	51 ± 1	6.0 ± 1.1
araA	250 ± 129	42 ± 4
araG	284 ± 40	3.0 ± 1.4

Cytostatic activity of test compounds against H9/wt and H9/dThd cell lines (dThd; deoxythymidine, ddC; 2′,3′-dideoxycytidine, araA; 9-β-D-arabinofuranosyladenine, araG; 9-β-D-arabinofuranosylguanine). Values represents the mean IC_{50} (μM) ± sd of triplicate measurements.

increased sensitivity towards araG as compared to H9/wt. Although araG has been shown to be incorporated into mtDNA it does not cause mitochondrial toxicity. It has however been demonstrated that the cytotoxicity of araG is caused by incorporation into nuclear DNA.[3] The sensitivity toward ddC and araA was found to be increased by 10- and 5-fold, respectively, in the H9/dThd cells compared to the H9/wt. ddC is a substrate for dCK and TK2 and causes cellular toxicity by incorporation into mtDNA. It has a low affinity for nuclear DNA polymerases. AraA on the other hand is not a substrate of any of the human deoxynucleoside kinases and little is known of the mechanism behind the toxicity of this compound.[5] AraA has mainly been studied as an anti-herpes-simplex virus agent.

A possible explanation of our findings is that less mtDNA may result in an increased incorporation of araG in nuclear DNA, thereby also increasing the cellular toxicity. Further studies are ongoing to elucidate how low levels of mtDNA in the H9/dThd cells affect araG toxicity.

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