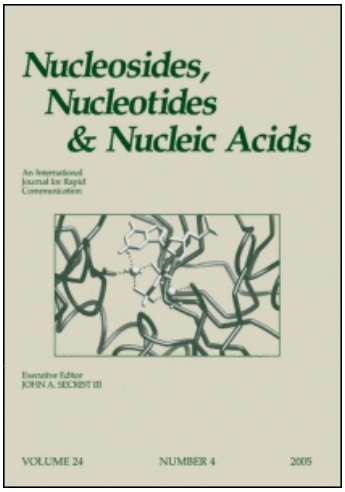

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5-Fluoro-2'-Deoxyuridine Has Effects on Mitochondria in CEM T-Lymphoblast Cells

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5-Fluoro-2′-Deoxyuridine Has Effects on Mitochondria in CEM T-Lymphoblast Cells

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ABSTRACT

Fluoropyrimidines are useful anticancer agents and the compound 5-fluoro-2′-deoxyuridine (FdUrd) plays an important role in chemotherapy of colon cancers. Several nucleoside analogs, such as 3′-azido-2′,3′-dideoxythymidine (AZT) and 2′,3′-dideoxycytidine (ddC), can be incorporated into and cause depletion of mitochondrial DNA (mtDNA). These drugs are known to cause mitochondrial toxicity after prolonged treatment in patients. In this study we demonstrate that FdUrd reduces the mtDNA content and the expression level of the mtDNA encoded cytochrome c oxidase (COX II) in a CEM T-lymphoblastic cell line.

Key Words: Fluorodeoxyuridine; Fluoropyrimidine; Metabolism; Mitochondrial DNA; Mitochondrial toxicity.

INTRODUCTION

Several nucleoside analogs interfere with mtDNA replication, and some adverse effects of these compounds are correlated to mitochondrial dysfunction. The adverse effects caused by these compounds range from mild reversible myopathy and

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neuropathy to multi-organ failure and death. FdUrd is a drug of choice in treatment of colon cancer but some patients given FdUrd display side effects such as neurotoxicity. The principal mode of FdUrd action is through inhibition of thymidylate synthase by its metabolite FdUMP, and thereby interfering with DNA synthesis. However, FdUTP may also be incorporated into DNA and FUTP may be incorporated into RNA, and thereby causing disruption of RNA processing and splicing, mRNA translation, DNA chain elongation and synthesis. FdUrd can be anabolized to FdUMP by cytosolic thymidine kinase 1 (TK1), but also by the mitochondrial thymidine kinase 2 (TK2). In contrast to TK1, which is only active during the S-phase of the cell cycle, TK2 is active also in terminally differentiated non-dividing cells. Since the role of mitochondrial enzymes in mitochondrial toxicity is not fully understood it is important to learn more about it. FdUrd has previously been shown to alter the mitochondrial morphology[1] and in this study we therefore investigated the effects of FdUrd on mtDNA content and expression of mtDNA encoded protein.

MATERIALS AND METHODS

Cell Culture

CEM T-lymphoblastic cells (ATCC) were cultured in RPMI medium supplemented with 10 % foetal calf serum (Gibco BRL), 100 U/ml penicillin, 0.1 mg/ml streptomycin, 4.5 mg/ml glucose, 110 μg/ml sodium pyruvate and 50 μg/ml uridine.

Quantification of mtDNA

CEM cells were grown in the presence or absence of 0.25 mg/ml ethidium bromide (EtBr), 1 μM ddC, 3nM FdUrd or 0.1 μM 9-β-D-arabinofuranosylguanine (araG), concentrations approximately ten times lower than the IC_{50}-values. Fresh drugs were added every two days. 5×10^4 cells were harvested after two, four or six days and subjected to slot blot, as described previously.[2] After hybridization to a heat-denatured mitochondrial DNA probe[3] the blots were subjected to IP-autoradiograpy.

Quantification of Cytochrome c Oxidase Subunit II (COX II) Expression

CEM cells were cultured in the absence or presence of EtBr, ddC, FdUrd or araG as described above. Crude protein cell extracts were prepared and 30 μg of the extracts were separated by 4–12% NuPAGE Bis-Tris gel electrophoresis (Invitrogen) and blotted onto PVDF membranes (Amersham-Pharmacia). The membranes were immunodetected following the instructions for PVDF membranes, using a primary antibody against COX subunit II (Molecular Probes) and a secondary alkaline phosphatase conjugate anti-rabbit IgG antibody (Sigma). The alkaline phosphatase immobilized on the membranes was visualized by incubation with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (Boehringer Mannheim).

Table 1. MtDNA content in CEM cells after treatment with FdUrd, as determined by slot-blot analysis.

	MtDNA content [% compared to control]		
Compound	2 Days	4 Days	6 Days
araG	131 ± 13	95 ± 19	112 ± 64
EtBr	23 ± 7.3	5.2 ± 3.6	3.6 ± 1.1
ddC	39 ± 13	14 ± 3.0	5.9 ± 1.1
FdUrd	129 ± 8.1	56 ± 7.3	17 ± 10

EtBr and ddC are known to cause mtDNA depletion and were included as positive controls and araG served as a negative control. The data are shown as % mtDNA content in treated compared to untreated cells (100%) and represent the means ± SD of three independent experiments.

RESULTS

FdUrd has been shown to change the mitochondrial morphology in cell culture, and patients being treated with FdUrd experience neurotoxicity, a common symptom in mitochondrial toxicity. We therefore decided to quantify the amount of mtDNA in CEM cells after incubation with FdUrd. EtBr and ddC are known to deplete mtDNA and were included in the study as positive controls. We have recently shown that araG has no acute effect on mtDNA content in CEM cells[2] and araG therefore served as a negative control. In contrast to araG which had no effect on the content of mtDNA, the cells incubated with subtoxic concentrations of EtBr or ddC as well as the drug of investigation, FdUrd, displayed decrease in their mtDNA contents (Table 1).

Since FdUrd depleted the mtDNA in the CEM cells we also measured the expression of the mtDNA encoded cytochrome c oxidase subunit II (COX II) following exposure to FdUrd. After six days of FdUrd exposure the expression of COX II decreased to ≈30%, a somewhat delayed effect in comparison to EtBr and ddC where

Table 2. Expression of the mtDNA encoded protein cytochrome c oxidase subunit II (COX II) after exposure to araG (negative control), EtBr, ddC (positive controls) or FdUrd.

	Expression of COX II [% compared to control]		
Compound	2 Days	4 Days	6 Days
araG	86	119	120
EtBr	23	5.8	2.2
ddC	62	10	8.2
FdUrd	91	67	33

The results are shown as % COX II expression in treated compared to untreated control cells.

prominent decreases in COX II expression were detected already after two and four days, respectively (Table 2).

DISCUSSION

In this study we have shown that subtoxic concentrations of FdUrd depletes the mtDNA and decreases the expression of COX II in CEM cells. The role of the mitochondrial nucleoside kinases for mitochondrial toxicity is not clear although there is evidence that they contribute to the mtDNA precursor synthesis.[4] A mitochondrial nucleotide carrier was recently identified[5] and accordingly nucleotides can be synthesized in the cytoplasm and then imported into the mitochondria. The mitochondrial location of TK2 has previously been suggested as a determinant of the mitochondrial toxicity of AZT and the uridine nucleoside analog FIAU.[6] Since FdUrd is a substrate of TK2 it is possible that TK2 is involved in delayed toxicity of FdUrd as well. FdUrd is also a substrate of thymidine phosphorylase, which catabolizes it to 5-fluorouracil (FU). FU is in turn partially converted to fluoro-β-alanine, which also displays neurotoxicity in patients. We have shown that FdUrd affects mtDNA and therefore it could be useful as a model drug to study effects on mtDNA and possibly also for studying the role of TK2 for toxicity in resting cells. Furthermore, since we have shown that FdUrd has effects on mtDNA it is possible that it could cause delayed mitochondrial toxicity in patients.

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