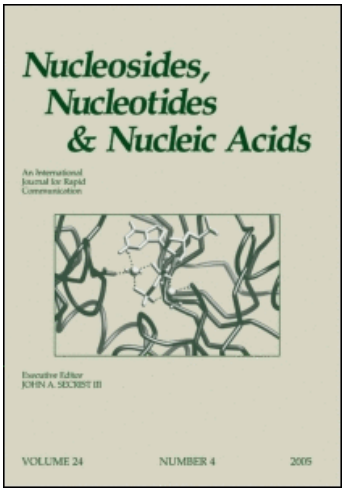

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Cellular Resistance Against Troxacitabine in Human Cell Lines and Pediatric Patient Acute Myeloid Leukemia Blast Cells

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To cite this Article Adema, A. D. , Zuurbier, L. , Floor, K. , Hubeek, I. , Kaspers, G. J. L. , Albertoni, F. and Peters, G. J.(2006) 'Cellular Resistance Against Troxacitabine in Human Cell Lines and Pediatric Patient Acute Myeloid Leukemia Blast Cells', Nucleosides, Nucleotides and Nucleic Acids, 25: 9, 981 — 986
To link to this Article: DOI: 10.1080/15257770600889212
URL: http://dx.doi.org/10.1080/15257770600889212

Nucleosides, Nucleotides, and Nucleic Acids, 25:981–986, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600889212

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CELLULAR RESISTANCE AGAINST TROXACITABINE IN HUMAN CELL LINES AND PEDIATRIC PATIENT ACUTE MYELOID LEUKEMIA BLAST CELLS

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□ *Troxacitabine is a cytotoxic deoxycytidine analogue with an unnatural L-configuration, which is activated by deoxycytidine kinase (dCK). The configuration is responsible for differences in the uptake and metabolism of troxacitabine compared to other deoxynucleoside analogues. To determine whether troxacitabine has an advantage over other nucleoside analogues several cell lines resistant to cladribine and gemcitabine were exposed to troxacitabine, while blast cells from pediatric leukemia patients were tested for cross-resistance with other deoxynucleoside analogues. The gemcitabine resistant AG6000 (IC_{50}: >3000 nM), and the cladribine resistant CEM (IC_{50}: 150 nM) and HL-60 (IC_{50}: >3000 nM) cell lines, all with no or decreased dCK expression, were less sensitive to troxacitabine than their wild type counterparts (IC_{50}; A2780: 410, CEM: 71 and HL-60: 158 nM). dCK protein expression in CEM was higher than in HL-60, which, in turn, was higher than in A2780. Catalytically inactive p53 seems to increase the sensitivity to troxacitabine. The patient samples showed a large range of sensitivity to troxacitabine, similar to other deoxynucleoside analogues. Cross-resistance with all other deoxynucleoside analogues was observed.*

Keywords Troxacitabine; Deoxycytidine kinase; Cross-resistance; Deoxynucleoside analogues; Leukemia

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INTRODUCTION

Troxacitabine is a cytotoxic deoxycytidine analogue with an unnatural L-configuration (see Figure 1). This configuration is responsible for differences in the uptake and metabolism of troxacitabine compared to other deoxynucleoside analogues.[1] In contrast to other deoxynucleoside analogues, the influx of troxacitabine into the cell might not be mediated by the human equilibrative nucleoside transporter (hENT) and human concentrative nucleoside transporter (hCNT) and might (partially) enter the cell by passive diffusion.[2] Because of the stereospecificity of cytidine deaminase (CDA), troxacitabine cannot be inactivated by deamination.[1] Like gemcitabine and cytarabine, troxacitabine needs to be phosphorylated to its monophosphorylated form by deoxycytidine kinase (dCK) thereby making this the rate-limiting step in the intracellular activation of troxacitabine.[1] Due to the lack of the hydroxyl group in the sugar ring, incorporation of troxacitabine into DNA leads to an immediate chain termination.[3] Damage introduced by troxacitabine is repaired by apurinic/apyrimidinic endonuclease (APE1).[4,5]

The aim of this study was to determine whether troxacitabine has a potential advantage over other deoxynucleoside analogues. We used various cell lines with a different sensitivity to other deoxynucleoside analogues to investigate potential resistance factors in troxacitabine resistance. In addition, in vitro sensitivity to troxacitabine was determined in the leukemia blast cells of 20 pediatric acute myeloid leukemia (AML) patients. Cross-resistance patterns with other deoxynucleoside analogues and non-nucleoside cytotoxic drugs were studied.

MATERIALS AND METHODS

In leukemic cell lines growth inhibition by troxacitabine in vitro was studied using the MTT cytotoxicity assay,[6,7] on wild type and cladribine resistant HL-60 and CEM human leukemic cell lines.[8] The IC_{50} value of the

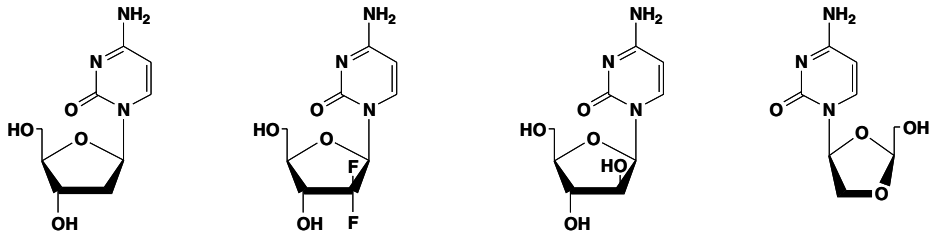

FIGURE 1 Structures of deoxycytidine and the analogues gemcitabine, cytarabine, and troxacitabine.

drug was determined in the different cell lines by interpolating the growth inhibition curves.

In solid tumor cell lines the role of dCK was studied using the SRB cytotoxicity assay on the A2780 and the gemcitabine resistant, dCK negative AG6000 cell lines.[9] The influence of p53 on troxacitabine induced growth inhibition was tested using the SRB cytotoxicity assay on the Lovo-B2 (empty vector transfectant), Lovo-Li (inactive p53) and the Lovo 175×2 (mutant p53) cell lines.[10,11] In these cell lines the protein levels of dCK and APE1 were determined with Western blotting.

Potential cross-resistance with other deoxynucleoside analogues was studied using the MTT cytotoxicity assay on bone marrow and/or peripheral blood samples from untreated children diagnosed with de novo AML.[12] Blast cells from patients were obtained after informed consent. Cross-resistance was determined to the deoxynucleoside analogues: cytarabine, cladribine (CdA), decitabine, fludarabine, thioguanine, gemcitabine, and other cytostatic drugs such as etoposide and daunorubin.

RESULTS AND DISCUSSION

Table 1 summarizes the sensitivity data of the different cell lines to troxacitabine. In order to determine whether the differences in sensitivity for troxacitabine were related with the dCK and APE1 expression we performed Western blotting (see Figure 2).

As expected the cytotoxicity and Western blot data show that the sensitivity of cell lines for troxacitabine is related to the expression of dCK. Loss of dCK protein expression results in a marked reduction in sensitivity to troxacitabine. As phosphorylation by dCK is also the rate-limiting step in the activation of other deoxynucleoside analogues commonly used in the treatment of leukemia, troxacitabine does not give an advantage compared to other deoxynucleoside analogues in view of dCK. The repair protein APE1 did not show differences and was thus not related to sensitivity to troxacitabine in the cell lines used in the experiments. The Lovo-Li cell line with inactive p53 was more sensitive to troxacitabine, while the Lovo 175×2

TABLE 1 IC_{50} Values (nM) of Troxacitabine Obtained with the Chemosensitivity Assays

Cell line	$IC_{50} \pm SEM$[1]	Cell line	$IC_{50} \pm SEM$[a]
CEM	71 ± 7	Lovo-B2	1917 ± 375
CEM-CdA	150 ± 50	Lovo-Li	750 ± 144
HL-60	158 ± 28	Lovo 175×2	2111 ± 365
HL-60-CdA	>3000		
A2780	410 ± 93		
AG6000	>3000		

[a]Highest concentration used 3000 nM. Experiments were performed at least 3 times.

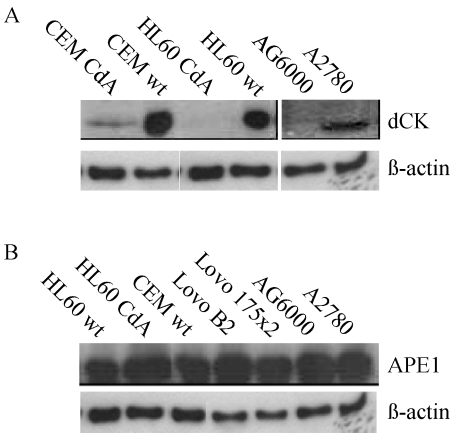

FIGURE 2 Protein expression of [a]dCK and [b]APE1 by Western blot analysis. Loading was checked with β-actin.

cell line with mutated p53 did not show a difference in sensitivity. This may indicate that cells with inactive p53 do not get arrested in the cell cycle after exposure to troxacitabine and thereby accumulate DNA damage faster.

Based on pilot experiments 6 concentrations of troxacitabine were chosen (0.0977–100 μM; 4 step dilution) to test samples from AML patients. Subsequently, samples from 20 patients for which the in vitro cytarabine sensitivity (LC_{50} value) had already been determined were selected.[13,14] On these samples the MTT-assay was performed, 19 out of 20 patient samples were tested successfully. One assay failure was caused by an insufficient number of blast cells after 4 days of culture. A large variation was seen in LC_{50} values, the median LC_{50} value for troxacitabine was 59.2 μM, with the 25th and 75th percentile being 3.9 and 82.6 μM, respectively. Although the concentration range was sufficient in pilot experiments we were unable to determine an LC_{50} value for troxacitabine in 5 out of 19 samples (4 samples >100 μM, and 1 < 0.0977 μM). The LC_{50} values obtained for troxacitabine were correlated with the LC_{50} values of the other cytotoxic drugs (see Table 2).

Cross-resistance of troxacitabine with all other cytotoxic drugs was observed in AML patient samples. Troxacitabine does not appear to circumvent in vitro resistance to dCK activated deoxynucleoside analogues, non-dCK activated anti-metabolites and nonnucleoside cytotoxic drugs indicating that downstream factors like DNA repair and apoptosis pathways might be involved.[14] This also is indicated by the similar sensitivity between CEM-CdA and HL-60 (see Table 1), while there is a large difference in dCK expression (see Figure 2).

These results show that both in AML patient samples and in tumor cell lines troxacitabine is not able to circumvent acquired dCK related resistance to other deoxynucleoside analogues. Since dCK does not seem to be a

TABLE 2 Cross-Resistance Between Troxacitabine, Deoxynucleoside Analogues, and Other Cytotoxic Drugs in Leukemic Blast Cells

Drug	Rho[a]	N
Cytarabine	0.76	16
Cladribine	0.83	12
Decitabine	0.75	13
Gemcitabine	0.85	13
Fludarabine	0.74	13
Etoposide	0.72	15
Daunorubicin	0.78	16
Thioguanine	0.71	16

[a]Spearman rho test (2-tailed), $p < 0.01$.

limiting factor in the sensitivity of AML to deoxynucleoside analogues,[15] the drug seems promising for future development in leukemia. Troxacitabine also can bypass resistance due to limited transport, a resistance parameter established in leukemia.[16] Because antitumor activity has already been described,[17] clinical studies are warranted.

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