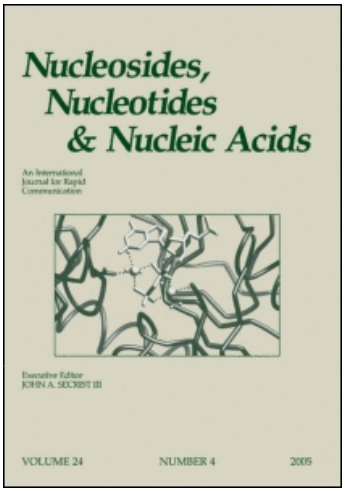

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EFFECTS OF *CYCLO*SAL-D4TMP DERIVATIVES IN H9 CELLS WITH INDUCED AZT RESISTANCE PHENOTYPE

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ABSTRACT: Cytotoxic and antiretroviral activity of *cyclo*Sal-d4TMP derivatives were tested in a new AZT-resistant H9 cell subline ($H9^rAZT^{250}$). The results showed, that *cyclo*Sal-d4TMP derivatives overcame resistance of HIV-1 to d4T in $H9^rAZT^{250}$ cells, which exert decreased thymidine kinase (TK) gene expression.

For inhibition of HIV-1 reverse transcriptase (RT) antiretroviral active 2',3'-dideoxynucleosides such as zidovudine (AZT) or stavudine (d4T) need to be activated intracellularly to their corresponding triphosphate[1]. Defective enzymatic phosphorylation of nucleoside analogs, may reduce antiretroviral efficiency in cells[2]. Nucleoside analog monophosphate prodrugs are an alternative strategy for overcoming cellular resistance mechanisms, such as decreased catalytic activity of first nucleoside kinases[3-5]. The aim of our study was to investigate antiretroviral activity of different *cyclo*Sal-d4T monophosphate prodrugs, which were designed to deliver the monophosphate form intracellularly, in $H9^rAZT^{250}$ TK deficient cells.

Antiviral agents: *Cyclo*Sal-d4TMP derivatives (FIG. 1) were synthesized as reported previously[6]. The drugs were dissolved in dimethylsulfoxide at a concentration of 10 mM and stored at –20°C. **Selection of AZT-resistant cell line:** AZT-resistant cell subline was established by the continuous cultivation of H9 cells in IMDM containing increasing

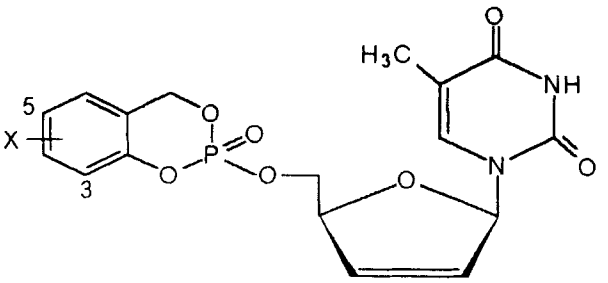

FIG.1: *Cyclo*Sal-d4TMP prodrugs
(x = H, Me, 3.5-Di-Me)

concentrations of AZT. The cell subline resistant against 250 μM AZT, designated $H9^rAZT^{250}$, was used in these experiments. **Determination of cytotoxicity:** Cytotoxic effects of *cyclo*Sal-d4TMP prodrugs were determined by MTT assay[7] as described previously[8]. **Antiretroviral assay:** Antiretroviral activity of different drugs was determined by the reduction of HIV-1 p24 antigen in cell culture supernatant using an ELISA test system (NEN Life Science Products, Boston, UK) as described previously[8]. **Determination of TK gene expression by RT-PCR:** RT-PCR was performed as described previously[8]. For the amplification of a region out of the TK mRNA, following primers were used: TK:5'-CAG GAT CCT CGG GTT CGT GAA C-3', TK2: 5'-TAG AAT TCG GCC CTT GCA GGT C-3'[9].

Continuous cultivation of T-lymphoid $H9^rAZT^{250}$ cells, in the presence of 250 μM AZT, resulted in five-fold lower expression of TK gene in comparison to parental cells (FIG. 2). Cytotoxicity of d4T and different *cyclo*Sal-d4TMP derivatives was comparable in parental as well as in $H9^rAZT^{250}$ cells, whereas AZT was toxic in parental H9 cells and has no cytotoxic effects in $H9^rAZT^{250}$ cells (TABLE 1). The very high EC_{50} and RI values of AZT and d4T in AZT-resistant $H9^rAZT^{250}$ cell subline showed no antiretroviral effects (TABLE 1). *Cyclo*Sal-d4TMP prodrugs overcame resistance mechanisms as demonstrated by significant antiretroviral activity in AZT-resistant $H9^rAZT^{250}$ cells.

In our study we found that *cyclo*Sal-d4TMP prodrugs are able to bypass TK-deficiency in $H9^rAZT^{250}$ cells and showed antiretroviral activity in parental H9 cells as well as in $H9^rAZT^{250}$ cells. However, cytotoxicity of d4T was comparable in AZT-resistant and parental cells. These findings pointed out, that HIV-1 infected cells may

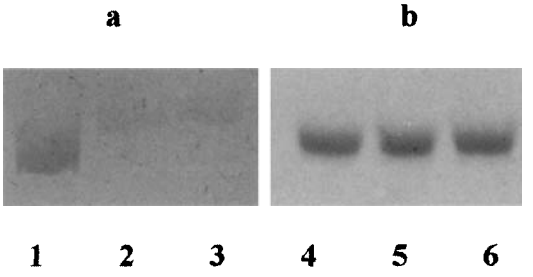

FIG.2: Specific PCR products from cDNA of TK-mRNA (765 bp) (a) and GAPDH-mRNA (126 bp) (b) separated by polyacrylamide gel electrophoresis. Lane 1,4: H9; lane 2-3, 5-6: $H9^rAZT^{250}$

TABLE 1 Cytotoxicity and anti-HIV-1 activity of different *cyclo*Sal-d4TMP derivatives in parental H9 and AZT-resistant $H9^rAZT^{250}$ cells

Drug	CC_{50}[a] [μM]		RI[b]	EC_{50}[a] [μM]		RI[b]
	H9	$H9^rAZT^{250}$		H9	$H9^rAZT^{250}$	
AZT	54.2 ± 8.8	> 2000	> 37	0.04 ± 0.05	> 100	> 2500
d4T	354.2 ± 38.4	336.3 ± 21.9	0.9	0.9 ± 0.013	26.0 ± 1.8	29
3.5-DiMe-*cyclo*Sal-d4TMP	122.8 ± 24.2	94.5 ± 16.5	0.8	0.9 ± 0.12	3.7 ± 0.4	4
3-Me-*cyclo*Sal-d4TMP	46.2 ± 10.8	34.7 ± 3.6	0.8	0.3 ± 0.04	1.0 ± 0.08	3
5-H-*cyclo*Sal-d4TMP	59.9 ± 14.4	39.5 ± 9.2	0.7	0.3 ± 0.03	0.5 ± 0.03	1.6

[a] Results represent mean value ± SD of three different experiments.
[b] Resistance-index (Ratio CC_{50} (EC_{50}) $H9^rAZT^{250}$: CC_{50} (EC_{50}) H9)

develop resistance mechanisms against anti-HIV-1 drug without developing resistance to cytotoxic effects of the drug.

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