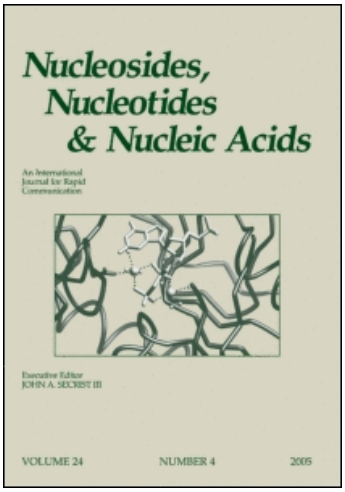

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The Cytotoxicity of Anti-PAI-I Oligonucleotides and Their Conjugates

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THE CYTOTOXICITY OF ANTI-PAI-1 OLIGONUCLEOTIDES AND THEIR CONJUGATES

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ABSTRACT: The cytotoxicity of anti-PAI-5 hexadecanucleotides (phosphodiesters and phosphorothioates) and their conjugates with lipophilic alcohols was tested in EA.hy 926 hybrid endothelial cells. Some cytotoxicity was found for cholesteryl and bornyl conjugates at concentrations higher than those used for antisense inhibition experiments.

In our recent studies we have shown that oligonucleotide 5'-conjugates of the general formula RO-P(S)(O^-)-[X]-$^{5'}$O-OLIGO are potent antisense inhibitors of PAI-1 biosynthesis in EA.hy 926 cell cultures.[1] The conjugates bear the residues of lipophilic alcohols (RO) derived of borneol, cholesterol, menthol and heptadecanol, connected (*via* the phosphorothioate linkage) either directly to the 5'-end of parent oligonucleotide or *via* a linker containing 3 tetraethylene glycol (TEG) units.[2] The parent oligonucleotide has a sequence 5'-GAGGGGTGGAGACATC, which was previously selected for maximal antiPAI-1 activity[1,3] and was used for conjugate synthesis either in phosphodiester (PO-16H) or phosphorothioate (PS-16H) form.[2]

In the light of enhanced anti-PAI-1 activity of the conjugates it was of interest to compare their toxicity towards the cells with that of parent oligonucleotides.

The cytotoxicity of bioconjugates and their parent oligonucleotides was measured in the EA.hy 926 human hybrid endothelial cell line. The constructs were incubated with the cells for 24 hrs at a final concentration of 5 and 10 μM. Thus, the cells were continuously kept at their logarithmic growth phase by diluting and supplying them with fresh culture medium every two or three days. Only cell cultures having less than 1% of dead cells were included in the study. Cellular vialibility was determined microscopically by Trypan Blue exclusion. The cytotoxicity data of the tested oligonucleotides and conjugates, expressed as the percentage of dead cells in comparison to control (untreated) cells (estimated accuracy ±10%), are presented in the Table. The inspection of

the data listed in the Table reveals, that at 10 μM concentration the cytoxicity of heptadecanyl and menthyl conjugates resembles that of unconjugated oligonucleotides (20-30%). The toxicity increases to 40-50% for conjugates of borneol without $(TEG)_3$ linker, and becomes the highest

Parent oligonucleotide	Lipophilic alcohol residue (RO)	X	Cytotoxicity at 5 μM (%)	Cytotoxicity at 10 μM (%)
PO-16H	-	-	5	32
PS-16H	-	-	2	27
PO-16H	Bornyl	-	12	50
PO-16H	Bornyl	$(TEG_{PO})_3$	0	30
PS-16H	Bornyl	-	0	42
PS-16H	Bornyl	$(TEG_{PS})_3$	10	24
PO-16H	Cholesteryl	-	0	0
PO-16H	Cholesteryl	$(TEG_{PO})_3$	40	75
PS-16H	Cholesteryl	-	0	30
PS-16H	Cholesteryl	$(TEG_{PS})_3$	80	90
PO-16H	Heptadecanyl	-	0	20
PO-16H	Heptadecanyl	$(TEG_{PO})_3$	0	20
PS-16H	Heptadecanyl	-	0	16
PS-16H	Heptadecanyl	$(TEG_{PS})_3$	0	0
PO-16H	Menthyl	-	0	20
PO-16H	Menthyl	$(TEG_{PO})_3$	0	20
PS-16H	Menthyl	-	0	30
PS-16H	Menthyl	$(TEG_{PS})_3$	0	15

(75-90%) for cholesteryl conjugates with $(TEG)_3$ linker. The same trend, with the highest cytotoxicity of bornyl and, especially, cholesteryl conjugates, can also be observed at 5 μM concentration. No measurable toxicity was observed for anti-PAI-I oligonucleotides and their conjugates at conditions identical to those used in PAI-1 inhibition experiments (conc. 1.25 μM, incubation time 24 h). Similarly, shorter incubation time (4 h) did not lead to any measurable toxic effect, even at 10 μM concentration of oligonucleotide constructs.

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