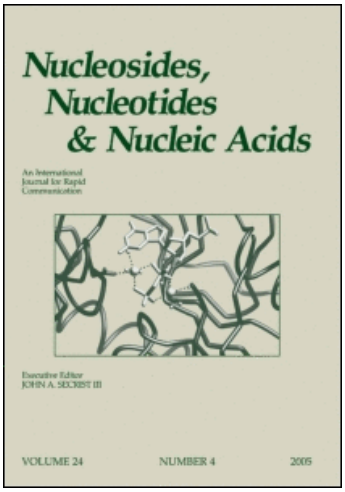


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# Cholesterol Conjugated Uniform and Gapmer Phosphorothioate Oligonucleotides Targeted Against PKC-α and C-*raf* Gene Expression

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# CHOLESTEROL CONJUGATED UNIFORM AND GAPMER PHOSPHOROTHIOATE OLIGONUCLEOTIDES TARGETED AGAINST PKC-α AND C-*raf* GENE EXPRESSION

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*In vitro* and *in vivo* antitumor activity of phosphorothioate antisense oligonucleotides targeted against two protein kinases within the mitogen-activated protein (MAP) kinase signaling cascade has been well documented by ISIS 3521/CGP 64128A (targeted against PKC-α protein) and ISIS 5132/CGP69846A (targeted against C-*raf*-1 kinase). For both of these compounds, cationic lipid formulations are necessary to observe any pharmacological activity in cell culture. In contrast, *in vivo* functional delivery of phosphorothioate oligonucleotides to cells in tissues does not appear to be a problem. These oligonucleotides have demonstrated reduction in either PKC-α or C-*raf* gene expression in tissues or human tumor xenografts following systemic administration.

Despite these successful results, it is apparent that delivery of phosphorothioate oligonucleotides to their intracellular targets can be improved for both cell culture based applications and *in vivo* applications: 1) not all cell types are susceptible to cationic lipid mediated delivery; 2) cationic lipid mediated delivery requires manipulation of the cell system; and 3) selective targeting of phosphorothioate oligonucleotides to specific cell types within tissues may be desirable for specific applications. Therefore, we evaluated the effect of cholesterol conjugated derivatives of ISIS 3521 and ISIS 5132 oligonucleotides.

**C-*raf* Compounds:** Cholesterol conjugated ISIS 5132, designated as ISIS 8006, was tested in cultured T24 cells. At 5 μm oligonucleotide treatment, in the absence of cationic lipids, ISIS 8006 was able to inhibit C-*raf* kinase mRNA expression while ISIS 5132 was inactive. In the same experiment, cholesterol conjugated ICAM-1 antisense oligonucleotide was inactive, supporting an antisense mechanism of action. In comparing the kinetics of C-*raf* kinase mRNA inhibition by ISIS 8006 in the presence and absence of cationic lipids, it was observed that inhibition is faster in the presence of cationic lipids.

**PKC-α Compounds:** ISIS 3521 is a potent, selective inhibitor of PKC-α gene expression, currently in Phase I clinical trials. PKC-α, a member of the multigene PKC family of

Table. Human PKC-a Oligos, Gapmers and their Cholesterol Conjugates

| | | |
|---|---|---|
| 3521 | GTT CTC GCT GGT GAG TTT CA | P=S; 20mer |
| 8007 | GU*T CTC GCT GGT GAG TTT CA | P=S; 20mer |
| 9520 | U*GTT CTC GCT GGT GAG TTT CA | P=S; 5'-Cholesterol, 21mer |
| 12373 | GTT CTC GCT GGT GAG TTT CA U* | P=S; 3'-Cholesterol, 21mer |

GAPMERS: 2'-F wings; deoxy eightmer gaps; uniform P=S

8469 GUU CUC GCT GGT GA GUU UCA (parent gapmer, analog of 3521)

9531 GUU CUC GCT GGT GA GUU UCA U (9520 Analog)

9532 GUU CUC GCT GGT GA GUU UCA U* (3'-hexylamine: Linker alone)

9533 GUU CUC GCT GGT GA GUU UCA U* (3'-cholesterol)

The gapmers have the added advantage of enhanced binding affinity to the target RNA due to 2'-F substitution ( an RNA mimic) in the wings.



signal transduction proteins, plays a role in regulating both normal and abnormal cell growth, including the growth of tumors. ISIS 3521works through an antisense mechanism of action to selectively inhibit PKC-α protein production.

In the compounds against PKC-α, three analogs of ISIS 3521 were tested either in A549 cells or in T24 cells: a) ISIS 8007, in which the nucleoside at position 19 was conjugated to cholesterol; b) ISIS 12373, in which cholesterol was conjugated to the 3' end of the oligonucleotide; and c) ISIS 9533, which is a gapmer (2'-F-containing nucleotides in the wings and 2'-deoxynucleotides in the gap). All of these compounds were tested at 10 μM concentration without cationic lipids and were able to reduce PKC-α mRNA levels in both cell lines while ISIS 3521 was inactive. All of the observed positive effects are in the absence of serum.

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