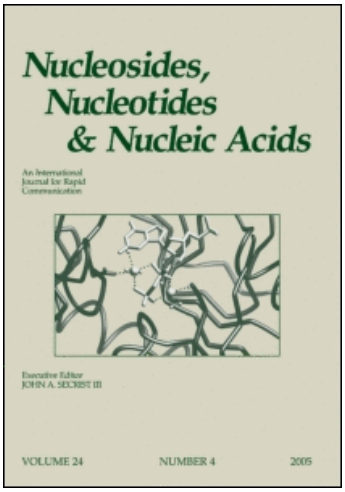

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Modification of a Phosphorothioate Oligonucleotide with Cholesterol Induces Association of the Oligonucleotide to Serum Lipoproteins and Affects Its Biological Fate

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MODIFICATION OF A PHOSPHOROTHIOATE OLIGONUCLEOTIDE WITH CHOLESTEROL INDUCES ASSOCIATION OF THE OLIGONUCLEOTIDE TO SERUM LIPOPROTEINS AND AFFECTS ITS BIOLOGICAL FATE

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ABSTRACT: A cholesterol-conjugated phosphorothioate ICAM-1 antisense oligonucleotide was evaluated for its binding to lipoproteins and its biodistribution. Our study indicates that the conjugate behaves differently from the parent compound.

INTRODUCTION

Oligonucleotides complementary to sequences in pathogenic genes (antisense oligonucleotides) can highly selectively affect the expression of these genes, which affords an exiting new strategy for therapeutic intervention. The initial drawback of lability of oligonucleotides in biological matrices has now large been overcome by the synthesis of nuclease-resistant analogues such as phosphorothioates. A remaining challenge is the improvement of permeation of target cells by antisense oligonucleotides *in vivo*. Several modifcations have been tried to enhance cellular uptake, and it has been found that coupling of cholesterol to phosphorothioate antisense oligonucleotides improves their efficacy *in vivo* [ref 1 and C.F. Bennett, unpublished]. The mechanisms involved are not entirely clear. Earlier work showed that cholesterol-modified phosphodiester oligonucleotides bind to serum lipoproteins, and are more slowly cleared from the circulation than underivatized oligonucleotides [2]. However, the pharmacokinetic- and protein binding characteristics of phosphodiester oligonucleotides are very different from those of the phosphorothioate compounds [3]. We here studied association of a phosphorothioate oligonucleotide and its cholesterol-modified derivative with blood constituents, and followed their fate after intravenous injection. We used a phosphorothioate oligonucleotide specific for adhesion molecule

ICAM-1 (ISIS-3082). It was derivatized with cholesterol at the 3'-end (ISIS-9388), which affords further resistance to 3'-exonucleases [M. Manoharan, unpublished].

RESULTS AND DISCUSSION

ISIS-3082 is a 20-mer phosphorothioate specific for murine ICAM-1 (sequence: TGC ATC CCC CAG GCC ACC AT). ISIS-9388 (Fig. 1A) has the same sequence as ISIS-3082, exept that a 3'-modified uridine is the 3'-end base instead of thymidine. 3'-O-(6-aminohexyl)-uridine was condensed with chloroformate and the resulting conjugate was attached to a solid support. Synthesis of ISIS-3082 using this modified solid support provided ISIS-9388 (Fig. 1B and ref. 4). To allow monitoring of the biological fate of both oligonucleotides, they were radiolabeled by exchange with tritiated water ase described by Graham et. al. [5].

To study the interactions of both oligonucleotides with blood constituents, [^{3}H]ISIS-3082 and [^{3}H]ISIS-9388 were incubated with human blood at 37 °C. After 60 min, blood cells and plasma were separated by centrifugation. The blood cells contained < 2.5% of the added amount of both oligonucleotides. The interaction of the oligonucelotides with plasma components was subsequently studied by subjecting the plasma to density-gradient centrifugation [6]. Fig. 2 shows that ISIS-3082 was almost exclusively recovered in the albumin/globulin fractions (fractions 1-6). Cholesterol-derivatized ISIS-9388, however, was mainly recovered in fractions containing high density lipoprotein (HDL) and low density lipoprotein (LDL). The distribution of ISIS 9388 over the LDL- and HDL fractions followed that of the phospholipids present in plasma, which indicates that the oligonucleotide divides over the available surface area of lipoproteins.

The biological fates of ISIS-3082 and ISIS-9388 were studied by monitoring radioactivity in plasma after intravenous injection of the radiolabeled oligonucleotides into rats. ISIS-3082 was cleared from the circulation with a half-life of 24.4 ± 5.3 min. The liver and kidneys were found to be the organs mainly responsible for the clearance of the oligonucleotide (35.0 ± 2.6% and 9.6 ± 2.0% of the dose at 90 min after injection, respectively). ISIS-9388, however, was cleared much slower from the circulation (half-life 46.3 ± 0.7 min) . Uptake by the liver was enhanced to 48.0 ± 3.0% of the dose at 90 min after injection, whereas the uptake by the kidneys was substantially reduced to 1.9 ± 0.0% of the dose.

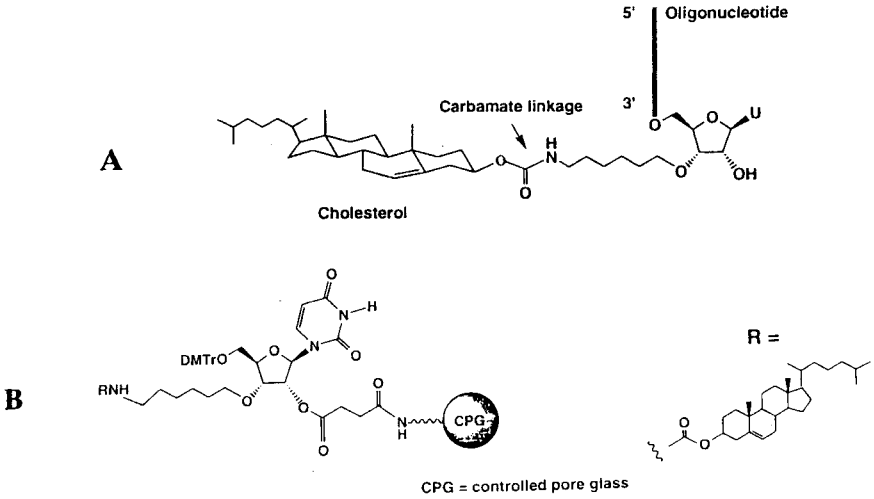

Figure 1: ISIS-9388 oligonucleotide (A) and its initial building block (B).

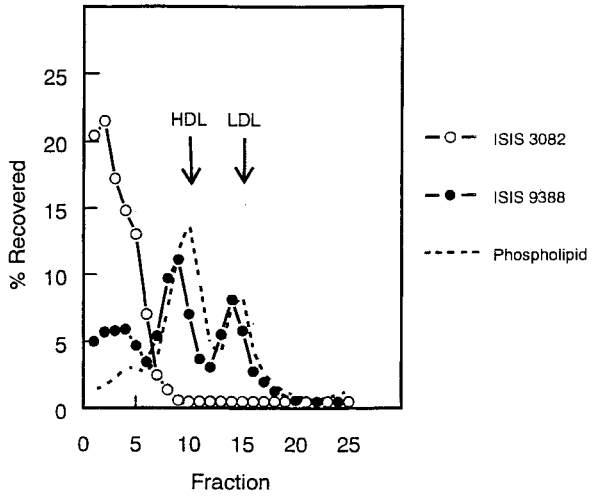

Figure 2. Density-gradient profile of ISIS-3082 and ISIS-9388 in blood plasma.

Human blood was incubated for 60 min at 37 °C with 2 μM [^{3}H]ISIS-3082 or 2 μM [^{3}H]ISIS-9388. After incubation, the blood was centrifuged and the plasma subjected to density gradient centrifugation. The gradients (12 ml) were fractionated from the bottom (fraction 1 highest density), and assayed for radioactivity and phospholipids (Boehringer enzymatic assay).

Table 1: Plasma clearance and tissue uptake of ISIS-3082 and ISIS-9388 in rats.

Rats were intravenously injected with [^{3}H]ISIS-3082 or [^{3}H]ISIS-9388 at a dose of 1 mg/kg body wt. Blood samples were taken up to 90 min after injection, and the plasma was assayed for radioactivity. At 90 min after injection the animals were sacrificied and the radioactivities in liver and kidneys were determined. Values are means ± S.E.M. of 2-3 rats.

Plasma clearance/Tissue uptake	ISIS-3082	ISIS-9388
Plasma half-life (min)	24.4 ± 5.3	46.3 ± 0.7
Blood plasma (%dose)	3.6 ± 1.3	22.5 ± 1.6
Liver (% dose)	35.0 ± 2.6	48.0 ± 3.0
Kidneys (% dose)	9.6 ± 0.2	1.9 ± 0.0

In conclusion, we found that ISIS-9388 associates to lipoproteins and has an altered metabolic fate as compared to ISIS-3082. The lipoprotein-associated oligonucleotide is not rapidly filtered by the kidneys and does probably not "leak" as rapidly into peripheral tissues as the underivatized oligonucleotides. As a result, ISIS-9388 circulates longer, which allows a longer exposure to its target.

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