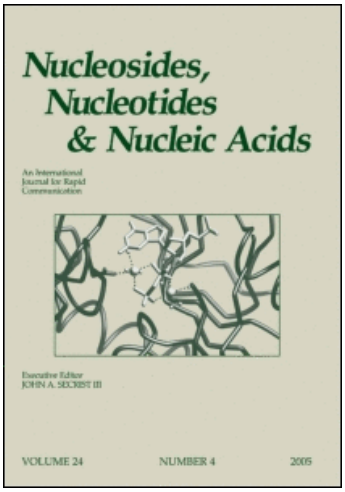

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Conjugation of Oligonucleotides with Steroid Structures and Their Interaction with the Low-Density Lipoprotein

Erik T. Rump[a]; Remco L. A. de Vrueh[a]; Erik A. L. Biessen[a]; Theo J. C. van Berkel[a]; Martin K. Bijsterbosch[a]
[a] Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, Leiden University, Leiden, RA, The Netherlands

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CONJUGATION OF OLIGONUCLEOTIDES WITH STEROID STRUCTURES AND THEIR INTERACTION WITH THE LOW-DENSITY LIPOPROTEIN.

Erik T. Rump, Remco L.A. de Vrueh, Erik A.L. Biessen, Theo J.C. van Berkel and Martin K. Bijsterbosch*

Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, Leiden University, P.O. Box 9503, 2300 RA Leiden, The Netherlands.

ABSTRACT: To induce association with low-density lipoprotein (LDL), antisense oligonucleotides were derivatized at their 3' terminus with several steroid structures. It was found that only highly lipidic steroids can complex the oligonucleotide with LDL.

LDL is an attractive carrier for the selective delivery of lipophilic drugs to tumor cells, as it has been found that many tumor cells take up more LDL than normal cells[1]. LDL is a spherical particle with a mean diameter of 23 nm, and serves as cholesterol transporter in blood. Its apolar core contains cholesterol-esters and triglycerides, and is surrounded by a monolayer containing phospho-lipids, some cholesterol and an apoprotein. LDL is internalized by cells via specific LDL-receptors that recognize its apoprotein moiety. It has been reported in several studies[2-5] that oligonucleotides conjugated with lipid structures can be complexed with LDL. These LDL-oligonucleotide complexes retain their recog-nition site for the LDL-receptor, and are taken up by cells via endocytosis. The complexation with LDL was shown to be the result of a lipophilic interaction between the lipid moiety (cholesterol) of the oligonucleotide and lipids of the lipoprotein[3]. It is our aim to use this approach to achieve enhanced uptake of

antisense oligonucleotides directed to the *c-myb* proto-oncogene in leukemic cells, which show an overexpression of the LDL-receptor[1]. We derivatized an 18-mer *c-myb*-specific antisense oligonucleotide with three different lipidic steroid structures **A**, **B**, and **C** (FIG. 1), and assessed which structure can induce association of the oligonucleotide with LDL. The lipidic structures were introduced at the 3' terminus of the oligonucleotide via the carboxylic acid function of **A** and **B**, or the C-3 hydroxyl of steroid **C**.

Conjugation of 18-mer phophodiester oligonucleotides (GTG CCG GGG TCT TCG GGC-3') with the steroid structures was performed under mild basic conditions. 3'-Amino tailed oligonucleotides (ODNs) were reacted with either pentafluorophenyl esters of the two litocholic-acid adducts (**A** and **B**), resulting in ODN-**A** (litocholic acid conjugate) and ODN-**B** (litocholic acid-3-oleate conjugate), or cholesterol chloroformate resulting in ODN-**C**. ODN-**A** was purified by reversed phase HPLC on a Nucleosil C-8 HPLC column (5μ, 100Å) using a gradient of 20-60% CH_3CN in 50 mM $LiClO_4$. The product eluted at 38% CH_3CN. ODN-**B** and ODN-**C** were purified by electrophoresis in a 1% agarosegel, which contained 0.1% tween 20. Using this technique, non-derivatized oligonucleotides were separated from the derivatized product[6]. The bands of interest were visualized by UV-shadowing, cut out and melted. After digestion of the melted agarose with agarase, the conjugated oligonucleotides were precipitated with acetone.

The interaction of derivatized oligonucleotides with LDL was studied by incubating freshly isolated LDL[7] (1.83 $mg.ml^{-1}$ in phosphate-bufferd saline /1 mM EDTA) with different molar equivalents of purified oligonucleotides for 1 h at 37 °C. Association of oligonucleotides with LDL increases the overall negative charge of the particle. This can be monitored by agarose gel electrophoresis. Incubation mixtures were therefore loaded on a 0.75% agarose gel in 75 mM Tris-hippuric acid buffer (pH 8.8), and were electrophoresed on ice. Lipoproteins were visualized by staining with Coomassie Brilliant Blue.

FIG. 2 shows that the relative mobility (R_f) of LDL was increased from 0.22 to 0.43 upon incubation with 10 molar equivalents of ODN-**B** or ODN-**C**. This finding indicates that a substantial proportion of these oligonucleotides associa-

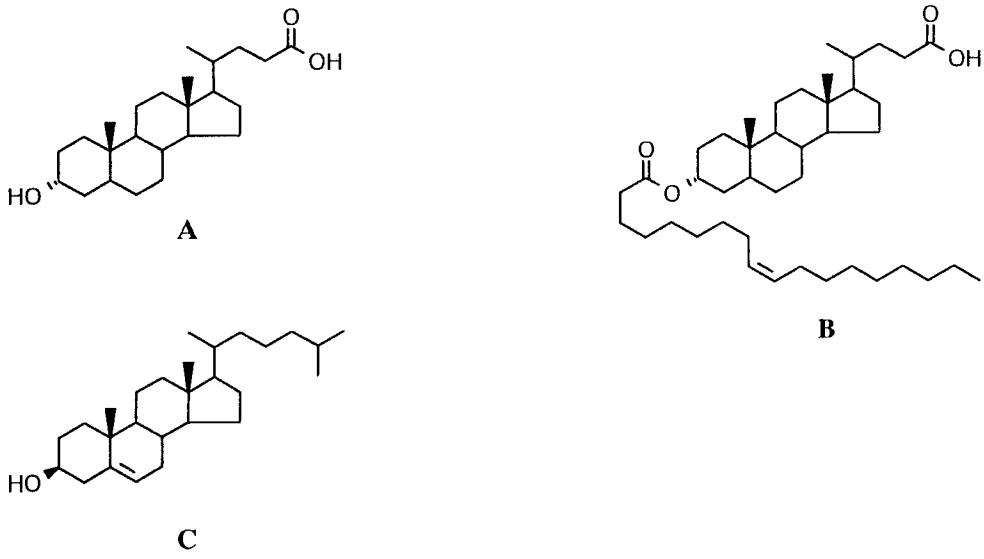

FIG.1 Lipophilic steroid structures
A : litocholic acid
B : litocholic acid-3-oleate
C : cholesterol

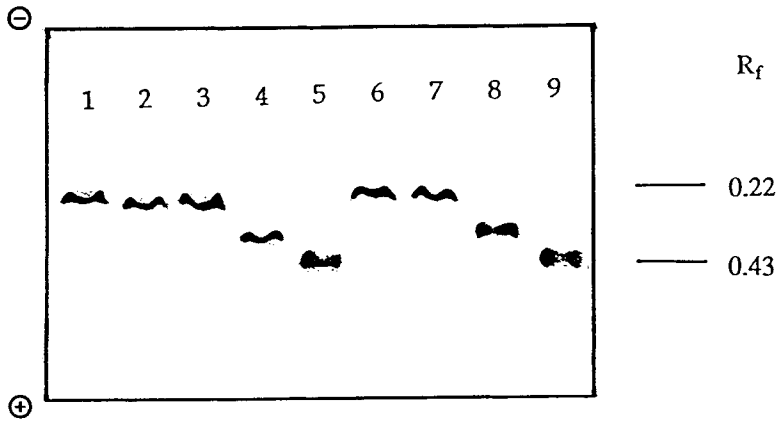

FIG. 2 agarose gel electrophoresis of LDL incubated with x molar equivalents of ODN. Lane 1, 3, 7: native LDL; lane 2: 5x ODN-A; lane 4: 5x ODN-B; lane 5: 10x ODN-B; lane 6: 10x ODN; lane 8: 5x ODN-C; lane 9: 10x ODN-C.

tes with LDL. On the other hand, incubation with ODN-**A** or non-derivatized oligonucleotides did not result in complex formation, since no R_f shift could be observed, even at a 10-fold molar excess.

Apparantly, the free hydroxyl function at C-3 of litocholic acid of ODN-**A** prevents LDL-complexation, probably due to repulsion by the polar shell of LDL. Esterification of this hydroxyl with oleic acid affected the lipid nature of this steroid significantly, and ODN-**B** could easily be complexed with LDL. ODN-**B** and ODN-**C** differ in their steroid orientation, as in ODN-**B** the lipid is attached to the oligonucleotide via the D-ring of the steroid, compared to the A-ring of the steroid in ODN-**C**. Orientation of the steroid ring in these experiments did not influence complex formation. Our results suggest that steroid lipids can complex oligonucleotides with LDL, provided that the steroid does not contain any hydrophilic functional groups.

REFERENCES

1) Firestone,R.A. *Bioconjugate. Chemistry.*,**1994**,*5*, 105-113.
2) De Smidt,P.C., Le Doan,T., De Falco,S. and Van Berkel,T.J.C. *Nucleic Acids Res.*,**1991**,*19*, 4695-4700.
3) Krieg,A.M., Tonkinson,J., Matson,S., Zhao,Q., Saxon,M., Zhang,L.-M., Bhanja,U., Yakubov,L. and Stein,C.A. *Proc. Natl. Acad. Sci. USA*,**1993**,*90*, 1048-1052.
4) Ramazeilles,C., Mishra,R.K., Moreau,S., Pascolo,E. and Toulme,J.J. *Proc. Natl. Acad. Sci. USA*,**1994**,*91*, 7859-7863.
5) Boujrad,N., Hudson,J.R. and Papadopoulos,V. *Proc. Natl. Acad. Sci. USA.* **1993**,*90*, 5728-5731.
6) Boutorine,A.S., Tokuyama,H., Takasugi,M., Isobe,H., Nakamura,E. and Hélène,C. *Angew. Chem. Int. Ed.*,**1994**,*33*, 2462-2465.
7) Redgrave,T.G., Roberts,D.L.K. and West,C.E. *Anal. Biochem.*,**1975**,*65*, 42-49.