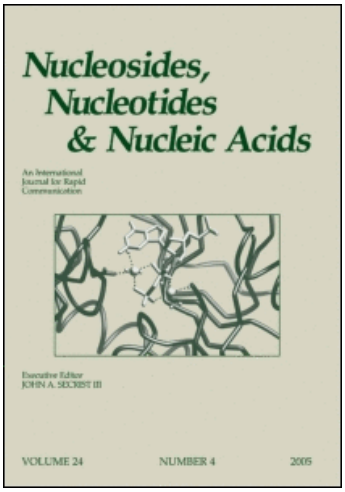

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Antiviral activity of antisense oligonucleotides linked to poly(L-lysine):targets on genomic RNA and/or mRNA of Vesicular Stomatitis Virus

Jean-Paul Leonetti[a]; Genevieve Degols[a]; Pierre Milhaud[a]; Corinne Gagnor[a]; Marc Lemaitre[a]; Bernard Lebleu[a]
[a] Laboratoire de Biochimie des Proteines, URA CNRS 199, Montpellier cedex, France

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Antiviral activity of antisense oligonucleotides linked to poly(L-lysine):targets on genomic RNA and/or mRNA of Vesicular Stomatitis Virus

Jean-Paul Leonetti, Genevieve Degols, Pierre Milhaud, Corinne Gagnor, Marc Lemaitre, Bernard Lebleu: Laboratoire de Biochimie des Proteines, URA CNRS 199, Universite de Montpellier 2, 34060 Montpellier cedex, France.

Abstract: Synthetic oligonucleotides provide a rational approach to viral genes control. Conjugation of antisense oligodeoxyribonucleotides (directed against several viral sequences) to poly(L-lysine) brings about specific protection against Vesicular Stomatitis Virus at concentrations lower than 1 uM.

Many groups have focused their activities on oligonucleotides and polynucleotides as potential antiviral agents. In our laboratory the antiviral properties of (2'-5')(A)n oligoribonucleotides which mimic interferon action through RNase L activation, of double stranded RNA interferon inducers, and of antisense oligodeoxyribonucleotides (oligo) are currently investigated.

However their difficulty to reach the intracellular target and their sensitivity to nucleases limit the potential usefulness of these compounds.

Efforts have been developed to overcome these difficulties and improve antiviral activity of these compounds:

-discrete modifications of (2'-5')(A)n (1) and of antisense oligos increase their stability towards nucleases.

-intracellular delivery techniques (**Tab. 1.**) as liposomes encapsulation (2), microinjection of r(I)n-r(C)n (3) and (2'-5')(A)n (4), administration of r(I)n-r(C)n with polycationic agents (5), and covalent linkage of (2'-5')(A)n to poly(L-lysine) (6) have been tested.

Poly(L-lysine) was previously described as a potential drug or macromolecule carrier (7). This delivery method has been adapted to functional internalization of synthetic oligos directed against the Vesicular Stomatitis Virus (VSV) (8).

Oligos were covalently linked to poly(L-lysine) (Mr=14,000) via a N-morpholine ring after oxidation of their 3' end ribose residue. This ribose initially added with T4 RNA

TABLE 1.

Antiviral synthetic oligo and polynucleotides.

Type	Target	Internalisation
(2'-5')(A)n	RNase L	microinjection (4) liposome encapsulation (2) poly(L-lysine) conjugation (6)
r(I)n-r(C)n	-interferon induction -double stranded RNA activated enzymes (2-5 A synthetases,..)	microinjection (3), polycationic agents (administration with DEAE dextran (5))
antisense oligos	complementary sequences in genomes and transcripts	poly(L-lysine) conjugation (8)

ligase (8), was later on introduced by synthesis of the oligo on an adenosine-derivatized support (9).

Most studies have defined the 5'-untranslated region as the best target for translation arrest. Our previous results with an oligo complementary to the 5' end of N protein mRNA, have shown a specific antiviral activity, at concentrations lower than 1 uM (8). So far indeed we do not know the target of this oligo which might act at the transcriptional and/or replicative level.

We have therefore tested oligos complementary to the intergenic untranscribed consensus region of VSV (10). These oligos, which have potential targets on the positive or negative strand of viral RNAs, inhibited virus multiplication at nearly the same concentration as the oligo complementary to the 5' end of N protein mRNA (Tab. 2.). Interference with VSV replication and/or VSV transcription could explain this strong antiviral activity.

Oligos complementary to an internal site of N protein mRNA , to the viral polymerase binding site , or to the initiation translation site of c-myc mRNA are devoided of any antiviral activity (Tab. 2.).

These results confirme the specificity of action attained with the conjugates. They also show that oligos directed to any potential target do not induce an antiviral effect necessarily. Moreover conjugation of oligos to PLL promoted efficient antiviral effect which was not observed with free oligo within the same range of concentration (8).

TABLE 2.

Antiviral activity of oligonucleotides poly(L-lysine) conjugates:

Target	Antiviral activity (*)
N protein mRNA translation initition site 5′ 3′ CATTTTGATTACTGTrA	- 2.2
internal site 5′ 3′ TTACACGGAGCAArA	-0.4
VSV intergenic region negative strand 5′ 3′ TGAAAAAAACTAACAGrA	-2.2
viral polymerase binding site 5′ 3′ CCATTATTATCATTArA	+0.3
control: human c-myc translation initiation site 5′ 3′ ACGTTGAGGGGCATCrA	+0.1

L929 cells were incubated for 2 hours with or without 1 uM conjugate and infected with VSV (mutiplicity of infection: 1). VSV titers (*) were measured by an end point method and expressed in log(infectious units/ml). The antiviral activity is expressed as the difference between the titers from cells incubated in the presence and the absence of conjugates.

The antiviral properties of oligo-poly(L-lysine) conjugates have been tested in L929 cells but also in other cell lines: wide differences in the level of antiviral response were observed (data not shown).

It was tempting to improve this technique by increasing the repression efficiency of the oligo-poly(L-lysine). Alpha anomeric oligos have been shown to possess favorable hybridization properties (11) and to exhibit an increased resistance to nucleases. In this regard alpha anomeric oligos (synthesized in the laboratory of Prof. J. L. Imbach; 12) have thus been coupled to poly(L-lysine) and compared with beta

anomeric oligo conjugates for their antiviral activity against VSV. We did not observe any reduction of VSV titer with these conjugates (data not shown). Nevertheless alternatives as the introduction at the 5' end of the oligo of intercalating agents (13), free radical generating groups (14), alkylating agents (15), or photoactivable groups (16), as well as the use of others oligo analogues (17), provide interesting prospects.

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