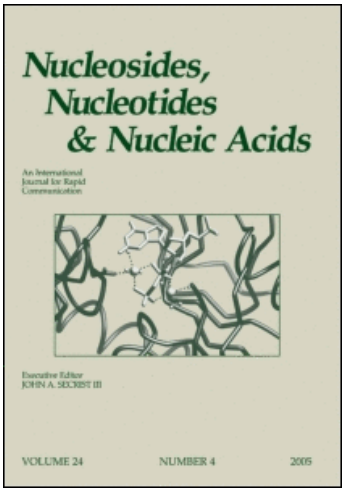



**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# In Vitro and In Vivo Anti-influenza A Virus Activity of Antisense Oligonucleotides

Takayuki Abe[a]; Tadashi Mizuta[b]; Shin-Ichi Suzuki[a]; Toshifumi Hatta[a]; Kazuyuki Takai[a]; Tomoyuki Yokota[b]; Hiroshi Takaku[a]
[a] Department of Industrial Chemistry, Chiba Institute of Technology, Chiba, Japan [b] Rational Drug Design Laboratories (R. D. L), Fukushima, Japan

**To cite this Article** Abe, Takayuki , Mizuta, Tadashi , Suzuki, Shin-Ichi , Hatta, Toshifumi , Takai, Kazuyuki , Yokota, Tomoyuki and Takaku, Hiroshi(1999) 'In Vitro and In Vivo Anti-influenza A Virus Activity of Antisense Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1685 — 1688
**To link to this Article: DOI:** 10.1080/07328319908044823
**URL:** http://dx.doi.org/10.1080/07328319908044823

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1685–1688 (1999)

# IN VITRO AND IN VIVO ANTI-INFLUENZA A VIRUS ACTIVITY OF ANTISENSE OLIGONUCLEOTIDES

Takayuki Abe[1], Tadashi Mizuta[2], Shin-ichi Suzuki[1], Toshifumi Hatta[1], Kazuyuki Takai[1], Tomoyuki Yokota[2], and Hiroshi Takaku[1]

[1]Department of Industrial Chemistry, Chiba Institute of Technology, Tsudanuma, Narashino, Chiba 275-0016, Japan
[2]Rational Drug Design Laboratories (R.D.L), Misato, Matsukawa, Fukushima 960-1242, Japan

**ABSTRACT:** We have demonstrated that antisense phosphorothioate oligonucleotides (S-ODNs) inhibit influenza virus A replication in MDCK cells. The liposomally encapsulated and the free antisense phosphorothioate oligonucleotides with four target sites (PB1, PB2, PA, and NP) were tested for their abilities to inhibit virus-induced cytopathogenic effects by a MTT assay using MDCK cells. The liposomally encapsulated S-ODN complementary to the sites of the PB2-AUG initiation codon showed highly inhibitory effects. Therefore, the antiviral effects of S-ODN-PB2-AUG and PA-AUG were examined in a mouse model of influenza virus A infection. PB2-AUG oligomer treated i.v. significantly prolonged the mean survival time in day (MDS) and increased the survival rates with does dependent manner.



## INTRODUCTION

Several reports have described the development of phosphorothioate oligonucleotides as potential anti-viral therapeutic agents. Although extensive studies of their molecular mechanisms have highlighted the potential value of this novel therapeutic strategy, very little is known about the *in vivo* pharmacokinetics and metabolism of these compounds. Another problem in the use of antisense oligonucleotides is their inefficient cellular uptake. They are found mainly in endosomes and lysosomes. The use employment of a delivery vehide with specific uptake by cells would increase the circulation half-life of the oligonucleotides and improve their efficacy. Liposome delivery addresses both of these concerns. Liposome delivery of oligonucleotides to cells in vitro has been shown to greatly reduce the effective dose as compared to the use of the free oligonucleotide.

In this report, we present a detailed analysis of the inhibition of influenza virus RNA polymerase (PB1, PB2, and PA) and nucleoprotein (NP) gene expression by antisense phosphorothioate oligonucleotides (S-ODNs), as determined by the virus-

induced cytopathic effects in MDCK cells. We also describe the antiviral effects of S-ODN-PB2-AUG and PA-AUG in a mouse model of influenza virus A infection.

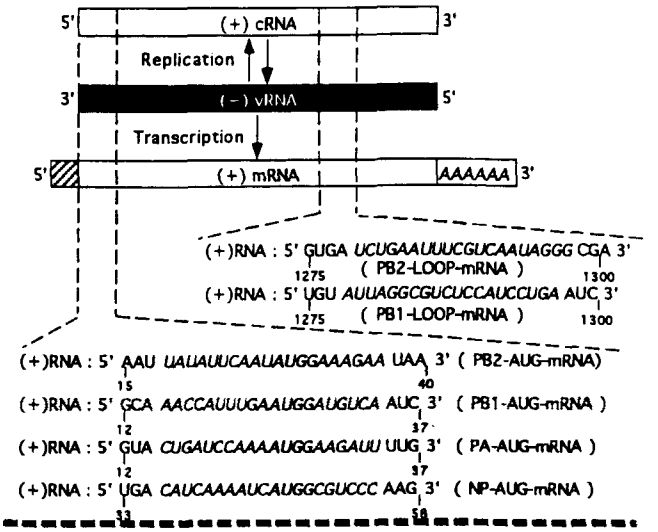
The *in vitro* antiviral activities of the antisense oligonucleotides were assessed on the basis of their inhibitory effects on the cytopathogenicities of influenza virus A. To clarify the sequence specificity, we tested the phosphorothioate oligonucleotides containing sense- and antisense sequences as targets of PB1, PB2, PA, and NP, and random sequences with the same base composition as the four targets, on the expression of the RNA polymerase genes in MDCK cells (Fig. 1). We also evaluated the inhibitory effects of free and liposomally encapsulated phosphorothioate oligonucleotides (S-ODN) on influenza virus RNA polymerase gene expression.

## RESULTS AND DISCUSSION

First, we evaluated the inhibitory effects of the encapsulated antisense phosphorothioate (S-ODN) oligonucleotides with lipofection reagent (DOTAP) on influenza virus RNA polymerase gene expression. The cells were washed with PBS, and each well was infected with a viral suspension at a multiplicity of infection (MOI) of 0.01. The cells were incubated for four days at 34℃ in a $CO_2$ incubator, and the cell viability was quantified.



The antisense phosphorothioate oligonucleotide with an AUG initiation codon (S-ODN-PB2-as), targeted to PB2 had an inhibitory effect, and caused more than 50% inhibition at 0.15-10 μM concentrations. However, the antisense oligonucleotide targeted to the loop-forming site (S-ODN-PB2-loop-as) did not lead to efficient inhibition. In contrast, the antisense phosphorothioate oligonucleotides (S-ODN-PB1-AUG-as), containing the AUG initiation codon targeted to PB1, showed lower anti-viral activity at 0.15-10 μM concentrations. The antisense phosphorothioate oligonucleotides (S-ODN-NP-AUG-as and SODN-PA-AUG-as), containing the AUG initiation codon sequence targeted to NP and PA, respectively, had inhibitory effects similar to that of the AUG initiation codon sequence targeted to PB2, at 8-10 μM concentrations. We could not detect any inhibitory effects on virus replication with the antisense phosphorothioate oligonucleotides (S-ODN-PB2-loop-as and S-ODN-PB1-loop-as), containing the loop forming site sequences. For the control sequences, the sense- and random-oligonucleotides, we could not detect any inhibitory effects on the target AUG initiation codon and the loop forming sites. These results suggest that the encapsulated antisense phosphorothioate oligonucleotide conferred sequence specific inhibition. However, when the antisense phosphorothioate oligonucleotide was not incorporated into the MDCK cells, it could still interfere with the absorption of virus particles to the receptor of MDCK cells.




| Oligonucleotide | Sequence |
|---|---|
| PB2-AUG-as | 5'-ATATAAGTTATACCTTTCTT-3' |
| PB2-AUG-sen | 5'-TATATTCAATATGGAAAGAA-3' |
| PB2-AUG-ran | 5'-TATATTCAATATGGAAAGAA-3' |
| PB2-LOOP-as | 5'-CCCTATTGACGAAATTCAGA-3' |
| PB2-LOOP-ran | 5'-ATGCTCACATACTGAAGTAC-3' |
| PB1-AUG-as | 5'-TTGGTAAACTTACCTACAGT-3' |
| PB1-AUG-ran | 5'-CCCATGTTGAGAAGTACGAG-3' |
| PB1-LOOP-as | 5'-TCAGGATGGAGACGCCTAAT-3' |
| PB1-LOOP-ran | 5'-GAGCATGAAGAGTTGTACCC-3' |
| PA-AUG-as | 5'-AATCTTCCATTTTGGATCAG-3' |
| PA-AUG-ran | 5'-TCATAAGTTCGACTTTCGTA-3' |
| NP-AUG-as | 5'-GGGACGCCATGATTTTGATG-3' |
| NP-AUG-ran | 5'-TTAGGTGGAGTCAACGCTGT-3' |

**FIG. 1.** Sequences of oligonucleotides tested for antiviral activity against influenza virus. Partions of the RNA sequences of the PB2, PB1, PA, and NP genes of influenza virus A/PR/8/34 are shown in the (+)-sense orientation. The oligonucleotide sequences correspond to the 5'- ends of the respective mRNAs and include the twenty nucleotides present upstream of the initiation codon. The phosphorothioate derivatives are denoted by "S" (underlined).Targeted mRNA: 5' to 3'(*italics*). Abbreviations: as, antisense-sequence; sen, sense-sequence; ran, random-sequence.

Next, we investigated the inhibition of influenza virus A replication by free antisense phosphorothioate oligonucleotides in MDCK cells, using the previously described assay system. Surprisingly, no viral inhibition was observed for the free antisense phosphorothioate oligonucleotides, S-ODN-PB2-AUG-as, S-ODN-PB1-AUG-as, S-ODN-PA-AUG-as, and S-ODN-NP-AUG-as, at 0.15-10 μM concentrations. This result suggests that the free antisense phosphorothioate oligonucleotides had very poor delivery efficiency into the MDCK cells. That is to say, the action of the antisense

**TABLE 1.** Effect of liposomally encapsulated S-ODNs (20-mer) i.v. on the survival of mice infected with influenza virus.

| Compound | Dose (mg/kg) | Liposome ( mg/kg) | No. of mice | MDS[a] | %Survival [b] |
|---|---|---|---|---|---|
| Control | | Tfx(1) | 24 | 4.1 | 0 |
| SODN-PB2-AUG | 20 | Tfx(1) | 8 | 8.1 | 25* |
| | 40 | Tfx(1) | 8 | 9.5 | 25* |
| SODN-PB2-AUG-ran | 40 | Tfx(1) | 8 | 3.9 | 0 |
| SODN-PA-AUG | 40 | Tfx(1) | 8 | 4.3 | 0 |
| SODN-PA-AUG-ran | 40 | Tfx(1) | 8 | 3.9 | 0 |
| SODN-PB2-AUG | 20 | Tfx(5) | 11 | 8.5 | 27* |
| | 40 | Tfx(5) | 11 | 11.1 | 45** |
| SODN-PA-AUG | 40 | Tfx(5) | 11 | 4.9 | 0 |

[a]MSD during the 14-day experimental period. [b]Percentage of mice surviving 14 days after the infection(*, $p<0.05$; **, $p<0.01$, $\chi 2$ analysis).



phosphorothioate oligonucleotides becomes exclusively sequence specific when directly delivered into the cell. It is worthwhile to note that the increased cellular association of the oligonucleotides can explain the enhanced antisense oligonucleotide effect in the presence of DOTAP.

Finally, we examined the antiviral effects of S-ODN-PB2-AUG and PA-AUG in a mouse model of influenza virus A infection (Table 1). When mice infected with 100LD50 of influenza A/PR/8/34 virus, control mice treated i.v. with PBS had MSDs of 4 days and 0% survival. The other control mice groups treated i.v. with liposomes No difference between vehicle (PBS) and liposomes-treated mices were observed with regard to the MSDs. The protective effect of S-ODN-PB2-AUG was demonstrated when infected mice were treated i.v. with the complex of liposomes (Tfx-10 or DOTAP). S-ODN-PB2-AUG and 40 mg/Kg had MSDs of 8.1 and 9.5 days, respectively ($p<0.05$) and 25% survival rates ($p<0.05$) in the presence of Tfx-10 1mg/Kg, and MSDs of 8.5 and 11.1 days ($p<0.05$) and 25% ($p<0.05$), and 45% ($p<0.01$) survival rates, respectively, in the presence of Tfx-10 5mg/Kg. However, the liposomally endocapsulated S-ODN-PB2-AUG-ran, S-ODN-PA-AUG, and S-ODN-PA-AUG-ran at 40mg/Kg showed no significant effect on both MSDs and survival.

## REFERENCES

1. Abe,T.; Suzuki,S.; Hatta,T.; Takai,K.; Yokota,T.; Takaku,H. *Antiviral Chem. Chemotherapy* **1998** 9, 253-262.
2. Hatta,T.; Takai,K.; Nakada,S.; Yokota,T.; Takaku,H. *Biochem.Biophys. Res. Commun.* **1997** 232, 545-549.
3. Hatta,T.; Nakagawa,N.; Takai,K.; Nakada,S.; Yokota,T.; Takaku,H. *Biochem. Biophys. Res. Commun.* **1996** 223, 341-346.