2nd International Electronic Conference on Medicinal Chemistry

1-30 November 2016

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Effects of oligoribonucleotides-D-mannitol complexes on the hemagglutinin-glycan interactions

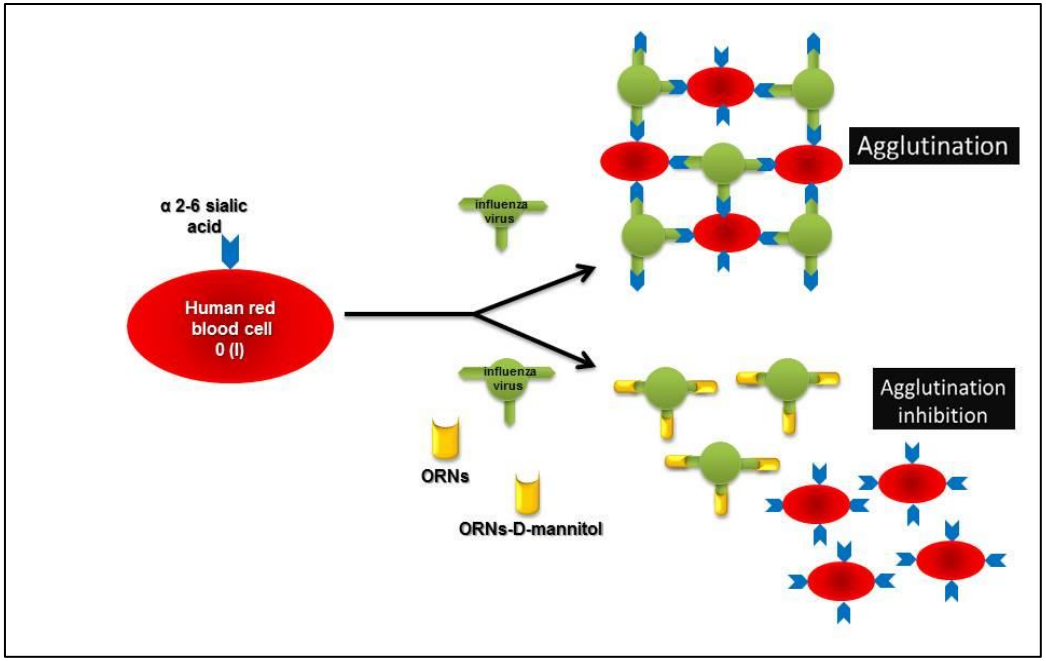
Natalia Melnichuk[1,*], Zenoviy Tkachuk[1], and Maryna Vivcharyk[1]

[1]Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine 150, Zabolotnogo Str., Kyiv - 143, Ukraine, 03680.

*natalia.melnichuk8@gmail.com

Effects of oligoribonucleotides-D-mannitol complexes on the hemagglutinin-glycan interactions

Our results showed that oligoribonucleotides-D mannitol complexes binds to HA of influenza virus and in this manner inhibit hemagglutinin-glycan interactions and reduces influenza A virus infectivity.



Agglutination of the human red blood cells 0 (I) by influenza virus with and without RNA drugs*

* Adapted to – Gopinath S.C.B,. Kumar P.K.R / Acta Biomaterialia (2013)

Abstract:

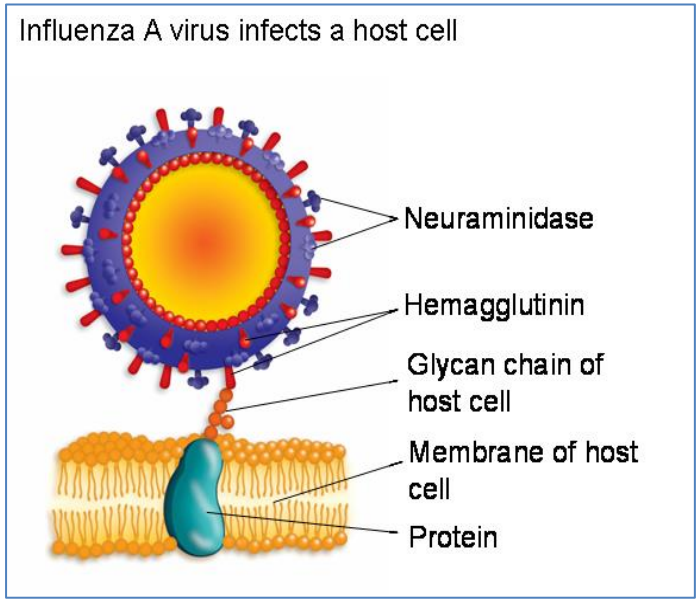
The influenza virus surface glycoprotein hemagglutinin (HA) is responsible for viral attachment to sialic acid-containing host cell receptors and it facilitates the initial stage of viral infection. Oligoribonucleotides (ORNs) – have a wide range of biological activities and can be used in antiviral treatments since they play a key role in antiviral activity and can change a conformation of some proteins. However, the mechanism of ORNs-D-mannitol antiviral activity is still not clear. In this work was studied interactions between RNA drugs and HA, effect of RNA drugs on the HA-glycan interactions activity, the influenza A virus infectivity and viability cells infected with the influenza A virus *in vitro*.

It was shown that decrease of HA activity and infectious titer of influenza A*(A/FM/1/47(H1N1))* virus by a factor 4 and 2 lgTCID$_{50}$ respectively were observed after incubation of virus with ORNs-D-mannitol in comparison to the virus control. Reduction of the fluorescence intensity of HA of flu virus in the presence of the ORNs-D-mannitol was observed. This effect may indicate that interactions between HA and ORNs-D-mannitol are responsible for conformation changes of HA.

Keywords: hemagglutinin; influenza A virus; RNA drugs

Introduction

Single-stranded DNA (ssDNA) or RNA sequences can bind with high affinity and specificity to a wide range of target molecules, such as proteins, cell surface receptors, and even whole cells as well as other organic or inorganic molecules such as ATP, dyes, amino acids, drugs, or simple small. Theoretically, they can be used therapeutically in any disease for which extracellular blocking of protein–protein interactions is required. Yeast total RNA (ORNs) with the dominant fraction of 3-8 nucleotides has antiviral activity and can change a conformation of some proteins.



The HA protein of influenza virus, a trimeric spike on the viral membrane, is responsible for hemagglutination and binding of the virus particles to the susceptible host cells. This binding mediates the subsequent entry of influenza viruses into host cells through membrane fusion, which is significant for initial viral infection.

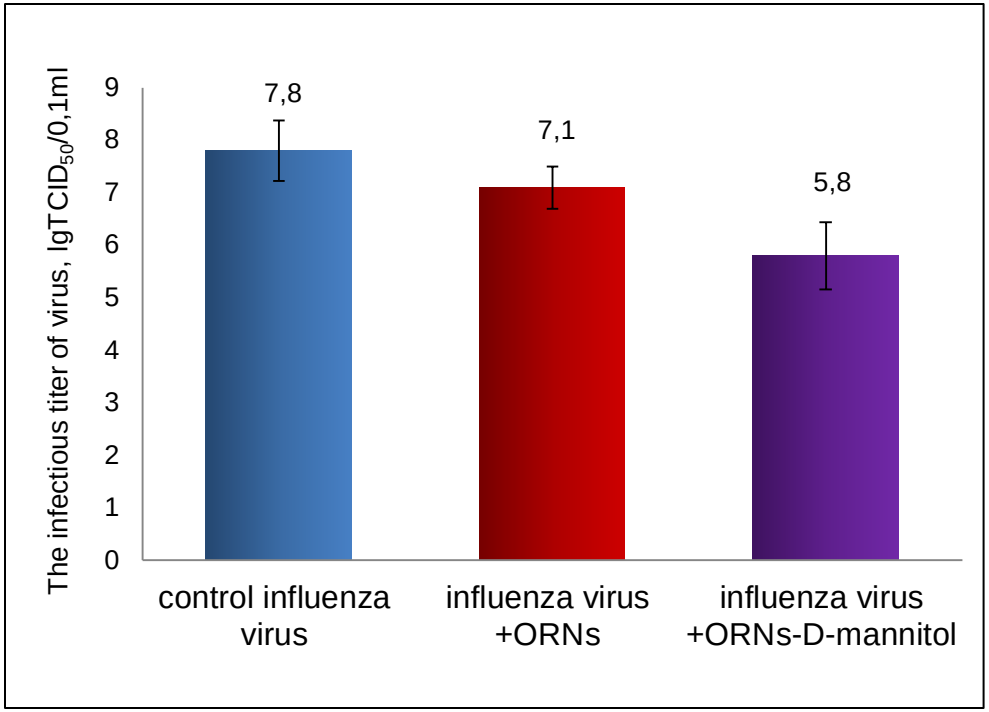
Results and discussion

RNA drugs effect on the infectivity of influenza A virus

Effect of RNA drugs on the infectivity of influenza A virus was studied on MDCK cells. The influenza A virus incubated with ORNs (2,5 mg/ml) and ORNs-D-mannitol (3,5 mg/ml) 30 min 20°C.

The infectious titer of influenza A virus was determined by cytopathic effect assay 48 hours after the influenza A virus infection.

It was shown that the infectious titer of influenza A virus decreased by 2 $lgTCID_{50}$ after incubation of virus with ORNs-D-mannitol at the concentration 3,5 mg/ml in comparison to the virus control.



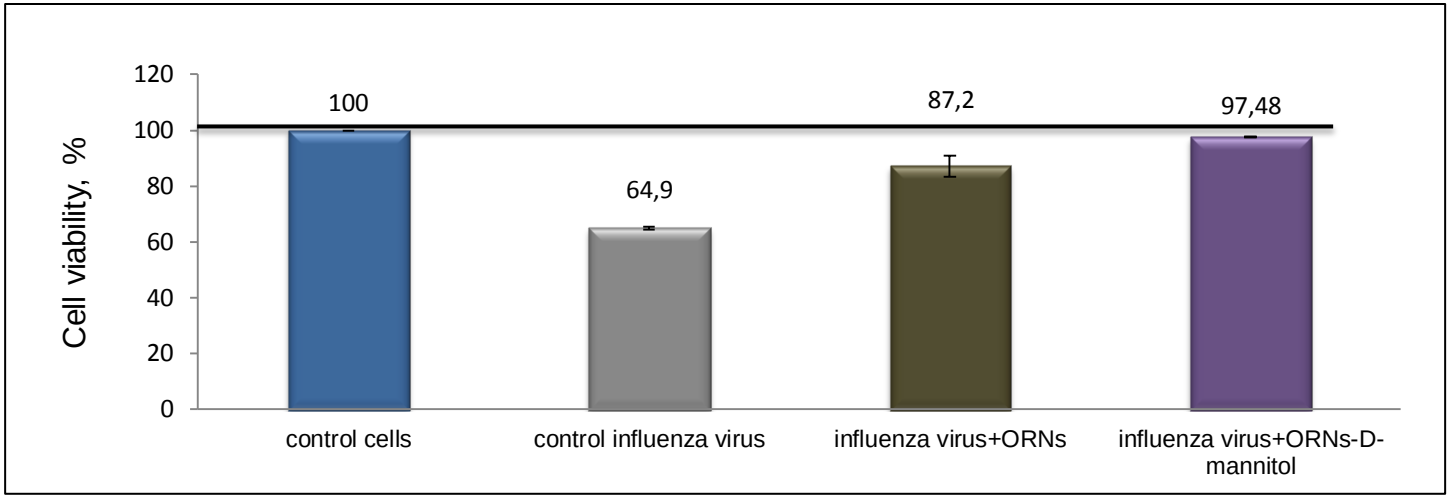
Decrease of the influenza A virus infectious titer after incubation with RNA drugs

Results and discussion

RNA drugs effect on the viability of MDCK cells infected with the influenza A virus

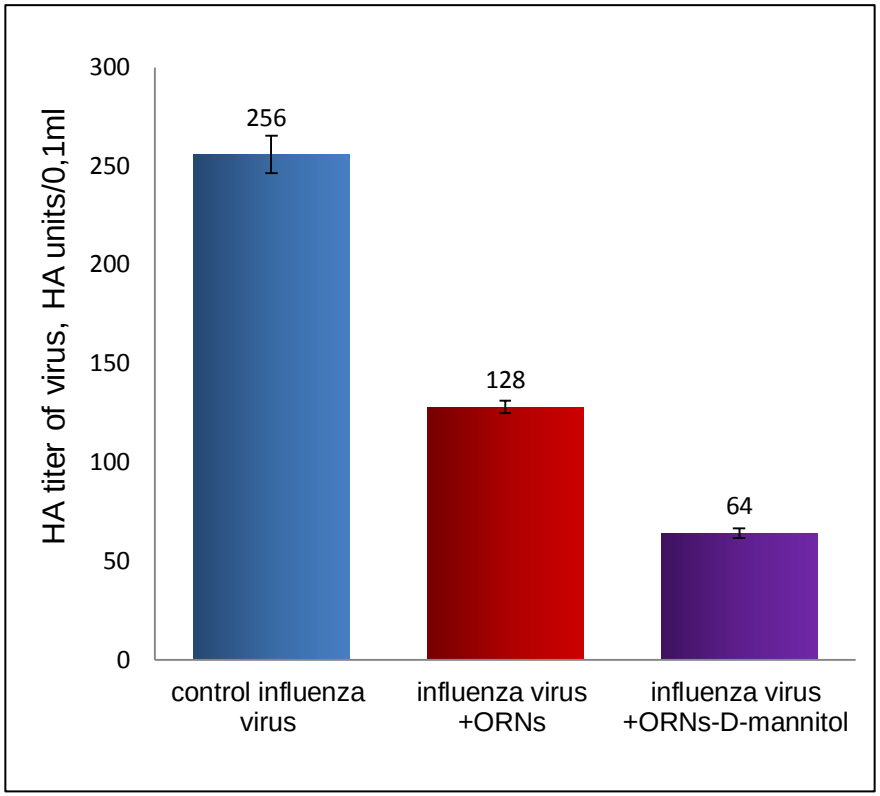
The influenza A virus was incubated 30 min 20℃ with ORNs and ORNs-D-mannitol at the concentration 2,5 mg/ml and 3,5 mg/ml respectively. Cell viability was determined by MTT-test 48 hours after the influenza A virus infection.

By suppressing influenza A viral infection, oligoribonucleotides-D-mannitol complexes increase cell viability at the concentration 3,5 mg/ml.



Enhanced viability of MDCK cells, which have been infected by the influenza virus, thanks to RNA drugs

Results and discussion



Inhibition of agglutination
of the human red blood cells 0 (I) by influenza A virus which were incubated with RNA drugs

RNA drugs effect on HA-glycan interactions activity

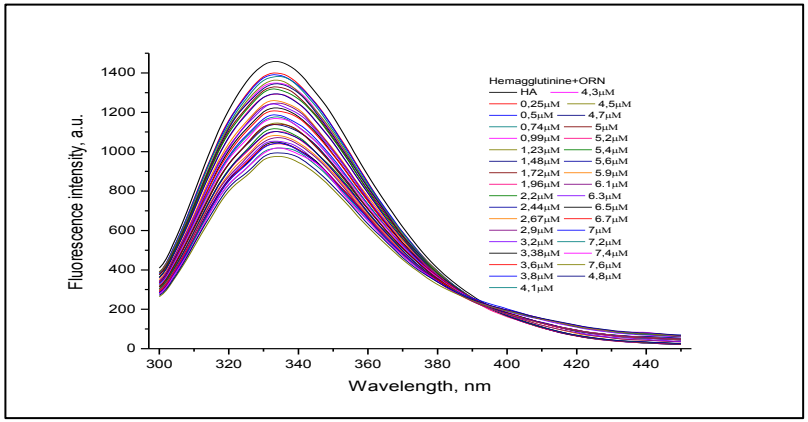
Effect of RNA drugs on the infectivity of influenza A virus was studied on human red blood cells 0 (I). The influenza A virus was incubated with ORNs (2,5 mg/ml) and ORNs-D-mannitol (3,5 mg/ml) 30 min 20°C. HA-glycan interactions activity was determined by agglutination assay.

Decrease of HA activity of influenza A virus by a factor 4 was observed after incubation of virus with ORNs-D-mannitol at the concentration 3,5 mg/ml in comparison to the virus control.

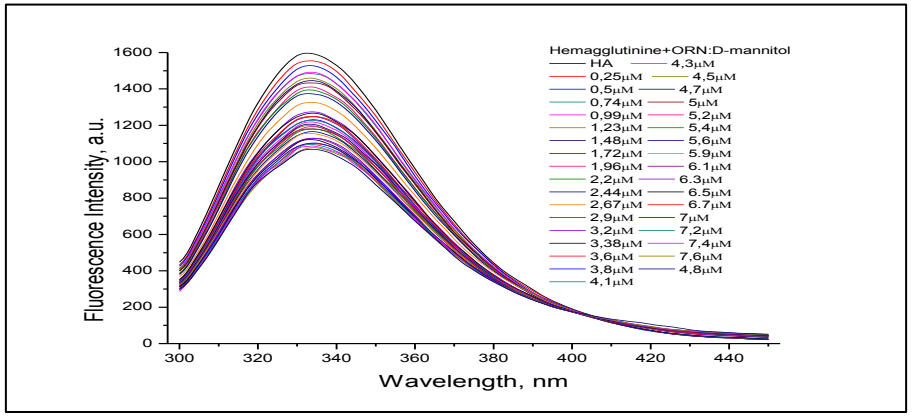
Results and discussion

RNA drugs effect on the fluorescence intensity of influenza A virus HA

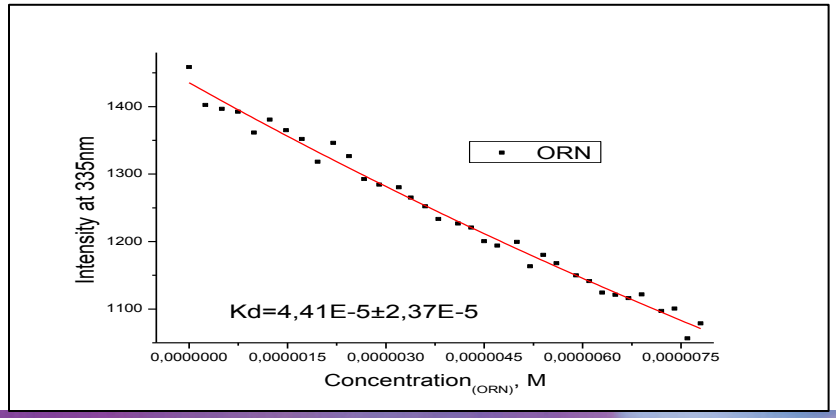
Fluorescence spectra HA under influence of the ORNs



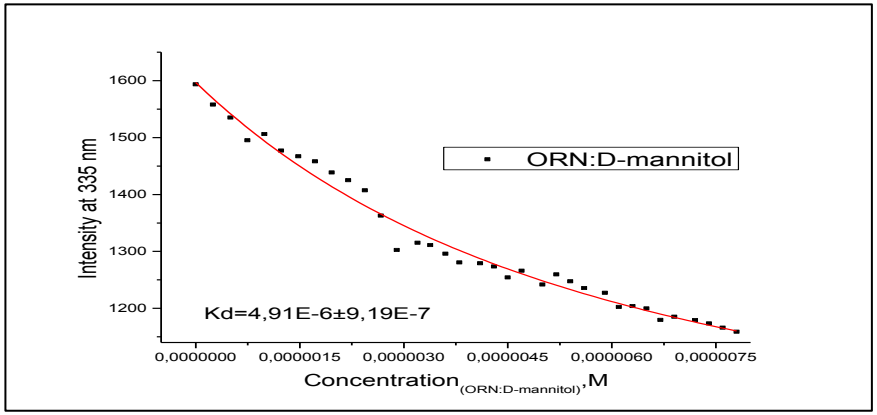
Fluorescence spectra HA under influence of the ORNs-D-mannitol



Dependence of the fluorescence intensity of the HA on ORNs concentration



Dependence of the fluorescence intensity of the HA on ORNs-D-mannitol concentration



Results and discussion

RNA drugs effect on the fluorescence intensity of influenza A virus HA

Spectrofluorometer Jasco FP-8200

The fluorescence melting curves were obtained from fluorescence spectra of protein-RNA solutions.

For estimating the dissociation constant (K_d) next formula was used where Θ is the fraction of bound to total protein at the stoichiometric point and P_0 is the total protein concentration in the cuvette.

$$K_d = \frac{(1-\theta)\times(D-(\theta\times P_0))}{\theta}$$

It was observed that the fluorescence intensity of HA of flu virus reduced in the presence of the ORNs-D-mannitol. This effect may indicate that interactions between HA and ORNs-D-mannitol are responsible for conformation changes of HA.

Conclusions

- Oligoribonucleotides-D-mannitol complexes reduce influenza A virus infectivity affecting the hemagglutinin-glycan interactions *in vitro*.

- By suppressing influenza A viral infection, oligoribonucleotides-D-mannitol complexes increase cell viability *in vitro*.

- Complexes of Oligoribonucleotides-D-mannitol interact with hemagglutinin of the influenza A virus, and in this manner inhibit HA-glycan interactions.