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## **Influence of the Oligoribonucleotides-D-Mannitol Complexes on Upexpression of some Genes Induced by Influenza Virus in vivo.**

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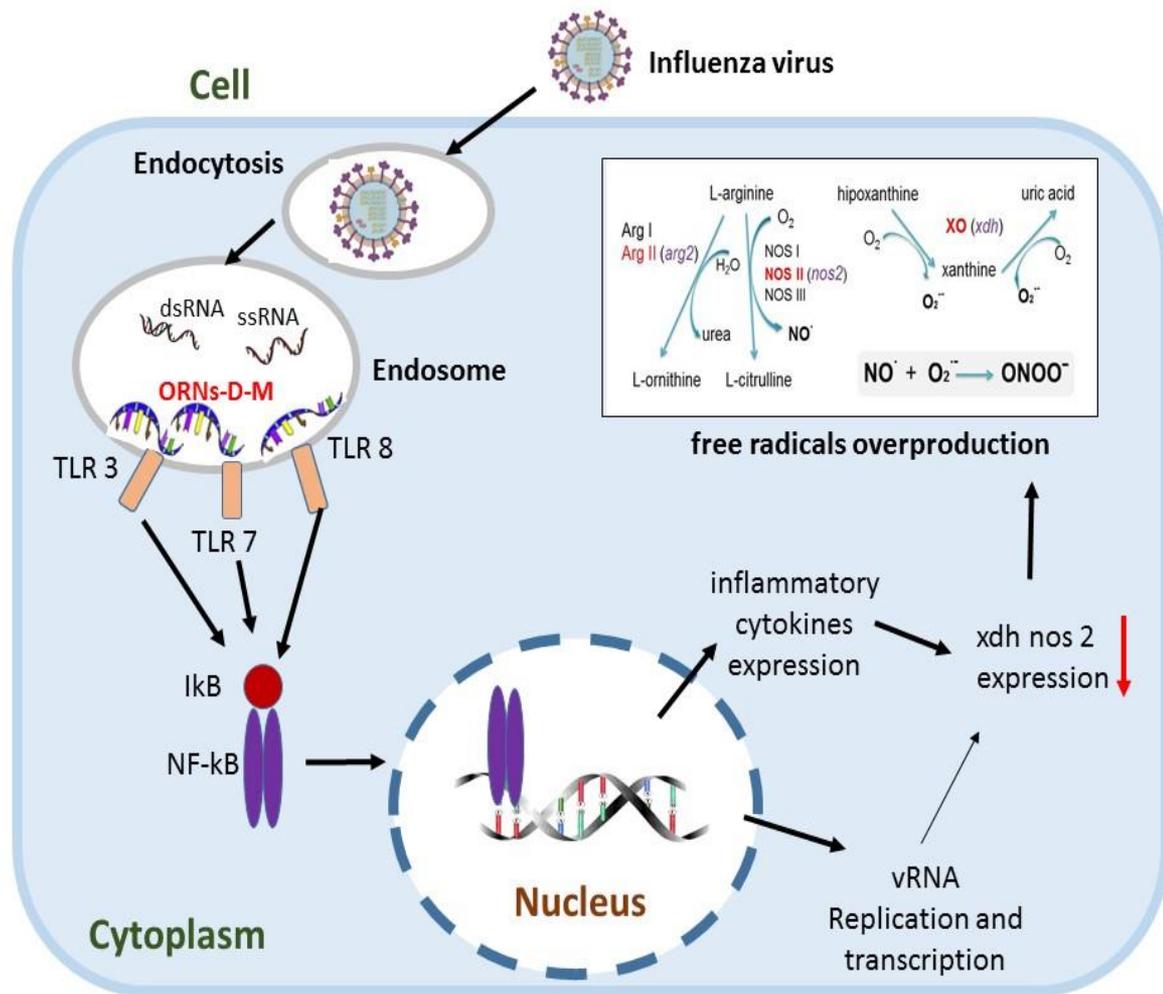
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# Title of the Presentation

Our data showed that by inhibiting the expression of *tlr3*, *tlr7*, *tlr8*, oligoribonucleotides-D-mannitol complexes can impair the upregulation of *nos2*, *xdh*, *nfkbia*, *nfk1* induced by influenza virus.



Schematic diagram of the mechanism of suppression of influenza virus-induced *nos2* and *xdh* genes by the ORNs-D-M



**Abstract:** The influenza (flu) virus infection induces an upexpression of *tlr3*, *tlr7*, *tlr8*, *nfkb1*, *nfkb1 $\alpha$* , *nos2*, *xdh* and other genes in mice lunge. One of mechanisms of oligoribonucleotides-D-mannitol complexes (ORNs-D-M) anti-influenza activity is direct virucidal action by blocking hemagglutinin–glycan interactions and inhibiting neuraminidase activity of flu virus. However other mechanisms of the ORNs-D-M anti-influenza activity have to be study.

Current research was aimed at study of the ORNs-D-M effects on expression of the *tlr3*, *tlr7*, *tlr8*, *nfkb1 $\alpha$* , *nfnb1* *nos2*, *xdh* genes in mice lung under flu virus infection. To achieve this goal we applied a two-step RT-PCR assay. In mice lunge after 48 h flu infection it was detected the overexpression of all investigated genes compared to the healthy ones. The ORNs-D-M injection for prevention reduced the mRNA level of *tlr3*, *tlr7*, *tlr8*, *nfkb1 $\alpha$* , *nfnb1* *nos2*, *xdh* expression vs. the virus-infected mice. And the ORNs-D-M injection for treatment reduced the mRNA level of *tlr3*, *tlr7*, *tlr8*, *nfkb1 $\alpha$* , *nfnb1* *nos2*, *xdh* expression vs. the virus-infected mice. Our results show that the expression of all investigated genes is modulated by the ORNs-D-M after injection for prevention and treatment of the flu virus infection *in vivo*.

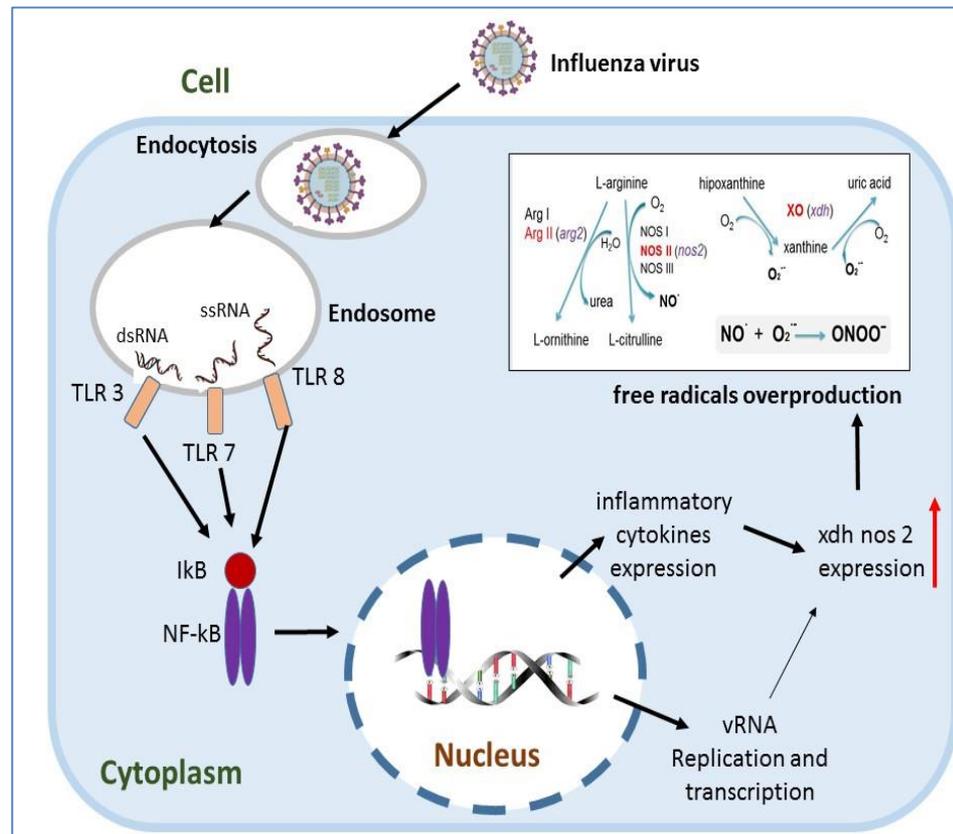
**Keywords:** influenza, NF- $\kappa$ B, oligoribonucleotides-D-mannitol complexes



# Introduction

Influenza virus is an important human pathogen, which causes worldwide epidemics and pandemics. The lung is one of the most widely investigated targets for influenza virus infection and potentially at high risk of injury mediated by oxygen-derived free radicals and lipid peroxidation products.

The influenza virus infection induces an upexpression of *tlr3*, *tlr7*, *tlr8*, *nfkb1*, *nfkbia*, *nos2*, *xdh* and other genes in mice lunge. The *nos2* and *xdh* upexpression causes overproduction of free radicals that lead to lung tissue damage.



Influenza-virus-induced signaling processes and their functions in the infected host cell.



# Introduction

The upexpression of *nfk1*, *nfbia* genes induce incorrect regulation of NF- $\kappa$ B that regulates expression of the immune, inflammation genes and takes part in influenza viral replication.

During influenza viral replication, single stranded (ss RNA) and ds RNA are intermediate molecules which are recognized by the toll-like receptors 3, 7, 8, 9 (TLRs) which are expressed by cells of the innate immunity including dendritic cells, natural killer cells and macrophages as well as on respiratory epithelium and elsewhere. When activated, they trigger immune and inflammatory responses to respond to these infectious agents.

The oligoribonucleotides-D-mannitol complexes (ORNs-D-M) possess antiviral activity against the influenza virus *in vitro* and *in vivo*. One of mechanisms of the ORNs-D-M anti-influenza activity is direct virucidal action by blocking hemagglutinin–glycan interactions and inhibiting neuraminidase activity of influenza virus. However other mechanisms of the ORNs-D-M anti-influenza activity have to be studied.



# Materials & Methods



## Experimental groups:

- **Control** – healthy mice (NaCl injection, 0,9%);
- **+ ORN<sub>S</sub>** – ORNs (15 mg/kg) injection in mice;
- **+ ORN<sub>S</sub>-D-M** – ORNs-D-M (15 mg/kg) injection in mice;
- **+ Influenza** – infection of mice with influenza virus;
- **+ ORN<sub>S</sub> + Influenza** – ORNs (15 mg/kg) injection 24 h before influenza virus infection;
- **+ ORN<sub>S</sub>-D-M + Influenza** – ORNs-D-M (15 mg/kg) injection 24 h before influenza virus infection;
- **+ Influenza + ORN<sub>S</sub>** – ORNs (15 mg/kg) injection 24 h after influenza virus infection;
- **+ Influenza + ORN<sub>S</sub>-D-M** – ORNs-D-M (15 mg/kg) injection 24 h after influenza virus infection.

The 8-10 weeks old **BALB/c mice**  
The mouse-adapted influenza virus  
**A/FM/1/47(H1N1)**, 4.0 lg LD<sub>50</sub>

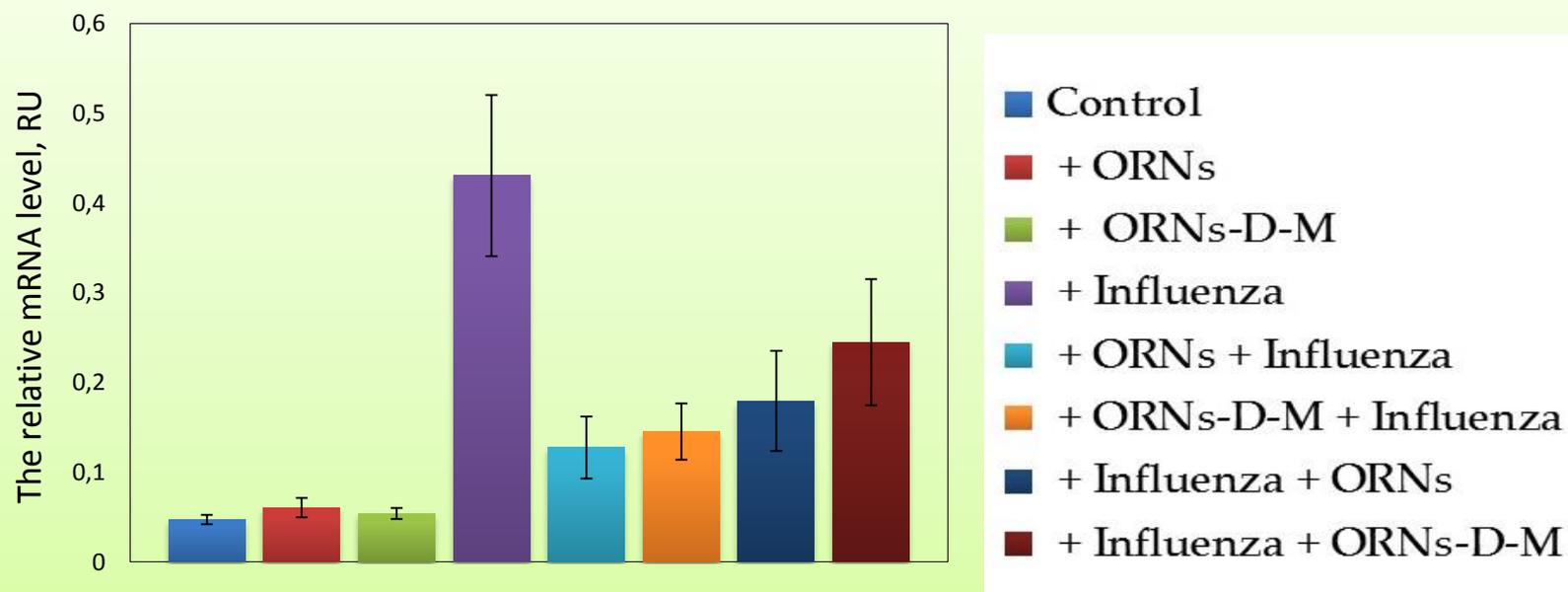
The mRNA level of investigated genes was tested by **RT-PCR assay**.  
Samples were normalised to *gapdh* as a control.



## Results and discussion

Effects of both prevention and treatment with the RNA drugs on the expression of *nos2* under influenza virus infection

The mRNA of *nos2* has been detected to increase in 6.7 times in mice lung after 48 h influenza virus infection in comparing to control. Increasing the mRNA of *xdh* in 2.8 and 4.8 times was detected after prevention and treatment with ORNs-D-M respectively in comparing to control. These data indicate that both prevention and treatment with the RNA drugs can decrease the expression of *nos2* during the influenza virus infection.



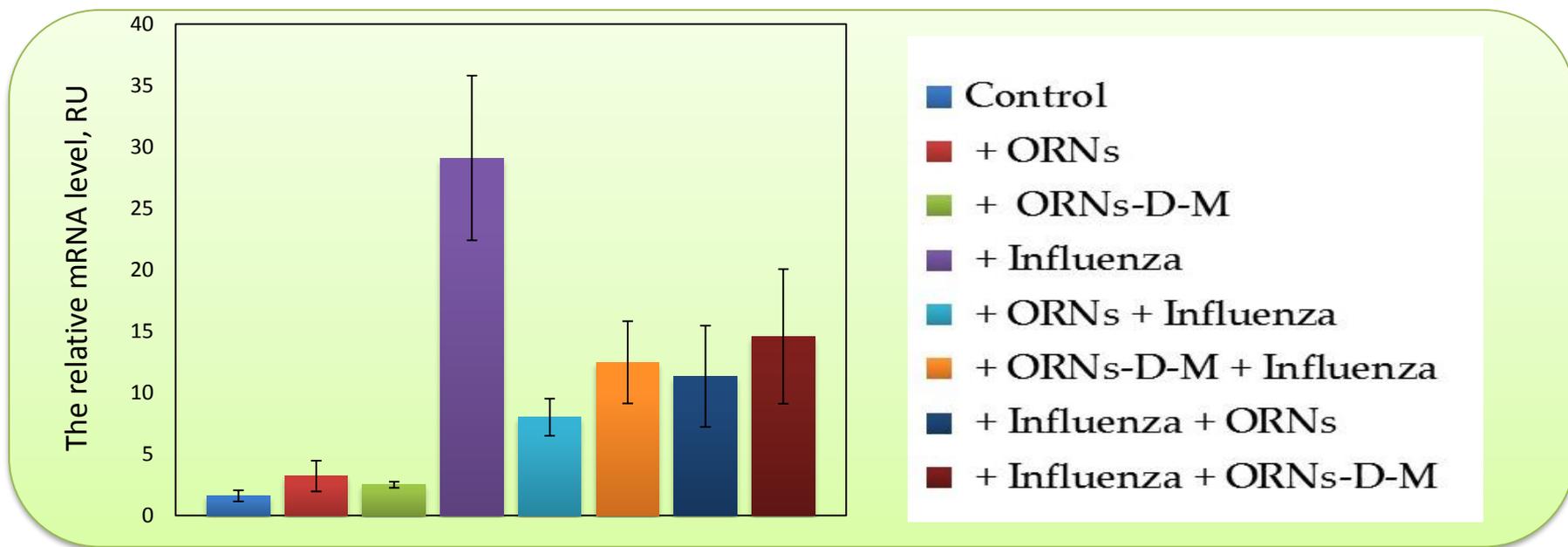
The mRNA level of *nos2* (NO synthase II) in mice lung.



## Results and discussion

Effects of both prevention and treatment with the RNA drugs on the expression of *xdh* under influenza virus infection

It was shown that the mRNA of *xdh* increased in 18 times in mice lung after 48 h influenza virus infection in comparing to control. However, the mRNA of *xdh* after prevention and treatment with ORNs-D-M increased in 7 and 9 times respectively vs control. These data indicate that both prevention and treatment with the ORNs-D-M can decrease the expression of *xdh* during the influenza virus infection.



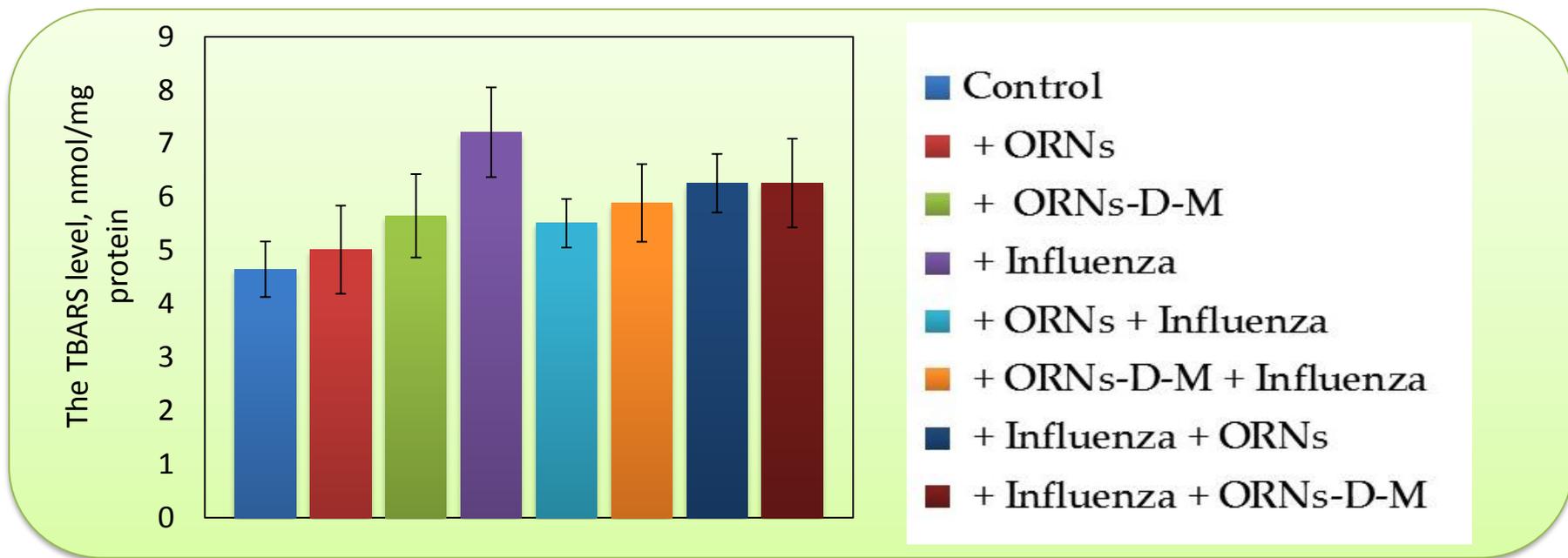
The mRNA level of *xdh* (xanthinoxidase) in mice lung.



## Results and discussion

Effects of both prevention and treatment with the RNA drugs on the level of **LPO products** under influenza virus infection.

In mice lung after 48 h influenza virus infection the level of thiobarbituric acid reactive species (TBARS) was found to be high by 48 % compared to control, whereas both prevention and treatment with the ORNs-D-M decreased the level of TBARS compared to influenza infected mice. These data indicate probable decrease of the protein level of *nos2* and *xdh* genes during both prevention and treatment with the ORNs-D-M.



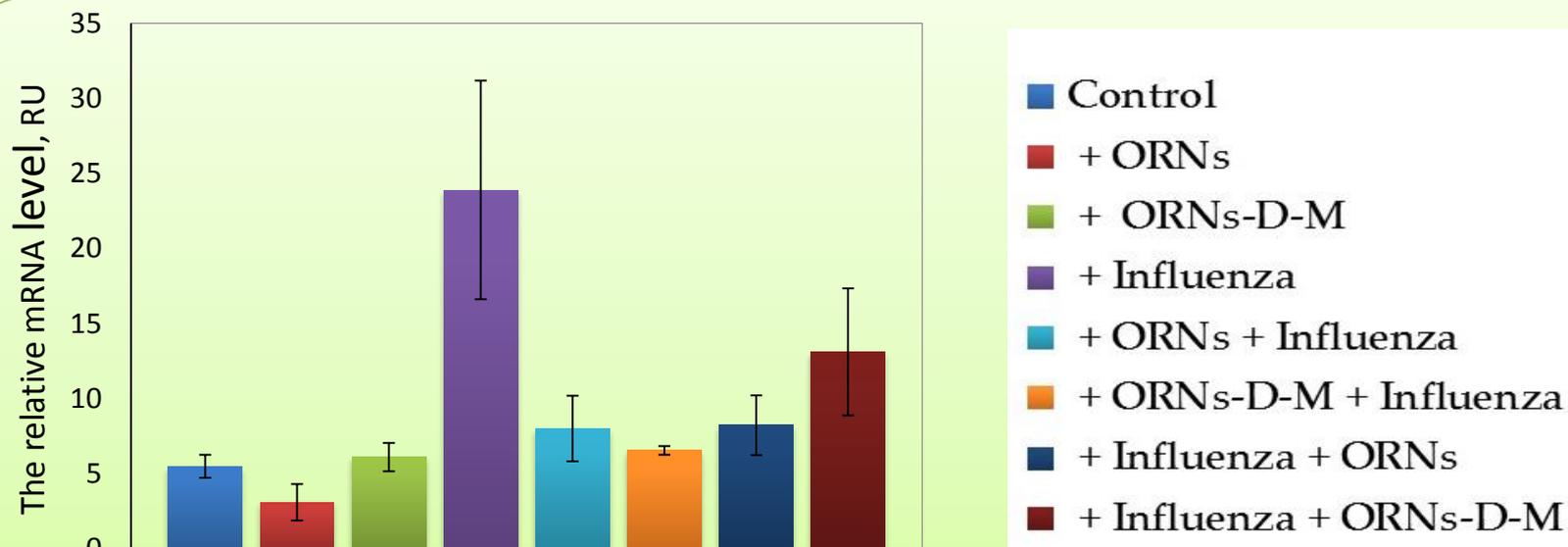
The level of TBARS in mice lung.



## Results and discussion

Effects of both prevention and treatment with the RNA drugs on the expression of *nfkbia* under influenza virus infection

Increasing mRNA expression of *nfkbia* was observed in 4.3 times in influenza infected mice lung compared with control. Whereas mRNA expression of this gene increased in 2.4 times in mice lunge after treatment with ORNs-D-M and unchanged in mice lunge after prevention with ORNs-D-M compared to control. These results indicate that the ORNs-D-M can reduce the expression of *nfkbia* at the influenza virus infection.



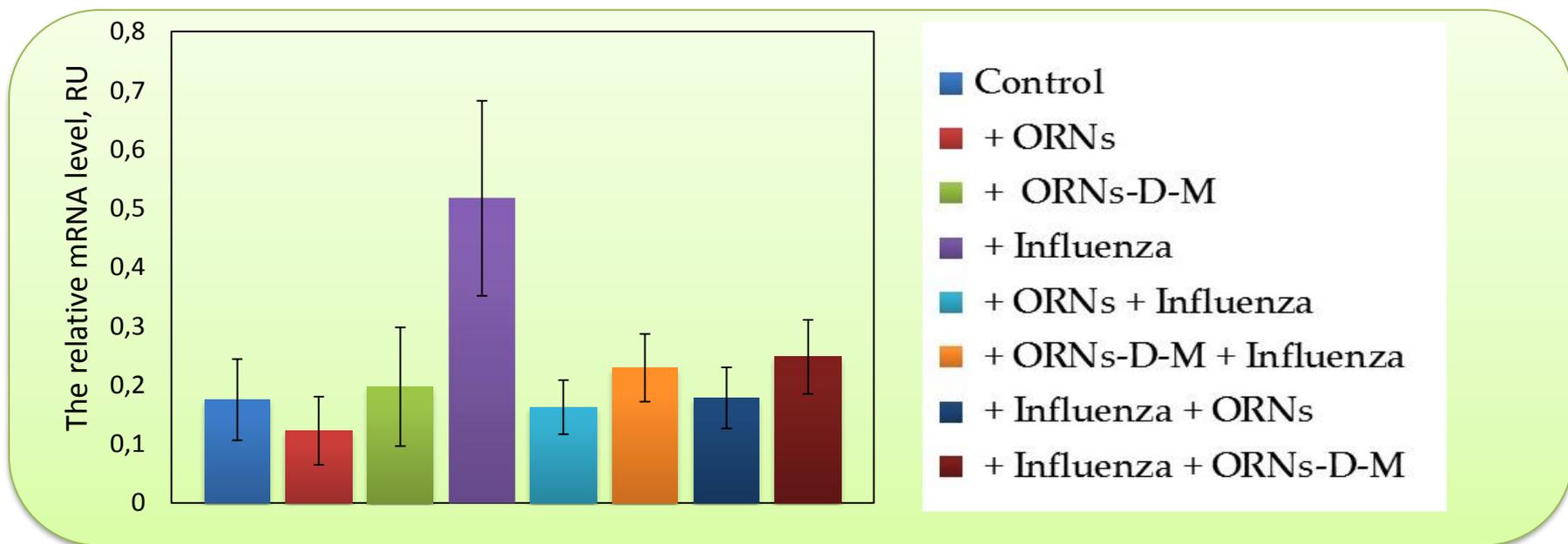
The mRNA level of *nfkbia* (NF- $\kappa$ B inhibitor  $\alpha$ , I $\kappa$ B) in mice lung.



## Results and discussion

Effects of both prevention and treatment with the RNA drugs on the expression of *nfkb1* under influenza virus infection

While investigating the mRNA level of *nfkb1* we observed the same tendency as during studding of the *nos2*, *nfkbia* and *xdh*. During 48 h influenza virus infection the mRNA level of *nfkb1* increased in 2.9 times and at both prevention and treatment with the ORNs-D-M of the influenza virus infection it increased in 1.3, 1.5 times respectively vs control. These data also indicate reducing the expression of *nfkb1* by the ORNs-D-M under influenza virus infection.



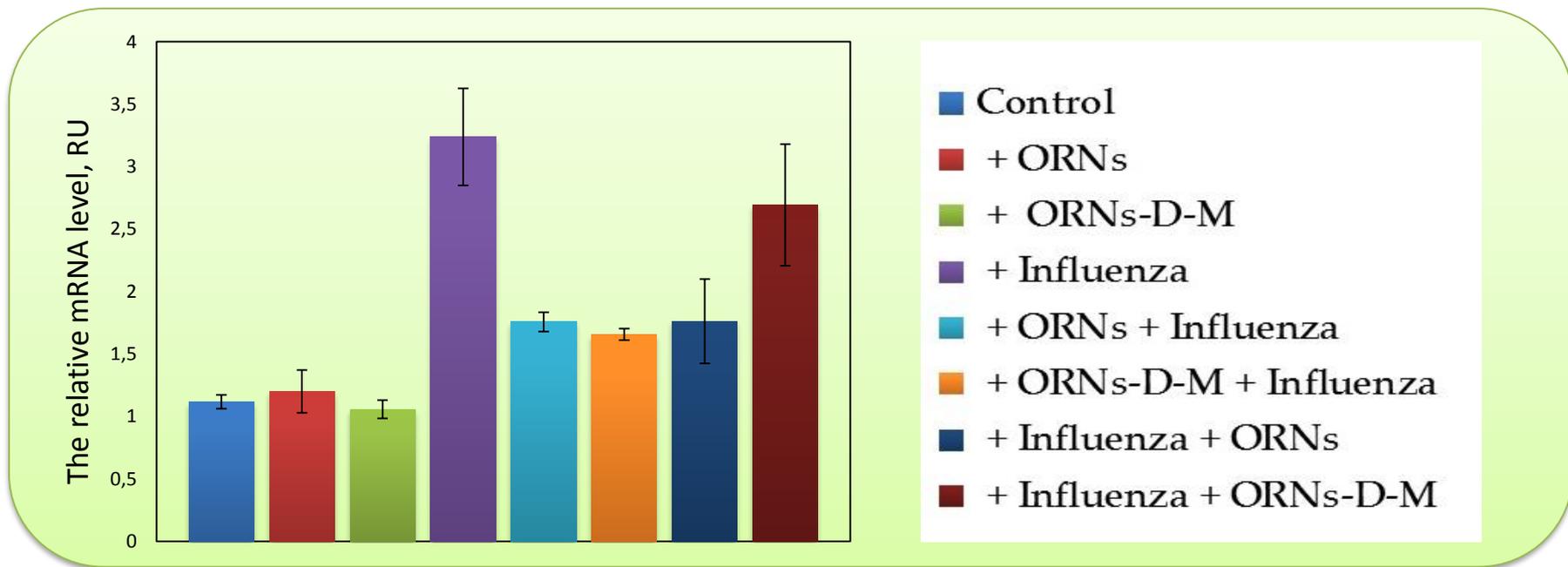
The mRNA level of *nfkb1* (p50 subunit of NF- $\kappa$ B) in mice lung.



## Results and discussion

Effects of both prevention and treatment with the RNA drugs on the expression of *tlr3* under influenza virus infection

The mRNA of *tlr3* has been found to increase in 2.3 times in influenza virus infected mice lung vs control. Increasing the mRNA of *tlr3* by 33% and in 1.8 times was detected after prevention and treatment with ORNs-D-M respectively in comparing to control. These data indicate that the ORNs-D-M can decrease the expression of *tlr3* at the influenza virus infection.



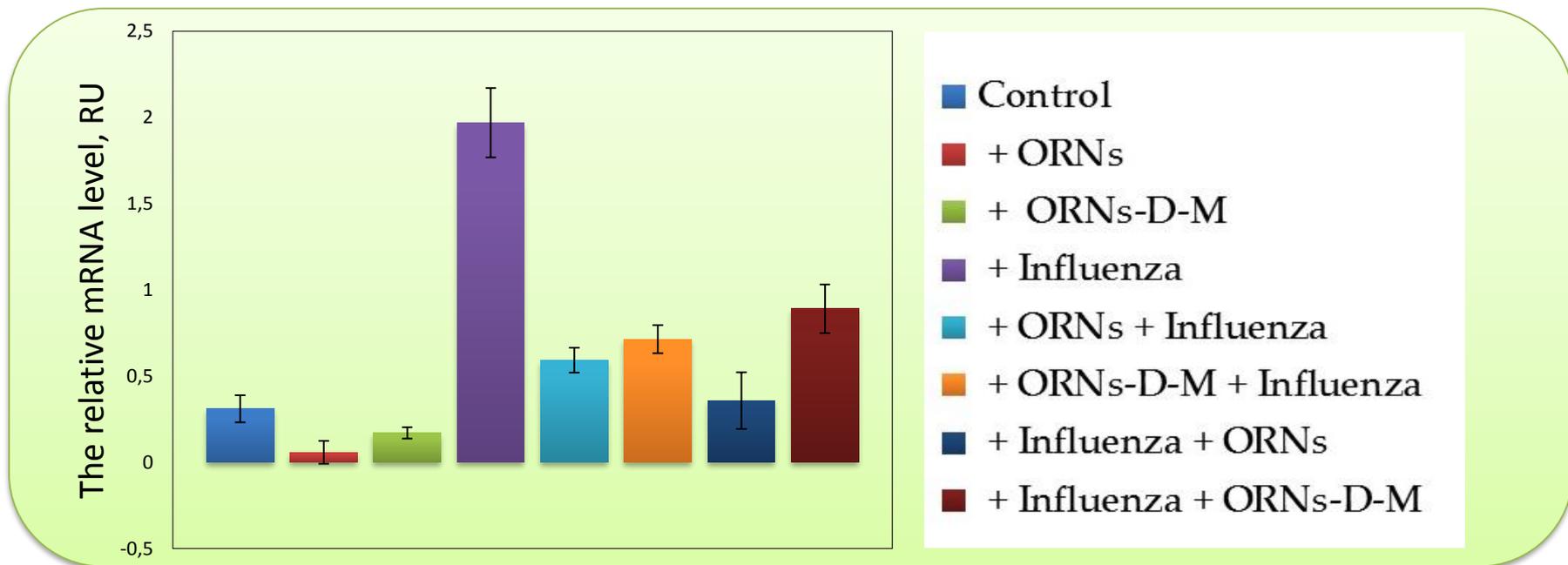
The mRNA level of *tlr3* (Toll-like receptors 3) in mice lung.



## Results and discussion

Effects of both prevention and treatment with the RNA drugs on the expression of *tlr7* under influenza virus infection

The mRNA expression of *tlr7* increased in 6.5 times in influenza virus infected mice lung vs control. Whereas mRNA expression of this gene increased in 2.3 and 3 times in mice lunge after prevention and treatment with ORNs-D-M compared to control. These results show that the ORNs-D-M can also reduce the expression of *tlr7* at the influenza virus infection.



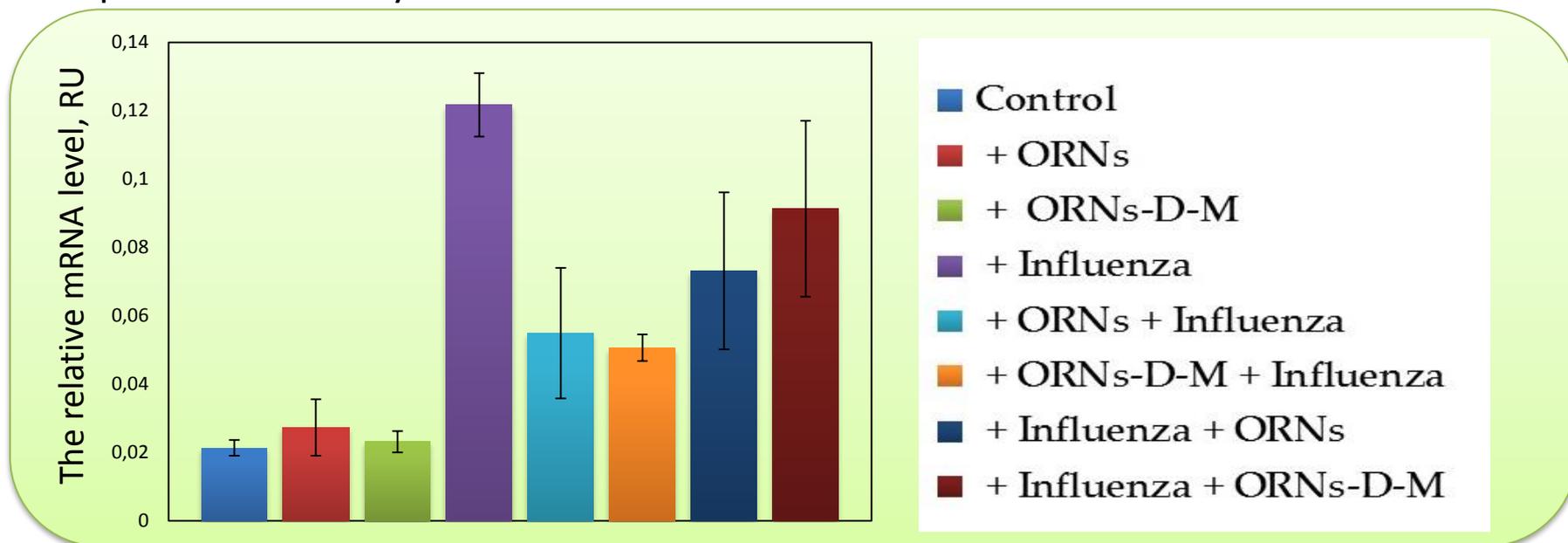
The mRNA level of *tlr7* (Toll-like receptors 7) in mice lung.



## Results and discussion

Effects of both prevention and treatment with the RNA drugs on the expression of *tlr8* under influenza virus infection

Investigating the mRNA level of *tlr8* we found the same tendency as during studying the mRNA level of *tlr3*, *tlr7*, *nfkbia*, *nfnb1* *nos2*, *xdh*. The mRNA expression of *tlr8* increased in 6 times in influenza virus infected mice lung vs control. Increasing the mRNA level of *tlr8* in 2.5, 4.5 times respectively was observed in mice lunge after prevention and treatment with the ORNs-D-M of influenza virus infection vs control. These results demonstrate a decreasing the expression of *tlr8* by the ORNs-D-M at influenza virus infection too.



The mRNA level of *tlr8* (Toll-like receptors 8) in mice lung.



## Conclusions

- The expression of *nos2*, *xdh*, *nfkbia*, *nfk b1*, *tlr3*, *tlr7*, *tlr8* genes is modulated by the ORNs-D-M injection for prevention and treatment of the influenza virus infection *in vivo*.
- By inhibiting the expression of *tlr3*, *tlr7*, *tlr8*, the ORNs-D-M impair the upregulation of *nos2*, *xdh*, *nfkbia*, *nfk b1* induced by influenza virus.
- The ORNs-D-M can be antagonists of the Toll-like receptors 3, 7 and 8.

