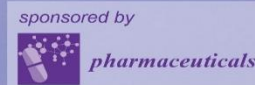




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chaired by Dr. Jean Jacques Vanden Eynde



## PROTEOLYSIS INHIBITOR AMINOCAPROIC ACID AS EFFECTIVE DRUG FOR PREVENTION AND TREATMENT OF INFLUENZA, OTHER ACUTE RESPIRATORY VIRAL INFECTIONS AND THEIR BACTERIAL COMPLICATIONS

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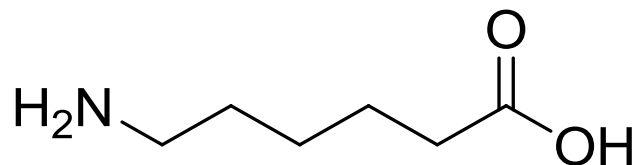
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# PROTEOLYSIS INHIBITOR AMINOCAPROIC ACID AS EFFECTIVE DRUG FOR PREVENTION AND TREATMENT OF INFLUENZA, OTHER ACUTE RESPIRATORY VIRAL INFECTIONS AND THEIR BACTERIAL COMPLICATIONS

## Graphical abstract



**Abstract:** We have established the efficacy of proteolysis inhibitor (PI) aminocaproic acid (ACA) in viral infections as a result of its impact on the etiological factor and pathogenesis of the infection. The Ministry of Health of Ukraine on the basis of our studies and clinical trials allowed using ACA for prophylaxis and treatment of influenza and other acute respiratory viral infections (ARVI). Including ACA to therapeutic complex for the treatment of influenza and other ARVI in children and neonates led to a decrease of the duration of symptoms of intoxication, catarrhal phenomena, and fever as well as to a decrease of the number of complications. The prevention effectiveness of ACA has also been established. The obtained results allow to recommend the use of ACA for the efficient prophylaxis of ARVIs and pneumonia in organized collectives in the period of increased incidences of these infections. Our research has shown that the combined use of drugs with different mechanisms of action - PI ACA and neuraminidase inhibitor Tamiflu- causes a synergistic effect. We also studied the antibacterial action of ACA on *S. aureus* strains with different sensitivity to antibiotics. ACA inhibits all these strains, and the combined use of ACA with antibiotics magnifies the antibacterial effect.

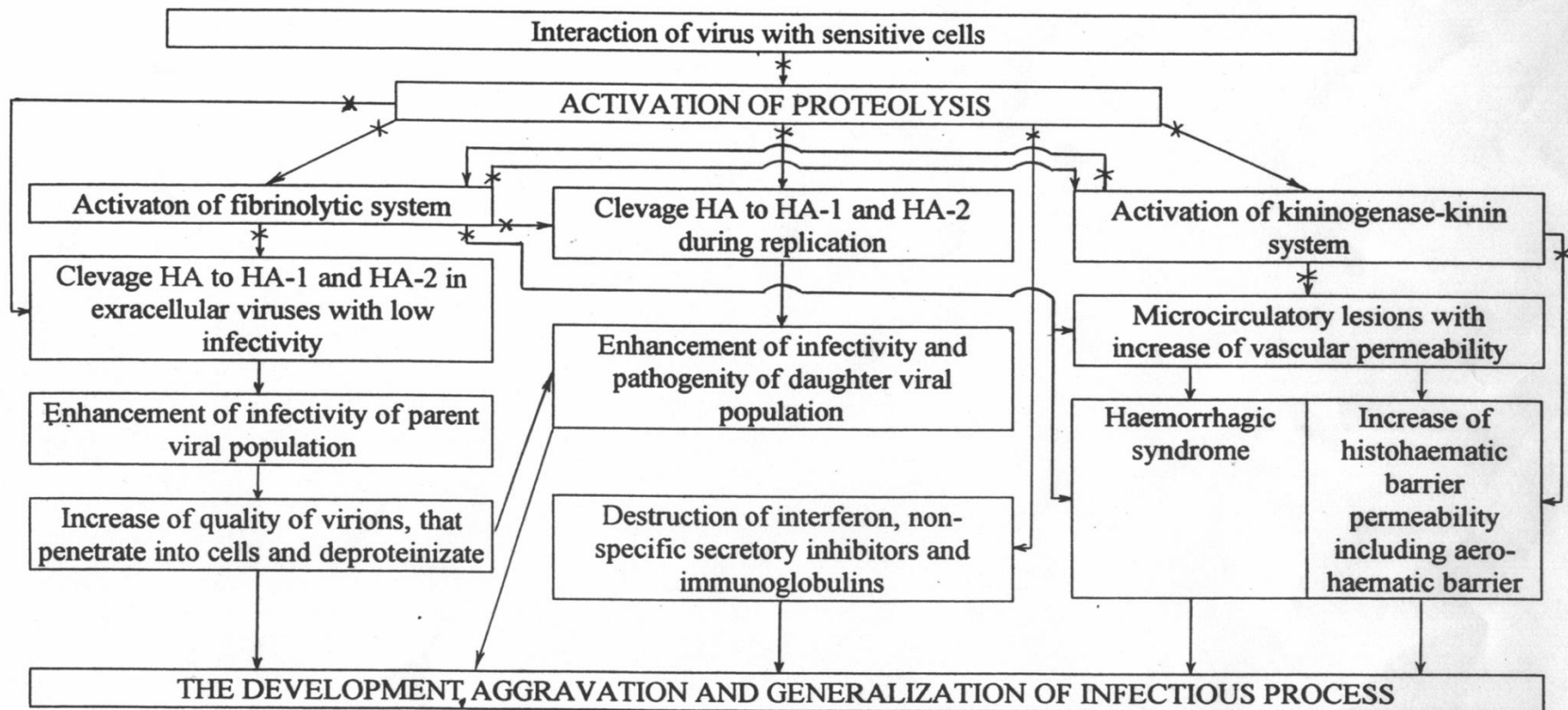


## INTRODUCTION

The proteolytic cleavage is a universal mechanism of specific proteins activation. The activation of proteolysis plays an important role in the pathogenesis of many diseases including viral infections. The results of our research and the scientific data have made it possible to formulate the concept of “vicious circle” formation: virus activates the proteolytic systems, which in turn assist the development, generalization and aggravation of the infectious process at the expense of influence on the etiological and pathogenetical factors.



The inhibitors of proteolysis (IP) may prevent the formation or destruction of this “vicious circle”.



THE PARTICIPATION OF PROTEOLYTIC SYSTEM DURING DEVELOPMENT OF INFLUENZA INFECTION AND AETHIOPATHOGENETIC ACTION OF PROTEASE INHIBITORS (x- SHOW THE POINTS OF INHIBITORS ACTION)



Generally PI  $\epsilon$ -aminocaproic acid (name of medicine “Aminocaproic acid” ACA) is used for hemostasis when fibrinolysis contributes to bleeding. ACA is a low toxic drug. The intravenous and oral LD<sub>50</sub> of ACA is 3.0 and 12.0 g/kg respectively in the mouse. An intravenous infusion dose of 2.3 g/kg was lethal in the dog.

## Toxicity of E-ACA (LD 50 g/kg)

Method of application	Rats	Mice
Oral	16.4	12
Intravenous	3.2	3



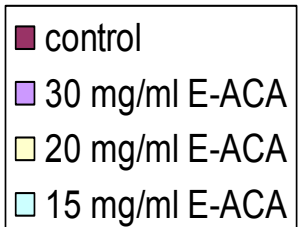
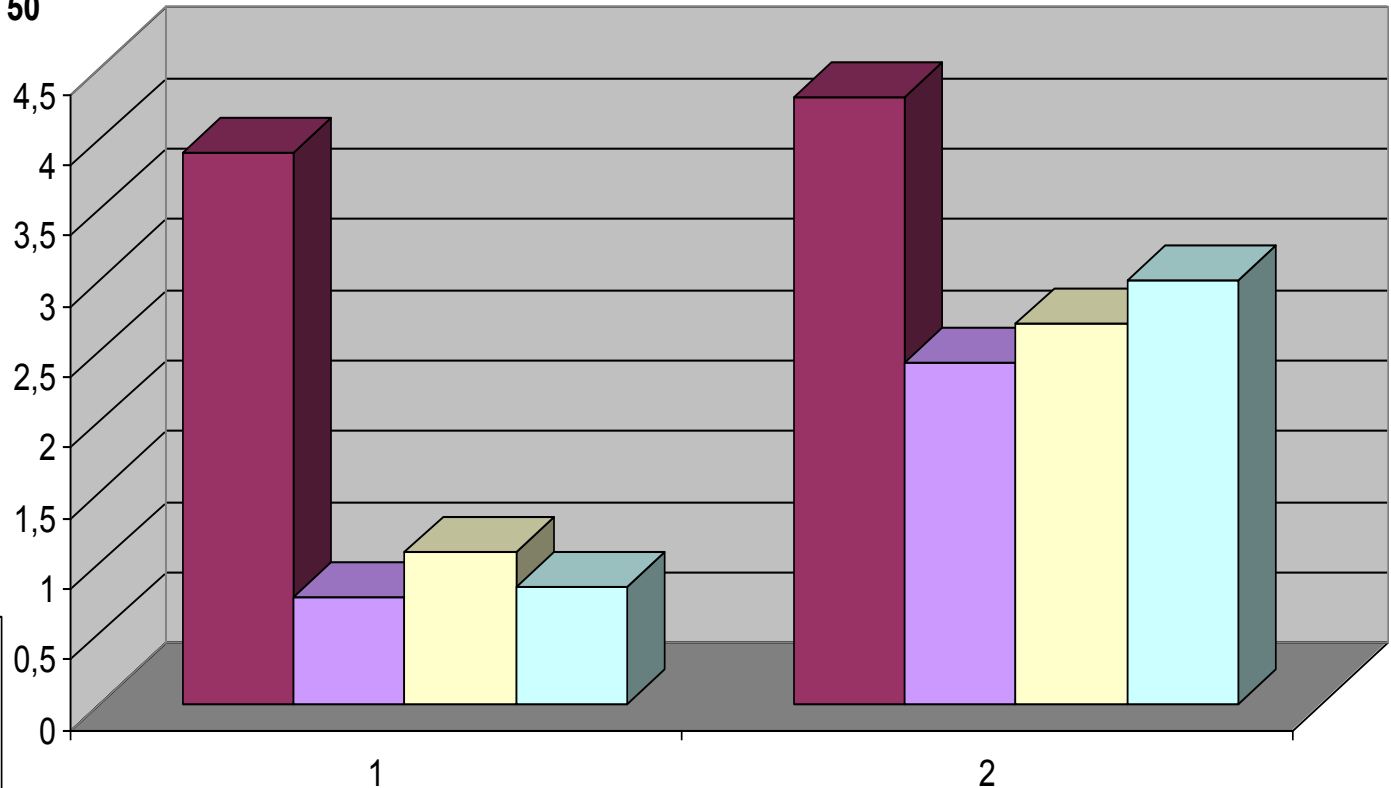
**ACA prevents the enhancement of proteolysis during the interaction of virions with cell membranes, decreases penetration of virions into sensitive cells. It brings down proteolytic cleavage of HA- precursor to HA-1 and HA-2 and reduces the infectious virus harvest. High levels of ACA anti-influenza efficacy *in vitro* were shown on subtypes H1N1; H2N2; H3N2 of human, H5N3 and H7N3 avian influenza A viruses and on influenza B viruses.**

**Results showed that both H5N3 and H7N3 avian influenza viruses are sensitive to ACA, but virus with H5 is more sensitive.**



## The influence of E-ACA on avian influenza virus H5H3 (1) and H7H3 (2) replication

- log<sub>10</sub> TID 50

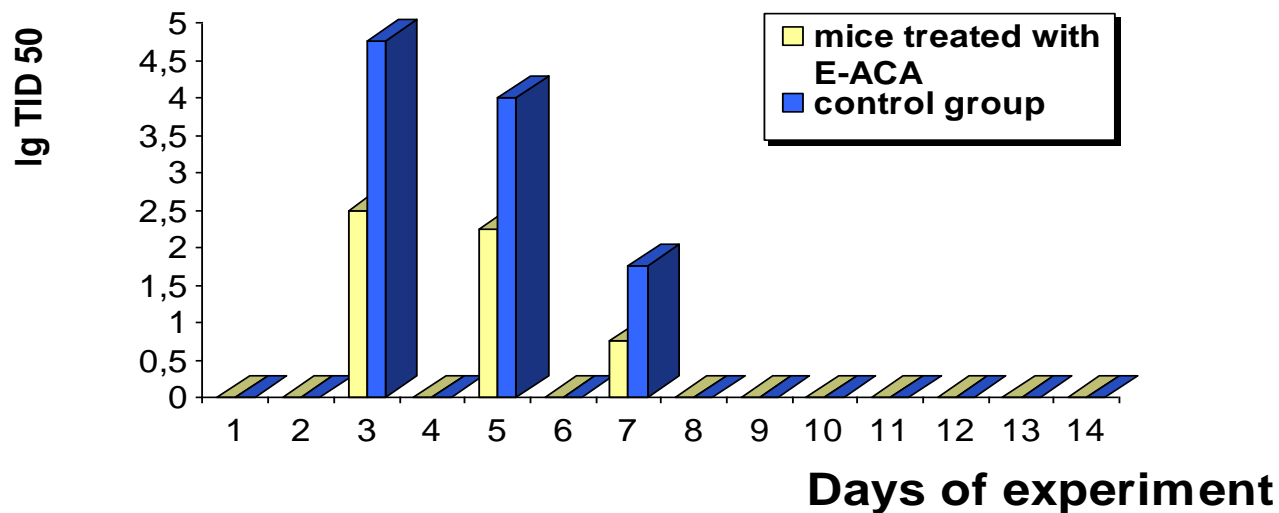




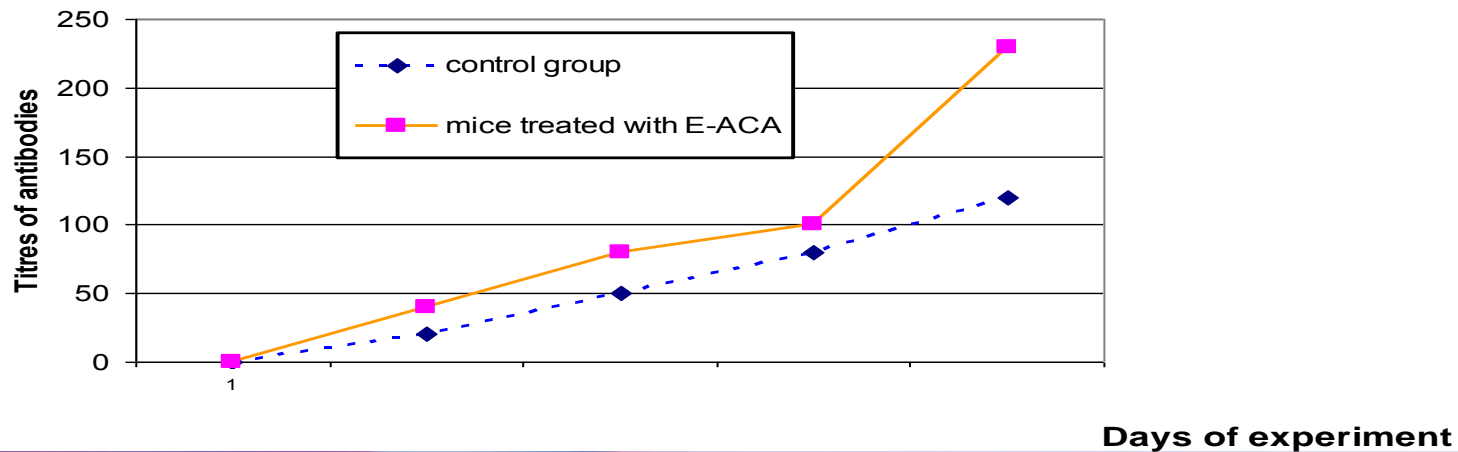
**ACA shows prophylactic and therapeutic effects during the enhancement of alkaline proteases activity in lung after infection. Treated by ACA after infection, mice were more protected to re-infection. ACA, when used in the treatment of influenza, decreased virus reproduction in lungs and also enhanced the humeral immune response. The titers of antibodies on days 14 to 21 post infection were significantly higher in the treated animals. On day 30 after challenge with the homologous strain (H3N2), the virus reproduced to lower level in the lungs of untreated convalescent mice, but no virus was detected in the lungs of mice that had been treated with ACA during primary infection. Significant increase of the antibody level was found in such mice. We believe that the immunomodulatory effect of ACA may play an important role in the increased resistance to challenge.**



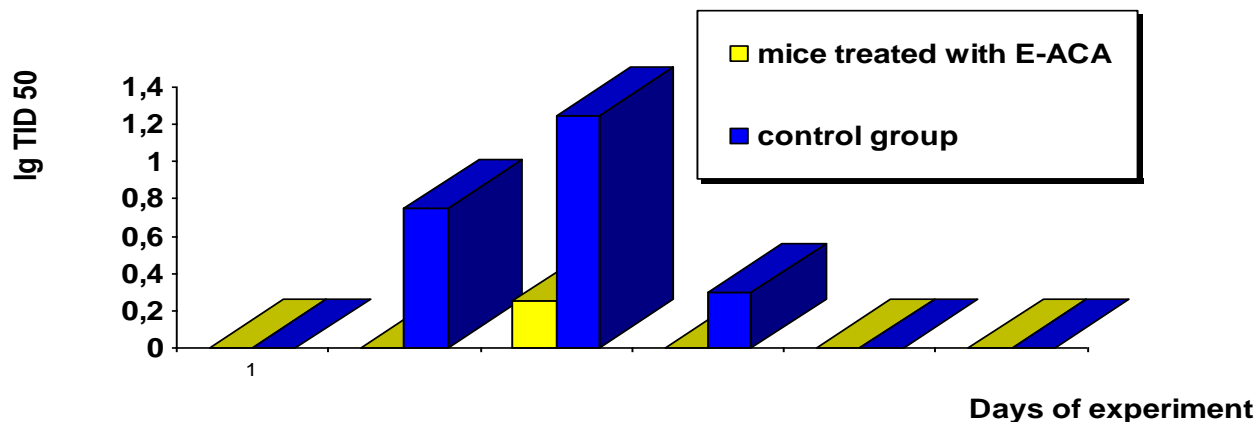
## Influence of therapeutic E-ACA usage on replication of virus A/Hong Kong/1/68 in murine lungs



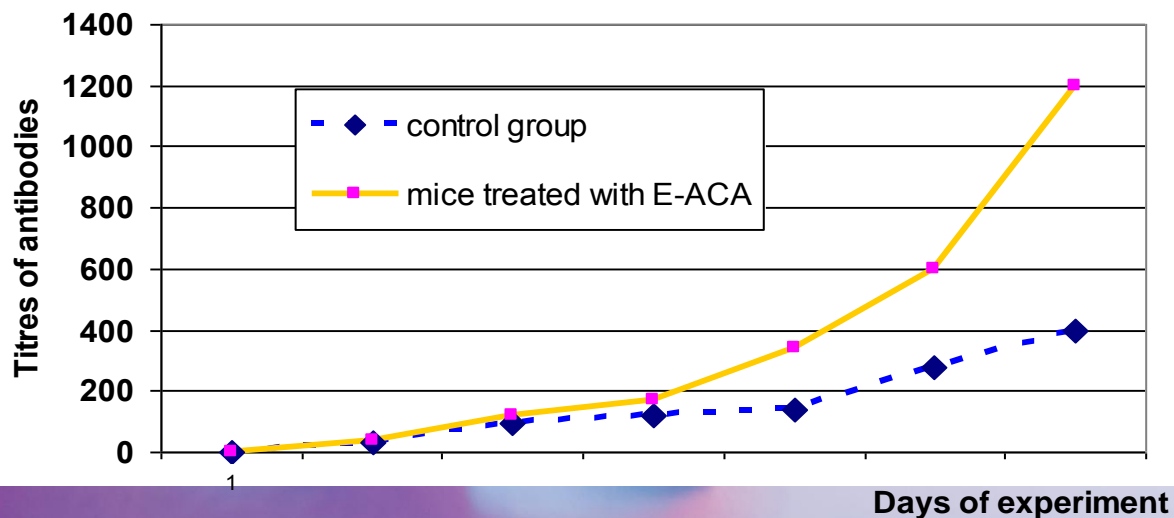
## Influence of E-ACA on titres of antibodies to influenza virus A/Hong Kong/1/68



## Influence of treatment with E-ACA of primary infection on replication of influenza virus A/Hong Kong/1/68 (H3N2) in murine lungs after reinfection



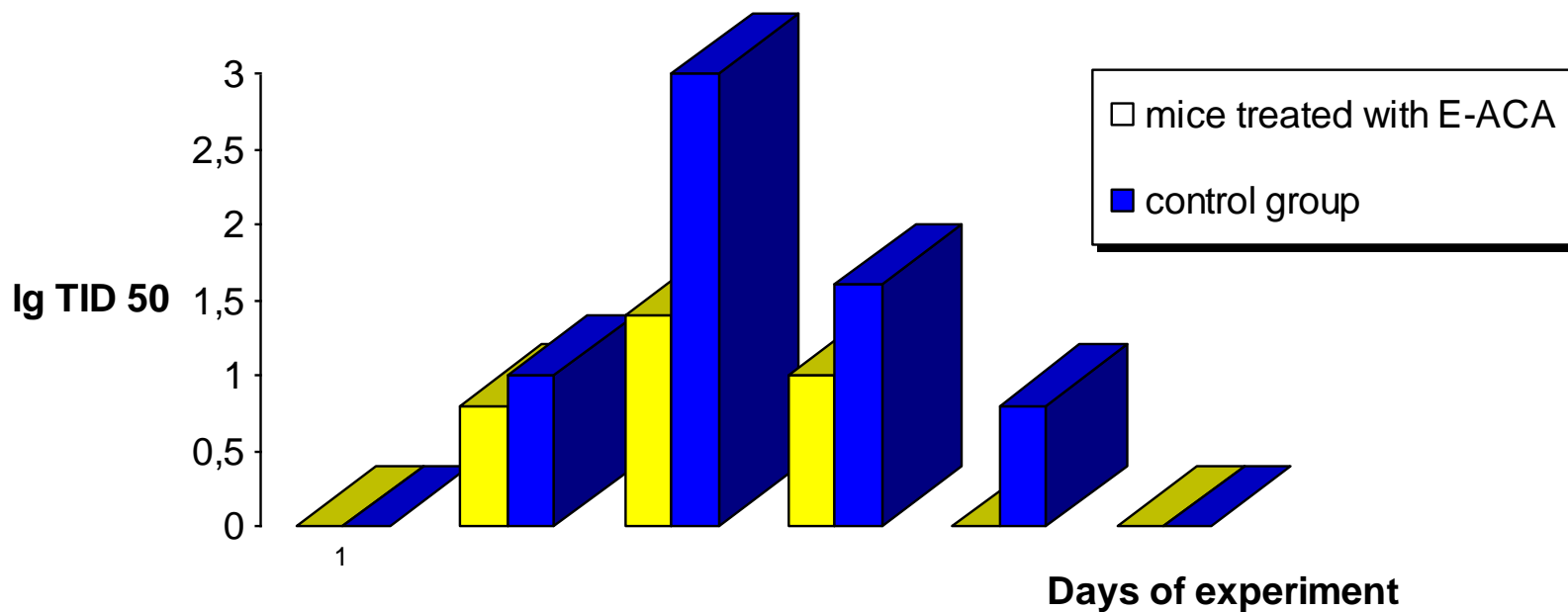
## Influence of treatment with E-ACA of primary infection on titres of antibodies of influenza virus A/Hong Kong/1/68 after reinfection



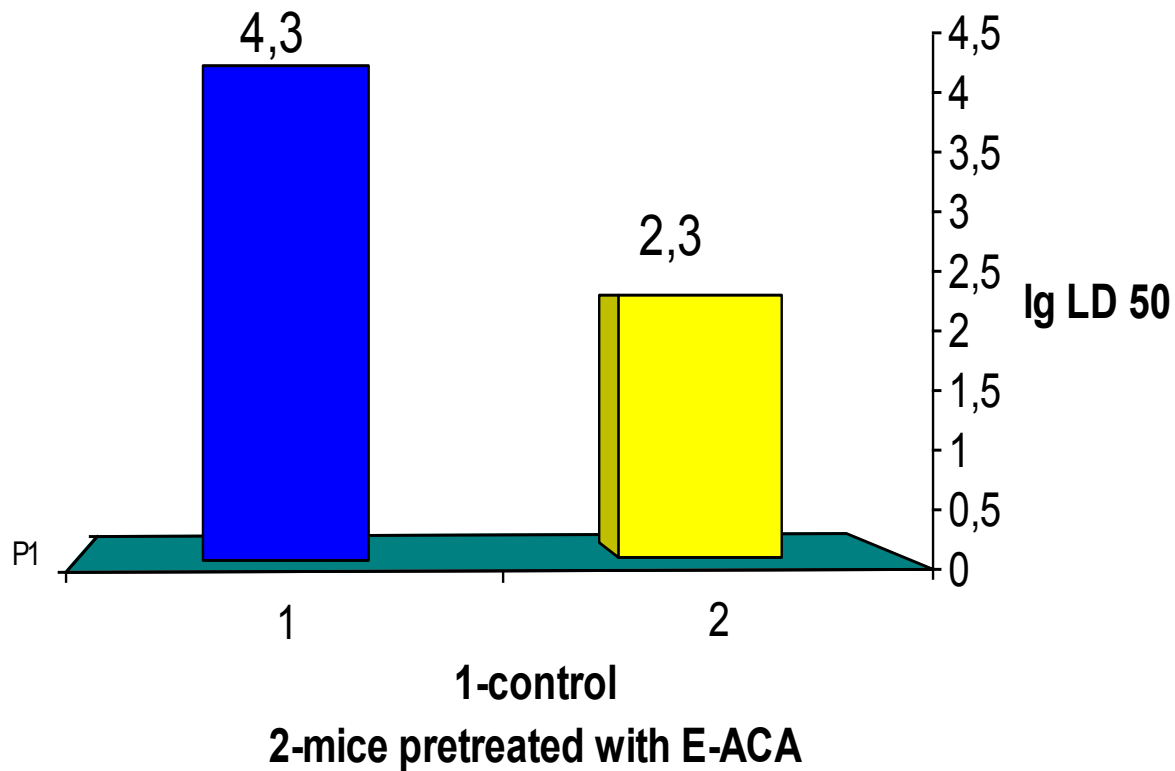
**The reproduction of influenza virus in the lungs was reduced 2 and 10 days after a single dose of ACA as well as 4 weeks after 5-day prophylactic treatment. This correlated with the ability of proteolysis inhibitor to stimulate early production of specific serum antibodies. The favorable effect of prophylactic administration of ACA was the most effective at experimental lethal influenza. The results of immunization by inactivated influenza virus were better when combined with ACA.**



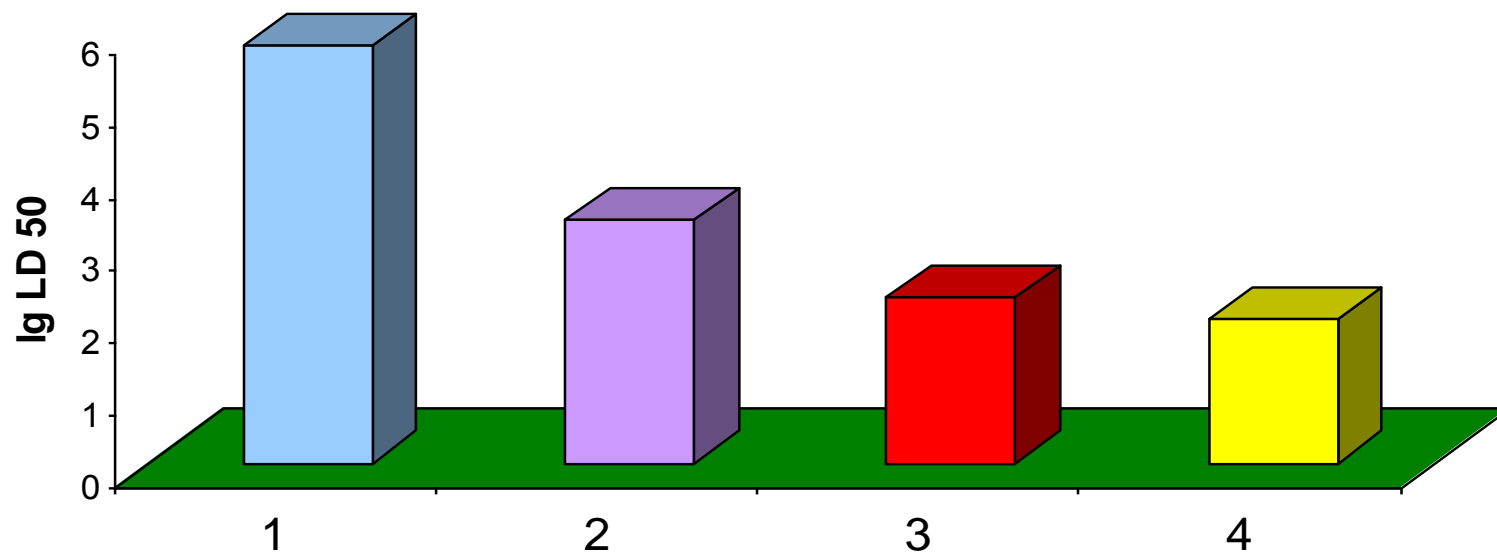
## Prophylactic influence of E-ACA on replication of virus A/Hong Kong/1/68 in murine lungs



## Action of prophylactic usage of E-ACA on death of infected with influenza virus A/PR/8/34 mice



## Influence of E-ACA on efficacy of immunization of mice with inactivated influenza virus



1 - control; 2 - immunization with inactivated influenza virus (512 HAU);  
3 - E-ACA (30 mg); 4 - immunization + E-ACA



**State Pharmacological Center and Ukrainian Ministry of Health on the basis of our experimental research and clinical trials have allowed the use of ACA as antiviral agent for prevention and treatment of influenza and other acute respiratory viral infections (ARVI) in children and adults. Recommendations for administration of ACA for treatment of influenza are as follows:**

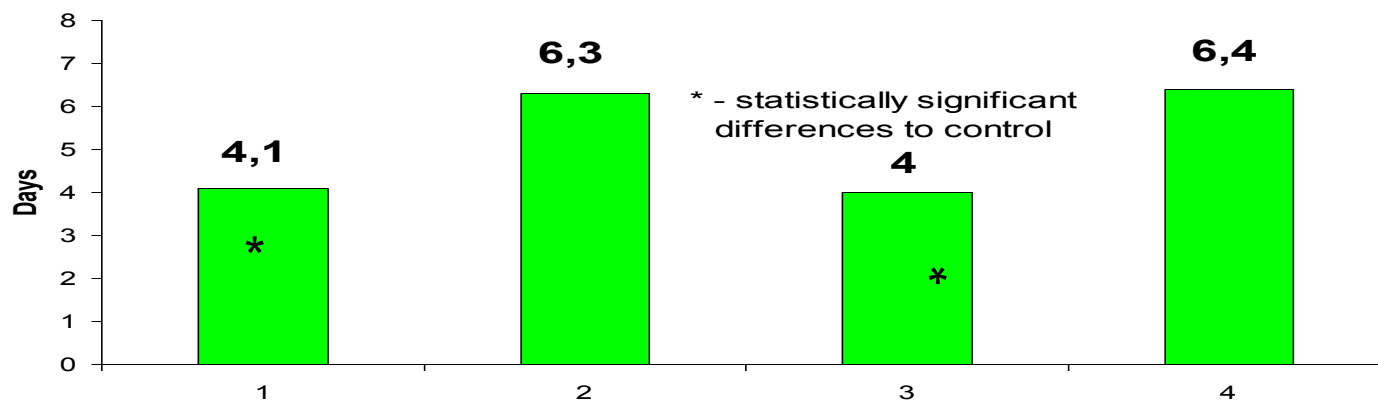
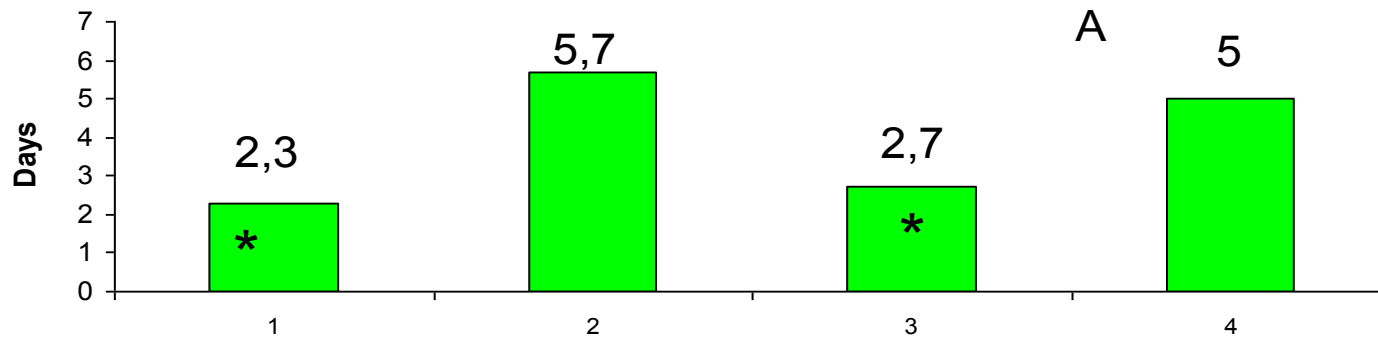
- ACA is used orally, intravenously, nasally or in inhalations during 3-7 days;**
- ACA is given orally 4 times a day in daily doses 25-75 mg/kg;**
- Besides that, intranasal instillations (every 4h) or inhalations (twice a day) of 5% E-ACA solution are expedient.**

**It is important to note that ACA is unique in the world of anti-viral medicine allowed for medical use in neonates and infants. We determined that proteolytic activity (pH 7,6) in blood of hospitalized patients was statistically higher than such activity in blood of healthy children. Therefore use of proteolytic inhibitor ACA was not only etiological but also pathogenetically justified. As results of including ACA in the therapeutic complex for treatment of influenza and other ARVI in children are decreases of duration of intoxication symptoms, of fever, and of catarrhal symptoms. Quantity of complications was reduced to 17%.**





## Influence of E-ACA on duration of intoxication symptoms in patients with non-complicated (A) and complicated (B) forms of influenza or other ARVI



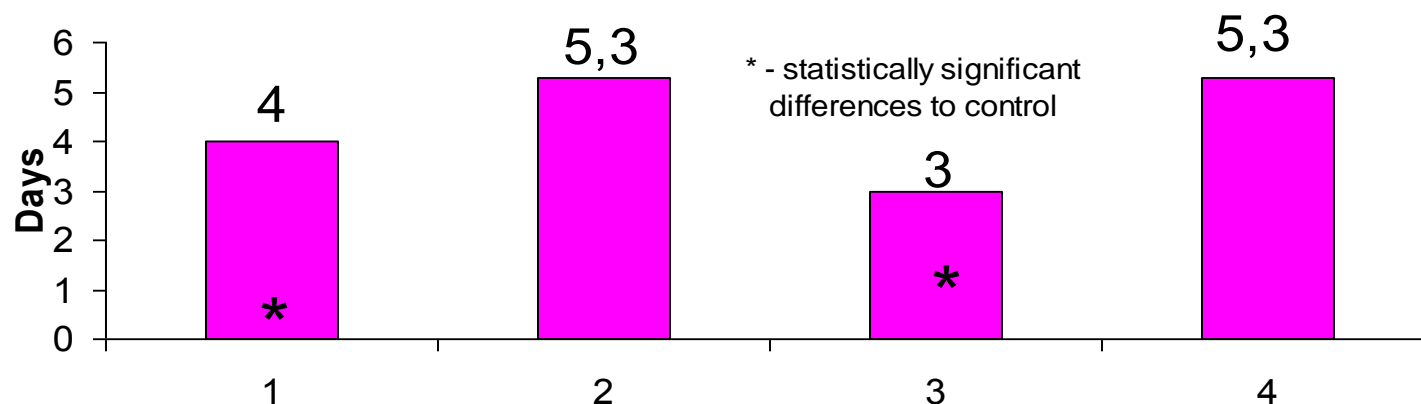
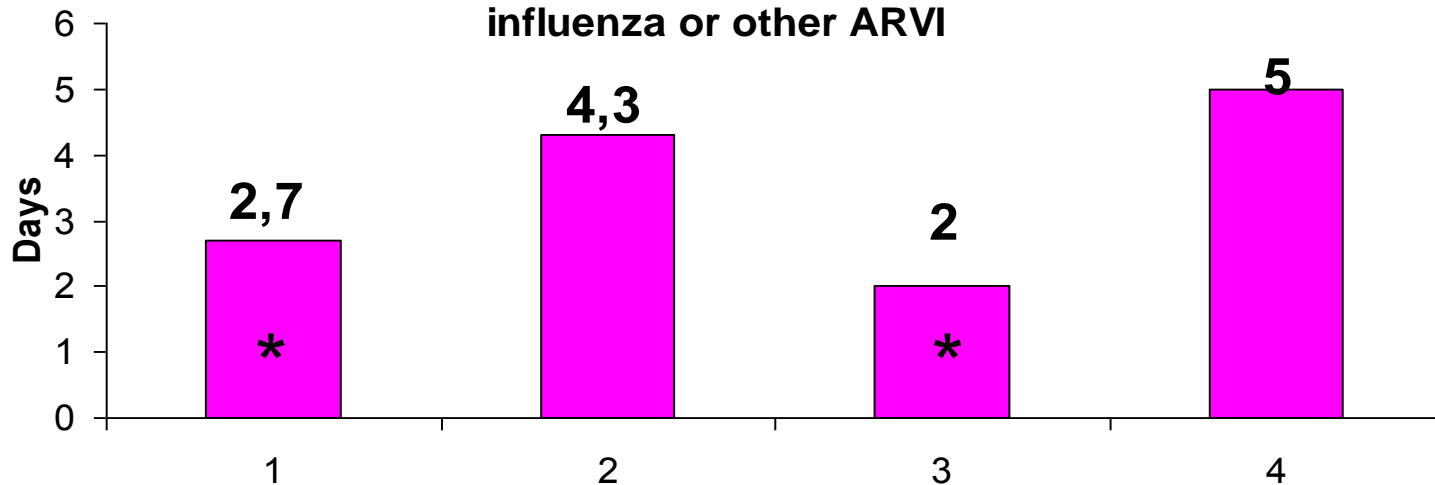
**Children under 1 years old:** 1.Treated with E-ACA;  
2.Non treated with E-ACA (control);  
**Children from 1 to 3 years old:** 3.Treated with E-ACA;  
4.Non treated with E-ACA (control)

\*

\*



## Influence of E-ACA on duration of fever in patients with non-complicated (A) and complicated (B) forms of influenza or other ARVI



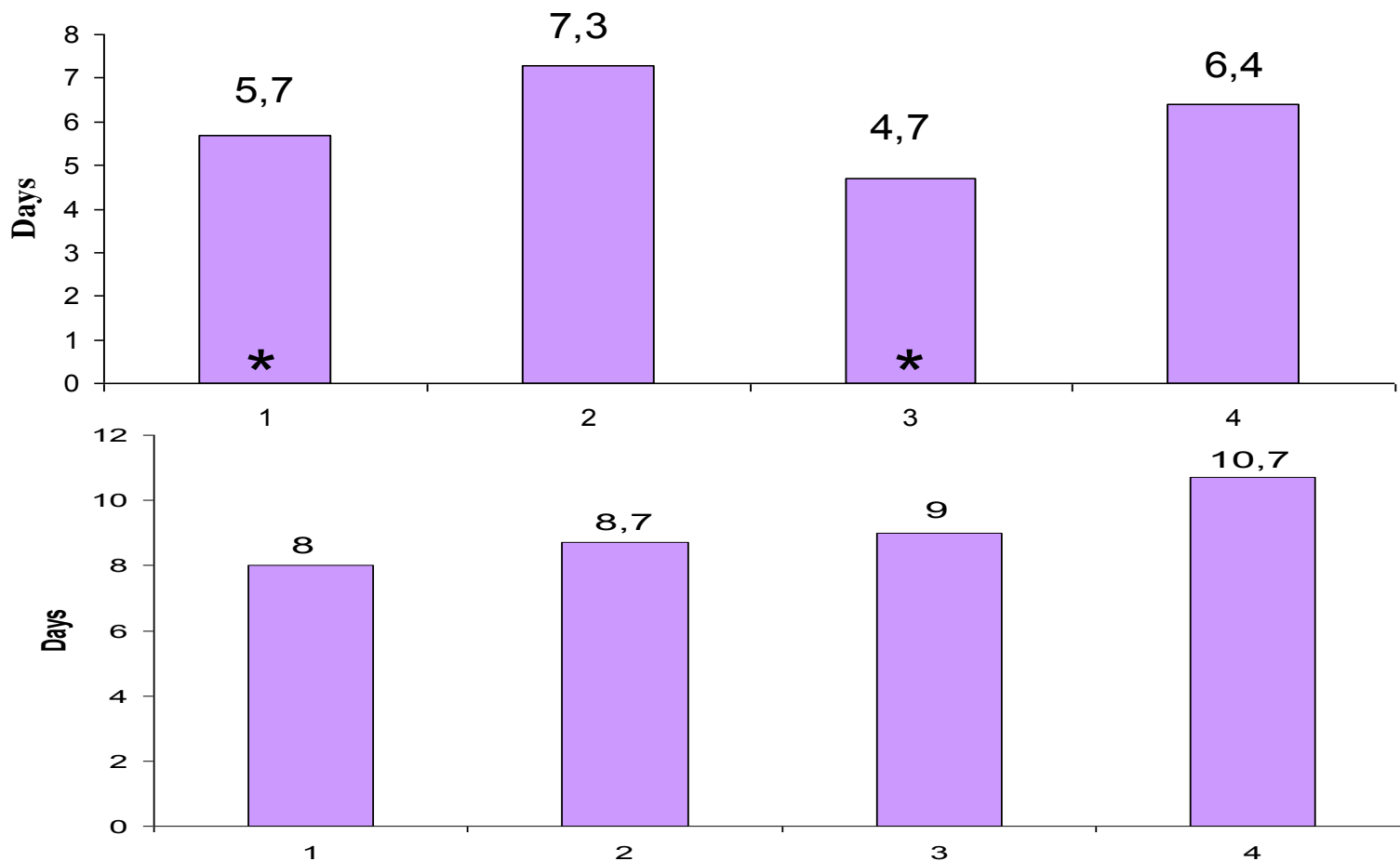
**Children under 1 years old:** 1. Treated with E-ACA;

2. Non treated with E-ACA (control);

**Children from 1 to 3 years old:** 3. Treated with E-ACA;



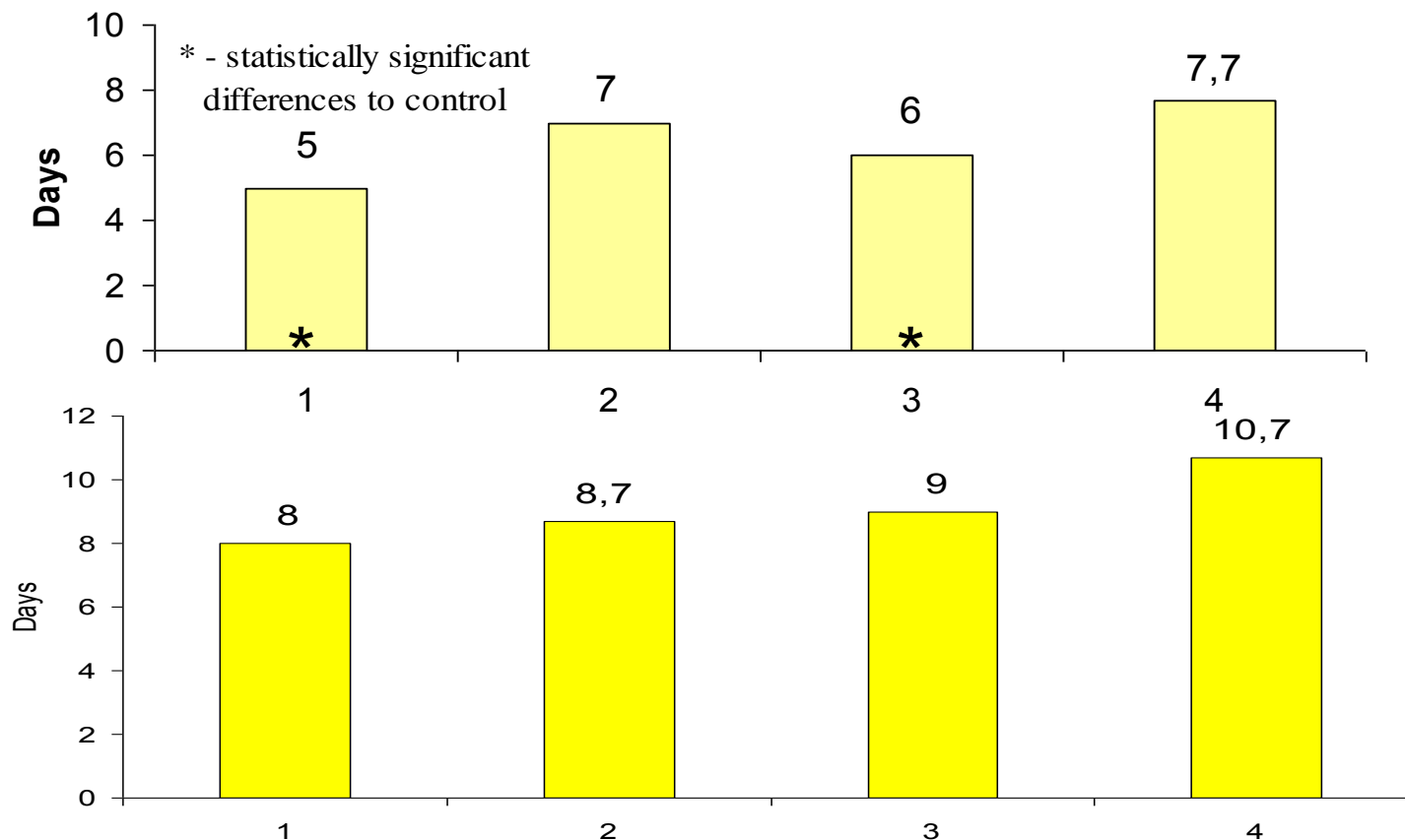
## Influence of E-ACA on duration of catarrhal symptoms in patients with non-complicated (A) and complicated forms (B) of influenza or other ARVI



**Children under 1 years old:** 1.Treated with E-ACA; 2.Non treated with E-ACA (control); **Children from 1 to 3 years old:** 3.Treated with E-ACA; 4.Non treated with E-ACA (control)



## Influence of E-ACA on duration of physical changes in lungs of patients with non-complicated (A) and complicated (B) forms of influenza or other ARVI



**Children under 1 years old:**

1. Treated with E-ACA; 2. Non treated with E-ACA (control);

**Children from 1 to 3 years old:** 3. Treated with E-ACA; 4. Non treated with E-ACA (control)



The prevention effectiveness of ACA has also been established. With the scope to study the ACA prophylactic activity in organized collectives, we have prescribed it per-orally in 2.0 g dose four times a day during a week. The monitoring was performed in two independent collectives (923 young adults males aged 18-19 years old) during acute respiratory diseases appraisal. As the reference, 4 groups were taken (2 from each collective) but without ACA application. The obtained results show that, compared to the high morbidity rates in the reference group of the first tested collective, in the group preventively treated with ACA, number of ARVI has decreased by two-fold. At the same time, compared to the morbidity rate growth of ARVI (by 15-27%), quinsy (by 14-21%) and pneumonia (by 6-7%) in the reference groups of the second tested collective, in the group treated with ACA, pneumonia morbidity rate has decreased up to five-fold, while the ARVI and quinsy levels were stabilized. The smallest number of cases of these infections in both studied teams was recorded in the period of ACA application. The obtained results allow to recommend the use of ACA for the efficient prophylaxis of ARD, quinsy and pneumonia in the organized collectives in the period of increased incidences of these infections.



**We also studied the influence of the simultaneous use of ACA on the development of complications during the treatment of influenza and acute respiratory diseases. Clinical observations were carried out in hospitalized patients. Patients received a comprehensive symptomatic and pathogenetic therapy. Patients of the main group also received 2.0 g of ACA, 4 times a day orally for 5 - 7 days.**

**Our results indicate that:**

**At ARVI with mild form of disease, in patients treated with ACA there was no case of pneumonia, whereas in the comparison group in 5% of cases the disease was complicated by pneumonia. Sinusitis in the comparison group also developed at a higher level of 3%.**

**At ARVI with moderate disease course, in patients treated with ACA sinusitis developed in 2,5-fold, and pneumonia in 2-fold less likely than in patients who did not receive the drug.**

**The number of uncomplicated cases of ARVI with a mild disease in patients treated with ACA was 8% higher than in the group that had not been treated with the drug. These differences reached 20% at ARVI with moderate disease course.**

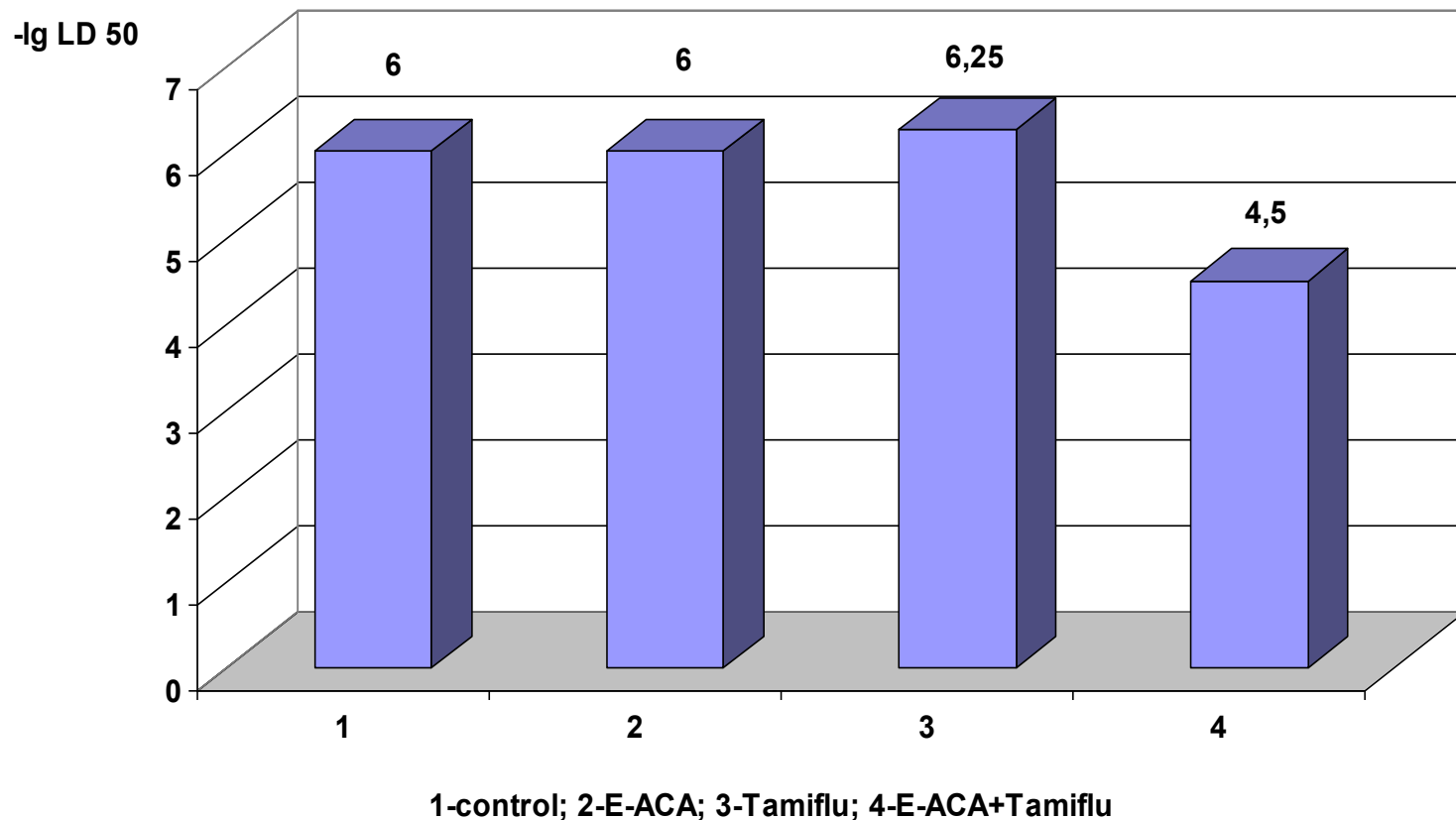


**Use of agents with different mechanisms of antiviral actions may result in a synergistic effect. Neuraminidase inhibitor (NI) Tamiflu (Tm) is the most popular anti-influenza agent but its toxicity is higher than that of ACA. Therefore we studied the efficacy of ACA & Tm combined action for optimization of anti-influenza therapy.**

**Synergistic inhibitory effect of ACA with Tm on reproduction of A/HK/1/68, A/PR/8/34 (H1N1) and H5N3 viruses took place *in vitro*.**

**During modeling of lethal infection in mice, only combination of ACA with Tm has shown strong protection effect when used in preparations containing lower doses than the minimal effective doses.**





**So the combined use of protease and neuraminidase inhibitors has good perspectives in anti-influenza protection and therapy**





It is known that *Staphylococcus aureus* is often the cause of bacterial complications of the influenza. We studied the sensitivity to ACA of:

- S. aureus* strain ATCC 25923 (commonly used as a control strain for susceptibility testing to antibiotics - Abs - and as a quality control strain for commercial drugs);
- S. aureus* strain 2781 (highly sensitive to ABs);
- S. aureus* strain Kunda (multi-resistant to ABs).

We established that ACA inhibits the reproduction of all studied strains of *S. aureus*.

We found that combined use of ABs with ACA increases the antibacterial efficacy against pathogens. ACA not only has itself antibacterial properties, but also can enhance the antimicrobial effects of such ABs of different nature like gentamicin, doxycycline, penicillin, lincomycin, streptomycin, rifampicin.



**According to our hypothesis, the proteolysis inhibitors (PIs) may block or decrease the activity of bacterial enzymes directed to ABs destruction and enhance the sensitivity of the microorganisms to ABs.**

**Thus, the combined use of PIs with ABs could cause changes of microorganisms metabolism and in such a way decrease the effective doses of ABs. Consequently such a combination can decrease the toxicity of the treatment.**



## CONCLUSIONS

- The results of our long-term research had made it possible to formulate the concept of “vicious circle” formation: virus activates the proteolytic systems, which in turn assist the development, generalization, and aggravation of infectious process at the expense of influence on the etiologic and pathogenetic factors. The inhibitors of proteolysis may prevent the formation or destruction of this “vicious circle”. State Pharmacological Center and Ukrainian Ministry of Health on the basis of our experimental research and clinical trials have allowed and recommended use Aminocaproic acid (ACA) as antiviral agent for prevention and treatment of influenza and other acute respiratory viral infections (ARVI) in children and adults.



## CONCLUSIONS

- **Combined application of protease and neuraminidase inhibitors may be very effective because they together could “shut the doors” for influenza viruses entrance to sensitive cells (PIs) and for their exit (NIs).**
- **The use of ACA in treatment of influenza also prevents bacterial complications.**
- **ACA used for the treatment of bacterial complications of influenza enables to increase the sensitivity of the microorganisms to antibiotics.**



# ACKNOWLEDGEMENT

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