CURRENT APPROACHES OF PREVENTION AND TREATMENT OF INFLUENZA OF PROTEOLYSIS INHIBITORS

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The grippe till now is a mass disease resulted in hospitalization of thousands of sick persons and high mortality rate. In Ukraine some 10 -1 4 million of people fell ill annually which constitute 25-30% of the general morbidity rate. Number of fatalities does not decrease, on the contrary it has a tendency to stabilization and even increase.

The objective. To excrete inhibitor of trypsin – like proteinases of industrial wastes of human gamma-globulin manufacturing as this anti-enzyme takes part in blocking of hemagglutinin of virus grippe cleavage.

Materials and methods. Strains of influenza virus: A/PR/8/34 (H1N1), grown on a 9-day chicken embryos, were obtained at the D.I. Ivanovsky Research Institute of Virology, Academy of Medical Sciences of Russia and strain AO/32 (H1N1) - from the Influenza Research Institute of St. Petersburg, Russia; white mice and hybrids; chicken embryos; white rats, Wistar line. Influenza virus AO/32 (H1N1) with infectious titers 7.0 log $EID_{50/0,2}$ml and hemagglutinin (HA) -1:256. To obtain influenza virus preparations we used 10-11-day chicken embryos. The virus was accumulated by infecting chicken embryos in a volume of 0.2 ml, diluted to 10^{-3} with infectious material. Infected chicken embryos were incubated for 48 hours at +36 °C. Then they were cooled for 18 hours at +4 °C and then the virus-containing fluid was collected, purified and concentrated with centrifugation. We used the wastes of the first stage of gamma-globulin manufacture (II + III) from donors' blood which contained a great amount of this inhibitor and the latter was excreted by ion-change chromatography on Diethylaminoethyl (DEAE)-cellulose.

Results. Cleaning and concentration of flu virus with centrifugation does not exempt virus from proteins with proteinaceous activity. Additional cleaning of flu virus in saccharobiose's ladder-shaped gradient showed that the main quantity of flu virus and the greater part of proteolytic activity has been localized in the area of 38-43% saccharobise's concentration. Primarily associated with flu virus proteinase in sacchorobiose's gradient was divided into four isoforms, and proteinase from normal horionallantoisny membranes – into three isoforms. The latter were 345 times lower than virus-inductive forms. At experimental animals infection by flu virus there was violation of fermental and inhibitory balance, especially during the first hours after infection. Virus-inductive cellular inhibitor plays an important role in proteinase blocking during first hours after infection. After its exhaustion the leading role in the development of infection is assumed by trypsin-like proteinases which started to split hemagglutinin and in this connection there was the growth of an infectious titer. From the lungs of healthy mice six isoforms of trypsin- like proteinase have been discharged. To them anti-protease immune sera were obtained and the treatment of animals with their use was carried out. 60% of the mice survived only at the effect of anti-serum to the III-rd isoform. Inhibitor of trypsin-like proteinases emitted from the lungs of healthy mice, protected the experimental animals from death for 80% and is a perspective anti-influenza drug. It has been established that the greatest number of trypsin-like proteinase and its inhibitor contained in the fractions of waste of the I-st and II-nd stages of industrial manufacturing of gamma-globulin and albumin from donor blood which are utilized.

Derivation the pure inhibitor included the following stages: the enzyme excretion, ultrasonic disintegration of cells, ion-change chromatography on DEAE –cellulose, dialysis, lyophilic drying. The method described allows to obtain five isoforms with inhibitory activity. The greatest amount on trypsin-like proteinases was registered in the isoform of the V-th fraction.

Conclusion. The V-th isoform with high inhibitory activity was used for the study of therapeutical properties and the experimental grippe A at white mice. The isoform mentioned showed a promoted protective effect.

Key words: flu, trypsin-like proteinase, inhibitor of proteinase, vaccine, human blood.