antiviral effect of a novel low-molecular polyvalent hemagglutinin inhibitor, containing Sia2-3Gal disaccharide motifs, on avian influenza A virus. *Methods*: Low-molecular polyvalent sialoside was studied as viral hemagglutinin inhibitor of avian influenza A (H5N1, H5N2, H5N3) virus strains in the inhibition assays of virus binding with fetuin molecules (FBI) and infectious focus forming in MDCK cells. To investigate the protective effect of hemagglutinin inhibitor in an animal model, we have infected mice with highly pathogenic influenza virus strain A/Chicken/Suzdalka/2005 (H5N1), isolated from poultry and wild birds in Western Siberia. To study the development of viral resistance to novel inhibitor we have conducted serial influenza virus passages in MDCK cells in the presence of increasing concentrations of hemagglutinin inhibitor. *Results*: The values of 50% inhibiting concentration (IC50) of hemagglutinin inhibitor obtained in MDCK cells and in FBI assay ranged from 1.5 to 10.0 μM for different influenza A virus strains. Intranasal administration of inhibitor (3.6 mg/kg) completely protected mice from infection of highly pathogenic avian strain A/Chicken/Suzdalka/2005 (H5N1). The results of passaging experiments indicated that hemagglutinin inhibitor showed no tendency to induce viral resistance. *Conclusion*: The data obtained in vitro and in vivo suggest that novel low-molecular polyvalent hemagglutinin inhibitor may be useful for the treatment of avian influenza virus infection.

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In Vitro Anti-Influenza Virus Effect of a Protease Inhibitor from a Streptomyces Strain

Julia Serkedjieva[1,*], Lidia Angelova-Duleva[1,2], Iskra Ivanova[2]

[1] *Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria;* [2] *Department of Microbiology, Sofia University, Sofia, Bulgaria*

Influenza viruses are important pathogens, causing infections both in humans and domestic animals. The virulence of these viruses depends on the ability of the hemagglutinin precursor (HA0) to be cleaved post translation to subunits HA1 and HA2 by trypsin-like proteases of the host. The inhibition of this cleavage by exogenous protease inhibitors may result in inhibition of subsequent rounds of viral replication. In the search for novel alternative approaches for the treatment of influenza infection we have studied the in vitro anti-influenza virus effect of a novel proteinaceous protease inhibitor (SS 34-1), isolated from the culture supernatants of a Streptomyces strain. The influenza virus-inhibitory effect was further studied with respect to the specificity and selectivity of viral inhibition. As a first approach we assessed the susceptibility of representative influenza viruses to the inhibitory action of SS 34-1; most sensitive to inhibition were A/Germany/34, strain Rostock (H7N1) and A/PR8/34 (H1N1). By the use of complementary virological assays it was demonstrated that the expression of the viral haemagglutinin on the surface of infected cells, the virus-induced cytopathic effect and the infectious virus yields, used as measures of A/Rostock virus growth, were all reduced at non-toxic concentrations of SS 34-1. In addition in preliminary experiments it the preparation protected mice from mortality in the experimental influenza A/Aichi/2/68 (H3N2) virus infection. All experiments were performed in parallel with the known proteolytic inhibitors ε-aminocaproic acid and aprotonin. The isolated novel protease inhibitor was purified by anion-exchange chromatography and reversed phase-HPLC analysis. It was a hydrophobic and a termostable protein, had a molecular mass of 11.2 kDa, isoelectric point of 7.5 and a high content of hydrophobic amino acids and proline. The N-terminal sequence demonstrated its homology to the *Streptomyces* subtilisin inhibitors family. The present results are in accordance with the findings that protease inhibitors of microbial origin could be used for the control of influenza virus infection.

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Protective Effect of a Fungal Superoxide Dismutase, Combined with a Plant Polyphenol Extract and Rimantadine Hydrochloride in the Murine Experimental Influenza Virus Infection

Julia Serkedjieva*, Maria Angelova

Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

The protective effect of a fungal Cu/Zn-containing superoxide dismutase, produced from *Humicula lutea* 103 (HL-SOD), applied in combination with a polyphenol extract, isolated from *Geranium sanguineum* L. (PC) or with rimantadine hydrochloride (Rim), was evaluated in the experimental influenza virus infection (EIVI) in mice, induced with virus A/Aichi/2/68 (H3N2). Preliminary results showed that HL-SOD caused neither acute nor chronic toxicity in the experimental animals, treated 4-fold with doses of 500 U/mouse/day. Rimantadine hydrochloride is an established selective antiinfluenza virus agent; in the dose 40 mg/kg, administered orally 24 and 2 h before and 24, 48 and 72 h after virus challenge, it protected mice from mortality (protective index, PI = 85.5%). The plant extract PC exhibited a pronounced antiinfluenza virus effect applied orally 3 h before viral infection in the dose 10 mg/kg (PI = 80.0%). HL-SOD, applied intravenously fourfold from 4 to 7 days after viral challenge in the dose 500 U/mouse/day also protected mice in the EIVI, PI = 86.1%). The intraperitoneal application of HL-SOD, a much more convenient way of treatment, was less effective. The combined application of HL-SOD, both intravenously and intraperitoneally, and PC or Rim in doses, which by themselves did not defend significantly mice, resulted in a synergistically increased protection, determined on the basis of protective indices and the amelioration of lung injury. Lung weights and consolidation as well as infectious lung virus titres were all decreased significantly parallel to the reduction of mortality rates; lung indices were raised. The excessive production of reactive oxygen species by alveolar macrophages as well as the elevated levels of the lung antioxidant enzymes superoxide dismutase and catalase, induced by EIVI, was brought to normal. For comparative reasons the combined protective effect of PC and the antioxidant vitamin C was investigated; synergistic enhancement of protection was observed. The results support the findings that the appropriate use of antiviral agents with alternative modes of action is a promising approach for the treatment of influenza virus infection.

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Discovery of New Inhibitors of the Influenza H5N1 Virus

William Severson[1,*], Xi Chen[1], Joseph Maddry[1], Yong-Kyu Chu[1], Colleen Jonsson[1], Alexis McBrayer[1], Ronald Tapp[1], Donald Smee[2], Clinton Maddox[1], Subramaniam Ananthan[1], James Noah[1], Leland Black[1], Blake Moore[1], Melinda Sosa[1], Lucile White[1], Lynn Rasmussen[1]

[1] *Southern Research Institute, Drug Discovery Division, Birmingham, USA;* [2] *Institute for Antiviral Research, Logan, USA*

We screened the NIH Molecular Libraries Screening Centers Network (MLSCN) 100,000 compound library against influenza