and, in particular, its neutral charge and steric bulkiness, were shown to be important determinants of activity. The relatively narrow structure-activity relationship points to a highly specific interaction with the target protein, which, based on preliminary time-of-addition studies, is thought to be related to viral binding (i.e., interaction of the influenza virus hemagglutinin with its cellular receptor). The observation that influenza virus fully retained its sensitivity to 8e after eleven sequential virus passages in MDCK cells in the presence of 8e (at concentrations up to 25 μM) agrees with such a highly conserved interaction site. Compound 8e proved to be inactive against several unrelated RNA and DNA viruses, except for varicella-zoster virus, against which a favorable activity was noted (EC_{50}: 0.55 μM and SI: 180; determined in human embryonic lung fibroblasts). The aglycoristocetin derivative 8e represents a new class of potent and broad-acting influenza virus inhibitors with potential therapeutic relevance. [Supported by a grant from the International Consortium on Anti-Virals (ICAV)].

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Cinnamon Fraction Neutralizes Avian Influenza H5N1 Both In Vitro and In Vivo

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Influenza is one of the most prevalent and significant viral infections. The aim of this research was to examine the ability of the Cinnamon Extract fraction CEppt to inhibit avian influenza VNH5N1-PR8/CDC-RG, after we previously demonstrated this antiviral activity against human influenza H1N1 [Barak, I., Ovadia M., 2005. Antiviral Research 65(3), 65]. The ability of CEppt to neutralize the avian influenza virus was tested in vitro on human erythrocytes by the hemolysis assay and in vivo on white mice. Both activities of the virus were inhibited. A dose of 50–100 μg of the crude extract was sufficient to achieve total neutralization of 128 HAU of the virus within one minute. CEppt has a long shelf-life of at least two years in the refrigerator or at room temperature. It still retained its antiviral activity after dialysis in bags with a cut-off of 10 KD or after heating at various temperatures up to 121 °C. The antiviral activity was also stable at a wide range of pH between 1 and 12. Moreover, the antiviral agent neutralized the virus after it was already attached onto the erythrocytes and prevented its subsequent hemolytic activity. The CEppt has also proved its ability to inhibit the virus *in vivo*. When the virus was mixed with CEppt prior to infection of 25-day-old mice, the mice did not develop the disease (strong hemorrhage in the lungs leading to death) nor did they lose weight or die. In conclusion, CEppt has exhibited an effective antiviral activity against avian influenza VNH5N1-PR8/CDC-RG virus both in vitro and in vivo. The cinnamon has been used in the human diet for thousands of years and should therefore not be an obstacle to introducing the isolated antiviral fraction for human and animal use. Virus sample was gifted by CDC, Atlanta, Georgia, USA. International Patent Application PCT/IL2004/001161.

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Comparative Study of the Efficacy of Low- and High-Molecular Inhibitors of Influenza Virus Hemagglutinin

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Synthetic analogs of the cellular receptors, which are able to compete with natural cellular receptors, have potential for development of anti-influenza therapeutics. Synthesis of low-molecular inhibitors of influenza virus is more preferable for the development of drugs by reason of possible toxicity, immunogenicity and incomplete biodegradation of polymeric compounds. The objectives of this study were to compare the anti-influenza activity and to clarify the mechanisms of action for low-molecular and high-molecular compounds, containing Sia2-6Gal disaccharides. *Methods*: We conducted the comparative study of the antiviral effect of low- and high-molecular hemagglutinin inhibitors on influenza A (H1N1, H2N2, H3N2) and B viruses in the inhibition assay of infectious focus forming in MDCK cells. To characterize efficacy of inhibitors in vivo we have investigated a mouse model, based on measuring the value of 50% respiratory infectious dose for mice. To elucidate mechanism of action for hemagglutinin inhibitors we examined influenza virion morphology by the negative contrast techniques. *Results*: The values of 50% inhibiting concentration (IC50) obtained in MDCK were 0.03 (±0.005) μM for low-molecular hemagglutinin inhibitor and 0.1 (±0.01) μM for high-molecular hemagglutinin inhibitor. Intranasal administration of 0.25 mg/kg low-molecular inhibitor or 1.25 mg/kg polymeric inhibitor completely protected mice from influenza virus A/Aichi/2/68 (H3N2) infection. Electron microscope study of the virion morphology showed direct damage of influenza virus particles by low- and high-molecular compounds, and allowed us to propose mechanism of virucidal action of hemagglutinin inhibitors. *Conclusion*: These data show significant antiviral effect of low- and high-molecular hemagglutinin inhibitors on influenza viruses at low micromolar concentration. Although we do not consider high-molecular hemagglutinin inhibitor as candidate anti-influenza drug due to the probable toxicity and limited biocompatibility of polymer, low-molecular hemagglutinin inhibitor has potential for the prevention of influenza virus infection as specific virucidal therapeutic.

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Antiviral Effect of a Novel Inhibitor of Influenza Virus Hemagglutinin on Influenza A (H5N1) Virus

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Background: The initial step of influenza virus infection is the attachment of the viral glycoprotein hemagglutinin to sialic acid-containing receptors of the host cells. Human and avian influenza A viruses differ in their recognition of host cell receptors: the former preferentially recognize receptors with saccharides terminating in Sia2-6Gal, whereas the latter prefer those ending in Sia2-3Gal. An attractive approach for the prevention of avian influenza infection involves inhibition of virus attachment to susceptible cells by synthetic analogs of cellular receptors on the basis of Sia2-3Gal. The goal of this investigation was to study the

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

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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

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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

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