combinations is important since ST-246 and CMX001 are the most advanced candidates under development for the treatment of orthopoxvirus infections and potentially could be used together in the clinic to increase efficacy or minimize the emergence of drug resistance. Combination assays were performed in human foreskin fibroblast cells using the Western Reserve (WR) strain of vaccinia virus. Results from these studies revealed robust synergistic inhibition of viral replication with combinations of 4′-thioIDU and ST-246. Combinations of 4′-thioIDU and CMX001 also exhibited modest, but significant synergistic inhibition of vaccinia virus replication. Simultaneous cytotoxicity controls did not reveal any increased toxicity and suggested that the synergistic effects were not the result of increased toxicity. The use of drug combinations with different mechanisms of action is advantageous because the combinations can offer improved efficacy at lower dosages and minimize the development of drug resistance. The results of these experiments indicate that combinations of 4′-thioIDU with ST-246 or CMX001 are particularly effective in the treatment of orthopoxvirus infections in vitro and suggest that combined therapy may be useful in the treatment of these infections in animals and humans.

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Induction of Interferon Gamma Inducible Protein 10 by SARS-CoV Infection, Interferon Alfacon 1 and Interferon Inducer in Human Bronchial Epithelial Calu-3 Cells and BALB/c Mice

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SARS-CoV has been identified as the causative agent of an emerging human infectious disease, SARS. Its immunopathological mechanisms have not been fully characterized. One hypothesis is that the pathogenesis of SARS-CoV is caused by a disproportionate immune response, illustrated by elevated levels of inflammatory cytokines and chemokines, such as IP-10, MCP-1, IL-6 and IL-8. SARS-CoV has been shown in vitro to induce changes of cytokines and chemokines in various human and animal cells. We previously reported that interferon (IFN)-alfacon 1 was more active against SARS-CoV infection in Calu-3 cells than in African green monkey epithelial cells on day 3 post-infection. In the current study, we evaluated its efficacy of IFN-alfacon 1 in Calu-3 cells during the first 7 days of virus infection compared to its efficacy in Vero 76 cells, in which a more productive virus infection occurs. Calu-3 cells appeared to be more responsive to the antiviral effects induced by exogenous IFN than did Vero 76 cells. Furthermore, IP-10, an IFN-inducible white cell chemoattractant, was detected in Calu-3 cells after SARS-CoV infection. Interestingly, IP-10 expression was shown to be significantly elevated when SARS-CoV-infected Calu-3 cells were treated with IFN-alfacon 1. To our knowledge, this is the first time that the IP-10 expression has been clearly demonstrated in Calu-3 cells after SARS-CoV infection. Since IP-10 seems to be coordinated with a protective response in cells, we evaluated the efficacy of antivirals directed against SARS-CoV infection in BALB/c mice. IP-10 expression was detected in the lungs of SARS-CoV-infected BALB/c mice. Significantly high levels of mouse IP-10 in BALB/c mice was also detected when SARS-CoV-infected mice were treated with the interferon inducer, poly IC:LC. Our data might provide an important insight into the mechanism of pathogenesis of SARS-CoV and these properties might be therapeutically advantageous.

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Anti-influenza Efficacy of Combination Apply of Proteolytic Inhibitor E-aminocaproic Acid with Neuraminidase Inhibitor Tamiflu

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Our investigations have shown antiviral activity of proteolysis inhibitor E-aminocaproic acid (E-ACA). Tamiflu (Tm) is neuraminidase inhibitor and the most popular anti-influenza (AI) agents. But its toxicity is higher than E-ACA. We investigate efficacy of E-ACA and Tm combine action for optimization of AI therapy. AI activity in vitro was studied in tissue culture of chorioallantoic membranes chick embryos. Influenza virus strains A/HK/1/68 (H3N2), A/PR/8/34 (H1N1) and avian influenza H5N3 were used. Both Tm in doses 2 mkM/ml and 1 mkM/ml and E-ACA in doses 10 mg/ml and 15 mg/ml have displayed regular AI activity to A/PR/8/34 and H5N3 accordingly if the preparations were used separately. Combination action of these preparations was more effective. Combination action of Tm (1 mkM/ml) and E-ACA (10 mg/ml) has demonstrated synergistic effect on inhibition of reproduction A/HK/1/68 virus. Synergistic effect took place during experimental infection with influenza virus A/PR/8/34 in mice too. Only combination using of Tm and E-ACA have shown strongly protected effect. The results of this study have demonstrated the expediency of combination using of proteolytic and neuraminidase inhibitors for AI protection and therapy.

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Structure–Activity Relationship of a Novel Class of Aglycoristocetin Derivatives with Potent and Broad Activity Against Influenza Viruses

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Attachment of a hydrophobic substituent to a glycopeptide backbone structure was previously reported to offer favorable pharmacological (i.e. antibacterial and antiviral) properties. We here report on the in vitro anti-influenza virus activity of aglycoristocetin derivatives containing hydrophobic side chain-substituted cyclobutenedione. In Madin-Darby canine kidney (MDCK) cells, the lead compound 8e displayed an antivirally effective concentration (EC_{50}) of 0.4 μM, which was consistent amongst influenza A/H1N1, A/H3N2 and B viruses. The concentration producing 50% inhibition of cell proliferation was 67 μM, yielding an antiviral selectivity index (SI) of 167. Structural analogues derived from aglycovancomycin were completely inactive. The hydrophobic side chain

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