yields in eggs and in MDCK cells. NA enzyme inhibition assay revealed differences in IC_{50} values with A/duck/Laos/25/06 being the most sensitive and A/Turkey/65-1242/06 less sensitive. Determination of the NA enzyme parameters revealed that avian-like NAs possess significantly higher levels of enzymatic activity (V_{max}) compared to human-like NAs of the same subtype. NA kinetic analysis demonstrated different affinities for the MUNANA substrate (K_m, ranged from 64 to 300 μM) and for oseltamivir carboxylate (K_i, ranged from 0.1 to 7.9 nM). In mice, all viruses replicated systemically and caused lethal infection, although different lethality was observed. Susceptibility to oseltamivir in mice was dependent at least in part on the pathogenicity of the H5N1 virus. Oseltamivir treatment with 20 mg/(kg day) for 5 days against less virulent A/chicken/Jogiakarta/BBVet/IX/04 virus resulted in 100% survival, and prevented death in 60–80% of animals infected with three other H5N1 viruses. Higher-dose oseltamivir regimen was required to achieve protection of mice against infection with A/Turkey/65-1242/06 virus. Notably, this H5N1 virus strain was characterized by high expression of pro-inflammatory cytokines/chemokines (IL-1α, IL-6, INF-α, MCP-1) in mouse lungs. We conclude that multiple factors can affect the optimal strategies of antiviral therapy for infection with highly pathogenic H5N1 influenza viruses.

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In Vivo Synergistic Combination Effect of Rimantadine and Oseltamivir Against Influenza A(H3N2) is Manifested in Several Dose Ratios

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Objectives: Previous studies of ours demonstrated a marked synergistic combination effect of rimantadine and oseltamivir in 100:1 compounds doses ratios in experimental infection with influenza A(H3N2) in mice when the treatment course onset was on the day of virus inoculation. Considering these data we studied combination effect of both compounds in 50:1 and 25:1 ratios in order to determine the dose ratios scope preserving a high efficacy.
Methods: Male white mice 16–18 g were inoculated intranasally with 0.05 ml/mouse of influenza A/Aichi/2/68 (H3N2) virus. Rimantadine hydrochloride and oseltamivir phosphate were administered per os in 5-day treatment course beginning 4 h before or 24 h post-virus inoculation with 20–30 MLD_{50}. Protection index (PI) and mean survival time (MST) were determined through 14 days post-infection. Infectious virus titers were determined in Madine-Darby canine kidney cells. Lung consolidation score and lung index were evaluated.
Results: Combinations of selected doses of 5, 10 and 20 mg/(kg day) rimantadine and 0.2, 0.4 and 0.8 mg/(kg day) oseltamivir were combined in doses ratio 25:1. PI up to 75% and 79.6% and MST up to 12.9 and 13.1 days for certain combinations were evaluated, while the individual effects of the same doses were from 0% to maximum 33% PI and 8.2–9.8 days MST, respectively. Determination of lung virus titers and lung parameters in combination-treated groups also proved the synergistic effect of both antivirals.
Conclusions: Oseltamivir and rimantadine at daily doses up to 50 times lower than optimal effective one for oseltamivir and 8–16 times lower for rimantadine in 1:25 ratio demonstrated synergistic effect when administered in combination in experimental infection with influenza virus A(H3N2) in mice.

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Development of Novel Selective Parainfluenza Virus Hemagglutinin–Neuraminidase Inhibitors

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BCX 2798 and 2855 are novel selective inhibitors of human parainfluenza viruses (hPIVs), whose design was based on the three-dimensional structure of the catalytic-binding site of the hemagglutinin–neuraminidase protein of Newcastle disease virus. Both compounds are derivatives of Neu5Ac2en in which the O4 hydroxyl group has been replaced either with an azido (BCX 2798) or a dichloromethanesulfonylamino (BCX 2855) group. Inhibitors were characterized for their antiviral activity in *in vitro* and in *in vivo*. Both compounds had a striking inhibitory effect on a spectrum of hPIVs as determined by hemagglutination and neuraminidase inhibition tests as well as inhibition of virus growth in LLC-MK_2 cells. However, BCX 2798 exhibited highest inhibition toward hPIV-1 while BCX 2855 showed superior activity toward hPIV-3. To evaluate efficacy of BCXs in a mouse model, we rescued and used the recombinant Sendai viruses whose HN genes were replaced with that of hPIV-1 (rSeV[hPIV-1HN]) or hPIV-3 (rSeV[hPIV-3FHN]). The ectodomain of F protein was also substituted in rSeV(hPIV-3FHN). Both recombinant viruses replicated robustly in the lungs of infected mice causing severe illness. A dosage of 10 mg/kg daily of BCX 2798 administered intranasally (IN) for five consecutive days starting 4 h before lethal rSeV(hPIV-1HN)-infection protected 100% of mice from death and significantly increased both the mean day to death and survival in mice infected with a non-lethal dose of rSeV(hPIV-3FHN). Treatment with 10 mg/kg daily of BCX 2855 in the same regimen was effective in reducing weight loss and virus lung titers in mice infected with non-lethal doses of either virus. In delayed (24, 48 and 72 h) treatment models with either non-lethal recombinant virus infection, 10 mg/kg daily of either compound administered IN significantly lowered the mouse viral lung titers. However, the effect observed with BCX 2798