coronavirus (SARS-CoV), killed more than 800 people and posed significant challenges for the public health systems and hospital workers during the outbreak in 2002–2003. SARS may still remain a threat to the public health since the natural reservoir of SARS-CoV remains largely unknown and there are no specific treatments and effective vaccines available for SARS-CoV infection. To further identify molecular targets for the development of novel strategies against SARS-CoV infection, we have employed siRNA mediated RNA interference technology to examine the potential effects of a panel of siRNA molecules on the viral entry and/or replication of SARS-CoV by targeting the genes encoding the human angiotensin-converting enzyme 2 (ACE2) receptor or the viral nucleocapsid protein (NP). We first found that some of the siRNA duplexes that were treated by Yan Xin Life Science and Technology (YXLST) could dramatically and specifically down-regulate the cellular ACE2 receptor or viral NP expression in a dose-dependent manner in human 293T cells. We then showed that the siRNA directed against ACE2 receptor could potently suppress the viral entry of the spike protein pseudotyped viruses. We further demonstrated that these siRNA molecules targeting ACE2 or NP genes could also markedly suppress the cytopathic effect (CPE) of the SARS-CoV infected cells, and potently inhibit the viral replication. Therefore, our study has identified two highly conserved molecular targets for the siRNA-mediated RNA interference against SARS-CoV infection.

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Thiazolides: A New Class of Broad-Spectrum Antiviral Drugs Targeting Virus Maturation

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Nitazoxanide [2-acetolyloxy-*N*-(5-nitro-2-thiazolyl)benzamide, NTZ] is a new anti-infective thiazolide used in the United States for treating Giardia- and Cryptosporidium-originated enteritis. We have recently shown that NTZ is effective in reducing clinical symptoms in hospitalized pediatric patients with severe rotavirus infection (Rossignol, J.F., et al., 2006. Lancet 368, 124–129). We now report that NTZ and its active circulating metabolite tizoxanide [2-hydroxy-*N*-(5-nitro-2-thiazolyl)benzamide, TIZ], have a broad-spectrum antiviral activity, effectively inhibiting the replication of several RNA and DNA viruses in experimental in vitro models. The thiazolides were found to be effective at low micromolar concentrations, which were non toxic to uninfected cells, against viruses belonging to seven different families including: simian SA11 and human Wa G1P1A rotaviruses, influenza A (PR8 and WSN strains) viruses, Sendai virus (SV), respiratory syncytial virus (RSV), coronavirus (CCoV), vesicular stomatitis virus (VSV), adenovirus (Ad5) and herpes simplex virus type 1 (HSV-1). IC_{50} and S.I. varied between 0.5 and 2 μg/ml and, 25 and >100, respectively, in the different experimental models examined. In the case of rotavirus and paramyxovirus infection, both drugs were found to protect the host cell from the cytophatic effect caused by the virus for at least 24 h p.i. Approximately, 20 NTZ derivatives have now been tested for antiviral activity, some of which were found to be more effective than the parent compound. The mechanism of the antiviral activity was studied in cells infected with rotaviruses and influenza viruses. Thiazolides do not inhibit viral RNA transcription and do not cause a general block of virus protein synthesis, but act at post-translational level interfering with the correct processing of selected viral glycoproteins, thus hindering the formation of mature viral particles.

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Oseltamivir-Ribavirin Combination Therapy for Highly Pathogenic H5N1 Influenza Virus Infection in Mice

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The unusual severity of disease caused by H5N1 influenza viruses in humans raises concern that contemporary antiviral drugs may be ineffective against systemically replicating highly pathogenic viruses. Combination therapy with drugs that interfere with different stages of the virus replication cycle and/or affect different aspects of virus pathogenicity may provide several advantages over single-drug treatment. To test this hypothesis, we studied the effect of combinations of oseltamivir (neuraminidase inhibitor) and ribavirin (non-specific inhibitor of viral polymerases) against two highly pathogenic H5N1 viruses (A/Vietnam/1203/04 and A/Turkey/15/06) representing two different clades of the H5 phylogenetic tree. BALB/c mice were treated with oseltamivir (10, 50 or 100 mg/kg/day), ribavirin (37.5, 55 or 75 mg/kg/day), or combinations of the two drugs twice daily for 8 days by oral gavage, starting 4 h before inoculation with 5 MLD_{50} of each H5N1 virus. Single-drug oseltamivir produced a dose-dependent antiviral effect against both H5N1 viruses ($P < 0.01$). A higher dose was required for the greatest effect against A/Turkey/15/06 virus (90% survival rate), whereas oseltamivir 10 mg/kg/day resulted in 70% survival of mice infected with A/Vietnam/1203/04 virus. Single-drug ribavirin showed a similar dose-dependent effect against both strains: dosages of 37.5 and 75 mg/kg/day significantly delayed death and provided 10% and 50% survival rates, respectively ($P < 0.01$). The mode of drug interaction in vivo was characterized by the three-dimensional model of Prichard and Shipman. The combination of two drugs produced additive-to-synergistic effects against A/Turkey/15/06 (H5N1) virus, with no enhancement of host toxicity. Combination treatment with 10 mg/kg/day oseltamivir and 37.5 mg/kg/day ribavirin completely inhibited

virus replication in mice infected with A/Vietnam/1203/04 virus ($P < 0.05$ compared to single-drug treatment) and protected 100% of the animals from death. Our results suggest that drugs with different antiviral mechanisms can exert a beneficial fashion of interactions with respect to inhibition of H5N1 influenza virus infection in vivo.

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Intramuscular Administration of Neuraminidase Inhibitor Peramivir Promotes Survival Against Lethal H5N1 Influenza Infection in Mice

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Human H5N1 influenza virus infections have been documented in 10 Eurasian countries, with a mortality rate >50%. Although, person-to-person transmission remains limited, the rapid evolution, genetic diversity, unprecedented geographic spread and changing ecology of the virus raise pandemic concerns. Antiviral drugs will be an important intervention strategy at early stages of a pandemic when strain-specific vaccines are unavailable. The objective of this study was to achieve complete protection against lethal H5N1 virus infection in mice by examining different schedules of administration of neuraminidase inhibitor peramivir. Five drug schedules were evaluated that differ by: (1) duration of administration (1 day versus 8 days); (2) route of administration (intramuscular [i.m.] injections alone or i.m. injections followed by oral administration); (3) frequency of administration on first day (once versus twice). In all regimens studied, BALB/c mice were administered peramivir 1 h after intranasal inoculation with 5 MLD_{50} of A/Vietnam/1203/04 (H5N1) influenza virus. A single i.m. injection of peramivir at 30 mg/kg resulted in 40% survival rate of mice with a mean survival of 12.8 days. Survival of mice increased to 60% when administered two i.m. injections of 30 mg/kg of peramivir. The single i.m. injection did not completely inhibit H5N1 virus replication in the lungs and spleen, but did decrease spread of virus to the brain. The analysis for the emergence of variants resistant to peramivir is in progress. The most beneficial protection was achieved when peramivir was administered i.m. for 8 days. This drug schedule inhibited the replication of virus in lung, brain and spleen at days 3, 6, 9 post-inoculation and resulted in 100% survival rate with no weight loss. These results indicate that duration of treatment is directly related to survival rate in this model of H5N1 influenza infection. Peramivir is an effective treatment when injected i.m. to control H5N1 infection in mice, supporting the use of this drug to control influenza in the event of pandemic.

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Treatment of Paralysis Caused by West Nile Virus in Hamsters

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WNV-specific humanized monoclonal antibody (hE16) was used to treat WNV-induced poliomyelitis and fatal WNV-aerosolization when administered as a single intraperitoneal (i.p.) injection days after the virus had infected the spinal cord of rodents. The 50% effective dose was 0.25 mg/kg when administered at 5 days post-subcutaneous (s.c.) viral injection (dpi). The hE16 was effective when administered at 5 dpi either i.p. or by direct delivery into the pontine of the mid brain after the virus had infected neurons. It lost activity when delivered i.p. at 6 dpi, but retained activity when delivered into the brain at 6 dpi, which demonstrated that the antibody was acting directly in the brain and not by simply inhibiting peripheral virus. The hE16 improved survival of mice aerosolized with WNV when administered i.p. at 5 days post-exposure long after the brain had been infected. Since disease signs of hamsters injected s.c. with WNV varied widely, a rodent model of uniform paralysis was developed. WNV was injected directly into the spinal cord at T8–T9 vertebra. At 6–8 dpi, all rodents developed overt hind limb paralysis. The WNV-infected neurons of paralyzed animals were stained strongly by TUNEL assay, a marker for apoptosis, whereas the inflammatory response compared to other viral encephalitides was mild. In this paralysis model, hE16 remarkably improved paralysis and survival when administered i.p. as late as 3–4 dpi when there was a robust WNV infection in the spinal cord. Overall, the versatility of hE16 was demonstrated by treatment of West Nile virus in rodents that was introduced by three routes of infection; subcutaneously, intra-spinal cord injection, and aerosolization. The efficacy of hE16 was limited when treatment was delayed further during advanced stages of neurological disease were apparent.

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