accumulation, cytotoxicity and antiviral activity of Nano-NRTIs. HIV-1 RT activity was measured following the 2–4 h preincubation of MDM with nanoformulations and viral infection. Mitochondrial DNA levels were determined by SYBR Green real-time PCR after multiple treatments of HepG2 cells used for evaluation of mitochondrial toxicity by Nano-NRTI. Nanogels were efficiently captured by MDM, demonstrated low cytotoxicity, and had no effect on viral infection without drugs. Nanoformulations with the highest inhibition of HIV-1 activity and the lowest toxicity were selected, and up to 12-fold reduction in efficient drug concentrations (EC90) was observed for Nano-NRTIs as compared to free drugs. Cytotoxicity (IC50) of Nano-NRTIs began at 200-fold higher concentrations. Antiviral activity of the nanoencapsulated dimer was the same as the one observed for both AZTTP and ddITP. Peptide modification of Nano-NRTIs did not affect their antiviral efficacy. The loss of mitochondrial DNA, a major cause of neurotoxicity, was reduced 2-fold in comparison to free drugs at application of selected Nano-NRTIs. Nano-NRTIs demonstrated important advantages over free nucleoside analogs and therefore held a great promise in the development of potent and low neurotoxic antiviral drug formulations for systemic targeting of HIV-1 infected macrophages.

doi:10.1016/j.antiviral.2010.02.388

79

Oseltamivir Protection of Oxidative Damages in Mice Experimentally Infected by Influenza Virus

Milka Mileva*, Angel S. Galabov

The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria

Oseltamivir is a neuraminidase inhibitor with a specific action against influenza A and B viral infection. As a structural analogue of neuraminic acid oseltamivir competitively binds the active site of the enzyme neuraminidase on the influenza virus surface. The present study was designed to investigate the effect of oseltamivir on the oxidative damages in lung and liver of influenza virus infected mice. It was established that supplementation of mice with oseltamivir leads to protection against the oxidative stress in lung and liver of mice experimentally infected with influenza virus A/Aichi/2/68 (H3N2) (1.5 LD 50). As markers of oxidative damages we use two products of lipid peroxidation—malondialdehyd, and fluorescent lipofuscine-like products, as well as the levels of natural antioxidants vitamin E and glutathione on the 5th and 7th day after virus inoculation. The results showed that influenza virus infection A/Aichi/2/68 (H3N2) was accompanied by a significant increase of the markers of lipid peroxidation and decrease of natural antioxidants (vitamin E, glutathione). The changes of CYP system are as follows—decrease in cytochrome P-450, NADP.H-cytochrome *c*-reductase activities, and liver monooxygenases (aniline hydroxylase, ethylmorphine-*N*-demethylase, analgin-*N*-demethylase and amidopyrine-*N*-demethylase) as compared to the controls. We find out that oseltamivir treatment led to decrease of the products of lipid peroxidation on days 5 and 7 after the inoculation as well as on the positive changes on the compounds of CYP system. The antioxidant properties of oseltamivir were investigated by measuring the ability of the drug to influence the lipid peroxidation and to scavenge superoxide radicals in some model system. From these experiments we could conclude that oseltamivir does not show scavenging properties and does not influence lipid peroxidation.

doi:10.1016/j.antiviral.2010.02.389

80

Discovery and Treatment of Respiratory Neurological Sequelae in West Nile Virus Infected Hamsters

John D. Morrey[1,*], Venkatraman Siddharthan[1], Hong Wang[1], Neil E. Motter[1], Jeffery O. Hall[1], Robert D. Skinner[2]

[1] *Institute for Antiviral Research, Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, USA;* [2] *Center for Translational Neuroscience and Department of Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock, USA*

Based upon respiratory distress observed in WNV-infected human patients, we addressed the hypothesis that respiratory distress is caused by lesions in the central nervous system. In rodents, arterial oxygen hemoglobin saturation (SaO2) was slightly suppressed in alert WNV-infected C57BL/6 mice and anesthetized golden Syrian hamsters. To determine if the cause was neurological, electromyographs (EMGs) were measured longitudinally from the diaphragms of alert WNV-infected hamsters. The amplitudes of EMGs in hamsters injected subcutaneously (s.c.) were significantly less than sham-infected animals, beginning with suppression at day 3 and continuing to beyond day 17 after viral challenge. To further confirm the neurological cause, immunohistochemistry (IHC) was performed on hamster tissues known to control respiration, i.e., lung, diaphragm, cervical spinal cord, brain stem, and the carotid or aortic bodies sensing pH, O_2, or CO_2. At various times after viral challenge, viral foci in some animals with EMG suppression were detected in the medulla oblongata, but not in the spinal cord, or the carotid or aortic bodies, which suggested that the offending lesions were primarily located in the medulla, which contains areas of respiratory function. WNV injected directly into the ventral medulla or the cervical cord suppressed EMG amplitude. EMG, SaO2 and IHC data indicated that lesions in the ventral medulla, and possibly the cervical cord, can cause respiratory dysregulation. These markers for respiratory function were improved upon treatment with a therapeutic antibody, MGAWN1 (hE16) or cyclosporine A administered intraperitonally after the virus had infected the central nervous system (>5 days). Moreover, these data demonstrated that WNV infection in the medulla, and possibly the cervical cord, results in EMG dysregulation in WNV-infected hamsters.

Funding: 1 U54 AI-065357, NO1-AI-30063, and RR020146.

doi:10.1016/j.antiviral.2010.02.390

81

Breaking Tolerance with CLDC-HBsAg in HBV Transgenic Mice

John D. Morrey[1,*], Jeff Fairman[2], Stella Chang[2], Neil E. Motter[1]

[1] *Institute for Antiviral Research, Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, USA;* [2] *Juvaris BioTherapeutics, Inc., Burlingame, USA*

Immune tolerance to hepatitis B virus (HBV) is thought to play a role in the maintenance of chronic hepatitis. This study tested the hypothesis that CLDC/antigen complexes can break immune tolerance in transgenic mice expressing HBV. Previous *in Vivo* studies suggest that administration of CLDC/antigen complexes induce robust antibody and T-cell responses versus the target antigen. These adaptive immune responses have been shown to be therapeutic in a wide variety of viral, bacterial, and cancer model systems. In this study, male and female transgenic mice expressing HBV were block-randomized across groups and administered with combinations of HBV antigen (HBsAg) and CLDC-adjuvant (JVRS100) at days 1, 22, and 43. At the end of the

treatment, serum was collected for the determination of ALT and anti-HBsAg antibodies. Splenocytes were incubated with HBsAg for 48 h to allow for T-cell activation. These cell culture supernatants were tested for interferon-gamma as a surrogate marker for CD4 and/or CD8 T-cell activation. Two vaccine injections consisting of HBsAg/JVRS100 administered intramuscularly (IM) or a combination of HBsAg/JVRS100 administered IM and JVRS100 administered intravenously (IV) broke tolerance as evidenced by significantly ($P < 0.001$) increased HBsAg-specific IgG total, IgG1 and IgG2c, and IFN-gamma. The other treatment groups (JVRS100 and HBsAg) were not statistically different from the non-treatment group. The combination of HBsAg/JVRS100 administered IM and JVRS100 administered IV resulted in statistically increased serum ALT and decreased serum HBsAg. These data indicate that CLDC-adjuvant may be informative as to the potential for efficacy of a therapeutic HBV vaccine in human clinical trials.

Funding: NO1-AI-50036.

doi:10.1016/j.antiviral.2010.02.391

82

Pro-drugs of Strand Transfer Inhibitors of HIV-1 Integrase: Inhibition Data, Structure–Activity Analysis and Anti-HIV Activity

Vasu Nair*, Byung Seo, Malik Nishonov, Maurice Okello, Sanjay Mishra

University of Georgia, Athens, USA

HIV integrase is encoded at the 3′-end of the pol gene of HIV and catalyzes the integration of viral DNA into the host cell genome in two key steps, which are 3′-processing in the cytoplasm and strand transfer in the nucleus. HIV integrase is essential for the replication of this virus and is a significant biochemical target for the development of anti-HIV therapeutic agents. At present, there is only one FDA-approved integrase inhibitor, Raltegravir, for the clinical treatment of HIV-AIDS. As resistance and toxicity are issues that are regularly encountered with anti-HIV drugs targeted at various viral replication points of intervention, the discovery of new classes of integrase inhibitors remains a significant scientific challenge. This presentation will focus on the discovery of integrase inhibitors assembled on modified nucleobase scaffolds that were found to be potent inhibitors of the strand transfer step of HIV-1 integrase ($IC_{50} \leq 10$ nM). However, while the integrase data were compelling, a significant disconnect existed between the enzyme inhibition data and the cell culture data for anti-HIV activity. A possible explanation of this disconnect may be issues of cellular permeability. Thus, a pro-drug investigation was undertaken to enhance cell permeability. Pro-drug SAR will be explained and illustrated. For example, an active integrase inhibitor of this investigation had an enzyme IC_{50} of 6 nM. Its pro-drug showed an EC_{50} in cell culture of 9 nM and a CC_{50} of 135 μM. These and other data will be presented.

doi:10.1016/j.antiviral.2010.02.392

83

Triple Combination Antiviral Drug (TCAD) Regimen Composed of Amantadine, Ribavirin, and Oseltamivir Imposes a High Genetic Barrier to the Development of Resistance Against Influenza A Viruses *In Vitro*

Jack Nguyen[1,*], Justin Hoopes[2], Elizabeth Driebe[3], Kelly Sheff[3], David Engelthaler[3], Minh Le[1], Amy Patick[1]

[1] *Adamas Pharmaceuticals, Emeryville, USA;* [2] *Utah State University, Logan, USA;* [3] *TGen North, Flagstaff, USA*

Background: Virtually all circulating influenza A viruses are resistant to one of the two classes of approved antivirals. The continued use of antivirals as monotherapy could result in the emergence of strains resistant to both classes of approved drugs. Here, we evaluated the effects of a triple combination antiviral drug (TCAD) regimen composed of amantadine (AMT), ribavirin (RBV), and oseltamivir carboxylate (OSC) on the emergence of resistance in vitro.

Methods: Influenza A viruses were serially passaged in MDCK cells in the presence of fixed, clinically relevant concentrations of AMT and OSC as single agents and in double combination, and the TCAD regimen, or under escalating concentrations of each drug regimen. The emergence of genotypic resistance was determined by mismatch amplification mutational analysis for the M2 (codons 27, 30, and 31) and neuraminidase (NA; codon 274) genes, or by Sanger sequence analysis for the M2, hemagglutinin, and NA genes.

Results: Serial passage of influenza at fixed concentrations of AMT or OSC alone or in double combination resulted in the early breakthrough of viruses with resistance-associated mutations, with the resistant variants rapidly becoming predominant (>90% by passage 3). In contrast, treatment with the TCAD regimen resulted in sustained suppression of the resistant virus population (<35% at passage 5). Under escalating concentrations, the TCAD regimen imposed a high genetic barrier to the development of resistance, inhibiting virus replication at concentrations below the EC_{50} of each drug for up to 31 days in culture. For the double combination and single agents, the presence of resistance-associated mutations enabled virus replication at concentrations of up to 275-fold greater than the EC_{50} of each drug.

Conclusion: These data demonstrate that the TCAD regimen composed of AMT, RBV and OSC imposes a high genetic barrier to resistance and suppresses the replication of resistant influenza viruses in vitro, and support the use of TCAD therapy for the treatment of influenza A infection.

doi:10.1016/j.antiviral.2010.02.393

84

Inhibition of Hepatitis C Virus Replication by Semisynthetic Derivatives of Glycopeptide Antibiotics

Susan Obeid[1,*], Maria N. Preobrazhenskaya[2], Leen Delang[1], Svetlana S. Printsevskaya[2], Eugenia N. Olsufyeva[2], Svetlana E. Solovieva[2], Jan Balzarini[1], Alfons Van Lommel[3], Johan Neyts[1], Jan Paeshuyse[1]

[1] *Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium;* [2] *Gause Institute of New Antibiotics, Russian Academy of Medical Sciences, Moscow, Russia;* [3] *Department of Morphology and Molecular Pathology, K.U. Leuven, Leuven, Belgium*

Glycopeptide antibiotics (teicoplanin and eremomycin) are used as antibacterial agents. We here report on the anti-HCV activity of hydrophobic teicoplanin and eremomycin derivatives. Analogue LCTA-949 resulted in the most selective anti-HCV activity three dif-