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Activity of Certain 5-Substituted-4′-Thio Pyrimindine Nucleosides against Orthopoxvirus Infections

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As part of a program to identify new compounds that have antiviral activity against orthopoxviruses, a number of 4′-thionucleosides were synthesized and evaluated for efficacy against vaccinia and cowpox viruses. Seven compounds were identified that were active at about 1 μM in human cells against both viruses without significant toxicity. The 5-iodo analog, 1-(2-deoxy-4′-thio-β-D-ribofuranosyl)-5-iodouracil (4′-thioIDU), was selected as a representative molecule for additional studies involving resistance and mechanism of action. This compound inhibited viral DNA synthesis at less than 1 μM but only partially inhibited the replication of a recombinant vaccinia virus that lacked a thymidine kinase. This compound retained complete activity against cidofovir and ST-246 resistant mutants and was synergistic in combination with CDV or ST-246 against vaccinia virus infection of tissue culture cells. To determine if these compounds had activity in an animal model, mice were infected intranasally with vaccinia or cowpox virus and treated with 4 of the active compounds including 4′-thioIDU, 5-bromo-4′-thio-2′-deoxyuridine (4′-thioBrDU), 5-trifluoromethyl-2′-deoxy-4′-thiouridine (4′-thioCF_3DU) and 1-(2′-deoxy-4′-thio-β-D-ribofuranosyl)-thymine (4′-thioT). The compounds were given orally, twice daily, at 15, 5 or 1.5 mg/kg beginning 24–120 h post-infection and continued for 5 days. Almost complete protection was observed when 1.5 mg/kg of 4′-thioIDU or 4′-thioBrDU was begun 72 h post-infection and significant protection and was still obtained with 4′-thioIDU when 5 mg/kg was initiated at 96 h. Under these conditions, 4′-thioCF_3DU and 4′-thioT were inactive. In mice treated with 4′-thioIDU, virus titers in liver, spleen and kidney were reduced by about 4 $\log_{10}$ in mice infected with vaccinia virus and about 2 $\log_{10}$ in mice infected with cowpox virus. These results indicate that 4′-thioIDU and 4′-thioBrDU are potent, nontoxic, inhibitors of orthopoxvirus replication in cell culture and experimental animal infections and suggest they may have potential for use in the treatment of orthopoxvirus infections in animals and man.

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Motor Unit Number Estimation as a Therapeutic Marker in Acute and Persistent West Nile Virus Infection in Hamsters

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Since West Nile virus (WNV) damages motor neurons in the spinal cord, we anticipated that motor units would have suppressed function, which might serve as a clinical or therapeutic marker for neurological disease. To measure the health of motor neurons, electrophysiological techniques were employed, of which the motor unit number estimation (MUNE) assay was identified as a sensitive marker for neuropathology in the spinal cord of WNV-infected hamsters. MUNE was found to be suppressed beginning at day 9 out to day 92 in hamsters injected subcutaneously with WNV. MUNE suppression at day 10 correlated with the loss of neuronal function as indicated by reduced choline acetyltransferase-staining. Between days 10 and 26, some α-motor neurons had died, but further neuronal death was not detected beyond day 26. MUNE was a marker for the degree of paralysis as indicated by paralyzed limbs yielding the lowest MUNE values. MUNE measurements facilitated the detection of persistent infection and neuropathology long after the acute phase of WNV infection. WNV RNA and foci of infected cells were identified in the central nervous system (CNS) of all hamsters tested from 28 to 86 days. Neuropathology, such as encephalitis, meningitis, lymphocytic infiltration, perivascular cuffing, gliosis, and axonal swelling and degeneration in the cauda equina persisted in these animals. WNV-positive staining co-localized with the neuropathology, which indicated that the persistent WNV infection contributed directly to neuropathogenesis. The results suggest that WNV can persistently infect neurons to cause dysfunction, that this chronic dysfunction is nearly a universal event in WNV-infected hamsters, and that MUNE is a very sensitive marker for this suppression in the spinal cord. MUNE was used to evaluate WNV-specific antibody efficacy. In as much as WNV-infected humans can also experience a poliomyelitis-like disease where motor neurons are damaged, MUNE may also be a useful clinical or therapeutic marker for those patients.

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Novel Imino Sugar Derivatives Demonstrate Potent Antiviral Activity against Dengue Virus

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Imino sugars, such as N-butyl-deoxynorimycin (NBDNJ) and N-nonyl-DNJ (NNDNJ), are glucose analogues that selectively inhibit cellular a-glucosidase I and II in the endoplasmic reticulum and exhibit antiviral activities against many types of enveloped viruses. Although these molecules have broad spectrum activity, their development has been limited by lack of efficacy and/or selectivity. We have previously reported that, OSL-95-II, a DNJ derivative with a hydroxylated cyclohexyl side chain, has an antiviral efficacy against dengue virus (DENV) similar to NNDNJ, but significantly less toxicity. Building upon this observation, a family of imino sugar derivatives containing oxygenated side chains and terminally restricted ring structures were synthesized and shown to have low cytotoxicity and superior antiviral activity against members of flaviviridae family, such as bovine viral diarrhea virus (BVDV), dengue virus (DENV) and West Nile virus (WNV) in culture. Of particular interest is that DENV is especially sensitive to imino sugar treatment, several of these novel imino sugar derivatives potently inhibit DENV infection with EC_{90} values at submicromolar concentrations and selectivity index of greater than 800. Therefore, these imino sugar derivatives represent the best in vitro activity in their class. Pharmacokinetic data indicated that plasma levels of one of the compound, CM-9-78, following oral administration maintained above 15 mM for more than 10 h which are markedly higher than that of intraperitoneal injection. Animal efficacy study using DENV-infected AG129 mice showed that oral administration of CM-9-78 significantly reduced viremia in a dose-dependent manner. Our

study suggests that these novel imino sugar derivatives may offer realistic candidates for the development of antiviral therapeutics against human dengue virus infection.

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Using the C57BL/6 and SKH1 Strains to Evaluate the Efficacy of CMX001 Following Lethal Respiratory Infections with Ectromelia Virus

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We are currently faced with the potential use of variola and monkeypox viruses as biological weapons, as well as the natural emergence of human MPXV. Such outbreaks would require therapeutic and prophylactic intervention with antivirals. Cidofovir, an antiviral approved for the treatment of cytomegalovirus retinitis in AIDS patients, has activity against poxviruses, but must be administered intravenously and is associated with nephrotoxicity. An ether lipid analogue of CDV, CMX001 (HDP-CDV), has excellent oral bioavailability, minimal nephrotoxicity, and potent in vitro and in vivo antiviral activity against poxviruses. Furthermore, the ST-246 antiviral has also been shown to be efficacious at preventing lethal respiratory infections in mice. Using the A/Ncr and C57Bl/6 mousepox model we have evaluated the optimal, delayed, dosing regimen of CMX001 required for providing protection following a lethal intranasal challenge. Furthermore, we have evaluated biomarkers to stage disease progression and monitor the efficacy of a single dose treatment with CMX001. Ectromelia virus infections of the A/Ncr and C57BL/6 mice result in a highly fulminant diseases with rapid mortality before the onset of rash, whereas human respiratory infections with variola and monkeypox viruses typically result in death after onset of rash. Here we describe a mousepox model using the SKH1 mouse strain that results in an extended disease course with the majority of deaths occurring after the onset of rash. To our knowledge this is the first description of a lesion model of mousepox following a respiratory infection. We also show that rash development in this model can be used to initiate efficacious CMX001 treatment.

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Novel 9-Arylpurines, as Selective Inhibitors of *In Vitro* Enterovirus Replication

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Enteroviruses (family of the Picornaviridae) are implicated in a wide spectrum of illnesses ranging from mild respiratory syndromes, herpangina, hand-foot-mouth-syndrome and common cold to potentially life-threatening disorders such as pancreatitis, myocarditis, meningitis, encephalitis and exacerbations of COPD and asthma. A number of 9-arylpurines have been identified as selective inhibitors of the replication of various enteroviruses. A representative example of this series of compounds is 9-(3-acetylphenyl)-6-chloropurine [TP219] that emerged as one of the most potent congeners in this series. The antiviral activity against Coxsackievirus B3 of TP219 was further assessed by (i) MTS-based CPE assays, (ii) virus yield reduction assays, (iii) real-time quantitative PCR and (iv) antigen detection. Also potential effects of the compound on the accumulation of viral (+)ssRNA and on polyprotein processing were determined. Drug-resistant variant were selected that were at least a 10-fold less susceptible to TP219 than the wild-type virus. TP219 did not prove cross-resistant to other classes of enterovirus inhibitors (including 3A, 2C and a 3D inhibitor). Genotyping of the drug-resistant variants revealed that 2–3 mutations, both in genes encoding for structural and non-structural proteins, may be responsible for the drug-resistant phenotype. To study whether or not individual mutations are sufficient to confer resistance, either single mutations or multiple mutations are being introduced in the wild-type genome. Further genotypic and phenotypic characterization of drug-resistant mutants will help to understand the mechanism by which TP219 exerts its anti-enterovirus activity.

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Oral Session 6: Mini-Symposium: Perspectives and Challenges in the Development of Topical Microbicides

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A Microbicide Perspective: Past, Present, and Future

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Microbicide Product Development: What Is A Microbicide?

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Formulation of Compounds for Vaginal and Rectal Delivery

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Development of Microbicides with Broad Based Anti-Infective Action

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