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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