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Nitazoxanide is an Effective Antiviral Agent Against Both HBV and HCV replication in vitro

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Nitazoxanide (NTZ), a thiazolide, is an anti-infective drug marketed in the United States for treating gastroenteritis caused by *Cryptosporidium parvum* and *Giardia lamblia* and is in late stages of development for treating *Clostridium difficile* and rotavirus-associated diseases. NTZ and its active circulating metabolite, tizoxanide (TIZ), were tested against Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) replication in cell culture. NTZ (EC_{50}, 0.2 μM; SI, 462) and TIZ (EC_{50}, 0.03 μM; SI, 300) exhibited potent and selective antiviral effects against HCV sub-genomic, genotypes 1b and 1a replicons. Moderate synergistic interactions against HCV were observed between nitazoxanide and either alpha interferon 2b, or 2′C-methyl cytidine in combination treatments. Activity against NS5b S282T, and NS3 A156S/V/T drug-resistant mutants, as well as a genotype 2a replicon are being investigated. Both compounds also inhibited intracellular HBV replication and virion production by 2.2.15 cells (EC_{50}, 1.5 μM, SI, 172), and NTZ exhibited moderately synergistic anti-HBV interactions with either lamivudine (LAM) or adefovir dipovoxil (ADV). NTZ was equally effective in inhibiting the replication of several LAM and ADV-resistant mutants. Most notably, NTZ induced a reduction in the production of several HBV proteins (HBsAg, HBeAg, HBcAg) without a corresponding reduction in HBV RNA, indicating a post-transcriptional mechanism. NTZ is currently in clinical trials against HBV and HCV infection. Preliminary reports have demonstrated multi-log declines in HBV viremia (and improvement in serum ALT levels) with NTZ monotherapy, and in HCV viremia with both monotherapy and combination therapy with interferon alpha, during 24–48 weeks treatment regimens. Detailed studies of mechanisms against both HBV and HCV are in progress. Nitazoxanide is a promising new antiviral agent that, due to its probable novel mechanism of action, has substantial potential for use as an adjunct to current and future therapies to enhance sustained response rates against chronic hepatitis virus infection and disease.

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Efficacy of Cationic Lipid-DNA Complexes (CLDC) on Hepatitis B Virus in Transgenic Mice

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Cationic lipid-DNA (non-coding) complexes (CLDC) are activators of the innate immune response inducing substantial TH1 cytokine production as well as natural effector mechanisms. CLDC rapidly induces efficacious natural immune responses that increase survival of rodents with some acute viral infections and cancers. CLDC were evaluated in transgenic mice carrying an infectious clone of hepatitis B virus (HBV). Mice used in the studies were restricted as nursing pups from solid food, because the expression of HBV DNA in the liver was increased above background levels in some mice with this restriction. Survival surgery was performed on these mice to obtain liver biopsies from which to determine their pre-experiment HBV DNA levels. Only animals with suitable levels of liver HBV DNA were entered into the experimental protocols. Intravenous administration of 50 μg/mouse of CLDC on days 1, 7 and 13 reduced liver HBV DNA to similar low levels achieved with the positive control, adefovir dipivoxil. In a subsequent experiment, the same treatment schedule was used to determine that the minimal effective dose was between 0.5 and 0.05 μg/mouse. Selective cytokines were increased in the livers of CLDC- compared to placebo-treated mice in a dose-responsive manner. CLDC were effective in reducing liver HBV DNA and could be considered for further evaluation in other hepatitis models.

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Mononuclear Cells as a Transfer Vehicle For Herpes Simplex Virus (HSV) or Vaccinia Virus (VV) Infection of Epithelial Cells Grown in 3D

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We have previously shown that organotypic raft cultures of human keratinocytes isolated from neonatal foreskins can be infected with different dermotropic viruses, including α-herpesviruses [i.e. Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) and varicella-zoster virus] and poxviruses [i.e. vaccinia virus (VV), cowpox virus (CPV) and orf virus]. Normal keratinocytes stratify and fully differentiate in a manner similar

to the normal squamous epithelial tissues. Typical cytopathic changes identical to those found in the squamous epithelium in vivo were observed throughout the raft following infection with the different viruses. We have now assessed the feasibility of using mononuclear cells (MCs) as viral carriers to infect organotypic epithelial raft cultures. For this purpose, mononuclear cells were isolated from human umbilical cord blood by Ficoll-Hypaque density gradient centrifugation, stimulated with phytohemaglutinin and incubated in the presence of IL-2. MCs were infected with HSV-1, HSV-2 or VV at a multiplicity of infection of approximately 0.01, incubated overnight and then washed to remove the viral inoculum. The MCs were added on top of the raft cultures after the epithelial cells were allowed to differentiate for 6 days. MCs were able to transfer the virus to the epithelium and histological changes characteristic of HSV infection could be observed. When the anti-herpesvirus agents, acyclovir, penciclovir, HPMPC (cidofovir) or HPMP-5azaC were added to the culture media at the time the MCs carrying HSV were added on top of the rafts, the epithelium was protected from the virus-induced cytopathic effect in a dose-dependent manner. The antiviral activity was quantified by measuring viral titers by plaque assay. Similarly, HPMPC and HPMP-5-azaC were able to protect the epithelial cells from VV spread by the MCs. Our results show the possibility of using MCs as a transfer vehicle for HSV and VV infections of epithelial cells grown in 3D and suggest that MCs could be responsible for the spread of the virus infection.

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Structural Basis for the Expanded Substrate Specificity of Vaccinia Virus Thymidine Kinase: Insight from the Crystal Structure

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Vaccinia virus, like other orthopoxviruses, encodes an enzyme with thymidine kinase (TK) activity. This enzyme is closely related to the human cytosolic enzyme, TK1, and both belong to the type 2 family of TK homologs. Previous studies with *N*-methanocarbathymidine (*N*-MCT), a new antiviral agent with activity against cowpox virus replication, demonstrated that the compound was much less effective in TK negative strains of the virus and suggested that the enzyme could preferentially phosphorylate this compound. A similar TK dependence was also observed with a series of 5-substituted deoxyuridine analogs. These results were unanticipated since the viral TK and TK1 share 70% identity at the amino acid level and were predicted to exhibit very similar substrate specificities. This was investigated further by purifying both enzymes in bacteria and comparing their relative ability to phosphorylate a number of thymidine analogs. This analysis revealed that the viral enzyme exhibited a marked preference for a few compounds including *N*-MCT and fialuridine. The crystal structure of recombinant TK at 2.9 Å resolution explains the structural basis for these differences. A comparison of this structure with the previously published structure of human TK1 revealed significant differences in the catalytic site. In particular, the active site of the viral enzyme appeared to be more open than the human homolog. These results have important implications in the development of antiviral therapies to orthopoxvirus infections. Thymidine analogs, such as (*N*)-MCT that are selectively phosphorylated by this enzyme in infected cells could be developed as potent and highly selective inhibitors of orthopoxvirus infections.

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Inhibition of an Innate Antiviral Response by Human Cytomegalovirus UL97 Kinase is Antagonized by Maribavir

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Nuclear aggresomes are cellular structures that function to degrade highly ordered protein aggregates in the nucleus, such as viral structural proteins and are an important innate antiviral response. The formation of nuclear aggresomes occurs around PML oncogenic domains (PODs, ND10 sites). Many viruses including human cytomegalovirus disrupt PODs, presumably to protect against the sequestration and degradation of viral proteins in the nucleus. One previously described facet of the UL97 negative phenotype was the formation of large nuclear aggregates, which also formed when cells were infected with the wt virus in the presence of the UL97 kinase inhibitor, maribavir (MBV). Interestingly, the kinase was shown to inhibit the nuclear aggregation of pp65 when both proteins were expressed transiently in COS7 cells. Here, we report that the kinase also reduced the nuclear aggregation of the tegument protein pp71, as well as cellular protein that is a marker for nuclear aggresomes. Thus, it appeared that the inhibition of nuclear aggregations was more general than previously suspected. To investigate this further, we examined the effect of the kinase on PODs and observed that it disrupted their formation in a manner similar to IE1, which was used as a positive control. This effect did not occur either with a point mutant of UL97 that has no kinase activity, or if the kinase activity was inhibited by MBV. These results taken together suggest that one important function of UL97 is to inhibit the formation of PODs and the degradation of structural proteins by nuclear aggresomes. In the absence of UL97 kinase activity, at least 90% of the mass of viral structural proteins is sequestered in nuclear aggresomes resulting in a significant impairment of viral morphogenesis. We conclude that the inhibition of the kinase by MBV impairs the ability of the virus to inactivate this innate