and disoxaril-dependent Cox B1 mutants have been developed in vitro, in FL cells. Phenotypic characteristics, VP1 genome sequencing and VP1 protein sequence deduction of disoxaril mutants have been determined. Sequence changes gave satisfactory explanation for mutant resistance and on the unusual effect of inhibitor-dependence. Combination effects of anti-enteroviral agents with different modes of action have been carried out in cell culture experiments and a series of synergistic combinations have been selected. Administration of antivirals in synergistic combinations could be considered as a prospective approach to decrease the level of drug-resistance and to improve the chemotherapy efficacy. A new scheme of application of the partners in the synergistic combinations was developed on the model of experimental coxsackievirus B1 infection in newborn mice. The maximum protective effect was reached with the combination disoxaril/guanidine.HCl/oxoglaucine.

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74

Arenaviral Inhibitory Activity of MY-24, a Novel Aristeromycin Derivative

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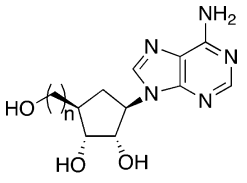
Several arenaviruses are known to cause viral hemorrhagic fever (HF), a syndrome often associated with a fatal outcome. The only option for antiviral intervention is ribavirin, a drug that has had mixed success in treating severe arenaviral HF, and has significant toxicity. Inhibitors of S-adenosyl-L-homocysteine hydrolase such as Aristeromycin (1) have shown promise as antivirals by disrupting essential viral macromolecular methylation processes. A search for new lead compounds based on 1, where structural variation occurred at the 5'-center, led to the 5'-homo derivative 2 (MY-24). Developing an efficacious synthesis of MY-24 allowed for evaluation of antiviral activity in cell culture systems based on infection with Pichinde, Tacaribe, or Junin arenaviruses, and in vivo against Pichinde virus (PICV) infection in hamsters. By virus yield reduction assay, the 90% inhibitory concentration against these viruses ranged from 0.64 to 3.54 μM, with therapeutic indexes ranging from 10 to 42. MY-24 was well-tolerated in hamsters up to the highest tested dose of 175 mg/kg/day. In PICV-challenged hamsters, MY-24 repeatedly extended survival and significantly reduced liver disease as measured by serum alanine aminotransferase, but no remarkable reduction in viral load was observed. Activity in cell culture against several arenaviruses and limited efficacy in treating hamsters infected with PICV may warrant further exploration of related Aristeromycin derivatives that may have improved activity in vivo. Funded by NO1-AI-15435, NO1-AI-30048, NO1-AI-30063, and U19-AI-56540, Virology Branch, NIAID, NIH.



1, X = CH_2, n = 1
2, X = CH_2, n = 2

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76

Pyrazine Derivative Treatment of Phleboviral Infection in Cell Culture and Rodent Model Systems

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Several pyrazine derivatives have been reported to be effective in treating viral disease in mouse and hamster models. Remarkably, T-1106 has been found to be more effective than T-705 in treating yellow fever virus infection in hamsters. Based on these findings, we hypothesized that T-1106 may be a better choice for treating hepatotropic viral disease such as that caused by Punta Toro virus (PTV), a phlebovirus related to the highly pathogenic Rift Valley fever virus (RVFV). T-705 and T-1106 efficacy against several phleboviruses was evaluated in cell culture by cytopathic effect and virus yield reduction assays. Both compounds were also tested in the mouse and hamster PTV infection models. For additional comparison, T-705 and T-1106 were evaluated in the hamster Pichinde arenavirus infection model, as disease is based on diffuse pantropic infection. The compounds were administered orally, twice daily ranging from 25 to 100 mg/kg/day, for 5–7 days. In cell culture, the inhibitory effects of T-705 and T-1106 against PTV, sandfly fever virus, and RVFV (vaccine strain) were comparable with 50% and 90% inhibitory concentration ranges from 3 to 55 μM for T-705 and 8-75 μM for T-1106. In PTV-challenged hamsters, a model that generally presents with high liver viral burden, T-1106 was more effective at reducing mortality than T-705 when compared on a molar basis. In contrast, T-705 had better activity than T-1106 in preventing mortality in a Pichinde arenavirus infection in hamsters. Both compounds were equally effective in treating mice infected with PTV. The data support the idea that T-1106 may be a more effective treatment for severe viral diseases that predominantly target the liver. Supported by contracts NO1-AI-15435, NO1-AI-30048, and NO1-AI-30063 from the Virology Branch, NIAID, NIH.

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77

FGI-104: A Broad-Spectrum Small Molecule Inhibitor of Viral Infection

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The treatment of viral diseases remains an intractable problem facing the medical community. Most conventional antivirals focus upon selective targeting of virus-encoded targets. However, the plasticity of viral nucleic acid mutation, coupled with the large number of progeny that can emerge from a single infected cells, often conspire to render conventional antiviral ineffective as resistant variants emerge. Compounding this, new viral pathogens are increasingly recognized and it is highly improbable that conventional approaches could address emerging pathogens in a timely manner. Our laboratories have adopted an orthogonal approach to combat viral disease: Target the host to deny the pathogen the ability to cause disease. The advantages of this novel approach are many-fold, including the potential to identify host pathways that

are applicable to a broad-spectrum of pathogens. The acquisition of drug resistance might also be minimized since selective pressure is not directly placed upon the viral pathogen. Herein, we utilized this strategy of host-oriented therapeutics to screen small molecules for their abilities to block infection by multiple, unrelated virus types and identified FGI-104. FGI-104 demonstrates broad-spectrum inhibition of multiple blood-borne pathogens (HCV, HBV, HIV) as well as emerging biothreats (Ebola, VEE). We also demonstrate that FGI-104 prevents lethality from Ebola in vivo. Altogether, these findings reinforce the concept of host-oriented therapeutics and present a much-needed opportunity to identify antiviral drugs that are broad-spectrum and durable in their application.

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78

Derivatives of Tunicamycin as Effective Inhibitors of Classical Swine Fever Virus

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Classical Swine Fever Virus (CSFV) is often used as a surrogate model to elucidate the role of envelope glycoproteins of HCV. These two viruses are homologous in genomic organization, replication and protein function. Glycoproteins E2, E0 (E^{rns}) and E1 of CSFV play a major role in the initial stages of viral infection. They are detected on the external part of viral particles. It has been found that some glycosylation inhibitors, such as tunicamycin, which act at the early stages of glycan chain processing, can influence, not only glycosylation, but also the stability of E2 and E0 glycoprotein, effectively inhibiting the formation of glycoprotein complexes and the yield of the virus. Because tunicamycin is relatively toxic to the cells, we have synthesized a number of inhibitors mimicking tunicamycin structure or a part of this structure. The main aim of this work was to study the influence of tunicamycin derivatives on penetration and propagation of CSF virus, and on maturation of viral envelope glycoproteins. To this end we have investigated the formation of glycoprotein dimers by immunoperoxidase monolayer assay and by immunoblotting (Western blotting). Some of inhibitors effectively arrested viral growth without significant toxicity for mammalian cells. These inhibitors were further studied in order to elucidate the molecular mechanism of their antiviral effect using different mammalian and insect cell lines and it has been found that most of them inhibit N-glycosylation at the stage of glycan modification characteristic for mammalian cells. These results for CSFV were used in the initial characterization of the effect of the inhibitors on recombinant HCV glycoproteins.

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79

Anti-Picornavirus Activity and Other Antiviral Activity of Sulfated Exopolysaccharide from the Marine Microalga Gyrodinium impudicum Strain KG03

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The sulfated exopolysaccharide p-KG03, which is produced by the marine microalga Gyrodinium impudicum strain KG03, had a molecular weight of 1.87×10^6, and was characterized as a homopolysaccharide of galactose with uronic acid (2.96%, w/w) and sulfate groups (10.32%, w/w). Like other sulfated polysaccharide exhibited impressive antiviral activity in vitro against several enveloped viruses such as influenza virus and herpes simplex virus type 1 and type 2. It is a strong immunoinducer and showed antiviral activity against several picornaviruses such as encephalomyocarditis virus (EMCV) and Coxsackie B type 3 virus which are known as naked virus. Antiviral activities of p-KG03 against various picornaviruses and also other viruses will be reported and compared to other sulfated polysaccharides. The biological activities of p-KG03 suggest that sulfated metabolites from marine organisms are a rich source of antiviral agents. The p-KG03 polysaccharide may be useful for the development of marine bioactive exopolysaccharides for use in biotechnological and pharmaceutical products.

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80

Assay Development for Antiviral Drug Efficacy Evaluation Against Dengue Virus

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Dengue disease is an arthropod-borne disease, and Dengue virus is transmitted from person to person by Aedes aegypti in the domestic environment. Dengue virus (DENV), a NIAID Category A priority pathogen, is the most important mosquito-borne viral disease affecting humans currently. More than 2.5 billion peoples now live in areas at risk of infection. Annually, there are 50–100 million people being infected, with about 50,000 reported cases. The case-fatality rate of Dengue hemorrhagic fever is about 5%, and most fatal cases are among children and young adults. Currently, there is no efficient vaccine, no effective vector control measures, and no effective antiviral drugs against DENV diseases. With the rapid expansion of DENV disease in most tropical and subtropical areas of the world, it is urgent to develop antiviral drugs for Dengue disease control. To identify novel antivirals targeting DENV, we developed an assay for the evaluation of an antiviral efficacy against DENV, including serotype-1, -2, -3 and -4. This assay is a cytopathic effect (CPE)-based assay which has been used to evaluate the efficacy of recently identified antivirals against DENV-2 virus. The assay conditions, including the multiplicity of infection (M.O.I.) and cell density, were optimized and validated in 96-well plates. DENV-2-induced CPE can be observed and detected in BSR cells using CTG reagent (Promega) between 3 and 5 days post infection (d.p.i.) with M.O.I of 1. Antiviral efficacy studies were carried out using ten concentra-