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.

doi:10.1016/j.antiviral.2009.02.082

78

Derivatives of Tunicamycin as Effective Inhibitors of Classical Swine Fever Virus

Ewelina Krol[1,*], Ilona Wandzik[3], Boguslaw Szewczyk[1], Grzegorz Grynkiewicz[2], Wieslaw Szeja[3]

[1] *Department of Molecular Virology, University of Gdansk, Gdansk, Poland;* [2] *Pharmaceutical Institute, Warsaw, Poland;* [3] *Silesian Technical University, Gliwice, Poland*

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.

doi:10.1016/j.antiviral.2009.02.083

79

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

Chong-Kyo Lee[1,*], Hae Soo Kim[1], Ju Ryung Nam[1], Myung-Jin Lee[1], Joung-Han Yim[2], Hong Kum Lee[2], Erik De Clercq[3]

[1] *Pharmacology Research Center, Korea Research Institute of Chemical Technology (KRICT), 305-600 Daejeon, South Korea;* [2] *Polar Biocenter, Korea Polar Research Institute (KOPRI), 406-840 Incheon, South Korea;* [3] *Rega Institute for Medical Research, K.U. Leuven, 3000 Leuven, Belgium*

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.

doi:10.1016/j.antiviral.2009.02.084

80

Assay Development for Antiviral Drug Efficacy Evaluation Against Dengue Virus

Qianjun Li

Division of Infectious Diseases, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, USA

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-

tions dose responses starting at 20 μM psot the infection of DENV-2. Future optimization and validation of the assay in 384-well plates is currently in process, and follow-up studies for these promising antiviral leads, including the mechanism of action studies and analogs synthesis and analysis, is also designed.

doi:10.1016/j.antiviral.2009.02.085

81

Structural Basis of the Disoxaril Resistance and Dependence of Coxsackievirus B1

Ivanka Nikolova[1,*], Angel S. Galabov[1], Rumena Petkova[2], Stoyan Chakarov[3], Boris Atanasov[4]

[1] *Institute of Microbiology, Bulg. Acad. Sci., Sofia, Bulgaria;* [2] *Scientific Technological Service Ltd., Sofia, Bulgaria;* [3] *Department of Biochemistry, Sofia University, Sofia, Bulgaria;* [4] *Institute of Organic Chemistry, Bulg. Acad. Sci., Sofia, Bulgaria*

Disoxaril (WIN) inhibits replication of a broad spectrum of entero- and rhinoviruses through bonding the hydrophobic pocket within VP1 coat protein, thus stabilizing the virion and blocking its uncoating. Using selection approach disoxaril-resistant mutants of the Coxsackievirus B1 (CVB1/RES) from the wild disoxaril-sensitive strain (Connecticut 5, variant Sofia, CVB1/SOF) were obtained. Nine consecutive passages of CVB1/RES mutant in the presence of disoxaril lead to obtaining of disoxaril-dependent mutant (CVB1/DEP). Timing-of-addition study on CVB1/DEP replication demonstrated that the lack of disoxaril stop the virus particle assembly only. All CVB1 disoxaril mutants were phenotypically characterized. A parallel comparative analysis of the VP1 sequences of CVB1/RES and CVB1/DEP mutants were studied with using the existed GenBank sequence as a reference structure. Amino acid sequence in a large VP1 195-255 peptide of CVB1/RES is highly different. A crucial important change in disoxaril-resistant mutant was two point mutations – M213H and F237L – both in the ligand-binding pocket. 3D-alignment of CVA9 over CVB3/B1 allows explicit transferring of two WIN-ligand atomic coordinates into CVB1 "canyon". The second site is forbidden for ligand in CVB1/SOF. It was generated more than 100 models and all they were treated with 'clashing analyses' for side chain rotamers. CVB1/RES has mainly steric and less energetic nature. In CVB1/DEP occupation of site-1 is restricted but site-2 can be filled. WIN molecule in site-2 interact the neighboring VP2 protein and all capsomers becomes chained in the pentamer. This is a good explanation of the finding that CVB1/DEP mutant needs WIN compound to provoke coating.

doi:10.1016/j.antiviral.2009.02.086

82

Antiadenoviral Activity of 6-Azanucleoside Analogues

Lidiya Nosach[1,*], Olga Povnitsa[1], Inna Alexeeva[2], Larisa Palchikovska[2], Nadezhda Nesterova[1]

[1] *Institute of Microbiol. & Virol., NAS of Ukraine, Kiev, Ukraine;* [2] *Institute of Mol. Biol. & Genetic NAS of Ukraine, Kiev, Ukraine*

Human adenoviruses have long been recognized as pathogens, causing a broad spectrum of diseases, including upper and lower respiratory tract infections, gastroenteritis, conjunctivitis, keratoconjunctivitis and disseminated infection in immunodeficient patients, including bone marrow and solid organ transplant recipients. We had previously demonstrated the antiadenoviral activity of 6-azacytidine (6-AC) in vitro in cell cultures and in vivo on the model of disseminated adenoviral infection in newborn Syrian hamsters. The high antiadenoviral activity of 6-AC was the basis for studying of activity derivatives 6-AC and role of the separate molecule fragments in antiviral activity. The antiadenoviral activity was investigated in Hep-2 and Hela cells against adenoviruses of types 2 and 5 by reduction of the quantity of infected cells. It has been shown that D-ribofuranosylic fragment 6-AC is necessary for antiadenovirus effect. The elimination of OH-group in the sugar moiety of nucleosides decreased their inhibitory effects. Thus the furanoic ring structure and 5′-OH group must be preserved in the molecule compounds. Commutation sugar moiety into D-xylose, D-glucose, L-arabinose leads to loss activity. The high antiadenoviral activity had N,O-tetraacetyl-6-AC (EC_{50} = 0,125 μg/ml); 2-thio-6-AC (EC_{50} = 2 μg/ml); 2′,3′-"seco"-5-methyl-6-AC; 2′-deoxy-6-AC and 2′,3′-dideoxy-2′,3′-didehydro-6-AC (EC_{50} = 8 μg/ml). The activity of N_4-aminoacid 6-AC derivatives was dependent on the amino-acid side chains nature. Newly synthesized N_4-alkyl-, allyl- and heteryl-derivatives showed the promising activity: N_4-methyl-6-AC (EC_{50} = <0.02 μg/ml); N_4-allyl-6-AC (EC_{50} = 0.2 μg/ml); N_4-(pyridin-3-yl-methyl)-6-AC and N_4-[2-(dimethylamino) ethyl]-6-AC (EC_{50} = 8 μg/ml). The results suggest that at least one of compounds (N_4-methyl-6-AC: EC_{90} = 8 μg/ml; EC_{50} = <0.02 μg/ml; SI = 15,660) is potential clinical antiadenoviral agent that need to be further studied.

doi:10.1016/j.antiviral.2009.02.087

83

Antiviral Activity of Octadecyloxypropyl Esters of 3-Hydroxy-2-(Phosphonomethoxy)Propyl Nucleosides Against Adenovirus In Vitro

J.P. Roth[1,*], T.Z. McLean[1], N. Valiaeva[2], J.R. Beadle[2], K.Y. Hostetler[2], D.L. Barnard[1]

[1] *Institute for Antiviral Research, Utah State University, Logan, USA;* [2] *Division of Infectious Disease, University of California, San Diego, La Jolla, USA*

The majority of human adenovirus serotypes cause respiratory infections while other serotypes cause gastroenteritis, conjunctivitis, rash illness, and cystitis. Most adenovirus infections are mild, but a re-emerged serotype, adenovirus 14 (Ad14), was reported to cause severe and fatal pneumonia in rare cases of people of all ages. No antiviral compounds have been approved for the treatment of adenovirus infections and vaccines have been developed for only two serotypes, 4 and 7, to prevent acute respiratory disease (ARD) in military personnel. In this study, four nucleoside analog compounds, 2′,3′-dideoxycytidine, ODE-HPMPA, ODE-HPMPC, and ODE-HPMPG, were evaluated against several adenoviruses. Neutral red uptake assays were used to test the potency of each compound in vitro. For adenovirus 1 (Ad1), the 50% antiviral efficacy values (EC_{50}) ranged from 5.3 to 29 nM for the ODE-HPMPA/C/G compounds and 7.1 μM for 2′,3′-dideoxycytidine. For adenovirus 5 (Ad5), the EC_{50} values ranged from 21 to 42 nM for the ODE-HPMPA/C/G compounds and 17 μM for 2′,3′-dideoxycytidine. For Ad14, the EC_{50} values ranged from 3.8 to 9.5 nM for the ODE-HPMPA/C/G compounds and 13 μM for 2′,3′-dideoxycytidine. The virus yield reduction assay was used to validate the results. For Ad1, the 90% antiviral efficacy values (EC_{90}) ranged from 3.5 to 9.2 nM for the ODE-HPMPA/C/G compounds and 3.6 μM for 2′,3′-dideoxycytidine. For Ad5, the EC_{90} values ranged from 1.6 to 9.2 nM for the ODE-HPMPA/C/G compounds and 6.2 μM for 2′,3′-dideoxycytidine. For Ad14, the EC_{90} values ranged from 6.5 to 20 nM for the ODE-HPMPA/C/G compounds and 12 μM for 2′,3′-dideoxycytidine. The 50% inhibitory concentrations for each compound on A549 cells were >3900 μM for 2′,3′-dideoxycytidine,