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,