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

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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

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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

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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,

420 nM for ODE-HPMPA, 1400 nM for ODE-HPMPC, and >7800 nM for ODE-HPMPG. Time of addition studies revealed that the potency of each compound was constant for up to 8 h following adenovirus attachment. The results of this study support the further testing of these compounds as candidates for the clinical treatment of adenoviruses.

doi:10.1016/j.antiviral.2009.02.088

84

Indole Derivatives Are Potent Inhibitors of HIV Integrase

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HIV Integrase is a recently validated novel therapeutic target for designing potential antiviral agents for combating HIV/AIDS. Indole is versatile lead molecule for invention of newer class of anti-HIV therapeutic agents. The indole derivative Delarviridine (NNRTI) has been approved for the treatment of HIV/AIDS and another indole derivative 5CITEP was also investigated for inhibition of HIV integrase activity. Based on those prior findings, the present work is to design novel indole derivatives as HIV integrase inhibitors. Synthesized compounds were investigated for inhibition of crucial steps in HIV Integrase activity such as the 3′-Processing and Strand transfer reactions. Our results will show that lead molecules were not active against HIV integrase. But their three derivatives were found to be more active against both step, with some selectivity for the strand transfer step. Among the tested compounds compound B inhibits the 3′-processing at the concentration of 5.7 μ M and strand transfer at the concentration of 4.5 μM. Details of synthesis and HIV Integrase activity data will be presented.

doi:10.1016/j.antiviral.2009.02.089

85

Antiproliferative Effects of Octadecyloxyethyl-Phosphonomethoxyethylguanine (ODE-PMEG) on the Growth of Human Papilloma Virus Positive Cervical Carcinoma (ME-180) Cells In Vitro and Solid Tumors in Athymic Nude Mice

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Cidofovir (CDV) inhibits HPV DNA positive cervical cancer cell proliferation by reducing levels of HPV E6 protein which results in upregulation of p53. We previously showed that ODE-CDV was several logs more active than CDV in vitro versus Me-180 cervical cancer cells (HPV 69). To extend these findings, we have synthesized ODE-PMEG and compared its activity versus Me-180 cervical cancer cells with ODE-CDV. In primary human fibroblasts, ODE-CDV and ODE-PMEG have 50% inhibitory concentrations of 4.2 and 5.0 μM, respectively. However, against the human cervical cancer cell line, Me-180, ODE-CDV and ODE-PMEG had IC_{50}s of 0.39 and 0.002 μM, respectively. ODE-PMEG has a selectivity for cervical cancer cells of 2500 versus only 10 for ODE-CDV. Therefore, we decided to evaluate the in vivo effect of ODE-PMEG versus Me-180 solid tumors in athymic nude mice. We used 24 female mice injected subcutaneously with 5×10^6 Me-180 cervical cancer cells. The tumors were allowed to become established for 14 days. Tumor measurements were taken in two dimensions and multiplied to get a total tumor volume. Baseline tumor volume measurements were approximately 30–35 mm^2. Mice were then randomized into three groups of eight mice each. The mice were dosed by intratumor injection of 25 μg (1 mg/kg) of ODE-PMEG every other day for a total of 21 days. Control mice received 50 μl of 0.9% saline solution every other day. Tumor volume measurements and body weights were taken 3 times a week during the 21 day dosing period and continued to 39 days. Control mice injected with vehicle showed tumor size increases of 298%. Mice treated with 25 μg of ODE-PMEG every other day for 21 days (10 doses) resulted in a 38% decrease in tumor volume which remained stable after dosing was stopped. In conclusion, control Me-180 cervical cancers treated with vehicle increased by 298% while mice in the 1 mg/kg group showed a 38% decrease in tumor volume which was stable after dosing stopped. No significant changes in weight loss or other toxic effects were observed in the treated group.

doi:10.1016/j.antiviral.2009.02.090

86

Antiviral Activity of Geneticin Against Dengue Virus

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The aminoglycoside, Geneticin (G418), was recently shown to have antiviral activity against bovine viral diarrhea virus (BVDV). Since BVDV, dengue virus (DENV) and yellow fever virus (YFV), all belong to the Flaviviridae family, it seemed possible that a common step in their life cycle might be affected by this aminoglycoside. Here it is shown that Geneticin prevented the BHK cells killing (CPE) resulting from DENV-2 infection, in a dose-dependent manner with an EC50 of 3 mcg/ml. Geneticin had no detectable effect upon YFV in BHK cells. Geneticin also inhibited DENV-2 viral yield with EC50 of 2 mcg/ml and EC90 of 20 mcg/ml. Selectivity index of anti-DENV activity of Geneticin in BHK cells was established to be about 70. Furthermore, 25 mcg/ml of Geneticin nearly completely blocked plague formation induced by DENV-2, but not YFV. In addition, Geneticin, inhibited DENV-2 viral RNA replication and viral translation. Gentamicin, Kanamycin, and the guanidinylated Geneticin showed no anti-DENV activity. Neomycin and Paromomycin demonstrated weak antiviral activity at high concentrations. Finally, aminoglycoside-3′-phosphotransferase activity of neomycin-resistant gene abolished antiviral activity of Geneticin. Our data indicate that, although the antiviral activity of Geneticin is broad, with activity against DENV-2, BVDV and other viruses, it is selective. That is, Geneticin has no activity against YFV. The work here also shows that the antiviral mechanism of Geneticin against DENV replication and translation is different from its ability to inhibit assembly and release of BVDV, suggesting that the drug can broadly target different viral functions. Furthermore, similar to its anti-BVDV activity, we conclude that the anti-DENV activity of Geneticin is likely to be due to its ring I and II. Overall, our data suggest that Geneticin represents a potentially novel class of virus-selective antivirals with broad-spectrum antiviral activity.

doi:10.1016/j.antiviral.2009.02.091