correspondently. Modulating influences of the lipotropic (cage-hydrocarbon) pharmacophores on the anti-CMV activity were observed only under the viricidal and prophylactic experimental schemes, where the lipid membranes of cells and/or viral envelope are involved. But the dominant role in the AVA antiviral activity was played by the sulf-anionic modulation. The negative charge accumulation on AVA macromolecules, seems, amplifies a potential for electrostatic blocking virions/virus-cell adsorption, and agonistic stimulated cell resistance or antagonistic competition of this synthetic polyacids with the viral nucleic acids. The most promising compounds have been selected for the future explorations of mechanisms of the anti-CMV and anti-HIV activity.

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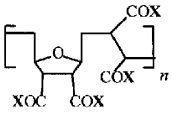

Where -X =
-OH/-O⁻ Na+, carboxyacid (CA), in part negative charged
-NH-Spacer$_1$-Adamantane (Ad), cage-tricyclic, mebranotropic
-NH-Spacer$_2$-exo-Norbornene (Nb), cage-bicyclic, mebranotropic
-O-Spacer$_3$-SO$_3^-$ Na+, sulfoacid (SA), full negative charged

AVA code	Various kind side groups (X), mol. ratio, CA : Ad : Nb : SA	Cytotoxicity [a], CC_{50}, μg/ml		Selectivity Index of Anti-CMV[b] activity SI = CC_{50}/EC_{50} (3 days)		
		1 day	3 days	Viricid.[c]	Prevent.[d]	Therap.[e]
ÀS. 470	1.00 : 0.00 : 0.00 : 0.00	3 750	3 500	< 10	< 10	< 10
ÀS. 473	0.94 : 0.06 : 0.00 : 0.00	3 200	2 500	< 10	< 10	< 10
ÀS. 632	0.93 : 0.07 : 0.00 : 0.00	3 600	2 400	41	< 10	< 10
ÀS. 504	0.92 : 0.00 : 0.08 : 0.00	3 200	1 700	< 10	< 10	< 10
ÀS. 677	0.86 : 0.00 : 0.08 : 0.06	-	1 440	66	22	< 10
ÀS. 678	0.79 : 0.00 : 0.08 : 0.13	1 800	1 420	355	189	< 10
ÀS. 679	0.67 : 0.00 : 0.08 : 0.25	1 000	500	**5 000**	91	< 10
ÀS. 688	0.60 : 0.00 : 0.00 : 0.40	4 000	3 000	**7 500**	**250**	**4 286**

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A Mass Spectrometry-based Method to Detect Antiviral Drug Resistance in Human Cytomegalovirus

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During antiviral therapy, the emergence of viral escape mutants that are resistant against the drug of choice is a major problem. Monitoring the introduction of resistance-associated mutations into the viral genome population can facilitate antiviral therapy management, including the selection of the most effective drug(s) in a given situation. We are currently assessing whether a mass spectrometry (MS)-based re-sequencing method (iSEQ by Sequenom, Inc., San Diego, USA) can improve the accuracy, speed, and sensitivity of the identification of resistance-associated mutations in the human cytomegalovirus genome (HCMV). Briefly, the assay employs four base-specific cleavage reactions of an amplicon of a relatively small region of the viral genome. The resulting four MS data sets are compared to *in silico* derived cleavage patterns from a database of reference sequences. Differences between the spectra derived from patient samples and those derived from viral reference sequences are indicators of sequence variations and can reveal potential resistance mutations. As resistance mutations against ganciclovir frequently occur in the phosphotransferase gene of HCMV (UL97), this gene was chosen to obtain proof of principle. A collection of patient samples was used to produce amplicons of 300–700 base pairs representing the 3′ half of the gene, which were analyzed using the Sequenom approach. The results were verified by traditional sequence analysis of the same samples. Our first data confirmed that detection of mutations or polymorphisms by SNP discovery is faster, but equally accurate compared to identification by regular sequencing. A more extensive comparison will be performed in the coming months.

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L-Analogs of 1-Beta-D-Ribofuranosyl-2-Bromo-5,6-Dichlorobenzimidazole (BDCRB) Inhibit Human Herpesvirus-6 Replication

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Benzimidazole nucleoside analogs have proven to be an abundant source of molecules with highly specific antiviral activity against human cytomegalovirus (HCMV). One analog, 1H-β-D-ribofuranosyl-2-bromo-5,6-dichlorobenzimidazole (BDCRB) inhibits the packaging of viral DNA, whereas a related L-ribosylnucleoside, 1H-β-l-ribofuranosyl-2-isopropylamino-5,6-dichlorobenzimidazole (maribavir) inhibits the HCMV UL97 protein kinase and is currently in Phase III clinical trials. Human herpesvirus-6 (HHV-6) is a related betaherpesvirus that is inhibited to a limited extent by maribavir but is insensitive to BDCRB. Therefore we hypothesized that other L-sugars in this series would be specific inhibitors of HHV-6. Of several compounds tested, two L-analogs of BDCRB (L-ribosyl BDCRB and (−)-carbocyclic BDCRB) have been identified that have good activity against the A variant of HHV-6 (EC_{50} = 2.6 and 2.4 μM, with selective indices of 11 and 5, respectively). Both compounds also inhibited HCMV in this concentration range (EC_{50} = 1.3–3.8 μM). These data differ with results for D-ribosyl analogs that were active against HCMV, but not HHV-6. Additional studies were conducted to examine their mechanism of action. Neither compounds inhibited viral DNA synthesis at high multiplicities of infection and no inhibition of HHV-6 U69 kinase activity was detected. Both results are consistent with a mechanism of action that is similar to that of BDCRB against HCMV and suggest that certain L-benzimidazole analogs have a mechanism of action similar to the D-benzimidazole analog BDCRB and differ from that of the L-analog maribavir. The results substantiate the prior observation that both the sugar moiety and the substituent in the 2-position of the heterocycle affect the mechanism of action and antiviral specificity of benzimidazole nucleosides.

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Comparative Efficacy of Treatment with CMX001 Versus Acyclovir in BALB/c Mice Infected with Herpes Simplex Virus

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Previous reports have shown excellent activity of CMX001 both in vitro and in vivo against vaccinia virus, cowpox virus, cytomegalovirus (CMV) and herpes simplex virus, Type 1 and 2 (HSV-1 and HSV-2). In cell culture, CMX001 has proven to be

a more potent inhibitor of HSV replication than acyclovir (ACV) and is also active against ACV resistant strains of the virus. In the current studies, treatment with CMX001 was compared with acyclovir in murine models of herpes encephalitis and neonatal herpes. Compound was suspended in 0.4% carboxymethylcellulose to yield desired dosages in a 0.2 ml volume. Mice were lethally infected intranasally with HSV-1, strain E-377, or HSV-2, strain MS and treatments were initiated 24 h post-viral infection. CMX001 was administered orally once daily at 5 mg/kg beginning 24 h post-HSV infection and continued for 7 days. ACV was administered twice daily beginning 24 h post-HSV infection at 100 mg/kg and continued for 7 days. Treatment with CMX001 significantly reduced mortality of HSV-1 and HSV-2 infected mice at 5 mg/kg doses ($P < 0.001$). ACV was also effective in reducing or eliminating mortality. The reduction of viral replication of 5 $\log_{10}$ PFU/g tissue by CMX001 in cerebral cortex, cerebellum, pons, medulla and diencephalon in mice infected with HSV-2 was superior to the effect observed by ACV. During ACV treatment, viral replication was reduced in some areas of the brain by 2–5 $\log_{10}$ PFU/g tissue compared to vehicle controls; however, CMX001 reduced viral replication below the limits of detection. In addition, when ACV treatment was discontinued, viral replication rebounded to levels higher than in the vehicle treated mice. In contrast, in CMX001 treated mice virus titers remained below the limits of detection. In these studies, CMX001 given at 5 mg/kg given once daily was more efficacious than ACV at 100 mg/kg given twice daily and suggests that CMX001 may have potential for use in the treatment of herpes encephalitis, neonatal herpes or other severe HSV infections in humans.

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Compounds that Target Host Cell Enzymes Prevent Varicella-zoster Virus Replication In Vitro, Ex Vivo, and in SCID-hu Mice

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Varicella-zoster virus (VZV) replicates in T cells, neurons, and skin cells that are typically quiescent in humans. In cultured dermal fibroblasts (HFFs), VZV induces host cyclin expression and cyclin-dependent kinase (CDK) activity without causing cell cycle progression. We found that CDK1/cyclin B1 phosphorylates the major transactivator protein of VZV, and that a CDK inhibitor, roscovitine, prevents virus mRNA transcription and replication. Here, we investigated the antiviral effects of additional compounds that target CDKs or other host cell enzymes involved in cell cycle progression. Compounds were tested in vitro (cultured HFFs), ex vivo (human skin organ culture) and in vivo (SCID-hu mice implanted with human skin). First, cytotoxicity and cell growth arrest doses in HFFs were determined by Neutral Red dye uptake assay. Then, antiviral effects were evaluated in HFFs by plaque assay, genome copy number, and bioluminescence. Positive controls were acyclovir (400 mM) and phosphonoacetic acid (PAA, 1 mM). Test compounds were roscovitine, aloisine A, and purvalanol A (CDK inhibitors), aphidicolin (inhibits human and herpesvirus DNA pol), mimosine (inhibits host DNA pol), and DRB (inhibits CKI, CKII, CDK2, and -7). All had antiviral effects below the concentrations required for cell growth arrest. The selective indices showed 3 ranges of potency: at low SI (<20) were aloisine A, Rosco, and DRB; at intermediate SI were PAA and mimosine; and at high SI (>250) were acyclovir, aphidicolin, and purvalanol A. Next, compounds were tested in skin organ culture at EC_{99} doses; all prevented VZV replication in skin except aloisine and purvalanol. This surprising result shows that skin organ culture is a useful system for evaluating antiviral compounds prior to animal studies. Preliminary experiments in SCID-hu mice with skin implants demonstrated that Rosco (1.5–2.8 mg/(kg day)) was as effective as PAA (35.6 mg/(kg day)) in vivo. Additional drugs against VZV are needed because current treatments must begin soon after onset and acyclovir-resistant strains exist. Targeting host cell functions makes developing resistance unlikely. The screening systems described here will be important models for evaluating novel antiviral drugs for VZV.

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Evaluation of Octadecyloxyethyl Esters of 3-Hydroxy-2-(phosphonomethoxy)propyl Nucleosides Against HCMV, HSV and Poxviruses

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Acyclic nucleoside phosphonates (ANPs) are an important group of broad spectrum antiviral agents. Using unmodified phosphonic acid forms, systematic studies of the relation between antiviral selectivity and the structures of the heterocyclic base and side chain of ANPs established that the 2-(phosphonomethoxy)ethyl (PME-) and 2-(phosphonomethoxy)propyl (PMP-) side chains exhibit selective antiviral activity against retroviruses while the 3-hydroxy-2-(phosphonomethoxy)propyl (HPMP-) side chain confers broad spectrum anti-DNA-viral activity. Work from our laboratory exploring the use of phosphonate-masking alkoxyalkyl esters to improve the oral bioavailability of ANPs has shown that this esterification, in some cases, imparts significant activity to phosphonates previously considered inactive, affording an opportunity to expand the therapeutic utility of ANPs. For example, we synthesized the octadecyloxyethyl esters of five HPMP-nucleosides and reported that several are potent inhibitors of HIV-1 replication in MT-2 cells, whereas the corresponding unmodified phosphonates were not active against HIV-1. We describe here additional in vitro evaluation aimed at elucidating the full spectrum of antiviral activity in this series. The HPMP-compounds were evaluated in cells infected with vaccinia (VV), cowpox (CV) and ectromelia viruses. ODE-(S)-HPMPA was the most active analog against VV and CV with EC_{50}s 0.003 and 0.008 μM, respectively. Against ectromelia virus, ODE-(S)-HPMPA and ODE-(S)-HPMPC had similar activity. The guanine and diaminopurine analogs of the series also showed significant activity (0.06 and 0.03 μM) while ODE-(S)-HPMPT was less active against each poxvirus. Against HSV ODE-(S)-HPMPA, the most potent compound, exhibited an $EC_{50} < 10$ picomolar and a selectivity index $> 6 \times 10^5$. HCMV assays showed similar potent antiviral activity. The in vitro activity of HPMP-nucleosides is increased when they are esterified with an octadecyloxyethyl group and this modification may lead to new broad spectrum antiviral therapies.

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