116

Effects of Antivirals via Intratympanic Delivery in GPCMV-related Hearing Loss

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Congenital cytomegalovirus (CMV) infection affects 1% of all newborns and is one of the most common causes of sensorineural hearing loss (SNHL). Approximately 90% of newborns infected with CMV are asymptomatic at birth. Yet, 20% of those go on to show SNHL. The therapies available to treat CMV infection have proven to be toxic and have severe side effects. Therefore, the need is to find a safe and effective treatment for CMV induced SNHL.

Cidofovir (CDV) and ganciclovir (GCV) have shown to stabilize and improve hearing loss in infants infected with CMV. However, the potential toxic side effects of these antivirals are the major deterrents for their clinical use. Therefore, intratympanic (IT) delivery warrants investigation as a non-toxic alternative to systemic treatments. Our proposal is that the antivirals delivered via IT injection will treat CMV-related hearing loss.

Previous work has shown that direct inoculation of guinea pig cytomegalovirus (GPCMV) into the bulla of a guinea pig (GP) is a consistent and reliable model of CMV infection. The similarities in the anatomy and physiology of the GP and human ear make this model extremely relevant. In these studies, various concentrations of the antivirals at different time points were injected IT into the GP inner ear. The pharmacokinetics data of GCV shows that it is present in the inner ear and experiments confirm no hearing impairment or toxic effects at 1 mg/ml and the hearing experiments of CDV show no impairment. The ongoing studies for both antivirals will include hearing tests, inner ear histopathology, serum and cochlear viral load, toxicity and drug levels of both the infected and saline control animals. The results of these studies will determine the safety and effectiveness of IT injections for the treatment of GPCMV-related hearing loss.

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117

Inhibition of Herpes Simplex Virus by Polyamines

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Cationic polysaccharides were synthesized by conjugation of various oligoamines to oxidized polysaccharides by reductive amination and tested for their activity against herpes simplex virus. Polycations of dextran, pullulan and arabinogalactan, grafted with oligoamines of 2–4 amino groups, were examined for their ability to inhibit formation of plaques of herpes simplex virus in BS-C-1 cells. Structure–activity relationship revealed that the grafted oligoamine identity of the polycation has an essential role in the antiviral activity. The most potent compound was dextran-propane-1,3-diamine (DPD) which resulted in significant inhibition of herpes simplex type 1 (HSV-1) and type 2 (HSV-2) growth. This inhibition of HSV was virus specific, since DPD did not inhibit poliomyelitis and vaccinia viruses, grown in the same host cells. While DPD did not affect the infectivity of HSV when the virus was directly exposed to this compound, the growth of the virus in BS-C-1 cells was efficiently inhibited when DPD was added to the cells 1 h prior to infection. DPD efficiently inhibited the adsorption and penetration of HSV to the host cells.

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118

Characterization of Cowpox Virus (CPV) Mutants Arising under Pressure with Different Acyclic Nucleoside Phosphonates

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We have previously reported on the characterization of vaccinia virus (VACV) mutants arising under pressure with different ANPs. We describe here the phenotypic and genotypic characterization of cowpox virus mutants selected under pressure with HPMPC (CDV, HPMPC), HPMPDAP {(S)-1-[3-hydroxy-2-(phosphonomethoxypropyl)-2,6-diaminopurine]} and HPMPO-DAPy {6-[3-hydroxy-2-(phosphonomethoxy)propoxy]-2,4-diaminopyrimidine}. CPV was grown in increasing concentrations of each ANP for approximately 40 passages in human embryonic lung (HEL) cells. Sequencing of the complete DNA polymerase genes of plaque-purified mutant viruses was performed and drug-susceptibility was determined by CPE reduction assay in human embryonic cells. The HPMPC^r virus presented the A314T and the A684V changes in the DNA polymerase while the HPMPDAP^r virus harbored the L924F substitution and an insertion of nine amino acids at position 408. The HPMPO-DAPy^r presented an insertion of the amino acid leucine at position 851. Other amino acid changes observed in the resistant viruses were related to genetic polymorphism. These amino acid changes were confirmed in several plaque-purified viruses. These findings highlight the importance of residues 314, 684 and 851 in the poxvirus DNA polymerases for the acquisition of resistance to ANPs since we have previously shown that substitutions at these positions were associated with resistance to HPMPC and other HPMP derivatives in VACV [J. Virol. 2006; 80:9391–9401; J. Virol. 2008; 82:12520–12534]. Furthermore, changes at these amino acid positions have been found in VACV mutants isolated following independent rounds of selection with different ANPs.

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119

Interactions Between the Human Oligopeptide Transporter, hPepT1 and Serine Side-chain-linked Cidofovir Prodrugs

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Cidofovir (HPMPC) is a broad spectrum antiviral agent that has limited therapeutic value due to ionization of the phosphonate group at physiological pH. To improve the oral bioavailability of HPMPC, we have masked the first P-OH group by conversion to the equipotent cyclic form (cHPMPC). The second P-OH is then esterified by the hydroxyl group of a serine dipeptide. Transport studies in a rat model showed enhanced levels of cHPMPC in plasma after oral dosing of the prodrugs. The enhanced bioavailability of valaciclovir (conjugated in a different way, by an ester bond between the terminal hydroxy of the drug and the amino acid carboxyl group) has

been attributed to hPepT1-mediated transport. In electrophysiology experiments on *Xenopus laevis* oocytes over-expressing hPepT1, we have not detected a significant signal with Val-Ser-OMe cHPMPC amino acid L/D stereoisomers, although stereoisomers having an L-configuration at the N-terminal amino acid potently inhibited Gly-Sar binding. Single side-chain ester-linked amino acid conjugates of cHPMPC have now been synthesized and investigated for transport and affinity in the oocyte model. An L-Val L-Val dipeptide analogue of acyclovir was also evaluated. The 'monopeptide' conjugates exhibited little or no hPepT1-mediated transport in the model, and had reduced affinity compared to dipeptide analogs. In some cases, TFA salts of the analogues produced weak positive signals in the model, whereas the HCl salts gave no signals, indicating the importance of using the latter form of the prodrug in these assays. The results suggest that one or more alternative mechanisms play a role *in vivo* to facilitate transport of the cHPMPC dipeptide prodrugs.

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120

Compounds Designed to Bind Conserved Regions of Human Papillomavirus (HPV) DNA show Broad-spectrum Activity Against High-risk Genotypes

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Cervical infections by the "high-risk" human papillomaviruses (HPVs), including HPV16 and 18, are usually not treated upon their discovery, but are flagged for later "follow-up." Traditional approaches to antiviral design for HPV have failed for a variety of reasons including the lack of traditional antiviral targets. Therefore, novel antivirals designed to specifically reduce viral persistence are needed. A series of pyrrole-imidazole polyamides was optimized via medicinal chemistry based on an original lead compound designed against a sequence within the ori of HPV16. A set of improved polyamides was prepared, including compounds that potently reduced both HPV16 and HPV31 copy number (compared with vehicle-control) in cells maintaining these genomes as episomes. Keratinocytes maintaining either HPV16 or HPV31 episomes were treated with increasing concentrations of polyamide or vehicle-control for 48 h in order to study dose–response behavior. Loss of episomal DNA was measured by Q-PCR. Of the 46 polyamides tested, including 16 control polyamides not derived from our core lead structure, 12 gave pseudo-IC50s 200 nM against both genotypes, while 4 reduced HPV16 and HPV31 episomal DNA copy number to undetectable levels. Southern blot analysis confirmed these decreases. Broad-spectrum activity is likely achieved due to high conservation in A-T rich regions among high-risk HPV genotypes and the binding degeneracy of polyamides. Treatment of cells with a lead polyamide, followed by removal of compound and passage of cells, resulted in a moderate rebound of viral DNA that did not return to control levels after 6 additional days in culture. Extension of the polyamide treatment period resulted in a remarkably effective delay and inhibition of episomal DNA rebound. These results illustrate that targeting of the HPV ori with polyamides has the potential for potent and long-lasting effects on HPV DNA load.

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121

Synthesis and Biological Studies of Mutagenic Ribonucleoside Analogues as Potential Inducers of *Error Catastrophe* of Riboviruses

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The development of effective treatments against riboviruses (causing many human diseases, i.e. common cold, haemorrhagic fever, AIDS, Hepatitis C, and SARS) is hampered by their ability to rapidly adapt by mutation and to acquire resistance to antiviral drugs. Riboviruses exhibited an extremely high mutation frequency, and this suggests that the viral population exists near the threshold for viral viability. Maintaining such a high mutation frequency is dangerous for the virus. An increase in mutation could result in a lethal increase in the already high proportion of defective viruses.

An antiviral strategy called *lethal mutagenesis* attempts to exploit this high mutation frequency by increasing the mutation rate even further and driving the virus population into "error catastrophe" (lethal accumulation of errors). This new strategy was validated with the demonstration that virus extinction can be achieved with the mutagenic nucleoside analogue ribavirin. Therefore, RNA virus mutagens may represent a promising new class of antiviral drugs.

We describe here the synthesis and biological studies of potential mutagenic ribonucleosides that may be incorporated into the viral genome during replication and, by mispairing, induce lethal mutagenesis. These ribonucleosides bear universal bases with ambiguous hydrogen bonding properties. We have documented various degrees of inhibition of the replication of foot-and-mouth disease virus (FMDV), encephalomyocarditis virus and lymphocytic choriomeningitis virus in BHK-21 cells by several base and ribonucleoside analogues.

VPg uridylylation (initiation of FMDV RNA synthesis) is inhibited by halogenated pyrimidine-triphosphates. The inhibitory activities cannot be accounted for by the toxicity of the drugs on BHK-21 cells. We are currently carrying out experiments to identify the steps in the life cycle of these viruses that may be affected by the drugs.

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122

Antiflogistics as Viral Inhibitors

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Virus infection of cells induces cytokines production. Inflammatory cytokines increase a permeability of cell membranes and promote virus penetration. Antiflogistics hamper a synthesis of proinflammatory interleukines and therefore may show antiviral properties. (Iso)nicotinic acids derivatives such structure: C_5H_4N–3(4)-$CONH(CH_2)_m$–$(NHCO)_n$–Ar, where m, $n = 0$ or 1 are known as antiflogistics. In this series we found that compounds display antiviral activity against several viruses if they inhibit carrageneen oedema not less than 30%. Weak antiflogistics does not