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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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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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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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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