ing with their viral DNA polymerase. In this context, analysis of the mechanisms through which poxviruses acquire resistance to ANPs is an important concern. The molecule HPMPDAP has been shown to be one of the most effective ANPs for the inhibition of poxvirus replication in vitro. The mutations within the viral DNA polymerase gene (*E9L*) involved in the resistance phenotype to HPMPDAP have only been described for vaccinia virus. In this study, camelpox viruses (strains Iran and Dubai) were passaged 30 times in medium containing an escalating dose of HPMPDAP, which selected for mutant viruses exhibiting an approximately 28- to 45-fold-increase in resistance to the drug. HPMPDAP-resistant clones were isolated following plaque purification. The antiviral activities of several ANPs, as well as of phosphonoacetic acid [PAA] and of ST-246 were determined by plaque reduction assays against the different clones. As a general conclusion, it appears that these HPMPDAP-resistant clones exhibit cross-resistance to other ANPs, including cidofovir, and that they also show hypersensitivity to two molecules: 6-[2-(phosphonomethoxy)ethoxy]-2,4-diaminopyrimidine] [PMEO-DAPy] and PAA, a direct viral DNA polymerase inhibitor. Interestingly, (S)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]-3-deazaadenine [3-deaza-HPMPA] retained marked activity against most of these resistant clones. Also, all the resistant clones were as susceptible as the wild type clones to ST-246, a poxviral egress inhibitor. The sequencing of the viral DNA polymerase genes of both wild type and resistant camelpox viruses is currently ongoing, and our results will be compared to those published for HPMPDAP-resistant vaccinia viruses. In conclusion, our studies provide additional insights in the mechanism of action of ANPs at the level of the viral DNA polymerase. Further in vivo experiments are still needed to evaluate the pathogenicity of such resistant viruses.

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Effects of Anti-human Papillomavirus (HPV) Disease Agents on HPV Episome Levels In Vitro: Cidofovir, Podophyllotoxin, and Pyrrole–Imidazole Polyamides

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Many in vitro studies of anti-HPV agents have misguidedly focused upon cells maintaining integrated rather than episomal copies of human papillomaviruses (HPV). HPV displays genotype-specific tissue tropism and causes hyperproliferative diseases of both cutaneous and mucosal epithelia. Persistent infection with "high risk" HPVs may lead to malignancy. We have taken a novel approach to design a series of pyrrole–imidazole polyamides against the sequences located in the ori of high-risk HPV genotypes. The compounds specifically reduce HPV episome levels in cells maintaining high-risk HPV genomes. In this study, we compared the effects of our targeted polyamides against Cidofovir, which is currently being used off label for treatment of HPV-related disease including recurrent respiratory papillomatosis (RRP), and podophylotoxin which is commonly used to treat cutaneous warts. Monolayer cultures of human foreskin keratinocytes maintaining HPV31 were treated for 48 h with a range of doses of each compound. The effect of this treatment on HPV31 episome levels was measured via Q-PCR normalized to DNA input. Cell viability was also assessed in parallel using an MTT assay. A 50% reduction in HPV31 genome copy number was achieved at a concentration of 1 μM of polyamide NV1020 with no observable cytotoxicity up to the highest dose tested (10 μM). Cidofovir caused a dose-dependent decrease in HPV31 DNA at high doses of compound, although a 50% reduction in viral genomes was never reached for this compound up to 500 μM. The observable loss of HPV31 episomes due to Cidofovir correlated with losses in cell viability. Podophyllotoxin had no effect on HPV31 episome levels, however there was a significant dose-dependent reduction in cell viability (TD50 = 80 μM). These studies demonstrate the following: that podophylotoxin does not effect HPV episome levels but acts primarily via a cytotoxic mechanism; that Cidofovir appears to have an anti-HPV effect that is associated with cytotoxic activity; and that NV1020 effects on HPV episome levels occur in the absence of measurable cytotoxicity.

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Identification of the Type of Cells Responsible for Transfer of Herpes Simplex Virus (HSV) and Vaccinia Virus (VACV) Infection to Epithelial Cells Grown in 3D

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We have previously shown that organotypic raft cultures of human keratinocytes isolated from neonatal foreskins can be infected with different dermotropic viruses and these cultures can be used as a model to evaluate the activity of antiviral compounds. We have also demonstrated the feasibility of using mononuclear cells (MCs) as viral carriers to transfer infection to organotypic epithelial raft cultures. We have now determined the population of cells responsible for carrying herpes simplex virus (HSV) and vaccinia virus (VACV) infection to the epithelial cells. For this purpose, MCs were isolated from human umbilical cord blood by Ficoll-Hypaque density gradient centrifugation and they were infected with HSV-1, HSV-2 or different VACV strains at a multiplicity of infection of approximately 0.01 and incubated overnight. MCs were washed three times to remove viral inoculum and were used to infect organotypic epithelial raft cultures. A part of the MCs was processed for confocal microscopy. Double-staining with anti-HSV or anti-poxvirus antibodies, and different cell differentiation marker-specific antibodies was performed to identify infected cell types. HSV and poxvirus infection was detected in CD45+ (leukocytes) and CD14+ (monocytes). Purified CD14+ cells either differentiated into macrophages by treatment with phorbol myristate acetate and hydrocortisone or not differentiated were able to support viral replication and transfer the infection to the epithelial cells suggesting that monocytes/macrophages may be considered as a vehicle to transfer infection to epithelial cells.

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Drug Resistance Mutations in HSV-1 UL5 Selected using a Helicase–Primase Inhibitor: Frequency and Effects on Virus Growth and Pathogenicity

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Helicase–primase inhibitors (HPIs), e.g. BAY 57-1293, are extremely active against HSV in cell culture and animal infection models. They target the helicase–primase (HP) complex which is involved in virus DNA replication. Using BAY 57-1293 at inhibitory concentrations (e.g. 10–100 times the IC50) it was possible to detect HPI-resistant viruses in two different laboratory working stocks of

HSV-1 following a single passage with the inhibitor in Vero cells. Furthermore, resistance selection occurred when the inhibitor was continuously present from prior to virus inoculation suggesting that certain resistance mutations may pre-exist in virus populations at relatively high frequency. PCR data will be presented to confirm these observations. It was shown subsequently that 2 out of 10 recent clinical isolates of HSV-1 also contained BAY 57-1293-resistant variants at 10^{-4} to 10^{-5} p.f.u. This is similar to the laboratory isolates and 10–100 times the previously reported spontaneous rate for HPI-resistance mutations (10^{-6}) in plaque-purified HSV-1 strains. The most common resistance mutations involved three amino acid residues just down-stream from the predicted helicase motif IV in HSV-1 UL5 and one residue near the C-terminus of the primase (UL52). We also showed that certain HPI-resistance mutations in UL5 are associated with increased or decreased virus growth in tissue culture with concomitant effects on pathogenicity.

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Murine Model of Recurrent Vaginal HSV-2 Shedding and Effect of Acyclovir

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Genital herpes simplex type 2 (HSV-2) infection is common and leads to latency, reactivation, recurrent shedding and transmission. A murine model of recurrent genital HSV-2 shedding would be useful for characterization of the immune response to recurrent HSV-2 and for testing of novel antiviral therapies; however, genital HSV-2 infection of mice is lethal. In our studies, Swiss Webster mice were infected intravaginally with HSV-2 (strains 186 or MS) and treated with acyclovir (ACV) (100–150 mg/kg) beginning at 3 days post-infection (dpi) for 10 days. Animals became infected as evidenced by vaginal virus replication, developed local symptoms and survived (>80%) to 35 dpi. No replicating virus was detected in the dorsal root ganglia (DRG) after 30 dpi, yet all DRG samples were positive for HSV-2 DNA by PCR, indicating the establishment of latency. To determine whether recurrent HSV-2 vaginal shedding occurred, vaginal swabs were collected every 3 days beginning at 20 dpi and analyzed by PCR and plaque assay. Replicating virus was not detected in the vaginal samples, however, 47/59 mice (80%) shed virus as detected by nested PCR on at least 1 day, and virus was detected on approximately 15% of days. In another study, surviving ACV-treated mice were divided so that one group was again treated with ACV from 21–31 dpi and one group was not retreated. Retreated mice had increased survival, indicating that viral replication contributes to the eventual death of the mice. Mice receiving the second ACV treatment exhibited significantly less recurrent vaginal shedding during treatment (0.8% of swabs were HSV-2 positive) compared to 7.3% in mice not receiving ACV during this period ($P = 0.01$) supporting the validity of the recurrence model and its utility to evaluate antiviral therapies. Furthermore, initially ACV-treated mice receiving cyclophosphamide from d35–38 experienced increased mortality, supporting a role for immune control of the persistent infection. This model will be useful for evaluating anti-HSV therapies and will allow further evaluations of the immune mechanisms that control recurrent infections.

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Activation of Cyclopropavir Involves Unique Phosphorylation by Guanylate Kinase

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Human cytomegalovirus (HCMV) is a widespread pathogen that can cause severe disease in immunologically immature and immunocompromised individuals. Cyclopropavir (CPV), a second generation methylenecyclopropane guanosine nucleoside analog, demonstrates in vitro activity against HCMV and MCMV with EC_{50}s of 0.27–0.49 mM [J. Med. Chem. 47: 566, 2004] and in vivo activity with a 2–5 log reduction in titers of virus in SCID mice (Antimicrob. Agents Chemother. 48: 4745, 2004). We have found that resistance of HCMV to CPV maps to a mutation in the viral UL97 gene resulting in a truncated UL97 protein devoid of both the ATP binding region and kinase activity domain (Antiviral Res. 78: A54, 2008). UL97 is the protein responsible for the initial phosphorylation of certain nucleoside analogs, all of which must be converted to a triphosphate to elicit antiviral activity. Consequently, it has been hypothesized that CPV must be converted to a triphosphate (CPV-TP) to give antiviral activity. Once CPV-MP is formed by UL97, we further hypothesize that guanylate (GMP) kinase is responsible for the conversion of CPV-MP to CPV-DP. Preliminary studies demonstrated that the S–(+)-enantiomer of CPV-MP was the preferred substrate for GMP kinase and was used for all subsequent experiments. Incubation of CPV-MP with bovine GMP kinase gave a linear increase of CPV-DP for over 30 min. Surprisingly, another compound consistent with CPV-TP was formed as well. We also observed a species variation in the formation CPV-TP. With bovine GMP kinase, formation of CPV-TP was the rate-limiting step whereas the conversion of CPV-MP to CPV-DP was rate limiting with porcine GMP kinase. There was a greater accumulation of CPV-TP with the porcine enzyme at 30 min (∼25% CPVTP, ∼5% CPVDP), while the opposite was true with the bovine enzyme (∼5% CPVTP, ∼70% CPVDP). In control experiments in which the enzyme was incubated with its natural substrate GMP, only GDP was formed. We conclude that unlike other antivirals such as acyclovir, cyclopropavir can be converted to its active triphosphate by a single cellular enzyme once the monophosphate is formed by a viral kinase.

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EBNA1—A virally encoded protein binds cellular host promoters in a unique sequence and directly interferes with cellular gene expression. Implications for genomics approaches in drug design

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Although most EBV carriers are healthy, several cancers were identified to be associated with EBV. One EBV protein of particular interest is EBNA1. It was found to be expressed in all forms of EBV infections and tumors and it is the only EBV protein being expressed in the common latency type I infection. It was demonstrated by others that specific inhibition of EBNA1's expression