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