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Antiviral Activities of New Cidofovir Analogs Against Camelpox Virus, Used as a Model of Variola Virus, in Human Skin Equivalent Cultures

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Variola virus (VARV) is a member of the *Orthopoxvirus* genus that also includes camelpox virus (CMLV). Growing concerns over the possible release of VARV as a biological weapon have stimulated the development of new antiviral therapies to treat post-exposure VARV infection. CMLV is the etiologic agent of an orthopoxvirus infection in camels and dromedaries and has been shown to be the closest known orthopoxvirus to VARV. In this study, we have evaluated the potency of the three classes of acyclic nucleoside phosphonates (ANPs) against CMLV in human embryonic lung (HEL) cells and primary human keratinocyte (PHK) monolayers, as well as in a three-dimensional skin equivalent model for epitheliotropic viruses. The concentrations required to inhibit 50% of viral replication (IC_{50}) were determined by plaque reduction assays. We found seven active compounds from the 1st class of ANPs including cidofovir (Vistide®) and from the 2nd class of ANPs that includes the DAPy derivatives with IC_{50} values ranging from 0.04 μg/ml to 2.78 μg/ml in both HEL and PHK cells. The 3rd class of ANPs containing a 5-azacytosine moiety were active against CMLV in HEL cells with IC_{50} values ranging from 0.04 μg/ml to 6.40 μg/ml, giving selectivity indices from 20 to 130. The antiviral activities of the relevant molecules were confirmed in virus yield assays in monolayers. We finally used human organotypic epithelial rafts cultures to evaluate the antiviral activities of the compounds. This model gives histological pictures comparable to those described for the skin biopsy specimens of the corresponding diseases. Complete suppression of cytoplasmic ballooning of the keratinocytes caused by CMLV was observed at the highest concentrations of the ANPs tested so far. In conclusion, we have established an in vitro camelpox virus infection model for the evaluation of anti-poxvirus compounds.

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Cytopathic Maporal Hantavirus Infection of Vero E6 Cells

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Hantavirus cardiopulmonary syndrome (HCPS) is an acute human disease with remarkably high case fatality rates (30–50%). Maporal virus (MAPV), recently isolated from western Venezuela, is most similar phylogenetically to hantaviruses known to cause HCPS in southern regions of South America. There is no evidence that MAPV can productively infect humans and cause severe disease, yet infection in hamsters closely resembles disease manifestations associated with human cases of HCPS. In general, hantaviruses, produce little, if any, cytopathic effect (CPE) in cultured cells. Unexpectedly, we found that MAPV produces dramatic CPE in Vero E6 cells resulting in rapid and complete monolayer destruction. Cell death was triggered through the induction of apoptosis as demonstrated by caspase 3/7 activation and TUNEL staining following infection. Blockade of apoptosis by the caspase inhibitor Z-VAD-FMK during MAPV infection limited cytopathology and cell death, with little effect on viral burden. Induction of apoptosis may require active viral replication as inhibitory effects of ribavirin and consensus a-interferon protected cells challenged with MAPV. As with other pathogenic hantaviruses, MAPV was found to utilize the αvβ3 integrin for cellular entry suggesting that it may also be a human pathogen. Since infectivity could not be entirely blocked with specific antibodies, other receptors may be involved. Highly cytopathic MAPV infection in Vero E6 cells will greatly facilitate the development of high-throughput cell-based screening assays needed to identify effective antivirals for the treatment of severe hantavirus infections.

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Comparison of Anti-Proliferative Activity of Selected Antiviral Agents in Various Assay Systems

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Determining toxicity of experimental compounds is an important step in the antiviral screening process. While bone marrow clonogenic assays are considered the most predictive for drug induced bone marrow suppression, they are expensive and not