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Virus replication in brain tissues of mice infected intracerebrally with a avirulent drug–resistant mutant of HSV-1. C-K. Lee, J.H. Kim, PK Bae and H.S. Kim. Pharmaceutical Screening Center, Korea Research Institute of Chemical Technology, Taejon 305-600, Korea

We have isolated and characterized various drug-resistant mutants of HSV-1 strain F. One of them, AR1 showed 1,000 fold reduced virus titers in mouse derived cell lines, BALB/3T3 and C3H/3T3 cells but not in Vero and 143B cells. It was extremely avirulent in various mouse infection models. LD_{50} of AR1 was >1,000,000 PFU/mouse while that of its parental type was 100 when 5 week-old female BALB/c mice were intracerebrally infected. We have measured tissue virus titers of the mice infected with virus and treated with acyclovir - 0, 5, 10, 25 mg/kg b.i.d., i.p. for 6 days. Various brain tissues such as cerebrum, cerebellum and brain stem, etc. were isolated everyday for 6 days. Dose responses in survival rate were observed in F-infected mice but only in 25 mg/kg group significantly reduced tissue titers observed. Cerebra and cerebella especially showed high titers and titers of cerebra increased continuously and the mice eventually died. Virus replication in various tissues was also observed in AR1-infected mice. But virus titers of cerebra increased until 2 day p.i. and decreased from 3 days p.i. in all the groups. AR1 is TK-deficient mutant. It has not been successful in recovering recombinant viruses replicating well in mouse cell lines in genetic recombination experiment with AR1 DNA and a plasmid DNA containing F-TK gene. AR1 may be a useful tool to elucidate the pathogenesis of HSV-1.

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Keratinocyte Organotypic Cultures as a Model for Studying Viral Infections

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Organotypic cultures of keratinocytes, isolated from human foreskins, grown at the interface air-liquid medium in a CO_2 incubator at 37°C give after 12-14 days of incubation a fully differentiated epidermis-like epithelium, including keratin formation. Such cultures are generally used for the study of human papillomaviruses (HPV). We have now used organotypic cultures to evaluate the ability of various DNA viruses to infect such cultures at different stages of their differentiation. Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) gave cytopathic effects resulting in ballooning and reticular degeneration of the keratinocytes and the occurrence of intranuclear eosinophilic inclusion bodies. Varicella-zoster virus (VZV) also led to morphological alterations, the nature of the lesions depending on the stage of differentiation of the cell cultures. The organotypic cultures also supported infections by vaccinia virus (VV) and the simian polyomavirus SV40 that resulted in vacuolation of the keratinocytes. In contrast, infection of the organotypic cultures with human cytomegalovirus (HCMV) did not lead to any morphological sign of infection even if the cultures were infected at very early stages of differentiation. Thus, the keratinocyte organotypic culture is an ideal model for the study of HPV, HSV, VZV, poxvirus and simian polyomavirus infections, that allows in the same culture, under the same experimental conditions, a comparative morphological and immunohistochemical study. In addition, this system also allows the evaluation of antiviral drugs as well as drug metabolism and biodisponibility. We are currently evaluating the pathogenicity of several drug-resistant mutants of VZV and HSV-1 in organotypic cultures at various stages of differentiation.

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Antiviral Activity of Different Anti-herpesvirus Compounds on Keratinocyte Organotypic Cultures Infected with Herpes Simplex Virus and Varicella-Zoster Virus

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The three-dimensional organotypic "raft" culture system of keratinocytes recreates important features, both morphological and physiological, of epithelial differentiation *in vitro*. Human primary keratinocytes, isolated from neonatal foreskin, grown at the air-liquid interface, stratify and differentiate with the expression of specific keratins within 12-14 days. These cultures can be used as a model for skin tissue, the main viral replication site during both primary and recurrent herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections. We have shown that infection of cultures with HSV results in the production of cytopathic effect (CPE) independently of the stage of differentiation of the cultures. In contrast, infection with VZV after 8-10 days of differentiation resulted in reduced CPE compared to cultures infected after 2, 4 or 6 days of differentiation. In order to evaluate the effect of various anti-herpesvirus drugs, organotypic cultures were infected with HSV-1 and HSV-2 after 8 days of differentiation and with VZV after 6 days of differentiation. At the moment of infection the cultures were incubated in the presence of medium containing different concentrations of the test compounds (acyclovir, brivudin, cidofovir and foscarnet). The cultures were incubated for 12 days, fixed and processed for histology. Morphological analysis of the organotypic cultures showed that treatment with cidofovir, acyclovir and brivudin at 40, 4 and 0.4 µg/ml and foscarnet at 200 µg/ml complete protected the epithelium against virus-induced CPE. As expected, only cidofovir and foscarnet showed activity against thymidine kinase-deficient viral strains. The evaluation of antiviral compounds in organotypic cultures may be of particular interest for VZV, given the fact that there is no *in vivo* model for VZV.

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Phospholipid Prodrugs of Antiviral Nucleosides: Synthesis and Activity of Octadecyloxyethyl, -propyl and -butyl esters of Ganciclovir Phosphonoformate.

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Phosphonoformate (PFA) and ganciclovir (GCV) are potent antivirals that might be useful in combination against human cytomegalovirus (HCMV) or herpes simplex virus (HSV-1). However, PFA is not active orally and GCV oral uptake is low (6-9%). Previously, we prepared lipophilic prodrugs of both PFA and GCV and showed that their oral bioavailability in mice was increased relative to the parent compounds, and that antiviral activity in vitro was enhanced. We have now synthesized a new series of prodrugs wherein ganciclovir is coupled to lipophilic alkoxyalkyl phosphonoformates, creating conjugates with the potential to deliver both PFA and GCV to infected cells. These combined prodrugs were active when evaluated for activity in HCMV- and HSV-1-infected MRC-5 cells in vitro (IC_{50} = 0.21 µM and 0.004 µM, respectively, when n = 0). Because of their structural similarity to previously studied PFA and GCV prodrugs, they may prove orally active in animal models of disease, and perhaps efficacious in man.