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Antiviral susceptibility of penciclovir resistant mutants derived from herpes simplex virus type 1 in various cell lines. J.H. Kim, P.K. Bae, H.S. Kim, J.K. Ahn*, and C.K. Lee. Pharmaceutical Screening Center, Korea Research Institute of Chemical Technology, and Department of Microbiology, Chungnam National University*, Taejon , Korea

To elucidate drug resistant mechanism in herpes simplex virus type 1 (HSV-1), we have isolated 5 penciclovir (PCV) resistant mutants, PR1-PR5 derived from strain F (F) in cell culture system. PCV is phosphorylated by a virus specific thymidine kinase (TK). PCV monophosphate is then phosphorylated by host enzymes to PCV diphosphate and further to triphosphate, a selective inhibitor of viral DNA synthesis. PR1 and PR2 were deficient in the TK activity. Antiviral activity of various compounds, mainly nucleoside analogues was examined in Vero, 143B and FTK143B cell lines by using CPE inhibition assay. PR1 and PR2 were highly resistant to acyclovir and ganciclovir, etc in Vero and cellular TK-deficient 143B cells. But they lost the resistance in FTK143B cells expressing viral TK of F. PR3, PR4 and PR5 were resistant to ganciclovir but not to acyclovir in all the cell lines. PR3 showed resistant to phosphonoformic acid and phosphonoacetic acid. PR1 and PR2 showed frame shift mutation causing premature translation of the TK gene and PR3, PR4 and PR5 did not show any base change. We considered that PR3, PR4 and PR5 have mutation(s) in DNA polymerase. Drug resistant mechanism induced by PCV is quite different from acyclovir which is caused by mainly TK mutation.

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Cross Susceptibility Patterns and Neuro-Virulence of Acyclovir-Resistant Herpes Simplex Virus (HSV) Isolates Collected in a National Surveillance Study. E.A. Harden[1], R.J. Rybak[1], C. Hartline[1], J.W. Gnann[1], C.A. Hodges-Savola[2], N.T. Wetherall[2], E.R. Kern[1], and The Task Force on Herpes Simplex Virus Resistance. [1]The University of Alabama School of Medicine, Birmingham, Ala., and [2]ViroMed Laboratories, Minneapolis, Minn, USA.

Acyclovir (ACV) has been widely used for treatment of genital HSV infections for the past 15 years. There are concerns, however, that resistance to ACV may become a problem, particularly in patients who are immunocompromised due to infection with HIV. To determine the frequency of ACV-resistant HSV, a Task Force was organized to carry out surveillance in HIV-positive individuals visiting geographically diverse outpatient clinics. Samples collected from patients with clinically suspected HSV disease were sent to a central laboratory for virus isolation and susceptibility testing to acyclovir. Isolates that required ≥1.0 μg/ml of ACV for inhibition of HSV replication were sent to 2 additional laboratories for confirmation. In our laboratory (UAB), all isolates were re-tested for ACV susceptibility, and those that were confirmed to be ACV-resistant were also tested for susceptibility to penciclovir (PCV), ganciclovir (GCV), cidofovir (CDV), and phosphonoformate (PFA). A total of 918 samples from HIV-positive subjects were collected, 419 were positive for HSV and 34 were ACV-resistant by plaque reduction assay at a cut-off level of 1.0 μg/ml. All but 2 isolates were HSV-2. Additional testing confirmed that 23 were definitely resistant to ACV at levels greater than 2.0 μg/ml (5.0 – >100 μg/ml) and 22/23 were also resistant to PCV. Of the 23 ACV-resistant isolates, 16 (70%) were GCV-resistant, 3 (13%) were CDV-resistant, and 2 (9%) were PFA-resistant. Assays for neurovirulence in mice indicated the individual isolates had differing levels of neurovirulence depending on the type of mutation, i.e. TK-deleted, TK-altered, polymerase, etc. In this national surveillance program the frequency of ACV resistance (6%) in the immunocompromised host has not changed from that reported in previous studies, however there is a need to continue to survey these populations in order to more readily detect changes in the susceptibility of HSV to antiviral drug therapy.

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Characterization of Acyclovir-Resistant Herpes Simplex Virus Collected by the Task Force on Herpesvirus Resistance. M. G. Davis, A. Root, C. L. Talarico, W. K. Lawrence, and the Task Force on Herpesvirus Resistance, Glaxo Wellcome, Inc. Research Triangle Park, NC, USA.

In an effort to monitor the incidence of acyclovir-resistant (ACV^r) HSV, the Task Force on Herpesvirus Resistance has established a surveillance program. To date >2200 HSV clinical isolates collected from urogential lesions have been screened with twenty-three HSV2 isolates identified as ACV^r (IC_{50}>2 μg/ml by plaque reduction assay). Of the twenty-three, twenty were from immunocompromised individuals and three were from immunocompetent individuals. Further characterization of these clinical isolates included thymidine kinase (Tk) functional studies using ^{14}C-thymidine and ^{125}I-iodo-2'-deoxycytidine plaque autoradiographies. Homogeneous ACV^r virus populations were isolated by plaque purification. In two cases, a sensitive isolate from a separate outbreak was also purified. DNA sequencing of the Tk open reading frame was performed to determine the ACV^r-associated genotype. Seventeen sequences contained single nucleotide insertions or deletions (compared to wt) that led to a predicted protein truncation, and cells infected with virus strains carrying these alterations were negative for ^{14}C -thymidine incorporation, a thymidine kinase deficient (Tkd) phenotype. Five sequences had nucleotide substitutions leading to predicted amino acid changes in the Tk protein. Viruses carrying these substitutions had an altered susceptibility (Tka) phenotype, in which both ^{14}C-thymidine and ^{125}I-iododeoxycytidine were incorporated into the nascent viral DNA, but the incorporation was greatly reduced as compared to wt HSV. Two isolates were resistant to ACV and PFA, a phenotype consistent with mutations in the DNA polymerase gene. Both by DNA sequence and phenotypic analysis, one of these viruses had a wild-type Tk, and the other had an altered Tk . Confirmation of suspected polymerase alterations by sequence analysis of the DNA polymerase genes of these two viruses is in progress. Overall the virus isolates from surveillance samples are similar to those previously reported for clinical and laboratory strains.

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Herpes simplex virus type 1 (HSV-1) variants arising after selection with an antiviral carrageenan. E.B. Damonte, M.J. Carlucci, L.A. Scolaro. Laboratorio de Virología, Dpto. Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1428 Buenos Aires, Argentina.

Carrageenans of diverse structural types (λ, μ/ν and κ/ι) isolated from the red seaweed *Gigartina skottsbergii* proved to be potent inhibitors of HSV types 1 and 2, by blockade of the initial virus binding to the host cell. The μ/ν-carrageenan 1C3 was tested in vitro for its ability to generate resistant variants by serial passage of HSV-1 strain F in Vero cells in the presence of increasing concentrations of 1C3. After 11 passages, there emerged viruses which were 2-10 fold more resistant to 1C3 inhibition than parental virus and formed large plaques with an altered syncytial phenotype (1C3-syn). Plaque-purified syncytial variants isolated from passages 13 and 14 have shown variable levels of cross resistance to the three types of carrageenans as well as to heparin and dextran sulfate, but all the clones were susceptible to acyclovir. All the 1C3-syn variants formed large syncytia in Vero and CV-1 cells, with more than 600 nuclei/syncytium, but did not induce cell fusion in other cell types. The syn phenotype was not related neither to drug-resistance nor to variant ability to bind Vero cells. After intracerebral or intraperitoneal inoculation of mice, 1C3-syn variants were as virulent as parental virus. The syncytial properties of 1C3-syn variants were not affected by cyclosporin A or melittin, suggesting that an alteration on glycoprotein gB could be responsible of the syn phenotype induced by 1C3 and, consequently, gB would be a main target for the antiviral activity of this natural carrageenan.