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Antiviral Drug Susceptibility of Herpes Simplex Type 1 (HSV-1) Strains Emerging Under the Selective Pressure of (S)-1-(3-Hydroxy-2-Phosphonylmethoxypropyl)Cytosine (HPMPC) *in vitro*

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Emergence of HSV-resistant strains in immunocompromised patients, particularly those suffering from AIDS, is an increasing problem in the management of viral infections in such patients. Acyclovir-resistant (ACV^r) strains have been isolated and characterized, and so have been foscarnet-resistant (PFA^r) strains and dually (ACV^r, PFA^r)-resistant strains. We report here the development of HSV resistance under the selective pressure of HPMPC. The resistant strains were obtained by serial passages of the HSV-1 KOS strain in increasing concentrations of HPMPC in Vero cell cultures. After reaching the highest possible concentration (40 μg/ml), a last passage was done in drug-free medium in order to obtain a virus stock. Drug sensitivity of the strains was determined by a viral CPE reduction assay. The strains obtained under the pressure of HPMPC (or HPMPA) were only resistant to these two compounds [IC_{50} of HPMPC: 2.7 μg/ml, as compared to 0.12 μg/ml for the KOS strain (IC_{50} of HPMPA: 1.8 μg/ml, as compared to 0.11 μg/ml for the KOS strain)]. No cross-resistance was detected toward acyclovir (ACV) or other phosphonate derivatives such as PMEA [9-(2-phosphonylmethoxyethyl)adenine] and PMEDAP [9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine]. Strains obtained under the pressure of PMEA or PMEDAP were cross-resistant to each other, but also cross-resisted to PFA and phosphonoacetic acid (PAA) [IC_{50} of PFA: 114 μg/ml, as compared to 30 μg/ml for the KOS strain (IC_{50} of PAA: 70 μg/ml, as compared to 15 μg/ml for the KOS strain)]. Our results point to differences in the mechanism of action of the HPMP and PME derivatives. In addition, the long lasting activity of acyclic nucleoside phosphonates, allowing infrequent administration and therefore a decreased selective pressure, together with the difficulty to obtain drug-resistant mutants *in vitro*, may be predictive of a low rate of *in vivo* selection of virus resistance to these compounds.

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Isolation and Initial Characterization of a Herpes Simplex Virus Type 2 (HSV-2) Strain With Decreased Sensitivity to Cidofovir.

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Cidofovir (CDV; (S)-(1)-(3-hydroxy-2-phosphonylmethoxy-propyl)cytosine, HPMPC) is an acyclic cytosine nucleotide analog with potent *in vitro* and *in vivo* activity against a broad spectrum of herpes viruses. By gradually increasing the concentration of CDV in tissue culture over an extended period we have selected a strain of HSV-2 (CDV-1) with a four-fold decrease in its sensitivity to CDV in MRC-5 cells. This virus exhibits no change in sensitvity to other anti-herpetic agents including acyclovir, ganciclovir, foscarnet, and 9-(2-phosphonylmethoxyethyl)-adenine (PMEA), an acyclic adenosine nucleotide analog. The plaque-purified virus does not revert to the parental phenotype even after ten passages in the absence of selective pressure, nor does it further decrease its sensitivity to CDV in response to increased pressure. In the absence of selective pressure, CDV-1 and the wild type virus grow at similar rates in tissue culture. However, in animal models, CDV-1 is much less virulent than the wild type virus. CDV-1 requires a 700-fold higher titre of virus for lethality following intracerebral injection in mice, and it is not able to infect, replicate, or cause mortality after intravaginal inoculation of mice. DNA sequence analysis of the DNA polymerase gene of CDV-1 identified a single amino acid change from the wild type virus (HSV-2, MS strain). This amino acid is located near, but not contained within, sequences conserved among other DNA polymerases and thought to be involved in substrate binding. Construction of a recombinant virus containing this mutation is in progress. We are also comparing the kinetic properties of DNA polymerases purified from CDV-1 and the parent strain to determine if there is a decreased sensitivity to CDV at the enzyme level.