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ACYCLOVIR, AS A TOOL TO DIFFERENTIATE BETWEEN MUTANTS OF HERPES SIMPLEX VIRUS, IN MIXED INFECTIONS. Ehud Katz and Kazem Keywan. Department of Virology, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

The growth of several couples of strains of herpes simplex virus type 2 (HSV-2), in BSC1 cells, was followed. The yield of the viruses in the co-infected cells was evaluated by plaque assay. In order to distinguish between the strains, one of the viruses used in every system was HSV-2-ACG, an acyclovir (acycloguanosine, ACG)-resistant mutant. While the titer of the resistant mutant was only slightly effected by the drug (100μM), the other virus used, was highly inhibited by it. The cells were first infected with HSV-2 (Curtis strain), and at time-intervals thereafter, infection with HSV-2-ACG, took place. The cultures were harvested 22 hrs after the initial infection. In the absence of the drug, HSV-2 was inhibited by more than 90% when HSV-2-ACG super-infected the cells, until 1 hr following the first infection, but proceeded normally when HSV-2-ACG was added on, or after, the third hr post-infection. As to HSV-2-ACG, it was decreased by 50% only, and just when added during the first 1/2 hr following infection with HSV-2. However, in the presence of ACG, the growth of both viruses was completely inhibited. The results suggest that in the presence of the drug, the capability of HSV-2 to inhibit the growth of HSV-2-ACG is highly increased and does not depend on the time of its addition to the HSV-2 infected cultures. We also wished to find out whether the capability of one strain of HSV to inhibit the growth of the other, depends on the ability of the virus to inhibit the host-cell protein synthesis. Strain G of HSV-2 and its HG52 mutant, which is deficient in gene 41, responsible for the shut-off of host-cell protein synthesis, were chosen for this study. The results show that these two strains inhibit equally well the growth of HSV-2-ACG, which is subsequently infecting the cells. This finding suggests that the inhibition of the second virus, is not only a consequence of the inhibition of the host-cell protein synthesis induced by the first, but also due to another mechanism.

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Intracellular Metabolism of the Novel Anti-Herpesvirus Agent 2-Amino-7-[1,3-dihydroxy-2-propoxy)methyl]purine Compound S2242 in Non-Infected and Herpesvirus-Infected Cells.
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We investigated the intracellular metabolism of S2242, the first known antivirally active acyclic nucleoside analogue with the side chain substituted at the N7 position of the purine ring. Uptake of S2242 by CEM cells increased linearly with increasing extracellular concentrations of the compound and was blocked by inhibitors of nucleoside transport. S2242 was phosphorylated in a time- and concentration-dependent manner to its mono- di- and triphosphate metabolites. Intracellular half-life of the di- and triphosphate in CEM cells was ~ 3-6 hr. In accord with the findings that (i) the cytostatic potential of S2242 was reversed by dCyd and (ii) that the growth of dCyd kinase (dCK)-deficient cells was relatively refractory to the action of S2242, the metabolites formed from S2242 in the dCK-deficient cell line were about one hundredth that of the wild-type cultures. The observation that purified dCK phosphorylated S2242 to its monophosphate, further corroborates these results. The anti-HSV, anti-VZV and anti-HHV6 activity of compound S2242 was reversed by 50-to 100-fold upon addition of exogeneous dCyd. In contrast, exogeneously added dCyd had no effect on the anti-HCMV activity of S2242. Compound S2242 was not preferentially phosphorylated in HSV-1-, VZV- or HHV6-infected cells (in Vero, HEL and HSB-2 cells, respectively), and exogeneously added dCyd substantially reduced the formation of S2242 metabolites in these infected cells. In HCMV-infected HEL cells, a 10- to 25-fold increase in S2242 metabolite formation was observed as compared to the non-infected cells, suggesting that a HCMV-encoded or -induced enzyme caused the specific phosphorylation of S2242. Compound S2242, at concentrations 40- to 200-fold higher than ganciclovir, had, however, no effect on the increased phosphorylation of the latter in HCMV-infected cells.