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Inhibitory Effects of (*S*)-1-(3-Hydroxy-2-Phosphonylmethoxypropyl)Cytosine and 9-(1,3-Dihydroxy-2-Propoxymethyl)Guanine on Human Cytomegalovirus Replication and DNA Synthesis

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Human cytomegalovirus (CMV) is an important pathogen, especially in immunosuppressed patients. The only drug which has been approved for the treatment of CMV infections is 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG, ganciclovir) Recently, (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine (HPMPC) was shown to be a potent and selective inhibitor of CMV replication *in vitro*. We have now evaluated the inhibitory effects of HPMPC and DHPG on viral DNA synthesis in CMV-infected human embryonic lung (HEL) cells. Both compounds achieved a dose-dependent inhibition of CMV DNA synthesis within the concentration range of 0.04 to 4 μg/ml. At a concentration of 4 μg/ml, HPMPC and DHPG reduced viral DNA synthesis by 100% and 96%, respectively. At a concentration of 0.04 μg/ml they inhibited viral DNA synthesis by 30% and 16%, respectively. A short exposure time (from 0 to either 6 or 24 hr post infection) of CMV-infected cells to HPMPC sufficed to afford a pronounced and prolonged inhibition of viral DNA synthesis and viral replication. In contrast, DHPG did not effect more than a weak and transient inhibition of CMV DNA synthesis and CMV replication after it had been exposed to the cells from 0 to either 6 or 24 hr post infection. Neither DHPG nor HPMPC inhibited cell proliferation and cellular DNA synthesis unless they were added at a concentration (100 μg/ml), that is 1000-fold higher than the concentration required to inhibit viral DNA synthesis.

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Mechanism of Cellular Uptake of Phosphonylmethoxyalkyl Purine Derivatives

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Cellular uptake of tritium-labelled (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA) and 9-(2-phosphonylmethoxyethyl)adenine (PMEA) was studied in a number of cell lines of different origin, i.e. macrophage, lymphocyte, fibroblast and epithelial cells. The compounds showed a virtually identical pattern of permeation in all cells, as indicated by kinetic experiments with inhibitors of the nucleoside transport system. Cellular uptake did not occur via facilitated diffusion, as for natural nucleosides, but through a process involving endocytosis, which was temperature-dependent and sensitive to endocytosis inhibitors such as sodium azide and cytochalasin B. Uptake was significantly higher in cells of the macrophage type than in the other cell lines. The amount of cell-associated drug increased with time, until at about 2 hr an equilibrium was established between endo- and exocytosis. At this time, intracellular drug molarity exceeded extracellular drug molarity by two- or three-fold. Cellular uptake of the drugs was inhibited by an excess of cold HPMPA or PMEA, and by ssDNA, which suggests that the endocytosis process is of the absorptive type. It is probably mediated by the receptor sites that recognize nucleic acids based on their negative charges. This conclusion is substantiated by the observation that a significant proportion (≈ 10%) of the cell-associated drug was TCA-precipitable.