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The Anti-Herpes Simplex Virus (HSV) Activity of *n*-Docosanol Includes Inhibition of the Viral Entry Process. *L.E. Pope, *J.F. Marcelletti, **M.L. Parish, **P.G. Spear and *D.H. Katz. *LIDAK Pharmaceuticals, La Jolla, CA, USA and **Northwestern University Medical School, Chicago, IL, USA

n-Docosanol-treated cells are resistant to infection by a variety of lipid-enveloped viruses, including HSV, that enter cells by fusion with the plasma membrane. Detailed study of the HSV system revealed that, although *n*-docosanol had no effect on the binding of radiolabeled HSV to target cells, the compound inhibited subsequent cellular entry by HSV. An inhibitory effect of *n*-docosanol on fusion between the HSV envelope and plasma membrane was indicated by a 76% reduction in fusion-dependent dequenching of a lipophilic fluorescent probe, octadecyl rhodamine B chloride, that had been inserted in the HSV envelope. Inhibitory effects on HSV entry were also evidenced by reduced release into cells of virion-associated regulatory proteins, as indicated by an 80% reduction in the expression of beta-galactosidase from target cells carrying a stably transfected lacZ gene under control of an HSV immediate-early promoter. Nuclear localization of the HSV genome was also reduced to less than 1% of levels observed in untreated cells following *n*-docosanol treatment, as shown by monitoring the fate of ^{3}H-TdR-labeled HSV. Consistent with the inhibition of viral entry, there was a reduced expression of viral genes in *n*-docosanol-treated cells including an 80% reduction in the expression of a reporter gene under the control of a constitutive promoter that had been inserted into the viral genome. Thus, inhibition of fusion between the plasma membrane and the HSV envelope, and the subsequent lack of replicative events, as shown by these studies, appears to be a predominant mechanism for the anti-HSV activity of *n*-docosanol.

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In Vitro Antiherpetic Activity Of Brivudin: Effect Of Variable Exposure Times And Evaluation Of Drug Interaction

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The antiherpetic activity of Brivudin (BVDU) has been widely described in literature. In order to mimic more closely the conditions found in humans after oral dosing of the drug, we studied the effect exerted by Brivudin on Herpes Simplex Virus -1 (HSV-1) at different stages of its replication. We also investigated the antiviral activity of BVDU in combination with antiviral drug commonly used in chemotherapy. The "in vitro" anti-HSV-1 effect of BVDU was evaluated in cell cultures at different stages of viral replication. Infected cell cultures were exposed to BVDU or Acyclovir (ACV) for varying time intervals (1 to 4 h) either immediately post infection (early exposure) or 5 h after virus addition (late exposure). Antiviral drug potency was assessed by measuring cytopathic effect, production of infectious virus (viral yield) and viral DNA synthesis. The results demonstrate that the drug susceptibility is greater when the treatment starts late in the viral replication cycle: late exposure of 2 h with Brivudin 1μM inhibited viral yields by 90%, while an early exposure of same length led to an inhibition near 30%. Combination of BVDU with other antiviral agents was assessed by inhibition of HSV-1 induced cytopathic effects in Vero cells. Antiretroviral compounds as AZT and ddI, antiherpetic drugs such as ACV and Foscarnet were employed in a wide range of doses up to 100 μg/ml with BVDU 0.03 μg/ml. In our experimental model, BVDU exhibited an additive interaction with ACV and Foscarnet. When employed at high concentrations (>10 μg/ml) , AZT was found to diminish the activity of BVDU , while ddI had no interference. BVDU showed no interaction with AZT and ddI, when these drugs were used at a concentration similar to their peak plasma level in man, respectively 0.4 and 0.5 μg/ml. Cytotoxicity on uninfected cells was not increased by combined treatment.

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Metabolism Of Brivudin (BVDU) In HSV-1 Infected Cells

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By using ^{14}C-BVDU and its synthetic phosphorylated derivatives, BVDU-MP, BVDU-DP and BVDU-TP, we have evaluated the intracellular metabolism of Brivudin, an uracil based nucleoside exerting potent virostatic activity against VZV and HSV-1. Vero cells were either mock infected or infected with HSV-1 (HF strain) at 0.01 PFU/cell for 20 hr. After washing off the inoculum, cells were exposed to ^{14}C--BVDU (5μM) for variable intervals (20 min. up to 24 hr). Two cell fractions were obtained by extracting the washed cells with ethanol-buffer and SDS. The former fraction corresponds to the cytosol, while the second contains DNA and proteins. Radioactivity was determined by scintillation counting while BVDU-MP, -DP and -TP were assayed by reverse phase HPLC using both a UV and radio-detector. During the first 20 min of incubation a massive transfer of radioactivity from the medium to the cytosol occurred (approx. 35%); most of the radioactivity (80%) corresponded to BVDU-MP, while BVDU-TP and BVDU represented 10% each of total radioactivity. In the SDS fraction total radioactivity rose slowly during the first 2 hr of incubation and then rapidly up to 24 hr. BVDU-DP was undetectable in cell fractions at any time point. In mock infected cells uptake was negligible. Infected Vero cells were also exposed to ^{14}C--BVDU for a short interval (2 hr. pulse treatment) and reincubated in drug-free medium for various time intervals (up to 24 hr). Radioactivity present in the cytosol, identified as BVDU-MP and BVDU-TP, declined biphasically with a terminal half-life of 10 hr for both compounds. After pulse with ^{14}C--BVDU, the incorporation into the SDS fraction rose steadily up to 24 hr. These findings indicate that BVDU is avidly taken up by HSV-1 infected cells, immediately converted in the cytoplasm to BVDU-MP and BVDU-TP, and more slowly incorporated into viral DNA. The long terminal half-life of intracellular BVDU phosphorylated derivatives is consistent with the temporal pattern of inhibition of viral synthesis.

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Anti-HSV-1 Activity of Phenolic Polymers Derived from p-Diphenolic Starting Compounds.

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Humic acid-like phenolic polymers derived from o-diphenolic starting compounds are potent inhibitors of herpes simplex virus type 1 (HSV-1) replication (Neyts et al., 1992, Helbig et al., 1996, in press). Referring to these results, we synthesized a new series of 13 polymers by oxidation of *p-diphenolic* compounds. Using the XTT-based tetrazolium reduction assay EZ4U, both the low-molecular starting compounds and the synthesized polymers were examined for their antiviral and cytotoxic activities in HSV-1-infected Vero cells. The results show that most of the starting compounds do not exhibit any antiviral activity to HSV-1, but strongly reduce the viability of cells. In contrast to this, polymers are well tolerable by cells and inhibit virus replication to different degree. The antiviral activity was strongly dependent on the substitution of the aromatic or quinoid ring systems of starting compounds. Best results were obtained with polymers derived from gentisinic acid (GENOP: IC_{50} 3.7 μg/ml, CC_{50} >128 μg/ml) and from 2,5-dihydroxyphenylacetic acid (2,5-DHPOP: IC_{50} 4.7 μg/ml, CC_{50} >128 μg/ml).