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Antiviral Effect of the Extract of Culture Medium of Lentinus Edodes Mycelia on the Replication of Human Cytomegalovirus in Human Cells.

S. Chatterjee[1], J. Koga[2] and R.J. Whitley[1]. [1]Department of Pediatrics, University of Alabama School of Medicine, Birmingham, Ala., USA; [2]JCR Pharmaceuticals, Kobe, Japan.

An extract of culture medium of Lentinus edodes mycelia, JLS-S001, blocked the replication of human cytomegalovirus (HCMV) in human fibroblast cells. The block in replication was due to neither the effect of JLS-S001 on the attachment of HCMV to the human fibroblast cells nor the toxic effects of JLS-S001 on human fibroblast cells. Furthermore, an in situ DNA hybridization experiment using a biotinylated HCMV DNA probe revealed no major inhibition of HCMV DNA synthesis in JLS-S001-treated human cells. Immunoblot analysis of JLS-S001-treated cells, however, showed a significant block in the expression of HCMV-specific proteins in treated human cells. Consistent with the above observation, electron microscopy demonstrated few intracellular or extracellular viral particles. These results suggest that JLS-S001 may affect some specific step(s) in the complicated transcription-translation cascade that regulates HCMV gene expression in human cells. Studies are now in progress to determine more clearly the stage(s) at which virus replication in human cells is inhibited by JLS-S001.

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Inhibitory Effect of Cytokines in Breast Milk for the Reactivation of Human Cytomegalovirus

K. NUMAZAKI*, H. ASANUMA, and S. CHIBA. Sapporo Medical University School of Medicine, Sapporo, 060, Japan

Breast-feeding is the major factor during the first year of life with human cytomegalovirus (HCMV) excretion rates of more than 50% observed in countries where the majority of women were seropositive and breast-feed their infants. Studies in our and other laboratories provided compelling evidence that virolactia was more common in samples collected during 1 week to 3 months postpartum than in those collected during the first week. We collected colostrum, breast milk and serum from 65 HCMV IgG antibody-positive mothers who breast-fed at 3 days and a month after delivery. When investigated by indirect immunofluorescence assay (IFA), any concentration of liquid supernatant of colostrum without cytotoxicity from 10 different mothers was not found to exert inhibitory effect on HCMV-infected MRC-5 cells. The activities of tumor necrosis factor (TNF)-α were detected in HCMV DNA-negative colostrum and breast milk. These activities were not detected from HCMV DNA-positive milk and not from sera. Interferon (IFN)-γ activities were detected in colostrum. Serum levels of cell free soluble interleukin-2 receptor (sIL-2R) titers of one month after delivery were significantly elevated than those of three days. It is likely that presence of cytokines such as TNF-α and IFN-γ in colostrum and early breast milk are related to inhibit the reactivation of HCMV which occurs locally in the mammary gland of the lactating mother after delivery.

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Kinetic and Mechanistic Studies of the Interactions of Ganciclovir Triphosphate with Human DNA Polymerases α, β, γ and Human Cytomegalovirus DNA Polymerase. X. F. Xiong and M. S. Chen. Gilead Sciences, Foster City, CA, U.S.A.

Ganciclovir, 9-[(1,3-dihydroxy-2-propoxy)methyl] guanine (GCV) is a potent inhibitor of herperviruses, including cytomegalovirus (CMV). The primary mechanism of action of ganciclovir against CMV is inhibition of the replication of viral DNA by GCV triphosphate (GCVTP), the putative antiviral metabolite of GCV. In this study, we use defined synthetic oligodeoxynucleotide primer-templates to study the mechanisms and kinetics of the interactions of ganciclovir triphosphate with human DNA polymerases α, β, γ and human CMV DNA polymerase. Human DNA polymerase α, γ and human CMV DNA polymerase can incorporate one GCV monophosphate (GCVMP) molecule and continue to elongate the DNA chain. None of the three enzymes can incorporate two consecutive GCVMP molecules. Human DNA polymerase β can not incorporate any GCVMP. For human DNA polymerase α, γ, and CMV DNA polymerase, the Km values of GCVTP are 9.3 ± 2.9 μM, 9.6 ± 2.9 μM, and 14.2 ± 1.2 μM, respectively. The ratios of Vmax/Km values of GCVTP to dGTP have also been determined for these enzymes. A primer-template set containing one GCVMP molecule at the 3' end of the primer is used to study the effect on incorporation of the next nucleotide. Km and Vmax/ Km values of the GCVMP terminated primer-template have been compared with that of regular primer-template.

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Effects of Temporal Treatments of Nucleoside Analogs on Replication of Epstein-Barr Virus

E.-C. Mar and J.-C. Lin. Centers for Disease Control, Atlanta, GA 30333

We assessed effects of different temporal treatments of inducers and nucleoside analogs on the replication of Epstein-Barr virus (EBV) *in vitro*. Two groups of anti-EBV drugs were chosen: 1) acyclovir and ganciclovir; 2) (+)-*B*-D-dioxolane-cytosine and (-)-*B*-L-dioxolane-cytosine. Three protocols were assessed for drug effects. First, when cells were treated with effective dose of drugs and inducers for 7 days, a 90 to 98% of inhibition of EBV genome copy numbers was observed. Due to prolonged exposure of inducers, cell numbers decreased after 7 days of treatment, making the assay difficult. Second, when cells were treated with inducers for 1 day, washed, and resuspended in fresh medium plus drugs for 7 days, a partial inhibition (10 - 20% for dioxolane and 40 - 50% for acyclovir group) was observed, although cells proliferated under these conditions. Third, when cells were co-treated with drugs and inducers for 1 day, washed, and refed with fresh medium plus drugs for 7 days, a similar degree of inhibition as in the first protocol, but without affecting cell growth, was observed. Thus, the last protocol appears to be an effective system for drug study.