118

Effect of CTC-23 on the Replication of Herpes Simplex Virus Type 1 in Human Fibroblast Cells. S. Chatterjee, P. Burns, R.J. Whitley and E.R. Kern, Department of Pediatrics, University of Alabama School of Medicine, Birmingham, Alabama 35294, U.S.A.

CTC-23 is a crystalline, cobalt containing complex with activity against herpes simplex virus type 1 (HSV-1). We utilized CTC-23 to investigate the mechanism of action of this compound against HSV-1 infection in human fibroblast cells. In the course of these studies two distinct mechanisms for inhibition of HSV-1 replication were observed. When CTC-23 was incubated directly with HSV-1, the infectivity of the virus for tissue culture cells was neutralized. In addition to having the capacity to inactivate HSV-1 directly, this compound also interfered with viral replication after the virus infected susceptible cells. Pretreatment of human fibroblast cells with 5 μg/ml of CTC-23 blocked the release of infectious HSV-1 by approximately 90%. The mean EC_{50} of CTC-23 added 1 hr post-infection was approximately 1 μg/ml. Analyses of early events following virus infection showed that CTC-23 did not interfere with the adsorption of HSV-1 to the target cells. Consistent with this observation, an in situ DNA hybridization experiment demonstrated no significant inhibition in DNA synthesis in CTC-23-treated human cells. Preliminary analyses of the intracellular proteins produced in CTC-23-treated human cells by immunoblot technique demonstrated no significant changes in the expression of virus-specific capsid proteins. However, at higher concentration (10 μg/ml), the expression of some nucleocapsid proteins and glycoproteins B and D were reduced in CTC-23-treated cells. These results indicated that the CTC-23-induced block in HSV-1 replication appeared to be at a late stage in the virus replication cycle. Studies are now in progress to determine more clearly the stage(s) at which virus replication in human cells is inhibited by CTC-23.

119

Molecular Effects of Papaverine on Human Cytomegalovirus Replication. D. Millinoff, I. Boldogh and T. Albrecht. The University of Texas Medical Branch, Galveston, Texas, U.S.A.

In this study, we examined the effect of papaverine on the synthesis of specific CMV macromolecules. Papaverine is a potent inhibitor of CMV replication (ED_{90}=1.6 μM) which appears to disrupt virus-induced cellular physiological responses closely related to efficient virus replication. Papaverine did not affect the abundance of CMV immediate early (IE) proteins while the abundance of CMV early and late protein was decreased in a dose-dependent manner. This pattern of inhibition suggested that CMV DNA synthesis, which requires CMV early gene products and mediates the expression of CMV late genes, might be compromised by papaverine treatment. Slot blot hybridization analysis confirmed that papaverine (3 μM) inhibited CMV DNA synthesis by 90% (96 hr postinfection, PI). Northern blot analysis of RNA isolated from CMV-infected cells (at 96 hr PI) indicated that the expression of CMV DNA polymerase gene was inhibited 6-fold by papaverine (3 μM). Furthermore, kinetics analysis demonstrated that papaverine behaves as a non-competitive inhibitor of CMV DNA polymerase activity (K_i=2.3 μM). The reduced expression of CMV early genes, including CMV DNA polymerase, suggested the possibility that papaverine inhibited posttranslational modification of CMV IE proteins, which are required for transactivation of CMV early gene expression. Phosphorylation of CMV IE1 and IE2 proteins was substantially decreased in the presence of papaverine. Therefore, it appears that the extraordinary antiviral potency of papaverine may be related to inhibition of the synthesis of several classes of CMV macromolecules through interference with posttranslational modification of CMV-encoded regulatory proteins.