63

Inhibition of Human Immunodeficiency Virus Envelope Glycoprotein-Mediated Cell Fusion By Synthetic Phospholipid Analogs.

L.KUCERA,[1] J.THOMPSON,[1] N.IYER,[1] R.PODUVAL,[1] S.MORRIS-NATSCHKE,[2] K.ISHAQ,[2] Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157[1] and The University of North Carolina, Chapel Hill, NC 27599[2] USA.

Phospholipid (PL) analogs of platelet activating factor have potent and selective activity against HIV-1 envelope glycoprotein (gp120)-CD4 interaction in productively infected CEM-SS cells measured by inhibition of cell fusion. Fusogenic activities in the presence of PL were further analyzed using a highly versatile recombinant vaccinia virus (vPE16) expressing gp120 after 15 hrs infection of nonhuman monkey kidney (Vero) cells and subsequently cocultured with uninfected CD4+ CEM-SS cells for 6-24 hrs.. Results indicated that at a moi = 10 the efficiency of vaccinia virus infection averaged 73% of the cells measured by DAPI staining of intracytoplasmic inclusions. When PL CP-51 treatment was started with vaccinia virus infection or delayed until establishment of coculture with uninfected CD4+ CEM-SS cells fusogenic activity was inhibited 82% or 80%, respectively, indicating that CP-51 inhibition does not involve gp120 glycosylation. CP-51 and PL-AZT conjugate (CP-92) inhibited fusogenic activity in a dose-dependent manner with a IC_{50} of 0.41 and 5.0uM, respectively. Inhibition of fusogenic activity also occurred following CP-51 treatment of vaccinia virus infected (73% inhibition) or CP-51 or CP-92 treatment of uninfected (74% or 67% inhibition) CEM-SS cells for 15hrs prior to coculture. In summary, these results indicate that PL inhibition of gp120-CD4+ interaction most likely is a post-transcriptional event that can occur in nonhuman or human cells expressing the HIV-1 envelope gp120.

64

Characterization of siamycin I, an HIV fusion inhibitor

P.-F. Lin, H. Samanta, C. M. Bechtold, C. Deminie, M. Alam, K. Riccardi, R. E. Rose, A. K. Patick, R. White and R. J. Colonno Dept. of Virology, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492

The HIV fusion inhibitor Siamycin I, a 21-mer tricyclic peptide, was isolated from a Streptomyces culture using a cell fusion assay involving cocultivation of Hela-$CD4^+$ cells and monkey kidney cells (BSC-1) infected with a vaccinia vector expressing gp160 (Ref. Tsunakawa, Hoshino, Detlefson, Hill, Furumai, Nishio, Kawano, Yamamoto, Fukagawa, Oki, submitted to J. Antibiotics). Siamycin I is effective against acute HIV-1 and HIV-2 infections in a cell protection assay with ED_{50s} of 0.1-0.6 μM and a CC50 of 150 μM in CEM-SS cells. Inhibition appears to be specific, since Siamycin I will inhibit fusion between HIV chronically-infected CEM-SS and $CD4^+$-C8166 cells (ED_{50} of 0.08 μM), while having no effect on Sendai virus-induced fusion or murine myoblast fusion. Siamycin I seems to bind non-covalently, since inhibition of HIV-induced fusion by this compound is reversible. Siamycin I does not inhibit gp120-CD4 binding in either gp120 or CD4 capture ELISAs or I^{125}I-gp120-CD4 binding assays. To determine the anti-viral target of this compound, a variant of HIV resistant to Siamycin I was selected by *in vitro* passage of virus in the presence of increasing concentrations of the compound. Drug sensitivity studies of chimeric virus and pseudovirus containing the envelope gene from resistant virus suggest that the gp160 domain may be responsible for resistance. Further DNA sequencing of the envelope gene from the resistant and parent viruses revealed a total of 6 amino acid changes in the gp160 domain. Confirming the significance of these substitutions in resistance development using site-directed mutagenesis is in progress.