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Influence of Hsp70 on HIV-1 Infection

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Introduction: HIV-1 virions are reported to be formed by assembly of Gag protein multimers which come into contact with cell membrane and undergo budding and release from the cell. During this process, it has been reported that APOBEC3G (A3G) and Heat shock protein 70 (Hsp70) are incorporated into HIV-1 virions. A3G is a host protein with anti-HIV-1 activity and Hsp70 is one of molecular chaperones which assist protein folding. Nowadays, molecular chaperones are said to be related with control of cell functions such as protein synthesis, transport and quality control. In addition, they are widely involved in signal transduction and cellular immunity. In this study, we investigated the interaction of A3G, Hsp70 and HIV-1 Gag, and influence upon HIV-1 virion formation and infectivity.
Methods: In order to investigate the interaction among A3G, Hsp70 and HIV-1 Gag, we co-transfected 293T cells with HIV-1 infectious molecular clone and the A3G expression vector, and then performed I.P and I.F. Next, the quantity of A3G incorporation to HIV-1 virions and infectivity after interaction of A3G and Hsp70 were investigated by co-transfection of 293T cells with Hsp70-specific siRNA or Hsp70 expression vector, A3G expression vector and the HIV-1 infectious molecular clone. After that, Western blot analysis and infection assay were performed.
Results and conclusion: According to the results of I.P and I.F, it was verified that A3G, Hsp70 and HIV-1 Gag interact each other within cytoplasm. The amount of A3G incorporation into HIV-1 virions decreased when Hsp70 was knock downed, whereas it increased when Hsp70 was over expressed. Moreover, HIV-1 virion yield also decreased when Hsp70 was knock downed and virion yield increased when Hsp70 was over expressed. However, HIV-1 infectivity increased when Hsp70 was knock downed and when it was over expressed, HIV-1 infectivity declined, showing possibility that over expression of Hsp70 could lead to inhibition of HIV-1 and we can suggest the possibility of HIV-1 therapy.

doi:10.1016/j.antiviral.2008.01.098

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Suppression of HCV RNA Replication by Baculovirus-mediated shRNA Expression Vectors

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Hepatitis C virus (HCV) core protein has been reported to interact with a variety of cellular protein and to influence numerous host cell functions. Recently, RNAi is a highly specific mechanism of posttranscriptional gene silencing mediated by double-stranded siRNAs ranging in size from 21–27 nt, which can be targeted to any gene of interest. The HCV core gene is a potential target for RNAi technology. On the other hand, the baculovirus autographa californica multiple nucleopolyhedrovirus (AcMNPV) can infect a variety of mammalian cells, facilitating its use as a virus vector for gene delivery in viral entry into cells. In this study, we describe the suppression of HCV replication by baculovirus-mediated shRNA expression vectors. We identified an effective site on the core region for suppression of the HCV core protein. This was carried out by core protein-shRNA expression Baculovirus vectors (core-shRNA-452, -479, and -523). Especially, the core-shRNA-452 containing sequence of 452–472 nt, as the target of the HCV core gene dramatically inhibited the expression of HCV core protein in replicon cells. Our results support the feasibility of using shRNA-based gene therapy to inhibit HCV core protein production.

doi:10.1016/j.antiviral.2008.01.099

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Synthesis and Evaluation of Octadecyloxyethyl Esters of Five 3-Hydroxy-2-(Phosphonomethoxy)Propyl Nucleoside Phosphonates in HIV-1 Infected Cells

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Acyclic nucleoside phosphonates (ANPs) having the (*S*)-3-hydroxy-2-(phosphonomethoxy)-propyl (HPMP) side chain are an important group of potent and selective anti-DNA virus agents that includes cidofovir and (*S*)-HPMPA. Addition of a phosphonate-masking alkoxyalkyl group containing about 20 atoms (e.g. hexadecyloxypropyl, HDP) enhances the antiviral activity, oral absorption and pharmacokinetics of cidofovir, (*S*)-HPMPA and all other ANPs that our group has studied. Octadecyloxyethyl (ODE) esters are generally more active than HDP esters. We have shown previously that the

HDP and ODE esters of (*S*)-HPMPA are potent inhibitors of HIV-1 replication in vitro ($EC_{50} = 0.4–7.0$ nM) whereas the unmodified phosphonic acid (*S*)-HPMPA and other unmodified HPMP series ANPs are generally inactive against HIV-1. To expand our approach to other HPMP nucleosides, we synthesized the ODE esters of (*S*)-HPMP-guanine (HPMPG), (*S*)-HPMP-thymine (HPMPT), (*S*)-HPMP-cytosine (HPMPC) and (*S*)-HPMP-2,6-diaminopurine (HPMPDAP) and evaluated these compounds against HIV-1 infection in MT-2 cells using a p24 reduction assay. ODE-(*S*)-HPMPA, ODE-(*S*)-HPMPDAP, and ODE-(*S*)-HPMPC were the most active compounds in this series with EC_{50}'s ≤ 1 nM. ODE-(*S*)-HPMPG also suppressed HIV-1 replication with EC_{50} value of 9 nM. However, ODE-(*S*)-HPMPT was inactive. Our results indicate that HDP-(*S*)-HPMPA, ODE-(*S*)-HPMPC, ODE-(*S*)-HPMPG and ODE-(*S*)-HPMPDAP should be evaluated further as potential therapies against HIV-1 infection. Alkoxyalkyl analogs of (*S*)-HPMPA and (*S*)-HPMPC are orally bioavailable and effective in animal models of various viral diseases such as vaccinia, ectromelia, HCMV and HBV infection. With the exception of ODE-(*S*)-HPMPT, ODE esters of HPMP-nucleosides are generally quite active and selective in MT-2 cells infected with HIV-1 and further studies of their possible utility in HIV infection are being pursued.

doi:10.1016/j.antiviral.2008.01.100

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Dysregulation of the Antioxidant Enzyme Defense in Hispanic Women with HIV-associated Neurocognitive Disorder

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HIV-associated neurocognitive disorders (HAND) remains prevalent during HAART. Activated and HIV-infected monocytes are known to cross the blood brain barrier and contribute to the release of virus and inflammatory mediators including cytokines, chemokines, free radicals, and viral proteins. These neurotoxins and inflammatory mediators can induce oxidative stress and neuronal death. We hypothesize that monocyte innate immunity is defective therefore contributing to oxidative stress in the CNS in patients with HAND. Studies from our laboratories have demonstrated different protein profiles in blood monocyte-derived macrophages and CSF that are related to HAND. We confirmed differential expression and activity of Cu/Zn Superoxide Dismutase (SOD-1) in the CSF and monocytes in a cohort of Hispanic women with HAND using HAART. The present study investigates activity of glutathione peroxidase (GPx) in the CSF, monocytes, macrophages, and plasma of 36 Hispanic women with or at risk of developing HAND. GPx activity was measured indirectly by the change in absorbance at 340 nm upon oxidation of glutathione and NADPH. We found that CSF from women with cognitive impairment (CI, MSK ≥ 1) had a significant decrease in GPx activity ($p = 0.001$). Monocytes showed a decreased Gpx activity although not significant ($p = 0.124$). Monocyte-derived macrophages showed a higher activity in the asymptomatic group of patients ($p = 0.056$) which decreased with HAND severity. Interestingly, we found a higher activity of GPx in plasma of patients with CI ($p < 0.05$). Our conclusion is that Hispanic women with CI have decreased protection from oxidative damage in the central nervous system. The reduction of GPx and SOD activity in monocytes may contribute to the significant low antioxidant activity found in the CSF of patients with CI. Our data supports the need for complementary therapy to antiretroviral drugs that increase the activity of the cellular antioxidant enzymatic complex. This combination will contribute to the prevention of development and or progression of HAND.

doi:10.1016/j.antiviral.2008.01.101

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Characterization of Small Molecule Inhibitors of West Nile Virus NS3 Serine Protease

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Characterization of inhibitors of West Nile Virus NS3 Serine Protease. The members of Flaviviridae encode a serine protease (NS3-pro) that is essential for polyprotein processing and thus for virus replication, making it an attractive target for inhibitor design. This study details the biochemical characterization of inhibitors of WNV NS3-pro identified in a high throughput screen (HTS) followed by further analysis of selected compounds and their derivatives. Two of these compounds exhibited IC50 values of 3.74 ± 0.17 μM and $\sim 5.146 \pm 0.54$ μM and Ki values of $\sim 2.2 \pm 0.36$ μM and $\sim 5 \pm 0.46$ μM. Inhibitory values of structurally related compounds were also determined. The potency and selectivity of the compounds using cell-based and infectivity assays will be reported. The information derived from this study could be useful in designing potent inhibitors for therapeutic use.

doi:10.1016/j.antiviral.2008.01.102