71

Antiviral Effect of Ingenol and Gingerol During HIV-1 Replication in MT4 Human T Lymphocytes

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We tested various natural products to screen the novelanti-human immunodeficiency virus type 1 (HIV-1) agents. Among 17 natural products, ingenol purified from Euphorbiaingens and gingerol derived from Zingiber officinale showed inhibitory function against HIV-1 replication during various stages of the virus life cycle in infected MT4 human T lymphocytes. Cell viability of MT4 cell was measured after 24 h, and Anti-HIV infection activity was measured after 6-day infection of HIV-1 in presence of serially diluted ingenol and gingerol. MT4 cells were infected with HIV-1 (IIIB) at multiplicity of infection (MOI 0.002–0.016) with 1.95–250 μM ingenol and gingerol. Cell cycle and apoptosis levels were measured using propiodiumiodide (PI), Anexin V staining methods after 3- or 6-day infection. Both ingenol and gingerol effectively maintained high cell viability. CD50 of ingenol was >1 mM for ingenol and 587.5 μM for gingerol. ED50 of ingenol was 5.06 uM for 100 TCID, 16.87 μM for 200 TCID of HIV-1. ED50 of gingerol for 100 TCID and 200 TCID were not reached 50% effective dose. Interestingly, anti-HIV infection activity of ingenol showed 20% lower than control of Azidothymidine (AZT) treatment. Viral p24 antigen was not remarkably reduced by ingenol or gingerol, either IFN-γ production was not induced at sixth day after HIV-1 infection. As a result, ingenol and gingerol help CD4+ T cells to maintain high cell viability against HIV-1 infection without ruling viral replication, and both can be considered as safe and effective candidates for immune boosting therapy for AIDS patients.

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72

Cystatin B Associates with STAT-1 in Monocyte-derived Macrophages and Placental Macrophages: A Possible Link to HIV Activation

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Cystatin B, a cysteine proteinase inhibitor has been found to induce nitric oxide production in macrophages and secreted in HIV infected monocyte-derived macrophages (MDM) supernatants. We found increased intracellular levels of cystatin B in HIV-infected MDM when compared with uninfected cells. Cystatin B was even higher in both uninfected and HIV-infected MDM when compared to uninfected and HIV-infected placental macrophages where HIV replication is restricted compared to MDM. We wanted to elucidate the signaling pathway that could play a role in viral replication or cell activation upon HIV infection of PM and MDM. We analyzed cystatin B after immunoprecipitation of uninfected and HIV-infected MDM and PM cell culture lysates with a specific anti-cystatin B monoclonal antibody. Using western blotting the immunoprecipitated cystatin B was tested for STAT activation with anti-STAT-1, STAT-3, and STAT-5 antibodies. We found that STAT-1 co-immunoprecipitated with cystatin B. Our study demonstrated the direct interactions of cystatin B and STAT-1 suggesting a possible mechanism for nitric oxide activation. We posit that STAT-1 interaction with cystatin B can enhance viral replication by increasing levels of nitric oxide. Therefore the study of STAT-1 in the context of cellular interaction with other proteins during the HIV infectious process could shed important information for the development of future antiviral therapies.

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73

Use of AX-4 Cells for the Study of the Pharmacodynamics of Oseltamivir Carboxylate for Influenza Viruses in an *In Vitro* Hollow Fiber Infection Model System

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MDCK cells have been used to determine the susceptibilities of influenza viruses to antiviral drugs. However, MDCK cells gave variable results when determining the susceptibilities of influenza viruses to neuraminidase inhibitors. To avoid this problem, we obtained MDCK cells transfected with the human β-galactoside α-2,6-sialyltransferase 1 gene (AX-4 cells) to determine the drug susceptibilities of influenza viruses to neuraminidase inhibitors. We also used these cells to determine the pharmacodynamically linked variable of oseltamivir carboxylate for influenza viruses in our in vitro hollow fiber infection model system. For dose ranging studies, six hollow fiber units were charged with 10^2 virus infected AX-4 cells and 10^8 uninfected AX-4 cells. Each unit was continuously infused with a different concentration of drug for 6 days. For dose fractionation studies, five hollow fiber units containing 10^2 virus infected AX-4 cells and 10^8 uninfected AX-4 cells were set up. One unit received no drug, one unit received 2× EC_{50} oseltamivir carboxylate by continuous infusion, one unit received the same AUC by bolus once daily, one unit received the same total dose in two equal fractions every 12 h, and one unit received the same dose in three equal fractions every 8 h. Each dose was followed by a no drug washout with the appropriate half-life for this drug. The dose fractionation experiment was carried out for 6 days. The effect of drug on virus replication was determined by sampling the units once a day and measuring the amount of infectious virus present by plaque assay. The dose ranging study performed in the hollow fiber infection model system showed that the EC_{50} value for oseltamivir carboxylate for this influenza A virus was 0.05 nM. The dose fractionation study showed that all treatment arms suppressed virus replication by essentially the same extent suggesting that the pharmacodynamically linked variable was the $AUC_{0–24}/EC_{50}$ ratio. These results suggest that AX-4 cells expressing high levels of sialyl-2,6-galactose receptors may be useful for determining the pharmacodynam-

ically linked variable of neuraminidase inhibitors for influenza viruses.

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74

Sub-optimal Protease Inhibition of HIV-1: Effects on Virion Morphogenesis and RNA Maturation

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During or soon after release of HIV-1 from an infected cell the virion initiates the process of maturation. The viral protease becomes activated, leading to the subsequent cleavage of the viral polyproteins Gag and GagPol into their constituent parts. As a result, an internal conical core condenses surrounding the viral nucleic acid and the particle becomes infectious. Concomitant with this global alteration in virion morphogenesis is a conformational change in the viral genomic RNA from a loosely associated dimer into a more thermodynamically stable form. Protease defective viruses are capable of virus release and viral RNA encapsidation, but these particles are non-infectious and immature due to an inability to carry out proteolytic cleavage. Within these particles the viral RNA is also observed to be in an immature state, demonstrating a link between the proteinaseous maturation and that of the nucleic acid. We have used sub-optimal concentrations (IC50 and IC90) of two protease inhibitor drugs (Lopinavir and Atazanavir) to demonstrate their effect on the Gag polyprotein processing and RNA properties of the treated virions. The results were then correlated to their effects on virion morphogenesis as determined by EM. The results show that even with high levels of viral inhibition (IC90) most of the viral protein is processed. However, a slight but significant increase in processing intermediates was detected upon drug exposure and a small decrease (2–3 °C when 50% of dimers remained) in overall thermostability of the viral RNA dimer was also observed. These defects correlated with an increase in immature particles as observed by EM, but the numbers of immature particles did not adequately account for the level of viral inhibition. These data suggest that the presence of small quantities of residual processing intermediates, within the viral particles, is capable of disproportionately inhibiting the viral replication cycle, without having comparative effects on either RNA maturation or virion core condensation.

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75

Significance of 3b-dehydroxysterol-D24-reductase (DHCR24) in life cycle of Hepatitis C virus

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Hepatitis C virus (HCV) causes persistent infection often progressing to hepatocellular carcinoma (HCC). We previously reported that full HCV genome-expressing HepG2 cells enhanced their clonogenic capacity after 44 days of passage (M6 44 days cells). We established monoclonal antibodies (MoAbs) against surface antigens on these cells. One of the MoAbs specifically recognized the molecule which was overexpressed in the cancerous region of livers of all HCV-positive HCC patients. It was identified as 24-dehydrocholesterol reductase (DHCR24), which was reported to be involved in cholesterol biosynthesis and hydrogen peroxide-induced cytotoxicity. The full-length HCV upregulated the transcription of DHCR24 in human liver cells in the presence of p53. Expression of HCV induced the upregulation of DHCR24 and p53, and was sustained in M6 44 days cells. However, activity of $p21^{WAF1/CIP1}$ promoter in response to hydrogen peroxide was impaired in M6 44 days cells. This might be induced by the post-translational modification of p53, which was regulated by DHCR24. Thus, DHCR24 plays a critical role in the regulation of the response to HCV and hydrogen peroxide, and this pathway is a target of HCV during its persistent expression.

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76

Inhibition of Human T-Cell Lymphotropic Virus Type-1 Integrase by Dicaffeoylquinic Acids Extracted from Coffee (*Coffea arabica*) Seeds

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Human T-cell lymphotropic virus type-1 (HTLV-1) replication depends on the viral enzyme integrase (IN) that mediates integration of a DNA copy of the virus into the host cell genome. Integrase represents a novel target to which antiviral agents might be directed. The C-terminal part of the HTLV-1 pol gene is predicted to encode the HTLV-1 IN; however, this protein has not yet been detected in virions or infected cells. In order to evaluate compounds with anti-HTLV IN activity, we extracted dicaffeoylquinic acids (DCQAs) from coffee (*Coffea arabica*) seeds. Using a baculovirus system we expressed a 38-kDa IN