ically linked variable of neuraminidase inhibitors for influenza viruses.

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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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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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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

protein from an HTLV-1 carrying human T-cell line in insect cells of Mamestra brassicae. We further purified the enzyme under native conditions using affinity chromatography. The purified IN carried out activities characteristic of retroviral integrases when it was evaluated for processing and strand-transfer reactions. The extracted 3,5-DCQA inhibited the recombinant IN activities in biochemical assays at 20 nM. Additionally, docking studies supported the hypothesis of an enzyme induced allosteric change by interaction with the caffeoyl groups of 3,5-DCQA and a lysine in position 159 (K159) in the domain outside the active site of IN. Thus, dicaffeoylquinic acids represent an important class of antiviral agents that may contribute to the understanding of the molecular mechanism of viral integration and the design of HTLV therapeutics.

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Serine Side-Chain-linked Peptidomimetic Prodrugs of Cidofovir and Cyclic Cidofovir: C-Ester Effects on Transport and Activation

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Cidofovir (HPMPC, **1**) and its equivalently potent cyclic form (cHPMPC, **2**) are active against orthopox virus infections, but are limited in this role by low oral bioavailability. In contrast to some alternative promoieties, peptides offer low toxicity together with versatility in tuning transport and activation pharmacology. We previously reported the synthesis and biological evaluation of several prodrugs of cyclic cidofovir in which the phosphonic acid group of **2** was esterified by the free serine side-chain hydroxyl group of an X-Ser-CO_2Me dipeptide. Val-Ser-CO_2Me cHPMPC (**3**) was stable at pH 3–5, but rapidly released the active drug in cell and tissue homogenates, while exhibiting enhanced transport versus the parent drug in a rat model. Incorporation of D-amino acids, especially at the N-terminus, resulted in increased stability and improved transport, consistent with our present finding that co-dosing with the aminopeptidase inhibitor, bestatin, results in enhanced transport of **3**. Seeking an optimal balance of transport and efficient activation, we are exploring the influence of the carboxyl ester group in these prodrugs. Val-Ser-CO_2iPr cHPMPC (**4**) was synthesized and was also found to be a useful synthon for preparation of its acyclic analogue, Val-Ser-CO_2iPr HPMPC (**5**). LC and LC–MS analysis of **3–5** stability in buffer and in cell and tissue homogenates provides evidence that the activation efficiency and pathway are strongly dependent on the ester structure. The results further demonstrate the potential of this peptidization approach in the development of an orally effective form of cidofovir.

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Check of Antiviral Activity of Nanocomposites with Active Check of Antiviral Activity of Drugs Based on Nanocomposites, Which Contained Oligonucleotides for Direct Splitting Viral Genome of Influenza Virus Type A

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Influenza is a mass infection, which yearly registered at different countries and inflict huge economic damage. Main characteristics of influenza virus type A are rapid spreading and high sickness rate. During epidemy falled ill about 20–30% of children and about 5–10% of adult people, during pandemic they are up to 40–60%. Yearly influenza virus and its complications causes death of 250,000–500,000 people in developed countries [Reichert, T.A., Sharma, A., 2001. WER, 2005]. For research new antiviral drug we used nanocomposites. They contain antisense oligonucleotides (as immunostimulating components and facilities for inhibition of NP gene expression of birds' influenza virus) and TiO_2-nanoparticles, which helps penetration of complex into mammal cell. Influence of TiO_2-nanoparticles on different cellular enzymes activity was explored and shown possibility of principle protection of antisense oligonucleotides against nucleases. Cytotoxic tests of TiO_2 shown that TiO_2 is non-toxic for cells at concentrations lower than 100 mg/ml. We test antiviral activity of conjugants which based on TiO_2-nanoparticles. To do this we infected MDSK cells and used nanocomposites. We discovered that conjugants have definitely antiviral activity. When we used nanocomposites amount of survived cells increased by 3.5 times. Thus we show obvious antiviral activity of conjugants against influenza virus type A (H5N1). In perspective, methods we developed can be used to make antiviral drugs against influenza virus type A for humans.

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