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An Anti-Influenza Peptide That Inhibits Attachment Through Aggregation

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Influenza viruses continue to cause widespread morbidity and mortality on a yearly basis. Protection is often afforded by vaccination programs and treatment with either of two classes of antivirals: adamantanes or neuraminidase inhibitors. In recent years, the highly pathogenic avian H5N1 influenza viruses have displayed characteristics suggesting increasing resistance to both classes of antivirals, supporting the need for development of new alternatives. We previously reported that a 20 amino acid peptide, Entry Blocker (EB), inhibited the attachment of influenza viruses to host cells at low micromolar concentrations (3 to 30 μM). Furthermore, we showed that the EB peptide interacts with hemagglutinin (HA), possibly facilitating the inhibition of attachment. In vivo, the EB peptide prevented clinical signs of infection when mice were administered EB-treated H5N1 influenza virus, and morbidity was delayed in mice given EB post-infection. Based on these studies, we hypothesized that the binding of EB to HA leads to viral aggregation. In support of this hypothesis, we demonstrate that the EB peptide directly interacts with HA and inhibits the attachment of HA to red blood cells. The binding of the EB peptide is independent of the carbohydrate moieties present on HA. Further, we demonstrate that treatment of virion with the EB peptide led to delayed mobility though a continuous 30-60% sucrose gradient during ultracentrifugation. Aggregation of virion was confirmed by electron microscopy. Our studies suggest that the binding of EB to HA leads to viral aggregation and decreased attachment to host cells. Aggregation is a common theme in pathogen clearance, with molecules such as surfactants facilitating aggregation mediated phagocytosis of influenza virions. Ongoing studies will continue to examine the mechanism of EB antiviral activity, in addition to understanding the effect of EB induced aggregation on viral clearance in vivo.

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Synergistic Inhibition of H1N1 and H3N2 Influenza Viruses by Aurintricarboxylic Acid With Amantadine, But Not With Interferon

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Aurintricarboxylic acid (ATA), a potent inhibitor of cellular nuclease, kinases and phosphatases, is known to have inhibitory properties against the growth of several families of viruses, including human immunodeficiency virus, vaccinia virus and coronavirus (SARS-CoV). The mechanisms involved in the antiviral activities of ATA are not well understood but might be related to disruption of both cellular and viral factors. Here we investigated the potential anti-viral effects of ATA against two strains of influenza A viruses, namely A/Puerto Rico/8, A/New Caledonia/20, (both H1N1 subtype) and A/New York/55 (H3N2 subtype). The mean 50% cytotoxic concentration (CC50) of ATA in MDCK cells was approximately 0.2 mg/mL as measured by neutral red uptake assay. At 0.01 mg/mL, ATA was found to significantly protect Madin Darby Canine Kidney (MDCK) cells against the aforementioned influenza virus subtypes as determined by electron microscope, neutral red cytotoxicity assay, and antigen quantitation using enzyme linked immunosorbent assay (ELISA). In addition, amplification of viral NP mRNA using RT-PCR revealed a substantial reduction of the viral transcripts, suggesting a block at the viral transcriptional level. Importantly, treatment of H1N1-infected cultures with non-toxic doses of ATA resulted in defective viral particles characterized with irregular matrix protein shells and RNP structures, strongly suggesting a direct effect of ATA on the viral structural proteins. We further determined whether ATA could synergistically inhibit influenza virus replication with other known anti-viral agents including amantadine and interferon gamma. ATA was found to synergistically inhibit virus infectivity with amantadine, but not with interferons. These novel findings could shed light on the mechanisms underlying the morphology and structure of the viral particles upon exposure to the aforementioned anti-viral agents. Furthermore, the defective viral particles generated in the presence of ATA might also be worthy of being investigated for its potential as candidate vaccines.