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Combinations of Thiovir and Neuraminidase Inhibitors Exert Synergistic Antiviral Activity on Human, Equine and Avian Influenza In Vitro

Shani Waninger*, Silvestre Ramos, Joan Robbins

ADVENTRX Pharmaceuticals, San Diego, CA 92121, USA

Although neuraminidase inhibitors constitute an important treatment option for influenza virus, reports of the emergence of drug resistant avian influenza strains make the development of new anti-influenza molecules a priority. Thiovir™ (thiophosphonoformic acid) is a prodrug of the broad-spectrum antiviral drug foscarnet. Foscarnet has limited therapeutic usage, in part because of its intravenous route of delivery. In contrast, Thiovir has increased bioavailability that enables oral delivery. Thiovir is a pyrophosphate analogue that inhibits viral polymerases, a novel mechanism among current influenza therapeutics. We examined the antiviral activity of Thiovir against multiple influenza virus types, including human, equine and avian. In addition, Thiovir was paired with current influenza treatments to evaluate potential synergistic anti-influenza activity.

Methods: Thiovir activity against influenza virus infection of MDCK or bronchial epithelium cells was determined by ELISA assay. For combination drug activity assays, Thiovir was paired with neuraminidase inhibitors, such as oseltamivir phosphate (Tamiflu™) and activity (synergistic, additive, or antagonistic activity) assessed using median effect principal.

Results: Thiovir showed dose-dependent antiviral activity against human (H1N1 and H3N2), equine (H3N8) and avian (H5N2) influenza virus in single drug assays of extracellular virus in multiple cell types. Thiovir efficacy was approximately equal to foscarnet with IC_{50} in the micromolar range. In addition, combination indices of Thiovir and neuraminidase inhibitors indicate synergistic inhibition of viral replication.

Conclusions: Thiovir efficacy against multiple subtypes of virus from various animal species suggests broad-spectrum anti-influenza activity. Combination therapy with two or more drugs that have different modes of action and synergistic activity may have advantages, including increased clinical efficacy, reduced drug dosage and reduction of resistance to a single drug. Our results suggest that Thiovir and neuraminidase inhibitor combinations may offer a promising therapeutic option in the event of an avian influenza pandemic.

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Development of a Cell-Based Assay for Identification of Viral Entry Inhibitors Against SARS-CoV by High Throughput Screening (HTS)

Chengsheng Zhang[1,*], Yan Feng[1], Gillian Wong[1], Liping Wang[2], Jonathan Cechetto[3], Jan Blanchard[3], Eric Brown[3], James Mahony[1]

[1] Department of Pathology & Molecular Medicine, McMaster University, Canada; [2] Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA; [3] Department of Biochemistry and High Throughput Screening Laboratory, McMaster University, Canada

Severe acute respiratory syndrome (SARS) is caused by a newly identified human coronavirus named SARS coronavirus (SARS-CoV). Viral entry is one of the most important early events in the replication cycle of a coronavirus. Previous studies have demonstrated that the spike (S) protein of SARS-CoV binds specifically to the human angiotensin-converting enzyme 2 (ACE2) receptor, and mediates the viral entry into host cells. In this study, we first constructed and produced the S-protein pseudotyped viruses that carry the luciferase reporter gene, and confirmed that the viral entry was dependent on the human ACE2 receptor. We subsequently infected a variety of human cell lines with the pseudopyted virus, and found that human Huh-7 cells are highly permissive to the pseudopyted viral infection. To develop an assay for high throughput screening (HTS), we further optimized this viral entry assay in 96-well plate format, and demonstrated that soluble human ACE2 protein or anti-human ACE2 antibodies could potently block the S-ACE2 mediated viral entry. We are currently employing this sensitive and quantitative viral entry assay to screen a small molecule library consisting of 190,000 compounds at McMaster HTS Laboratory to identify novel molecules that may disrupt the S-ACE2 interaction and block viral entry of SARS-CoV.

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The Inhibitory Effects of Medicinal Herbs on SARS-CoV Entry In Vitro

Min Zhuang[1,*], Hong Jiang[2], Peng Xiao[1], Yasuhiro Suzuki[1], Toshio Hattori[1]

[1] Division of Infectious and Respiratory Diseases, Department of Internal Medicine, Tohoku University, Japan; [2] Institute of Laboratory Animal Science, Chinese Academy of Medical Science, Beijing, China

Purpose: Severe acute respiratory syndrome (SARS) is an atypical type of contagious pneumonia with a high mortality rate, however no effective drugs and vaccine were established. To identify an entry inhibitor of severe acute respiratory syndrome coronavirus (SARS-CoV), we have examined the effects of medicinal herbs which were found to be effective