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

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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)

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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

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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

in SARS patients, on infection of SARS virus to cell line cells.

Methods: The water extracts of seven medicinal herbs and five Kampo medicines (traditional Japanese herb medicines), which were reported to be used for the treatment of SARS patients, were studied using HIV/SARS-CoV S pseudotyped virus assay. The effects were also confirmed by wild type SARS-CoV infection assay. We also examined whether these herbs inhibit the bindings of anti-S or anti-ACE2 antibody to cells using FACS analysis.

Results: Cinnamomi Cortex extract (CCE) and Caryophylli Flos extract (CFE) showed inhibitory activities against HIV/SARS-CoV S pseudovirus. The 50% inhibitory concentration (IC_{50}) of the former was lower then those of the latter, both of them were less than 60 mg/ml. We also confirmed that both extracts inhibited wild-type infection in the plaque reduction assay. The selective index (SI) of CCE was also higher than CFE. We further examined four fractionated samples from Cinnamomi Cortex (CC). The SI of the fraction 2 (Butanol fraction) in the plaque reduction assay was higher than those of CCE. To analyze the mechanism of inhibitory effects of herbs we examined whether the herbs inhibit the binding of anti-S protein and anti-ACE2 antibodies to cells. These extracts failed to inhibit the bindings of antibodies to S and ACE2 on cells surface.

Conclusion: These results strongly indicate that CC and Caryophylli Flos (CF) contain a potent inhibitor of SARS-CoV entry, and the inhibitor could be enriched in the Butanol fraction of CC. However they apparently did not interfere S/ACE2 interactions.

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Uptake and Metabolism of Cidofovir and Oleyloxyethyl-cidofovir in Human Papillomavirus Positive ME-180 Human Cervical Cancer Cells

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Nearly all cervical cancers are caused by the high-risk subtypes of human papillomavirus (HPV) which express the E6 and E7 oncoproteins. Several groups have shown previously that cidofovir (CDV) inhibits HPV^+ cervical cancer cell proliferation in vitro by lowering levels of E6, upregulating p53, and increasing susceptibility to apoptosis. CDV has been reported to have antiproliferative effects against HPV^+ cancers, both in animals and man. We found that the antiproliferative activity of alkoxyalkyl esters of CDV, such as oleyloxyethyl-cidofovir (OLE-CDV), is 1000–2700 times greater than CDV in CaSki, ME-180, HeLa, and SiHa human cervical cancer cell lines. To evaluate the mechanism involved, we studied the cellular uptake of ^{14}C-labeled CDV and OLE-CDV in the cervical cancer cell line, ME-180. ME-180 cells were exposed to 3 μM CDV or OLE-CDV for 24 h. Cellular uptake of OLE-CDV was 270-fold greater than that of CDV in ME-180 cells. The cellular levels of CDV, CDVp and CDVpp were measured by Partisil SAX HPLC. Levels of the active metabolite, CDVpp, were 183 times greater with OLE-CDV than with CDV. These findings may explain, at least in part, the previously reported multi-log increase in the antiproliferative effects of OLE-CDV versus CDV in HPV^+ human cervical cancer cell lines. These results indicate that alkoxyalkyl esters of CDV, such as OLE-CDV, are of interest for further evaluation as agents for the treatment of cervical cancer.

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Selection of Human Cytomegalovirus Resistant to a Second Generation Methylenecyclopropane Purine

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We recently described a second generation of methylenecyclopropane purines (the bis-hydroxys) that have potent and selective activity against HCMV and MCMV (Zhou et al., 2004. J. Med. Chem. 47, 566). The guanine analog cyclopropavir was effective in vitro against HCMV and MCMV with IC_{50}'s of 0.27–0.49 μM and no cytotoxicity at 100 μM. It was active when administered orally in animal models for CMV infections (Kern et al., 2004. Antimicrob. Agent. Chemother. 48, 4745). To investigate its mechanism of action, HCMV resistant to this compound was selected by passage of Towne strain HCMV in the presence of 0.625 μM cyclopropavir until 50% CPE was evident (2 weeks). Cells were harvested, recombined with supernatant, and used to infect 25 cm^2 flasks of HFF cells. Cultures were maintained with periodic replacement of drug-containing media until CPE was >50% (6 weeks) or nearly 100% (10 weeks). Medium from 10-week cultures was used to infect fresh HFF cells. After 4 weeks in the presence of 2.5 μM cyclopropavir, supernatant virus was plaque purified in the presence of 2.5 μM drug. An initial survey of drug resistance showed that IC_{50}'s for virus from five plaque isolates were approximately 10-fold greater than for wild-type (wt) HCMV. More extensive dose-response experiments gave IC_{50}'s of 22 and 42 μM for cyclopropavir and ganciclovir compared to IC_{50}'s of 0.9 and 1.5 μM, respectively, for wt virus. DNA sequencing and marker transfer studies with HCMV resistant to a first generation analog (synadenol) have now shown that mutations M460I and C603Y in gene UL97 are necessary and sufficient to give resistance to both synadenol and cyclopropavir. Sequencing UL97 and UL54 from cyclopropavir-resistant HCMV is being used to determine if the cause of resistance to this second generation drug is similar to that responsible for resistance to the first generation compound.