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Antiviral Drug Efficacy Evaluation Against Pandemic Influenza a (H1N1) Viruses in MDCK Cells

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New and emerging influenza virus strains, such as the pandemic influenza A (H1N1) virus, require constant vigilance for antiviral drug sensitivity and resistance. These studies evaluated eight isolates of pandemic influenza A virus against seven antiviral drugs: oseltamivir, peramivir, zanamivir, T-705 (favipiravir), ribavirin, amantadine and rimantadine. The pandemic viruses tested include isolates from California, New York, Utah, and an oseltamivir-resistant isolate from Hong Kong. In addition, a mouse-adapted A/CA/04/2009 virus was evaluated. EC_{90} values for each drug were determined by virus yield reduction assay in three replicate experiments. A broad range of drug sensitivities was observed among the H1N1 viruses tested. However, significantly higher ($P < 0.05$) EC_{90} values for the neuraminidase inhibitors were only observed for the drug-resistant A/Hong Kong/2369/2009 virus. As expected, all isolates showed resistance to the adamantanes. Dose–response curves also showed that the mouse-adapted strain had consistently higher EC_{90} values compared to its non-adapted counterpart. We also evaluated the effects of antiviral drug combinations on drug-resistant influenza A/Hong Kong/2369/2009 virus. Drug combinations included T-705 plus one of the neuraminidase inhibitors: oseltamivir, peramivir, or zanamivir. Synergism was observed involving three doses of T-705 (1.0, 3.2, and 10.0 μM) for all three neuraminidase inhibitors. Oseltamivir and zanamivir were synergistic with T-705 at three concentrations (3.2, 10, and 32 μM for oseltamivir, or 0.1, 0.32, and 1.0 μM for zanamivir) and peramivir showed synergism with T-705 at two concentrations (0.32 and 1.0 μM). These studies demonstrate that pandemic influenza A virus isolates from different regions remain sensitive *in vitro* to commonly used antiviral drugs, and that combination chemotherapy holds promise as a treatment strategy for drug-resistant influenza virus.

Acknowledgments: This work was supported by Contract N01-AI-30048 from the Virology Branch, DMID, NIAID, NIH.

doi:10.1016/j.antiviral.2010.02.414

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In Vitro Combination of ANA598 with Other Anti-HCV Agents can Eliminate the Emergence of Resistant Colonies

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ANA598 is a novel HCV non-nucleoside polymerase inhibitor that is currently in a Phase II combination trial with IFN/RBV in HCV infected patients. Clinical trials in HCV are beginning to explore combinations of two or more direct antivirals, which may provide additional benefit in certain populations and may reduce or eliminate the need for interferon or ribavirin. To better understand the potential of combinations utilizing ANA598, co-selection studies of ANA598 with IFN-α and clinically advanced direct antiviral agents were evaluated *in vitro*. The results provide support for future clinical exploration of combination regimens that include ANA598.

Co-selection studies were conducted using Huh7-Luc/neo wt 1b dicistronic replicon cells. Combinations of ANA598 with IFN-α, the HCV NS3/4 protease inhibitor telaprevir, and the NS5B nucleoside polymerase inhibitor PSI-6130 were evaluated. These direct antiviral agents have distinct mechanisms of action and non-overlapping resistance profiles. The cells were selected with G418 in the absence or presence of either one or two agents at combinations between their EC_{50} and EC_{99}. After 3 weeks in culture, cells were either fixed and stained or total cellular RNA was extracted. The NS5B and NS3 coding sequences were determined through direct sequencing of PCR products.

In vitro combination of ANA598 with IFN-α, telaprevir, and PSI-6130 after 3 days in the replicon system showed that the antiviral interaction between the compounds was additive to synergistic (see Patel et al. ICAR 2010 abstract). Treatment of the replicon cells for 21 days with ANA598 or telaprevir alone selected for resistant colonies. The co-selection studies demonstrate that at the EC_{95} of ANA598, which is readily achievable in the clinic, combination with IFN-α, telaprevir or PSI-6130 led to clearance of the replicon from the cells rather than emergence of resistant colonies.

The *in vitro* co-selection studies demonstrate an elimination of resistant variants and additive to synergistic antiviral effects when ANA598 is combined with other anti-HCV agents. Clinical use of such combinations may produce a greater viral load reduction and potentially allow durable clearance of virus prior to the emergence of drug resistance in most patients.

doi:10.1016/j.antiviral.2010.02.415

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Development and *In Vitro* Evaluation of Gel-formulated Saquinavir as Vaginal Microbicide: Anti-HIV-1 Activity and Pharmaceutical Availability in Biorelevant Media

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The HIV protease inhibitor saquinavir is an interesting microbicide candidate for its potent anti-HIV-1 activity and favorable resistance profile. Here, we wanted to evaluate the formulate-ability of this poorly water-soluble compound in vaginal microbicide gels in terms of anti-HIV-1 activity and pharmaceutical availability in biorelevant media. Saquinavir mesylate was dissolved in an aqueous hydroxyethylcellulose gel (pH 4.6) at a concentration up to 2 mg/g (10^5-fold in vitro anti-HIV-1 IC_{50} values). The in vitro anti-HIV-1 activity of saquinavir was preserved in the formulation: comparable mean IC_{50} values were obtained for formulated and native saquinavir mesylate (10 and 13 ng/ml, respectively). In the vaginal environment, potential precipitation of saquinavir in vaginal fluid or semen may reduce its availability. Therefore, saquinavir concentrations were monitored upon adding the formulation to vaginal fluid simulant (VFS) or semen simulant (SS) (1:1 dilution). Dilution of the saquinavir mesylate solution with VFS resulted in preservation of its antiviral activity, both at low (0.1 mg/g) or high (1 mg/g) dose, without noticeable precipitation. Comparable data were obtained for low dose saquinavir in combination with VFS and SS. However, dilution of high dose saquinavir (1 mg/g) with VFS and SS caused immediate precipitation of saquinavir with subsequent reduction in anti-HIV-1 activity (5-fold increase in IC_{50} value). Inclusion of the solubilizing excipients polyethylene glycol 1000 (12%) and hydroxypropyl-b-cyclodextrin (2.5%) to the formulation reduced the extent of precipitation of saquinavir and restored its antiviral potency. In conclusion, while the mesylate salt of saquinavir allows the for-