The BAC technology has the potential to increase bioavailability of a wide range of active compounds using oral administration, thereby increasing the accessibility and potentially improving the safety profile of the drug product.

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Lymphocytic Necrosis in Hamsters Inoculated with Western Equine Encephalitis Virus

Aaron Olsen[1,*], John Morrey[1], Justin Julander[1], Jeffery Hall[2], Ramona Skirpstunas[2], Robert Sidwell[1]

[1] Institute for Antiviral Research, Utah State University, UT, USA; [2] Utah Veterinary Diagnostic Laboratory, Utah State University, UT, USA

We hypothesized that disease in hamsters inoculated with the California strain of Western Equine Encephalitis virus (WEEV) follows a biphasic pattern, an initial systemic phase and a secondary neurologic phase. Furthermore, we hypothesized that the cause of death during the systemic phase was primarily inflammatory in nature. To test these hypotheses, we inoculated hamsters with WEEV and evaluated clinical, histopathological and virological parameters. Following inoculation animals displayed fever and weight loss, and the majority of animals, which died due to virus infection succumbed by 108 h post-virus inoculation (hpi). Animals developed necrosis in lymphocytic organs by 96 hpi, but, no pathological lesions could be observed in the brain at this time. Animals also developed lymphopenia, the severity of which appeared to be correlated to outcome. Animals that died during the initial phase had significantly decreased lymphocyte counts at 72 and 84 hpi compared to animals, which survived. Administration of the non-steroidal anti-inflammatory drug Flunixin Meglumine (FM) to hamsters inoculated with WEEV significantly improved survival. Over the course of multiple experiments placebo treated animals had an average survival rate of 4% at 120 hpi, while animals treated with FM had a survival rate of 30% ($p < 0.001$). Hamsters, which survived the initial stages of WEEV infection sometimes progressed to a neurological stage of the disease, showing overt signs of nervous system involvement, such as hind limb paralysis. Histopathological analysis of animals dying after 120 hpi revealed inflammation and necrosis within the central nervous system. These data indicate that WEEV in hamsters results in an initial systemic phase with lymphocytic necrosis and death due to an overwhelming inflammatory response, which may be partially blunted by administration of anti-inflammatory agents. Animals that survive the systemic phase may develop an encephalomyelitis.

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Combined Anti-Influenza Virus Effect of a Plant Polyphenol-Rich Extract and Ribavirin

Julia Serkedjieva*, Ani Teodosieva

Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

The anti-influenza virus activity of the extract, obtained from the plant *Geranium sanguineum* L (PC) has been studied intensively. It was shown that its in vitro antiviral effect was strain-dependent, consistent with a selective antiviral action (Serkedjieva and Hay, 1998). In one-cycle experiments of viral growth of A/Germany/34, strain Rostock (A/Rostock) in CEF PC inhibited the synthetic stages of viral replication. PC exhibited a pronounced protective effect in the lethal murine experimental influenza A/Aichi/2/68 (H3N2) virus infection (Serkedjieva and Manolova, 1992). Here, we present the results from the investigation of the combined virus-inhibitory effects of PC with ribavirin (Rib), a selective viral inhibitor. The in vitro combined application resulted in enhancement of the inhibitory effect of PC on the replication of A/Rostock in MDCK cells. The antiviral activity was determined by the difference in the infectious titers of control and treated viruses and the combined effect was defined on the base of infectious viral yields. As a rule the combinations showed increased virus-inhibitory effects with respect to the individual compounds. Most of the combinations proved to be synergistic. Administration of PC in combination with Rib in the course of the experimental influenza infection in mice produced a synergistic protective effect: mortality rate was significantly decreased, MST was markedly prolonged. A pronounced reduction of the lung lesions and of lung virus titres was achieved. The presented results together with the data from others suggest that the combined application of natural and synthetic viral inhibitors may be used successfully to potentate the antiviral efficacy of the plant preparations and may enable dose reduction of their toxic components.

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Anti-Influenza A Synergistic Combination Effect of Rimantadine and Oseltamivir in Mice

Lora Simeonova*, Angel S. Galabov, Galina Gegova

The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, BG-1113, Sofia, Bulgaria

The combination effect of rimantadine hydrochloride and oseltamivir phosphate was examined on infection with 10 and 20 MLD_{50} influenza A/Aichi/2/68 (H3N2) virus in mice. Doses of 2.5, 5.0 and 7.5 mg/kg/day of rimantadine and 0.05, 0.1 and 0.2 mg/kg/day of oseltamivir were selected and combined in a chess-board order, as an initial study. Compounds were administered in 5-day-treatment course, beginning 4 h pre-infection. Significant differences were observed comparing combination-

treated to individually treated and placebo groups. The following values of the protection index were recorded: 34–41% for the combination of oseltamivir with 5 mg/kg rimantadine, 43–87% for oseltamivir with 7.5 mg/kg rimantadine, while the individual effects were 0–10% for oseltamivir, 0% for 5 mg/kg rimantadine and 18.7–29.6% for 7.5 mg/kg rimantadine. The mean survival time was lengthened by up to 6.9 days in the combination groups, up to 1.9 days in oseltamivir groups and to 3.2 days in rimantadine groups. The combination effect was characterized by three-dimensional method of Prichard and Shipman as synergistic. Lung virus titer in MDCK cells, lung index and consolidation score proved the high effectiveness of the combination of 5 mg/kg rimantadine and 0.05 mg/kg oseltamivir. At the peak of virus growth, 48–60 h post infection, the titer was 2.8 $\log_{10}$ 50% cell culture infectious dose ($CCID_{50}$) lower than in placebo control. In rimantadine and oseltamivir separately applied groups a decrease of only 1.1–1.4 $\log_{10}$ and 0.1–1.0 $\log_{10}$ $CCID_{50}$, respectively, was established. These data emphasize the high anti-influenza A potential of the combination.

Experiments on the effect of combinations of 10, 20 and 40 mg/kg/day rimantadine and 0.2, 0.4 and 0.8 mg/kg/day oseltamivir, and other rimantadine/oseltamivir doses' ratios (50:1 and 25:1) as well as on different delay schemes of drug application experiments are in progress.

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Phosphorodiamidate Morpholino Oligomer—Mediated Inhibition of Influenza A Virus in Mice

Thomas Voss[2,*], Kelly Warfield[3], Rebecca Brocato[2], Joseph Barbercheck[2], Bryan Kaplin[2], David Stein[1], Sina Bavari[1], Patrick Iversen[1]

[1] AVI BioPharma Inc., 4575 SW Research Way, Corvallis, OR 97333, USA; [2] Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans 70112 LA, USA; [3] US Army Medical Research Institute for Infectious Diseases, Fort Detrick, Frederick, MD 21702, USA

Phosphorodiamidate morpholino oligomers (PMO) are single-stranded nucleic acid-like antisense agents that can reduce gene expression by sterically blocking complementary RNA sequence. PMO are water-soluble and nuclease resistant, and they readily achieve uptake into cells under standard conditions. Two PMO, were evaluated for their ability to inhibit influenza A viruses (H1N1) and (H3N2) replication in a murine model of infection. The PMO were designed to base pair with FLUAV RNA sequences that are highly conserved across viral subtypes and considered critical to the FLUAV biological-cycle, such as gene segment termini and mRNA translation start site regions. Several PMO previously shown to be highly efficacious in cell culture models of influenza infection were evaluated in a murine model of infection with influenza. Two PMO, one designed to target the AUG translation start site region of PB1 mRNA and the other the 3′-terminal region of nucleoprotein viral genome RNA, proved to be effective against influenza infection of mice reducing clinical signs (loss of body weight) and virus titers in the respiratory tract. These results, taken together with in vitro results, suggest these oligomers may represent a broad-spectrum therapeutic approach against a high percentage of known influenza A strains.

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Practical Synthesis of (−)-Carbocyclic Cytosine (Carbodine) and its In Vitro Antiviral Activity against Venezuelan Equine Encephalitis (VEE) Virus and Yellow Fever Virus

J.R. Rao[1,*], J.G. Julander[2], R.W. Sidwell[2], C.K. Chu[1]

[1] The University of Georgia College of Pharmacy, Athens, GA 30602, USA; [2] Utah State University Institute Antiviral Research, Logan, UT 84322, USA

Natural as well as synthetic carbocyclic nucleosides are well known for their interesting biological activities, including antitumor as well as antiviral activities against a wide variety of RNA and DNA viruses. The carbocyclic analogue of cytosine (carbodine **2**) was previously prepared as a racemic mixture and has been shown to possess significant antitumor (lymphoid leukemia L1210 in mice) and antiviral activities against human influenza type A virus, measles, vesicular stomatitis virus and herpes simplex viruses (HSV-1 and HSV-2). These interesting biological properties of carbodine, prompted us to synthesize enantiomerically pure (−)-carbodine (**2**) for biological evaluations. Herein, we report an efficient and practical synthesis of (−)-carbodine (**2**) as well as its antiviral activities against VEE and yellow fever virus (Fig. 1).

The key intermediate, chiral cyclopantanol **1**, was achieved from a chiral enone by a 1,4-addition reaction in a multi-gram scale. The cyclopentanol intermediate **1** was reacted with protected cytosine under Mitsunobu reaction conditions, however, it provided only O-alkylated product instead of the desired N-alkylated product. Therefore, the desired heterocycle, cytosine was constructed by the linear approach to afford the target nucleoside, (−)-carbodine (**2**) in a gram scale. The (−)-carbodine (**2**) showed potent antiviral activity against Venezuelan equine encephalitis virus (TC-83 virus strain, EC90 0.3 μM) with the high selective index >333 and yellow fever virus (17D virus strain, EC90 2.2 μM) in Vero cells.

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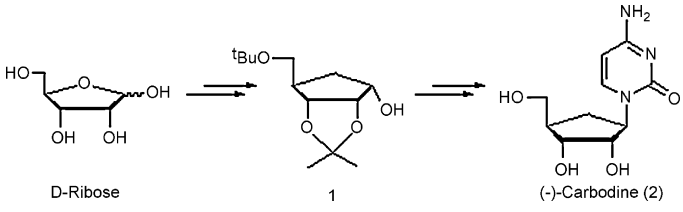

Fig. 1.

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