

through 7 days post-virus challenge at a dose of 100 mg/kg/day was effective in significantly improving survival ($P < 0.001$), weight change ($P < 0.01$) and mean day to death ($P < 0.01$) as compared with controls. No death or significant weight change was observed in toxicity control mice. Treatment with 200 mg/kg/day (–)-carbodine resulted in a higher survival rate as compared with 100 mg/kg/day treatment, although this difference was not significant. A statistically significant improvement in weight change ($P < 0.01$), brain virus titer ($P < 0.05$) and mean day to death ($P < 0.01$) was also observed with 200 mg/kg/day treatment as compared with placebo-treated controls. Some toxicity was observed as determined by a significant ($P < 0.05$) weight change in toxicity controls treated with 200 mg/kg/day. Treatment i.p. with 200 mg/kg/day (–)-carbodine initiated 24 h after virus challenge resulted in significantly improved survival ($P < 0.001$), weight change ($P < 0.001$) and mean day to death ($P < 0.001$). Prophylactic and therapeutic (–)-carbodine treatments were effective in improving disease in VEEV-infected mice, suggesting the potential utility of this compound in the treatment of natural VEEV infections.

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Poster Session I: Retrovirus, Respiratory Virus, West Nile Virus and Hepatitis Virus, and Antiviral Methods

43

Intranasal Protollin Formulated Recombinant SARS-CoV S Protein Elicits Respiratory and Serum Neutralizing Antibodies

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A truncated recombinant spike protein (Δ TM S protein) of SARS coronavirus (SARS-CoV) was investigated as a vaccine in formulation with either the proprietary adjuvant Protollin (ProT) or with Alhydrogel. In young mice intranasal (i.n.) immunization with Protollin-formulated Δ TM S protein elicited a high level of specific serum IgG and neutralizing antibody. Intramuscular (i.m.) administered Alhydrogel vaccine achieved similar results. In a challenge study, mice immunized i.n. with the Protollin-formulated vaccine had significant levels of lung IgA, while i.n. immunized aged mice had no detectable virus titers after challenge with infectious SARS-CoV (strain Urbani). In contrast, young mice immunized i.m. with Alhydrogel-adsorbed vaccine did not show any detectable lung IgA, while virus titers in lungs of aged mice after challenge were comparable to those observed in control mice immunized with buffer or Protollin alone. The most protective immunization regimen appeared to

be 30 μ g Δ TM S protein administered i.n. in the presence of ProT adjuvant, based on average neutralizing antibody titers, average total IgG titers, and lack of detectable infectious virus in the lungs of challenged animals. Alhydrogel achieved protection from wild type virus challenge as well, although infectious virus was detected in the lungs of some animals in contrast to the Pro T adjuvant vaccine. The antibody was also neutralized the SARS-CoV Tor-II strain in vitro. Cytokines released by in vitro restimulated splenocytes collected from mice immunized with Protollin-formulated vaccine represented a balanced Th1/Th2 phenotype while a Th2-biased cytokine profile was observed for the intramuscular Alhydrogel-adsorbed vaccine. These data suggest that i.n. administered Protollin-formulated Δ TM S protein can elicit protective immunity against SARS-CoV infection in mice. Such a vaccine may serve as a useful template for the development of a SARS-CoV for humans.

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44

Bile Acid Conjugates Improve the Oral Bioavailability of the Neurominidase Inhibitor Zanamivir

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With the concern for an avian influenza pandemic increasing, there is a need to develop antiviral therapies with improved bioavailability. We are developing an enhanced oral delivery platform for anionic small molecule antiviral drugs using zanamivir as our investigational drug. While zanamivir has proven to be a potent and effective inhibitor of influenza neuraminidase and inhibitor of influenza virus replication in vitro and in vivo, it has been difficult to translate into a successful clinical treatment for influenza, due primarily to its poor oral bioavailability. Zanamivir, therefore, is currently administered by inhalation, a route of administration is not acceptable as the oral route. At TSRL, we have created carrier molecules, termed bile acid conjugates (BAC) that enhance the oral bioavailability of poorly absorbed, charged molecules.

In vitro characterization of the BACs have shown the ability of most BACs to form micelles with the critical micellar concentration (CMC) in the range of the parent bile acid (0.8–4.4 mM for chenodeoxycholic acid). BAC's also increase the octanol:water partition coefficient for zanamivir in a dose dependent manner with increasing BAC concentrations.

Testing of the oral bioavailability of zanamivir in mice with several of the BAC's showed a rapid increase in plasma serum levels peaking 30–60 min after dosing by gavage. Peak plasma concentrations were three-fold increased from control plasma concentrations for zanamivir dosed without BAC. Increasing the molar ratio of BAC to zanamivir from 1:1 to 2:1 increased the oral bioavailability approximately two-fold further. This increase in bioavailability in mice was observed both in fed as well as fasted animals.

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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45

Lymphocytic Necrosis in Hamsters Inoculated with Western Equine Encephalitis Virus

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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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46

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

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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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47

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

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The combination effect of rimantadine hydrochloride and oseltamivir phosphate was examined on infection with 10 and 20 MLD₅₀ 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-