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

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

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A truncated recombinant spike protein (ΔTM 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 ΔTM 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 μg ΔTM 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 ΔTM 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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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 it's 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.