Coceleate Lipid-Crystal Nano-Particles Significantly Enhance the Immune Response in Murine Models of Influenza

Raphael A. MANNINO and RUYING LU – Matinas BioPharma, Inc.

ABSTRACT

Background: Coceleate Lipid-crystal Nano-particle (LCNP) technology demonstrates non-invasive delivery of a broad range of compounds. New formulations of the influenza vaccine with this technology may provide less invasive administration options and increase delivery efficiency. Objectives: To demonstrate that oral/intranasal coceleate LCNP formulations of the influenza vaccine (HCNP H1N1) can provide significant antibody responses with associated viral protection at lower doses. Methods: LCNP H1N1/NA were prepared by incorporating extracted influenza virus haemagglutinin (HA) and neuraminidase (NA) protein into the coceleate lipid crystal matrix. In three experiments, mice were dosed at week zero, three, and 13.5 ± 1, full and quarter of the indicated dose (n=5 mice/group). Results: Flu Nano-crystal H1N1/NA formulation was administered to BALB/c mice comparing 50µg IM to oral dosing. Antibody isotype plasma titers were measured at week 14. Exp. 2: assays in lungs and trachea by oral administration of LCNP H1N1/NA were tested at doses of 10µg, 25µg, 125µg, 1250µg, 6250µg, and 6250µg, followed by intranasal viral challenge at week 16. Exp. 3: neutralizing plasma antibody titers were assessed at week 20 comparing a commercial influenza vaccine versus LCNP of this commercial vaccine after intranasal doses of 1.25µg and 12.5µg. Results: Exp. 1: Intranasal administration of higher IgM and IgG antibody titers than IM administration, with both reaching significant levels (IgG titers of 200,000 versus 25,000 intranasal doses of 1.25µg and 12.5µg, respectively). IgG was much higher upon oral than IM dosing (titers 640 oral vs. 10 IM suggesting stronger protection. Exp. 2: By week 14 all mice demonstrated plasma titers with full long-term detection at doses of 12.5µg and higher and with 22 out of 25 mice receiving 6µg or higher having no virus present in the trachea. Exp. confirmed the dose efficiency of the coceleate nano-particle formulation with neutralizing antibody titers more than 10x higher than unformulated influenza vaccine at oral dose level. Conclusions: These studies demonstrate that oral or intranasal administration of LCNP influenza vaccines stimulate systemic and mucosal, antibody and cell mediated responses, and provide a high level of protection from viral infection.

WHAT ARE COCHELEATES?

- A drug delivery system
- Nano-scaled crystals
- Formulated with simple, naturally occurring materials
- Can be inhaled
- Can be ingested

COCHELEATE TECHNOLOGY

- Formulation of Drugs
- Vehicle technology that encapsulates drugs
- Can be administered by inhalation
- Can be given orally
- Can be given intranasal
- Can be given subcutaneously

How Coceleates Encapsulate Drugs

Coceleate delivery vehicles have been shown to mediate oral bioavailability for lipopolysaccharide drugs, nicotine, and significantly enhance intranasal drug delivery. Coceleates are stable, lipid, nano-particle compound of simple, naturally occurring materials: phosphatidylcholine and calcium. They have a unique multilayered structure consisting of a single, outer shell, lipid bilayer (nested) rolled up in a spiral or stacked sheets, with no internal aqueous space. This unique structure provides protection from degradation for "impenetrable" molecules. Components within the coceleate remain intact, even through the outer layer of the coceleate may be exposed to harsh environmental conditions or enzymes.

Cell-Targeted Delivery

- Microscopic readily engulf coceleates and their cargo
- Once inside the macrophage, the low level of calcium in the cytoplasm causes the coceleate to open, releasing the cargo molecules

Mechanism of Drug Delivery

Different calcium concentrations in serum and mucosal secretions are such that the coceleate structure is maintained. Hence, the majority of coceleate associated molecules are present in the inner layers of a solid, stable, impermeable structure. Once within the interior of a cell, however, the low level of calcium in the cytoplasm causes the opening of the coceleate and release of the entrapped API.

Protection of Mice from Influenza Virus Challenge – Lung and Trachea - Oral Administration

Initial doses of 3µg, 12.5µg, 50µg, or 100µg flu coceleates, were administered orally to groups of mice at 0 and 3 weeks. The third immunization, given at 21 days, was administered by intranasal route and the fourth and final immunizations were given by oral route. These mice were challenged with intranasal administration (white cocker) of 5x10^6 particles of influenza virus at one week after the fourth immunization. Three weeks post-challenge, mice were examined, and the entire lung and trachea were removed and the tissue was fixed in formalin. A high degree of protection from viral replication in the trachea was achieved. The 20 µg dose of the single highest dose (1 µg) resulted in the highest level of protection against viral replication in the lungs. All mice that received 12.5 µg or higher, were protected against challenge. In addition, a low level of viral replication was also observed in the 3 µg group, 6 out of 5 mice were infected. All five of the unvaccinated mice were infected.

The oral protein coceleate vaccine also provided excellent protection against viral replication in the lungs. All mice that received 12.5 µg or higher, (20 out of 20), were negative for virus. The 3 µg and 12.5 µg dose groups had reduced viral loads in the lungs when compared to the controls, (data not shown).

Induction of Influenza Virus Serum Antibody Isotypes – Oral vs. IM Immunization

Induction of IgG, IgA, and IgE IgG levels were given at zero, three, and 13 weeks by intranasal (50 µg, 250 µg, 1250 µg, and 6250 µg) and oral administration (1250 µg). Controls were given IM after 21 days and given a booster at week 26 comparing a commercial influenza vaccine versus LCNP of this commercial vaccine after oral administrations of 1.25µg and 12.5µg. The sera were also measured in experiments with fish and other protein formulations. The results show that the induction of specific IgG antibodies was achieved at higher than those generated by intranasal immunization (400 bleedings at 14 weeks). Similarly, the induction of antibodies against the A/H3N2 component was achieved at lower than those generated with subsequent vaccinations. This antibody sub-type distribution indicates the induction of both Pre-H3 and Post-H3 responses in A/H3N2 protein conjugates, and correlates with results of cytokine secretion assays. Interestingly, the ratios of IgG1 to IgG2a sub-types are skewed towards the immune response.