Formulation of Orally and Intranasally Administered Influenza Vaccine in Cochleate Lipid-Crystal Nano-Particles Significantly Enhances Immune Response in Murine Models of Influenza

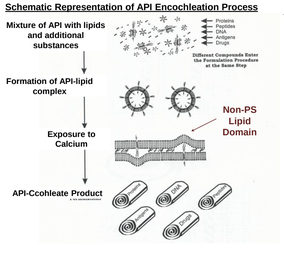
RAPHAEL J. MANNINO and RUYING LU – Matinas BioPharma, Inc.

ABSTRACT

Background: Cochleate 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 dosed LCNP formulations of the influenza vaccine (LCNP HA/NA) provide significant antibody responses with associated viral protection at lower doses.
Methods: LCNP HA/NAs were prepared by incorporating extracted influenza virion hemagglutinin antigen (HA) and neuraminidase antigen (NA) protein into the cochleate lipid-crystal matrix. In three experiments, mice were dosed at week zero, three, and 13-15 at full, full and quarter of the indicated dose level respectively. Exp1: FLU-LCNP HA/NA formulation was administered to BALB/c mice comparing 50µg IM to oral dosing. Antibody isotype plasma titers were measured at week 14. Exp2: assess in lungs and trachea by oral administration of LCNP HA/NA formulations at doses of 100µg, 50µg, 25µg, 12.5µg, 6µg, 3µg, and 0µg, followed by intranasal viral challenge at week 16. Exp3: neutralizing plasma antibody titers were assessed at week 20 comparing a commercial influenza vaccine versus LCNP of this commercial vaccine after intranasal doses of 1.2µg and 12 µg.
Results: Exp1: intramuscular injection resulted in higher IgM and IgG antibody isotype titers than oral administration, with both reaching significant levels (IgG titers of 200,000 versus 25,000 respectively). IgA was much higher upon oral than IM dosing (titers 640 oral vs. 10 IM) suggesting strong protection. Exp2 confirmed this level of protection, with full lung protection 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. Exp3 confirmed the dose efficiency of the cochleate nano-particle formulation with neutralizing antibody titers more than 10x higher than unformulated influenza vaccine at each 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 COCHLEATES

Schematic Representation of API Encochleation Process



What are Cochleates?

Unique drug delivery technology that can formulate and stabilize a variety of molecules:

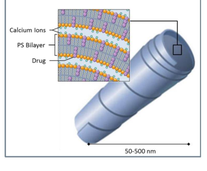
- Pharmaceutical drugs
- Proteins (e.g. insulin, vaccines, etc.)
- Peptides (e.g. daptomycin, etc.)
- DNA plasmids (e.g. gene therapy, etc.)
- siRNAs (e.g. miraviresen, etc.)

Benefits

- Makes oral delivery possible
- Makes intra-nasal delivery possible
- Can reduce toxicity
- Can increase efficacy
- Reduces costs
  - Allows for room temperature storage
  - Composed of safe, naturally occurring materials
  - Inexpensive cost of goods and manufacturing

Cochleate Formulations

- Solid, crystalline structure made up of calcium and a soy-based phosphatidylserine (PS) bilayer
- Cargo molecules, such as drugs, are protected within the cochleate

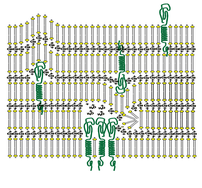


Scanning Electron Micrographs

Control Cochleates

Influenza Virus Protein Cochleates

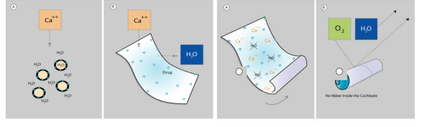
Membrane Protein Cochleates



COCHLEATE TECHNOLOGY

How Cochleates Encapsulate Drugs

Cochleate delivery vehicles have been shown to mediate oral bioavailability for injectable drugs, reduce toxicity, and significantly enhance intracellular drug delivery. Cochleates are stable, lipid-crystal, nano-particles composed of simple, naturally occurring materials: phosphatidylserine and calcium. They have a unique multilayered structure consisting of a large, continuous, solid, lipid bilayer sheet rolled up in a spiral or as stacked sheets, with no internal aqueous space. This unique structure provides protection from degradation for "encochleated" molecules. Components within the interior of the cochleate remain intact, even though the outer layers of the cochleate may be exposed to harsh environmental conditions or enzymes.



Formation of Stable Drug-Liposome Intermediate

Calcium Interaction with Negatively Charged Lipid

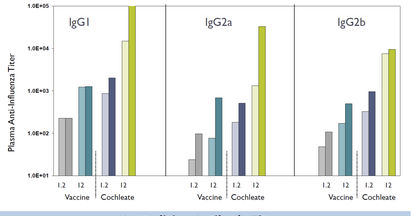
Formation of Stable Drug-Cochleate Nano-Crystal

- The API is associated with the negatively charged lipid.
- The addition of calcium creates a calcium-phospholipid anhydrous crystal.
- Nano-crystals are composed of layers of a lipid-calcium complex.
- The API is trapped in or between the layers protecting the API from harmful environmental elements

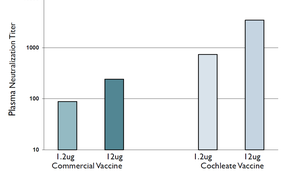
COCHLEATE FORMULATIONS OF A COMMERCIAL FLU VACCINE RESULTS IN A 10X ENHANCEMENT OF EFFICACY

Comparison of Commercial Influenza Vaccine to Cochleate Formulation of Commercial Vaccine – Serum Antibody Titers
Intranasal Immunizations, Day 0; Day 109 – Bleeds, Day 103; Day 137 (mice)

Serum Antibody Titers

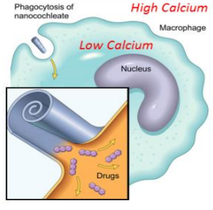


Neutralizing Antibody Titers



Cell-Targeted Delivery

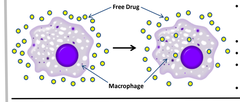
- Macrophage readily engulf cochleates and their cargo
- Once inside the macrophage, the low level of calcium in the cytoplasm causes the cochleate to open, releasing the cargo molecule



Divalent cation concentrations *in vivo* in serum and mucosal secretions are such that the cochleate structure is maintained. Hence, the majority of cochleate associated molecules are present in the inner layers of a solid, stable, impermeable structure. Once within the interior of a cell, however, the low calcium concentration results in the opening of the cochleate crystal and release of the entrapped API.

Cochleates Can Change the Pharmacokinetics and Biodistribution of Drugs

Traditional Model of Drug Delivery

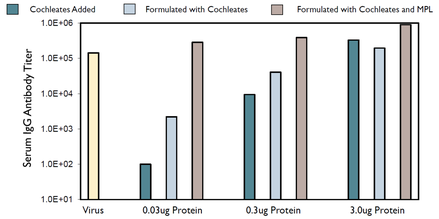


- Free drug in the extracellular milieu must cross the cell membrane in order to be effective against intracellular microorganisms.
- High plasma and interstitial drug levels are needed.
- A relatively low percentage of circulating drug enters the cell.
- Drugs with these properties have difficulty treating intracellular infections.
- High circulating drug levels can result in nonspecific toxicity.

Model of Drug Delivery - The "Trojan Horse" Hypothesis

- High calcium concentrations in gastrointestinal secretions, serum and interstitial fluid stabilize the drug-cochleate crystal.
- Drug cochleates enter the circulatory system, diffuse into tissues and/or are taken up by "activated" and/or infected cells.
- Intracellular levels of drug-cochleates increase and reach high levels.
- The low intracellular calcium concentration causes the drug-cochleates to open releasing their cargo the cochleates.
- Lower plasma levels are required to reach efficacious intracellular drug concentrations.
- These lower plasma levels may result in less systemic toxicity.

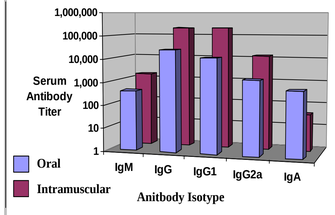
ADJUVANTS INCLUDED IN COCHLEATE FORMULATIONS RESULT IN A > 1000X ENHANCEMENT OF EFFICACY



IMMUNE RESPONSES TO INFLUENZA VIRUS PROTEIN COCHLEATE VACCINES

Cochleates were formulated with the surface glycoproteins from influenza virus integrated in the lipid bilayer. Influenza virus was grown in and harvested from embryonated chicken eggs. The glycoproteins and lipids from the envelope of influenza virus were extracted and mixed with phosphatidylserine and cholesterol to form influenza virus protein cochleates. BALB/c mice were immunized by gradually dispensing 0.1 ml of a liquid cochleate suspension into the mouth and allowing it to be swallowed.

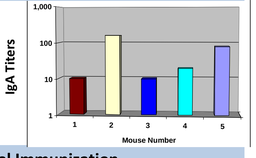
Induction of Influenza Virus Serum Antibody Isotypes – Oral vs. IM Immunization



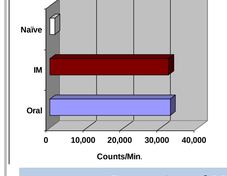
Immunizations of 50ug, 50ug, and 12.5ug of glycoproteins were given at zero, three, and thirteen weeks, respectively. Intramuscular immunization supported higher circulating antibody titers than oral. Importantly, however, the oral route also gave strong circulating IgG titers (25,600 at 14 weeks). Very significantly, and consistent with induction of immune responses at mucosal surfaces, the circulating IgA levels observed are extremely high following oral administration, and substantially higher than those generated by intramuscular immunization (a titer of 640 versus 10 at 14 weeks). Subtype analysis of serum from influenza cochleate immunized animals demonstrates the production of both IgG_1 and IgG_{2a}. The pattern seen in numerous experiments with flu and other protein cochleates is higher IgG1 titers, but very substantial IgG2a titers, both increasing with subsequent immunizations. This antibody subtype distribution indicates the induction of both T helper cell type 1 and type 2 responses (supporting IgG2a and IgG1, respectively), and correlates with results of cytokine secretion assays. Interestingly, the ratios of IgG1 to IgG2a are similar whether the cochleates are given orally or intramuscularly.

Mucosal Antibody - Induction of Influenza Virus Specific IgA Titers in Saliva – Oral Immunization

Secretory IgA antibody is perhaps the most important component of the mucosal defense system. Secretory IgA can protect against pathogens which replicate on or enter via mucosal surfaces, by binding to infectious organisms and blocking their attachment to or invasion through mucosal surfaces.
Influenza glycoprotein cochleates were given to mice orally at zero, three, and ten weeks (50, 50, and 12.5ug). At twelve weeks, all five mice had significant influenza glycoprotein specific salivary IgA, with titers ranging from 10 to 160.



Induction of Influenza Virus Specific Spenocyte Proliferation – Oral Immunization

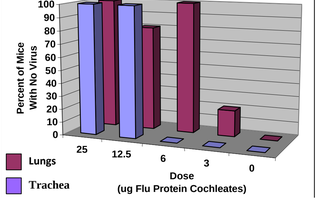


The ability to stimulate antigen specific cell-mediated memory is crucial for successful vaccination. Balb/c mice were immunized at weeks 0 and 3 with 50ug of influenza protein-cochleates. At week 13 they received a 12.5ug boost, and were sacrificed at week 14. Splenocytes were incubated *in vitro* with 16ug/ml UV-inactivated influenza virus. Proliferation was determined by measuring the uptake of ^{3}H-TdR into DNA.
Influenza Specific Splenocyte Proliferation
These data demonstrate that strong proliferative responses (up to 25 fold stimulation index) can be obtained with either oral or IM administration of antigen cochleate formulations.

Protection of Mice from Influenza Virus Challenge - Lungs and Trachea - Oral Immunization

Initial doses of 3ug, 6ug, 12.5ug, 25ug, 50ug, or 100ug flu cochleates, were administered orally to groups of mice at 0 and 3 weeks. The third immunization, given at 15 weeks, was at one-fourth the dose used for the initial two immunizations. Mice were then challenged by intranasal application (while awake) of $2.5x10^9$ particles of influenza virus at one week after the final boost. Three days after viral challenge, mice were sacrificed, and lungs and trachea were obtained. The entire lung or trachea was triturated and sonicated, and aliquots were injected into embryonated chicken eggs to allow amplification of any virus present. After three days at 37°C, allantoic fluid was obtained from individual eggs, and hemagglutination (HA) titers were performed to quantitate virus.

A high degree of protection from viral replication in the trachea was achieved. Of the 25 mice that received the five highest doses (100ug to 6ug), 22 had no virus in the trachea. In the 3ug dose group, 4 out of 5 were infected. All five of the unvaccinated mice were infected.



The oral protein cochleate vaccine also provided excellent protection against viral replication in the lungs. All mice that received 12.5ug or higher, (20 out of 20), were negative for virus. The 3ug and 6ug dose groups had reduced viral burdens in the lungs when compared to the controls, (data not shown).