Efficacious and Commercially Viable DNA Vaccines: Plasmids Formulated into Lipid-Crystal Nano-Particles for Oral and Systemic Immunization
Raphaelo J. Mannino and Ruying Lu - Matinas BioPharma, Inc.

ABSTRACT

Background: The use of DNA plasmids for protective and therapeutic vaccine applications continues to progress. One of the major hurdles with using DNA vaccines is the development of an efficient delivery system. Matinas BioPharma (MBP) has developed a DNA plasmid delivery technology that is cost-effective and commercially viable. Cochleates are a unique stable, multi-tiered, essentially anhydrous lipid-crystal nano-particle that, following either oral or systemic delivery, safely and efficiently delivers nucleic acids to target tissues. MBP’s oral-administered, encochleated formulation of amphotericin B (CAMBIVIR) has commenced a Phase 2b clinical study, funded and led by MBP, designed to evaluate the ability of cochleates to deliver antifungal agents, in early-stage trials, cochleates have been found to mediate rapid uptake in vitro and in vivo delivery and efficacy of potential oligonucleotide based therapies, including DNA plasmids.

Results: Oral Administration of HIV-1 DNA cochleates; Encocochleated formulations of plasmid CMV HIV-1 containing 3.5 µg or 17 µg of DNA were given to BALB/c mice by intramuscular or intranasal routes. Cytokinyt Oral administration of two 3 µg or 17 µg doses yielded strong splenocyte cytolytic responses (73 to 255% specific lysis at an E:T ratio of 100:1) analogous to intramuscular injection. Oral administration of a higher dose of 50 µg plasmid was inactive. Proliferation Low doses (3.5µg) of orally administered encochleated DNA induced antigen specific splenocyte proliferation at E:T ratio 8-10 below background, similar to intramuscular. Naked DNA was inactive. Adjuvant Enhancement: HIV-2 DNA Cochleates II-12 Cochleates: Mice were immunized intramuscularly with HIV-2 DNA, 25µg/dose, (pc DNA 3.1 vector backbone) and two IL-12 plasmids (equal mixture of the pED and pcDNA 3.1 vector backbone) and two IL-12 plasmids (equal mixture of the pED and pcDNA 3.1 vector backbone). Results: - Encocochleation of gD 12 µg plasmids induced 2X greater HIV-specific cytotoxic T cell responses than gD 12 µg, as well as HIV-specific antibody responses. CD4+ T cells co-administration of cytokines can enhance the immunogenicity of a DNA-based vaccine. Naked DNA was inactive. Conclusion: Plasmid-Cochleate formulations promise as viable commercially available oral and systemic vaccines.

COCHLATE TECHNOLOGY

How Cochleates Encapsulate Drugs

Cochleates are stable, lipid-crystal, nanoparticle composed of simple, non-uniformly microphased phospholipids and calcium. They have a unique multi-layered structure of a combination of a continuous, solid, lipid sheet layer rolled up in a signal lipid-stacked sheets, with no internal aqueous space. This unique structure gives this delivery platform its characteristic features, including stability and shelf-life, efficient cellular uptake, and the ability to encapsulate both hydrophilic and hydrophobic molecules. Components within the layers are membrane-like, and therefore, even though the outer layers of the cochleate may be exposed to harsh environmental conditions or enzymes.

Experimental Design

DNA Cochleates

Flamson of HIV-1 gp140, the HIV-1 proteins env (gP160), and tat, in mammalian cell lines. DNA (50µg) of plasmid or 17µg of plasmid were given to BALB/c mice by IM administration. A second dose was administered 2 weeks after the primary dose. Naïve control animals received a vehicle injection without DNA. Animals were sacrificed two weeks after the second immunization.

DNA COCLEATES FORMULATED WITH PLASMIDS EXPRESSING HIV-1 PROTEINS – ORAL ADMINISTRATION

Encochleation of HIV-1 DNA Plasmids Enhances HIV-1-1 Cell Proliferation

Proliferation was measured as the ability of Th1 cells to proliferate in the presence of antigen specific splenocyte proliferation at E:T ratio 8-10 below background. The response to oral administration was similar to intramuscular. Vaccination with two 3 µg doses of plasmid DNA 30 fold greater than cochleate associated with encochleation following oral administration

Conclusions

Plasmid-Cochleates show promise as commercially viable effective oral and systemic vaccines.

- Stable lipid-crystal nanoparticle formulations. Efficacious through oral and systemic delivery.
- Drives both Th1 and Th2, antibody and cellular immunity.
- Induces both Th1 and Th2, antibody and cellular immunity.
- Antimicrobial cochleate formulations are currently in Phase I and Phase II human trials.
- Commercial scale manufacturing processes.