Safe and Efficacious Therapeutic Peptide Delivery Using Nano Emulsion

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Abstract: Nano emulsions have been used as drug delivery systems with several advantages. Cao et al have reported a successful adjuvant-free vaccine with peptide only and peptide sensitized DCs as vaccine and polymer encapsulated peptide vaccine against Alzheimer’s disease using Aß1-42 peptide and its derivatives. We report here a new method of peptide delivery for AD vaccination using nano emulsion approach incorporating Amyloid beta or alpha synuclein peptide fragments. We used different oils to look at the safety of the delivery systems. Our result indicated that our method can effectively encapsulate hydrophobic peptide into nano emulsion. The olive oil generated smaller particles than soybean oil solvent. These nanoparticles can induce low antibody response and there is no inflammation response. We conclude here that nano emulsion can encapsulate hydrophobic peptides and can be good candidate for therapeutic vaccine. More work is warranted to expand the application of these delivery systems.

Introduction: Currently, there is no cure or preventive methods for Alzheimer’s disease and Parkinson’s disease. Nanoemulsions are good carriers for hydrophobic drugs. Aß peptide or alpha synuclein protein aggregation is considered as the major pathological factor of AD or PD. Both vaccine and antibody targeted on these proteins are proved effective methods for the treatment of AD/PD in the mouse model. Due to the high hydrophobic property, it is very difficult to use this peptide as vaccine. Using oil based nano emulsion delivery to deliver the peptide and also allows peptide continuing its aggregation within the particles.

Materials and Methods: Aß peptides (1-35) with or without mutation, and alpha synuclein peptides are purchased from Biomer Technology (CA); Olive oil and soybean oil (food grade) are purchased from the local store; Mice (15 months old) are from our breeding colony. ELISA kit is an in-house product; Cytokine and Ig isotyping kits are purchased from Millipore. Nano emulsion were prepared by separately mixing all the oil dispersed ingredients (Olive or soybean oil, Lecithin, peptide) and water dispersed ingredients (methyl paraben, propyl paraben, Pluronic F 68, glycerin and propylene glycol). Both the phases were vortexed for 12 to 15 minutes to get a primary emulsion. Later, the primary emulsion was subjected to ultrasonic mixing in three cycles of 15 minutes. The nano emulsions were stored in brown bottles at room temperatures. Peptide is reconstituted in DMSO at 5 mg/ml and then mixed together with the oil (1:9) and submit for sonication for 1 hour until the unified particle formed. The control group is the same procedure but no peptide. The particle size distribution of the nano emulsions and zeta potential was determined using the Malverm Nano/zeta sizer using laser diffraction technique. pH of the nano emulsion was also determined.

Vaccination and blood collection: Three day before vaccination and two weeks after each injection, blood was collected from mandibular vein puncture, and collected with EDTA tube, and then plasma was separated by centrifugation and stored in -80C with screw capped tube. Each mouse received 200 µl nano immersions (vaccine group containing 100 µg peptide) by i.p. at two weeks interval for 4 injections.

Results and Discussion: Nano emulsion peptide particle formation in different oil formula: We used Soybean oil and Oliver oil as solvent to emulsified amyloid beta peptide and alpha synuclein peptide fragments, and the particle sizes were detected and shown in figure 1.

Figure1. Nano emulsion peptide particle formed unified particle size after being processed with emulsion procedures. Figure 1a is the particle comparison of soybean oil only versus oil plus peptide, and figure1b is the particle comparison between Oliver oil only versus oil plus peptide. Oliver oil based emulsion has smaller particle size than soybean oil based emulsion. Antibody responses: Mice produced low antibody responses after two injections, but failed to be boosted after that.

Ig isotyping result: It is very important to identify Th1 versus Th2 response for a vaccine, and one of the most popular assays is to use the subtype of immunoglobulin level by comparing the change of among different time points.

Cytokine responses: Inflammation is always a major concern to a vaccine against neurodegenerative diseases, so it is pivotal to evaluate inflammation related cytokines (figure 4)
Figure 2. Antibody response after injection with Nano emulsion peptides and oil controls: As demonstrated in the graphs, all mice injected emulsion peptides produce antibodies. Oliver oil particle seems more potent than soybean oil. There are four mice in each group. Plate is coated with the naked peptide, and detected with HRP conjugated anti-mouse antibody.

Figure 3. Ig isotyping result of anti-sera from nano emulsion particle injected mice: Four different IgG subtype were quantified with Luminex Ig isotyping kits. The major subtype of antibody produced by mice is IgG2b. With the boosting (4th versus 2nd injection), IgG2b increased, indicated this is a specific response to the peptide nano emulsion.

Figure 4: Cytokine expression profile after vaccinations: A panel of 8 cytokines is detected for inflammation and anti-inflammation evaluation (IL1a, IL6, IFNγ, TNFa, IL10, IL12p70, IL17, GCSF). Most of them are undetectable, so we only show those detectable cytokine levels in these graphs. Figure 3a and 3b are two time points’ cytokine expression results. There is no cytokine level increased with continuous injection, so there is no inflammation occurred with the application of nano emulsion peptide particles.

Conclusions: Nano emulsion has been mainly used for drug delivery purpose, and several groups have reported their use as carrier for anti-cancer drugs. We want to compare the different function of oils and the ability of encapsulation to hydrophobic peptide, and further to test the potential to generated isoform specific vaccine. We have successfully formulated a nano emulsion vaccine with amyloid beta and alpha synuclein peptide. This new vaccine has no inflammation effect. We also discovered that nano emulsion vaccine mainly induce IgG2b production instead of IgG1 or IgG2a. This is quite different from traditional vaccinations. We can’t have result for the isoform recognition due to the low antibody titer induced in this study. We will test this vaccine with younger mice in the future. Some major conclusions include
1. Nano emulsion can encapsulate hydrophobic peptide, and different oil gives different size of particle; 2. Vaccine with nano emulsion particle can reduce inflammation in vivo; 3. Nano emulsion is a safe and good delivery system for low water insoluble drugs. 4. Nano emulsion peptide particle can induce IgG2b production. 5. Oliver oil based emulsion has been immune-response than soybean oil based emulsion.


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