A polymeric particulate vaccine for Zika for transdermal immunization using microneedle patch

Akanksha Kale[1], and Martin D'Souza[1]

[1]Mercer University College of Pharmacy, Atlanta, GA - 30341

Statement of Purpose:

The world witnessed a Zika outbreak in the year 2015-2016, with the World Health Organization declaring it as a public health emergency of international concern. Zika fever is a zoonotic disease transmitted to humans through bites of the *Aedes aegyptii* mosquito species. Apart from mosquito bites, the Zika virus can also be transmitted through sexual contact, blood transfusions, and from mother to fetus during pregnancy. If transferred from mother to fetus, it can result in congenital Zika virus syndrome and microcephaly in the new-born. Zika has also been associated with Guillain-Barré Syndrome. Hence, there is a need for a safe and effective vaccine for Zika. Our approach focuses on the formulation of a microparticulate vaccine using biodegradable polymer encapsulating inactivated Zika virus (strain PRVABC59) as the antigen. Subsequently, the microparticulate vaccine is loaded into dissolving microneedle patches and administered via the transdermal route to provide a pain-free immunization.

Methods:

The inactivated Zika virus-loaded microparticles were formulated by a double emulsion solvent evaporation method using poly(lactic-co-glycolic) acid as the polymer. The microemulsion was lyophilized to obtain the vaccine microparticles. Adjuvanted microparticles containing a combination of Alhydrogel® and MPL-A® were formulated following a similar method. Microparticles were characterized for size, charge, morphology, encapsulation efficiency, *in vitro* release, and antigen integrity. *In vitro* safety, immunogenicity, and the ability of vaccine microparticles to induce autophagosomes were assessed in murine dendritic cells. Subsequently, microparticles with or without adjuvants were loaded in the dissolving microneedle patches. The microparticulate vaccine with or without adjuvants was either injected via intramuscular route or administered using microneedle patches via transdermal route to Swiss Webster Mice. Serum samples were collected every two weeks and monitored for IgG, IgG2a, and IgG1 antibody titers.

Results:

The size of microparticles was 573.4 ± 10.18 nm with a polydispersity index of 0.294 ± 0.133. The zeta potential of microparticles was -22.6 ± 0.503 mV. The encapsulation efficiency was in the range of 55-70%. Scanning electron microscopic images showed that the microparticles were spherical. *In vitro* release study showed that 50% of the encapsulated antigen was released in 24 hours. SDS-PAGE confirmed that the formulation process did not affect antigen integrity. Cell viability assay in murine dendritic cells showed that microparticles were not cytotoxic when compared to dimethyl sulfoxide - a known cytotoxic agent. Murine dendritic cells pulsed with the adjuvanted microparticulate Zika vaccine produced a significantly higher amount of nitrite, a marker of innate immunity, as compared to untreated cells. The microparticulate vaccine with and without adjuvants induced 20-fold more autophagosomes necessary for antigen presentation than inactivated Zika in solution. The mice immunized with the microparticulate vaccine with and without adjuvants via both the intramuscular and transdermal routes produced significant antibody titers when compared to naive mice. Total antibody titers of groups receiving adjuvanted vaccine via transdermal route were comparable to groups receiving vaccine via the intramuscular route. Vaccinated mice produced significantly higher IgG2a and IgG1 antibody titers when compared to naive mice.

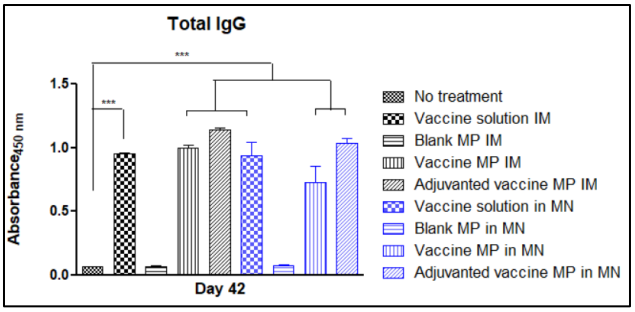

*Figure: Total antibody titers in different groups after immunization with a prime dose on day 0 and booster doses on day 15 and 29 (Abbreviations: IM: Intramuscular, MP: Microparticles, MN: Microneedle, Data represented as Mean ± SEM. Kruskal-Wallis test, ***P< 0.001)*

Conclusion:

The microparticulate Zika vaccine was successfully formulated and was found immunogenic and non-cytotoxic *in vitro*. The *in vivo* immunization in Swiss Webster mice via both intramuscular as well as transdermal route revealed that the microparticulate vaccine was able to generate a humoral immune response. Moreover, induction of IgG2a and IgG1antibodies indicates a balanced Th1/Th2 immune response. This study established the feasibility of the development of transdermal microneedle-based vaccine for Zika. Future studies will comprise the evaluation of cell-mediated immune response and memory response induced by the microparticulate Zika vaccine.