Cairo University
Faculty of Veterinary Medicine
Department of Virology

Studies on Biodegradable Nanoadjuvants for Mucosal Vaccine Delivery

A dissertation presented by

Walaa Abd El Moneim El Sayed Mohammed
M. V. Sc. (2008), Cairo University

For the degree of

Doctor of Philosophy in Veterinary Medical Sciences (Virology)

Under the supervision of

Prof. Dr. Ahmed Abd El-Ghani El-Sanousi
Professor of Virology, Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Mohamed Abd EL-Hamid Shalaby
Professor of Virology
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Ismail Mohammed Reda
Professor of Virology
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Mohammed Sami Saber
Professor of Virology
Faculty of Veterinary Medicine
Cairo University

Dr. Abo Zeid Ahmed Abo Zeid
Chief researcher
Serum and Vaccine
Research Institute

2017

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

6 CONCLUSIONS

In conclusion, the described results demonstrate that local adjuvants can be produced with relative ease and cost effectiveness. It also shows that it is possible to improve commercial vaccines by incorporating some additional components without major modification of the production process. More importantly, this research demonstrates that important steps were made towards the development of a local mucosal adjuvant (better than the commercial mucosal adjuvant tested in this experiment). Further studies are needed to achieve higher protection rates by the described mucosal adjuvant preparation. This research is considered an important milestone in the complete independence of the national vaccine industry. It is recommended that the national vaccine production industry headed by VSVRI should consider establishment of an adjuvant production factory to cover national vaccine production needs.

7 SUMMARY

The current study was initiated with the intent to prepare biodegradable nanoadjuvants that can be delivered both mucosal surfaces of chicken. The study also targeted the development of other adjuvants that can used to deliver inactivated viral antigens through the percutaneous route. In parallel, the study targeted improvement of the immune response to current commercial vaccines and to reduce the cost of production of these vaccines in a manner that will sever national interests. Therefore, this study was conducted on several stages.

Stage one (the preparation of some chemical compounds and biological material to be tested as adjuvants)

a. The following material was prepared:
b. Micro-sized Chitosan particle (> 6 um).
c. Oily nanoemulsion (within the 100 nm range).
d. Nonoaluminum hydroxide (within the 130 nm range).
e. IgY preparations that contain antibodies to NDV antigens (known to act as a natural immunomodulatory material).

Propagation of NDV in 9-11 days old SPF ECE then titrated in 9-11days old SPF ECE.

Virus purification and inactivation

Stage two included

Preparation of formulations

Physical and visual characterization of those experimental formulations.

The critical point for equilibrium was determined for the NDV-IgY complex.

The optimum method for virus inactivation that would facilitate further downstream antigen loading and final vaccine formulation within the nanosize range was determined.

Stage three (preliminary experiments to investigate the optimal methods, concentrations, and delivery routes to achieve maximum adjuvant effect using the Newcastle disease virus model)

SPF-origin seronegative chicken or SPF chickens were used. The experimental inactivated virus vaccine preparations were given via different routes. Each preparation contain the same proportion of inactivated virus of the local isolate of NDV genotype VIId and LaSota strain antigens and different types of the proposed adjuvants, delivered by different route. Samples were collected at immunization and weekly afterwards. After 21 days, challenge was conducted using 10^6 EID_{50}/bird of the virulent of NDV genotype VIId in isolators.

The preliminary results using IgY-antigen preparation on the mucosal surfaces was 0 seroconversion and protection. Protection and seroconversion using nanoaluminum hydroxide-antigen preparations containing 0.7 mg nanoaluminum hydroxide was achieved in only 50% of treated chicks.

Groups that received the nanoemulsion at 20% emulsion of the whole dose delivered also failed to induce protection despite the fact that seroconversion was sometimes achieved in birds receiving

percutaneous treatments; but birds that received the formula via the mucosal route seroconversion was somewhat low than percutaneous (as measure by HI).

Another major experiment conducted on three hundred and fifty five chicks of SPF origin that were tested to be seronegative were kept until they reached 6 weeks of age, then divided into 26 groups, each given one dose of the inactivated experimental NDV vaccine containing of equal amounts (7.68 X 10 $^{6}EID_{50}$/bird) of the local isolate of NDV genotype VIId and LaSota strain antigens except one group and different types of the formulated adjuvants administered either percutaneously or on mucosal surfaces. Appropriate controls were included. The birds were observed for 10 days after delivery and samples were collected before and after immunization (after one week and after 10 days). Birds were challenged only 10 days post immunization. Serum HI antibody levels were measured, and protection was calculated following challenge. The results revealed important improvements over the imported commercial vaccines. Results obtained were as follows:

For groups that received inactivated NDV and Chitosan at a concentration of 1.5 mg, with or without “Bacterial component” and/or Natural lipid:

- Formulations containing Chitosan and “Bacterial component” gave 71.42 % protection when taken by injection. The same formulation without stimulant “Bacterial component” resulted in only 60% protection. The formula failed to stimulate protective immunity when given mucosal (0% protection).

- Formulations containing the inactivated antigen and both “Bacterial component” and Natural lipid resulted in the production of 70% protection when given by injection, and 14.28% when given on mucosal surfaces.
- Formulations containing only chitosan without antigen failed to induce antibody production, and birds were killed following challenge.
- Birds receiving double the dose of the inactivated antigen showed some seroconversion and only 10% birds survived challenge.

Formulations containing the inactivated antigen, the nanoaluminum hydroxide with or without stimulant Bacterial component or with andwithout both “Bacterial component” and natural lipid resulted in:

- 86.67% protection when formulations contained the “Bacterial component” stimulant only given by injection, and 0% when given on mucosal surface.
- 75% protection when formulations contained the “Bacterial component” and Y stimulants were given by injection, and 10% when given on mucosal surfaces.
- 80% protection when formulations contained only nanoaluminum hydroxide without “Bacterial component” and natural lipid were given by injection.
- Formulations containing only nanoaluminum hydroxide without antigen failed to induce antibody production, and birds were killed following challenge (0% survival).

Formulations containing the inactivated antigen, the antigen-IgY complex at the proper equilibrium ration with 40% nanoemulsion with or without stimulant Bacterial component, or with and without both “Bacterial component” and natural lipid resulted in:

- 40% protection when the formulation contained the nanoemulsion and the antigen with “Bacterial component”, and was delivered by injection. The same formulation resulted in the production of 15.38% protection when it was given via the mucosal routes.
- 46.66% protection when the formulations contained the nanoemulsion and the antigen with “Bacterial component”, “natural lipid”, and nanoaluminum hydroxide delivered via injection.

Formulations containing the inactivated antigen properly mixed with Montanide ISA 71 resulted in protection percentages of 80% under the same experimental conditions of the rest of the experiment.

Formulations containing the inactivated antigen properly mixed with Montanide Gel O1 delivered via the mucosal route failed to stimulate protective responses (0% survival) when conducted under the same conditions of the rest of the experiment.

Formulations containing the inactivated antigen properly mixed with the Rehydragel aluminum hydroxide gel adjuvant resulted in protection percentages of 71.42 % under the same experimental conditions of the rest of the experiment.

Formulations containing the inactivated antigen properly mixed with Montanide ISA 71 resulted in protection percentages of 100% when the formula contained properly balanced IgY-Antigen complex was delivered by injection under the same experimental conditions.

Protection was 0% when the formulations contained the properly mixed IgY-antigen complex together with nanoaluminum hydroxide, “Bacterial component”, and “natural lipid” in 40% nanoemulsion delivered via mucosal surfaces.

40% protection was achieved when the formulations contained the properly mixed IgY-antigen complex together with nanoaluminum hydroxide, “Bacterial component” stimulant, “natural lipid”, in 40% nanoemulsion, contained twice the amount of antigen and, was delivered via mucosal surfaces.

Proper controls were included. 0% protection was observed when formulations contained no antigen.

Stage four included characterization of select formulations and evaluation of final formulations.

In this stage, characterization of different formulations and final experimental evaluation of different formulation in birds.

Characterization of the prepared components of formulae using high resolution electron microscope, and a Zeta potential analyzer.

Final experiment for evaluation of different formulations in SPF chicks

Proper controls were included. Birds were observed for 21 days after administration of the treatment. Serum samples were collected

one and two weeks post immunization, and assessed for the presence of specific antibodies using HI. After 21 days the birds were challenged as before and protection percentages were recorded. The approach used for assessment was that adopted by the national regulatory authorities for the release of vaccine for market use.The results detailed below show that the reported experimental formulations produced similar or better results when compared to control imported adjuvants. Results obtained from each group were as follows:

The results detailed below show that the experimental formulations gave comparable or better results compared commercial vaccines used for comparison. Results obtained from each group were as follows:

- Formulations containing the inactivated antigen and the nanoaluminum hydroxide without any additional stimulant elicited the production of protective immunity in all vaccinated chicken (100% protection).
- Formulations containing the inactivated antigen, the nanoaluminum hydroxide, and stimulant “Bacterial component” elicited the production of protective immunity in 95% of vaccinated chicken.
- All formulations containing adjuvant(s) without the inactivated antigen failed to protect birds (0% survival after challenge).
- Formulations that contained antigen-IgY complex at the proper equilibrium with 40% nanoemulsion with both “Bacterial component” and natural lipid, and given 2X the regular dose elicited immunological response that lead to the protection of 50% of challenged birds following mucosal delivery of the formula.

- Formulations containing the IgY, and “Bacterial component” and natural lipid without the inactivated antigen failed to protect birds (0% survival after challenge).
- Formulations containing the inactivated antigen properly mixed with Montanide ISA 71 resulted in protection percentages of 90% under the same experimental conditions of the rest of the experiment.
- Formulations containing the inactivated antigen properly mixed with Rehydragel aluminum hydroxide gel adjuvant resulted in protection percentages of 90% under the same experimental conditions of the rest of the experiment.
- Formulations containing the inactivated antigen properly mixed with Montanide ISA 71 resulted in protection percentages of 100% when the formula contained properly balanced IgY-Antigen complex, and was delivered by injection under the same experimental conditions of the rest of the experiment.
- Formulations containing the inactivated antigen properly mixed with nanoaluminum hydroxide elicited responses that lead to protection percentages of 80% when the formula contained properly balanced IgY-Antigen complex, and was delivered by injection under the same experimental conditions of the rest of the experiment.

This research is considered an important milestone in the complete independence of the national vaccine industry. It is recommended that the national vaccine production industry headed by VSVRI should consider establishment of an adjuvant production factory to cover national vaccine production needs.