Immunological Effects of a Single Dose of PLGA Nanoparticles Encapsulated Peptide in Broilers in Comparison to Traditional Vaccines against Infectious Bursal Disease

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ABSTRACT

Infectious bursal disease virus (IBDV) is a viral poultry disease that causes economic losses. This study aimed to evaluate the white blood cells count (WBCs count), differential count, antibody (Ab) titration against IBDV and interferone gamma (INF-γ) responses in broiler administered traditional and prepared nanovaccines. A total of 98 broiler chicks (Ross 308) were used to evaluate the immunological responses. They were divided into G1 (control); G2 (traditional vaccine); G3 (PLGA nanoparticles); G4 (160 µg of peptide loaded PLGA); G5 (80 µg of peptide loaded PLGA); G6 (40 µg of peptide loaded PLGA) and G7 (20 µg of peptide loaded PLGA) by oral administration. At day 19 of broiler age, the chicken was administered orally with traditional and prepared nanovaccines and the blood was collected for Ab titration at day 10 (for maternal immunity), day 29 and day 42 of broiler age. At the end of experiment, whole blood was collected for WBCs and differential count and for INF-γ level. The results indicated that there were no significant (p≥0.05) differences among experimental groups in WBCs count while there were significant (p<0.05) increase in lymphocyte count in G3 (NPs) in compared with G2 (traditional vaccine). Also there was a significant (p<0.05) increase in heterophil count in G4 (NPs + 160 µg peptide) as compared to control. For Ab titration, at day 29, data indicated significant (p<0.05) increase in G3 and G7 as compared to G2 while at day 42, there was a significant (p<0.05) increase in each of G3, G4, G5 and G6 groups in compared to control and traditional vaccine. For INF-γ level, G4 recorded significantly (p<0.05) the highest level as compared to other groups. These immunological responses were explored in order to evaluate the prepared nanovaccine for IBD in broiler.

Key words: Ab Titration, Brioler, INF-γ, Nanovaccine, PLGA nanovaccine, Wbcs Count.

INTRODUCTION

Infectious bursal disease virus (IBDV) is the causative agent of a highly contagious disease in young chickens, and has a major impact on the economy of the poultry industry worldwide. IBDV multiplies rapidly in developing B-lymphoid cells, resulting in the destruction of the precursors of antibody-producing B cells in the bursa of Fabricius, resulting in immunosuppression, which leads to vaccination failures and susceptibility to other infections (Safi, 2008). IBDV infected Chickens show suppression of both humoral and cellular immunity (Pradhan et al, 2014).

Traditional vaccines are mainly composed of attenuated or heat-inactivated viruses often generating many unwanted side effects. Subunit protein and peptide vaccines are generally very safe vaccines with well-defined components. However, proteins and peptides are often poorly immunogenic (Seth et al, 2015) and thus require the use of adjuvants to induce adequate immunity. Therefore, particulate adjuvants have been widely investigated in vaccine delivery systems (Peek et al, 2008).

Nanoparticles based on polymers were the most widely studied nanosystems in the field of immunization because nanomaterials are able to enhance the responses of B and T cells because of their similarity with viruses in terms of size and surface characteristics (Peleteiro et al, 2018). In addition, because of their long-term exposure to the immune system, these nanoparticles can produce long-term immune responses thereby, providing the possibility of reducing the number of doses required to achieve protective Abs. levels. Moreover, the nanoparticle compositions demonstrated the ability to avoid hyperactivity in the immune response and, at the same time, stimulate the production of proinflammatory cytokines (Bachmann and Jennings, 2010).

1- In particular the use of nanotechnology in vaccinology lead to “nanovaccinology” birth (Gill, 2013). Nanoparticles are used in both prophylactic and therapeutic approaches, as either a delivery system or an immunostimulant adjuvant. Therapeutic nanovaccinology is applied for treatment of cancer (Kennedy et al, 2011), Alzheimer (Champion et al, 2009), hypertension (Praetorius and Mandal, 2007) and nicotine addiction (Fifis et al, 2004). Nanotechnology is used in creating a new generation of effective vaccines (i.e., nanovaccines) which capable of transcending many
biological barriers in the body (Mozafari et al., 2009). Nanosized particles have similarly been used for vaccine construction against microbial agents (Champion et al., 2009). The experiment is designed to compare between broilers immunized with the prepared nanovaccine against those immunized with the traditional Gumbaro vaccine in vivo and investigate the physiological and immunological effects of both vaccines.

**MATERIALS AND METHODS**

**Preparation of PLGA nanoparticles loaded with peptide**

PLG nanoparticles encapsulating peptide (GDQMSWSASG SLAVT) were prepared by solvent evaporation method (McCall and Sirianni, 2013; Kumar, 2014), with some modification, which can be summarized: 200 mg of PLGA dissolved in 2 ml of DMSO and left at room temperature overnight. PVA was prepared by dissolving 2g in 100 ml of dH2O. The PLGA solution added drop wise to PVA and left overnight on stirrer then the product solution centrifugal and wash 3X and the precipitate collected and lypholized for 72hr.

**Animals**

Ninety eight day old (40.5 gr. Weight), unsexed broiler (Ross) chicks were brought from a local hatchery and randomly divided equally into 7 groups (14 chick) fed on an ordinary diet, starter from day 1 to 20 (22 % crud protein, 2926 K Cal / Kg) and finisher from day 21 to 42 (19 % crud protein, 3109 K cal / Kg) ad libitum. The experimental animal were divided into: G1(Control) were received dH2O by oral administration; G2- were received the traditional vaccine by oral administration; G3 were received PLGA nanoparticle by oral administration; G4 were received prepared vaccine (160 µg of peptide loaded PLGA) by oral administration; G5 were received prepared vaccine (80 µg of peptide loaded PLGA) by oral administration; G6 were received prepared vaccine (40 µg of peptide loaded PLGA) by oral administration; and G7 were received prepared vaccine (20 µg of peptide loaded PLGA) by oral administration. At day 10 of chicks’ age blood was collected from wing vein randomly from 18 chicks and the serum separated for detecting antibody titration for ND and IBDV (maternal immunity) to design vaccination schedule. The exp. was carried out in private poultry farm from 3/10/2018 until 13/11/2018 (42 day).

**Total white blood cells count, differential W.B.C. Count and H/ L ratio**

Blood was collected from wing vein at the end of the experiment (42 day old) in tubes with anticoagulant (EDTA). The count was done in Veterinary laboratories/ hematology laboratory by using Orphee appliance (Switzerland).

**Antibody Titer for IBD**

To determine the Ab titration for IBD blood was collected from wing vein in 10,29 and 42 days old chick. The serum was separated by using a centrifuge (800B centrifuge, China) and the determination was done according to the directive of manufactured company (Synbiotics Corporation) (appendix1). And the below mention equation was applied:

\[ \log_{10} \text{TITER}\times=1.172\times \log_{10}\text{Sp}+3.614 \]

\[ \text{TITER=ANTILOG OF LOG10 TITER} \]

**Chicken Interferon Gamma (IFN-γ) by (ELISA)**

Interferon Gamma (IFN-γ) was detected in the serum of broiler at the end of study, depending on the direction of manufactured company (Cloud-Clone Corp.).

**Statistical analysis**

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA and least significant differences (LSD) post hoc test were performed to assess significant differences among means. (P < 0.05) was considered statistically significant (SAS, 2010).

**RESULTS AND DISCUSSION**

**Total White blood cells count, differential W.B.C count, and H/ L ratio**

Fig (1A) represents the mean values of total leukocytes count among groups. The figure shows no significant (p<0.05) differences between groups that received ordinary vaccine (G2), PLGA nanoparticles (G3), PLGA+160µg of peptide (G4), PLGA+80µg of peptide (G5), PLGA+40µg of peptide (G6) and PLGA+20µg of peptide (G7) compared with control (G1). On the other hand, broilers that received PLGA nanoparticles alone (G3) revealed significant (p<0.05) increase in lymphocyte number with a significant (p<0.05) decrease in heterophils number (Fig1B) and H/ L ratio (Fig1C). The number of heterophils and H/ L ratio showed higher significant (p<0.05) value in group of broilers that received PLGA+160µg of peptide (G4).

Incorporation of antigens in nanoparticles can be achieved by encapsulation (physical entrapment) or by conjugation (covalent functionalization) (Chattopadhyay et al., 2017). Studies have demonstrated that nanoparticles could protect the native structure of antigens from proteolytic degradation and/or improve antigen delivery to antigen-presenting cells (APCs) (Pachioni-Vasconcelos et al., 2016). In addition, nanoparticles incorporating antigens can exert a local depot effect, ensuring prolonged antigen presentation to immune cells (Fredriksen and Grip 2011). Interestingly, nanoparticles have also shown intrinsic immunomodulatory activity (Mamo and Poland 2012). For instance, nanoparticles such as poly (lactic-co-glycolic acid) (PLGA) have been reported to induce NLRP3-associated inflammasome activation (Zhu et al., 2014) which subsequently activates the formation of the inflammasome complex (He et al., 2016). Subsequently, interleukins are produced as downstream signaling events, leading to the recruitment and/or activation of immune cells (Scharf et al., 2012). Taken together, these properties advocate that nanoparticles are promising antigen carriers and immune cell activators for vaccination.
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Fig 1: Effect Of PLGA Nps and PLGA loaded with different concentration of peptide on the H/ L ratio in broilers. G1=received dI_H2O, G2= ordinary vaccine, G3=PLGA nanoparticle, G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide,G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide. WBCs count (A); Lymohocyte and Heterophil count (B) and H/ L ratio(C).

Also these polymers have the ability to activate cellular immune response in the host by interact and activate various toll like receptors, thus involving several innate immune system players in immune response, this increase as a result of enhancing in IL6 and IFNγ (El Naggar et al., 2017). The delivery of antigens to B cells via NPs has a co-stimulatory effect on Th cells, which helps to trigger specific antibody responses. The crosstalk between B cells and T cells widens the potential of utilizing NPs as vaccine adjuvants or vaccines (Du et al., 2017).

In our study there were no significant differences among groups and thus is in lins with the finding of Kumar (2014), who observed no considerable changes after administration of NPs to mice with endometriosis. Also Krucinska et al. (2017) recorded no significant changes in WBCs and thrombocytes parameters in rabbits. There were significant increase in lymphocyte count and that may be attributed to the increasing IFNγ concentration which play an important role in the cellular immunity and antiviral process and stimulate lymphocyte proliferation specially T- lymphocyte (Gao et al., 2011). Saroja et al. (2011) and Bolhassani et al. (2014) showed that PLGA microspheres are rapidly taken up by M-cells and translocated toward the underlying lymphatic tissue within 1 h. For instance, the loading of Hepatitis B core antigen into PLGA NPs (300 nm) induced a stronger cellular immune response as compared with Hepatitis B core antigen alone in a mouse model.

Antibody of IBD
The available data reported in Figure (2) denote a significant (p<0.05) increase in the Ab titration for IBD in broilers subjected to PLGA Nps (G3) at 29 and 42 days of age. Besides that, the table shows variable results of increasing Ab titer at 29 and 42 days of broilers age for other treated groups.

The humoral immune response is an essential component of protection against IBDV. In order to evaluate the immune response triggered by oral IBDV administration, Carballeda et al. (2011) monitored the specific antibody levels in serum of IBDV inoculated chickens at 0, 7, 14, 21 and 35 dpi and showed that a specific anti-IBDV humoral immune response began to be detected at 7 dpi, which reached the maximum titre at 21 dpi. The level of antibodies remained high until 35 dpi. In our immunization trial, we also observed the similar antibody pattern. High levels of antibody response to PLGA nanoparticles have been previously reported by many workers (Raghuvanshi et al., 2002; Gutierrez et al., 2002).

Binjawadagi et al. (2014) had shown that the cross protective immune response in pigs can be produced by adjuvanted poly (lactic-co-glycolic) acid nanoparticles entrapped inactivated porcine reproductive and respiratory syndrome virus vaccine.

Many researches have indicated that polymeric nanoparticles (NPs) can be used as potent adjuvants as
part of a "nano" mucosal immune delivery system. NPs are biodegradable and biocompatible, have low toxicity and protect the antigen or DNA from damage (Zhao et al., 2014).

Among all the polymers, polyesters based on polylactic acid, polyglycolic acid, and their copolymers, poly(lactic-co-glycolic) acids (PLGAs), have attracted the most attention and have been used as carriers for a wide range of vaccines (Hsu et al., 2011). Also Noh (2013) said that intranasal immunization of C57BL/6 mice resulted in antigen-specific increases in IgG, IgA and IFN-γ in sera. Ayari-Riabi (2016) mention that used of polymeric NPs as a safe adjuvant to deliver a venom fraction causing increased antibody levels in mice serum and resulted in increased immunity. Zhao et al. (2010) previously reported that PLGA-NPs that were prepared with a water/oil/water double emulsion-solvent evaporation method did not change the encapsulated plasmid DNA but promoted the sustained release of the plasmid DNA and induced stronger mucosal immune responses than for nonencapsulated plasmid DNA.

This indicated that delivery of vaccine antigen through PLGA nanoparticles was able to provide a sustained immunity as compared to the commercial vaccine.

**Interferon gamma (INF-γ) level**

From the data presented in Fig (3), it was observed that the INF-γ has highly significant (p≤0.05) mean in serum of broilers received PLGA+160 µg (G4) at day 42 of age as compared with control and other treated groups.

Interferons (IFNs) are important members of host innate arm of immunity to prevent virus infection by their antiviral effect (El-Naggar et al., 2017). IFN-γ produced by CD4+ T helper cell type 1 (Th1) lymphocytes, CD8+ cytotoxic lymphocytes and NK cells (Schroder et al., 2004). However, there is now evidence that other cells, such as B cells, NKT cells and professional antigen-presenting cells (APCs) secrete IFN-γ (Harris et al., 2000). IFN-γ production by professional APCs (monocyte/macrophage, dendritic cells (DCs) acting locally may be important in cell self-activation and activation of nearby cells (Frucht et al., 2001). IFN-γ secretion by NK cells and possibly professional APCs is likely to be important in early host defense against infection, whereas T lymphocytes become the major source of IFN-γ in the adaptive immune response (Sen, 2001).

Noh (2013) said that intranasal immunization of C57BL/6 mice resulted in increases in IFN-γ in sera. Shakya and Nandakumar (2012) show that the nanoparticles and polymers vaccine adjuvant could enhance the efficacy of avian mucosal vaccination against infectious diseases by increasing the expression of IFN-γ and IL6 genes to provide insights into the role of innate immune response in protection against infectious disease in chicken. Also Hu et al. (2012) observed increase the phagocytic activity as a result of enhancing in IL-6 and IFN-γ. Jahan et al. (2018) observed that the highest amount of IFN-γ was secreted after DC stimulation with OV-NPs in compared with the stimulation of free OV.
CONCLUSION

Infectious bursal disease in poultry is an economic disease causes economic losses due to the weight loss and secondary infection associated with the disease. The results of the experiment indicate that the use of nanoparticles in the preparation of a vaccine against IBD cause a significant increase in the number of lymphocytes and significant decrease in heterophil, especially in G3, which orally administered with PLGA NPs and recorded this group the lowest level in the proportion of H/L ratio. For the level of antibodies, it is noted that the highest values were recorded in G3, G4, G5 and G6 compared to the other groups on the 29th and 42 of the experiment. INF-γ recorded the highest level at the end of the experiment in G4. This shows that the use of PLGA NPs as adjuvante act as stimulator and stimulate immunity in both cellular and humoral arms in poultry better than traditional vaccine.

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