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Full Article

Immunogenicity of commercial, formaldehyde and binary ethylenimine inactivated Newcastle disease virus vaccines in specific pathogen free chickens

Razmaraii*[1&3], N., Toroghi[2], R., Babaei[3]. H., Khalili[1], I., Sadigh-Eteghad[1], S., Froghy[4], L.

1. Department of Molecular cell biology, Razi Vaccine and Serum Research Institute, Marand, Iran
2. Department of Biotechnology, Razi Vaccine and Serum Research Institute, Mashhad, Iran
3. Pharmacology Lab, Drug Applied Research Center of Pharmacy Faculty, Tabriz University of Medical Science, Tabriz, Iran.
4. Student Research Committee,Tabriz University of Medical Science,Aras Branch, Tabriz, Iran.

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ABSTRACT

Newcastle disease (ND) is one of the most important diseases that affect birds; the epizootic nature of the disease has caused severe economic losses in the poultry industry worldwide. In this experiment ND virus (NDV) was inactivated by two different chemicals binary ethylenimine (BEI) and formaldehyde. Formaldehyde was used at 0.1%, while BEI was used at concentrations of 1 to 4 mM. NDV inactivation with BEI was done in various incubation temperatures and periods and the best result (30 °C, 4 mM BEI and 21 hrs treatment) used as an experimental vaccine. Prepared inactivated NDV vaccines and a commercial vaccine were tested for their efficiency in generating humoral immune response in different groups of specific pathogen free (SPF) chicks. Test groups received 0.2 ml formaldehyde inactivated NDV (NDVF), BEI inactivated NDV (NDVEI) and Razi institute produced NDV vaccine (NDVR) subcutaneously respectively. HI Log 2 total mean titer of NDVEI group (8.42 ± 0.12) were significantly higher than NDVF (7.64 ± 0.16) and NDVR (7.86 ± 0.11) groups ($p<0.05$). BEI-inactivated vaccine gave higher antibody titers than formaldehyde-inactivated vaccine and preserves both structural integrity and antigenicity of the virus. Thus, it might be possible to use these compounds as an inactivator agent for commercial NDV inactivated vaccines in future.

Keywords: Newcastle disease virus, vaccine, binary ethylenimine, formaldehyde

INTRODUCTION

Newcastle disease (ND) is caused by the ND virus (NDV) and it is one of the most important diseases that affect birds in particular chickens (Fenher *et al* 1987). NDV is a member of the avian paramyxovirus type 1 viruses that belong to the *Avulavirus* genus of the *Paramyxoviridae* family (Jeon *et al* 2008, Knipe & Howley 2001). The epizootic nature of the disease has caused severe economic losses in the poultry industry

*Author for correspondence.Email: n.razmaraii@rvsri.ir

worldwide since the 1920s (McFerran & McNulty 1993).

Both live attenuated and inactivated vaccines are used routinely to protect chicken against ND during the entire life of bird (Koppad *et al* 2010). The inactivated ND vaccines that are currently on the market contain viral antigen which has been inactivated either by formaldehyde or by β-propiolactone (BPL) treatment (Nathanson 2001). The mechanism of inactivation is different for the two chemical substances. BPL mainly attacks nucleic acids whereas formaldehyde mainly reacts with proteins (Jagt *et al* 2010)

Binary ethylenimine (BEI) an aziridine compound has been used for inactivation of adventitious viruses in biological preparations (Berhane *et al* 2006, Lubroth *et al* 2007, Pyke *et al* 2004). BEI reacts with viral nucleic acids while preserving conformation and accessibility of epitopes to a much greater extent than formalin and BPL (Bahnemann 1990).

BEI has been used to successfully inactivate various viruses for vaccine production including infectious bursal disease virus (Mudasser *et al* 2006) Rabies Virus (Larghi & Nebel 1980) foot and mouth disease virus (Aarthi *et al* 2004) and arbovirus (Pyke *et al* 2004).

In the present study NDV was inactivated with formaldehyde and BEI. The potency of both prepared vaccines and a commercial vaccine were established by HI test in specific pathogen free (SPF) chickens.

MATERIALS AND METHODS

Virus preparations. NDV (V4 vaccine strain/ Razi research and vaccine institute) was used to inoculate in 10-day old SPF embryonated chicken eggs. The eggs were observed for 24-72 hrs post inoculation. Allantoic fluid of the inoculated eggs was harvested, centrifuged 1200 rpm, 30 min and supernatant was collected.

Egg infective dose 50 (*EID*50) of the virus was calculated by the method of Reed & Muench (1938) in fertile eggs using 9-day-old embryos. The calculated *EID*50 was 9.67.

HA assay was performed in U-bottom 96-well plates with 1% chicken red blood cell as described with Burleson *et al* (1992).

Vaccine preparations. Binary ethylenimine (BEI) treatment: BEI was prepared as 0.1 M solution by 2-bromoethylamine hydrobromide (Merck, Germany) in NaOH (Merck, Germany). The prepared BEI was added to the virus (*EID*50= 9.67) suspension at the rate of 1, 2, 3 and 4% to make final concentration of 1 to 4 mM. The treated material was incubated at 25, 27.5 and 30 °C, and sampling was done after 2, 4, 6, 8, 12, 15, 19 and 21 hrs of treatment. The residual BEI was hydrolyzed in samples by treatment with sodium thiosulfate (Merck, Germany) added at a concentration 10 times the final BEI concentration.

Formalin treatment:

Formaldehyde (Merck, Germany) solution was diluted in DDW. The final concentration of 0.1% (King 1991) was attended by virus solution. A control with no added formalin was indicated. The viruses were mixed and incubated for 16 hrs at 37 °C.

Infectivity assays. All of the prepared samples, checked for their pathogenicity by inoculation into ten-day old embryonated SPF eggs. Each sample was inoculated into 5 eggs at the rate of 0.2ml/egg. All eggs were sealed with wax and incubated for 120 hrs at 37 °C. Eggs were candled daily. Allantoic fluid from surviving embryos was testing for HA activity. The samples with no unwanted effects were replicated for approve and best result was elect as a vaccine for experimental trial.

Experimental design. One-day SPF chickens (n=300) were used in this study. Before the chickens were introduced into the laboratory experimental facilities, they were tested with Jeon *et al* (2008) method for the major viral diseases (NDV, Influenza, bronchitis, infection bursal disease) that affect chickens. The SPF birds were maintained in air-filtered bio-security isolation units with feed and water *ad libitum* for the 9 weeks. All the procedures were carried out under the ethical guidelines of Tabriz University of Medical sciences (Code Number: 916).

After 21 day of acclimatization, the birds randomly divided in 6 groups (NDVF, NDVEI, NDVR, NS, F and EI) of 50 birds each. NDVF, NDVEI and NDVR groups received 0.2 ml formaldehyde inactivated NDV, EI inactivated NDV and Razi institute produced NDV vaccine (HI: 7.4, Batch No: P118601) subcutaneously (S.C.) respectively. NS, F and EI groups received normal saline, formaldehyde 0.1% (vol:vol in DDW) and EI in the same volume and method.

Blood samples were collected directly from heart in randomly selected 5 birds of each group at the day 0 (vaccination day) and end of each week (until 6 week) post inoculation. Serum was separated, heat inactivated and stored at -20 °C for future use.

Serology. Serum titers to NDV were determined by HI tests. Two-fold dilutions of test sera in PBS were mixed with the same volume of antigen in U-bottom 96-well plates. Then the mixture was incubated at 22 °C for 30 min. 50 µl of 0.5 % chicken RBC in PBS was then added. The end point was determined as HI titer.

Statistical analyses. Data were examined using a commercially available statistical package (SPSS version 17 for Windows) and comparisons were made using the descriptive statistics and one way ANOVA tests.

RESULTS

The multiple concentrations of BEI were tested for their abilities to inactivate NDV at various temperatures using a different incubation period. Complete inactivation of BEI was found in 30 °C, 4 mM BEI and 21 hrs treatments. Mean of HI titers in the study groups are shown in Figure 1. No significant differences were seen for HI titer, between NDVR and NDVF groups. The mean of HI titer in the NDVEI group was higher than that of the NDVR and NDVF groups during all study weeks. Compared to the normal saline group, the titer was significantly higher in all other treatment groups. HI Log 2 total mean titer of NDVEI group (8.42 ± 0.12) were significantly higher than NDVF (7.64 ± 0.16) and NDVR (7.86 ± 0.11) groups ($p<0.05$) (Figure 2). None of the sham (F and EI) or control (NS) groups had detectible HI antibody to NDV during the study days.

DISCUSSION

For many years most of the viral vaccines with inactivated antigen were prepared with formaldehyde as inactivating agent (Bahnemann 1990). For example, formaldehyde was used to inactivate foot and mouth disease vaccine virus for many years but suffers from the disadvantage that the kinetics of the inactivation process is not first-order (Barteling 2002). Similar results were obtained for the formaldehyde inactivating of various viruses by Kai & Chi (2008), Mudasser *et al* (2006) and Jagt *et al* (2010). For this reason, formaldehyde was replaced by aziridin compounds, in particular BEI. BEI is superior in safety and antigenicity to other commonly used viral chemical inactivates (Rueda *et al* 2001).

In this study, NDV was completely inactivated by 4 mM BEI when the allantoic fluid was incubated for 21 hrs. In the similar study, 10 mM of BEI was used for best inactivation of NDV (King 1991). Multiple studies indicated the BEI is a good inactivating agent even in the lower concentrations (Mondal *et al* 2005, Kamaraj *et al* 2008). In our study, the reaction of aziridines will be more effective when incubated at 30ºC comparing to the reaction at lower temperatures. The chemical agents will faster insert into viral particles when the temperature is rising (Burrage *et al* 2000). Mudasser *et al* (2006) studied the inactivation activity of BEI on infectious bursal disease virus and immunogenic properties and stability of the prepared vaccine. They concluded that vaccine inactivated with BEI was highly immunogenic and stable.In other study, Formalin and BPL inactivated NDV and AVI vaccines show lower HI titers in contrast of BEI (king 1991). According to results of this study, differences between total mean HI Log 2 titer of NDVEI group were significantly higher than other groups ($p<0.05$) (Figure 2). HI Log 2 total mean titer in NDVEI group was 8.2 ± 0.7, whereas in

the NDVF and NDVR groups were 7.7 ± 0.6 and 7.9 ± 0.7 respectively. Jeon *et al* (2008) assessed the ability of commercial inactivated vaccines derived from La-Sota or Ulster 2C NDV strains to protect chickens from challenge with Kr-005/00 (Korean epizootic genotype VII strain).

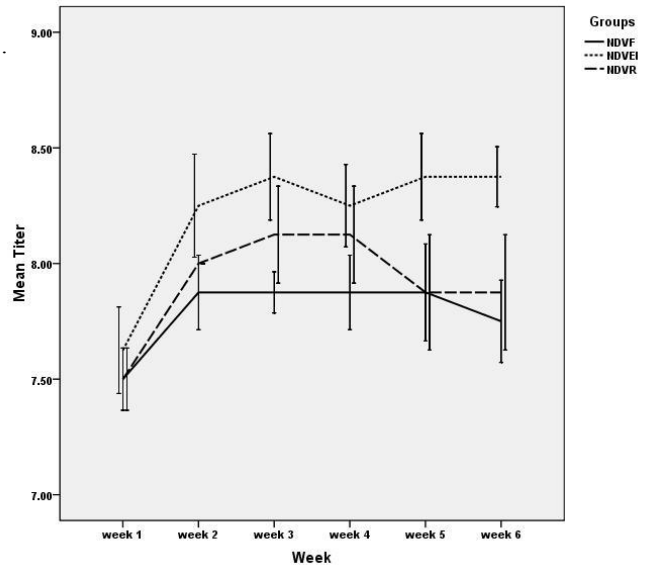

Figure 1. Mean HI log2 titers in the serum of chickens vaccinated with various vaccines during post vaccination days.

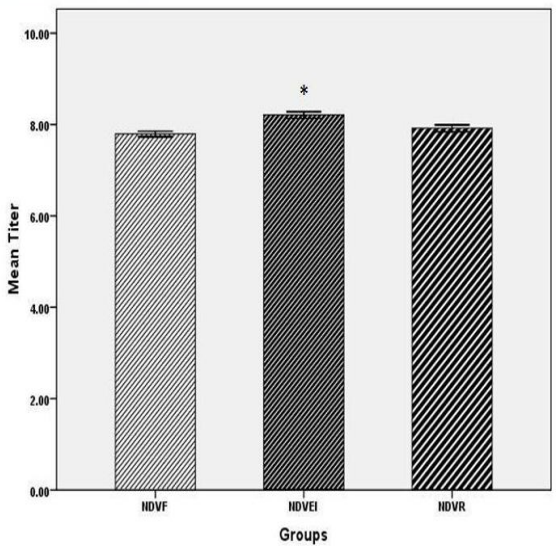

Figure 2. Total means of HI log2 titers in the study groups. * ($p<0.05$).

Average log 2 HI titers of the La Sota-vaccinated birds were 4.3 to 5.2 and Ulster-vaccinated birds were 6.6 to 7.5. In the Mudasser *et al* (2006) study, infectious bursal disease virus was inactivated with formaldehyde and BEI; BEI-inactivated vaccine gave relatively higher antibody titers and was almost twice as efficient as formaldehyde-inactivated one. Formalin and high concentration of BPL had variable effect on serum HI titers. In contrast BEI-inactivated material had no adverse effect on the virus antigens or titers (King 1991).

Although most of the viral vaccines with inactivated antigen were prepared using formalin, in recent years an aziridine compound, BEI was recommended as a superior inactivation agent to formalin. Since BEI does not react with proteins, the vaccines of many RNA viruses and DNA viruses prepared with BEI were reported to be antigenicically superior to the vaccines inactivated by formalin (Kai & Chi 2008). Generally BEI-inactivated vaccines gave higher antibody titers than formaldehyde-inactivated vaccines. This inactivator agent is inexpensive, easy to prepare and less hazardous to handle and protocol for BEI inactivation is simple and preserves both structural integrity and antigenicity of the virus. Thus, it might be possible to use these compounds as an inactivator for commercial NDV inactivated vaccines in the future.

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