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Original Article

Immune Response to Inactivated Newcastle Disease Virus by Electrolysed Catholyte Anolyte and Binary Ethylenimine in Specific Pathogen Free Chickens

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ABSTRACT

This study aimed to investigate the immunogenicity of Newcastle disease virus (NDV) in specific-pathogen-free (SPF) chickens. The NDV was inactivated using either Binary ethyleneimine (BEI) or Electrolysed water-Catholyte-Anolyte (ECA). Complete inactivation of NDV occurred after 24 hours with either BEI or ECA. Prepared inactivated NDV vaccines 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 BEI inactivated NDV (NDVBEI) and ECA (NDVECA) subcutaneously. No significant ELISA total mean titer between NDVBEI group (11303 ± 4515) and NDVECA (12131 ± 1932) ($p<0.05$) at two week post inoculation. BEI and ECA inactivated vaccine gave higher antibody titers 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, Water-Catholyte-Anolyte

INTRODUCTION

Newcastle disease virus (NDV) is one of the most infectious diseases of birds, in particular chickens (Fenher et al, 1987). NDV is a member of the avian paramyxovirus type 1 viruses belonging to the genus of the Paramyxoviridae family Avulavirus (Jeon et al 2008, Knipe & Howley 2001). The epidemic nature of the disease has caused serious economic losses in the poultry industry worldwide since the 1920s (Mcferran & McNulty 1993). Both live attenuated and inactivated vaccines are routinely used to protect chickens against ND during the lifetime of bird (Koppad et al, 2010). The inactivated ND vaccines currently on the market contain viral antigen or inactivated by formaldehyde or β-propiolactone (BPL) treatment (Nathanson 2001).

Binary ethyleneimine (BEI) an aziridine product was used for the inactivation of adventitious viruses in biologicals (Berhane et al 2006, Lubroth et al 2007, Pyke et al 2004). BEI reacts with viral nucleic acids with retention of conformation and the accessibility of epitopes to a greater extent than formalin and BPL (Bahn Mann 1990). BEI has been used to successfully inactivate different viruses for vaccine production including infectious bursitis virus (Mudasser et al, 2006) Rabies virus (Larghi & Nebel 1980) foot and mouth disease virus (Aarthi et al, 2004) arbovirus (Pyke et al, 2004). Moreover, electrolyzed water Catholyte-Anolyte (ECA) is an activated anolyte solution with a highly oxidized as a fast-acting antimicrobial agent that viruses and other micro-organisms destroyed. Studies have suggested that the hypochlorous acid in the course of the production can microbial cell membranes penetrate, and in turn anti-microbial activity to be exercised by the oxidation of the most important metabolic systems (Barrette et al, 1989). In the current study NDV was inactivated with the BEI and ECA. The strength of the two vaccines prepared and commercial vaccine were determined by the ELISA test, in certain pathogen free (SPF) chicks.

MATERIALS AND METHODS

Virus preparations

10-day-old SPF embryonated chicken eggs were inoculated with NDV. The eggs were checked for 24-72 hours after injection. Allantoic fluid of the inoculated eggs was harvested, centrifuged 1200 rpm, 30 min, and the supernatant was collected. Egg dose 50 (EID 50) of the virus was calculated by the method of Reed and Muench (1938) into fertilized eggs in 9-day-old embryos. The calculated EID50 was 9.5.HA assay in U-bottom was 96-well plates with 1% chicken red blood cells, as described Burleson et al (1992).

Binary ethylenmine (BEI) Treatment

Bromoethylamine (BEA) (Sigma, USA) was converted to BEI by adding 2.05g BEA to 100 mL 0.175N NaOH (0.7g/100 mL deionized water) warmed to 37°C for one hour. The BEI preparation (2%) was added to the virus suspensions with the virus titer of $EID_{50}=10^{9.5}$. A control group without addition of BEI was included. The residual BEI was hydrolysed in samples by the addition of 1 mol/L sterile Na

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thiosulfate (Merck) solution at 10% of the volume of the BEI used.

Electrolysed water-Catholyte-Anolyte (ECA) Treatment

ECA solution, an anolyte with pH 2.2 was used to inactivate the virus by adding 0.5 mL of viral suspensions at $EID_{50}=10^{9.5}$ to 4.5 mL of the anolyte solution to make 1/10 dilution. A control group without addition of ECA was included.

Determination of Time Required to Inactivate Virus

Samples from BEI and ECA as the treated viruses and the control group were incubated for different times namely as 6, 12, 24, 30 and 36 hours at 37.5°C to determine the inactivation time of the virus. After incubation period, the treated virus was inoculated in to 5, 10-day-old SPF embryonated chicken eggs. After inoculation, all eggs were sealed with melted wax and were re-incubated at 37.5°C.

Perparation of Killed- Virus Oil Emulsion

One volum of killed virus suspecsion treated with BEI and ECA was mixed with an equal volume of *Freund's incomplete adjuvant* (Sigma, USA) by using a Waring blender at the highest speed 20000 rpm for 15 minutes. An equal volume of 2% Tween-80 was added to the mixture and the emulsion was mixed again for 15 minutes giving a final 1:3 dilution of virus suspension

Experimental Design

A total of 30, 42-day-old SPF chickens, were divided three groups namely the B, E and C and with 10 birds in each group. B group (BEI), E group (ECA) and C (control negative). The inoculum in the groups B and E were mixed with Freund's incomplete adjuvant, and injected at 42-day-old chickens subcutaneously (0.2mL/dose).

Statistical analysis

Data was analysed statistically using analysis of variance (two way ANOVA) followed by Duncan's multiple range test was used as the post hoc produced by using SPSS version 15 for windows (Norusis, 2004)

RESULTS

Clinical Signs

No clinical signs and mortality were observed in any chickens in the groups C, B and E throughout the experiment.

Gross Lesions

The chickens in the groups C, B and E did not shown any gross lesions of IBD after two weeks post inoculation.

Antibody Titer (ELISA)

There was no significant difference ($p>0.05$) between the mean antibody titer at two weeks post inoculation in the two types of inactivate NDV (BEI and ECA): 11303 ± 4515 (NDVBEI) and 12131 ± 1932 (NDVECA) (Table 1).

Table 1: Antibody titers to NDV determined by ELISA in the inactivated NDV by BEI and ECA after two weeks of post inoculated

Antibody titer (Mea SD)	
Time (Weeks)	
Group	2 weeks pi
NDVBEI	11303 ± 4515 a
NDVECA	12131 ± 1932 a

[ab] Values with different subscripts within rows differ significantly at ($p<0.05$)

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 and ECA. BEI is superior in safety and antigenicity to other commonly used viral chemical inactivates (Rueda et al 2001).

In this investigation, NDV was successfully inactivated by BEI and ECA 24 hours after the treatment. 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 37.5ºC 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).

On the basis of humoral immune response, the results showed that the inactivated NDV in all the treatment groups (BEI and ECA) were immunogenic with increased in antibody titers in all inoculated groups 2 weeks pi. It is evident that groups NDBEI and NDECA had no significant titres ($p>0.05$).

Although most of the viral vaccines with inactivated antigen were prepared using formalin, but 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).

It was conclude that BEI and ECA inactivated vaccines gave higher antibody against NDV. These inactivator agents are inexpensive, easy to prepare, less hazardous to handle and preserves both structural integrity and antigenicity of the NDV. Thus, it might be possible to use these compounds as an inactivator for commercial NDV inactivated vaccines in the future.

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