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Original Article

Partial Quality Control of Inactivated Split Human Influenza Vaccine 2008-9

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Abstract

Background and Aims: Influenza vaccination is one of the best way to prevent and control influenza worldwide. It is manufactured by WHO-licensed companies based on the WHO expertise committee annually. The aim of this study was partial quality control of the commercial human influenza vaccine 2008-9 and its matching with the circulating strains.

Materials and Methods: The trivalent imported vaccine was cultured in bacterial and fungal media, injected to the mice and inoculated into the allantoic cavity of Embryonated Chicken Eggs (ECEs). Hemagglutination-Inhibition (HI) assay was carried out on pre and post vaccination serum samples. The bacterial endotoxin was assessed by LAL assay. The Hemagglutinin (HA) content of the vaccine was measured using SRID. Heterogenecity of the circulating influenza strains during 2008-9 seasons in Tehran in comparison to the vaccine strains was determined.

Results: No bacterial contamination and no occurrence of mortality and morbidity in animal was observed. The mean fold increase of HI antibody titer in subjects without previous vaccination for H1N1, H3N2 and B strains were 6.7, 3.3 and 1.8 respectively, while in subjects with previous vaccination were 4, 1.6 and 1.1 for same strains. Amino acid variation was found in Tehran H1N1 isolates but the H3N2 isolates showed higher genetic resemblance to the 2008-9 vaccine strain.

Conclusion: The sterility, safety, and efficacy of the vaccine were approved and there was some variation in A/H1N1 but not in A/H3N2 isolates in comparison with the vaccine strain.

Keywords: Human influenza vaccine; Phylogenetic Analysis; Hemagglutination-Inhibition (HI)

Introduction

Influenza virus is a globally important respiratory pathogen causing a high degree of morbidity and mortality annually. Influenza viruses are divided into three types A, B and C on the basis of antigenic differences of internal proteins, nucleoprotein (NP) and matrix protein (M).Type A viruses are further classified based on two external glycoproteins hemagglutinin (HA) and neuraminidase (NA) (1). Vaccination before the start of the influenza season is the best way to prevent and control the disease worldwide (2). The vaccine is trivalent based on the predicted circulating strains, two subtypes of

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influenza A (H1N1 and H3N2) and an influenza B virus recommended by the World Health Organization (WHO). Two kinds of human influenza vaccine, inactivated and live attenuated, are commercialized. Inactivated vaccines are produced by propagation of strains of influenza virus in embryonated eggs or in mammalian cells (1). There are three predominant types of inactivated influenza vaccines: whole virus, Split and subunit formulation which are administered intramuscularly or subcutaneously. The split vaccines are widely used for seasonal vaccination (3). The aim of this study was partial quality control of the inactivated split human influenza vaccine 2008-9.

Methods

Efficacy of vaccine:

Efficacy of the vaccine was evaluated by analyzing antibody induced by the vaccine in animal models as well as in vaccinated people (4).

Human samples: Blood samples were collected from 11 individuals before and 3 weeks after vaccination. Hemagglutination Inhibition (HI) assay was performed on sera according to WHO protocol (5). Two parameters were investigated to evaluate humoral immune response against influenza vaccine: the geometric mean titre (GMT) of HI antibody and the mean-fold increase in antibody titres (MFI) (6). Subjects were grouped based on previous year vaccination.

Animal model: 6-8 week-old female Balb/c mice (Animal Facility, Pasteur Institute of Iran) were immunized intramuscularly twice at a 3-weeks interval with 0.1 ml of the human influenza vaccine. Control mice received 0.1 ml PBS. Blood samples were collected from 10 mice in each group at days 10 and 21 after the first dose and day 10 after the second dose of the vaccine. The sera were stored at -20° C to be used for HI assay. The amount of antibodies against influenza hemagglutinin was measured in sera according to the WHO protocol with a small modification. To eliminate non-specific reactions of the mice sera, heat-inactivated serum was adsorbed to 3 volumes 25% of kaolin/PBS solution and incubated at room temperature for 20 min. After centrifugation (6500×g for 2 min), the supernatant was used for assays (7,8). A twofold serial dilution of kaolin-treated sera was made in 96-well U bottom plate. Subsequently, 50 µl of 4 hemagglutinin unit (HAU) of viral suspension was added to each well followed by incubation at room temperature for 40 min. Finally, 1% chicken red blood cell (RBC) suspension (50 µl) was added and HI titres were determined after 1 h incubation at room temperature. HI antibody titres were expressed as the reciprocal of the highest serum dilution producing complete inhibition of hemagglutination.

Heterogenecity of circulating influenza strains during 2008-9 season in comparison to the vaccine strains:

Nasopharyngeal swabs (n=142) were collected from patients with influenza and influenza-like illness referred to three medical centers in Tehran. Typing and subtyping of the isolates were performed using multiplex RT-PCR and phylogenetic analysis was carried out for hemagglutinin genes of the influenza virus (9).

Safety of the vaccine:

To evaluate safety of the influenza vaccine, 1 dose of it (0.5 ml) was injected intraperiotenally to each of 5 healthy mice and 2 healthy guinea-pigs followed by 7 days observation.

Residual infectious virus in the inactivated influenza vaccine: 0.2 ml of the vaccine was inoculated into the allantoic cavity of each of 10 embryonated chicken eggs (ECES) and were incubated at 35˚C for 3 days. 0.5 ml of the allantoic fluid from each surviving embryo was harvested and pooled. 0.2 ml of the pooled fluid was inoculated into a further 10 fertilised eggs and were incubated at 35˚C for 3 days. Finally 0.1 ml of the allantoic fluid from each surviving embryo was harvested and examined individually for the virus titration by Heamagglutination assay.

Sterility of the vaccine: FTM (Fluid Thioglycollate Medium) and SCDM (Soybean Casein Digest Medium), two standard and commercial media,were used to determine anaerobic and aerobic bacteria and fungi contaminationof the final products. Since the

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Table 1. HI antibody response according to viral strain.

Vaccine strain	HI titers, GMT		MFI (Mean Fold Increase)
	Pre Vac	Post Vac	
Subjects with previous vaccination (7/11)			
H1N1	262.50	95	4
H3N2	195.04	35	1.6
B	97.52	10	1.1
Subjects without previous vaccination (4/11)			
H1N1	113.13		6.7
H3N2	134.54	45	3.3
B	67.27	11	1.8

Pre Vac: prevaccination; Post Vac: postvaccination

influenza vaccine is propagated in embryonated chicken eggs, RAPPAPORT medium is necessary to confirm the absence of Salmonella in the vaccine. Thus, to evaluate the sterility, the vaccine was inoculated in FTM, SCDM and RAPPAPORT medium incubating at 37°C (to detect bacteria) and 25°C (to detect fungi) for 14 days (10, 11).

Hemagglutinin content of the vaccine: The content of hemagglutinin antigen of H3N2 subtype in the vaccine was determined by single radial immunodiffusion (SRID) assay (12). Sheep anti A/Brisbane/10/2007/H3N2 antibody and standard A/Brisbane/10/2007/ H3N2 antigen were used as reference antibody and antigen, respectively. Samples were diluted and loaded into wells of 1% agarose gel containing anti-HA sheep reference antibody and allowed to diffuse for 18-30 hour in moist chamber. Then, the agarose gel was incubated in PBS for 30 min to remove un-bound proteins, dried and stained with Coomassie blue. The antigen-antibody precipitation ring's diameter was measured (13,14).

Bacterial Endotoxin Assay: The vaccine was assayed for endotoxine content using a limulus amebocyte lysate (LAL) assay (Lonza) kit according to the manufacturer's instructions. The vaccine was diluted using the negative control solution as diluent. The vaccine dilutions were added into the test vials containing lysate. After mixing and 1 hour of incubation at 37°C in a water bath, the tubes were gently inverted. Formation of a firm gel was considered a positive result. A weak gel which could break was scored ±, whereas watery fluid result was designated as negative (15).

Results

Efficacy

Human samples: In subjects without previous vaccination (36.36%) MFI was calculated 6.7, 3.3 and 1.8 for H1N1, H3N2 and B subtypes, respectively while the subjects with previous vaccination who had high pre-immunisation antibody levels, the MFI was 4, 1.6 and 1.1, respectively (Table1). These data shows

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Table 1. Serum HI antibody titers against influenza vaccine strains in BALB/c mice.

Virus Strain	H1N1			H3N2			B		
	post- priming		post-boosting	post- priming		post-boosting	post- priming		post-boosting
Group	10 days	20 days	10 days	10 days	20 days	10 days	10 days	20 days	10 days
Control	<40	<40	<40	<40	<40	<40	<40	<40	<40
Vaccine	254	278.57	359.18	380.54	640	905	201.58	242.51	403.17

higher antibody response against A/ H1N1 subtype than A/ H3N2 and B.

Animal Model: HI antibodies against all three strains of the virus were detected at day 10 and increased up to day 20 after the first dose of vaccine. The second immunisation significantly boosted the HI antibody response. HI titres were presented as GMT of 10 mice per sampling point. Control mice received only PBS, exhibited HI titre<40 (Table 2).

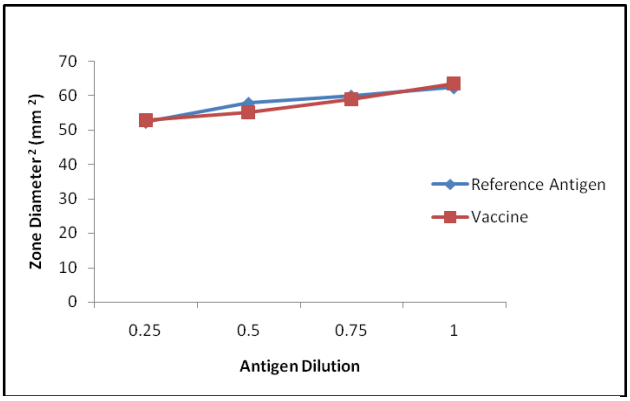

Fig. 1. SRID assay of A/Brisbane/10/2007/H3N2 antigen in inactivated split human influenza vaccine 2008-9. Detergent-disrupted and diluted reference antigen and vaccine were added to wells of 1% agarose gel containing anti-HA sheep reference antibody. SRID responses and the resulting dose response graph are shown.

Molecular evaluation: Fifty out of 142 samples were positive for influenza A virus, and no influenza B virus was detected. Out of those 50 positive samples, 15 H1N1 and only 2 H3N2 were detected. Phylogenetic analyses revealed that the A/H1N1 isolates were related closely to A/Brisbane/59/2007, and the A/H3N2 isolates were close to A/Brisbane/10/2007 vaccine strains.

Sterility of the vaccine: No growth of microorganisms (bacteria or fungi) was observed following culture of the vaccine in FTM Medium and SCDM as well as RAPPAPORT medium. Bacterial endotoxin test indicated that the vaccine was endotoxin free.

Safety of the vaccine: Evaluation of safety in animal model revealed no occurrence of mortality, signs of illness or weight loss in any of the examined animals. Also surviving embryos in the inoculated ECEs and negative

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HA results, revealed lack of infectious virus in the vaccine.

Hemagglutinin content of the vaccine:

The HA content of A/Brisbane/10/2007/H3N2 antigen in the vaccine was measured by comparing the dose-response slope of the graph showing diffusion zones (Fig. 1). Both vaccine and reference antigens gave clearly defined diffusion zones, with the size of the zones (diameter^2) reflecting HA concentration.

Discussion

Vaccination has usually direct effect on induction of protective immunity in vaccinated people leading to social protection by reduction of the disease transmission among individuals. The vaccine efficiency to induce protection is assessed in different ways including detection of antibodies and increasing the GMT after receiving the vaccine. Induction of antibody against the circulating influenza strains is considered as a good sign of the vaccine efficiency (16).

Previous studies have shown that preferential induction of antibodies against one of the three virus strains may occur in the elderly (17, 18). In this study more significant increase in immune response was observed against H1N1strain compared with H3N2 and B strains. The greater antibody response to the H1N1 strain may be a result of repeated natural exposure to the similar viruses previously.

Measurement of the antibody produced by vaccination in animal model is another way to evaluate the effectiveness of influenza vaccine (19). lncreased titers of antibodies against all three vaccine strains in the vaccinated BALB/c mice show the effect of the vaccine on induction of the immune response.

According to recent surveys presence of endotoxin in influenza vaccines is a serious factor to increase the production of antibody against unrelated antigens. It is known that ability of endotoxin to increase the permeability of the blood-brain barrier, may allow proteins having destructive neurogenic effects to enter into the nervous system. These factors effect on autoimmune conditions induced by vaccination and emergence of Guillain-Barre syndrome (20, 15). Negative result of endotoxin test confirmed the absence of endotoxin in the evaluated vaccine.

Safety is an important issue for a vaccine. Live attenuated vaccines were considered as a threat for vaccinated people since they are not killed or incomplete detoxification may cause some problems (21). In order to confirm the complete inactivation of the viruses, the vaccine was inoculated into Embryonated Chicken Eggs. Our results indicated the absence of residual infectious virus in the vaccine. In addition, safety test on animal model and lack of clinical symptoms confirmed harmless of the vaccine.

SRID is a technique for measurement of soluble antigens by precipitation in semi-solid medium containing specific antibodies. SRID is more suitable to measure hemagglutinin contents of split and subunit vaccines since it does not depend on the physical status of the antigen whether it is soluble or is assembled as a whole vaccine (22, 23). A further advantage of the SRID technique is that no cross-reaction between HA antigen of different influenza types or subtypes has been observed in this test (24, 23). In this project, antigen concentration of H3N2 strain in the vaccine was evaluated using standard antigen and antibody associated with the same strain taken from NIBSC and by comparing the dose-response slope of the vaccine with the reference antigen.

Analysis of the circulating influenza virus and identification of antigenic variation has an important role to determine the vaccine strains annually. Each year, WHO recommends the most suitable composition of the influenza vaccine strains for the next season based on information gathered from reference laboratories around the world (25). Since the HA1 subunit of hemagglutinin protein is an important factor in virus binding to epithelial cells and as target antigen for antibodies, evaluation of antigenic drift in this section is necessary (26). It was demonstrated that the A/H1N1 was predominant subtype of human influenza virus among the studied patients in Tehran during 2008-9 winter season. In addition, some amino acid variation was found in Tehran/2008/H1N1 isolates from the 2008-9

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vaccine strain but the H3N2 isolates showed higher genetic resemblance to the vaccine strain (9).

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