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The long-term immunogenicity of an inactivated split-virion 2009 pandemic influenza A H1N1 vaccine: Randomized, observer-masked, single-center clinical study☆

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ABSTRACT

The aim of this study is to investigate the long-term immunogenicity of inactivated split-virion 2009 pandemic influenza A H1N1 vaccine after a single immunization. We recruited 480 adults, aged 18–60 years, for a placebo-controlled, observer-masked, single-center clinical study. We randomly assigned subjects into four groups: 15 μg, 30 μg and 45 μg of hemagglutinin (HA) dosage groups, and a placebo control group. Finally, 259 subjects completed the entire study. The rates of seroconversion and seroprotection and the geometric mean increase (GMI) fulfilled the criteria of the European Medicines Agency (EMEA) for influenza vaccine for 180 days after vaccination in all three dosage groups. However, the seroprotection rates of all dosage groups were below 70% at day 360 post vaccination, while the seroconversion rates and the GMI continued to meet the licensure criteria at this time point. In conclusion, a single dose of 15 μg HA vaccine could induce a protective immune response persisting for at least six months in adults. This study could be beneficial for the future development of influenza vaccines conferring long-term immunity.

1. Introduction

Influenza is a serious public health problem, causing severe illness and death in humans. In April 2009, a new swine-origin influenza A (H1N1) virus emerged in Mexico and the United States, and then spread rapidly to more than 200 countries and regions, causing human infections and tens of thousands of deaths throughout the world [1,2]. This novel H1N1 virus is responsible for the first influenza pandemic of the 21st century [3].

Vaccines are considered to be one of the most effective tools, not only to prevent the spread of influenza virus but also to mitigate the severity of illness and the impact of the disease [4]. The risk presented by the pandemic influenza A (H1N1) virus prompted a new monovalent vaccine to be actively developed and clinically assessed by several vaccine manufacturers throughout the world, and mass immunization programs have been implemented by many countries. A variety of vaccines are being thoroughly evaluated for their safety and immunogenicity in humans, including inactivated whole virus vaccines, split vaccines with or without adjuvant and live attenuated vaccines [5,6]. The results of clinical trials showed that these vaccines had good levels of safety and that single-dose vaccination could induce strong immune responses in healthy people [7–11]. Furthermore, the indicators all met the EU criteria for assessing seasonal influenza vaccines [12]. It has now been reported by many studies that 2009 pandemic influenza A H1N1 vaccine can provide effective protection in humans [7–11]. Clinical trials were completed in China in August 2009. In these clinical trials, 15 μg of hemagglutinin antigen as a two-dose regimen was administered to vaccine subjects of different age groups and the results showed that the vaccine was safe and effective [13]. Despite the fact that the current influenza epidemic has reached a peak in many areas and that the incidence rate is now declining, the influenza A H1N1 (2009) virus continues to cause a threat and remains the predominant cause of seasonal influenza virus infection [14]. The WHO has added the 2009 pandemic influenza A H1N1 virus to the recommended composition of influenza virus vaccines for use as a seasonal influenza vaccine candidate [15]. Although the WHO has announced the end of the pandemic of influenza A H1N1 (2009) virus, we cannot rule out the possibility of local epidemics of this virus. The WHO has also advised the continued administration of the influenza A H1N1 vaccine. It is important to study the long-term immunogenicity of the 2009 pandemic influenza A H1N1 vaccine and to determine the potential need for re-vaccination during extended epidemics.

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The primary aim of this study was to investigate the immune responses and the persistence of immunogenicity induced by a single dose of the 2009 pandemic influenza A H1N1 monovalent split-virion vaccine among adults aged 18–60 years. We also compared the effects of dosage on the long-term immunogenicity and efficacy of the split-virion H1N1 vaccine, with the aim of determining the optimum dosage and regimen for the vaccine for long-term immunization.

2. Methods

2.1. Study design

From July 2009 to July 2010, we carried out randomized, double-blind, single-center clinical trial in Hengdong County of Hunan Province (China) on 480 subjects. The Center for Disease Control and Prevention (CDC) in Hunan Province was responsible for the clinical trial and the CDC in Hengdong County participated in the clinical trial. The study was sponsored by the Shanghai Institute of Biological Products (China). The CDC in Hunan Province and Hengdong County were mainly responsible for data collection during the clinical trial. The Central South University (Changsha, Hunan, China) was responsible for data analysis and statistical processing. All of the pilot programs, clinical manuals and other materials used in this study were consistent with the Declaration of Helsinki and the quality control requirements for clinical trials, and were approved by the Ethics Committee of Hunan Province.

All 480 participants received a single dose injection of the vaccine or a placebo. The immunologic end points were determined by detecting the hemagglutination-inhibition (HI) antibody positive rates on day 28, day 90, day 180 and day 360. After serum samples were collected on day 180, the subjects in control group were received a supplementary injection of vaccine and the serum samples were not collected on day 360. The subjects were monitored and their systemic and local reactions were recorded after vaccination.

2.2. Vaccines

The inactivated split-virion vaccine against the H1N1 (2009) virus was developed by the Shanghai Institute of Biological Products, and the seed virus was prepared from the reassortant vaccine virus A/California/7/2009 NYMC X-179A, as recommended by the WHO [16,17]. The vaccine was prepared in embryonated chicken eggs according to the standard techniques used in the production of seasonal influenza vaccine. In brief, the virus was amplified in chicken embryos, then harvested and inactivated with formaldehyde. The viral cultures were then concentrated and purified for use as the final vaccine. The vaccine was approved for clinical use by the Chinese National Institute for the Control of Pharmaceutical and Biological Products (China).

The experimental vaccines were split-virus products containing 15 μg, 30 μg or 45 μg of hemagglutinin antigen per dose (0.5 ml). The placebo consisted of phosphate-buffered saline (PBS).

2.3. Participants

All subjects participated voluntarily in the clinical trials and their written informed consents were obtained once they fully understood the study procedures. All subjects were 18–60 years old. The main exclusion criteria included: a history of infection with the 2009 pandemic influenza A H1N1 virus; receipt of any influenza vaccine within six months; inoculated with any other prevention products in the last week; a history of allergy or contraindications of vaccination.

Injections were given intramuscularly in the deltoid muscle. After an on-site safety observation within 30 min of the injection, subjects were asked to record data on systemic and local adverse reactions at 6, 24, 48 and 72 h and on day 7, day 14 and day 21. Serum samples were collected on day 28, day 90, day 180 and day 360 after vaccination.

2.4. Randomization and masking

We recruited 480 subjects, aged from 18 to 60. Subjects were randomly divided into four treatment groups in a 1:1:1:1 ratio which were administered 15 μg, 30 μg or 45 μg of hemagglutinin or placebo, respectively. Each treatment group comprised 120 subjects with a male to female ratio of 1:1. The blind testing was designed by a third party at Central South University, who was not involved in other elements of the clinical trials.

2.5. Assays

The antibody titers against the vaccine strain were determined by HI assays of the anti-homologous strain of X-179A, performed in accordance with established measures using turkey erythrocytes [18,19]. In brief, sera were firstly treated with receptor destroy enzyme at 36°C for 16 h. The titers of HI antibody that were below the detection limit (i.e., <1:10) were recorded as a value of 1:5, and titers above 1:10,240 were recorded as a value of 1:10,240. The seroconversion rate represented a post-vaccination titer ≥1:40 in subjects with a pre-vaccination titer of <1:10 or a ≥4-fold titer increase in subjects with a pre-vaccination titer of ≥1:10. All serum samples were assayed in a blinded manner, in duplicate, and were checked in parallel by the Chinese National Institute for the Control of Pharmaceutical and Biological Products.

2.6. Statistical analysis

For safety assessments, the frequency, severity, mean time of appearance and duration of all the local and systemic adverse events were calculated in all groups in accordance with the requirements for influenza vaccines published by the Division of Microbiology and Infectious Diseases of the US National Institutes of Health [20,21].

For immunogenicity assessments, the seroconversion rate represented either a post-vaccination titer ≥1:40 (in accordance with the requirements for seasonal influenza vaccines by the European Committee for Proprietary Medicinal Products) in subjects with a pre-vaccination titer of <1:10 or a ≥4-fold titer increase in subjects with a pre-vaccination titer of ≥1:10. The seroprotection rate represented the proportion of subjects with a post-vaccination titer ≥1:40. A seroprotection rate >70% was considered to provide protection. In addition, the geometric mean increase (GMI) was the ratio of the titer after vaccination to the titer before vaccination. All the serum data analyzed in this research were from the subjects who received five times blood collections [22].

Hypothesis testing was conducted using two-sided tests, with an alpha value of 0.05 considered to indicate statistical significance. All statistical analyses were performed using the SPSS software package (version 11.5).

3. Results

3.1. Study participants

Recruitment visits were attended by 493 participants (Fig. 1). A total of 480 subjects between 18 and 60 years of age participated in the clinical trial and 480 serum samples were collected initially. Some subjects were gradually withdrawn from the clinical trial, so only 454 serum samples were collected on day 28, 416 serum samples were collected on day 90, 377 serum samples were collected on day 180. In addition, we only collected 259 serum samples in vaccine groups on day 360, because the subjects in control group were received a supplementary injection of vaccine on day 180 and the serum samples were not collected on day 360. In all vaccine groups, 259 subjects completed the entire study and provided five serum samples.