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Original Article

Immunogenicity and Safety of a China-Made Monovalent Pandemic (H1N1) 2009 Influenza A Vaccine in Healthcare Workers in Guangzhou, China

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SUMMARY: Because healthcare workers played an important role in the battle against novel pandemic (H1N1) 2009 influenza, a clinical study was conducted to examine the immunogenicity and safety of a single dose of a China-made monovalent, split-virus, pandemic (H1N1) 2009 influenza vaccine in this special high-risk population. Healthcare workers in the First Affiliated Hospital of Guangzhou Medical College who received the pandemic (H1N1) 2009 influenza vaccine were prospectively enrolled. Antibody titers were measured at enrollment and 14 days later using hemagglutination-inhibition (HI) and microneutralization assays. Adverse events were recorded within 7 days and 6 months after vaccination. Double sera were provided by 51 of 65 enrolled subjects. Postvaccination titers of 1:40 or more on HI assay were observed in 96% of recipients. Seroconversion or a significant increase in titer on HI assay occurred in 59% of subjects. The factor increase in GMTs was 4.3. The majority of complaints were mild to moderate in intensity. Although more than half of healthcare workers seemed immune to the pandemic (H1N1) influenza A virus before vaccination, most of the subjects still showed a fast, robust immune response to a single 15-μg dose of a monovalent, split-virus unadjuvanted pandemic H1N1 2009 influenza vaccine.

INTRODUCTION

In April 2009, the Centers for Disease Control and Prevention (CDC) in the United States (1) and the General Directorate of Epidemiology (GDE) in Mexico (2) identified several human cases of infection with pandemic (H1N1) 2009 influenza A virus (pandemic H1N1, also called novel H1N1, swine-origin influenza virus, or swine flu).

The first cases of pandemic H1N1 infection in China and Guangzhou were documented on May 10 and May 20, 2009, respectively. Pandemic H1N1 infection became prevalent beginning in September 2009 in Guangzhou (3), where influenza was uncommon during autumn and winter (4). It was deemed an unseasonal influenza virus in Guangzhou and worldwide because of its outbreak time in Mexico and the United States (1,2). The virus was subsequently determined to be a descendent of the 1918 pandemic strain (5), and was characterized by a unique triple reassortment of gene segments that had never before been identified in humans, pigs, or birds (6,7). There was concern that little protective immune memory exists in the general human population in China (8). The rapid spread of the virus worldwide in less than 2 months (9) led the WHO to raise the alert level of influenza pandemic from phase 3 to phase 6 (10).

Social intervention, i.e., contaminant policy or closure of schools, has been shown to be effective during the early period of a pandemic (11). However, it becomes useless once the influenza virus is prevalent in the community. An influenza pandemic will not wane until an immune barrier is set up in the majority of the public. Therefore, vaccination was considered to be the most effective weapon in the battle against the influenza pandemic and epidemic. The availability of safe and effective vaccines was critical for the prevention of pandemic H1N1 infection.

Healthcare workers are recommended targets for vaccination during epidemics and pandemics due to their special role in the battle against influenza (12). Vaccination can reduce the probability of nosocomial pandemic H1N1 infection caused by healthcare workers to a large degree (12). Inapparent infection, a critical and potential source of infection, has a high probability occur-
rence in healthcare workers because of cross-immunoreactivity or preexisting partial immunity against pandemic H1N1 influenza virus (13-15). Additionally, healthcare workers work on the frontline of care for pandemic H1N1 infected patients, and pandemic H1N1 infection-related absence from work may result in an interruption of services for pandemic H1N1 patients.

On September 2, 2009, China was the first country in the world to announce the successful development of a pandemic H1N1 influenza vaccine (pandemic vaccine). Later, a pandemic vaccination campaign was conducted for the first time for healthcare workers by the Chinese government. Due to the novelty of this pandemic H1N1 strain, we undertook a clinical study of healthcare workers in the First Affiliated Hospital of Guangzhou Medical College (Guangzhou, China) to further examine the immunogenicity and safety of a single dose of a China-made monovalent, split-virus, pandemic vaccine.

METHODS

Study design: Healthcare workers in the First Affiliated Hospital of Guangzhou Medical College, Guangzhou, China who received the China-made pandemic vaccine during the pandemic vaccination campaign were prospectively enrolled in this study. We excluded subjects with influenza-like illness (ILI) before and within 14 days after vaccination and those who had received an experimental influenza vaccine 6 months prior to enrollment.ILI was defined as a body temperature ≥38°C or feverishness, accompanied by at least one constitutional or respiratory symptom. A baseline blood sample was collected at the time of enrollment and a postvaccination blood sample was taken 14 days later to determine the serologic response to the vaccine. Individuals who did not provide double sera were excluded from immunogenicity analysis, but were further analyzed for safety. Demographic data and history of acute respiratory infection (ARI) since the outbreak of pandemic H1N1 were also recorded at the enrollment. The study was approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical College, and all subjects provided written informed consent.

Vaccine: The pandemic vaccine, a monovalent, unadjuvanted, inactivated, split-virus vaccine, was produced by Tianyuan Bio-Pharmaceutical Company (Zhejiang, China). The vaccine was manufactured by the same methods used for the production of the company’s seasonal trivalent inactivated vaccine. The seed virus was prepared from the reassortant vaccine virus NYMC X-179A (New York Medical College, N.Y., USA), derived from the A/California/7/2009 (H1N1) virus, one of the candidate reassortant vaccine viruses recommended by the WHO (16,17). Pandemic vaccine was prepared in embryonated chicken eggs and was filled into syringes or vials at 0.5 ml for single-use. Each subject received 1 dose of vaccine with 15 μg of hemagglutinin antigen per 0.5 ml dose.

Laboratory assays: Titors of anti-pandemic H1N1 influenza antibody were measured at enrollment and 14 days after vaccination. The immunogenicity of the pandemic H1N1 vaccine was evaluated by using hemagglutination-inhibition (HI) and microneutralization (MN) assays, which have been described elsewhere (18,19).

Primary end points: The three primary immunogenicity end points after vaccination were as follows: the proportion of subjects with antibody titers of 1:40 or more on HI assay, the proportion of subjects with either seroconversion or a significant increase in antibody titer, and the factor increase in the geometric mean titers (GMTs). They were selected according to international guidelines used to evaluate influenza vaccines (20,21).

Safety assessments: We collected common complaints of local (including any local symptoms, redness, induration, pain, pruritus, and ecchymosis) and systemic (including any systemic symptoms, headache, myalgia, fatigue, coryza, sore throat, cough, gastrointestinal symptoms, and fever) adverse events within 7 days after vaccination. Adverse events were rated on a 4-point scale: absent, mild (work and life were not affected), moderate (between mild and severe), or severe (work and life were both affected). Other special rare influenza vaccine-related adverse events, including nervous system disorders, i.e., encephalitis and Guillain-Barré syndrome (GBS), immune-system disorders, and other disorders, were collected within 6 months after vaccination. Any serious adverse events or special adverse events were to be reported to the vaccine administrators within 24 h.

Statistical analyses: Quantitative characteristics were presented as mean or median and interquartile range (IQR). Otherwise, the categorical variables were reported as frequencies and percentages.

RESULTS

Study population: From October 30 to November 18, 2009, 65 subjects (11 males and 54 females) were enrolled, and all were available for safety analysis. The median (IQR) age was 25 (24–33) years (age range, 21–56 years). Fifty-one subjects provided double sera for further laboratory analysis. Ultimately, 51 subjects (9 males and 42 females) were available for immunogenicity analysis and were defined as the immunogenicity population. The median (IQR) age of immunogenicity population was 25 (24–29.5) years. All subjects in the immunogenicity population denied a history of seasonal influenza vaccination and ARI episodes during the 6 months prior to pandemic H1N1 vaccination.

Immunogenicity: At baseline, 31 of 51 (61%) subjects had an antibody titer of 1:40 or more on HI assay, and 24 (47%) subjects had an antibody titer of 1:40 or more on MN assay. An immune response was seen in a majority of subjects (Tables 1, 2 and Fig. 1). Postvaccination titers of 1:40 or more on HI assay were observed in 96% (95% CI, 90.6–101.4%) of vaccine recipients. Seroconversion or a significant increase in titer on HI assay occurred in 59% (95% CI, 45.5–72.5%) of subjects. After vaccination, there was a substantial rise in GMTs on HI assay (Tables 1 and 2). In general, the patterns of antibody responses, as measured by the MN assay, were similar to those measured by the HI assay (Table 1 and Fig. 1).

The baseline serostatus seemed to influence the immune response to H1N1 vaccination. Subjects with a baseline titer of <1:40 (on HI or MN assay) had GMTs with a higher impact factor after vaccination than those with a baseline titer of 1:40 or more (Table 2). In sub-
Table 1. Immune response after pandemic H1N1 vaccination, as measured by hemagglutination-inhibition (HI) and microneutralization (MN) assays

<table>
<thead>
<tr>
<th></th>
<th>Subjects with antibody titer</th>
<th>Subjects with seroconversion or significant increase in titer</th>
<th>Geometric mean titer value (95% CI)</th>
<th>Factor increase in geometric mean titer value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (95% CI)</td>
<td>% (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HI assay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>61 (47.6–74.4)</td>
<td>—</td>
<td>45.8 (31.2–67.3)</td>
<td>—</td>
</tr>
<tr>
<td>After vaccination</td>
<td>96 (90.6–101.4)</td>
<td>59 (45.5–72.5)</td>
<td>196.2 (146.0–263.5)</td>
<td>4.3 (3.0–6.1)</td>
</tr>
<tr>
<td><strong>MN assay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>47 (33.3–60.7)</td>
<td>—</td>
<td>31.3 (20.3–48.4)</td>
<td>—</td>
</tr>
<tr>
<td>After vaccination</td>
<td>88 (79.1–96.9)</td>
<td>39 (25.6–52.4)</td>
<td>180.8 (124.5–262.7)</td>
<td>5.8 (3.6–9.0)</td>
</tr>
</tbody>
</table>

—, data not available.

Table 2. Immune response after pandemic H1N1 vaccination in subjects with different baseline antibody titer, as measured by hemagglutination-inhibition (HI) and microneutralization (MN) assays

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Subjects with seroconversion or significant increase in titer % (95% CI)</th>
<th>Geometric mean titer</th>
<th>Factor increase in geometric mean titer value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>After vaccination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>value (95% CI)</td>
<td>value (95% CI)</td>
</tr>
<tr>
<td><strong>HI assay</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1:40</td>
<td>51</td>
<td>95 (85.4–104.6)</td>
<td>11.1 (8.5–14.4)</td>
</tr>
<tr>
<td>≥1:40</td>
<td>51</td>
<td>35 (18.2–51.8)</td>
<td>114.4 (84.2–155.5)</td>
</tr>
<tr>
<td><strong>MN assay</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1:40</td>
<td>51</td>
<td>27 (40.4–77.6)</td>
<td>8.4 (6.6–10.6)</td>
</tr>
<tr>
<td>≥1:40</td>
<td>51</td>
<td>24 (2.0–32.0)</td>
<td>138.5 (124.0–184.3)</td>
</tr>
</tbody>
</table>

Fig. 1. Reverse cumulative distribution curves of antibody titers in serum before vaccination and 14 days after vaccination on the hemagglutination-inhibition (HI) and microneutralization (MN) assays.

...jects with a baseline titer of <1:40, the proportion who achieved seroconversion was 95% on the HI assay and 59% on the MN assay, this was higher than in subjects with a baseline titer of 1:40 or more (P < 0.001 by HI assay and P = 0.002 by MN assay) (Table 2).

Adverse events: Generally, the pattern and frequency of adverse events after vaccination in the study were mild. No deaths, serious adverse events, or special rare influenza vaccine-related adverse events were reported within 6 months. At least one local adverse event was reported by 15 of 65 (23%) subjects, and at least one systemic adverse event was reported by 11 of 65 (17%) subjects. The most common complaints were pain in injection site for local events and coryza for systemic events (Fig. 2). Although systemic events were less frequent than local adverse events, they were a little more severe in intensity. The majority of adverse events were mild to moderate in intensity. None of subjects reported ILI within 14 days after vaccination.

DISCUSSION

A single 15-μg dose of China-made unadjuvanted 2009 H1N1 vaccine showed a fast, robust immune response in healthcare workers. HI titers of 1:40 or more were seen in 96% of subjects only 14 days after vaccination, which was identical to other published data (22,23). However, approximately 61% of subjects had baseline antibody titers of 1:40 or more on HI assay. It may influence the actual immunogenicity of the pan-
mune reactivity (13-15) or long-term T-cell-mediated immunity (24) against pandemic H1N1 influenza, and may contribute to the high prevalence of antibody titers of 1:40 or more. It is unfortunate that contemporaneous serostatus was not available in the general population.

The pattern and frequency of adverse events after vaccination in this study were mild, and were consistent with those reported for seasonal influenza vaccines in adults (12,25). However, because of the novelty of pandemic H1N1 influenza virus, the full safety profile of pandemic H1N1 vaccine should be clearly defined when mass vaccination is conducted in the general population, especially to assess rare adverse events. A temporal association between immunization and GBS development within 6 weeks was found during the 1977 swine influenza immunization program, during which approximately 430 GBS cases occurred among 41 million vaccines, an estimated 7-fold higher rate than expected (26). Reported associations of influenza immunization with systemic vasculitis, recurrent GBS, adverse ocular effects, and pericarditis remain unproven. In our study, no rare long-term adverse events occurred in the 6 months after vaccination. Nevertheless, only a small number of subjects were enrolled in our study. A much larger scale study is needed to correctly assess the incidence of rare long-term adverse events for novel pandemic influenza vaccination.

In general, the MN assay is more sensitive than the HI assay in detection human antibodies to influenza virus in infected or immunized individuals. However, it was just the opposite in our study, with similar tendencies between HI and MN assays. Similar data were also seen in the study by Greenberg et al. (22). Higher passages (more than 100) of the MDCK cells used maybe responsible for this phenomenon.

There were several limitations of the present study. Although all subjects were observed for 6 months, rare events still may be underestimated due to the low enrollment numbers. In addition, we did not include a placebo control group since it was not a randomized control study, but only an observational study. Inapparent or asymptomatic infection due to cross-immunoreactivity or preexisting partial immunity against pandemic H1N1 influenza virus (14,15) may exist and may account for the high antibody titers at baseline. Furthermore, all subjects were inoculated with only a single dose, without comparing different dose level. Finally, since the studies were conducted on healthcare workers, trials also need to be conducted in general populations that may have different baseline antibody titers and different responses to the vaccine, such as healthy adults in other professions. The elderly, children, and those with impaired immunity also need further study.

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Conflict of interest None to declare.

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