Comparison of Immunogenicity Between Inactivated and Live Attenuated Hepatitis A Vaccines Among Young Adults: A 3-Year Follow-up Study

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A randomized clinical trial of hepatitis A vaccines (1 or 2 doses of inactivated vaccine [Healive] or 1 dose of live attenuated vaccine [Biovac]) was conducted among adults to evaluate seroprotection rates and geometric mean concentrations of antibody against hepatitis A virus for 36 months. High rates of seroprotection persisted for at least 36 months among adults who received 1 or 2 doses of inactivated hepatitis A vaccine but not among adults who received 1 dose of live attenuated hepatitis A vaccine. The long-term serial monitoring of immunogenicity induced by 1 dose of inactivated hepatitis A vaccine is needed to determine an effective alternative to a 2-dose schedule.

Keywords. inactivated hepatitis A vaccine; live attenuated hepatitis A vaccine; immunogenicity.

Hepatitis A is a viral liver disease that causes asymptomatic-to-severe illness [1]. The infected adults develop signs and symptoms of illness more often than children, and the disease severity and mortality increase in older age groups. Hepatitis A vaccine is the most effective way to combat the disease [2]. Traditional immunization schedules for hepatitis A vaccine are 2 doses administered 6–18 months apart for the inactivated vaccine, and 1 dose for the live attenuated vaccine. The 1-dose schedule of inactivated vaccine, as a potential alternative to the 2-dose schedule, removes barriers to the use of vaccines for public health purposes and may be easily introduced into national immunization programs by most developing countries [3]. In addition, the single-dose vaccination has potential value for use in immunizing travelers and to control outbreaks of hepatitis A. A World Health Organization position paper on hepatitis A vaccines suggested that the duration of the protection induced by 1-dose and 2-dose schedules of the inactivated vaccine should be regularly monitored. In particular, the possible use of a single-dose schedule should be accompanied by monitoring and evaluation plans [4]. For the live attenuated vaccine, the manufacturer’s recommended dose and schedule are the same for children and adults. We previously conducted a clinical trial involving children to compare the immunogenicity of 1 dose of inactivated vaccine, 2 doses of inactivated vaccine, and 1 dose of live attenuated vaccine [5]. In the present study, a randomized clinical trial among healthy young adults was performed to explore the 1-dose regimens of inactivated vaccine or attenuated vaccine as an alternative to the 2-dose regimen of inactivated vaccine.

METHODS

Vaccines
The inactivated and live attenuated hepatitis A vaccines are licensed by Chinese Food and Drug Administration for routine use in humans. The inactivated hepatitis A vaccine (Healive; Sinovac Biotech, Beijing, China) was formulated for individuals aged ≥16 years, contained 500 U/mL hepatitis A virus (HAV) antigen, and was administered through the intramuscular route in the deltoid region. The live attenuated hepatitis A vaccine (Biovac; Pukang Biotechnological; Zhejiang, China) was given subcutaneously in the deltoid region with a titer of 6.5 log 50% cell culture infective doses (CCID50). The inactivated and attenuated vaccines were stored at 4°C, with the cold chain maintained during transport.

Study Design and Subjects
A randomized clinical trial was conducted on young adults at colleges in Nanchang City, China. The Peking University Institutional Review Board approved the trial protocol. The procedure of the trial was in accordance with the ethical standards of the Helsinki Declaration. A total of 239 college students aged 16–21 years were enrolled in 2008. Informed written consent was obtained from subjects before their inclusion in the study. Exclusion criteria were the same as those described in a
similar study on children [5]. The first screening blood samples were collected before immunization. Thirty-seven of 239 subjects were excluded because they tested positive for anti-HAV immunoglobulin G (IgG).

The 202 remaining subjects were each randomly assigned to one of 3 groups. Two groups were administered the inactivated vaccine with a 1-dose or 2-dose schedule (at 0 and 6 months), and the third group was inoculated with 1 dose of the live attenuated vaccine. At 1.5 months (7 months for the 2-dose regimen of inactivated vaccine), 12 months, 24 months, and 36 months after the first dose, serum specimens were collected from subjects. The flow diagram of the study is shown in Figure 1.

The randomization code was prepared using SAS software, version 9.0 (SAS Institute, Cary, North Carolina), and code numbers were assigned to subjects in chronological order by the investigators. The participants were blinded to vaccination allocations during the study.

Safety Assessment
Safety was evaluated among the 202 vaccinees. Any adverse events within 30 minutes after injection were recorded, and axillary temperature, injection-site reactions, and systemic reactions during the 3-day period after injection were recorded.

Adverse reactions were graded according to the standard guideline for adverse reactions grading in vaccine clinical trials [6]. Grade 1 reactions were defined as mild symptoms or signs, such as an axillary temperature of 37.1–37.5°C or injection-site redness with a diameter of <15 mm, that disappeared without medical treatment within 48 hours of appearance.

Laboratory Tests
Quantitative testing of anti-HAV IgG was performed using a microparticle enzyme immunoassay (HAVAB 2.0; Abbott Laboratories, Chicago, Illinois) throughout the 3-year follow-up. An anti-HAV IgG titer of ≥20 mIU/mL was considered seroprotective, as defined elsewhere [7].

Statistical Analysis
Seroprotection rates, geometric mean concentrations (GMCs) of anti-HAV IgG, and their 95% confidence intervals (CIs) were calculated. Pearson $\chi^2$ or Fisher exact tests were used to compare seroprotection rates based on anti-HAV IgG titers, and one-way ANOVA was used to compare GMCs among the 3 vaccine groups. A $P$ value of <.05 (2-tailed) was considered statistically significant. SPSS 18.0 software (SPSS, Chicago) was used to perform the analyses.

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**Figure 1.** Follow-up of subjects in the 3 hepatitis A vaccination groups. Abbreviation: HAV, hepatitis A virus.
RESULTS

Recruitment of Subjects
Results of the first year of follow-up of our study have been published in Chinese [8]. Comparison of the demographic characteristics of the 202 subjects in the 3 vaccine groups revealed no significant differences in age ($P = .494$), height ($P = .891$), weight ($P = .910$), sex ratio ($P = .866$), and race ratio ($P = .715$) at 1.5 (or 7) months in the full analysis set (FAS). Similar results were observed for the safety analysis set at 0 month and for the FAS at 12, 24, and 36 months (data not shown).

Immunogenicity
Seroprotection rates and GMCs of anti-HAV IgG at 1.5 (or 7), 12, 24, and 36 months of follow-up in the 3 vaccination groups are shown in Table 1. At 1.5 (or 7) months of follow-up, there were no significant differences in seroprotection rates among the 3 groups ($P > .05$). For GMCs, the 2-dose inactivated vaccine group was significantly higher than those for both 1-dose groups ($P < .05$). The GMC in the 1-dose inactivated vaccine group was also significantly higher than that in the attenuated vaccine group ($P < .05$). At 12, 24, and 36 months of follow-up, seroprotection rates and GMCs in the 2-dose inactivated vaccine group were significantly higher than that in both 1-dose groups ($P < .05$). Seroprotection rates and GMCs in the 1-dose inactivated vaccine group were serially higher than those in the attenuated vaccine group ($P < .05$).

The dynamic changes of seroprotection rates were stable for the 2-dose and 1-dose inactivated vaccine groups from the first to the fourth follow-up time point. However, a fast decline in the seroprotection rate, from 90.5% at 1.5 months to 65% at 36 months, was found in the attenuated vaccine group. A tremendous decrease in the GMC was observed in the 2-dose inactivated vaccine group, with values of 4812.71 mIU/mL at 7 months, 1637.88 mIU/mL at 12 months, and 704.25 mIU/mL at 24 months, and the same trends were also showed in both 1-dose groups. The GMCs declined rapidly during the first 2 years after receipt of the primary dose and declined slowly from 24 months to 36 months for all 3 vaccine groups.

Safety
Adverse events were observed in 5 of 202 subjects (2.5%), all cases of which were grade 1 fever. There were no significant differences in the adverse event rates among the 3 vaccine groups (0.0% for the 2-dose inactivated vaccine group, 4.3% for the 1-dose inactivated vaccine group, and 3.2% for the attenuated vaccine group; $P > .05$).

DISCUSSION
Two doses of inactivated hepatitis A vaccine delivered on a schedule of 0 and 6 months is widely used as a routine regimen for children and adults. The seroprotection duration of >5 years yielded by the 2-dose schedule of inactivated hepatitis A vaccine

<table>
<thead>
<tr>
<th>Time After Vaccination, Immunogenicity Measure</th>
<th>Inactivated Vaccine, 2 Dose</th>
<th>Inactivated Vaccine, 1 Dose</th>
<th>Live Attenuated Vaccine</th>
<th>$P$ Value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 mos or 7 mos$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects, no.</td>
<td>69</td>
<td>70</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Seroprotection, % (95% CI)</td>
<td>100 (93.4–100)</td>
<td>91.4 (81.6–96.9)</td>
<td>90.5 (79.8–96.6)</td>
<td>.848</td>
</tr>
<tr>
<td>GMC, mIU/mL (95% CI)</td>
<td>4812.71 (4011.22–5774.36)</td>
<td>194.58 (122.36–309.43)</td>
<td>99.78 (65.73–151.47)</td>
<td>.034</td>
</tr>
<tr>
<td>12 mos</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects, no.</td>
<td>69</td>
<td>69</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Seroprotection, % (95% CI)</td>
<td>100 (93.4–100)</td>
<td>88.4 (77.9–94.9)</td>
<td>82 (69.6–90.6)</td>
<td>.002</td>
</tr>
<tr>
<td>GMC, mIU/mL (95% CI)</td>
<td>1637.88 (1326.42–2022.47)</td>
<td>154.2 (109.44–217.27)</td>
<td>89.99 (59.60–135.89)</td>
<td>&lt;.001</td>
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<tr>
<td>24 mos</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Subjects, no.</td>
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<td>61</td>
<td>56</td>
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</tr>
<tr>
<td>Seroprotection, % (95% CI)</td>
<td>100 (92.8–100)</td>
<td>90.2 (79.1–96.5)</td>
<td>66.1 (52.1–78.1)</td>
<td>&lt;.001</td>
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<td>GMC, mIU/mL (95% CI)</td>
<td>704.25 (560.52–884.82)</td>
<td>103.5 (74.23–144.32)</td>
<td>43.57 (30.28–62.49)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>36 mos</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects, no.</td>
<td>43</td>
<td>43</td>
<td>40</td>
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<tr>
<td>Seroprotection, % (95% CI)</td>
<td>100 (89.8–100)</td>
<td>93 (79.9–99.2)</td>
<td>65 (48.3–79.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>GMC, mIU/mL (95% CI)</td>
<td>805.68 (565.93–1146.99)</td>
<td>93.67 (61.15–144.10)</td>
<td>42.05 (26.09–67.77)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.

$^a$ Pearson $\chi^2$ or Fisher exact tests were used to compare seroprotection rates of anti-HAV IgG, and one-way ANOVA was used to compare GMCs among the 3 vaccine groups.

$^b$ For the 2-dose formulation of inactivated vaccine.
has been demonstrated by many clinical trials. Van Herck et al reported on the 2 longest follow-up studies, initiated in 1992 and conducted in adults, which demonstrated the persistence of anti-HAV antibodies up to at least 17 years after receipt of a monovalent inactivated hepatitis A vaccine (Havrix; GlaxoSmithKline Vaccines) on a 2-dose schedule (0 and 6 months or 0 and 12 months) [9]. Data from these 2 follow-up studies have been used in several statistical modeling analyses to estimate the long-term persistence of vaccine-induced antibodies [10, 11]. Recently, Hens et al [12] used a linear mixed model to analyze 17-year observational data and estimated that 95% of 2-dose vaccinees will remain seropositive for anti-HAV (defined as an anti-HAV IgG titer of \( \geq 20 \) mIU/mL) at 25 and 30 years. Beyond year 30, the model predicted a seropositivity rate of \( >90\% \) for up to 40 years. Moreover, the study showed that the linear mixed model yielded the highest level of agreement between observed and predicted antibody levels for the time points included in the analysis.

The authors pointed out that these estimations could not be extrapolated for 1-dose administration of inactivated hepatitis A vaccine. Therefore, the present clinical trial was conducted to monitor the persistence of anti-HAV induced by 1 dose of inactivated vaccine.

From our 3-year study, the same dynamic trends of immunogenicity were observed in both 2-dose and 1-dose inactivated vaccine groups. The GMCs declined rapidly in the first 2 years after receipt of the primary dose and then decreased slowly after 2 years. The seroprotection rates in both groups were stable through 3 years. Overbosch et al [13] also observed similar dynamic changes in immunogenicity in healthy adults who received a single dose of inactivated hepatitis A vaccine (Avaxim). Apparently, seroprotection rates in the single-dose inactivated vaccine group were comparable to those of the 2-dose group throughout the 3-year observation period. However, GMCs were significantly lower in the 1-dose group than in the 2-dose group during the same period. It can be projected that the duration of the persistence of anti-HAV associated with the 1-dose vaccine would be shorter than that of the 2-dose vaccine. To estimate the persistence of anti-HAV induced by 1 dose of vaccine and to explore whether 1-dose vaccination could be an effective alternative to 2-dose administration, long-term (ie, >5 years) serial monitoring of immunogenicity is needed in the future.

The live attenuated hepatitis A vaccine (H2 strain) was first licensed in China in 1992, and subsequently licensed in India (in 2005), Guatemala (in 2006), the Philippines (in 2008), and Thailand (in 2010) [14]. A large number of clinical trials were performed in children to evaluate live attenuated hepatitis A vaccine. However, the studies in adults were very limited. Until now, the same vaccine dose was recommended for children and adults (\( 10^{6.5} \text{CCID}_{50}/\text{mL/dose} \)), based on the results of short-term (<1 year) follow-up data [15]. Our study is the first report on the persistence of anti-HAV induced by the attenuated vaccine in adults for 3 years. Through 3 years of follow-up, the seroprotection rates of anti-HAV declined rapidly, from 90.5% at 1.5 months to 65% at 36 months, and the GMCs also decreased, from 99.78 mIU/mL at 1.5 months to 42.05 mIU/mL at 36 months. In children, the anti-HAV seroprotection rates and GMCs in the H2 vaccine group dropped slowly (from 92.2% and 126.2 mIU/mL, respectively, at 3 months to 75.0% and 80.8 mIU/mL, respectively, at 36 months) [16]. To avoid bias associated with missing data during follow-up and to confirm the immunogenicity in adults at 36 months, GMCs at the first time point were compared between subjects with and subjects without subsequent follow-up, and no significant differences were found (Supplementary Table 1). Based on the results of our study, a higher dose of the attenuated vaccine might be required for adults.

There are some limitations to our study: (1) the actual protection of hepatitis A vaccine and the effects of cellular immunity were not measured; (2) the effects of the natural exposure to HAV during the follow-up of vaccinees were not evaluated; (3) the effect of the epitopes inducing anti-HAV after receipt of inactivated versus attenuated vaccines was not analyzed; (4) the trial evaluated only healthy adolescents/young adults, and the results might not be applicable to other age groups or to persons with underlying illness; and (5) loss of subjects to follow-up could have resulted in selection bias.

In conclusion, 1 or 2 doses of inactivated hepatitis A vaccine but not 1 dose of the attenuated hepatitis A vaccine induced high rates of seroprotection persisting for at least 36 months among healthy young adults. The longer follow-up and higher dose of the attenuated vaccine for adults, as well as the benefits of even short-term persistence of seroprotection after receipt of a single dose of attenuated hepatitis A vaccine for travel or post-exposure prophylaxis, need to be further studied.

**Supplementary Data**

**Supplementary materials** are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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**Potential conflicts of interest.** All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


