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Japanese encephalitis vaccination in HIV-infected children with immune recovery after highly active antiretroviral therapy

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Abstract

HIV-infected children are vulnerable to infections by vaccine preventable pathogens. However, they have poorer responses to childhood immunization than healthy children. The objectives of this study are to determine the prevalence of Japanese encephalitis (JE) protective antibody in HIV-infected children with immune recovery after highly active antiretroviral therapy (HAART) and evaluate response to JE revaccination. JE neutralizing antibody titer of plasma was determined by a plaque reduction neutralization assay. An antibody titer of more than 1:10 was defined as protective antibody. Children who did not have protective antibody to JE were enrolled to receive a two-dose JE revaccination during the study. There were 96 children with mean age of 9.7 years (S.D. 2.6) and mean CD4 percentage of 25 (S.D. 5) who participated in the study. Forty-four children (46%) had protective antibody to JE. A two-dose JE revaccination was administered to 50 children who did not have JE antibody. At 1 month after revaccination, 44 children (88%) developed protective antibody. This study demonstrated that there is a low prevalence of JE protective antibody in HIV-infected children despite history of JE primary childhood vaccination. However, the majority of HIV-infected children with immune recovery after HAART can develop protective antibody after JE revaccination.

Keywords: Japanese encephalitis virus; Human immunodeficiency virus (HIV); Protective antibody

1. Introduction

Japanese encephalitis virus is a mosquito-borne flavivirus. Japanese encephalitis (JE) is the leading cause of viral encephalitis in Asia. JE occurs in annual epidemics in many Asian countries including China, Vietnam, Thailand, Laos, India, Sri Lanka and Indonesia [1]. The estimated incidence of JE in Thailand ranges from 1.5 to 2.5 per 10,000 populations. It is highest in the northern region, and lowest in the central region. The age specific rate is highest in children 5–9 years of age [2]. The case fatality rate of JE ranges from 17 to 25% in various region [3,4]. Approximately 50% of the survivors have neurologic sequelae with frank motor deficits, or severe cognitive and language impairment [3]. JE is preventable through immunization with safe and effective inactivated vaccines with efficacy of 91–94% [5,6]. Since 1992, inactivated JE vaccine has been included in the childhood Expanded Program on Immunizations (EPI) vaccination schedule for children in Thailand. The primary series consists of two doses given 1–2 weeks apart for children around 18 months of age. The first booster dose is administered 1 year later. The second booster dose is administered as an optional dose in the following 4–5 years.

HIV immunodeficiency virus (HIV) infection destroyed CD4+ T-cells which provided a critical help to B-cells in the production of antibodies against T-cell-dependent
antigens and in the differentiation of B-cells into memory cells [7]. Several studies report poorer immune response to vaccine in HIV-infected children compared to the general population [7,8]. Rojanasuphot et al. reported a low response to JE vaccine among HIV-infected children after primary vaccination with two doses of JE vaccine. The response rate was only 36% compared to 67% among uninfected children [9].

The introduction of highly active antiretroviral therapy (HAART) has resulted in immune recovery [10] and reduction of morbidity and mortality in HIV-infected children [11]. However, information about the persistence of JE antibody after primary series vaccination in these children is limited. Whether revaccination after immune recovery is necessary remains unknown. We hypothesized that the majority of HIV-infected children had no JE protective antibody and were at risk of JE infection even after the commencement of HAART. The aims of this study were (1) to determine the prevalence of JE protective antibody in HIV-infected children with immune recovery after HAART and (2) to assess efficacy of JE revaccination in HIV-infected children after receiving HAART.

2. Patients and methods

2.1. Study design and patient population

The study had a two-step design. The first phase was a cross-sectional study to determine the proportion of HIV-infected children who had a protective antibody to JE virus. Children who had no JE protective antibody were enrolled to the second phase of the study. The second phase was an intervention study to determine a proportion of children who were able to produce a protective antibody to JE virus after having received a two-dose JE revaccination.

This study was conducted at Chiang Mai University hospital, Chiang Mai, Thailand from March 2005 to March 2006. The inclusion criteria were (1) HIV-infected children aged >5 years, (2) had been severely immunosuppressed (nadir CD4 lymphocyte percentage ≤15), (3) had shown evidence of immune recovery, defined as CD4 lymphocyte percentage >15 for at least 3 months after receiving HAART and (4) had a history of JE vaccination. The exclusion criteria were children who (1) received immunosuppressive agents within 3 months or (2) received blood component transfusion within 6 months prior to the study. The study protocol was approved by the research ethics committee of Chiang Mai University. Written informed consent was obtained from each child’s parent or guardian before enrollment.

2.2. Study procedures

2.2.1. The first phase—to determine the prevalence of JE protective antibody

A cross-sectional study to evaluate the prevalence of JE protective level was performed in March 2005. Past illnesses and immunization data were collected by medical record review and caregiver interview. The history of HIV-related illness and antiretroviral treatment was obtained by medical record review. The clinical stage of HIV disease was determined according to the 1994 US Centers for Disease Control and Prevention revised classification [12]. CD4 lymphocyte count and plasma HIV RNA level before starting HAART and at 24-week intervals after HAART were abstracted from medical records. A single blood drawing was performed to measure JE neutralizing antibody. Patient who had no JE protective antibody, defined as a neutralizing antibody titer of ≤1:10, were enrolled to the second phase of a study.

2.2.2. The second phase—to determine the efficacy of JE revaccination

Two subcutaneous 0.5 mL doses of the inactivated JE vaccine—Beijing strain (produced by the Thai Government pharmaceutical organization) were given 6 months apart. Subjects had blood drawn at 2 months after first dose, prior to second dose and at 1 month after second dose of JE revaccination. Since response to JE vaccine might differ between children who had had dengue infection and those who had not, blood specimens were measured for both JE and dengue antibody levels.

2.3. Safety assessment

Vaccine safety and tolerability were monitored by the use of a vaccine report card supplied to parents or guardians. The cards solicited daily recording of injection-site adverse events and systemic adverse events on the day of vaccination and for 72 h thereafter. They were also asked to notify the study physician immediately if unexpected or severe reactions occurred.

2.4. Laboratory tests

JE and dengue neutralizing antibody titer of plasma were determined at the Center for Vaccine Development, Mahidol University, Bangkok by a plaque reduction neutralization (PRNT50) assay modified from Russell et al. [13]. Plaque count was determined by using LLC-MK2 plaque assay single overlay technique. Briefly, sera were thawed and heat-inactivated by incubation at 56 °C for 30 min. Serial dilutions of serum were made (1:10, 1:40 and 1:160). An equal volume of diluted Japanese encephalitis (Beijing), Dengue 1 (16007), Dengue 2 (16681), Dengue 3 (16562) and Dengue 4 (1036) viruses contain about 40–60 pfu/0.2 mL/well was added to each serum dilution tube. Following incubation at 37 °C for 60 min, 0.2 mL was removed from each tube and inoculated onto triplicate 6-well plates of confluent LLC-MK2. Each plate was incubated at 37 °C for 90 min and the monolayers were then overlaid with 4 mL of 3.0% carboxy methyl cellulose/MEM. Plates were incubated for 7 days at 37 °C with 5% CO2, then plaques were counted. The endpoint neutralizing plaque dilution was determined from the dilution series.
by probit analysis; a 50% reduction of titer was taken as an endpoint.

A neutralizing antibody titer of more than 1:10 is defined as evidence of protection to Japanese encephalitis infection. Children with a positive titer to one of the four dengue viruses were considered to have a natural dengue infection.

CD4 cell counts were assessed with use of a FACSCount apparatus (Becton-Dickinson). Plasma HIV RNA levels were measured by the Roche Ultrasensitive Amplicor assays, version 1.5 (Roche). These tests were performed at the Research Institute for Health Sciences, Chiang Mai University.

Table 1. Characteristics of study participants (N=96)

<table>
<thead>
<tr>
<th>Characteristics of study participants (N=96)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>9.7 ± 2.6</td>
</tr>
<tr>
<td>Male gender</td>
<td>47 (49)</td>
</tr>
<tr>
<td>Patient characteristics before receiving antiretroviral therapy</td>
<td></td>
</tr>
<tr>
<td>CDC clinical category</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>10 (10)</td>
</tr>
<tr>
<td>A</td>
<td>16 (17)</td>
</tr>
<tr>
<td>B</td>
<td>23 (24)</td>
</tr>
<tr>
<td>C</td>
<td>47 (49)</td>
</tr>
<tr>
<td>CD4 percentage</td>
<td>5 ± 5</td>
</tr>
<tr>
<td>CD4 cell count (cells/µL)</td>
<td>142 ± 178</td>
</tr>
<tr>
<td>HIV RNA level (log_{10} copies/mL)</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>Patient characteristics at the time of enrollment</td>
<td></td>
</tr>
<tr>
<td>CD4 percentage</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>CD4 cell count (cells/µL)</td>
<td>778 ± 237</td>
</tr>
<tr>
<td>HIV RNA level (log_{10} copies/mL)</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>Participants with plasma HIV RNA level &lt;1.7 log_{10} copies/mL</td>
<td>88 (92)</td>
</tr>
<tr>
<td>Duration of antiretroviral therapy (months)</td>
<td>24 ± 4</td>
</tr>
<tr>
<td>Duration of immune recovery (CD4 ≥ 15%) (months)</td>
<td>14 ± 7</td>
</tr>
</tbody>
</table>

Note: Data shown in mean ± S.D. or number (%); CDC, Centers for Disease Control and Prevention.

3. Results

3.1. Demographic and clinical characteristics

Ninety-six HIV-infected children were enrolled. The demographic and clinical data of the participants are shown in Table 1. The mean age was 9.7 years (S.D. 2.6). About half of them (49%) were in CDC clinical category C. Means of base-line CD4 cell percentage and plasma HIV RNA level were 5% (S.D. = 5) and 5.4 log_{10} copies/mL (S.D. = 0.4), respectively. The JE immunization status was documented by medical records in 59 children (61%) and by history in 37 children (39%). Among children who had medical records, there were 24 (41%), 28 (47%) and 7 (12%) children who have received two doses, three doses and four doses of JE vaccine, respectively.

3.2. Prevalence of JE protective antibody

Forty-four children (46%) had JE protective antibody. There was no difference between children who had JE immunization documented in the medical records (28 out of 59 children = 47%) and children whose guardian gave a history of JE immunization (16 out of 37 = 43%), p value = 0.69. However, there was a significantly different JE protective antibody among children who had different number of JE vaccine doses. Thirty-eight percent, 43% and 100% of children who received two, three and four doses of JE vaccine, respectively, had protective antibody level (p value = 0.003).

3.3. The efficacy of JE revaccination

3.3.1. Immunogenicity

Fifty out of 52 children (96%) who did not have JE protective antibody participated in the second phase of the study. The response to JE vaccine is shown in Table 2. The overall response was 88% at 1 month after receiving two doses of JE vaccine. Of the six children who did not respond to JE revaccination, five had had at least two doses of JE primary series vaccination documented in their medical records. Nineteen children (38%) had dengue antibody level prior to JE revaccination. There was no significant difference of JE vaccine response between children who had baseline dengue antibody level and those who did not (Table 2). There were no significant difference in age, gender, clinical parameters prior to HAART (namely CD4 cell percentage and HIV RNA level, duration of HAART, and duration of immune recovery) between children who responded to revaccination and those who did not.

3.3.2. Vaccine safety

Pain at the injection site was the most commonly reported adverse reaction. It was reported in 29 (58%) and 14 (28%) of participants after the first and second vaccine doses, respectively. The pain was mild and resolved spontaneously within 1–3 days without any treatment. One participant (2%) had swelling at the injection site, two (4%) had low grade fever and three (6%) had rash after the first dose of vaccine. Two participants (4%) had swelling at the injection site after the second dose of vaccine. None of the participants had hypersensitivity reactions or neurological complications after vaccination and none withdrew from the study because of vaccine-related adverse events.
Despite the universal coverage of JE vaccination in Thailand, we found that only 46% of HIV-infected children with immune recovery after HAART had protective antibody to JE. Northern Thailand is the endemic area of JE viral infection. Therefore, half of these children are at risk of acquiring disease despite having received JE primary immunization during childhood. At 1 month after the two doses of JE vaccine, 88% of children developed a protective antibody level. Revaccination in this population should be considered to ensure individual immunity against Japanese encephalitis.

A study from Bangkok showed a low response to JE vaccine among HIV-infected children 3 months after primary vaccination with two doses of JE vaccine. The response rate was only 36% compared to 67% among uninfected children [9]. The slightly higher prevalence of protective antibody (46%) in HIV-infected children at the mean age of 9.7 years in our cohort might be explained by additional booster doses (one or two doses after primary immunization) or natural booster effect in northern Thailand. There are no published data in Thai children who are not infected with HIV to compare with the prevalence of protective antibody of the children in our study. However, in a large seroepidemiologic study in Taiwan which has a similar JE vaccine EPI schedule as Thailand, 168 of 215 (78%) and 391 of 425 (92%) of healthy children aged 14–15 and 6–7 years, respectively, had protective antibody against JE [14]. The low prevalence of JE protective antibody in school-aged HIV-infected children in our cohort may be explained by low seroconversion rate to the primary series [9] or by the more rapid rate of antibody decline. We have reported similar findings with other childhood vaccines, e.g. hepatitis B [15] and measles [16]. In addition some of the 96 children in our study did not receive the full course of four injections of JE vaccine. Of the seven children who were documented to have received the full course, all had protective JE antibody.

HIV destroys CD4 cells which provide critical help to B-cells in the production of antibodies against T-cell-dependent antigens and in the differentiation of B-cells into memory cells [7]. The difference between vaccine responses among HIV-infected and healthy children may be explained by impairment of specific memory T-cells by HIV infection. HAART suppresses viral replication and most patients respond in increased number of CD4 lymphocytes [10]. This could restore immune response to vaccine. In our study, the number of children who responded to JE revaccination was significantly higher than that reported among untreated HIV-infected children (88% vs. 36%) [9] and nearly as high as that reported in healthy children (91–94%) [5,6]. This finding is similar to previous reports with other childhood vaccines, e.g. pneumococcal [17], varicella [18], hepatitis A [19], hepatitis B [20] and measles [21]. An immune response to vaccine can be restored by antiretroviral therapy to the same level as found in healthy children.

The response to JE revaccination was not significantly different between children who had dengue infection and those who did not (84% vs. 95%, p = 0.39). This is similar to a previous report by Quina and coworkers that the presence of dengue antibody in the pre-vaccination sera did not significantly influence the seroconversion rate and the geometric mean titer of post-vaccination JE neutralizing antibody [22].

No serious adverse reactions occurred as a result of JE revaccination. Only 28–58% of children reported pain at the injection site. The pain was generally mild and resolved spontaneously within a few days without treatment. The major adverse events reported from JE vaccination are neurological and hypersensitivity reaction presented as generalized urticaria with or without angioedema [23]. In our study, none had major adverse events from revaccination.

In conclusion, this report has shown that children with immune recovery after HAART are at risk of JE infection despite primary childhood immunization against JE. The response rate to two-dose JE revaccination is as high as 88%. Revaccination in this population should be considered to ensure individual immunity to this high-morbidity disease. Currently, there is no specific guideline for immunization against JE in HIV-infected children after immune recovery. Our data might be useful for guideline development.
Acknowledgements

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References


