Comparison of Two Hepatitis B Vaccines (GeneVac-B and Engerix-B) in Healthy Infants in India

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Hepatitis B is a major problem in many parts of the world. The WHO has recommended the inclusion of hepatitis B vaccines in routine immunization schedules. We wanted to compare two recombinant hepatitis B vaccines in an infant population for immunogenicity and reactogenicity when given at 6, 10, and 14 weeks of age. One hundred seventy-three infants meeting eligibility criteria were given either GeneVac-B (Serum Institute of India Ltd.) or Engerix-B (GlaxoSmithKline Beecham) in a random fashion. Three 0.5-ml doses of the vaccines were given at 6, 10, and 14 weeks of age along with diphtheria-pertussis (whole cell)-tetanus (DTPw) vaccine. Blood samples were collected at baseline and 1 month after administration of the third dose of the vaccines to measure anti-HBs antibody levels. Seroconversion was defined as a titer of more than 1 × 10^{-3} IU/ml, while seroprotection was defined as a titer of more than 10 × 10^{-3} IU/ml. Of the GeneVac-B recipients, 98% seroconverted versus 99% of the Engerix-B group. The anti-HBs geometric mean titer was slightly greater for GeneVac-B (229 × 10^{-3} IU/ml) than for Engerix-B (167 × 10^{-3} IU/ml), but the difference was not significant. The seroprotection rates were similar for both vaccines (96% and 95%, respectively). The most common systemic reaction events were mild to moderate fever, excessive crying, local swelling, rash, and irritability, and the local reactions were redness, induration, and edema, which most probably were caused by the simultaneously administered DTPw vaccine. All events were transient and resolved without sequelae. Reactogenicity was similar for the two vaccines. The present study shows that GeneVac-B is as immunogenic and as well tolerated as Engerix-B when administered with DTPw vaccine at 6, 10, and 14 weeks of age.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test vaccine GeneVac-B (n = 85)</th>
<th>Comparator vaccine Engerix-B (n = 88)</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seroconversion</td>
<td>83</td>
<td>97.65</td>
<td>0.001</td>
<td>0.976 (NS)</td>
</tr>
<tr>
<td>Seroprotection</td>
<td>81</td>
<td>95.29</td>
<td>0.32</td>
<td>0.788 (NS)</td>
</tr>
</tbody>
</table>

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MATERIALS AND METHODS

Vaccines. GeneVac-B (test vaccine) consists of purified surface antigen (Ag) of HBV obtained by culturing genetically engineered *Hausenula polymorpha* yeast cells expressing the surface Ag gene of the virus. There is no material of human or animal origin. Each pediatric dose of 0.5 ml contains 10 μg of surface Ag adsorbed on 0.125 mg of aluminum hydroxide, with ≤0.01% thimerosal added as a preservative. The commercial batch number was S-50304, and the manufacture and expiration dates were January 2003 and December 2004, respectively.

Also, Engerix-B (comparator vaccine) is a noninfectious recombinant DNA hepatitis B vaccine. It contains purified surface Ag from genetically engineered *Saccharomyces cerevisiae* cells, which carry the surface Ag gene of HBV. Each pediatric dose of 0.5 ml has 10 μg of Ag protein adsorbed on 0.25 mg of aluminum hydroxide with 1:20,000 thimerosal added as a preservative. The commercial batch number was ENG-3445A1, and the manufacture and expiration dates were February 2002 and January 2004, respectively.

The vaccines were stored at 2 to 8°C in a refrigerator. Use and storage of the vaccine were under the supervision of a responsible staff member participating in the study.

Setup. An open, randomized, comparative postmarketing trial was conducted among 173 infants meeting the eligibility criteria. The study center was the Indira Gandhi Institute of Child Health, Bangalore, India, and serology testing was done by the Department of Microbiology, Indira Gandhi Institute of Child Health. This study was approved by the Institutional Ethics Committee of Indira Gandhi Institute, and it was conducted between October 2003 and September 2004. Strict confidentiality about the subjects’ identities was maintained during the course of this study. This study was conducted according to the principles of Good Clinical Practices for Clinical Research in India and the World Medical Association Declaration of Helsinki.

The parents of healthy infants attending the institute were offered an opportunity to participate in the study. If the parents were interested, detailed information was given about the study and study procedures. Parents were encouraged to ask for clarification if questions were raised. If an agreement was reached, signed informed consent was obtained.

The subjects were screened by history and physical examination before entering the study by using the inclusion criteria of the child of either gender being a normal healthy infant at the age of 6 to 8 weeks. Subjects with acute febrile illness, any other infection, conditions associated with immunosuppression due to disease or therapy, prior hepatitis B vaccination, hypersensitivity to any component of the vaccine, seropositivity for HBs Ag and/or anti-HBs antibody, and participation in another clinical trial were excluded from the study.

The eligible subjects were randomized by systematic sampling to receive the test vaccine or the comparator vaccine. Three doses of 0.5 ml of either vaccine were administered intramuscularly at 6, 10, and 14 weeks of age.

Laboratory tests and clinical follow-up. A venous blood sample was collected prior to administration of the first vaccination and 1 month after administration of the third dose. Baseline HBs Ag status was checked by enzyme-linked immunosorbent assay with kits from DiaSorin S.p.A., Saluggia, Italy. Immunoglobulin G antibodies for HBs Ag (anti-HBs) were also assayed for by enzyme-linked immunosorbent assay with kits from the same company. The serum samples were tested blindly. The test results were interpreted according to the instructions supplied with the kits. Anti-HBs levels above $1 \times 10^{-3}$ IU/ml were defined as seroconversion, whereas seroprotection was defined as the presence of a postimmunization anti-HBs titer of $10 \times 10^{-3}$ IU/ml. When the titer of the undiluted serum sample was $>1,000 \times 10^{-3}$ IU/ml, the sera were tested after diluting the sample 1:50 in human sera negative for anti-HBs and HBs Ag.

For reactogenicity assessment, subjects were physically examined at all vaccination visits (weeks 6, 10, and 14) and the postvaccination blood sample visit (week 18). Parents were asked to report any adverse event assumed to be causally associated with vaccination until the total follow-up period of 12 weeks ended. In the case of such a report, the child was examined thoroughly and the details were recorded. Fever was recorded by measuring the child’s oral temperature with a standard mercury thermometer. Fever was classified as mild (37.0 to 37.9°C), moderate (38.0 to 38.9°C), or severe (>39.0°C).

Seroconversion and seroprotection rates after administration of the three vaccine doses were calculated. The anti-HBs concentrations were log transformed, and the antilog of the mean log values was calculated for the geometric mean titers (GMTs). Potential differences between vaccines were tested with the Z test for GMTs by t test and Mann-Whitney test.

RESULTS

In all, 226 infants were screened, 7 of whom were anti-HBs Ag positive. No subject was initially HBs Ag positive. Insufficient follow-up led to 45 dropouts. One subject gave too small a blood sample. Thus, there were 173 samples left for testing (85 of the test vaccine and 88 of the comparator vaccine).

The mean age was 47 days (standard deviation, 4.6 days) in the test vaccine group and 49 days (standard deviation, 4.2 days) in the comparator vaccine group, in which there were 58 and 65% males, respectively. Immunogenicity was assessed by determining seroconversion rates (Table 1). The seroprotection rates and GMTs for anti-HBs antibodies are listed in Tables 1 and 2. No significant difference was observed between the vaccines. Of the test vaccine recipients, 98% seroconverted.
versus 99% of the comparator vaccine group. The anti-HBs GMT was slightly greater for the test vaccine of \((229 \times 10^{-3} \text{ IU/ml})\) than for the comparator vaccine \((167 \times 10^{-3} \text{ IU/ml})\), but the difference was not significant. The seroprotection rates were similar for the two vaccines (96 and 95%, respectively).

The reported adverse events are given in Tables 3 and 4. Since the most common systemic reaction events were mild to moderate fever, excessive crying, rash, and irritability, one may assume that the events were mainly caused by the simultaneously administered DTPw vaccine. All events were transient and resolved without sequelae. No subject required hospitalization because of a vaccine-associated event. Postvaccination reactogenicity was similar for the test vaccine and the comparator vaccine.

**DISCUSSION**

The Indian recombinant hepatitis B vaccine proved highly immunogenic. After administration of three doses, there was no difference from the competitor vaccine in the seroconversion or seroprotection rate. According to the WHO expanded program for immunization, the doses were administered at 6, 10, and 14 weeks of age so that no extra visits were required.

Rather similar immunogenicity results have been obtained with HBV vaccines administered on different schedules. Administration of Engerix-B at 0, 1, and 6 months of age induces a seroprotection rate of 96% with a GMT of \(3,141 \times 10^{-3} \text{ IU/ml}\) \((4)\). An Ag amount of 5 \(\mu\)g seems to be as immunogenic as 10 \(\mu\)g \((6)\), seroprotection being the same at 98% for both doses with GMTs of \(8,062 \times 10^{-3} \text{ IU/ml}\) and \(3,732 \times 10^{-3} \text{ IU/ml}\), respectively. Two studies \((5, 7)\) used 5 and 10 \(\mu\)g of Engerix-B at 2, 4, and 6 months, and the seroprotection rate was 99% in both cases. Another vaccine, Recombivax (Merck), when given at a dose of 2.5 \(\mu\)g on the same schedule, showed a 95% seroprotection rate \((10)\). The seroprotection rates in our study were comparable to those results \((4, 5, 6, 7, 10)\) for both the test vaccine and the comparator vaccine.

The GMTs we achieved with our 6-, 10-, and 14-week schedule were somewhat lower for both vaccines than those achieved with a 0-, 1-, and 6-month or a 2-, 4-, and 6-month schedule \((4–7)\). This could be expected since the antibody levels are greater with a longer interval between the last two doses. However, the clinical relevance of our observation is unclear, especially since the study populations and laboratory tests differ between the studies. We assume that the slightly lower levels we achieved are not of major importance, especially when one realizes that avoiding an extra visit is likely to improve vaccination coverage.

No serious adverse events were found. Mild to moderate fever was reported, probably due to DTPw vaccine. We found no difference in reactogenicity between the two vaccines we tested here.

The present study shows that the test vaccine (GeneVac-B) is as immunogenic and as safe as the comparator vaccine when administered with DTPw vaccine at 6, 10, and 14 weeks of age. Moreover, genetically modified \(H.\ polymorpha\), which is the host system used for GeneVac-B production, has the advantages of expressing mitotically stable recombinant strains, faithful processing of the produced polypeptides, and higher productivity than the host system used for Engerix-B production, i.e., \(S.\ cerevisiae\) \((2, 3)\). This high productivity results in a low production cost, which in turn has an impact on the price of the vaccine. Because the Indian-made vaccine is significantly less expensive than the competitor brought from abroad \((\sim$1.19 U.S. versus \sim$7.31 U.S. per 1-ml dose) \((1)\), it may be used widely in India and other countries.

**REFERENCES**


**TABLE 4. Adverse events for all three doses for the entire period**

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Adverse reaction</th>
<th>Test vaccine GeneVac-B ((n = 85 \times 3 = 255) opportunities)</th>
<th>Comparator vaccine Engerix-B ((n = 88 \times 3 = 264) opportunities)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mild fever</td>
<td>114, 44.70, 38.50–51.04</td>
<td>99, 37.50, 33.64–43.64</td>
</tr>
<tr>
<td>2</td>
<td>Moderate fever</td>
<td>18, 7.06, 4.24–10.93</td>
<td>26, 9.85, 6.53–14.10</td>
</tr>
<tr>
<td>3</td>
<td>Excessive crying</td>
<td>5, 1.96, 0.64–4.52</td>
<td>8, 3.03, 1.32–5.88</td>
</tr>
<tr>
<td>4</td>
<td>Irritability</td>
<td>0, 0.0, 0.0–1.44</td>
<td>1, 0.38, 0.0–2.09</td>
</tr>
<tr>
<td>5</td>
<td>Local swelling</td>
<td>3, 1.18, 0.24–3.40</td>
<td>1, 0.38, 0.0–2.09</td>
</tr>
<tr>
<td>6</td>
<td>Local erythema</td>
<td>5, 1.96, 0.64–4.52</td>
<td>0, 0.0, 0.0–1.39</td>
</tr>
<tr>
<td>7</td>
<td>Local edema</td>
<td>4, 1.57, 0.43–3.97</td>
<td>0, 0.0, 0.0–1.39</td>
</tr>
<tr>
<td>8</td>
<td>Local induration</td>
<td>8, 3.14, 1.36–6.09</td>
<td>9, 3.41, 1.57–6.37</td>
</tr>
<tr>
<td>9</td>
<td>Rash</td>
<td>1, 0.39, 0.0–2.16</td>
<td>0, 0.0, 0.0–1.39</td>
</tr>
</tbody>
</table>

*There were three opportunities to record each confidence interval (CI). Nonoverlapping of CIs indicates that they are significantly different in these two groups, but if CIs overlap for all adverse reactions, we can conclude that they are not statistically significantly different at a 0.05 level of significance.


