Clinical trial of hepatitis B vaccine in a simplified immunization programme

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The immunogenic effect on Senegalese infants of two doses of hepatitis B vaccine with a 6-month interval followed by a booster dose after another 6 months was studied. Anti-HBs antibodies were detected in 65.8% of the infants 6 months after the first injection (geometric mean titre (GMT), 6.1 mIU/ml). At the time of the booster injection, 89.7% of the infants exhibited anti-HBs antibodies (GMT, 83.7 mIU/ml). Two months after the booster dose, 95.4% of the infants were anti-HBs positive (GMT, 348 mIU/ml). This anamnestic anti-HBs response is lower than that produced by two doses of the vaccine at a 2-month interval (GMT, 670 mIU/ml) or three doses at a 1-month interval (GMT, 1500 mIU/ml).

In developing countries, immunization schedules tend to mirror the practices followed in developed countries, and therefore consist of three separate sessions and a fourth booster dose. However, such a schedule is usually only possible in health centres located in the main urban areas of developing countries. In rural areas, in contrast, access to health care centres is more limited and mobile teams for immunization are required. The areas covered by these teams are large, in general, and long periods occur between sessions. For example, schedules comprising two or three injections at 6-month intervals or two injections at a 1-year interval have been used for tetanus, diphtheria, and pertussis immunizations, and similar procedures are being considered for polio immunization schedules (1-4). Clearly, a schedule involving a 6-month interval greatly facilitates the organization of immunization programmes and may give high returns in terms of disease prevention (2).

We have previously reported (5) that a schedule consisting of two doses of 5 μg HBSAg vaccine with a 2-month interval plus a booster dose 1 year later produces an immune response comparable to that obtained with the traditional protocol of three injections at a 1-month interval plus a booster 1 year later (6). Here, we describe the results of a study carried out in rural areas of Senegal to assess the immunogenic effect of two doses of hepatitis B vaccine with a 6-month interval followed by a booster dose after another 6 months, and compare them with those obtained using two doses of vaccine with a 2-month interval or three doses at 1-month intervals (5, 7).

MATERIALS AND METHODS

Vaccine

Hepatitis B vaccine 8 containing 5 μg of HBSAg was used and administered by subcutaneous injection into the upper arm.

Laboratory methods

Levels of HBSAg, 8 anti-HBs, 8 and anti-HBe 8 were determined by radioimmunoassay. The concentration of anti-HBs antibody (in mIU/ml) was determined using the method reported by Hollinger et al. (8).

8 Havac B 8 From Pasteur Vaccins, Marnes la Coquette, France.
8 Assia H. From Abbott Laboratories, North Chicago, IL, USA.
8 Ausab. From Abbott Laboratories.
8 Corab. From Abbott Laboratories.
**Immunization protocol**

Infants received three injections of hepatitis B vaccine at 6-month intervals (T₀, T₆, and T₁₂, respectively), with the third dose considered as a booster. The following vaccines were also administered to subsets of children: BCG and diphtheria/tetanus/pertussis-polio (DTP-polio) at T₀ and DTP-polio at T₆ and T₁₂ (9).

**Study population**

The study was carried out in the département of Fatick, Senegal. A total of 664 infants received the first dose of hepatitis B vaccine, 409 the second dose, and 177 the third. Blood samples were taken at the time of each injection (T₀, T₆, and T₁₂), and in the case of 89 infants also 2 months after the last (booster) dose (T₁₄). The follow-up loss was therefore high, since only 26.7% of the infants completed the entire series of injections (Table 1). Only results from infants who were seronegative at T₀ are shown, i.e., 281 infants at T₆, 116 at T₁₂, and 65 at T₁₄. At T₀ the mean age of the seronegative infants was 10.2 months, while that of the seropositive infants with anti-HBs antibodies was 7.4 months. The mean age of infants who were only anti-HBc-positive was 4.8 months and that of infants who were already HBsAg-positive at T₀ was 14.3 months.

The results obtained were compared with those reported previously for two other groups of Senegalese infants (5, 7). The first of these comprised 72 seronegative infants who were immunized using a protocol of two doses of hepatitis B vaccine with a 2-month interval, while the second consisted of 111 seronegative infants immunized using three doses at 1-month intervals. Both groups also received a booster 12 months after the first dose.

**RESULTS**

The anti-HBs response was determined 6 months after the T₀ dose of hepatitis B vaccine for the 281 infants who were seronegative. Of these children, 185 (65.8%) exhibited anti-HBs antibodies but the geometric mean titre (GMT) was only 6.1 mIU/ml. The

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**Table 1. Some characteristics of the infants in the study population**

<table>
<thead>
<tr>
<th>Event</th>
<th>No. of infants</th>
<th>Percentage of infants followed up</th>
<th>No. of seronegative infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>First dose (T₀)</td>
<td>664</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second dose (T₆)</td>
<td>409</td>
<td>61.6</td>
<td>281 (68.8)</td>
</tr>
<tr>
<td>Third dose (T₁₂)</td>
<td>177</td>
<td>26.7</td>
<td>116 (65.5)</td>
</tr>
<tr>
<td>Two months after the booster injection (T₁₄)</td>
<td>89</td>
<td>13.4</td>
<td>65 (73.0)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are percentages.

**Table 2. Immune response to hepatitis B vaccine among seronegative infants who received three doses at 6-month intervals**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>6 months after 1st dose (T₀)</th>
<th>8 months after 2nd dose (T₁₂)</th>
<th>2 months after booster dose (T₁₄)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of infants</td>
<td>281</td>
<td>116</td>
<td>65</td>
</tr>
<tr>
<td>No. with anti-HBs antibodies</td>
<td>185 (65.8)²</td>
<td>104 (89.7)</td>
<td>62 (95.4)</td>
</tr>
<tr>
<td>Geometric mean titre (mIU/ml)</td>
<td>6.1</td>
<td>83.7</td>
<td>348.0</td>
</tr>
</tbody>
</table>

* Figures in parentheses are percentages.
anti-HBs response of the 116 infants who received the second dose of vaccine was determined when the third (booster) injection was given (T_{12}): 104 were positive for anti-HBs (89.7%), and the anti-HBs GMT was 83.7 mIU/ml. Assay of blood samples from 65 infants 2 months after the booster dose (T_{14}) indicated that 62 (95.4%) had anti-HBs antibodies, the anti-HBs GMT reaching 348 mIU/ml (Table 2).

The geometric mean titres of anti-HBs antibodies in the 65 infants who were followed over 14 months increased from 5.6 mIU/ml (T_0) to 67.0 mIU/ml (T_{12}) and finally to 348 mIU/ml (T_{14}) (Fig. 1). The increase in titre was more important between T_0 and T_{12} than between T_{12} and T_{14}, which indicates that the second injection had a booster effect.

A subset of infants received concomitantly with the hepatitis B vaccine also BCG and DTP-polio vaccines at T_0 as well as DTP-polio at T_{12}. Seroconversion at T_0 occurred in 65.7% of the 166 infants who were immunized only against hepatitis and in 65.2% of the 115 infants who also received BCG and DTP-polio vaccine; the geometric mean titres for the two sets of infants were 6.3 mIU/ml and 5.8 mIU/ml, respectively (Fig. 2).

In all, 94% of infants who received only hepatitis B vaccine seroconverted 6 months after receiving the
second dose at $T_{12}$ (GMT, 84.3 mIU/ml). For infants who also received BCG and DTP-polio vaccine, the corresponding rate of seroconversion was 87.5% (GMT, 83.5 mIU/ml).

The distribution of anti-HBs titres found in the present study is compared in Fig. 3 with those of 72 seronegative infants who received two doses of hepatitis B vaccine at a 2-month interval and 111 seronegative infants immunized with three doses at 1-month intervals (both sets of infants also received a booster at $T_{12}$): the rates of seroconversion were similar at the end of each vaccination schedule ($T_{12}$) (89.7%, 93.1%, and 94.6%, respectively; GMT of anti-HBs: 84, 82, and 92 mIU/ml, respectively). However, at $T_{14}$ the geometric mean titre of the 65 infants in the present study (348 mIU/ml) was lower than that of the 47 infants who received three doses of the vaccine (1500 mIU/ml), as was also the proportion of infants with high anti-HBs titres.

Hepatitis B infection occurred in eight (2.8%) of 281 susceptible infants during the first 6 months of the study (Table 3), five of whom were HBsAg-positive. Over the second 6 months ($T_{9}-T_{12}$), 114 susceptible infants were followed up: none became HBsAg-positive, but two (1.8%) exhibited anti-HBc antibodies. No cases of hepatitis B occurred among the 63 susceptible infants studied during the 2 months following the booster injection ($T_{12}-T_{14}$).

No age-dependent anti-HBs response was observed for the 3–24-month-old infants at the time of the first injection. However, following the booster injection given 1 year after the initial dose in each protocol, the anamnestic response was lower for the two doses/6 months schedule (GMT, 348 mIU/ml) than for the two doses/2 months (GMT, 670 mIU/ml) or three doses/1 month schedule (GMT, 1500 mIU/ml). Since the level of anti-HBs decreases with time ($T_{10}$, $T_{11}$), the booster dose should be administered earlier than 12 months after the first injection.

The level of protection afforded by the two doses/6 months schedule, as indicated by the presence of 1.8% HBsAg-carriers at the time of the second injection, is approximately half that afforded by natural infection (6, 12, 13). This implies that the presence of only a small amount of anti-HBs is protective. The vaccine used therefore contained pre-S gene products (14), which induce highly protective antibodies (15). It should be noted, however, that the protective levels of anti-HBs will vary according to the particular vaccine used.

A schedule of immunization involving three vaccinations at 6-month intervals greatly facilitates the organization of immunization programmes in the rural areas of developing countries, since each village has to be visited only twice per annum. However, a disadvantage of such a schedule is that yellow fever and measles vaccines are then administered at the same time as the hepatitis booster to 15–18-month-old infants, which is relatively late for these two vaccines.

In order to reduce the disadvantages of the 6-month protocol, an immunization schedule of three injections with a 3-month interval is being studied. This would have the advantage of reducing the period of low protection between the first and the second doses, while the booster injection ($T_{6}$) would be given to 9–12-month-old infants.

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**Table 3. Results of follow up studies of infants who were seronegative at the initial dose of vaccine ($T_{0}$)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>$T_{0}-T_{6}$</th>
<th>$T_{6}-T_{12}$</th>
<th>$T_{12}-T_{14}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of seronegative infants</td>
<td>281</td>
<td>114</td>
<td>63</td>
</tr>
<tr>
<td>No. of HBsAg-positive infants</td>
<td>5 (1.8$^a$)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No. of cases of hepatitis B infection</td>
<td>8 (2.8)</td>
<td>2 (1.8)</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ Figures in parentheses are percentages.
ACKNOWLEDGEMENTS

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RÉSUMÉ

ESSAI CLINIQUE DU VACCIN ANTI-HÉPATITE B DANS LE CADRE D’UN PROGRAMME SIMPLIFIÉ DE VACCINATION

La réponse immunitaire anti-HBs a été étudiée chez des enfants sénégalais recevant deux doses de vaccin anti-hépatite B à 6 mois d’intervalle. Une dose de rappel était injectée 6 mois après la deuxième dose de vaccin. Les résultats obtenus ont été comparés à ceux obtenus lors de deux études antérieures qui utilisaient des protocoles à 2 doses injectées à 2 mois d’intervalle et à 3 doses injectées à 1 mois d’intervalle.

Les anticorps anti-HBs ont été détectés chez 65,8% des enfants 6 mois après la première injection de vaccin (le jour de la deuxième injection). Le titre moyen géométrique des anticorps était de 6,1 mUI/ml. Le jour de l’injection de rappel, 6 mois après la deuxième injection, 89,7% des enfants étaient anti-HBs positifs et le titre moyen géométrique des anticorps était de 83,7 mUI/ml. Ces résultats sont comparables à ceux observés avec les deux autres protocoles de vaccination. Après l’injection de rappel, 95,5% des enfants étaient anti-HBs positifs, avec un titre moyen géométrique des anticorps de 348 mUI/ml. Ce chiffre est plus faible que celui observé dans les deux autres protocoles de vaccination (670 et 1500 mUI/ml).

Les résultats montrent également que la protection est faible pendant les 6 premiers mois, c'est-à-dire avant la deuxième injection, et que la troisième injection intervient chez des enfants relativement âgés. Il est alors un peu tard pour associer cette troisième injection aux vaccins anti-amari et antirougeoleux. Un protocole à 3 injections à 3 mois d’intervalle devrait être plus approprié au conditions de terrain, tout en apportant une meilleure protection.

REFERENCES
