Antibody response to *Haemophilus influenzae* type b tetanus conjugate vaccine with two doses given at 3 and 5 months of age

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**Background:** In developed countries, the use of Hib conjugate vaccines has led to the near disappearance of invasive Hib disease, but costs have limited its use in developing countries. In order to identify more economical vaccination schedules, we carried out a trial to evaluate the immunogenicity of an alternative two-dose PRP-T regimen, based on a previous report in which carrier priming could be obtained with prior diphtheria–tetanus–pertussis (DTP) vaccination.

**Methods:** Healthy infants were enrolled to receive the PRP-T given at 3 and 5 months of age, with DTP vaccination given at 2, 4, and 6 months of age. Serum specimens were obtained at 3, 6, and 15 months of age. IgG anti-Hib titer determination was performed using enzyme-linked immunosorbent assay to evaluate serologic response and its duration.

**Results:** One-hundred and seventeen infants were enrolled. The geometric mean titer (GMT) of antibody to PRP was low in the pre-immunization samples (0.13 µg/mL), achieving high values after two doses of PRP-T (27.42 µg/mL), with all titers over 1 µg/mL; the GMT at 15 months was 5.45 µg/mL; 94.6% of infants had serologic responses after the two doses of vaccination, with average intervals of 27 and 22 days between DTP and PRP-T first-to-first and second-to-second administrations, respectively. However, these intervals were 11 and 3 days for infants who did not have serologic responses (P=0.0013 and 0.0030, respectively).

**Conclusions:** These results indicate that two doses of PRP-T can induce high antibody titers using the proposed schedule; moreover, the GMT assessed at 15 months of age was also protective. The enhanced immune response observed in the study could be explained by the previous administration of the DTP vaccine, since the longer the interval between DTP and PRP-T, the better the response to Hib vaccine. The PRP-T vaccine given at 3 and 5 months of age may be an economical alternative to the current proposed schedule, which could make the introduction of Hib vaccination in developing countries more feasible, considering the relatively high cost of this vaccine.

**Key Words:** children, *Haemophilus influenzae*, vaccination

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**INTRODUCTION**

The disease caused by *Haemophilus influenzae* type b (Hib) has worldwide distribution. Until recently, Hib constituted the principal pathogen causing meningitis in the USA, with an attack rate of 47 cases/100 000 children younger than 5 years old, and an attack rate of 130 cases/100 000 infants younger than 1 year old. On the other hand, the attack rate in Europe is approximately half that in the USA, with 20–30 cases/100 000 children younger than 5 years old.1

However, in developing countries there are reports of higher incidences: Gambia has an incidence per year of 60 cases/100 000 children younger than 5 years old.1

In total, 6 684 cases of Hib meningitis were reported to the Brazilian National Health Surveillance System between 1987 and 1991. Infants younger than 1 year old were mostly involved, with an attack rate of 17.7 cases/100 000 inhabitants. Attack rate sharply decreased to 4.8/100 000 at age 1–4 years.2 Probably, these numbers do not correspond to reality, due to lack of information from the surveillance system and difficulty in making the accurate diagnosis.

In the USA, only 40% of cases of Hib meningitis occur in children younger than 1 year of age, but in developing countries, 67–84% of cases of Hib meningitis occur in children younger than 1 year of age.1

An 11-year study of invasive *Haemophilus* infections in São Paulo, Brazil demonstrated that 48.6% of Hib infections were identified in children younger than 1
year of age, the median was 1 year of age, and the peak was observed at 7 months of age. The mortality also ranged from 1% to 8% in developed countries, compared to 30% or more in developing countries, and permanent sequelae (e.g. hearing and mental deficiency) were observed in 20% of the survivors. In Brazil, the overall fatality rate of Hib meningitis was 15.9%. These worldwide epidemiologic discrepancies and cost-effectiveness issues concerning Hib immunization reinforce the need to consider different vaccination schedule programs based on local epidemiologic data and economical standards.

Anti-Hib conjugated vaccines were introduced into routine vaccinations in several countries after they were licensed in January 1991. Vaccines from different manufacturers have provided significant reductions in the Hib attack rate worldwide. In the city of Curitiba, Brazil, there was a significant decrease in the attack rate of Hib meningitis in children younger than 5 years old after vaccination was started (from 35.4 cases/100,000 children in 1996 to 9.73 cases/100,000 in 1997). Although a single dose of vaccine given at 15 months of age can induce protection, the central issue is how to protect younger infants, who are the main target of the disease, with less doses than the proposed four-dose schedule starting at 2 months of age.

Different strategies have been tried to achieve this goal. In 1996, Chile became the second developing country to introduce the vaccine anti-Hib in their calendar, exploring ways to lower the cost of vaccination without compromising immunization effectiveness. Comparing 627 children allocated to receive one of the four proposed schedules—three full doses; three one-half doses; three one-third doses; and two full doses—Lagos et al concluded that all the schedules resulted in an appropriate serologic response, and the fractional doses (half or a third) were able to induce appropriate serum concentrations of antibodies (>0.15 μg/mL) in 91-100% of immunized infants. This rate was similar to the 93% rate achieved with three full doses.

The PRP-T vaccine is highly immunogenic in adults, infants and children older than 2 months of age. Although the immune response in children is correlated with age, one to three doses are required to induce high titers of antibodies in children younger than 6 months of age. Serum titers over 1 μg/mL are considered protective, and the estimated level of 0.15 μg/mL is now generally accepted. These antibodies can be passively acquired through maternal-fetal transmission, or, in the postpartum period, through natural exposure to Hib or specific immunization.

In a study by Hessel et al, where children were immunized at 3, 4 and 5 months of age, the geometric mean titers (GMTs) of anti-Hib were 6.8 μg/mL at 5 months of age and 8.8 μg/mL at 6 months of age. Recent studies in the USA, Finland and France showed GMTs of antibodies of 6-10 μg/mL in children immunized with three doses during the initial 6 months of life.

Since October 1992, PRP-T has been used in a program of accelerated immunization that consists of three doses at 2, 3 and 4 months of age in the UK, or two doses at 4 and 6 months of age in Ireland. These immunization schedules do not use boosters, because if the children are protected, a natural booster would occur without risk of developing disease. Moreover, previous vaccination with DT or diphtheria-tetanus-pertussis (DTP) enhanced the magnitude of the response of anti-PRP to this conjugated vaccine.

Hib vaccination has now been introduced into the routine Brazilian vaccination program, which fosters the evaluation of new, cost-effective immunization approaches. We conducted this study to evaluate the antibody response against Hib using a two-dose schedule of PRP-T at 3 and 5 months of age. The age group chosen for vaccination was based on the following: (1) the epidemiologic need for early protection; (2) knowledge of the priming effect of the tetanus toxoid, the carrier protein in the PRP-T, providing a better response to anti-PRP; and (3) the tetanus toxoid is in fact routinely administered at 2, 4 and 6 months of age, through the vaccine DTP. We chose an age group for anti-Hib vaccination such that the vaccine DTP had already been received.

MATERIALS AND METHODS

Patients and eligibility

We enrolled 127 children who received primary care at the Section of Vaccination of the Hospital do Servidor Público Estadual de São Paulo and who were eligible to participate. We obtained parents' or legally responsible guardians' informed consent. Exclusion criteria included known immunologic disorders or previous history of anti-Hib vaccination.

Vaccine

The vaccine used in this study was PRP-T (ActHIB, Pasteur Merieux) given in a dose of 0.5 mL intramuscularly in the anterolateral part of the thigh.

Immunization schedule

The immunization schedule used was a regimen with two doses given at 3 and 5 months of age without booster (with a tolerance of 15 days after 3 or 5 months of age).

The use of a small number of doses was decided upon to investigate the possibility of a reduction in costs. However, since the results of the serologic assays were not readily available, a booster was applied at 15 months of age without follow-up.
Blood samples and Hib serology

Venous blood samples were collected, separated and stored at -20°C until the assays. These samples were collected in three time-points: (1) before immunization; (2) at 6 months of age; and (3) at 15 months of age (with a tolerance of 30 days after 6 or 15 months of age), in order to evaluate the seroconversion rate and the duration of the acquired immunity (Figure 1).

IgG anti-Haemophilus influenzae type b polysaccharide was determined by an enzyme-linked immunosorbent assay (ELISA) employing microplates (Cova Link NH, Nunc) for antigen adsorption, as described by Zielen et al. with some modifications. Briefly, at the time of assay, 5 mg/L unconjugated Hib PRP was diluted with 1% 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (Sigma, USA) and incubated in the wells overnight at 37°C. After washing with phosphate-buffered saline (PBS) 0.05% Tween, the serum samples were incubated in four serial dilutions for 2 h at 37°C. After washing with PBS–Tween, HRPO-conjugated anti-IgG (Sigma, USA) diluted 1 : 3000 was added to the plates, which were incubated for 90 min at 37°C. After washing, the substrate solution containing 0.4 mg orthophenylene-diamine and 0.01% a final concentration of H2O2 in 0.1 M citrate–phosphate buffer was added. After 30 min at room temperature, the color reaction was stopped by the addition of 2.5 N H2SO4. The absorbances were read in the plate reader (Flow, USA).

Statistical analysis

The results were analyzed using Epi Info version 5.1.b (Centers for Disease Control and Prevention–World Health Organization) and Microsoft Excel version 5.0 software. Statistically significant differences were considered for P<0.05.

RESULTS

One hundred and twenty-seven children were enrolled in the study. Ten children were excluded from the analysis for the following reasons: three children had just received the first dose of the vaccine anti-Hib, and seven children did not have serologic follow-up. The final number was therefore 117.

Seven-one children were male (60.6%) and forty-six were female (39.4%).

Serology

The GMTs pre-immunization were available for 115 children; 7 (6.1%) had titers over 1.0 µg/mL, and the average was 0.13 µg/mL.

GMTs after the second dose of the vaccine anti-Hib and at 15 months of age were, respectively, 27.42 µg/mL and 5.45 µg/mL (Table 1).

While 93.9% of children were considered susceptible to Hib prior to vaccination, because they had titers of antibodies lower than 1.0 µg/mL, after vaccination with two doses, 94.6% of children had documented seroconversion. At 15 months of age, 80% of children still had protecting titers of anti-Hib, as shown in Table 2.

Interval between the doses of DTP and PRP-T

The interval between the first dose of vaccine DTP and the first dose of vaccine anti-Hib was, on average, 27 days. For second doses, this interval was, on average, 22 days.

Table 1. Geometric mean titer of anti-Hib antibodies prior to immunization, at 6 and 15 months of age

<table>
<thead>
<tr>
<th></th>
<th>GMT (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>Prior to immunization</td>
<td>0.13 µg/mL (0.02–2.2)</td>
</tr>
<tr>
<td>At 6 months of age</td>
<td>27.42 µg/mL (13.1–156.1)</td>
</tr>
<tr>
<td>At 15 months of age</td>
<td>5.45 µg/mL (2.3–58.1)</td>
</tr>
</tbody>
</table>

Table 2. Percentage of children with protecting antibody titers who received the anti-Hib vaccination with two doses of PRP-T

<table>
<thead>
<tr>
<th>Anti-Hib antibodies</th>
<th>N</th>
<th>&gt;0.15 µg/mL</th>
<th>&gt;1.0 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to vaccination</td>
<td>115</td>
<td>40% (N=46)</td>
<td>6.1% (N=7)</td>
</tr>
<tr>
<td>At 6 months of age</td>
<td>111</td>
<td>98.1% (N=109)</td>
<td>94.6% (N=105)</td>
</tr>
<tr>
<td>At 15 months of age</td>
<td>100</td>
<td>93% (N=93)</td>
<td>80% (N=80)</td>
</tr>
</tbody>
</table>

* Venipuncture

Figure 1. Vaccination and sample collection schedule.
Table 3. Averages of days between the vaccines DTP and PRP-T compared to seroconversion to Hib

<table>
<thead>
<tr>
<th></th>
<th>Average of interval between the first doses (days)</th>
<th>Average of interval between the second doses (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seroconversion (+)</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>6 months (N=105)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seroconversion (−)</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>6 months (N=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.0013</td>
<td>0.0030</td>
</tr>
<tr>
<td>Seroconversion (+)</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>15 months (N=80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seroconversion (−)</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>15 months (N=20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.12</td>
<td>0.03</td>
</tr>
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</table>

DISCUSSION

The capsular polysaccharide constitutes a major virulence factor of Hib. PRP conjugated vaccines are able to stimulate an appropriate immune response in infants, inducing protective antibodies against PRP of the bacteria. Several studies have demonstrated the safety and the immunogenicity of the PRP-T vaccine. These studies, mostly conducted in developed countries, adopted a three-dose schedule.

England and Ireland initiated immunization programs in early infancy, where the incidence of invasive disease has a substantial peak, without booster doses, aiming early protection. On the other hand, some studies in Latin America have suggested that carrier priming with DTP can make the schedule with two doses an acceptable alternative to the standard regimen. Our results agree with this hypothesis, because only two doses of the PRP-T vaccine were able to induce high and durable titers of antibodies.

The high titers of antibodies after the complete immunization were surprising. Recent studies of the PRP-T vaccine in children in the USA, Europe and Chile demonstrated anti-PRP GMTs ranging from 3.7 to 11.3 μg/mL. However, the GMT in our study was substantially higher (27.42 μg/mL).

A possible explanation for this finding is that the vaccines DTP and PRP-T have been applied at different time points. The administration of the DTP on average 25 days before the vaccine PRP-T may have resulted in a carrier priming phenomenon. Granoff et al demonstrated that infants vaccinated with DT at 1 month of age and subsequently vaccinated with PRP-T at 2, 4 and 6 months of age had their immune response increased threefold (GMT=11.5 μg/mL) when compared to infants who were not primed with DT. These authors suggested that priming can be useful to accelerate acquisition of immunity to Hib in children younger than 6 months of age, who are at the age of maximum risk for Hib disease.

This priming also explains the high titers found in Venezuelan children vaccinated with PRP-T at 2, 4 and 6 months of age preceded by DTP vaccine by, on average, 6.5 days. The anti-PRP GMT was 37.9 μg/mL, whereas that of children vaccinated simultaneously with DTP and PRP-T was 3.63 μg/mL (P<0.00001).

However, in our study, it is possible that the higher antibody titers reflect a longer interval between the priming with DTP and PRP-T vaccination. The statistically significant differences demonstrate that previous vaccination with DTP with a longer interval of time can enhance the immune response to Hib, when the vaccine PRP-T is used.

Other explanations should be considered, such as genetic factors and possible natural exposure.

Therefore, the vaccine anti-Hib with PRP-T given at 3 and 5 months of age, in addition to DTP vaccination at 2, 4 and 6 months of age, may constitute a safe and feasible alternative for introduction into the immunization program, with costs substantially lower, despite two extra visits to the immunization center. Although we have created a complex vaccine schedule, interposing 3- and 5-month doses of PRP-T between the 2-, 4- and 6-month doses of DTP without measuring compliance and indirect costs, we believe that this schedule makes sense when earlier protection (5 months versus 6 months) is desirable, given the local epidemiology. An alternative dosing schedule might have been 4 and 6 months. This would allow sufficient time following the infant tetanus priming dose and would minimize the number of immunization visits and associated costs. Further studies would be helpful to find more about the actual month-specific incidence of disease caused by Hib in this population.

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REFERENCES

2. CENEF/FNS/MS. A meningite por Haemophilus no Brasil. Doencas imunopreveníveis—Informe quinzenal 1993; VII–(no. 5).

Table 3. Averages of days between the vaccines DTP and PRP-T compared to seroconversion to Hib


