Controlled trial of immune response of preterm infants to recombinant hepatitis B and inactivated poliovirus vaccines administered simultaneously shortly after birth

Nehama Linder, Rachel Handsher, Boris German, Lea Sirota, Mike Bachman, Sigal Zinger, Ella Mendelson, Asher Barzilai

Abstract
Aim—The study was conducted to evaluate the immunogenicity of an early, extra dose of enhanced inactivated poliovirus vaccine (IPV) administered simultaneously with recombinant hepatitis B vaccine (HBV) to preterm infants shortly after birth.

Methods—Three groups were studied. Fifty preterm infants received IPV intramuscularly within 24 hours of birth, in addition to routine recommended childhood immunisations. Fifty two preterm infants and 35 full term infants received routine immunisations only (routine vaccination timing: HBV at birth, 1 and 6 months of age; IPV at 2 and 4 months; oral polio vaccine (OPV) at 4 and 6 months; diphtheria-tetanus-pertussis (DTP) at 2, 4, and 6 months; and Haemophilus influenzae B vaccine at 2 and 4 months). Blood samples were taken at birth, 3 and 7 months of age from all infants, and at 1 month of age from preterm infants only.

Results—At birth, a lower percentage of both study and control preterm infants had antipoliovirus type 3 titres $\geq 1:8$ than full term infants. At 1 and 3 months of age significantly more early IPV infants had antipoliovirus type 3 titres $\geq 1:8$ than routinely vaccinated preterm infants ($p < 0.05$). At 7 months of age there were no significant differences in percentage of antipoliovirus titres $\geq 1:8$ or geometric mean times (GMTs) between the early IPV group and the routinely vaccinated preterm group. At 3 and 7 months of age, the percentage of positive antihepatitis B titres ($\geq 1:10$) and the GMT of the early IPV preterm group did not differ significantly from those of preterm controls. There was no significant difference in percentage of positive antihepatitis B titres between the early IPV group and full term controls at any time. GMTs for hepatitis B antibodies were significantly lower in the early IPV preterm group than in full term controls at 3 and 7 months of age.

Conclusions—Administration of an additional dose of IPV simultaneously with routine HBV to preterm infants shortly after birth provides early protection from poliovirus and hepatitis B infection, and does not interfere with poliovirus antibody production at the age of 7 months.

Keywords: preterm infants; hepatitis B; poliovirus; vaccination; antibody

During the summer of 1988, an outbreak of poliovirus type 1 occurred in Israel. One of the victims was a 2 month old baby who had not yet been immunised.1 Two studies performed since then have shown that approximately 50% of preterm infants lack positive titres ($\geq 1:8$) to at least one of the poliovirus types, and may benefit from early vaccination with inactivated polio vaccine (IPV).2 3

In 1992 hepatitis B vaccine (HBV) was introduced in Israel for vaccination of all newborns, including preterm infants, soon after birth. Concomitant administration of HBV and IPV has been shown to be effective in full term infants,4 but there are no studies in preterm infants.

This study aimed to evaluate the immunogenicity of IPV and HBV administered simultaneously to premature infants within the first 24 hours of life.

Materials and methods
SUBJECTS
The study population included 177 infants: 127 preterm infants and 50 full term infants. Preterm infants were born between June and December 1994 (gestational age 30–35 weeks, weight > 2500 g), born consecutively in the morning hours between 1 June 1994 and 15 June 1994 were recruited as controls. Forty children were excluded: 22 preterm infants who received blood products, three preterm infants with sepsis, and 15 full term infants who were withdrawn by their parents after the first blood test.

The preterm infants were divided into a study group (group A; n = 50) and a control group (group B; n = 52) by 1:1 randomisation using a blinded envelope drawn by the parents. All full term infants were included in control group C.

The study protocol was approved by the hospital human ethics committee and informed parental consent was obtained for all subjects.

Infants in the study group A received an extra, early dose of IPV within 24 hours of birth, simultaneously with the routine HBV, followed by all routine vaccinations at the appropriate times (table 1). The early IPV was administered to one thigh and the HBV to the
other thigh. Preterm infants in group B and full term group C received only routine immunisations (table 1).

Blood samples for the determination of antibody titres were obtained from all infants within the first 24 hours of life and at 3 and 7 months of age, and additionally from infants in groups A and B at 1 month of age.

**SEROLOGY**

Sera were kept frozen at −20°C until used. A microneutralisation system for the three poliovirus antibody serotypes.5 Serial twofold dilutions of sera were prepared and divided into aliquots in duplicate into 96-well microtitre plates; 32–100 TCID₅₀ of poliovirus were added to each well. The mixtures were incubated at 36°C for 20 hours, and then 20,000 Hep-2 cells were added to each well. The plates were incubated at 36°C for four days. On the fifth day, cells were fixed, stained, and examined macroscopically for cytopathic effects.

Titres were determined as the highest dilution of serum protecting 50% of the cultures against challenge virus. Titres ≥1:8 were considered seropositive.

Hepatitis B surface antibodies (HBsAB) were tested for by radioimmunoassay and interpreted as instructed by the manufacturer (Abbott Diagnostics, Chicago, Illinois, USA). A positive HBsAB titre was defined as 1:10 miu/ml.

**STATISTICAL ANALYSIS**

Statistical analysis was performed using the BMDP Statistical Software Package.7 The proportion of infants in each group with detectable antibodies to poliovirus types 1, 2, and 3 was compared for each type of antibody using the Pearson χ² test. Seroconversion was defined as the appearance of neutralising antibodies in seronegative infants or a fourfold or greater increase over the titre expected following the decay of transplacentally acquired antibodies. The expected titre was calculated using an antibody half life of 28 days.8–9 The proportion of infants seroconverting was compared for each type of antibody using the Pearson χ² test.

The geometric mean titres (GMTs) for antipolio were derived from the mean values after log, transformations. Titres < 1:8 were calculated as 1:4, whereas titres > 1:8192 were calculated as 1:16 384. The GMTs for antihepatitis B were calculated from the log₁₀ of the titres. Titres < 1:10 were calculated as 1:5, while titres > 1:1000 were calculated as 1:2000.

To determine the significance of the differences in antibody levels, we applied analysis of variance to the log, values (antipolio titres) and the log₁₀ values (antihepatitis B titres) of the reciprocal antibody titres with Bonferroni’s correction for multiple comparisons. All statistical tests were two sided with a significance level of p < 0.05.

**Results**

Patient characteristics are presented in table 2. Groups A and B were similar for sex distribution, median gestational age, and mean birth weight. No local or systemic side effects to either vaccine were noted.

**POLIO**

The percentage of infants with antibody titres ≥1:8 and the GMTs for poliovirus types 1, 2, and 3 are shown in tables 3 and 4. At birth, a lower percentage of preterm infants (from both preterm groups) than full term infants had antipolio type 3 antibody titres ≥ 1:8 (p < 0.05). At age 3 months, group A had significantly higher early IPV group A premature infants had antipolio virus type 3 titres ≥ 1:8 than routinely vaccinated premature controls (group B) at 1 and 3 months (p <0.05). At age 3 months, group A had significantly higher GMT levels for poliovirus 2 and 3 than group B (317.4 v 106.2; p < 0.01 and 218.3 v 72.0; p < 0.05 respectively). At 7 months there were no differences in percentage of infants with positive antibody titres ≥ 1:8 or GMT levels among the three groups.

Lower antibody titres (< 1:128) at birth to each of the three poliovirus types were noted in preterm infants compared with full term infants. At 7 months, a significantly higher percentage of preterm infants had poliovirus types 1, 2, and 3 antibody titres ≥ 1:128 (p < 0.05), with GMTs being higher in the preterm groups.

**Table 1** Vaccination protocol: first six months of life

<table>
<thead>
<tr>
<th>Vaccination</th>
<th>Birth</th>
<th>One month</th>
<th>Two months</th>
<th>Four months</th>
<th>Six months</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPV</td>
<td>X*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPV</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTP</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>HiB</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

*Only study group A received IPV at birth.

**Table 2** Clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Preterm study group (A) (n = 50)</th>
<th>Preterm control group (B) (n = 52)</th>
<th>Full term control group (C) (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>23/27</td>
<td>22/30</td>
<td>17/18</td>
</tr>
<tr>
<td>Median (range) gestational age (weeks)</td>
<td>32 (30–35)</td>
<td>32 (30–35)</td>
<td>40 (38–42)</td>
</tr>
<tr>
<td>Mean (SD) birth weight (g)</td>
<td>1528 (384)</td>
<td>1597 (402)</td>
<td>2990 (676)</td>
</tr>
</tbody>
</table>

**Table 3** Percentage of infants with protective antibody titres ≥1:8 for the poliovirus types 1, 2, and 3

<table>
<thead>
<tr>
<th>Age</th>
<th>Poliovirus type 1</th>
<th>Poliovirus type 2</th>
<th>Poliovirus type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>24 hours</td>
<td>100</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>1 month</td>
<td>100</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>3 months</td>
<td>100</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>7 months</td>
<td>98</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*p < 0.05 (A v B); †p < 0.05 (B v C); ‡p < 0.05 (A v C); all significance values follow Bonferroni’s correction for multiple comparisons.

A, group A (n = 50); B, group B (n = 52); C, group C (n = 35); ND, not done.
Table 4  Geometric mean titres for antibodies to poliovirus type 1, 2, and 3

<table>
<thead>
<tr>
<th>Age</th>
<th>Poliovirus type 1</th>
<th>Poliovirus type 2</th>
<th>Poliovirus type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>24 hours</td>
<td>189‡</td>
<td>163‡</td>
<td>534</td>
</tr>
<tr>
<td>1 month</td>
<td>117</td>
<td>79</td>
<td>ND</td>
</tr>
<tr>
<td>3 months</td>
<td>98</td>
<td>81</td>
<td>59</td>
</tr>
<tr>
<td>7 months</td>
<td>1144</td>
<td>1121</td>
<td>1783</td>
</tr>
</tbody>
</table>

*p < 0.05 (A vs B); †p < 0.05 (B vs C); ‡p < 0.05 (A vs C); all significance values follow Bonferroni’s correction for multiple comparisons.

A, group A (n = 50); B, group B (n = 52); C, group C (n = 35); ND, not done.

Table 5  Geometric mean titres (GMT) for hepatitis B surface antibody and percentage of infants with titres > 1:10

<table>
<thead>
<tr>
<th>Age</th>
<th>GMTs for hepatitis B</th>
<th>% with antibody titres &gt; 1:10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>24 hours</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 month</td>
<td>21*</td>
<td>10</td>
</tr>
<tr>
<td>3 months</td>
<td>91‡</td>
<td>51‡</td>
</tr>
<tr>
<td>7 months</td>
<td>273‡</td>
<td>420</td>
</tr>
</tbody>
</table>

*p < 0.05 (A vs B); †p < 0.05 (B vs C); ‡p < 0.05 (A vs C); all significance values follow Bonferroni’s correction for multiple comparisons.

A, group A (n = 50); B, group B (n = 52); C, group C (n = 35); ND, not done.

Table 5 shows the percentage of infants with antibody titres > 1:10 and the GMTs for antihepatitis B. There was no significant difference between the proportion of early IPV infants reaching protective antihepatitis B titres in either of the control groups at 3 and 7 months of age. There was no difference in antihepatitis B GMT between group A and group B at 3 and 7 months of age. However, the early IPV (group A) GMT was significantly lower than that of full term controls at 3 and 7 months of age.

**Discussion**

During recent outbreaks of poliomyelitis in Israel and the Netherlands, several infants who had not yet received their first routine immunisation were affected. In developing countries where poliomyelitis and hepatitis B are endemic, infection with both viruses can occur simultaneously. Early, conjoint administration of viral vaccinations would be advantageous for preterm infants who are a particularly vulnerable group because of lower titres of maternally acquired antibodies. Good immune response to oral polio vaccine (OPV) and IPV given to preterm infants at two months of age has been reported. Pagano et al were the first to report a good response of preterm infants to immunisation against poliovirus 1 with OPV given soon after birth. Our group found previously that administration of IPV to preterm infants soon after birth significantly decreases the proportion of infants susceptible to poliovirus type 3, and raises the GMTs for poliovirus types 1 and 3 at the age of 3 months. Although the age when hepatitis B vaccination for preterm infants should be initiated is controversial, it is important that it is administered early to preterm infants living in countries where the disease is endemic.

The results of the present study confirm that immunisation of preterm infants with IPV and HBV soon after birth results in a significantly higher proportion of infants with positive antibody titres against poliovirus 3 at 1 and 3 months of age. In addition, early immunisation resulted in significantly higher GMT levels for antipoliovirus types 2 and 3 at age 3 months.

One of the problems associated with early immunisation is the possible interference of maternal antibodies in the infant’s immune response. Dong et al showed that the seroconversion is delayed in the presence of high maternal antibodies. This is true for poliovirus vaccine administered to full term infants during the first 24 hours of life. In this study, lower antibody titres at birth were associated with a better immune response to all the poliovirus types in both the study and the control groups.

Concurrent administration of HBV and IPV has been studied in full term infants. No impairment in the expected protective efficacy was noted when HBV was administered with: BCG and IPV, diphtheria-tetanus (DT) and diphtheria-tetanus-pertussis (DTP), OPV, group A meningococcus vaccine, measles vaccine, Japanese B encephalitis vaccine, and hepatitis A vaccine. Our study is the first to investigate early simultaneous HBV and IPV vaccination in preterm infants. The percentage of early IPV infants who reached protective antihepatitis B titres by 7 months of age was similar to full term controls, although the study group antihepatitis B GMT was significantly lower. The cause is unclear and is most likely the effect of gestational age.

We conclude that the addition of an early IPV to the vaccination protocol significantly decreases the proportion of preterm infants susceptible to poliovirus type 3 at 1 and 3 months of age. Simultaneous administration of HBV and IPV to preterm infants within 24 hours of birth appears to be safe and effective, providing early protection against the two diseases. This approach may be useful in countries where both hepatitis B and poliomyelitis are endemic.

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Preterm immune response to vaccines