DT5aP-Hib-IPV and MCC vaccines: preterm infants’ response to accelerated immunisation

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Preterm infants mount an immune response to the antigens contained in combined diphtheria/tetanus/whole cell pertussis (DTwP) vaccines equivalent to that seen in term infants, under differing schedules. In addition, we have shown acceptable responses to the protein antigens of a DT-3 component acellular pertussis (aP) vaccine given under an accelerated schedule. Immune responses to inactivated polio vaccine (IPV) and oral polio vaccine (OPV) are similar to those seen in term infants, and preterm infants are able to mount a protective neutralising antibody response to IPV given in the first 24 hours of life. The proportion of preterm infants achieving protective titres against poliovirus type 3, however, may be reduced when a schedule using both IPV and OPV is used.

We have investigated the response of preterm infants to a combined DT5aP-Hib-inactivated polio vaccine (IPV) containing aluminium phosphate adjuvant given at the same time as a meningococcal serogroup C vaccine under the United Kingdom’s accelerated schedule.

METHODS

Infants born at less than 32 weeks gestation were recruited from three UK neonatal units: St Mary’s Hospital, Portsmouth; Royal Hampshire County Hospital, Winchester; and Princess Anne Hospital, Southampton. The study had local research ethics committee approval in all three centres and took place between May 2002 and March 2003. Informed consent was obtained from the parents of the infants involved. As part of a larger randomised study, term infants were recruited from immunisation clinics in Hertfordshire and Gloucestershire, UK. This study was approved by the London Multi-centre Research Ethics Committee and by the local ethics committees for the sites involved.

Abbreviations: aP, acellular pertussis; DT5aP-Hib-IPV, diphtheria/tetanus/5 component acellular pertussis-Haemophilus influenzae type b inactivated polio vaccine (DT5aP-Hib-IPV) and meningococcal serogroup C conjugate vaccine (MCC) under accelerated schedule. To compare results with term infants immunised with DT5aP-Hib-IPV and with historical data from preterm infants immunised with a DT3 component aP-Hib vaccine.

Methods: Prospective observational study in preterm infants born at <32 weeks gestation with comparison to contemporary cohort of term infants. DT5aP-Hib-IPV and MCC vaccines were given at 2, 3, and 4 months.

Results: Fifty preterm infants (mean gestational age 28.5 weeks) completed the study. After three doses of vaccines Hib polysaccharide IgG geometric mean concentration (GMC) was 1.21 µg/ml with 80% ≥0.15 µg/ml; MCC serum bactericidal assay geometric mean titre (GMT) was 1245 with 100% ≥8. All infants achieved protective titres to diphtheria, tetanus, and the three poliovirus types with ≥80% achieving protective rises in IgG against the five pertussis antigens.

Conclusion: Preterm infants immunised with DT5aP-Hib-IPV and MCC vaccines show IgG responses to Hib and MCC greater than seen historically in both term and preterm infants with a DT3aP-Hib vaccine, and for pertussis antigens and poliovirus type 1 responses similar to that seen in term infants immunised with DT5aP-Hib-IPV. Responses to poliovirus types 2 and 3 are reduced, but all infants achieved protective titres.
antigen units of poliovirus type II, 32D antigen units of poliovirus type III, and equivalent of 10 μg of Hib polysaccharide conjugated to tetanus toxoid) was given into the right thigh. MCC (Meningitec, Wyeth, 0.5 ml dose containing 10 μg meningococcal serogroup C oligosaccharide conjugated to approximately 15 μg CRM197 protein) was given into the left thigh of the preterm infants. Menjugate (Chiron, 0.5 ml dose containing 10 μg meningococcal serogroup C oligosaccharide conjugated to approximately 15 μg CRM197 protein) was given into the left thigh of the term infants. Where post-third dose PRP IgG concentration was <1.0 μg/ml a fourth additional dose of Hib conjugate vaccine (Hiberix, GlaxoSmithKline, containing 10 μg of Hib polysaccharide conjugated to tetanus toxoid) was given.

**Assays**

Blood was taken by needle venepuncture into Microtainer Serum Separator tubes just prior to the first and 4–6 weeks after the third vaccine was given. Antibody concentrations to PRP, diphtheria, and tetanus were assayed at HPA Porton Down by enzyme linked immunosorbent assay (ELISA).1 Serum bactericidal assay (SBA) to MCC was determined by the HPA Meningococcal Reference Unit, Manchester using rabbit complement according to established techniques.15 Antibody concentrations to the pertussis antigens (FHA, Fim2&3, PRN, PT) were determined by ELISA and neutralising antibody titres to poliovirus types 1, 2, and 3 by neutralisation assay at the laboratories of AventisPasteur, USA. Where compared, assays for term and preterm infants were performed in the same laboratories.

In infants where there was insufficient serum available for all vaccine antigen assays to be performed, the order of priority (decreasing) was as follows: pertussis antigens, PRP, MCC, tetanus, poliovirus type 1, diphtheria, and poliovirus types 2 and 3.

**Statistical analysis**

Antibody concentrations and titres were log10 transformed to achieve normality. PRP geometric mean concentration (GMC) and diphtheria, tetanus, FHA, Fim2&3, PRN and PT IgG, MCC SBA, and poliovirus types 1, 2, and 3 neutralising antibody geometric mean titre (GMT) were calculated with 95% confidence intervals (95% CI). For computational ease infants with undetectable PRP responses were given a value of 0.08 μg/ml and a titre of 2 for undetectable MCC SBA responses. Single variable linear regression analysis was performed to evaluate the influence of gestational age and weight at birth, pre-immunisation IgG levels, age at third immunisation, and number of doses of antenatal steroids on the IgG response to each of the vaccine antigens. Variables with a p value <0.2 in single variable analysis were examined further using multivariable analysis.

**RESULTS**

Sixty term infants and 55 preterm infants were recruited to the study. Of the preterm infants, 50 completed their primary immunisations. Of the five who did not, two died following first immunisation, one from chronic lung disease and one from non-accidental injury. Two further infants were withdrawn from the study by their parents: one following an Epstein-Barr virus associated encephalitis and one who became unsettled in the week following the second immunisation. One infant was excluded following immunisation outside the study.

Mean gestational age at birth of the preterm infants who completed the study was 28.5 weeks (range 23.9–31.9 weeks) and mean birth weight 1144 g (range 520–2020 g). Mean age and weight at first immunisation were 63 days (range 54–89 days) and 2367 g (range 1230–4000 g) respectively.

For the preterm infants, PRP IgG GMC prior to first immunisation was 0.14 μg/ml (95% CI 0.11 to 0.17 μg/ml). This rose to 1.21 μg/ml (95% CI 0.73 to 2.03 μg/ml) following third immunisation (p < 0.0001). These results, and the proportions achieving protective titres, are shown in table 1. Of note, 20% of preterm infants had undetectable PRP IgG after the three primary immunisations and 40% had a concentration <1.0 μg/ml. Sixteen infants with a PRP IgG <1.0 μg/ml after the three primary immunisations received an additional fourth dose of Hib at a mean age of 6.6 months (range 5.6–8.0 months). PRP IgG GMC of these infants was 0.20 μg/ml (95% CI 0.11 to 0.37 μg/ml) post-third dose, rising to 1.09 μg/ml (95% CI 0.44 to 2.68 μg/ml) one month after a fourth dose (p = 0.002). After the 4th, additional, dose of Hib, six of the 16 infants had a PRP IgG concentration <1.0, with three infants <0.15 μg/ml.

Four infants received postnatal steroids for chronic lung disease. In two of these there was insufficient blood to determine PRP IgG post-third dose. The remaining two infants had PRP IgGs post-third dose of 3.67 and 1.67 μg/ml.

In the preterm infants MCC SBA GMC prior to first immunisation was 2.51 (95% CI 2.05 to 3.07). This rose to 1245.10 (95% CI 745.62 to 2079.20) following third immunisation. The remaining two infants had PRP IgG GMC/Ts of 1.0 and 0.15 μg/ml.

For the preterm infants, MCC SBA GMC prior to first immunisation was 2.51 (95% CI 2.05 to 3.07). This rose to 1245.10 (95% CI 745.62 to 2079.20) following third immunisation (p < 0.0001), with 100% of infants achieving a SBA titre ≥1:8. No significant association was found between MCC SBA response and patient variables.

The GMC/Ts and proportions achieving protective titres for Hib and MCC with Pediacel in this study and those achieved after three doses of a three component DTaP-Hib vaccine (Infanrix-Hib, GlaxoSmithKline) in a previous study16 are shown in table 1.

GMTs for the pertussis antigens FHA, Fim2&3, PRN, and PT, and for poliovirus types 1, 2, and 3 are shown in table 2. GMTs for these antigens achieved in term infants after three doses of Pediacel with MCC vaccine given concurrently at 2, 3, and 4 months are given for comparison.

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| Table 1 | GMC for PRP IgG and GMT for MCC SBA with 95% CIs and proportions achieving protective cut off levels after three doses of Pediacel and Infanrix-Hib,16 with MCC-CRM197 (Meningitec)given concurrently |
|--------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|        | Pediacel PRP: n = 45; MCC: n = 39 | Infanrix-Hib16 PRP: n = 103; MCC n = 105 |
| PRP IgG GMC μg/ml (95% CI) | 1.21 (0.73 to 2.03) | 0.27 (0.21 to 0.35) |
| IgG ≥0.15 μg/ml | 36 (80%) | 57 (55%) |
| IgG ≥1.0 μg/ml | 27 (60%) | 22 (21%) |
| MCC SBA GMT (95% CI) | 1245 (746 to 2079) | 398 (298 to 532) |
| SBA ≥8 | 39 (100%) | 104 (99%) |
| SBA ≥128 | 36 (92%) | 93 (86%) |
The availability of a combined DT5aP-Hib-IPV vaccine that elicits protective responses to its various antigens presents an attractive choice for use in the preterm infant population. Acellular pertussis vaccines have a reduced side effect profile and the concurrent administration with Pediacel of an MCC vaccine conjugated to CRM197 (Meningitec) in preterm infants immunised under an accelerated schedule. Of note, the SBA response to MCC seen in this study is increased compared to that seen when the same vaccine was given with Infanrix-Hib. Whether this represents a MCC batch associated difference or an enhancement effect when given with Pediacel is unclear.

The IgG responses to the pertussis components are similar to those achieved in term infants with Pediacel while those to poliovirus types 2 and 3 are reduced. However, this latter finding should be interpreted in the light of the fact that no infant had undetectable neutralising antibody to any of the three virus types after three doses of vaccine. The IgG responses to diphtheria and tetanus were similar to that seen with Infanrix-Hib, with all infants achieving protective titres.

Different vaccine combinations given concurrently have been shown to reduce immune responses to antigens. In addition, the concurrent administration with Pediacel of an MCC vaccine conjugated to tetanus toxoid in term infants has resulted in an increased PRP IgG GMC but a reduced SBA response to MCC seen in this study is increased which may indicate an MCC batch effect. While a combined DT5aP-Hib-IPV vaccine appears an attractive option it is clear that further research is needed to determine the optimum combination of aP, Hib, and MCC vaccines in the preterm infant. Future studies will also need to investigate the response to pneumococcal conjugates in this population and the effect such a vaccine might have on responses to other vaccines.

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Table 2 GMTs for FHA, Fim 2&3, PT, PRN, diphtheria, tetanus, and poliovirus types 1, 2, and 3 with 95% CIs and proportions achieving protective cut off levels after three doses of Pediacel for both preterm and term infants

|          | FHA EU/ml | Fim 2&3 EU/ml | PT EU/ml | PRN EU/ml | Poliovirus type 1 Titre | Poliovirus type 2 Titre | Poliovirus type 3 Titre |
|----------|-----------|---------------|----------|-----------|-------------------------|-------------------------|-------------------------|
|          | % rise    | % rise        | % rise   | % rise    | % ≥1:8                  | % ≥1:8                  | % ≥1:8                  |
| Preterm  |           |               |          |           |                        |                        |                         |
| GMT      | 65.9      | 242.13        | 76.82    | 38.83     | 258.79                  | 285.80                  | 287.34                  |
| [95% CI] | (55.31 to 78.53) | (178.27 to 328.87) | (62.35 to 94.64) | (26.43 to 57.04) | (185.39 to 361.25) | (172.93 to 501.34) | (171.32 to 481.94) |
| n        | 50        | 50            | 96%      | 50        | 22                      | 100%                    | 100%                    |
| %        | 90%       | 96%           | 96%      | 80%       | 80%                     | 100%                    | 100%                    |
| Term     |           |               |          |           |                        |                        |                         |
| GMT      | 68.1      | 309.0         | 86.4     | 34.1      | 338.8                   | 867.6                   | 1087                    |
| [95% CI] | (39.4 to 50.3) | (248.5 to 285.5) | (68.5 to 109.0) | (26.0 to 44.7) | (216.4 to 530.5) | (508.0 to 1481.9) | (700.4 to 1688.8) |
| %        | 67%       | 88%           | 92%      | 82%       | 73%                     | 100%                    | 100%                    |

to Hib with Pediacel is greater than that seen with Infanrix-Hib, a significant minority of infants had IgG concentrations below protective levels after three doses of Pediacel (20% <0.15 and 40% <1.0 μg/ml). We would recommend, therefore, that when Pediacel is used in an accelerated schedule provision should be made for the administration of a fourth additional dose of Hib conjugate in this group of infants.

In single variable regression analysis each patient variable gave a p value <0.2 for at least one IgG response and so all were included in the multivariable model. No significant association was found between variables and IgG responses to PRP, MCC, FHA, Fim 2&3, and poliovirus types 1, 2, and 3. The IgG response to diphtheria was correlated negatively with gestational age at birth (p < 0.001) and increasing number of doses of antenatal steroids given to the infants’ mothers (p = 0.01) and positively with birth weight (p = 0.001). IgG response to tetanus was correlated negatively to both gestational age at birth and pre-immunisation levels of anti-tetanus toxoid IgG (p = 0.002 and p = 0.003 respectively). IgG response to PRN was correlated negatively to pre-immunisation anti-PRN IgG levels and that to PT to birth weight (p < 0.01 and p = 0.02 respectively).

Diphtheria IgG GMC after third primary immunisation in preterm infants was 1.39 IU/ml (n = 27, 95% CI 0.98 to 1.98, 100% ≥0.1 IU/ml) and IgG GMC to tetanus was 1.47 IU/ml (n = 35, 95% CI 1.10 to 1.96, 100% ≥0.01 IU/ml).

In single variable linear regression analysis each patient variable gave a p value <0.2 for at least one IgG response and so all were included in the multivariable model. No significant association was found between variables and IgG responses to PRP, MCC, FHA, Fim 2&3, and poliovirus types 1, 2, and 3. The IgG response to diphtheria was correlated negatively with gestational age at birth (p < 0.001) and increasing number of doses of antenatal steroids given to the infants’ mothers (p = 0.01) and positively with birth weight (p = 0.001). IgG response to tetanus was correlated negatively to both gestational age at birth and pre-immunisation levels of anti-tetanus toxoid IgG (p = 0.002 and p = 0.003 respectively). IgG response to PRN was correlated negatively to pre-immunisation anti-PRN IgG levels and that to PT to birth weight (p < 0.01 and p = 0.02 respectively).
REFERENCES

1. Ramsay ME, Miller E, Ashworth LA, et al. Adverse events and antibody response to accelerated immunisation in term and preterm infants. Arch Dis Child 1995; 72: 230–2.

2. D’Angio CT, Maniscalco WM, Pichichero ME. Immunologic response of extremely preterm infants to tetanus, Haemophilus influenzae and polio immunizations. Pediatrics 1995; 96: 18–22.

3. Bernbaum JC, D’Angio CT, Ramsay ME, et al. Response of preterm infants to diphtheria-tetanus-pertussis immunizations. J Pediatr 1985; 107: 184–8.

4. Conway S, James J, Balfour A, et al. Immunisation of the preterm baby. J Infect 1993; 27: 143–50.

5. Slack MH, Schapira D, Thwaites RJ, et al. Acellular pertussis vaccine given by accelerated schedule: preterm infants’ response. Arch Dis Child Fetal Neonatal Ed 2004; 89: F57–60.

6. Adeniyi-Jones SC, Faden H, Ferdon MB, et al. Systemic and local immune responses to enhanced-potency inactivated poliovirus vaccine in premature and term infants. J Pediatr 1992; 120: 686–9.

7. Smolen P, Bland R, Helligsten E, et al. Antibody response to oral polio vaccine in premature infants. J Pediatr 1983; 103: 917–19.

8. Linder N, Handshier R, German B, et al. Controlled trial of immune response of preterm infants to recombinant hepatitis B and inactivated poliovirus vaccines administered simultaneously shortly after birth. Arch Dis Child Fetal Neonatal Ed 2000; 83: F24–7.

9. D’Angio CT, Maniscalco WM, Pichichero ME. Immunologic response of extremely premature infants to tetanus, Haemophilus influenzae, and polio immunizations. Pediatrics 1995; 96: 18–20.

10. Slack MH, Schapira D, Thwaites RJ, et al. Immune response of preterm infants to meningococcal serogroup C and combined diphtheria-tetanus-acellular pertussis-Haemophilus influenzae type b conjugate vaccines. J Infect Dis 2001; 184: 1677–80.

11. Shinefield H, Black S, Ray P, et al. Efficacy, immunogenicity and safety of heptavalent pneumococcal conjugate vaccine in low birth weight and preterm infants. Pediatr Infect Dis J 2002; 21: 182–6.

12. Goldblatt D, Richmond P, Millard E, et al. The induction of immunologic memory after vaccination with Haemophilus influenzae type b conjugate and acellular pertussis-containing diphtheria, tetanus, and pertussis combination. J Infect Dis 1999; 180: E38–41.

13. Eskola J, Olander RM, Hovi T, et al. Randomised trial of the effect of co-administration with acellular pertussis DTP vaccine on immunogenicity of Haemophilus influenzae type b conjugate vaccine. Lancet 1996; 348: 1688–99.

14. Lee CY, Thippawong J, Huang UM, et al. An evaluation of the safety and immunogenicity of a five-component acellular pertussis, diphtheria, and tetanus toxoid vaccine (DtaP®) when combined with a Haemophilus influenzae type b-tetanus toxoid conjugate vaccine (PRP-T) in Taiwanese infants. Pediatrics 1999; 103: 25–30.

15. Richmond P, Borrow R, Miller E, et al. Meningococcal serogroup C conjugate vaccine is immunogenic in infancy and primes for memory. J Infect Dis 1999; 179: 1569–72.

16. Olin P, Rasmussen F, Gustafsson L, et al. Randomised controlled trial of two-component, three-component, and five-component acellular pertussis vaccines compared with whole cell pertussis vaccine. Lancet 1997; 350: 1569–77.

17. McVernon J, Andrews N, Slack MP, et al. Risk of vaccine failure after Haemophilus influenzae type b/Hib combination vaccines with acellular pertussis. Lancet 2003; 361: 1521–3.

18. Dagan R, Eskola J, Leclerc C, et al. Reduced response to multiple vaccines sharing common protein epitopes that are administered simultaneously to infants. Infect Immun 1998; 66: 2093–8.

19. Miller E, Southern J, Kitchin N, et al. Interaction between different meningococcal C conjugate vaccines and the Hib component of concomitantly administered diphtheria/tetanus/pertussis/Hib vaccines with either whole cell or acellular pertussis antigens. European Society of Paediatric Infectious Diseases, 21st Annual Meeting, 2003, Abstract 272.