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Immunogenicity and safety of PRP-T conjugate vaccine given according to the British accelerated immunisation schedule

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Abstract

The immunogenicity and safety of a new *Haemophilus influenzae* type b conjugate vaccine, PRP-T, was studied in 107 infants from the Oxford district. The vaccine was given concurrently with diphtheria, pertussis, tetanus, and polio vaccines at 2, 3, and 4 months of age. Symptoms after immunisation were recorded by a parent. Sera were obtained before the first immunisation and at 5 months of age and the antibodies were measured by both radioimmunoassay and enzyme linked immunosorbent assay (ELISA). No serious adverse reactions were observed and there was no increase in the incidence of expected minor side effects. By radioimmunoassay, the geometric mean titre of serum anticapsular antibody increased from 0·09 μg/ml before immunisation to 5·01 μg/ml after three immunisations. Ninety eight per cent of children had antibody concentrations consistent with protection (≥0·15 μg/ml). IgG antibody concentrations measured by ELISA correlated well with total antibody concentrations measured by radioimmunoassay (r=0·864). These results provide encouragement that routine immunisation against *H influenzae* type b at 2, 3, and 4 months of age, could prevent most cases of disease in children in the UK.

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Haemophilus influenzae type b is a major cause of serious bacterial infection in early childhood, during which it is the most common cause of meningitis, epiglottitis, cellulitis, and septic arthritis.[1] Epidemiological surveillance in the Oxford region of the UK over the past seven years has underlined the continuing importance of *H influenzae* type b infection.[2] The cumulative risk of infection for children by their fifth birthday is one in 600.

Serum antibodies to the polyribosylribitol phosphate (PRP) capsule of *H influenzae* type b are protective.[3] However, purified polysaccharide vaccine (composed of PRP) is poorly immunogenic in children less than 2 years old,[4] the age group in which most infections occur. This age related susceptibility, first documented in the 1930s, was found to correlate with absent or low concentrations of serum antibodies to PRP, a finding that is typical of infants in their first and second years.[5] Enhancement of the immunoprotective response to polysaccharide antigen is possible through its covalent linkage (conjugation) to a carrier protein,[6] which results in T cell dependency and a boostable response, thereby offering the potential of antibody responses that would protect young infants. Four conjugate vaccines against *H influenzae* type b have been developed, conjugates of PRP respectively to diphtheria toxoid (PRP-D), tetanus toxoid (PRP-T), a diphtheria toxin mutant (HbOC), and an outer membrane protein of meningococcus (PRP-OMP).

Immunogenicity studies comparing these conjugate vaccines given according to current Finnish and American primary immunisation schedules (Finnish: 4 and 6 months of age; USA 2, 4, and 6 months of age), suggest that PRP-T is at least as immunogenic as other conjugate vaccines and it has been shown to result in a greater antibody response after one or two injections than PRP-D or HbOC.[7 8] Also, the booster response to PRP-T is significantly greater than that seen with PRP-OMP.[8] Thus PRP-T appears an acceptable candidate for inclusion in the British schedule of immunisation at 2, 3, and 4 months of age.

Routine immunisation against *H influenzae* type b of infants under 6 months of age has become part of the routine primary schedule in the USA and several European countries.[9] The Department of Health has indicated that the UK will implement routine immunisation in October 1992. It has been shown previously that the conjugate vaccine HbOC is highly immunogenic in UK infants using the primary schedule of 3, 5, and 9 months,[10] but in May 1990 the primary schedule in the UK was altered to 2, 3, and 4 months of age. The purpose of this study was to determine the safety and immunogenicity of the conjugate vaccine PRP-T when given concurrently with diphtheria, pertussis, tetanus, and polio vaccines and according to the accelerated schedule of primary immunisation at 2, 3, and 4 months of age. Antibody concentrations to PRP were measured by both radioimmunoassay and enzyme linked immunosorbent asasy (ELISA) as part of the validation of the ELISA used.

Patients and methods

One hundred and seven Oxfordshire infants were recruited over a two month period. Parents were approached on the second or subsequent

days after birth with information about the study. Written consent was not requested until the child was at least 6 weeks of age. Seventy five per cent of those invited agreed to take part. Infants were randomised to receive one of three batches of PRP-T (Pasteur Merieux Serum et Vaccins; batch numbers S2141, S2181, S2189). PRP-T is a freeze dried lyophilised conjugate vaccine which is reconstituted with 0·5 ml sodium chloride 0·4%. Immunisations took place in the family home at 2, 3, and 4 months of age. *H influenzae* type b vaccine was given by intramuscular injection in the right thigh and, at the same visit, diphtheria, pertussis, and tetanus vaccine (Wellcome) was given in the left thigh. Oral polio vaccine was also administered. Blood for serology was obtained at the first visit and again one month after completing the primary series. Blood samples were separated on the day of venepuncture and serum stored in 0·5 ml aliquots at −20°C until required.

Parents were given a form to document any symptoms in the three days after each immunisation. They recorded axillary temperatures daily and at any other time if the infant felt hot. The size of local redness or swelling was measured with a calliper. Parents were taught how to use a thermometer and the calliper and were telephoned within one to three days of immunisation to check on progress. They were not told the side on which a particular injection was given, so infants acted as their own control for local reactions.

Total anti-PRP antibodies were measured using Farr type radioimmunoassay[11] by Dr H Kayhty and L Saarinen at the National Public Health Institute in Finland with the standard reference serum from the Food and Drug Administration, USA (72 μg per ml).

IgG antibodies to PRP were measured in Oxford by an ELISA using a modification of the method described by Kumararatne *et al.*[12] All incubations were at room temperature for one hour unless otherwise stated and were followed by four washes with phosphate buffered saline plus 0·05% Tween 20 (PBS-T). PRP (provided by Pasteur-Merieux Serum et Vaccins, France) was covalently bound to poly-L-lysine[13] and diluted in 0·05 M carbonate buffer (pH 9·6) to approximately 1 μg/ml. This solution was added to flat bottomed microtitre plates (Immulon 1, Dynatech) using 100 μl per well and incubated overnight at 4°C. Aliquots of 100 μl of test or standard sera diluted in PBS-T were added to each well. After incubation, the wells were exposed to mouse antihuman IgG diluted in PBS-T, followed by peroxidase conjugated sheep antimouse IgG diluted in PBS-T and finally to substrate solution (0·4 mg/ml o-phenylenediamine dihydrochloride in 0·15 M citrate phosphate buffer, pH 5 with 1 μl hydrogen peroxide per 5 ml substrate solution). The colour was allowed to develop in the dark and the reaction was stopped after 30 minutes by the addition of 25 μl 10% sulphuric acid. The optical density was measured at 492 nm using a Multiscan ELISA reader. A log linear standard curve was plotted with antibody concentration against optical density, from which the antibody concentration of the test samples could be calculated. The lower limit of sensitivity of the assay was 0·16 μg/ml. A standard reference serum was obtained from Dr H Kayhty in Finland. Both the Oxford and Finnish laboratories take part in the European quality control scheme for standardisation of measurement of antibodies to *H influenzae* type b.

Approval for the trial was granted by the central Oxford research ethics committee.

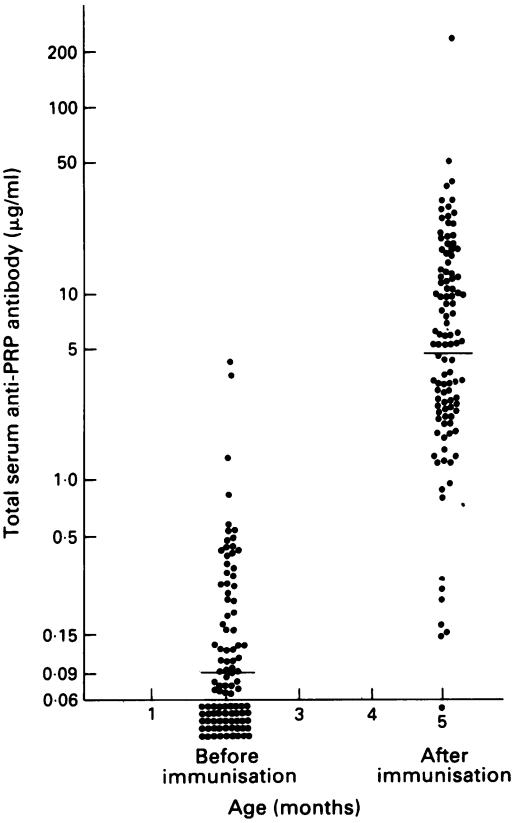
Statistical analyses were performed using Epi Info version 5. Analysis of variance was used for the comparison of response to vaccine batches and the Kruskal-Wallis test was used for comparisons of non-parametric data.

Results

All 107 infants tolerated immunisation for *H influenzae* type b well and none failed to complete the study. A comparison of side effects can be seen in table 1. Local reactions

Table 1 Number of infants with side effects after immunisation with H influenzae type b conjugate and diphtheria, pertussis, and tetanus vaccines (n=107)

	Immunisation dose		
	First	Second	Third
Redness or swelling at:			
Site of PRP-T injection	1	4	2
Site of diphtheria, pertussis, and tetanus injection	16	27	29
General reactions:			
Axillary temperature ≥38°C	6	5	6
Irritability >4 hours total	30	20	29
Inconsolable crying (maximum observed was six hours)	2	3	2



Concentration of serum antibodies specific for polyribosyl ribitol phosphate (PRP) in infants immunised at 2, 3, and 4 months with PRP-T conjugate vaccine. Horizontal bars=geometric mean titres (Results less than the lower limit of sensitivity of the assay were assigned a value of 0·03 for calculation of the geometric mean titre.)

(redness or swelling) were less common with PRP-T than with the diphtheria, pertussis, and tetanus vaccine. Systemic reaction rates were similar after each of the three primary immunisations. Two children had inconsolable crying and extensive local reactions to their first diphtheria, pertussis, and tetanus immunisations so pertussis was subsequently withheld. Their second and third immunisations were well tolerated.

Sufficient blood for analysis was obtained from 101 infants before immunisation and 107 infants after immunisation. The PRP-T vaccine was immunogenic, such that after the three dose course, 105 of the 107 children (98%) had antibody concentrations consistent with protection (≥0·15 μg/ml) as determined by radioimmunoassays. Ninety seven children (91%) had an antibody concentration of more than 1 μg/ml. The geometric mean titre of antibody to PRP rose from 0·09 μg/ml at 8 weeks of age to 5·01 μg/ml at 20 weeks of age (figure).

IgG antibody concentrations measured by ELISA showed a good correlation with total antibody concentrations measured by radioimmunoassay (r=0·864). By ELISA, only one child had a postimmunisation antibody concentration of <0·16 μg/ml and 90% of children had an antibody concentration of >1·0 μg/ml after immunisation.

All three batches of PRP-T vaccine elicited similar responses; analysis of variance showed no significant difference between batches in either the preimmunisation or postimmunisation antibody concentrations (p>0·1, table 2).

Among infants who had postimmunisation concentrations of ≥0·15 μg/ml by radioimmunoassay there were four who had less than fourfold rises in antibody concentrations. These all had anti-PRP antibody concentrations of >0·15 μg/ml before immunisation. The effect of maternally derived anti-PRP antibodies on the serum antibody response to PRP-T was therefore evaluated. This was done by dividing infants into two groups according to preimmunisation antibody concentrations using 0·15 μg/ml to divide the high and low antibody groups; postimmunisation antibody concentrations of the two groups were then compared. There was no significant differences (table 3; Kruskal-Wallis test, p>0·1). The antibody concentrations in the two infants not achieving 0·15 μg/ml postimmunisation was also <0·15 μg/ml before immunisation.

Table 2 Geometric mean anti-PRP antibody titres for three batches of PRP-T, before and after immunisation (μg/ml)

Vaccine batch	*Geometric mean titre*	
	Before	*After*
S2141	0·08	4·61
S2181	0·10	4·12
S2189	0·09	6·44
Overall	0·09	5·01

Table 3 Effect of maternally derived antibody on anti-PRP antibody responses evoked by PRP-T given at 2, 3, and 4 months of age in 101 infants. (There was insufficient serum in six infants for analysis of antibody titres before immunisation)

	Low group	*High group*
Maternally derived anti-PRP antibody concentration (μg/ml) before immunisation	<0·15	≥0·15
No of infants	30	71
Geometric mean anti-PRP antibody titre (μg/ml):		
Before immunisation	0·05	0·40
After immunisation	4·66	5·81

Discussion

This study demonstrates that an accelerated three dose series of PRP-T immunisation, which is completed by 4 months of age, is both immunogenic and well tolerated. Local reactions were uncommon and the rate of general reactions was similar to that previously reported for UK children immunised with diphtheria, pertussis, tetanus vaccine alone or diphtheria, pertussis, tetanus and *H influenzae* type b conjugate vaccine according to the 3, 5, and 9 months schedule.[10 14]

There was a good correlation between antibody concentrations measured by radioimmunoassay and by ELISA, the latter measuring only IgG antibodies. Similar findings have been reported previously, with total antibodies measured by both methods.[15] These results show that ELISA can be used as an alternative to the classical radioimmunoassay for reliable measurement of PRP antibodies. The ELISA has other potentially valuable uses such as measurement of isotype specific antibodies and IgG subclass antibodies.

An inhibitory effect by placentally transferred antibodies was suggested in a previous immunogenicity study of a prototype PRP-tetanus toxoid conjugate vaccine.[16] In contradiction, our study demonstrated that the immune response to PRP-T was not significantly affected by preimmunisation anti-PRP antibody concentrations.

The implementation of a new vaccine presents practical concerns, one of which is the present requirement to give *H influenzae* type b immunisation by separate injection. This was not a significant problem in our study as demonstrated by the high level of recruitment (75%) and the lack of dropouts. Preliminary observations from an implementation study now underway in the Oxford region suggests that vaccine uptake is minimally affected by the extra injection. However, studies are underway in which *H influenzae* type b and diphtheria, pertussis, and tetanus immunisations are given in the one syringe; in Finland, it is part of normal clinical practice to do so.

Although the initial response of young infants to PRP-T vaccine is clearly effective, the longevity of the protection is an important question and affects whether booster doses are required. Studies in Finland of PRP vaccine suggest that a serum anti-PRP antibody concentration of at least 1 μg/ml correlates with long term protection.[4] Given that over 90% of infants achieved antibody concentrations greater than 1 μg/ml, it is not certain that a booster dose will be required. In any case, it may be that the three dose course primes for a boostable (and thereby protective) response regardless of the absolute serum antibody concentration when the child is later exposed to *H influenzae* type b

organisms.[17] This question may abe addressed by long term follow up of antibody concentrations and ideally by an efficacy trial with vaccinations given at 2, 3, and 4 months of age but without a booster dose in the second year of life.

Epidemiological surveillance in Oxford has shown that, of *H influenzae* type b disease in children under 5 years, only 6% occurs in those under 4 months whereas 42% occurs before 12 months (unpublished data). This illustrates the rapid increase in risk of infection as maternally derived antibody declines. Given 95% vaccine uptake in the new 2, 3, and 4 month schedule, immunisation has the potential to prevent up to 90% of *H influenzae* type b infections in young children. This translates to the potential annual prevention of 60 deaths and 1170 cases of infection in the UK.[2] The encouraging immunogenicity and tolerability of PRP-T conjugate vaccine, demonstrated in this study, make it a candidate for inclusion in the routine national schedule. An open intervention study of PRP-T vaccine given at 2, 3, and 4 months of age is underway in the Oxford region. It is anticipated that 30 000 infants will be immunised and that clinical efficacy can be evaluated by late 1992 when immunisation against *H influenzae* type b disease is due to start in the national programme.

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