Haemophilus influenzae type b conjugate vaccine trial in Oxford: implications for the United Kingdom

G TUDOR-WILLIAMS,* J FRANKLAND,* D ISAACS,* R T MAYON-WHITE,† J A MACFARLANE,‡ D G REES,§ AND E R MOXON*

* University Department of Paediatrics, † Department of Community Medicine, Manor House, ‡ Department of Community Health, Radcliffe Infirmary, Oxford, and § Department of Computing and Mathematical Science, Oxford Polytechnic

SUMMARY The safety and immunogenicity of a Haemophilus influenzae type b conjugate vaccine was investigated in 103 infants immunised at 3, 5, and 9 months of age; the infants also received diphtheria, pertussis, and tetanus and polio vaccines. Side effects were compared with 99 matched infants receiving diphtheria, pertussis, and tetanus and polio vaccines only. No serious side effects were observed and the incidence of minor side effects was no greater in the recipients of H influenzae type b conjugate vaccine. Two doses of the vaccine (standard and low) were compared: geometric mean titres of serum anticapsular antibody rose from 0·11 μg/ml before immunisation to 26·4 μg/ml after three immunisations with the standard dose and 14·6 μg/ml with the low dose. The geometric mean titre among 21 unimmunised infants at this age was 0·06 μg/ml. Both doses therefore generated antibody concentrations likely to be protective after three immunisations. There were no non-responders.

Incorporation of an H influenzae type b conjugate vaccine into the primary immunisation schedule has the potential for preventing over 1000 cases of systemic H influenzae type b disease and 50 deaths each year in the United Kingdom.

Haemophilus influenzae type b causes potentially fatal systemic disease such as meningitis and epiglottitis and other serious infections affecting soft tissues, joints, and lungs. Recent studies1 2 suggest that the incidence of H influenzae type b disease in the United Kingdom is higher than previously reported.3

The polyribosyl-ribitol phosphate capsule, which is specific to serotype b H influenzae organisms, is a major virulence determinant.4 Serum antibody to the capsule has been shown to be protective in infants.5

Immunocompetence to polysaccharides matures more slowly than to proteins and lipopolysaccharides. Active immunisation using purified polyribosyl-ribitol phosphate is ineffective in children less than 18 months of age6; they constitute a major proportion of the population at risk. A conjugate of a polysaccharide hapten and a carrier protein gives T cell dependent properties to the polysaccharide7–9 with better immunogenicity in young children and predominantly IgG antibody responses that can be boosted.10 Concentrations of antibody induced by immunisation can be higher than those after natural infection at the same age.11 At least four different conjugates are undergoing clinical evaluation,12 13 and one large efficacy study has been published.14 It has been shown that a conjugate (HbO–C) of oligosaccharides derived from polyribosylribitol phosphate linked to a non-toxic mutant diphtheria toxin10 is more immunogenic than the polyribosylribitol phosphate-diphtheria toxoid conjugate used in the first efficacy trial (H Peltola, J Eskola, H Kayhty, PH Makela. Immunogenicity of Haemophilus influenzae oligosaccharide-protein conjugate vaccine (HbO–C) compared to polysaccharide-protein conjugate vaccine (PRP–D) in infancy. Personal communication, 1988). No conjugate has been tested in infants resident in the United Kingdom or according to the British schedule of primary immunisation at 3, 5, and 9 months of age and there is a need to define the optimal dose of polysaccharide.12 Our study aimed to examine the serum antipolyribosyl-ribitol phosphate antibody state of unimmunised infants in the United Kingdom and to provide safety and immunogenicity data

520
**Haemophilus influenzae type b conjugate vaccine trial in Oxford**

Comparing standard and low dose regimes using the HbO–C *H influenzae* type b conjugate vaccine.

**Patients and methods**

Two hundred and thirty two mothers living in and around the city of Oxford, with full term healthy babies born consecutively during a two month period, were invited to participate in the study of the *H influenzae* type b conjugate vaccine. Of these, 103 mothers (including one with twins) agreed to take part; thus 104 infants were enrolled into the ‘index’ group. The infants were randomised using batched envelopes to receive a 0.5 ml dose containing 10 µg or 2 µg oligosaccharide (*H influenzae* type b HbO–C conjugate vaccine lot No A2K61/22 without adjuvant (Praxis Biologics); 0.9% sodium chloride was used as a diluent). *H influenzae* type b vaccine was given intramuscularly on the same occasion as diphtheria, pertussis, and tetanus vaccine but in a different site (opposing anterolateral thighs) at 3, 5, and 9 months of age. Parents were given a standard form and asked to record any local or systemic reactions over the next five days and measure daily axillary temperatures. The families were routinely visited the next day. Venepuncture for serology was performed before immunisation at each visit and at 10 months. All venepunctures and immunisations were performed in the child’s home by one of us (GT–W) and only one attempt at venepuncture was made on any one visit.

Local reactions to *H influenzae* type b conjugate and diphtheria, pertussis, and tetanus vaccines could be compared for each index subject. To obtain comparative data for systemic reactions, 192 mothers from the same area giving birth to full term healthy infants in the month on either side of the recruiting period for the *H influenzae* type b conjugate vaccine were approached. Of these, 97 families including three with twins (100 infants) agreed to participate. This ‘comparison’ group received vaccine for diphtheria, pertussis, and tetanus (and oral polio) at home but were not given *H influenzae* type b conjugate vaccine or placebo. The child was visited the next day and the parents were requested to record reactions and measure temperatures as in the index group. The same batch of diphtheria, pertussis, and tetanus vaccine (A0726A, Wellcome) was used for both the index and comparison groups. Blood was taken from a random sample of 21 of these infants only once, at 10 months, to examine concentrations of antibody in United Kingdom children immunised with *H influenzae* type b conjugate vaccine.

One family from each group moved and were lost to follow up; therefore our results relate to 103 infants in the index group (52 received the 10 µg dose of vaccine) and 99 infants in the comparison group.

Serum samples were separated on the day of venepuncture and stored in 0.5 ml aliquots at −70°C. The Farr type radioantigen binding assay for total serum antipolyribosyl-ribitol phosphate antibody,16 17 using the recommended criteria of the Office of Biologics Research and Review,18 was performed in duplicate at three initial dilutions in both our own laboratories and those of Praxis Biologics. For neat sera with low concentrations of antibody, we interpreted the standard curves down to 0.05 µg/ml (7–8% binding in our assays). End point dilutions up to 1:1000 were performed to give estimates of high concentrations of antibody. Polyribosyl-ribitol phosphate intrinsically labelled with tritium radioantigen was supplied by Dr Porter Anderson, University of Rochester, USA, and Office of Biologics Research and Review lyophilised standard reference serum (70 µg/ml) was supplied by Dr Carl Frasch, Bureau of Biologics, Federal Drug Administration, Bethesda, USA.

The trial was approved by the Central Oxford Research Ethics Committee.

**STATISTICAL METHODS**

The proportion of children in the index and comparison groups were compared for all adverse reactions by χ² tests using Yates’s correction (or by Fisher’s exact test if expected values were <5). The proportion of unimmunised infants at 3 and 10 months of age whose antibody concentrations were above 0.05 µg/ml were compared by χ² test using Yates’s correction. Mann-Whitney U tests were used to compare antibody responses to the two doses of *H influenzae* type b conjugate vaccine at a given age. For each dose Wilcoxon signed rank tests were used to assess the responses to first, second, and third immunisations.

**Results**

The vaccine was well tolerated and highly immunogenic. After three immunisations, the 10 µg doses resulted in a rise in geometric mean serum antipolyribosyl-ribitol phosphate antibody titre from 0.11 at 3 months to 26.4 µg/ml at 10 months of age. The 2 µg doses generated lower responses at 10 months (*p*=0.007) although the geometric mean titre rose from 0.11 to 14.6 µg/ml, and at both doses all infants achieved greater than four fold increases in antibody titre and 98% achieved values above 1.0 µg/ml (figs 1 and 2).

The 10 µg dose resulted in significant increases in
antibody titre (p=0·0006) after one immunisation.
No increase was observed after the first 2 μg dose
(p=0·23). Geometric mean titres at age 5 months
were 0·19 and 0·10 μg/ml respectively. After the
second immunisation booster responses were noted
with both dosage regimes: geometric mean titres at 9
months rose to 2·05 and 0·75 μg/ml respectively. The
2 μg dose produced a wider scatter of results,
however, and four individuals had antibody concen-
trations below the limit of sensitivity of our assay.

In 21 infants from the comparison group who had
not received the H influenzae type b conjugate
vaccine, the geometric mean titre at 10 months was
0·06 μg/ml. There was a fall in antibody concentra-
tions (p<0·01) when compared with the combined
results for the index infants at 3 months of age in
samples taken before their first immunisation with
H influenzae type b conjugate vaccine (fig 3).

No major adverse reactions occurred in either
group and no complications from venepuncture
were observed. Adverse reactions did not differ
significantly by dose of H influenzae type b conjugate
vaccine and their results have been combined.
A comparison of side effects is shown in the table.
Local reactions defined as redness or swelling or
warmth >2 cm occurred in 2% of index infants after
administration of H influenzae type b conjugate
vaccine and 19% after diphtheria, pertussis, and
tetanus vaccine. Local reactions to diphtheria,
pertussis, and tetanus vaccine in the comparison
infants were of similar frequency. Systemic reactions
were similar or less frequent in the index children compared with those in the comparison group.

Discussion

The serum antibody concentrations observed in unimmunised infants at 3 months and 10 months are consistent with the decline in serum antibody observed in North American infants. Both are consistent with the relative protection from _H influenzae_ type b infection observed during the early months of life.

The _H influenzae_ type b conjugate vaccine was well tolerated with a low frequency of local reactions and no increase in systemic side effects when given with diphtheria, pertussis, and tetanus vaccine as compared with infants receiving diphtheria, pertussis, and tetanus vaccine alone. It has been shown that another conjugate (polyribosyl-ribitol phosphate-D) can be mixed with diphtheria, pertussis, and tetanus vaccine and given as a single injection without adverse effects on safety or immunogenicity.

The conventional dose of polysaccharide in polyribosyl-ribitol phosphate is 25 μg. A variety of doses from 10–25 μg have been employed in trials of _H influenzae_ type b conjugate vaccines. We found that 2 μg of oligosaccharide in the _H influenzae_ type b conjugate induces booster responses after a second immunisation at 5 months. The 2 μg dose, however, did not affect the decline in antibody concentrations after immunisation at 3 months, whereas a significant rise in antibody concentrations was observed after a 10 μg dose at this age. Given the pattern of susceptibility in young infants it is desirable to achieve immunological priming at as young an age as possible and our results suggest that a 10 μg oligosaccharide dose would be more appropriate than a 2 μg dose for the immunisation schedule in the United Kingdom.
There has been much debate as to what constitutes a 'protective' concentration of antibody: titres >1.0 μg/ml are widely accepted as correlating with long term protection after immunisation with unconjugated polyribosylribitol phosphate. The Finnish trial of polyribosylribitol phosphate-D, however, disclosed a higher protective efficacy than would have been predicted from the immunogenicity data. In view of the T cell dependent characteristics of responses to the conjugate vaccines it seems likely that the priming effect of one dose may be more important than achieving a designated post-immunisation antibody titre that correlates with protection but was based on experience with the unconjugated polysaccharide vaccine.

Our data and the results of studies of other candidate vaccines suggest that incorporation of immunisation for H influenzae type b into the schedule in the United Kingdom has the potential for preventing over 85% of systemic infection with H influenzae type b in children. Extrapolating from the incidence of systemic H influenzae type b disease in the Oxford region, this would represent 1105 cases with their attendant long term sequelae and 55 deaths each year.

This and even higher estimates of attack rates from other regions in this country make a compelling case for considering how active immunisation might be successfully implemented. The availability of safe, immunogenic, and protective conjugate vaccines strengthens the argument that wider intervention studies should proceed in the United Kingdom.

We thank all the families who took part in this study. We thank Dr WOC Cookson and Mrs Lin Barnetson for providing computing expertise and Miss Gail Davies for typing the manuscript. We thank Dr Porter Anderson for supplying the titrated polyribosylribitol phosphate and for his constructive criticism, and Praxis Biologies for materials.

References


Correspondence to Professor ER Moxon, University Department of Paediatrics, John Radcliffe Hospital, Headington, Oxford OX3 9DU.

Accepted 3 January 1989
Haemophilus influenzae type b conjugate vaccine trial in Oxford: implications for the United Kingdom.

G Tudor-Williams, J Frankland, D Isaacs, R T Mayon-White, J A MacFarlane, D G Rees and E R Moxon

Arch Dis Child 1989 64: 520-524
doi: 10.1136/adc.64.4.520

Updated information and services can be found at:
http://adc.bmj.com/content/64/4/520

Email alerting service

These include:
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/