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Randomised controlled trial of combined diphtheria, tetanus, whole-cell pertussis vaccine administered in the same syringe and separately with *Haemophilus influenzae* type b vaccine at two, three and four months of age

Ian G. Jones*, Helen Tyrrell†, Anthony Hill‡, Jean M. Horobin* and Brent Taylor§

An open randomised controlled multicentre study compared the immunogenicity and reactogenicity of three vaccines given by injection at two, three and four months of age. Children (89) received Haemophilus influenzae *type b (Hib) vaccine (SmithKline Beecham Biologicals [SB]) administered in the same syringe with combined diphtheria–tetanus–whole-cell pertussis (DTP_W) vaccine (Evans); 75 received Hib vaccine (SB) administered as a separate injection with DTP_W vaccine; 66 received Hib vaccine (Pasteur Merieux [PM]) administered as a separate injection with DTP_W vaccine. All subjects in both groups receiving Hib (SB) vaccine had levels of antibodies to the Hib polysaccharide polyribosylribitol phosphate (PRP) greater than 0.15 $\mu g\ ml^{-1}$ as did 97% of those receiving Hib (PM) vaccine 1 month after administration of the final vaccine dose. Subjects in all three groups demonstrated an immunological response to pertussis, diphtheria and tetanus antigens. The geometric mean titres of the group given Hib (SB) and DTP_W vaccine mixed in the same syringe were lower than the other groups. There were no apparent differences between the treatment groups in the incidence of local or systemic reactions, or serious adverse events. This study has confirmed that it is possible to halve the number of injections necessary to offer protection, with advantages to parents, children, doctors and nurses, using a combined DTP_WHib vaccine and in accordance with the UK's accelerated primary immunisation schedule at two, three and four months of age. © 1997 Elsevier Science Ltd.*

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Haemophilus influenzae type b (Hib) conjugate vaccines were included in the primary immunisation schedule in the UK in 1992. Until recently DTP_W and Hib vaccines have been given to children as two separate injections at two, three and four months of age[1], but in the UK these can now be given as a combined vaccine[2]. Although a number of trials has examined the immune response and reactions to combined vaccines, only a few have been reported with this schedule[3,4]. Both had important differences from the present investigation, one being an open non-random study with controls taken from previous trials[3], and the other a trial which used a smaller dose of Hib polysaccharide (7.5 μg) than normal[4]. Other reported trials have used different immunisation schedules.

The aim was to assess the comparative immunogenicity and reactogenicity of the Hib (SB) vaccine administered either in the same syringe or as a separate injection from DTP_W (Evans), and to compare these with Hib(PM) vaccine administered concomitantly with DTP_W vaccine in separate injection sites using the UK primary immunisation schedule mentioned above.

*Centre for Health and Social Research, Glenrothes House, Glenrothes, Fife KY7 5PB, UK. †SmithKline Beecham Pharmaceuticals, Welwyn Garden City, Herts AL7 1EY, UK. ‡Department of Public Health Medicine, South Humber Health Authority, Health Place, Wrawby Road, Brigg, North Lincs DN20 8GS, UK. §Department of Community Child Health, Royal Free Hospital School of Medicine, Pond Street, London NW3 2QG, UK. (Received 24 August 1996; revised version received 25 January 1997; accepted 8 May 1997)

METHODS

The study population included infants of either sex aged between 8 and 12 weeks, at the time of first immunisation, who were free from obvious health problems as evidenced by clinical examination prior to vaccination. They were recruited between November 1994 and August 1995 in Fife, Somerset and London, by their general practitioners, clinic staff or trained nurses who obtained written informed consent from parents.

The study was approved by the local research ethics committees and conducted in accordance with Good Clinical Practices and the Declaration of Helsinki as amended in Hong Kong (1989). The children were immunised in line with the recommended UK schedule. Vaccines were given not less than 28 days and not more than 35 days apart. Subjects with contra-indications defined in the protocol were excluded.

The vaccines used in this Phase III field trial comprised commercially available Evans vaccine (DTP_W), which contains at least 30 IU diphtheria toxoid and 60 IU tetanus toxoid and not more than 20×10^9 killed *Bordetella pertussis* organisms ('whole cell'); lyophilised Hib (SB) vaccine, which contains 10 μg polyribosylribitol phosphate conjugated to tetanus toxoid (PRP-T) in a 1:3, *w*/*w*, ratio; and Hib (PM) vaccine, which contains at least 10 μg lyophilised Hib polysaccharide (PRP) conjugated to tetanus toxoid.

The unit dose for all vaccines was 0.5 ml after reconstitution of the lyophilised Hib vaccines with the DTP_W vaccine or saline and all were given by intramuscular injection into the antero-lateral thigh. Allocation of subjects to the three regimens was formally randomised, using consecutive, unique numbers assigned to each packet of vaccination materials (containing all materials needed for a single subject), prepared by SB with computer-generated random numbers.

Antibody titre distributions and geometric mean titres (GMTs) were calculated from analysis of heel-prick blood samples (0.5 ml) taken prior to the first vaccine dose and one month after the third vaccine dose. Total antibodies to the Hib polysaccharide PRP were measured blind by radio-labelled antigen-binding assay (RABA) by SB Biologicals, Rixensart, Belgium, and the cut-off point for the assay was 0.15 μg ml^{-1}. All other serological measurements were analysed blind by the Centre for Applied Microbiology and Research (CAMR), Porton Down, Salisbury, England. Specific antibodies to diphtheria and tetanus toxoid were measured by ELISA techniques and the cut-off point of the assay was 0.01 IU ml^{-1}. IgG antibody titres to the pertussis components, pertussis toxin (PT), filamentous haemagglutinin (FHA), 69 kDa outer membrane protein (69 kDa) and agglutinogens 2 plus 3 (Agg 2+3) were also measured by ELISA techniques. The lowest level of detection for antibodies to the pertussis components by this test was $\geq$50. Thus for infants with undetectable levels of circulating antibodies prior to vaccination, a post-vaccination titre $\geq$50 demonstrated a vaccine response. Due to the gradual decrease in circulating maternal antibodies, for infants with a pre-vaccination titre $\geq$50, a vaccine response was defined as a post-vaccination titre equal to, or higher than, the pre-vaccination level.

Solicited adverse experiences, the incidence and severity of pain, swelling and redness of the injection site (local reactions), and temperature, unusual crying, vomiting, diarrhoea, loss of appetite, restlessness and sleepiness (systemic reactions), were assessed by recording on a diary card on the day of vaccination and for the following 7 days. A researcher contacted parents or guardians by telephone 24 and 48 h post-vaccination and on day 8 to check on the occurrence of any adverse experiences including both solicited (on the diary card) and unsolicited (not prompted for on the diary card). Parents returned the diary cards by post or handed them in on the next study visit. Serious adverse experiences, including life-threatening events, anaphylaxis, early onset vaso-vagal reaction and hypotonic hyporesponsiveness, and hospitalisation were monitored throughout the study.

Eighty vaccinees in each of the three groups was considered to be a sufficient number to detect a 15% difference in immunological response rate to Hib. Because of difficulty in recruitment during the study, however, the numbers were readjusted and at the time of termination 230 subjects had been recruited, 89 to receive DTP_W and Hib (SB) combined in the same syringe, 75 to receive DTP_W and Hib (SB) administered in opposite limbs, and 66 to receive DTP_W and Hib (PM) administered in opposite limbs.

Analysis of variance for GMT comparisons and Fisher's exact test for comparison of proportions with 95% confidence intervals (CI) were used to compare the three groups.

RESULTS

Table 1 shows the treatment allocation and demographic data. The groups were similar at randomisation except for a slightly higher number of females in the group receiving DTP_WHib (SB) in the same syringe. The most common reasons for protocol violations were immunisations given less than 28 or more than 35 days after the previous one, or outside the age range. Of the 17 subjects withdrawn from the study, three withdrew because of protocol violations, eight were lost to follow-up, three withdrew consent, two were withdrawn because of adverse events and one moved from the area.

All children who received three doses of vaccine except one given DTP_WHib (PM) had an immunogenic response in terms of titres $\geq$0.15 μg ml^{-1} for anti-PRP and 0.01 IU ml^{-1} for diphtheria and tetanus, and in terms of vaccine response for pertussis. There were no statistically significant differences between the treatment groups.

The GMTs one month after the final vaccine dose are recorded in *Table 2*. Children given the vaccines in the same syringe had a significantly lower Hib GMT than either the SB ($P = 0.005$) or PM ($P = 0.037$) vaccines when administered separately.

By the end of the study 264 children had received DTP_WHib (SB) in the same syringe, 221 DTP_WHib (SB) at different sites and 187 DTP_WHib (PM) at different sites. A summary of the incidence of local reactions, which included redness, swelling or pain at the injection site, is shown in *Table 3* and details of the general reactions in *Table 4*. There was no statistically

Table 1 Comparability of the three treatment groups

	DTP$_w$Hib (SB) same syringe	DTP$_w$Hib (SB) different sites	DTP$_w$Hib (Merieux) different sites
Number of subjects randomised	89	75	66
Number of subject withdrawals	2	6	9
Number of subjects completing treatment	87	69	57
Protocol violators	17	15	15
Evaluated per-protocol (visit 4)	70	54	42
Evaluated for safety	89	75	66
Male (%)	36	48	50
Age in weeks at study entry:			
Mean	9.5	9.2	9.3
Minimum	7.6	8.0	7.1
Maximum	12.4	12.0	12.0

significant difference in the incidence of one or more general reactions between those receiving DTP$_w$Hib (SB) in the same syringe and the other two groups. Those who received DTP$_w$Hib (SB) in the same syringe had a statistically significant lower incidence of one or more local reactions than either the group receiving DTP$_w$Hib (SB) at different sites (difference between proportions 12.8%, 95% confidence intervals 3.9% to 21.6%) or the group receiving DTP$_w$Hib (PM) at different sites (difference between proportions

Table 2 Geometric mean titres (GMT) and 95% confidence intervals (CI) one month after final vaccine dose for children who received three vaccine doses

	DTP$_w$Hib (SB) same syringe		DTP$_w$Hib (SB) different sites		DTP$_w$Hib (Merieux) different sites	
	GMT	(95% CI)	GMT	(95% CI)	GMT	(95% CI)
Hib	2.78	(2.05, 3.77)	5.42	(3.82, 7.69)	4.72	(3.19, 6.99)
Pertussis:						
PT	1049	(784, 1402)	871	(622, 1222)	981	(670, 1437)
FHA	1761	(1512, 2052)	1819	(1523, 2172)	1975	(1616, 2413)
69 kDa	6546	(5274, 8126)	7603	(5913, 9777)	5922	(4459, 7867)
Aggs 2+3	32731	(26236, 40835)	41922	(32413, 54225)	50898	(38067, 68057)
Diphtheria	2.08	(1.67, 2.60)	1.20	(0.93, 1.55)	1.56	(1.17, 2.09)
Tetanus	1.12	(0.94, 1.34)	1.50	(1.22, 1.86)	1.86	(1.46, 2.35)

Table 3 Summary of incidence of local reactions (injection site redness, swelling or pain), numbers and % of total vaccine doses administered

	DTP$_w$Hib (SB) same syringe		DTP$_w$Hib (SB) different sites		DTP$_w$Hib (Merieux) different sites	
	No.	%	No.	%	No.	%
Vaccinations given	264	100	221	100	187	100
One or more local reactions	118	45	127	57	117	63
Reaction at Hib site only	—	—	12	5	6	3
Reaction at DTP$_w$ site only	—	—	77	35	50	27
Reaction at both Hib and DTP$_w$ sites	—	—	38	17	61	33

Table 4 Incidence of general symptoms and reactions, numbers and % of total vaccine doses administered

	DTP$_w$Hib (SB) same syringe		DTP$_w$Hib (SB) different sites		DTP$_w$Hib (Merieux) different sites	
	No.	(/%)	No.	(/%)	No.	(/%)
Vaccinations given	264	100	221	100	187	100
One or more general reactions	189	72	154	70	133	71
Fever	17	6	17	8	19	10
Unusual crying	72	27	60	27	48	26
Vomiting	57	22	42	19	44	24
Diarrhoea	85	32	49	22	48	26
Loss of appetite	60	23	50	23	45	24
Restlessness	98	37	74	33	78	42
Sleepiness	84	32	81	37	79	42

17.9%, 95% confidence intervals 8.7% to 27.0%). This effect was due, at least in part, to the fact that in the latter two groups two rather than one injection was administered to each subject. Six subjects out of 230 (3%), including three who received DTP_W and Hib (SB) combined in the same syringe, one who received DTP_W and Hib (SB) administered in opposite limbs, and two who received DTP_W and Hib (PM) administered in opposite limbs, reported an unsolicited event on the day of immunisation prior to its administration (baseline events); 202 (88%) reported such events following immunisation (treatment emergent events). There was no significant difference between the treatment groups in the distribution of either baseline or treatment emergent events. The most common unsolicited adverse events were induration of the injection site (39%) and common cold (18%).

Overall, 18 of the 230 (8%) reported serious non-fatal adverse experiences, 6 out of 89 (7%) given DTP_WHib (SB) in the same syringe, 5 out of 75 (7%) given DTP_W and Hib (SB) at different sites, and 7 out of 66 (11%) given DTP_WHib (PM) at different sites. These differences were not statistically significant. The most common serious adverse experiences were diarrhoea 4 (2%), poor feeding 4 (2%), vomiting 3 (1%) and crying 2 (1%). Two subjects, both in the group which received DTP_WHib (PM) in separate sites, were withdrawn due to adverse events, one because of redness and swelling of the injection site of 9 cm by 5.5 cm after the first vaccination and the second because of hospitalisation for uncontrollable crying, screaming, refusal to breast-feed, diarrhoea and irritability following the second vaccination. The first was not considered to be serious but was considered to be related to the vaccination. The second was the only serious adverse effect considered to be possibly related to vaccination.

DISCUSSION

In this open, randomised controlled trial (RCT) all three vaccination schedules resulted in concentrations of anti-PRP of $\geq 0.15\ \mu g\ ml^{-1}$, concentrations of antibody to diphtheria and tetanus toxoids of $\geq 0.01\ IU\ ml^{-1}$ and an immunological response to pertussis antigens (PT, FHA, 69 kDa, Agg 2 plus 3) 1 month after administration of the final vaccine dose. All three groups showed a Hib antibody response above $0.15\ \mu g\ ml^{-1}$ in nearly all cases. The group given the vaccines in the same syringe had statistically significant lower GMTs to Hib. Moreover the group given the vaccines in the same syringe had no more general reactions than the group receiving the vaccines separately. The lower incidence of local reactions is probably explained by the lower number of injection sites in the group receiving the combined vaccine. Two other studies examined concomitant administration of DTP_WHib in the same syringe with a similar schedule[3,4], however, there were important differences in the design of these studies. In particular Begg et al. used the results from children in previous trials as controls in a non-random study[3]. The finding in their study of an increased immunological response to tetanus toxoid in those given the vaccines in the same syringe was not confirmed although an increased immunological response to diphtheria toxoid when the vaccines were given in the same syringe was observed in the present trial. Nevertheless the findings are similar in both of these studies since the combination of the vaccines in the same syringe produces an effective antibody response with no increase in adverse events.

A number of other trials of combined DTP_WHib according to other vaccination schedules has been reported[5–13]. Two trials of combined DTP_W oligosaccharide conjugate Hib vaccine (HbOC) at two, four and six months of age found that immunogenicity and reactogenicity were similar in children given the vaccine mixed in the same syringe or separately[5,6]. These trials both used different pertussis vaccines from that used in this study.

Other trials have examined DTP_WHib (PRP-T) in infants immunised at two, four and six months[7–13]. One study examined Hib antibody levels when DTP_WHib (PRP-T) was given in the same syringe and found that these were satisfactory[7]. Two reports noted no statistically significant differences in Hib antibody responses when the vaccines were combined. One compared DTP_WHib with DTP_W and found no difference in local or systemic reactions; 98% of subjects given the combined vaccine developed PRP antibody levels of $0.15\ \mu g\ ml^{-1}$ or more and 88% had a level of $1.0\ \mu g\ ml^{-1}$ or more, levels comparable to the present study; antibody responses to diphtheria and tetanus toxoid were comparable but one of the two lots of Hib vaccine when combined with DTP resulted in a statistically significant reduction in antibody response to pertussis toxin[8]. The second study compared DTP_WHib with DTP_W and Hib administered separately and found a slightly higher incidence of local reactions when the vaccines were combined but no difference in antibody response to PRP, diphtheria, tetanus or pertussis antigens[9]. One double-blind randomised controlled trial in Chile found that administering DTP_WHib in the same syringe resulted in lower responses of both PRP and tetanus antibodies and minor differences in reactions[10,11]. Two further open randomised controlled trials, one of which included inactivated poliomyelitis vaccine, have also demonstrated the efficacy and safety of DTP_WHib administered in the same syringe[12,13]. In both trials they noted some reduction in antibody responses to pertussis antigens and, in the trial incorporating inactivated polio vaccine, reduced antibody responses to PRP and tetanus toxoid, when the vaccines were mixed. When the vaccines were mixed local reactions were lower in the latter trial and higher in the trial which did not use inactivated polio vaccine, but systemic reactions were comparable.

The primary immunisation programme for infants in the UK has been extremely successful in recent years and over 90% now receive both DTP_W and Hib before their first birthday. Halving the number of injections necessary to offer protection has advantages to both parents and children on the one hand and doctors and nurses on the other. This study has confirmed that this is now possible using a combined DTP_WHib vaccine and in accordance with the UK's accelerated primary immunisation schedule at two, three and four months of age.

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