

A combined liquid Hib (PRP-OMPC), hepatitis B, diphtheria, tetanus and whole-cell pertussis vaccine: controlled studies of immunogenicity and reactogenicity

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Received 17 July 2000; received in revised form 18 September 2000; accepted 10 October 2000

Abstract

We evaluated the immunogenicity and reactogenicity of a new liquid pentavalent combination vaccine, which incorporates a diphtheria, tetanus and whole-cell pertussis vaccine (DTP) with Hib (PRP-OMPC) and hepatitis B vaccine (HB), in a series of three studies involving 2156 infants. The vaccination schedule was 2, 4, 6 and 18 months for all studies. In addition, subjects in the third study also received a dose of monovalent hepatitis B vaccine at birth. The principal study was a randomised double blind trial of two separate, but concurrently administered vaccines in each of three groups: pentavalent vaccine [DTP-Hib-HB] plus placebo (Group A, $n = 619$); quadrivalent vaccine [DTP-HB] plus Hib vaccine (Group B, $n = 620$); and bivalent vaccine [Hib-HB] plus DTP (Group C, $n = 226$). The second study (Group D, $n = 231$) was an open trial of three separate, but concurrently administered licenced control vaccines (DTP, Hib and HB). The third study (Group E, $n = 460$) administered a dose of monovalent hepatitis B vaccine at birth followed by pentavalent vaccine as for Group A. Subjects were bled prior to the 2- and 18-month vaccinations, and a month after the 6- and 18-month vaccinations. A diary card was used to record subject temperatures and other systemic and local clinical signs for 7 days after each vaccination. The pentavalent vaccine, whether or not preceded by a birth dose of hepatitis B vaccine, was generally well tolerated at all administration times, and had a reactogenicity profile similar to that observed for licenced vaccine controls. Diphtheria and tetanus antibody levels were substantially above protective levels in all study groups. The anti-HBs responses ($\% \geq 10$ mIU/ml) following the 6-month dose of vaccines were, respectively, for Groups A–E: 83.2, 91.7, 96.5, 98.8 and 93.9%, and following the 18-month doses: 87.9, 97.5, 98.8, 98.8 and 92.8%. Anti-PRP responses ($\% \geq 1.0$ μ g/ml) following the 6-month dose for Groups A–D were 86.0, 90.5, 91.2, and 74.4%, and after the 18-month dose for Groups A–E were 97.3, 98.3, 98.1, 97.0, and 99.5%. Consistently higher geometric mean titres (GMTs) for pertussis antibodies to agglutinogens (Agg2, Agg3) and pertactin were recorded for the pentavalent vaccine compared to the licenced control vaccine, though they were somewhat lower for pertussigen (PT). Except for the hepatitis B response, antibody responses induced by the pentavalent vaccine to all antigens with a schedule commencing at 2 months of age and completed at 18 months were equivalent to responses to the same antigens induced by the separate, but concurrently administered licenced control vaccines. A regimen of a birth dose of hepatitis B vaccine followed by pentavalent vaccine at 2, 4, 6 and 18 months was not countered by any clinically significant decrease in seroresponses. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Vaccine; Immunogenicity; Diphtheria; Hepatitis B; Pertussis; Tetanus; Hib-vaccine

1. Introduction

Vaccination programs with a combined preparation containing diphtheria and tetanus toxoids in addition

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to whole cell-derived pertussis components (DTP) have contributed substantially to the decline of diphtheria, tetanus and pertussis in developed countries. Hepatitis B poses a major public health threat throughout the world with an estimated 350 million carriers and 1–1.5 million deaths attributed annually to acute and chronic infection [1]. Universal vaccination of infants with hepatitis B vaccine (HB) is now recommended by WHO, the U.S. Advisory Committee on Immunisation Practices and the NH&MRC in Australia [2,3]. Impediments to the implementation of universal hepatitis B vaccination include a lack of suitable polyvalent vaccines that would reduce the number of injections required for an infant at any one visit and the potential cost of such vaccines. In addition, *Haemophilus influenzae* type b (Hib) conjugate vaccines are routinely administered in more than 20 countries and, since being made available to all children under 5 years of age in Australia in 1993, a reduction in meningitis incidence of more than 90% has been recorded [4].

If universal vaccination of infants against the five disease targets referenced above were to be implemented with a DTP vaccine, and monovalent HB and Hib vaccines, infants and young children would receive a course of up to 11 injections in the first 2 years of life. The Scientific Advisory Group of Experts (SAGE) of the WHO's Global program on vaccination has recently recognised the importance of combination vaccines in the future of immunisation [5]. However, difficulties with vaccine component interactions have slowed their development [6–8].

Following a preliminary pilot evaluation published previously [9], we now report the evaluation of a new liquid pentavalent combination vaccine for infant immunisation, which incorporates DTP with Hib (PRP-OMP) and hepatitis B vaccines. In order to delineate the performance of the pentavalent vaccine, a series of studies was designed to include comparison of immunogenicity and reactogenicity with other subset combinations of HB, Hib, and DTP vaccines.

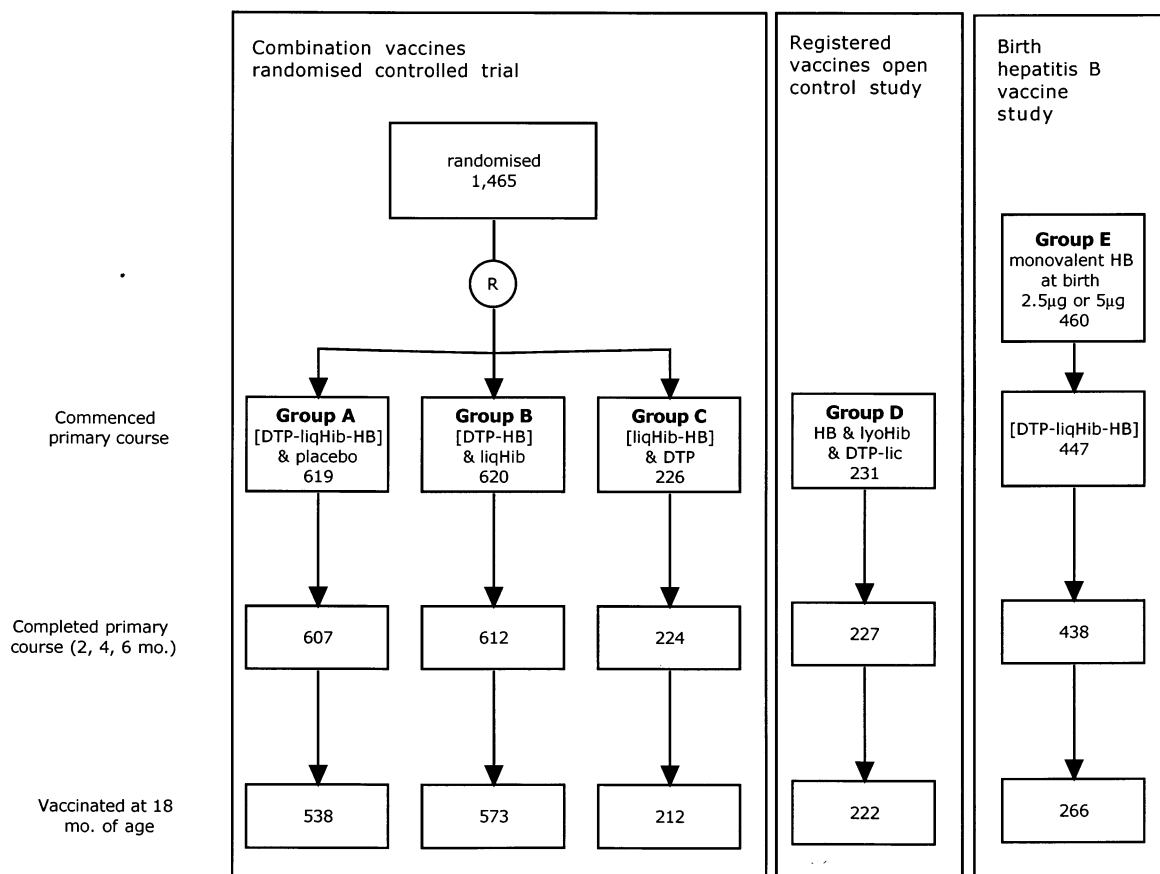


Fig. 1. Study design and subject numbers.

2. Method

2.1. Design

An integrated series of three separate studies in 2156 infants was conducted (Fig. 1) based on a primary course and booster schedule of 2-, 4-, 6-, and 18-month doses. The studies were conducted between October 1995 and April 1998. The principal study was a randomised double blind trial of two separate, but concurrently administered vaccines in each of three groups: pentavalent vaccine containing DTP, HB and liquid PRP-OMPC (liqHib) vaccines [DTP-liqHib-HB] plus placebo (Group A, $n = 619$), quadrivalent vaccine [DTP-HB] plus liqHib vaccine (Group B, $n = 620$), and bivalent vaccine [HB-liqHib] plus DTP (Group C, $n = 226$). The volume (0.5 ml) and appearance were the same for all study vaccines. The randomisation plan was designed to allocate subjects in a 3:3:1 ratio to Groups A, B and C. The randomised allocation was computer generated, with stratification by consent for immunogenicity testing and blocks of varying size. Vaccine packs were numbered according to the randomisation list and sequentially allocated to subjects.

In a contemporaneous but separate study (Control study) conducted in the same area, three separate, but concurrently administered control vaccines [licensed DTP (DTP-lic), lyophilised PRP-OMPC (lyoHib), and HB] were given in an open trial (Group D, $n = 231$: DTP + lyoHib + HB). This study was conducted as a separate study because adding such a control arm to Groups A–C in the principal study would have meant two placebo injections for Group A, and a placebo as a third injection for Groups B and C. Such a design was unlikely to have been acceptable to the institutional ethics committee and parents.

The third study (Group E, $n = 460$) involved administration of a dose of monovalent hepatitis B vaccine (randomised to either 5 or 2.5 μg) at birth followed by pentavalent vaccine at 2, 4, 6, and 18 months. At the 18-month booster stage, of the 404 subjects available for follow-up, the first 126 subjects to turn 18 months of age received pentavalent vaccine. The remaining 278 subjects were randomised to receive with equal probability either pentavalent vaccine or DTPa and a bivalent vaccine [HB-liqHib]. Analysis of baseline characteristics showed that there were no important differences between these subgroups. In total, 266 subjects received pentavalent vaccine and are reported here. Results from the latter group ($n = 138$) will be reported separately as they do not relate to the study vaccines. Oral poliomyelitis vaccine (OPV) was given to infants in all five groups concurrently with study vaccines at 2, 4 and 6 months of age. All studies were approved by appropriately constituted institutional ethics committees from the state health department

(Group D), Royal Children's Hospital (Groups A–C) and Royal Women's Hospital (Group E).

2.2. Subjects

Infants in good health were recruited through community maternal and child health clinics in greater Melbourne (Groups A–D) and from the maternity service of the Royal Women's Hospital in Melbourne (Group E). Written informed consent was provided by parents. Infants were ineligible for study if they had contracted pertussis, pertussis-like syndrome, or Hib disease; if they had immune deficiency syndromes; if their mothers were known to be hepatitis B carriers; or if they had received any blood products, prior vaccines for study disease targets, high dose corticosteroids, cytotoxic agents or radiotherapy.

2.3. Procedures

Home immunisation, follow-up, and 7-day symptom diary recording procedures have previously been described [9]. Oral paracetamol (15 mg/ml) was routinely administered prior to vaccination at 2, 4, 6 and 18 months. Further doses of paracetamol were given at the parents' discretion at 4-h intervals. Pre- and post-vaccination blood samples were obtained from approximately 75% of all subjects before the 2-month vaccination and 4–6 weeks after the third (6-month) and booster (18-month) doses. A cord blood sample was also collected for newborns in Group E. Infants in Groups A–C received 0.5-ml intramuscular injections of vaccine or placebo into the lateral aspect of each thigh or arm (booster). Group D received DTP and lyoHib vaccines in each thigh and HB into the deltoid of one arm. At 18 months they received DTP and HB into left and right arms and lyoHib into the thigh. Group E subjects received HB vaccine (within 7 days of birth) and subsequent pentavalent vaccine doses into a thigh, or arm (booster). For the principal study (Groups A–C), all study staff and parents/guardians were blinded to the vaccine administered for the duration of the study. The control study (Group D) was an open study, but parents/guardians were not aware of which vaccines were being administered in particular limbs. The third study (Group E) was an open study with regard to administration of the pentavalent vaccine.

2.4. Vaccines

2.4.1. Pentavalent vaccine

[DTP-Hib-HB] (Pentavax™, Merck & Co., Inc.) (adsorbed Diphtheria, Tetanus, Pertussis, hepatitis B and *H. influenzae* type b Vaccine) contained 30 Lf Diphtheria toxoid, 6 Lf Tetanus toxoid, not more than 20×10^9

inactivated *Bordetella pertussis* organisms, 5 µg hepatitis B surface antigen recombinant (HBsAg) and 7.5 µg *H. influenzae* type b PRP, 125 µg *Neisseria meningitidis* OMPC and 225 µg of aluminium as aluminium phosphate (423.5 µg) and aluminium hydroxide (379 µg) per dose. Thiomersal 0.01% w/v was added as a preservative. Investigational formulation.

2.4.2. Quadrivalent vaccine

[DTP-HB] (QuadriVax™, Merck & Co., Inc.) (adsorbed Diphtheria, Tetanus, Pertussis and Hepatitis B vaccine) contained 30 Lf Diphtheria toxoid, 6 Lf Tetanus toxoid, not more than 20×10^9 inactivated *B. pertussis* organisms, 5 µg hepatitis B surface antigen recombinant (HBsAg) and 225 µg of aluminium as aluminium phosphate (762.5 µg) and aluminium hydroxide (162.5 µg). Thiomersal 0.01% w/v. Investigational formulation.

2.4.3. Bivalent vaccine

[HB-liqHib] (CSL Ltd.) contained 7.5 µg *H. influenzae* type b capsular polysaccharide (PRP) conjugated to 125 µg *N. meningitidis* outer membrane protein complex (OMPC), 5 µg hepatitis B surface antigen recombinant (HBsAg), 225 µg aluminium as aluminium hydroxide (650 µg). Thiomersal 0.005% w/v was added as a preservative. Investigational formulation.

2.4.4. Liquid Hib vaccine

[liqHib] (CSL Ltd.) 7.5 µg *H. influenzae* type b capsular polysaccharide (PRP) conjugated to 125 µg *N. meningitidis* outer membrane protein complex (OMPC), and 225 µg of aluminium as aluminium hydroxide (650 µg). Thiomersal 0.005% w/v. Investigational formulation.

2.4.5. Lyophilised Hib vaccine

[lyoHib] (PedvaxHIB™, Merck & Co., Inc.) 15 µg *H. influenzae* type b capsular polysaccharide (PRP), conjugated to 250 µg *N. meningitidis* OMPC, 225 µg of aluminium as aluminium hydroxide, thiomersal at 1:20 000 and 2.0 µg of lactose in 0.9% sodium chloride.

2.4.6. DTP-lic vaccine

Licensed adsorbed diphtheria, tetanus and pertussis vaccine (Triple Antigen™, CSL Ltd.) contained 30 Lf Diphtheria toxoid, 6 Lf Tetanus toxoid, not more than 20×10^9 inactivated *B. pertussis* organisms, thiomersal 0.01% w/v as a preservative and aluminium phosphate.

2.4.7. DTP vaccine

As for DTP-lic but formulated to contain lower free phosphate concentration to enable the addition of other vaccine antigens. This vaccine was licensed in Australia and routinely distributed from January 1997.

2.4.8. Monovalent hepatitis B vaccine

[HB] (HB-Vax™II, Merck & Co., Inc.) 5 µg hepatitis B surface antigen recombinant adsorbed onto approximately 250 µg aluminium hydroxide and thiomersal 1:20 000.

2.4.9. Placebo

Aluminium (225 µg) as aluminium hydroxide (650 µg), Thiomersal 0.005% w/v.

2.5. Immunogenicity

All antibody assays were performed by CSL Ltd. except anti-PRP and anti-HBs which were carried out by Merck Research Laboratories. Assay techniques have been reported previously [9]. Geometric mean titres (GMT) were calculated for antibody responses to all study vaccine antigens. In Group E subjects, anti-HBs alone was assayed at birth, 2 and 7 months. For pertussis antibody responses at 19 months, results for Group E subjects were virtually identical to those recorded for Group A subjects and, for simplicity, are not presented in this report. Antibody titre thresholds used for calculating protected proportions were: anti-tetanus and anti-diphtheria 0.01 IU/ml, anti-HBs 10 mIU/ml, anti-PRP 1 µg/ml. Pertussis antibody responses [pertussigen (PT), filamentous haemagglutinin (Fha), agglutinin Type 2 (Agg 2), agglutinin Type3 (Agg 3), and pertactin] were expressed only as GMTs.

2.6. Statistical methods

Formal statistical comparisons of reactogenicity and immunogenicity data from subjects in Group A were made with data from subjects in Group B, Group C and Group D. Only informal comparisons were made between subjects in Group E and subjects in Groups A–D. Antibody responses were summarized both as proportion of subjects reaching recognized protective levels of antibody titre, with standard exact binomial confidence intervals, and as geometric mean titres with 95% confidence intervals, obtained in the standard way by applying normal-theory methods to log-transformed data. Geometric mean fold changes (ratios) comparing pre- and post-vaccination titres were also calculated, with confidence intervals. The analysis method and decision rules for judging equivalence on comparisons between vaccine groups were determined a priori. Equivalence was concluded if the upper bound of the CI on the observed difference in seroprotection rates between groups was ≤ 15 percentage points for each of the post-vaccination antibody responses to PRP, HBsAg, tetanus toxoid and diphtheria toxoid and if the upper bound of the CI on the observed difference in the GMT ratios was ≤ 2.5 -fold for each of the postvacci-

Table 1

Percentage of systemic and local clinical signs experienced by study groups during 48 h following vaccination^a

	Group				
	A [DTP-liqHib-hepB] + placebo	A Placebo	B [DTP-hepB] + liqHib	C DTP + [liqHib-hepB]	D DTP + lyoHib + hepB
Third vaccination (6 months)	<i>n</i> = 604	<i>n</i> = 604	<i>n</i> = 612	<i>n</i> = 224	<i>n</i> = 226
Redness > 25 mm	16.9* (14.0, 20.1)	1.2 (0.47, 2.4)	14.4 (11.7, 17.4)	12.1 (8.1, 17.1)	8.8* (5.5, 13.3)
Swelling > 25 mm	19.9* (16.8, 23.3)	1.2 (0.47, 2.4)	15.5 (12.7, 18.6)	10.3 (6.6, 15.0)	12.8* (8.8, 17.9)
Tenderness	53.3* (49.2, 57.3)	17.2 (14.3, 20.5)	48.9 (44.8, 52.9)	43.3 (36.7, 50.1)	43.4* (36.8, 50.1)
Temperature ≥ 38°C	26.6 (23.1, 30.3)		25.2 (21.8, 28.8)	25.4 (19.9, 31.7)	26.5 (20.9, 32.8)
Temperature ≥ 39°C	3.8 (2.4, 5.7)		3.6 (2.3, 5.4)	2.2 (0.7, 5.1)	4.0 (1.8, 7.4)
Excessive fussiness	21.5 (18.3, 25.0)		18.3 (15.3, 21.6)	16.5 (11.9, 22.0)	20.8 (15.7, 26.7)
Fourth vaccination (18 months)	<i>n</i> = 536	<i>n</i> = 536	<i>n</i> = 571	<i>n</i> = 212	<i>n</i> = 222
Redness > 25 mm	40.7 (36.5, 45.0)	1.7 (0.77, 3.2)	39.6 (35.5, 43.7)	33.5 (27.2, 40.3)	41.4 (34.9, 48.2)
Swelling > 25 mm	34.9 (30.9, 39.1)	1.7 (0.77, 3.2)	29.9 (26.2, 33.9)	27.5 (21.6, 34.0)	31.5 (25.5, 38.1)
Tenderness	78.4 (74.6, 81.8)	31.9 (28.0, 36.0)	79.9 (76.3, 83.1)	76.4 (70.1, 82.0)	82.0 (76.3, 86.8)
Temperature ≥ 38°C	39.6 (35.5, 43.9)		44.9 (40.8, 49.1)	50.9 (44.0, 57.9)	43.7 (37.1, 50.5)
Temperature ≥ 39°C	9.3 (7.0, 12.1)		9.9 (7.5, 12.6)	10.8 (7.0, 15.8)	9.0 (5.6, 13.6)
Excessive fussiness	19.1 (15.9, 22.7)		17.9 (14.8, 21.3)	17.0 (12.2, 22.7)	20.3 (15.2, 26.2)

^a Local clinical signs reported for limb receiving vaccine containing DTP; 95%CI in parentheses; *n* = number of subjects vaccinated. The denominator for each outcome varies slightly from this due to missing responses; Excessive fussiness, crying and could not be comforted at times or at all; temperatures by axillary thermometer.

* Statistically significant ($P < 0.05$) higher rates for Group A compared to Group D (see text for details).

nation antibody responses to pertussis antigens [10]. The frequency of adverse events was tabulated with 95% confidence intervals obtained by the binomial method. The cumulative frequencies of systemic and local clinical events at 48 h post-vaccination are reported because the common reactions associated with DTP vaccination cluster within this period [11]. Fever was defined as axillary temperature $\geq 38^\circ\text{C}$, and excessive fussiness as occurring in babies who were crying and could not be comforted at times or at all.

3. Results

3.1. Reactogenicity

Oral paracetamol usage was high ($\geq 93\%$ of subjects) during the first 48 h after each injection in all groups (A–E). Paracetamol usage declined substantially over the next several days post-vaccination and there were no notable differences among Groups. One hypotonic hyposponsive episode was reported (Group A). There were 10 subjects who experienced convulsions (eight of the 10 convulsions were assessed as febrile convulsions) following vaccination: two in Group A (one post dose 1, one post-booster), three in Group B (three post-booster), one in Group C (post-booster), one in Group D (post-booster, child had pre-existing epilepsy), three in Group E (post-dose 3). Table 1 summarises clinical events associated with vaccination in the 48-h period

following doses 3 and 4 for Groups A–D. Group E results were virtually identical to those recorded in Group A, and are not shown. For Group A comparisons, the local response was compared to the limb injected with DTP in other groups.

Following the first injection, subjects receiving pentavalent vaccine (Group A) experienced significantly lower rates ($P < 0.001$) of fever (25.9%), tenderness (45.9%) and drowsiness (55.4%) than those in the Control study (Group D) (37.2, 59.3, and 69.7%, respectively). There were no significant differences between Group A and D following dose 2.

Following the third vaccination (Table 1), there were significantly higher rates of local swelling ($P = 0.001$), and redness ($P = 0.004$) and a higher proportion with tenderness at the injection site ($P = 0.011$) in infants in Group A compared to Group D. There were similar rates of fever and excessive fussiness in these groups, and after the 18-month dose, the reactogenicity profile overall was also comparable. Placebo local adverse event rates after the third and fourth vaccinations for redness > 25 mm were, respectively, 1.2 and 1.7%, swelling > 25 mm 1.2 and 1.7%, and tenderness, 17.2 and 31.9%.

There were no significant differences in the clinical event parameters at 2, 4, 6 and 18 months for Group E subjects who received 5 μg compared to those who had received 2.5 μg of monovalent hepatitis B vaccine at birth (data not shown).

3.2. Immunogenicity

Antibody responses to tetanus and diphtheria toxoids (data not shown) induced by the pentavalent vaccine (Group A), quadrivalent vaccine (Group B), and reformulated DTP vaccine (Group C) produced seroprotection in all subjects and were equivalent to antibody responses to the same antigens induced by the licenced control vaccine (DTP-lic) in Group D. Table 2 presents the anti-HBs and anti-PRP responses for all study groups, and Figs. 2 and 3 illustrate reverse cumulative distribution plots for these responses. At 7 months, the proportion of subjects with a seroprotective response to hepatitis B was lower for the pentavalent vaccine compared to both Groups C and D, failing to reach the statistical equivalence criterion. In Group E, following their birth dose of monovalent HB vaccine and immediately prior to the 2-month dose of the pentavalent vaccine, 18% of infants had anti-HBs titres ≥ 10 mIU/ml.

Subsequently, 93.9% of these infants achieved titres ≥ 10 mIU/ml, approximating responses in Groups B and C but observationally lower than the 98.8% achieved in Group D. Anti-HBs GMTs reflected these

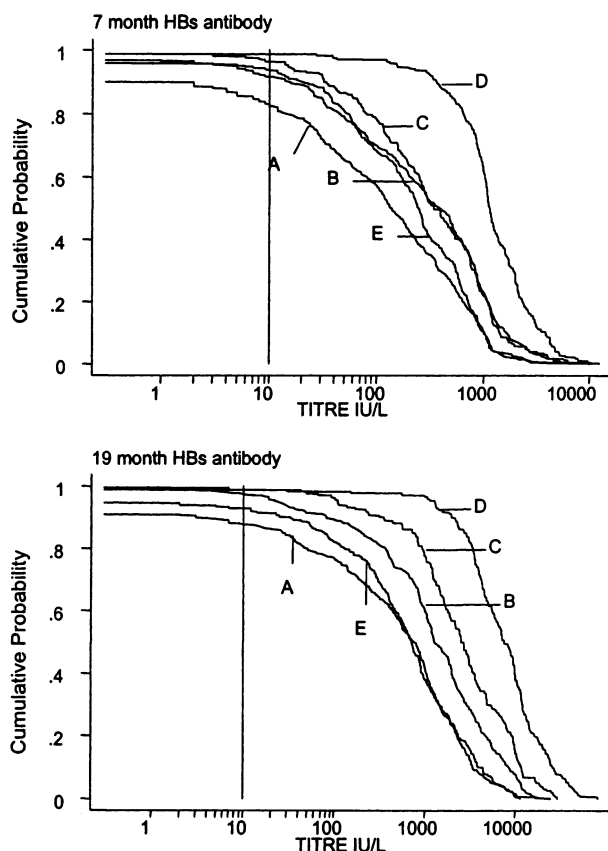


Fig. 2. Reverse cumulative distribution plots at 7 months (upper panel) and 19 months (lower panel) for anti-HBs antibody responses for study groups A–E (see text for explanation).

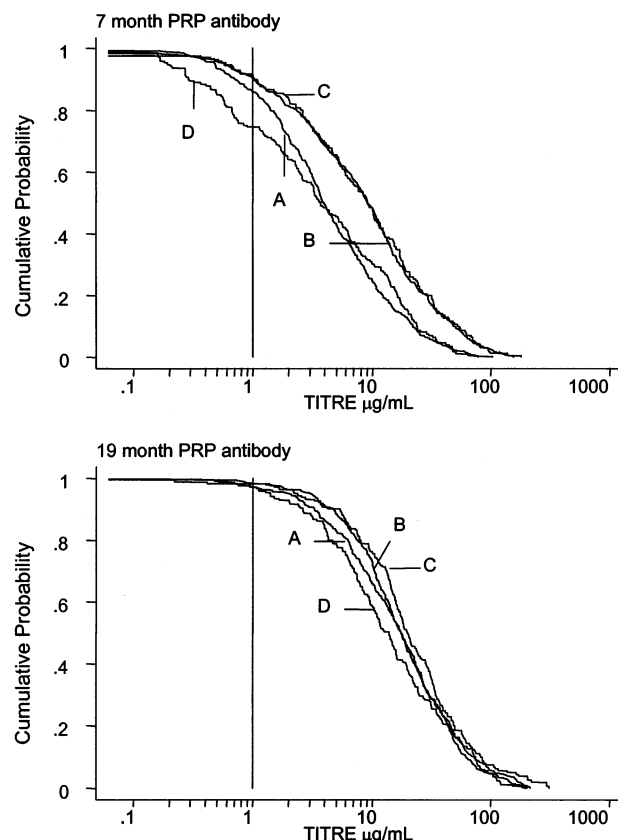


Fig. 3. Reverse cumulative distribution plots at 7 months (upper panel) and 19 months (lower panel) for anti-PRP antibody responses for study groups A–E (see text for explanation).

differences with the pentavalent GMT (Group A; no HB vaccine birth dose) being 10-fold lower than Group D. Administration of a HB vaccine birth dose (Group E) more than doubled the GMT at 7 months compared to no HB vaccine birth dose (Group A).

Following the 18-month dose, no comparison crossed the equivalence threshold. However, the pentavalent vaccine (Group A) anti-HBs response was still lower than all other groups (87.9%, GMT 294 mIU/ml). The Group E protected proportion was 92.8% (95%CI 88.5%, 95.5%), with a GMT of 424 mIU/ml representing a mean 33-fold increase in titre over the pre-booster level. For Group E children, there were virtually identical anti-HBs responses at both 7 and 19 months for subjects who received 5 µg compared to those who had received 2.5 µg of monovalent hepatitis B vaccine at birth (data not shown).

Anti-PRP responses (Table 2) were substantially higher in all three combination vaccine groups at 7 months than recorded for the licenced control group, with a protected proportion (≥ 1.0 µg/ml) of 86% (95%CI 82.3%, 89.2%) for subjects receiving the pentavalent vaccine. Following the 18-month booster dose, there was substantial boosting of titres in Groups A–D, with similar post-booster GMTs and proportions of subjects at or above the protective threshold.

Table 2
Antibody responses to HBsAg and PRP in the vaccine groups and comparison between the groups^a

	A [DTP-liqHib-hepB] + placebo	B [DTP-hepB] + liqHib	B versus A	C DTP +[liqHib-hepB]	C versus A	D DTP-lic + lyoHib + hepB	D versus A	E birth hepB [DTP-liqHib-hepB]
<i>7 months</i>								
✕ HBsAg (mIU/ml)	<i>n</i> = 417	<i>n</i> = 433		<i>n</i> = 171		<i>n</i> = 172		<i>n</i> = 347
% ≥ 10 mIU/ml	83.2 (79.3, 86.7)	91.7 (88.7, 94.1)	8.5 (4.0, 12.9) S	96.5 (92.5, 98.7)	13.3 (8.8, 17.8) U	98.8 (95.9, 99.9)	15.6 (11.7, 19.6) U	93.9 (90.9, 96.2)
GMT	78.5 (62.0, 99.5)	218 (180, 266)	2.8 (2.0, 3.8)	284 (222, 365)	3.6 (2.4, 5.4)	1066 (877, 1295)	13.6 (9.2, 20.0)	167 (137, 203)
PRP (μg/ml)	<i>n</i> = 415	<i>n</i> = 431		<i>n</i> = 170		<i>n</i> = 172		
% ≥ 1 μg/ml	86.0 (82.3, 89.2)	90.5 (87.3, 93.1)	4.50 (0.13, 8.80) S	91.2 (85.9, 95.0)	5.2 (−0.3, 10.6) S	74.4 (67.2, 80.8)	−11.6 (−18.9, −4.3) U	*
GMT	4.0 (3.6, 4.6)	7.6 (6.6, 8.8)	1.9 (1.6, 2.3)	7.8 (6.2, 9.8)	1.9 (1.5, 2.5)	3.4 (2.6, 4.3)	0.83 (0.65, 1.07)	*
<i>19 months</i>								
HBsAg (mIU/ml)	<i>n</i> = 373	<i>n</i> = 401		<i>n</i> = 160		<i>n</i> = 166		<i>n</i> = 221
% ≥ 10 mIU/ml	87.9 (84.2, 91.1)	97.5 (95.5, 98.8)	9.6 (5.9, 13.2) S	98.8 (95.6, 99.8)	10.8 (7.1, 14.5) S	98.8 (95.7, 99.9)	10.9 (7.2, 14.6) S	92.8 (88.5, 95.8)
GMT	294 (222, 389)	1028 (852, 1241)	3.5 (2.5, 4.9)	2192 (1706, 2817)	7.5 (4.7, 11.8)	6345 (5009, 8037)	22 (14, 34)	424 (310, 578)
PRP (μg/ml)	<i>n</i> = 373	<i>n</i> = 401		<i>n</i> = 160		<i>n</i> = 165		<i>n</i> = 221
% ≥ 1 μg/ml	97.3 (95.1, 98.7)	98.3 (96.4, 99.3)	0.94 (−1.15, 3.02) S	98.1 (94.6, 99.6)	0.81 (−1.86, 3.47) S	97.0 (93.1, 99.0)	−0.35 (−3.44, 2.74) S	99.5 (97.5, 100)
GMT	15 (14, 17)	18 (16, 20)	1.14 (0.97, 1.35)	19 (16, 24)	1.3 (1.0, 1.6)	13 (11, 16)	0.85 (0.68, 1.07)	21 (18, 24)

^a 95% confidence interval in parentheses; for group differences in % > threshold: S, satisfied equivalence criteria (lower bound 95% CI ≥ −15% and upper bound CI ≤ 15%); U, unable to meet equivalence criteria (lower bound 95% CI < −15% or upper bound CI > 15%); GMT comparisons are ratios.

* Not assayed at 7 months.

Table 3 shows pertussis antibody responses for Groups A–D. GMTs for PT in the three combination vaccine groups (A–C) were about half those of the licenced control (Group D) following the third and fourth doses. For the comparison of pentavalent vaccine versus licenced control at 7 months, the PT response did not reach the statistical equivalence threshold. The FHA GMTs were similar for all four groups. FHA responses were similar across all four groups. Higher GMTs for Agg2 and Agg3 were recorded for both pentavalent and quadrivalent vaccine groups compared to licenced controls, but they were well within the statistical equivalence criterion at both time points. A similar pattern of response was observed for pertactin.

4. Discussion

Acceptance of a paediatric combination vaccine requires that the obvious benefits derived from reducing the number of injections are not offset by a clinically meaningful increase in vaccine reactogenicity or a reduction in the protective efficacy against any of the disease targets.

Our studies demonstrate that the pentavalent vaccine was generally well tolerated during infancy and the second year of life. Moreover, prior administration of a birth dose of HB vaccine had no untoward effect on the reactogenicity of the pentavalent vaccine. Compared to the licenced control vaccines, there was a minor increase in local reactions after the 6-month dose, but this trend was not observed after the 18-month dose. Conversely, there were slightly lower rates of tenderness after the 2-month dose. For systemic reactions, there were slightly lower rates of fever after the 2-month dose, but similar rates after the remaining doses. Overall, the reactogenicity profile of the pentavalent vaccine was broadly comparable to the simultaneous administration of its component vaccines documenting that the addition of hepatitis B and Hib components to DTP did not result in increased reactogenicity. This observation is consistent with the historical experience with reactogenicity in the development of paediatric combination vaccines [7].

Since most paediatric combination vaccines are composed of licenced component vaccines, many of which have well-established immunologic correlates of protection, comparative immunogenicity between the combination vaccine and its components is generally accepted as the efficacy endpoint. For pertussis vaccines, there are no established immunologic correlates of protection. In our studies of the pentavalent vaccine, we used comparative immunogenicity as the efficacy endpoint for all components including pertussis since the same pertussis components and doses were included in both

the pentavalent vaccine and the licenced control vaccines.

A substantial obstacle to combination vaccine development has been inhibition of antibody responses to component antigens [7]. Such inhibition may result from chemical or immunologic interactions, but the precise mechanisms remain unknown. The most consistent inhibition of antibody responses has been observed with combination Hib/DTaP vaccines, which have generally induced substantially lower anti-PRP responses compared to those induced by control monovalent Hib conjugate vaccines [6–8]. The pentavalent vaccine induced a robust anti-PRP response which was comparable to that induced by the licenced control vaccine and there was no suggestion of inhibition of the anti-PRP response. Moreover, in our pilot study [9] of the pentavalent vaccine, the post dose 1 anti-PRP response ($\% \geq 1.0 \mu\text{g/ml}$) was 81%, indicating that the unique first-dose response [12] to PRP-OMPC was preserved within the matrix of the pentavalent vaccine. Accordingly, the pentavalent vaccine is expected to provide the same high level of efficacy and effectiveness as the monovalent PRP-OMPC vaccine.

Antibody responses induced by the pentavalent vaccine to all other target antigens were equivalent to responses induced by the separately and concurrently administered licenced control vaccines with the exception of responses to PT and hepatitis B. With regard to PT, the pentavalent vaccine induced an anti-PT response which failed to meet the equivalence criterion compared with DTP-lic (Group D) at the post-primary series time point (7 months), but there was little difference compared to DTP (Group C). Furthermore, at the post-vaccination time point (19 months), although the pentavalent vaccine induced an anti-PT response that remained lower compared to that induced by DTP-lic, it did meet the equivalence criterion. Our assessment is that the pentavalent vaccine will afford a similar level of protection as DTP-lic vaccine against pertussis infection.

Unexpectedly, there was notable inhibition of the anti-HBs response to the pentavalent vaccine, and this observation is in contrast to data from recent studies of acellular pertussis vaccine and hepatitis vaccine in combination [13]. Our studies evaluated a monovalent hepatitis B vaccine, a bivalent vaccine, a quadrivalent vaccine and a pentavalent vaccine. A comparison of the anti-HBs responses demonstrates the impact of combining monovalent hepatitis B vaccine with the Hib vaccine or the DTP vaccine individually, or with both vaccines. When the monovalent hepatitis B vaccine was combined with either Hib (bivalent vaccine, Group C) or with DTP (quadrivalent vaccine, Group B), there was slight inhibition of the anti-HBs responses (seroprotection rate and GMT) compared to anti-HBs responses to the monovalent HB vaccine at the

Table 3
Antibody responses (GMT U/ml) to each of the five pertussis antigens by vaccine group and comparison^a

	A [DTP-liqHib-hepB] + placebo	B [DTP-hepB] + liqHib	B versus A	C DTP+[liqHib-hepB]	C versus A	D DTP-lic+lyoHib+hepB	D versus A
<i>7 months</i>	<i>n</i> = 375	<i>n</i> = 383		<i>n</i> = 151		<i>n</i> = 166	
PT	3.9 (3.2, 4.6)	4.2 (3.6, 5.0)	1.09 (0.85, 1.39) S	4.6 (3.6, 6.0)	1.20 (0.87, 1.67) S	8.6 (6.7, 11.0)	2.2 (1.6, 3.1) U
FHA	15 (14, 16)	19 (17, 20)	1.3 (1.1, 1.4) S	14 (12, 17)	0.95 (0.80, 1.12) S	18 (16, 20)	1.2 (1.0, 1.4) S
Agg2	23 (20, 28)	29 (25, 34)	1.2 (1.0, 1.6) S	15 (12, 19)	0.64 (0.48, 0.86) S	18 (15, 23)	0.78 (0.59, 1.03) S
Agg3	45 (39, 53)	47 (41, 54)	1.04 (0.86, 1.26) S	34 (27, 44)	0.76 (0.57, 1.00) S	34 (27, 43)	0.75 (0.57, 0.98) S
Pertactin	15 (13, 17)	18 (16, 21)	1.21 (0.99, 1.49) S	16 (13, 20)	1.07 (0.80, 1.42) S	8.9 (7.1, 11.2)	0.60 (0.45, 0.79) S
<i>19 months</i>	<i>n</i> = 334	<i>n</i> = 381		<i>n</i> = 148		<i>n</i> = 158	
PT	25 (21, 30)	23 (20, 27)	0.92 (0.72, 1.16) S	24 (18, 31)	0.94 (0.69, 1.28) S	43 (35, 53)	1.7 (1.3, 2.3) S
FHA	52 (47, 57)	65 (60, 70)	1.3 (1.1, 1.4) S	56 (49, 64)	1.08 (0.91, 1.28) S	64 (57, 71)	1.2 (1.1, 1.4) S
Agg2	107 (92, 126)	132 (115, 153)	1.2 (1.0, 1.5) S	75 (59, 94)	0.70 (0.53, 0.93) S	60 (48, 75)	0.56 (0.42, 0.73) S
Agg3	97 (85, 111)	101 (91, 113)	1.04 (0.88, 1.23) S	90 (74, 109)	0.92 (0.73, 1.17) S	92 (76, 110)	0.94 (0.75, 1.19) S
Pertactin	41 (35, 48)	45 (40, 52)	1.10 (0.90, 1.36) S	39 (31, 49)	0.95 (0.72, 1.26) S	28 (23, 34)	0.68 (0.52, 0.88) S

^a 95% CI in parentheses; PT, pertussis toxin; FHA, filamentous haemagglutinin; Agg2 and Agg3, agglutinogens 2 and 3; For inter-group ratios of GMTs: S, satisfied equivalence criteria (lower bound 95% CI ≥ 0.4 and upper bound CI ≤ 2.5); U, unable to meet equivalence criteria (lower bound 95% CI < 0.4 or upper bound 95% CI > 2.5).

post-primary series time point (7 months). At the post-vaccination time point (19 months), the seroprotection rates (% of subjects with anti-HBs ≥ 10 mIU/ml) were similar and robust (98.8% for the bivalent vaccine; 97.5% for the quadrivalent; 98.8% for the monovalent HB vaccine), although the GMTs were somewhat lower for the two combined vaccines (2192 mIU/ml for the bivalent; 1028 mIU/ml for the quadrivalent; 6345 mIU/ml for the monovalent hepatitis B vaccine). However, when the HB vaccine was combined with both Hib and DTP (pentavalent vaccine, Group A), the post-vaccination (19 months) seroprotection rate was 87.9% with a GMT of 294 mIU/ml suggesting a synergistic inhibition of the anti-HBs response by the Hib and DTP components.

A post-vaccination seroprotection rate of 87.9% following four doses of the pentavalent vaccine was considered inadequate for routine vaccination of healthy infants against HBV infection. Accordingly, the same regimen was evaluated in children who had received a birth dose of monovalent HB vaccine (birth, 2, 4, 6 and 18 months). Addition of this birth dose improved the anti-HBs response to the pentavalent vaccine and resulted in a post-vaccination (19 months) seroprotection rate of 92.8% with a GMT of 424 mIU/ml. The anti-HBs responses (seroprotection rate and GMT) with this regimen approximated the anti-HBs responses observed after a traditional three-dose monovalent HB vaccine regimen in infancy [14–18].

The pentavalent vaccine has not been evaluated in infants born to HBsAg positive mothers (high risk infants) and is not recommended for completion of the HB vaccine regimen in this cohort. For high risk infants, the rapid development (within 6–8 months after birth) of immunologic protection is essential to prevent the development of the chronic HBsAg carrier state [2,19]. The anti-HBs responses to the pentavalent vaccine are less robust in a dose-to-dose comparison to monovalent HB vaccine and completion of the hepatitis B vaccination regimen using the pentavalent vaccine does not occur until 18 months of age.

Because of the need for a birth dose of monovalent HB vaccine and the long duration (18 months) of the hepatitis B vaccination regimen with the pentavalent vaccine, the vaccine is suitable for use only in areas where a birth dose of monovalent HB vaccine is routinely administered, and where there is low endemicity of HBV infection and well-implemented systems in place for universal screening of pregnant women for HBsAg.

Finally, routine hepatitis B vaccination in infancy in areas with low endemicity of HBV infection will ideally result in sustained protection through the adolescent years when a much higher risk of exposure to HBV is anticipated [2]. The duration of immunity after hepatitis B vaccination has not been conclusively established

but studies are underway to address this issue. Studies in both children and adults have shown that, when a HB vaccine induced a primary immune response, even if the anti-HBs level subsequently decayed below 10 mIU/ml, there was persistent immunologic memory that enabled an individual to rapidly respond when later exposed to HBsAg [20,21]. For the pentavalent vaccine, subjects (Group A) who received the four-dose regimen (but not the birth dose of monovalent HB vaccine) were evaluated for persistence of anti-HBs and immunologic memory at approximately 3 years of age. At that time, the seroprotection rate (and GMT) had declined from a peak post-vaccination level of 87.9% (294 mIU/ml) to 54% (9 mIU/ml). HB vaccine (5.0 μ g H-B-VAX™ II-Merck and Co., USA) was administered to 388 subjects and a post-vaccination blood sample was obtained 4–6 weeks later. Of 335 subjects with an available post-vaccination anti-HBs result, the seroprotection rate was 94% with a GMT of 1190 mIU/ml (unpublished data), indicating a robust anamnestic response and the presence of immunologic memory. These data also suggest that children who miss the birth dose of monovalent HB vaccine yet receive the four-dose regimen of the pentavalent vaccine could complete the hepatitis B vaccination regimen with a single dose of monovalent HB vaccine at a later date.

In summary, this pentavalent vaccine given in a schedule that incorporated a birth dose of monovalent hepatitis B vaccine was well tolerated and should successfully vaccinate children against five disease targets with a course of five injections instead of 11. This vaccine is suitable for use only in areas with low endemicity of HBV infection. The liquid, single-dose form offers enhanced sterility during both vaccine storage and delivery and may help to overcome potential confusion and errors associated with handling multiple vaccine formulations.

Acknowledgements

Valuable assistance in study planning, execution and evaluation was provided by Ian Gust, Darryl Maher, Jillian Bennet, David Dawson, John Varigos and Paul Fahey of CSL Ltd. and Cindy Marselis, Linda Schaffer and Holly Matthews of Merck & Co., Inc. Serology was carried out by the CSL Ltd. Quality Control Biochemistry Department under the supervision of Georgina McKay and at Merck Research Laboratories under Peter Kniskern. Bill Woods, Serge DeBartolo and the Clinical Trials Logistics Group at CSL Ltd. provided assistance to field staff and ensured that the vaccine cold chain was maintained. We thank the parents of subjects, the maternal and child health nurses in the participating areas, and staff from the health departments of the municipalities of Banyule, Boroorn-

dara, Darebin, Greater Geelong, Glen Eira, Kingston, Knox, Manningham, Monash, Stonnington, Whitehorse and Whittlesea. Nursing staff at the Royal Women's Hospital in Melbourne provided assistance with cord blood collection. This study was supported by a grant to the Royal Children's Hospital Research Foundation from CSL Ltd. and Merck and Co., Inc. Study doctors were Anne Altmann, Dianne Brown, Gillian Cameron, Michael Creati, Sharon Goldfeld, Sarah Kennedy, Len Hartman, Audrey Penington, Jenny Proimos, Derreck Sim, and Joanne Smart. Research assistants were Alice Bond, Michele Bond, Janet Briggs, Louise Buchanan, Anne Clare, Jacinta Clifford, Noreen Condon, Susan Dickie, Anne Ferguson, Margaret Flood, Jennifer Foord, Catherine Gaucher, Rosemary Gehrig-Mills, Jenny Grimshaw, Angela Harris, Trina Laffy, Nhiem Lam, Elisabeth Maloney, Elizabeth McGrath, Kate Minkoff, Lyn Pearson, Susan Proctor, Susan Smith, Susan Thorpe, Marian Ussher and Suzanna Vidmar. Study phlebotomists were Patricia Barbante, Patricia Boyd, Tracey Brimley, Deborah Gercovich, Jane Franich, Kathleen Lanigan, Charan Sandhu and Deborah Saunders.

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