Original Article

Primary Vaccination of Infants Against Hepatitis B can be Completed Using a Combined Hexavalent Diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliomyelitis-*Haemophilus influenzae* Type B Vaccine†

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Abstract

Introduction: Children in Singapore receive vaccination against hepatitis B virus (HBV) at 0, 1 and 5 or 6 months of age, and vaccination against pertussis, diphtheria, tetanus, and polio at 3, 4 and 5 months of age. Parents often choose to vaccinate with the combined acellular-pertussis-inactivated polio-Hib vaccine (DTPa-IPV/Hib). We investigated whether a combined hexavalent vaccine, DTPa-HBV-IPV/Hib, could replace the separate administration of DTPa-IPV/Hib and HBV for the final vaccination at 5 months of age (Trial DTPa-HBV-IPV-075). Materials and Methods: In an open study, 150 children were randomised to complete their vaccination schedule with DTPa-IPV/Hib + HBV or DTPa-HBV-IPV/Hib. Results: One month after the final vaccination, there was no difference between groups in seroprotection rates or antibody concentrations against HBV. Seroprotection rates against diphtheria, tetanus, Hib and polio, as well as vaccine response rates to pertussis antigens were also similar between groups. Local and general symptoms occurred at a similar rate after the third dose of either vaccine. Conclusion: The immunogenicity and reactogenicity of the hexavalent vaccine DTPa-HBV-IPV/Hib (*Infanrix hexa,* GSK) group is comparable to that of separately administered DTPa-IPV/Hib and HBV vaccines. Combined hexavalent vaccine, DTPa-HBV-IPV/Hib, could replace the separate administration of DTPa-IPV/Hib and HBV for vaccination at 5 months of age, thereby reducing the number of injections required.

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Key words: Combined vaccine, Hepatitis B vaccine, Hexavalent vaccine

Introduction

Before the introduction of mass vaccination of newborns against hepatitis B virus (HBV) in 1987, almost 10% of children <6 years of age and almost 55% of adults showed evidence of HBV infection in Singapore.[1] Like other Asian countries, the primary mode of HBV transmission in Singapore was perinatal.[1,2] Interruption of perinatal transmission through vaccination at birth has had a major effect on the epidemiology of HBV in Singapore, with marked reductions in the incidence of acute HBV disease and in chronic carrier rates.[1,3,4]

In Singapore, monovalent HBV vaccines are administered at birth and at 1 and 5 or 6 months of age. Whole-cell based pertussis and oral polio vaccines are provided free for the routine vaccination of infants at 3, 4 and 5 months of age. A significant number of parents choose instead to pay to immunise their children with combined acellular pertussis vaccines also available in Singapore. The features of combined diphtheria-tetanus-acellular pertussis-inactivated poliomyelitis-*Haemophilus influenzae* type b vaccine (DTPa-IPV/Hib) that appeal to parents and practitioners include: fewer adverse reactions after vaccination compared to whole-cell pertussis preparations,[5] the inclusion of IPV, thereby avoiding the rare but potentially devastating complication of vaccine-associated paralytic polio associated with the use of oral polio vaccines; the inclusion of Hib protection; and finally but perhaps of most significance to parents and practitioners, the provision of DTPa, Hib and IPV in a single injection.

Although the use of DTPa-IPV/Hib vaccine for routine

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vaccination reduces the number of injections required at each visit, an additional injection with HBV continues to be needed at either the fifth or sixth month when infants receive their final HBV immunisation. The combined hexavalent DTPa-HBV-IPV/Hib vaccine builds on the features of DTPa-IPV/Hib, with the additional inclusion of HBV in the vaccine formulation. We examined the immunogenicity and reactogenicity of the hexavalent vaccine when used at 5 months of age in the Singapore vaccination schedule.

Materials and Methods

This study was conducted according to Good Clinical Practice and the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the KK Women's and Children's Hospital. Written informed consent was obtained from each parent/guardian prior to the subject's entry into the study.

This was an open study conducted in a single centre in Singapore. Healthy infants were randomised into 1 of 2 groups that differed only in the vaccine they received at the final vaccination visit. All subjects received HBV vaccination at birth and at 1 month of age, as well as DTPa-IPV/Hib vaccine at 3 months and 4 months of age. At 5 months of age, subjects in Group DTPa-IPV/Hib + HBV received a third and final dose of DTPa-IPV/Hib and HBV vaccines as separate injections, while subjects in Group DTPa-HBV-IPV/Hib received the combined hexavalent vaccine DTPa-HBV-IPV/Hib. The study was conducted in an open fashion because the 2 groups received a different number of vaccines at the final visit.

Healthy infants 11 to 17 weeks of age at the time of the first dose of DTPa-IPV/Hib were eligible for enrolment if they had received routine HBV vaccination at birth and at 1 month of age. Infants were excluded if they had received, or planned to use any other vaccines or investigational product, within 30 days preceding the first dose of the study vaccine, had a family history of hereditary immunodeficiency, immunosuppressive conditions including iatrogenic immunodeficiency, major congenital defects, or acute disease at the time of enrolment, had received or planned to receive any blood products including immunoglobulin since birth or during the study period, had allergic disease likely to be exacerbated by any component of the vaccine, or any neurological disorders.

Vaccines

All vaccines administered during the study were manufactured by GlaxoSmithKline (GSK) Biologicals (Rixensart, Belgium). Each dose of HBV vaccine (*Engerix*-B) contained 10 µg recombinant HBV surface antigen (HBsAg). The DTPa-IPV/Hib vaccine contained ≥30 IU diphtheria toxoid, ≥40 IU tetanus toxoid, 25 µg pertussis toxin (PT), 25 µg filamentous haemagglutinin (FHA), 8 µg pertactin (PRN), 40D, 8D and 32D antigen units of poliovirus types 1, 2 and 3 respectively, reconstituted with 10 µg Hib polyribosyl-ribitol-phosphate (PRP) conjugated to tetanus toxoid. The DTPa-HBV-IPV/Hib (*Infanrix hexa*) contained identical antigen components to the DTPa-IPV/Hib vaccine along with 10 µg recombinant HBsAg.

All vaccines were administered into the antero-lateral region of the thigh. DTPa-containing vaccines were given on the left side and HBV vaccine on the right side.

Assessment of Immunogenicity

Two blood samples were collected from all subjects at 3 months of age prior to administration of the first dose of DTPa-IPV/Hib, and 1 month after the final vaccination. Samples were stored at -20ºC until shipment.

Anti-diphtheria and anti-tetanus, anti-PT, anti-FHA, anti-PRN antibodies and anti-HBV surface antibody (HBs) were measured by ELISA. Antibodies against the 3 polio virus types were measured by a virus micro-neutralisation assay.[6] For diphtheria, tetanus, HBV and polio, antibody concentrations/titres equal to or above the assay cut-off were considered to be indicative of seroprotection. The cut-offs for each assay were 0.1 IU/mL for diphtheria and tetanus, 10 mIU/mL for HBV and 1/8 dilution for polio types 1, 2 and 3. Since the assay cut-off for the diphtheria ELISA assay is conservatively set at 0.1 IU/mL, subjects seronegative to anti-diphtheria antibodies by ELISA testing 1 month after the last vaccination were re-tested using a more sensitive in vitro neutralisation assay on Vero cells, with a cut-off of 0.016 IU/mL.

The cut-off for anti-pertussis antibodies was 5 EL.U/mL; however, as there is no established correlate of protection against pertussis a vaccine response was defined as the appearance of antibodies in initially seronegative subjects, or the maintenance of antibody concentrations in subjects seropositive prior to vaccination.

All antibody testing, with the exception of the neutralisation assay on Vero cells, was performed at MEP (Michael E Pichichero) laboratories (University of Rochester). The measurement of neutralising antibodies to diphtheria was performed at a GSK laboratory in Belgium.

Assessment of Reactogenicity

Local symptoms of pain, redness and swelling, and systemic symptoms of drowsiness, fever (axillary temperature ≥37.5°C), irritability/fussiness and loss of appetite were actively solicited for 4 days following each vaccination (Day 0 to 3). Symptoms that occurred were graded by the investigator on a 3-point scale where "Grade 3" was defined as: cries when limb is moved/spontaneously painful (pain); a diameter >20 mm (swelling and redness);

axillary temperature >39.0°C (fever); crying that could not be comforted/prevented normal activity (irritability/fussiness); not eating at all (loss of appetite); preventing normal activity (all other symptoms). All other symptoms that occurred within 30 days of each vaccination were recorded. Serious adverse events were recorded from the first dose of DTPa-IPV/Hib until 30 days after the last vaccination.

Statistical Analysis

All analyses were descriptive. At each blood sampling time point, antibody seroprotection/seropositivity rates against all vaccine antigen components were computed with 95% confidence intervals (CI). Vaccine responses to pertussis antigens were calculated with 95% CI at the post-vaccination time point. Antibody geometric mean concentrations/titres (GMC/T) with 95% CI were calculated by taking the log-transformation of individual concentrations/titres and calculating the anti-log of the mean of these transformed values. Antibody concentrations/titres below the cut-off of the assay were given an arbitrary value of half the cut-off.

The incidence and intensity of individual solicited symptoms over the 4-day follow-up period and the incidence of unsolicited symptoms within 30 days of vaccination were tabulated with 95% CI. Serious adverse events and discontinuation due to adverse events were described in detail.

The analysis of immunogenicity was performed on the according-to-protocol (ATP) cohort for immunogenicity. The analysis of safety was performed on the total cohort.

With a sample size of 75 infants per group and anti-HBs seroprotection rate of 95%, the expected 95% CI was [86.0; 98.4].

Results

One hundred and fifty subjects were enrolled between September 2001 and September 2002. One subject (Group DTPa-HBV-IPV/Hib) did not complete the study after migrating from the study area after Dose 1. Twenty-three subjects (14 in Group DTPa-HBV-IPV/Hib) were eliminated from the ATP cohort for immunogenicity. The reasons for their elimination were: being older than the protocol-defined age limit at enrolment (1 subject), having received medication forbidden by the protocol during the study period (1 subject), non-compliance with the vaccination schedule (17 subjects) and non-compliance with the blood sampling schedule or insufficient sample for testing (4 subjects). A total of 66 and 61 subjects in group DTPa-IPV/Hib + HBV and DTPa-HBV-IPV/Hib respectively were included in the analysis of immunogenicity. The demographic characteristics of the Total and ATP cohorts are summarised in Table 1.

Immunogenicity

Anti-HBs antibody response: All subjects received 2 doses of HBV vaccine (at birth and at 1 month of age) at the time of the first blood sample collected at 3 months of age. As a result, almost 70% of subjects in both groups had seroprotective anti-HBs antibody concentrations at 3 months of age (Table 2). This increased to at least 96.7% 1 month after the completion of primary vaccination in both groups.

Response to other vaccine antigens: One month after completion of the primary vaccination course, at least 98%

Table 1. Demographic Characteristics of the Total Cohort and the ATP Cohort for Immunogenicity

	Total cohort (N = 150)						ATP cohort for immunogenicity (N = 127)					
	Total	Female	Age (weeks)				Total	Female	Age (weeks)			
Group	N	n	Mean	SD	Min	Max	N	n	Mean	SD	Min	Max
DTPa-IPV/Hib + HBV	75	38	12.8	0.84	12	17	66	32	12.8	0.87	12	17
DTPa-HBV-IPV/Hib	75	47	13.0	1.07	12	18	61	42	12.9	0.90	12	16

Max: maximum age; Min: minimum age; N: number in the relevant cohort; n: number of female subjects; SD: standard deviation

Table 2. Hepatitis B Antibody Seroprotection Rates and GMCs in Subjects Vaccinated with HBV Vaccine at Birth and 1 Month of Age, Followed By a Dose of Either HBV or DTPa-HBV-IPV/Hib Vaccine at 5 Months of Age (ATP Cohort for Immunogenicity)

		Group DTPa-IPV/Hib + HBV						DTPa-HBV-IPV/Hib					
Antibody	Time-point	n	%SP	95% CI		GMC	95% CI	n	%SP	95% CI		GMC	95% CI
Anti-HBs	Pre	46	69.6	54.2	82.3	30.6	18.7 50.1	41	68.3	51.9	81.9	29.7	17.9 49.2
≥10 mIU/mL	Post	66	98.5	91.8	100.0	938.1	606.6 1450.7	60	96.7	88.5	99.6	1128.6	722.4 1763.1

95% CI: 95% confidence interval; %SP: percent of subjects with antibody concentration above the specified cut-off; GMC: geometric mean concentration; n: number of subjects with available results; Pre, Post: blood sample collected at 3 months of age (corresponding to 2 months after the second dose of HBV vaccine) and 1 month after completion of primary vaccination at 6 months of age

of subjects in both groups had seroprotective antibody concentrations against Hib, tetanus, diphtheria (by ELISA or neutralisation assay on Vero cells), and polio types 1, 2 and 3 (Table 3). All subjects who received the DTPa-HBV-IPV/Hib vaccine for the third dose had a vaccine response to PT, FHA and PRN.

Antibody GMCs for all vaccine antigens were similar after the third vaccination, evidenced by overlapping 95% CIs, with the exception of the diphtheria antibodies, which were significantly higher in subjects in Group DTPa-IPV/Hib + HBV (95% CIs did not overlap).

Total cohort analysis: As 15% of the enrolled subjects were eliminated from the ATP cohort for immunogenicity, a second supplementary analysis of the Total cohort was performed. The results of the analysis of the Total cohort were consistent with that of the ATP analysis with the exception of the diphtheria response: in the Total cohort analysis the 95% CIs of the anti-diphtheria antibody GMCs overlapped, suggesting that a true difference between groups may not exist. It should be noted that this study was not powered to detect differences between groups.

Reactogenicity: All subjects returned completed symptom sheets after each vaccine dose they received. Since all subjects received the DTPa-IPV/Hib vaccine for Doses 1 and 2, only data concerning symptoms that followed Dose 3 are presented.

Pain, redness and swelling occurred in up to 16.2% of vaccinees after Dose 3 and the incidence of all local symptoms was similar in subjects who had received the DTPa-IPV/Hib + HBV vaccines and in those who had received the DTPa-HBV-IPV/Hib vaccine (overlapping 95% CIs in all cases, Table 4). No local symptoms of grade 3 intensity occurred after Dose 3 in any subject.

Irritability was the most commonly reported systemic symptom that occurred after vaccination at Dose 3 (Table 5). The incidence of systemic symptoms was similar between groups (overlapping 95% CIs). Grade 3 symptoms were rare and only occurred in 2 subjects, both of whom were in Group DTPa-IPV/Hib + HBV.

When all doses are considered, 57.3% and 54.7% of subjects in Group DTPa-IPV/Hib + HBV and DTPa-HBV-IPV/Hib respectively were followed by an unsolicited

Table 3. Antibody Seroprotection/Vaccine Response Rates and GMC/Ts Following Primary Vaccination (ATP Cohort for Immunogenicity)

		Group DTPa-IPV/Hib + HBV					Group DTPa-HBV-IPV/Hib				
Antibody	**Timing**	**n**	**%SP/VR**	**GMC/T**	**95% CI**		**n**	**%SP/VR**	**GMC/T**	**95% CI**	
Hib	Pre	64	57.8	0.2	0.1	0.2	61	52.5	0.2	0.1	0.2
≥0.015 μg/mL	Post	66	100.0	6.6	5.3	8.2	61	100.0	8.4	7.0	10.1
Diphtheria	Pre	63	17.5	0.1	0.1	0.1	61	18.0	0.1	0.1	0.1
≥0.016 IU/mL *	Post	64	100.0	1.2	0.9	1.5	61	98.4	0.6	0.4	0.8
Tetanus	Pre	64	46.9	0.1	0.1	0.2	61	59.0	0.1	0.1	0.2
≥0.1 IU/mL	Post	66	98.5	3.8	3.1	4.7	61	100.0	4.2	3.5	5.0
PT	Pre	64	-	3.1	2.7	3.5	61	-	2.8	2.6	3.1
VR	Post	63	96.8	38.1	31.4	46.3	61	100.0	45.0	38.0	53.4
FHA	Pre	64	-	3.5	3.0	4.1	61	-	3.5	3.0	4.0
VR	Post	63	98.4	85.6	74.8	98.1	61	100.0	86.4	77.8	95.9
PRN	Pre	64	-	3.1	2.6	3.5	61	-	2.9	2.6	3.2
VR	Post	64	96.9	175.0	144.1	212.4	61	100.0	156.2	131.7	185.2
Polio 1	Pre	58	58.6	10.0	7.6	13.2	61	62.3	9.7	7.6	12.4
≥1:8	Post	62	100.0	696.3	537.2	902.5	61	100.0	711.6	535.9	945.0
Polio 2	Pre	58	48.3	9.8	7.1	13.7	61	67.9	10.9	8.5	14.0
≥1:8	Post	62	98.4	423.5	318.8	562.5	61	100.0	491.8	382.4	632.5
Polio 3	Pre	58	27.6	6.3	4.9	8.1	61	20.8	5.5	4.5	6.7
≥1:8	Post	62	98.4	1138.8	829.1	1564.1	61	100.0	1606.8	1281.5	2014.6

95% CI: 95% confidence interval; %SP/VR: percent of subjects with antibody concentrations/titre above the specified cut-off or a vaccine response for PT, FHA, PRN; GMC/T: geometric mean antibody concentrations/titre; n: number of subjects with available results; Pre: prior to vaccination with a DTPa-based vaccine; Post: 1 month following the final vaccination

Vaccine response defined as: post-vaccination antibody concentration ≥5 EL.U/mL for initially seronegative subjects, or maintenance of antibody concentrations in initially seropositive subjects

Table 4. Incidence of Solicited Local Symptoms Reported During the 4-day Follow-up Period after Dose 3 (Total Cohort)

Symptom	Injection site		DTPa-IPV/Hib + HBV (n = 75)				DTPa-HBV-IPV/Hib (n = 74)*			
			n	%	95% CI		n	%	95% CI	
Pain	At any injection site	Any	10	13.3	6.6	23.2	7	9.5	3.9	18.5
		>20 mm	0	0.0	0.0	4.8	0	0.0	0.0	4.9
	DTPa-IPV/Hib	Any	10	13.3	6.6	23.2				
		>20 mm	0	0.0	0.0	4.8	-	-	-	-
	DTPa-HBV-IPV/Hib	Any	-	-	-	-	7	9.5	3.9	18.5
		>20 mm	-	-	-	-	0	0.0	0.0	4.9
	HBV	Any	9	12.0	5.6	21.6	-	-	-	-
		>20 mm	0	0.0	0.0	4.8	-	-	-	-
Redness	At any injection site	Any	12	16.0	8.6	26.3	9	12.2	5.7	21.8
		>20 mm	0	0.0	0.0	4.8	0	0.0	0.0	4.9
	DTPa-IPV/Hib	Any	12	16.0	8.6	26.3	-	-	-	-
		>20 mm	0	0.0	0.0	4.8	-	-	-	-
	DTPa-HBV-IPV/Hib	Any	-	-	-	-	9	12.2	5.7	21.8
		>20 mm	-	-	-	-	0	0.0	0.0	4.9
	HBV	Any	10	13.3	6.6	23.2	-	-	-	-
		>20 mm	0	0.0	0.0	4.8	-	-	-	-
Swelling	At any injection site	Any	5	6.7	2.2	14.9	12	16.2	8.7	26.6
		>20 mm	0	0.0	0.0	4.8	0	0.0	0.0	4.9
	DTPa-IPV/Hib	Any	5	6.7	2.2	14.9	-	-	-	-
		>20 mm	0	0.0	0.0	4.8	-	-	-	-
	DTPa-HBV-IPV/Hib	Any	-	-	-	-	12	16.2	8.7	26.6
		>20 mm	-	-	-	-	0	0.0	0.0	4.9
	HBV	Any	5	6.7	2.2	14.9	-	-	-	-
		>20 mm	0	0.0	0.0	4.8	-	-	-	-

Both groups received DTPa-IPV/Hib for Dose 1 and 2. Grade "3" pain: Cried when limb was moved/ spontaneously painful; 95% CI: 95% confidence interval; %: percentage of subjects reporting a specified symptom; n: number of subjects who received Dose 3
* One subject dropped out before Dose 3.

Table 5. Incidence of Solicited General Symptoms Reported During the 4-day Follow-up Period after Dose 3 (Total Cohort)

Symptom		DTPa-IPV/Hib + HBV (n = 75)				DTPa-HBV-IPV/Hib (n = 74)*			
		n	%	95% CI		n	%	95% CI	
Drowsiness	Any	15	20.0	11.6	30.8	5	6.8	2.2	15.1
	Grade "3"	0	0.0	0.0	4.8	0	0.0	0.0	4.9
Irritability	Any	19	25.3	16.0	36.7	14	18.9	10.7	29.7
	Grade "3"	1	1.3	0.0	7.2	0	0.0	0.0	4.9
Loss of appetite	Any	14	18.7	10.6	29.3	8	10.8	4.8	20.2
	Grade "3"	0	0.0	0.0	4.8	0	0.0	0.0	4.9
Temperature	≥37.5° C	9	12.0	5.6	21.6	9	12.2	5.7	21.8
(axillary)	>39.0° C	1	1.3	0.0	7.2	0	0.0	0.0	4.9

95% CI: 95% confidence interval; n: number of subjects who received Dose 3; n/%: number/percentage of subjects reporting a specified symptom
Both groups received DTPa-IPV/Hib for Dose 1 and 2. Grade "3" – drowsiness: drowsiness that prevented normal activity, irritability: crying that could not be comforted/prevented normal activity, loss of appetite: Not eating at all
*One subject dropped out before Dose 3.

symptom within 30 days after vaccination. None were of grade 3 intensity and none were considered by the investigator to be causally related to vaccination, with the exception of 1 case in each group of injection site reaction.

Seven serious adverse events occurred in 6 subjects from Dose 1 until 30 days after the last vaccination, of which 6 were reported after a dose of DTPa-IPV/Hib and 1 after a dose of DTPa-HBV-IPV/Hib. These events consisted of bronchitis (2 cases in 1 subject), urinary tract infection (1 case), gastroenteritis (2 cases), bronchiolitis (1 case) and head injury (1 case). None of these events were considered by the investigator to have a causal relationship to vaccination.

Discussion

Hexavalent vaccines are the largest combined vaccines currently available and provide antigens to 6 diseases in a single injection. The immunogenicity and reactogenicity of the hexavalent vaccine DTPa-HBV-IPV/Hib (*Infanrix hexa*) for use in primary vaccination of infants has been established.[7-10]

In this study, seroprotection rates and vaccine response rates against all vaccine antigens were similar in the 2 groups. Antibody concentrations were also comparable and although the anti-diphtheria antibody GMC was higher in group DTPa-IPV/Hib + HBV, this was not observed in the analysis of the Total cohort, suggesting that a true difference between groups may not exist. Importantly, when the results of test on the sensitive neutralising antibody assay on Vero cells is considered, there was no difference between the 2 groups in terms of the proportion of subjects with seroprotective anti-diphtheria antibody concentrations after vaccination.

Of particular note, the immune response to HBV in terms of seroprotection rates and antibody GMCs was the same in subjects who completed their primary HBV vaccination course with monovalent HBV vaccine (*Engerix*-B) at 5 months of age, and those who completed their vaccination course with HBV given as the DTPa-HBV-IPV/Hib combination.

The nature and intensity of local and systemic symptoms that occurred after vaccination with DTPa-HBV-IPV/Hib were not different from those experienced by subjects who received separate administration of DTPa-IPV/Hib and HBV. There were 7 serious adverse events but none were considered by the investigator to have a causal relationship to vaccination. *Infanrix hexa* was first licensed for use in 2000 and is currently registered in 70 countries. During the first 5 years of use, almost 12 million doses were distributed worldwide.[11]

In this study, the immunogenicity and reactogenicity of the hexavalent vaccine DTPa-HBV-IPV/Hib (*Infanrix hexa*) was comparable to that of separately administered DTPa-IPV/Hib and HBV vaccines. The combined hexavalent vaccine, DTPa-HBV-IPV/Hib, could replace separate administration of DTPa-IPV/Hib and HBV for vaccination at 5 months of age, thereby reducing the number of injections required.

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