Original Report

Comparison of Separate and Mixed Administration of DTPw-HBV and Hib Vaccines: Immunogenicity and Reactogenicity Profiles

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ABSTRACT

Objective: To investigate the immunogenicity, safety and tolerability of three doses of a pentavalent vaccine produced by extemporaneous mixing of diphtheria-tetanus-whole cell pertussis-hepatitis B virus (DTPw-HBV) and a lyophilized *Haemophilus influenzae* type b (Hib)-tetanus conjugate vaccine in one syringe in comparison with separate, concomitant administration of the same vaccine components in healthy infants at 1.5, 3, and 5 months of age, following a dose of hepatitis B vaccine at birth.

Methods: An open, randomized, controlled trial was undertaken with 269 children allocated to two groups to receive three doses of Hib and DTPw-HBV vaccines as either a syringe mix or as separate injections in opposite arms. Symptoms were solicited on 4-day diary cards to assess reactogenicity and immunogenicity based on serum samples drawn immediately before the first dose and 1 month after the third dose.

Results: There were fewer local and more general symptoms in the mixed vaccine group, but no statistically significant difference in reactogenicity between the two groups. There were no withdrawals due to adverse experiences. Seropositivity rates were similar for all antigens in the two groups, with no clinically relevant differences in titers.

Conclusions: The mixed DTPw-HBV-Hib vaccine was safe and well-tolerated, with high immunogenicity against all component antigens, and can be used to provide primary vaccinations of infants while increasing comfort.

Key Words: *DTPw-HBV and Hib vaccines, immunogenicity, reactogenicity, seropositivity*

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The use of combination vaccines to immunize against several diseases simultaneously is a recognized strategy for increasing vaccine coverage in childhood vaccination programs. By reducing the number of injections, compliance to vaccination is improved and administrative and logistic costs incurred while implementing recommended vaccination programs are decreased. However, although combination vaccines offer protection for children against more infectious diseases with fewer injections, a potential drawback is cross-interference between individual vaccine components when combined.

The best characterized of such vaccines is the diphtheria-tetanus-whole-cell pertussis (DTPw) combination, which has been in widespread use since the 1940s. A further addition to this combination is one of the recently developed conjugate vaccines against *Haemophilus influenzae* type b (Hib). *Haemophilus influenzae* type b disease is associated with high rates of morbidity and mortality caused by Hib meningitis, epiglottitis, and pneumonia. The development of Hib-conjugate vaccines has led to a dramatic decrease in the incidence of Hib disease in industrialized countries where Hib vaccination has been implemented.[1]

The World Health Organization (WHO) has actively encouraged the implementation of Hib vaccination in developing countries. In a recent clinical trial in The Gambia, which has a high incidence of invasive Hib disease in the very young, a DTPw-Hib mix vaccine had a 95% efficacy against all invasive Hib disease.[2] The inclusion of Hib vaccines in the DTPw combination is intended to increase compliance and hence decrease incidence of Hib disease. Currently, there are four commercially available Hib vaccines that can be combined with DTPw as one injectable vaccine.[3]

In addition to the injectable DTPw vaccines and the oral polio vaccines, the WHO Expanded Program of Immunization (EPI) recommends vaccination of all children against Hepatitis B virus (HBV) by 1997.[4] As part of the EPI the WHO recommended the development of a combined DTPw-HBV vaccine. SmithKline Beecham Biologicals (Rixensart, Belgium) has developed such a vaccine (Tritanrix™-HB) to fulfill this goal. The WHO-recommended schedule for such a vaccine is three doses at 6, 10, and 14 weeks of age, with the inclusion

of a hepatitis B vaccination at birth in areas of high endemicity where there is risk of perinatal transmission.[5] The aim of the present study was to investigate whether a pentavalent vaccine produced by the extemporaneous mixing of Tritanrix™-HB and a commercial Hib (Hiberix™) vaccine in one syringe would be immunogenic, safe, and well tolerated, in comparison with separate, concomitant administration of the same vaccines in opposite limbs. Such a combination of DTPw, Hib, and HBV vaccines would facilitate inclusion of the HBV vaccine into current national immunization schedules.

MATERIAL and METHODS

The study was approved by the local and national ethics review committees in Myanmar, and conducted at the investigating physician's center (Central Women's Hospital, Yangon, Myanmar), according to the Declaration of Helsinki and Good Clinical Practice guidelines effective at study initiation. Written informed consent in the local language (Burmese) was obtained from the parents or guardians of all children entered into the trial.

Trial Design, Vaccines, and Schedules

The study design was open, randomized, and controlled with two groups of healthy neonates. Neonates with an Apgar score of 7 or higher 5 minutes after birth and who had no concomitant administration of immunoglobulins were enrolled and randomly assigned to one of two groups to receive one intramuscular dose of a commercial Hepatitis B vaccine (Engerix™-B, SmithKline Beecham Biologicals) containing 10 μg recombinant hepatitis B surface antigen (HBsAg), within 24 hours of birth.

Between 5 and 8 weeks of age infants were eligible to continue in the study if they were not participating in other trials, had no acute disease, and were not receiving immunosuppressive therapy. Other exclusion criteria were a history of allergic disease; any symptom or sign of systemic dysfunction, especially of the central nervous system (CNS); and any previous vaccination with similar vaccines other than oral polio and BCG.

At approximately 1.5, 3, and 5 months of age and according to the local schedule, eligible subjects assigned to group 1 received three doses of the mixed vaccine: a tetravalent DTPw-HBV vaccine containing 30 International Units (IU) or more of adsorbed diphtheria toxoid, 60 IU or more tetanus toxoid, 4 IU or more of whole-cell pertussis antigen, and 10 μg recombinant HBsAg per 0.5 mL dose, which was used to resuspend one dose of the lyophilized Hib-tetanus conjugate vaccine (10 μg polyribose-ribitol phosphate [PRP] conjugated to about 30 μg tetanus toxoid adsorbed to 0.63 mg alumina). Following the same schedule, subjects in group 2 were administered the same DTPw-HBV and Hib vaccines as separate injections in opposite limbs; the Hib vaccine was reconstituted with 0.6 mL saline diluent in this case. All vaccines were manufactured by SmithKline Beecham Biologicals.

Data Collection

Demographic and reactogenicity data were collected from diary cards completed by parents or a study nurse on the day of vaccination and during the 3 days following each dose, and transferred to a Standard Case Report Form (CRF) by the investigator. The intensity of solicited local symptoms: pain on digital pressure and the size of redness and swelling at the injection site, and systemic symptoms: fever (axillary temperature measured with a supplied thermometer), irritability, drowsiness, unusual crying (persistent inconsolable screaming or crying for more than 3 hours within 48 hours of vaccination), feeding problems (not taking the bottle or breast-feeding as usual), diarrhea (loose stools), and vomiting, were recorded by parents. The parents or study nurse graded the symptoms as either absent, mild (easily tolerated), moderate (sufficiently discomforting to interfere with infant's daily activity), or severe (prevents normal daily activity). The relation of the solicited and unsolicited symptoms to the vaccinations was assessed by the physician at each subsequent visit of the vaccinee. Because the study was open to the investigator, diary cards and the subsequent transcription to the CRFs were checked by blinded clinical monitors from SmithKline Beecham to minimize possible discrepancies or bias.

Subsequent doses were not to be administered if any adverse experiences occurred: i.e., fever (axillary temperature) 40°C or higher within 48 hours after the vaccination, persistent crying for more than 3 hours within 48 hours after vaccination, or any symptoms and signs indicative of CNS disorders or hypersensitivity reactions to the vaccine. Any serious adverse events, including life-threatening events, or any case of hospitalization were to be reported by the physician to the sponsor within 24 hours of occurrence.

Serology

At birth sera were prepared from maternal cord blood for screening for HBsAg, anti-HBs and anti-Hepatitis B core antigen (anti-HBc). Before the first dose of the DTPw-HBV and Hib vaccines and 1 month after administration of the last dose (month 6), venous blood samples were drawn, and sera were prepared and stored for subsequent serologic assays.

All serologic testing was performed in a blinded fashion at SmithKline Beecham Biologicals. Antidiphtheria and antitetanus antibodies were determined by enzyme-linked immunosorbent assay (ELISA), with a cutoff of 0.1 IU/mL, above which there is a good correlation between the ELISA and in vivo neutralization tests.[6,7] *Bordetella pertussis* antibodies were determined using a whole-cell based commercial kit with an assay cutoff of 15 ELISA. U/mL

Table 1. Anti-HBs: Seropositivity and Geometric Mean Titers One Month after Administration of Combined Vaccines

	Postvaccination Seropositive Subjects (%)*		*Postvaccination GMT (mIU/mL) (95% CI)*
Status at Birth	*Month 1.5*	*Month 6*	*Month 6*
Group 1 (Mixed) (n = 127)	n = 47 (37)	n = 127 (100)	1124 (884–1430)
Seronegative (n = 63)	n = 1 (1.6)	n = 63 (100)	1426 (1030–1974)
Seropositive (n = 64)	n = 46 (71.9)	n = 64 (100)	890 (625–1267)
Group 2 (Separate) (n = 122)	n = 35 (28.7)	n = 120 (98.4)	1272 (971–1668)
Seronegative (n = 75)	n = 1 (1.3)	n = 74 (98.7)	1424 (1004–2020)
Seropositive (n = 47)	n = 34 (72.3)	n = 46 (97.9)	1063 (685–1649)

*Seroprotective titer ≥ 10 IU/mL.

(Labsystem, ICNFLOW, Helsinki, Finland). Anti-HBs antibody titers were measured using a commercial radioimmunoassay kit (AUSAB, Abbott Laboratories, North Chicago, IL) with a cutoff of 10 mIU/mL. A radiolabelled antigen binding assay (RABA) was used to measure the antibodies against the Hib polysaccharide, PRP, with a cutoff of 0.15 μg/mL. All cutoff values were considered to be seroprotective titers, with the exception of *B. pertussis*, for which there is no serologic correlate.

Statistical Analyses

The ratio of male to female subjects was compared between groups using the Fisher's exact test, and mean ages were compared between groups and sexes using a two-way analysis of variance (ANOVA). A one-way ANOVA was used to compare postvaccination geometric mean titers (GMT) between groups. Fisher's exact test was used to compare the percentages of vaccine recipients seropositive for diphtheria, tetanus, HBs, and PRP antibodies, and vaccine response rates against the pertussis component. A vaccine response was defined as the appearance of detectable antibodies in previously seronegative subjects and the maintenance of titers in previously seropositive subjects. This latter definition takes into account the expected decrease in maternal antibody titers in the period between pre- and postvaccination blood samples; the half-life for decay of maternal pertussis antibodies is approximately 40 days.[8]

RESULTS

Of 286 neonates screened and vaccinated with HBV vaccine at birth, there were 17 subjects excluded because subsequent vaccines were not administered according to protocol. The remaining 269 children eligible for the reactogenicity analysis were randomly assigned to group 1 (137) and group 2 (132). Of these, 249 were eligible for the immunogenicity analysis, because 16 did not comply with the required vaccination schedule and four did not comply with the blood sampling schedule, leaving 127 vaccine recipients in group 1 and 122 in group 2. All children who received the first dose of combined vaccine completed the full vaccination course. Both groups were statistically comparable according to gender and age.

Immunogenicity

The numbers of seropositive subjects and antibody GMTs postvaccination are shown in Table 1 for the hepatitis B vaccine components. There were no statistically significant differences in the prevaccination antibody status between the two groups. All vaccine recipients receiving the mixed vaccine (group 1) and 98.4% given separate vaccines (group 2) had anti-HBs titers indicative of seroprotection 1 month postvaccination (see Table 1). There was no significant difference between the GMTs of the two groups postvaccination. Although there was a tendency for the presence of maternal antibodies at birth to result in lower

Table 2. Postvaccination Seropositivity and Geometric Mean Titers for Mixed and Separate Vaccine Administrations

	Group 1 Mixed DTPw-HBV-Hib			*Group 2 Separate DTPw-HBV + Hib*		
Vaccine Component	*N*	*Seropositive Subjects (%) [95% CI]*	*GMT* [95% CI]*	*N*	*Seropositive Subjects (%) [95% CI]*	*GMT* [95% CI]*
Diphtheria	126	113 (89.7) [82.7–94.2]	0.652 [0.515–0.826]	120	117 (97.5) [92.3–99.4]	0.880 [0.733–1.056]
Tetanus	127	127 (100) [96.3–100]	2.618 [2.213–3.197]	122	121 (99.2) [94.9–100]	1.174 [0.962–1.433]
Pertussis	126	126 (100) [96.3–100]	140.7 [128.8–153.7]	122	122 (100) [96.2–100]	148.4 [134.2–164.1]
PRP (Hib)	127	125 (98.4) [93.9–99.7]	6.103 [4.842–7.693]	122	119 (97.5) [92.4–99.4]	5.722 [4.240–7.722]

*Geometric mean titers for diphtheria and tetanus in IU/mL, in ELISA U/mL for pertusis, and in μg/mL for PRP.

Table 3. Incidence of Symptoms per Number of Doses of Vaccine with which Reaction Was Observed

		Local Symptoms			
Number of Reactions Reported	*All Symptoms (%) (Doses)*	*DTPw-HBV-Hib (%) (Doses)*	*DTPw-HBV (%) (Doses)*	*Hib (%) (Doses)*	*Systemic Symptoms (%) (Doses)*
Group 1 (Mixed)					
397	66.8 (265)	38.5 (153)	–	–	61.7 (245)
Group 2 (Separate)					
385	62.9 (242)	–	41.3 (159)	19.7 (76)	56.6 (218)

postvaccination titers of anti-HBs antibodies, this did not result in significant differences between the subjects who were initially seropositive or seronegative.

The mixed vaccine group had a lower percentage of seropositive vaccine recipients (89.7% vs. 97.5%, P = 0.018) and a lower GMT for antidiphtheria antibodies (0.652 and 0.88 IU/mL in the mixed and separate groups, respectively (P = 0.0499) (Table 2), but the clinical relevance of these differences is limited as all subjects had titers above the seroprotective level (0.01 IU/mL). Conversely, the GMT for antitetanus antibodies was significantly higher in the mixed vaccine group (2.618 IU/mL) than the group given the vaccines separately (1.174 IU/mL) (P < 0.001), with no significant difference in the number of seropositive subjects for this antigen (100% and 99.2%, respectively).

One month after the third dose, all subjects were seropositive for pertussis with similar titers in groups 1 and 2 of 140.7 and 148.4 EL.U/mL, respectively. Similarly, there was no difference in the response to the Hib component between the two groups, with 98.4% and 97.5% having titers 0.15 μg/mL or higher, and 91.3% and 86.9% 1.0 μg/mL or higher in groups 1 and 2, respectively, with GMTs of 6.103 and 5.722 μg/mL, respectively.

Reactogenicity

There were no more reactions reported in the mixed vaccine group (66.8% of doses) than in the separate group (62.9%). There were no significant differences in the overall incidences of local or systemic symptoms between the two groups (Table 3). Incidences of individual local symptoms were slightly, but not significantly, lower in the mixed vaccine group than observed for DTPw-HBV alone (Table 4).

Systemic symptoms were reported in 61.7% and 56.6% of children in groups 1 and 2, respectively (see Table 3), of which fever, irritability, and unusual crying were the most frequent (see Table 4). Of the 396 diary cards returned by the mixed vaccine group, 183 (46.1%) contained reports of systemic symptoms considered to be related to the vaccination. As shown in Table 4, there were no significant differences between the two groups with regard to the total incidence of general symptoms, or the number considered to be related to the vaccination.

The most clinically relevant systemic symptom was considered to be fever. There were two reports of fever over 39°C in the mixed vaccine group, in vaccine recipients who developed intense swelling, redness, and pain at the injection site, which resolved in 7 to 10 days after treatment with antibiotics and antipyretics. Severe fever was associated with a similar injection site reaction at the DTPw-HBV site of one subject in the second group, the only serious adverse event reported, which developed after the last dose and required hospitalization. Following treatment with oral cloxacillin (250 mg/d), the child recovered within 4 days. Reports of severe irritability and unusual crying were 1% (mixed group, n = 4) and 1.6% (separate, n = 6) and 0.8% (mixed, n = 3) and 1.3% (separate, n = 5) respectively.

The incidences of local reactions were similar at the sites of the DTPw-HBV-Hib and DTPw-HBV injections,

Table 4. Incidence of the Most Frequent Systemic and Local Symptoms and of Those Assessed as Being Related to the Vaccination by the Investigator

	*Mixed DTPw-HBV–Hib (n = 396)**		*Separate DTPw-HBV+Hib (n = 385)*		
Symptom	*Total (%)*	*Related (%)*	*Total (%)*	*Related (%)*	*Total (%)*
Systemic					
Fever	219 (55.3)	166 (41.9)	201 (52.2)	152 (39.5)	–
Irritability	136 (34.3)	60 (15.2)	148 (38.4)	51 (13.2)	–
Unusual crying	48 (12.1)	22 (5.6)	65 (16.9)	25 (6.5)	–
Local					
Pain	127 (29.5)	All	127 (33.0)	All	65 (16.9)
Redness	103 (26.0)	All	110 (28.6)	All	42 (10.9)
Swelling	105 (26.5)	All	111 (28.8)	All	40 (10.4)

*One subject's diary card was not returned.
Mixed vs. separate: fever: P = 0.512; irritability: p = 0.474; unusual crying: P = 0.652.

the separate Hib vaccine being associated, as expected, with fewer reactions (see Table 3). Severe pain was reported after five (1.3%) of the doses of mixed vaccine, six (1.6%) of the DTPw-HBV doses, and four (1%) of the Hib doses given as separate vaccines. There were no reports of severe redness or swelling.

DISCUSSION

The administration of DTPw-HBV and Hib vaccines as a mixture in a single injection produced a lower total incidence of injection site symptoms and, numerically, slightly more general symptoms than separate administration of the two vaccines, although this was neither statistically nor clinically significant in either case. The incidence of local symptoms with the mixed vaccine was similar to that of the DTPw-HBV alone, showing that the extra Hib component did not increase the reactogenicity. Severe reactions, although infrequent, were also associated to the same extent with the mixed and separate administrations. The only serious adverse event, an inflammatory reaction, occurred at the site of a separate DTPw-HBV injection, and was successfully resolved following treatment with antibiotics. Although the study was open for the assessment of reactogenicity, in view of the similarity of the reactogenicity profiles in the two groups the authors are confident that no bias was introduced.

The DTPw-HBV mixed with Hib induced protective antibody titers against diphtheria, tetanus, hepatitis B, and *H. influenzae* and high antipertussis titers. Combinations of antigenic components from different organisms are known to cause variable effects on individual titers, decreasing one or other of the antibody titers.[9-17] Scheifele et al report that immunologic responses vary with the DPT preparation used to resuspend the PRP-T conjugated vaccine.[9] The present study showed no significant decrease in any of the antibody titers after mixing, with the exception of diphtheria. Indeed, the mixed vaccine induced equal or higher antibody responses against the other four antigens when compared to separate administration. Although a decrease in the anti-PRP response has been described when some DTPw and Hib vaccines were mixed in the same syringe,[1] mixing did not cause a reduction in titers of anti-PRP antibodies using the specific PRP-T and DTPw-HBV vaccines in the present study. Despite the smaller GMTs in the mixed group, the antidiphtheria titers were well above the protective cut-off titers in both groups. Significantly higher antitetanus titers were elicted in the mixed vaccine group compared to the separate injections.

The antibody titers elicited in this study are equivalent to those observed in other trials of the DTPw-HBV vaccine.[18-20] The WHO recommend that 95% or more of infants receiving DTPw and HBV vaccinations should have protective antibody titers against HBV (10 mIU/mL) after a three-dose primary vaccination course.[5] A further recommendation of the EPI is that three tetravalent vaccine doses of DTPw-HBV should be used as the primary vaccination course in countries with low HB prevalence, whereas in regions with high perinatal transmission a HB, monovalent vaccine dose should be given at birth followed by three doses of DTPw-HBV, as in this study. The mixed vaccine used in this trial fulfills the hepatitis B requirement in this situation.

The mixed pentavalent vaccine is thus proven to be as immunogenic and safe and better tolerated than separate, simultaneous injections due to the elimination of the extra injections for the separate Hib vaccine and, as could be shown, the attributable side-effects. The use of such a vaccine will allow the integration of hepatitis B vaccines into national childhood immunization schedules, as recommended by the WHO Expanded Program of Immunization, without the need to introduce a new vaccination. This will ensure that compliance is maintained, by improving patient comfort while also keeping to a minimum the increase in costs that a new vaccine entails in terms of administration, logistics, and storage. This study extends and confirms the results of recent reports describing the safety and immunogenicity of this combined DTPw-HBV vaccine.[18-20] The current study was intended to investigate the feasibility of using a DTPw-HBV-Hib combination vaccine as a primary course by extemporaneously mixing the DTPw-HBV and Hib vaccines in one syringe before administration, with the intention of developing such a formulation. These results support the possibility of syringe-mixing of currently marketed commercial vaccines as licensed in the European Union. This recommendation should not be generalized, however, as the data from this study apply only to the two specified vaccines, Tritanrix™ and Hiberix™, and alternative vaccines should be investigated with regard to non-interference before use as syringe mixes.

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