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A NEW COMBINED DTP-HBV-HIB VACCINE — STRATEGY FOR INCORPORATION OF HIB VACCINATION INTO CHILDHOOD IMMUNISATION PROGRAMMES

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Objectives. To evaluate the immunogenicity and reactogenicity of a pentavalent vaccine prepared by extemporaneously mixing diphtheria-tetanus pertussis-hepatitis B vaccine (DTP-HBV) and lyophilised *Haemophilus influenzae* type B (Hib)-tetanus conjugate vaccines in the same syringe, compared with the same vaccines given as separate, concomitant administrations.

Design. Open, randomised comparative study.

Setting. Durban, South Africa.

Subjects. A total of 120 healthy male and female infants were enrolled in the trial and randomised into two groups; group 1 received the combined administration (DTP-HBV-Hib), and group 2 received separate administrations of DTP-HBV and Hib vaccines. Vaccines were given as a three-dose primary vaccination course at 6,10 and 14 weeks of age.

Outcome measures. Antibody levels were measured using standard techniques and local and general solicited symptoms were recorded using diary cards.

Results. All subjects had seroprotective titres against diphtheria and tetanus; and antipolyribose-ribitol phosphate (PRP) titres ≥ 0.15 µg/ml 1 month after the final dose. A vaccine response (defined as post-vaccination titres ≥ 15 ELISA (EL).U/ml in initially seronegative subjects; and as post- vaccination titres ≥ pre-vaccination titres in initially seropositive subjects) against the pertussis component was seen in 83% and 85% of subjects in the groups receiving combined and separate administration. No differences were

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seen in any of the geometric mean titres (GMTs) between the two administrations either 2 months after the second dose or 1 month after the final dose. There was no observed increase in reactogenicity in the group receiving the mixed administration.

Conclusions. The results demonstrate that combined DTP-HBV-Hib vaccine is well tolerated and immunogenic.

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Diphtheria, tetanus and pertussis (DTP) vaccinations have been the cornerstone of infant vaccination for several decades. It is widely accepted that combination vaccines offer the most practical and cost-effective means of achieving ever-expanding immunisation goals.[1] Because DTP vaccination is mandatory in most childhood immunisation schedules, high coverage has been established. This, therefore, makes DTP a preferred combination partner. In recognition of this, the World Health Organisation (WHO) has already recommended the development of DTP-hepatitis B vaccine (HBV) combinations in order to facilitate the incorporation of hepatitis B vaccination into the Expanded Programme of Immunisation (EPI),[2] which is now being undertaken.[3-6]

In 1998, the WHO recommended the incorporation of *Haemophilus influenzae* type B (Hib) vaccination into routine infant immunisation programmes, as appropriate to national capacities and priorities.[7] The importance of Hib vaccination, especially in early childhood, is clear, particularly in developing countries where the disease occurs in young children, with most cases occurring before 12 months of age, and half of these before 6 months of age.[8] In addition infant immunisation has already reduced the rates of invasive disease in the developed world[9] and recent efficacy trials suggest that this could now be achieved in the developing world.[10-11] The addition of the Hib vaccine to the DTP-HBV combination vaccine would offer a means of incorporating Hib into childhood vaccination schedules with minimal cost increase and maximum ease.

Any new vaccine, and in particular combination vaccines, must be carefully evaluated in order to ensure that there is no loss of immune response to any of the components or any increase in reactogenicity. In consideration of these factors, we have evaluated the safety and immunogenicity of the pentavalent combination, whole-cell pertussis-based DTP (DTPw)-HBV-Hib, produced by the extemporaneous mixing of DTPw-HBV and Hib when given as a primary course to children at 6, 10 and 14 weeks of age.

MATERIALS AND METHODS

Study design and participants

The study was an open, randomised clinical trial and was conducted by the University of Natal in Durban, South Africa. The study received local ethical approval and was conducted according to the Declaration of Helsinki and the Good Clinical Practice Guidelines (International Conference on Harmonisation, April 1996). Before enrolment, written informed consent was obtained from the children's parents or guardians.

A total of 120 healthy male and female infants between 6 and 10 weeks of age were enrolled in the trial. Subjects were eligible to participate if they did not violate any of the exclusion criteria. These were: participation in another trial; immunosuppressive or immunoglobulin therapy; any congenital defects, chronic illness, acute condition or systemic dysfunction (especially relating to the central nervous system (CNS)); history of allergic reaction or immune disorder (including evidence of being born to an HIV-seropositive mother). In addition, during the trial both contraindications to vaccination (encephalopathy and hypersensitivity immediate anaphylactic reaction) and reactions (fever ≥ 40°C and persistent, inconsolable screaming or crying > 3 hours within 48 hours of vaccination, high-pitched screaming and crying, convulsions with or without fever) associated with whole-cell *Bordetella pertussis* vaccination according to the Advisory Committee on Immunization Practices (ACIP) recommendations[12] were considered as exclusion criteria.

Vaccines

Subjects were randomised into two groups. Group 1 received DTPw-HBV extemporaneously mixed with Hib in the same syringe, and group 2 received the same vaccines as separate, concomitant injections. A single, 0.5 ml dose of DTPw-HBV-Hib vaccine was prepared by resuspending the lyophilised Hib vaccine (10 µg of polyribose-ribitol phosphate (PRP) conjugated to ~ 30 µg tetanus toxoid, 10 mg lactose) in the contents of one vial of DTP-HBV (7.5 limit flocculation (Lf) diphtheria toxoid, 3.25 Lf tetanus toxoid, 15 IU whole cell pertussis, 10 µg recombinant hepatitis B surface antigen (HbsAg), 0.63 mg aluminium (as aluminium salts), 25 µg thiomersal, 150 mM sodium chloride and 0.5 mg phenoxyethanol (Tritanrix-HB, SmithKline Beecham Biologicals). For separate administration, a 0.5 ml dose of Hib (Hiberix, SmithKline Beecham Biologicals) was prepared by reconstituting the lyophilised pellet in saline solution (sodium chloride (NaCl) 9 mg/ml) supplied by the manufacturer. Vaccines were administered intramuscularly in the anterolateral region of the thigh at 6, 10 and 14 weeks of age.

Reactions

Diary cards were used by parents or guardians to record solicited local reactions (pain, redness and swelling) and general adverse experiences (irritability, unusual crying, drowsiness, feeding problems, diarrhoea and vomiting) on the day of vaccination and for 3 subsequent days. Any redness or

swelling was measured, a diameter > 20 mm being defined as severe. For all other symptoms 'severe' was defined as preventing normal daily activities. Parents were requested to contact study personnel for further assessment if their child experienced any severe or alarming symptom. The outcome of all adverse experiences was recorded and the relationship to vaccination was assessed.

Serology

Sera were taken from infants immediately before the first dose, approximately 2 months after the second dose, and approximately 1 month after the final dose. Samples were stored at – 20°C until analyses were performed at SmithKline Beecham Biologicals in a blinded fashion. Anti-hepatitis B surface antigen (HBs) antibodies were determined using a commercial radio-immunoassay (AUSAB, Abbott); the assay cut-off was 10 IU/ml.[13] Anti-diphtheria and antitetanus antibody titres were measured by enzyme-linked immunosorbent assays (ELISAs). Although antidiphtheria and antitetanus titres > 0.01 IU/ml are generally considered to be protective and a good correlation has been shown to exist between *in vivo* neutralisation tests and the ELISA results for this;[14,15] correlation may be reduced at antibody titres < 0.1 IU/ml. A titre of 0.1 IU/ml by ELISA was, therefore, conservatively set as the cut-off. Anti-whole-cell *B. pertussis* antibody titres (anti-*B. pertussis*) were measured by ELISA using an immunoglobulin G (IgG) enzyme immuno-assay (EIA) test kit (Labsystem, ICN-FLOW); the assay cut-off was 15 ELISA (EL).U/ml. The anti-PRP antibodies were measured using a radio-labelled antigen-binding assay; assay cut-off was 0.15 µg/ml.

Seroconversion was defined as titres greater than assay cut-off. All asay cut-offs were considered to be seroprotective, except for *B. pertussis* for which there is no serological correlate of protection. A vaccine response against *B. pertussis* in initially seronegative subjects was defined as the induction of antibody titres greater or equal to the assay cut-off value; and in initially seropositive subjects as a post-vaccination titre greater than or equal to the individual's pre-vaccination titre, thereby taking into account the half-life (approximately 40 days) of maternal antibodies.[16]

Statistical analysis

Geometric mean titres (GMTs) were calculated for all antibodies (titres below the assay cut-off value were given an arbitrary value of one-half the assay cut-off). All statistical analyses were performed using SAS with a type 1 error of 5%. Fisher's exact test was used to compare seroconversion rates and the confidence interval was used to compare GMTs between groups. The two-sided Fisher's exact test was used to determine the incidence of local reactions and general adverse reactions between groups.

RESULTS

Overall, the combined administration compared favourably with the vaccines given separately. Responses to each component in the combined preparation were similar to those obtained when the administration was separate (Table I). All subjects in both groups had antibody titres above the assay cut-off threshold 1 month after the final dose (Table I). Indeed, the percentage of subjects with protective titres for all vaccine components in both groups was extremely high (Table I).

Table I. Seroconversion rates post second and third dose

	Group 1 (combined) DTP-HBV-Hib (*N* = 49)		Group 1 (separate) DTP-HBV+Hib (*N* = 48)	
	P 11*	*P* 111†	*P* 11*	*P* 111†
Anti-HBs	89.6	100	91.7	100
Antidiphtheria	91.7	100	91.7	100
Antitetanus	97.3	100	93.8	100
Antipertussis	83.0	100	85.4	100
Anti-PRP	97.9	100	97.9	100

Comparison of P11 seroconversion rates between groups: anti-HBs ($P = 1.00$); anti-diphtheria ($P = 1.00$); antitetanus ($P = 0.62$); antipertussis ($P = 0.67$); anti-PRP ($P = 1.00$).
*P11 = 2 months after the second dose.
†P111 = 1 month after the third dose.

The GMTs for each component in either group are illustrated in Fig. 1, A-E. No differences were detected in the GMTs after the second and third dose of the vaccines in both groups, except for a significantly higher antitetanus antibody titre after the second dose in the combined group. The latter occurrence may be ascribed to chance as no plausible explanation exists for why this may have occurred. A month after the final dose, this difference had disappeared.

Table II provides data on safety; it is clear that there are no differences between the two groups for local reactions and

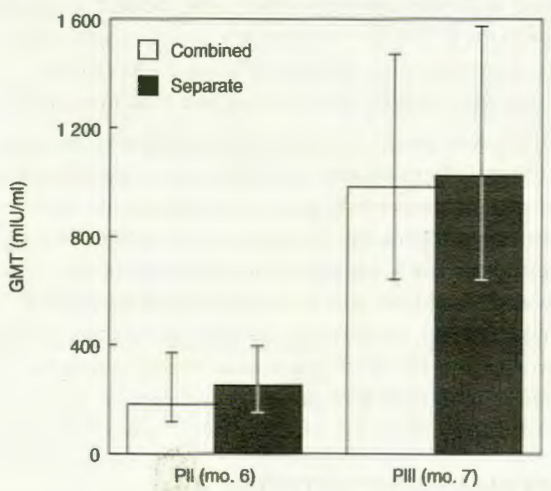

Fig. 1A. Anti-HBs GMTs post second and third dose (vertical bars represent standard error (SE)).

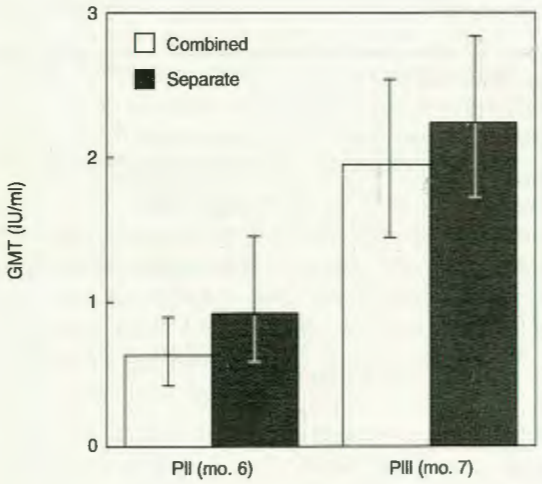

Fig. 1B. Anti-diphtheria GMTs post second and third dose (vertical bars represent standard error (SE)).

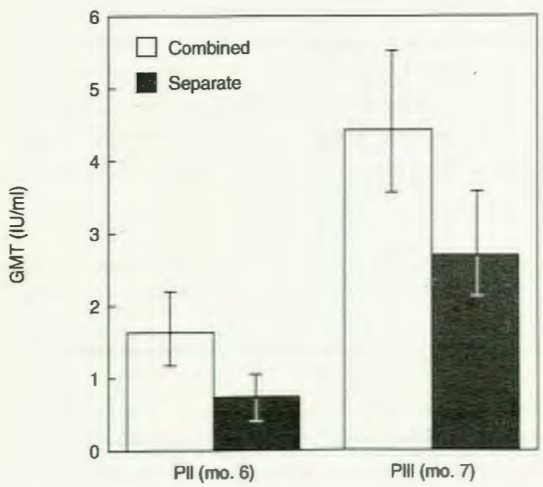

Fig. 1C. Anti-tetanus GMTs post second and third dose (vertical bars represent standard error (SE)).

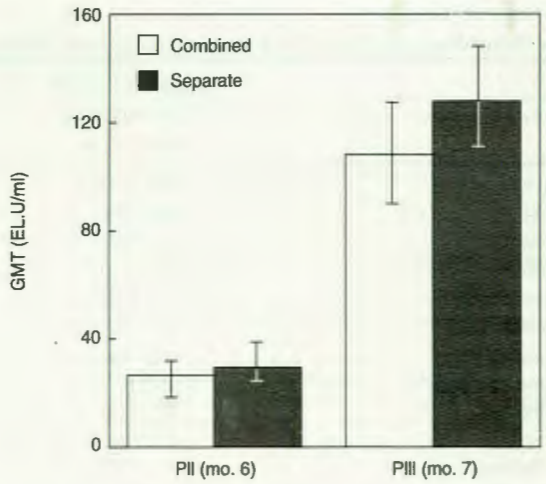

Fig. 1D. Anti-pertussis GMTs post second and third dose (vertical bars represent standard error (SE)).

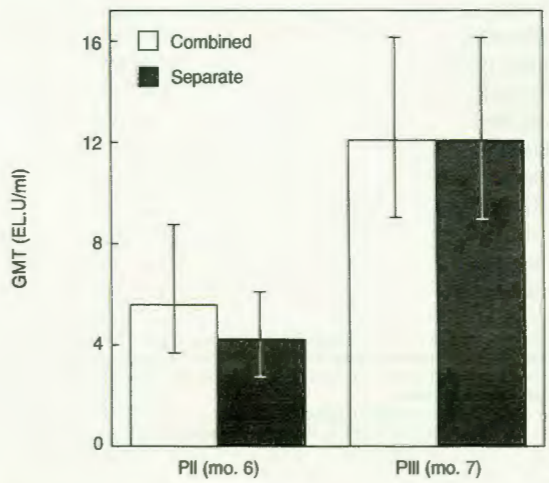

Fig. 1E. Anti-PRP GMTs post second and third dose (vertical bars represent standard error (SE)).

generalised adverse effects. Although fever is a common reaction, quantitative measurement of fever is not common practice on the part of parents and guardians. To ensure protocol compliance, therefore, quantitative measurements were restricted to further assessments by study personnel when consulted by parents. One child suffered an afebrile convulsion. She was not hopitalised, but 6 days later underwent a computed tomography (CT) scan which was normal and she was said to be clinically well. We judged the episode to be 'possibly related' to vaccination. Subsequent follow-up revealed normal development with no sequelae.

Only one other serious event was reported during the trial which was not considered to be related to vaccination, and no drop-out was due to a vaccine-related event. The majority of drop-outs (7 subjects) occurred as a result of subjects having been lost to follow-up. Another subject dropped out following a severe episode of influenza (unrelated to vaccination). In addition, three parents did not want blood to be taken from their children and therefore withdrew their consent.

DISCUSSION

The benefits of combination vaccines are evident, the most apparent being the decreased discomfort to the child and simplified administrative and delivery logistics. There is, owever, one concern that has been raised during recent years, viz. the lower apparent immune response to some components, as manifested by lower antibody titres.[17,18]

Generally such effects are small and inconsistent and it has, therefore, been difficult to attach any clinical significance to such observations.[17,18] Furthermore, these observations are more common with acellular pertussis (DTPa)-based vaccines than

Table II. Incidence of solicited local reactions and general adverse reactions

	Group 1 (N =172)* DTP-HBV-Hib (right thigh)	Group 2 (N = 168)* DTP-HBV (right thigh)	Hib (left thigh)
Local symptoms	Total % (N)†	Total % (N)†	Total % (N)†
Pain	43.6 (75)	37.5 (63)	32.1 (54)
Severe	8.7 (15)	8.3 (14)	5.4 (9)
Redness	32.0 (55)	33.3 (56)	22.6 (38)
> 20 mm	2.3 (4)	3.0 (5)	0.6 (1)
Swelling	45.3 (78)	39.3 (66)	22.6 (38)
> 20 mm	8.1 (14)	6.0 (10)	2.4 (4)
General symptoms	DTP-HBV-Hib	DTP-HBV+Hib	
Diarrhoea	5.2 (9)	2.4 (4)	
Severe	1.7 (3)	0.6 (1)	
Related	0.0 (0)	0.0 (0)	
Drowsiness	9.9 (17)	11.9 (20)	
Severe	1.2 (2)	0.6 (1)	
Related	0.0 (0)	0.0 (0)	
Feeding problems	17.4 (30)	12.5 (21)	
Severe	2.9 (5)	3.5 (6)	
Related	0.0 (0)	0.0 (0)	
Irritability	27.3 (47)	31.0 (52)	
Severe	5.8 (10)	8.9 (15)	
Related	0.5 (1)	0.0 (0)	
Unusual crying	33.1 (57)	32.1 (54)	
Severe	13.9	12.5 (21)	
Related	1.2 (2)	0.0 (0)	
Vomiting	5.8 (10)	5.4 (9)	
Severe	0.6 (1)	0.6 (1)	
Related	0.0 (0)	0.0 (0)	

Comparison of the incidence of symptoms between groups: pain (P = 0.27); redness (P = 0.82); swelling (P = 0.27); diarrhoea (P = 0.26); drowsiness (P = 0.60); feeding problems (P = 0.23); irritability (P = 0.48); unusual crying (P = 91); vomiting (P = 1.00).
There was no statistical difference in the comparison of any other individual symptom between groups.
*N = number of doses given.
N = number of doses reported for a given symptom.

with DTPw vaccines.[17,19,20] This may be due, in part, to the adjuvant effect of the whole-cell pertussis component.[21] In this study we saw no diminished response to any component in the combined administration group (group I) compared with group 2. All subjects in both groups had titres above the assay cut-offs 1 month after the final dose, as shown in Table I. With regard to the antidiphtheria, antitetanus, anti-HBs and anti-PRP responses, these values are considered to be indicative of protection. Notable then, is the high percentage of subjects in whom protective titres had been achieved 2 months after the second dose. This may be of benefit, especially in developing countries where travel to health centres is not always easy and therefore full compliance is not always achieved. In addition, no differences in post-second or third dose GMTs were seen between groups, with the exception of the antitetanus GMTs post-second dose (Fig. 1, A-E). The post-second dose anti-tetanus GMTs were significantly higher in the group receiving the combined administration; however, there was no difference between the groups 1 month after the final dose.

In this trial the anti-PRP response following separate administration of monovalent Hib was compared with the response seen following the DTP-HBV-Hib combination. This design would then be the most likely to elucidate an effect due to combination if it existed. The findings here show that the combination had no effect on either the seroconversion rates (at 0.15 µg/ml or 1.0 µg/ml — data not shown), or the post-vaccination GMTs (post second or third dose). This observation confirms the results of an earlier trial where the same vaccines were used.[22]

This study also assessed the comparative safety of the two vaccine administrations (data shown in Table II). There was no difference in the incidence of any symptom between the two groups. Furthermore, the incidence of symptoms was similar to those reported for DTPw vaccines,[23] and in addition there was a low number of severe reactions (maximum incidence

was 8.9% for severe irritability). The whole-cell pertussis component is responsible for the majority of reactions following DTPw vaccination,[23] and in the past has been associated with a number of serious adverse events.[12] 'Unusual crying' has been described as a common occurrence following whole-cell vaccination. Although the incidence of unusual crying was 33.1% and 32.7% in groups 1 and 2 respectively, only an incidence of 1.2% and 0% respectively were considered to be related to vaccination on the basis of further questioning of the parents regarding any association of the crying episode to nonspecific events such as hunger, teething, etc. This was because we felt that parents were not strictly adhering to the definition of unusual crying.[12]

The results of this trial show that the novel pentavalent vaccine, DTP-HBV-Hib, is well tolerated and immunogenic and the addition of Hib does not adversely affect the reactogenicity or the immunogenic profile. Having demonstrated the clinical feasibility, it is worth reiterating the economic and logistical advantages. The cost-benefits of monovalent Hib vaccination, in terms of the savings associated with treatment costs, have already been demonstrated.[24,25] This, coupled with cost savings that can be made by combining the Hib vaccine with DTP-HBV, lends further support to the use of the combined vaccine and constitutes an attractive option to health care providers.

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