Available online at www.sciencedirect.com
SCIENCE DIRECT®
Vaccine 23 (2005) 2602–2606
Vaccine
www.elsevier.com/locate/vaccine

Immunogenicity and safety of a combined hepatitis A and B vaccine administered concomitantly with either a measles–mumps–rubella or a diphtheria–tetanus–acellular pertussis-inactivated poliomyelitis vaccine mixed with a *Haemophilus influenzae* type b conjugate vaccine in infants aged 12–18 months

V. Usonis[a,*], S. Meriste[b], V. Bakasenas[c], I. Lutsar[b], F. Collard[d], M. Stoffel[d], N. Tornieporth[d]

[a] *Centre of Paediatrics, Vilnius University, P.O. Box 2561, 2009 Vilnius, Lithuania*
[b] *Tartu University Children's Hospital, Lunini 6, 51014 Tartu, Estonia*
[c] *Centre for Communicable Diseases Prevention and Control, Roziu Avenue 4a, 2600 Vilnius, Lithuania*
[d] *GlaxoSmithKline Biologicals, Rixensart, Belgium*

Received 27 January 2004; received in revised form 1 November 2004; accepted 9 November 2004
Available online 22 December 2004

Abstract

Two studies were undertaken to investigate the concomitant administration of combined hepatitis A/B vaccine with a diphtheria–tetanus–acellular pertussis-inactivated poliomyelitis vaccine mixed with *Haemophilus influenzae* vaccine (DTPa-IPV/Hib), or with a measles–mumps–rubella vaccine (MMR), during the second year of life. On completion of the vaccination course, all subjects were seropositive or seroprotected against all antigens except for one subject who was seronegative for anti-PT. Seropositivity and seroprotection rates for all other antibodies were comparable to reference values for each vaccine component, indicating that the immunogenicity of MMR, DTPa-IPV/Hib and combined hepatitis A/B vaccines is not impaired by co-administration. All vaccines were well tolerated.

Keywords: Hepatitis A/B vaccine; DTPa-IPV/Hib; MMR; Concomitant vaccination

1. Introduction

Co-administration of multiple antigens is probably the most effective strategy for infant immunisation [1,2]. Combined vaccines have proved effective in childhood vaccination programs [3,4] and the addition of combined hepatitis A/B vaccine would represent a further advance, in particular for those countries which do not have a routine hepatitis B vaccination programme at birth or during the first year of life [5].

2. Materials and methods

Subjects were recruited from one centre in Lithuania (study 1) and two centres in Estonia (study 2). Written informed consent was obtained from parents/guardians. The study populations comprised healthy infants aged 12–15 months (study 2) and approximately 18 months (study 1) born to mothers not known to be seropositive for HBsAg, hepatitis B antigen or anti-HBc antibodies (Abs). All subjects had received diphtheria–tetanus–pertussis (DTP) and inactivated polio virus (IPV), those in study 2 had not been vaccinated against measles–mumps–rubella (MMR).

* Corresponding author. Tel.: +370 5 2720368; fax: +370 5 2720368.
E-mail address: usonis@ktl.mii.lt (V. Usonis).

0264-410X/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2004.11.062

In study 1, a combined hepatitis A/B vaccine (Ambirix™[1] or Twinrix™ Adult, GlaxoSmithKline Biologicals) was administered concomitantly with DTPa-IPV mixed with *H. influenzae* vaccine (Infanrix™-IPV/Hib, GlaxoSmithKline Biologicals), by intramuscular injection in the left and right thigh, respectively. In study 2, the same hepatitis A/B vaccine was given in the left thigh with an MMR vaccine (Priorix™, GlaxoSmithKline Biologicals) administered subcutaneously in the right arm. A second dose of hepatitis A/B vaccine was given after 6 months in both studies.

Blood was taken at entry, 1 month after the first vaccination (month 1) and 1 month after the second dose of the combined hepatitis A/B vaccine (month 7). Commercial radioimmunoassay kits were used to measure anti-HBs Abs (AUSAB®, Abbott Laboratories), HBsAg (AxSYM® or AUSAB® 11-125, Abbott laboratories), anti-HBc Abs (AxSYM® or CORAB®, Abbott laboratories) and anti-HAV Abs (Enzymum Kit®, Boehringer). Anti-tetanus, anti-diphtheria, anti-PRP titres and IgG Abs against the pertussis components PT, FHA and PRN were measured by ELISA. The assay cut-off limits were set at ≥1 mIU/ml for anti-HBs, ≥0.1 IU/ml for anti-tetanus and anti-diphtheria, ≥33 mIU/ml for anti-HAV and ≥0.15 mcg/ml for anti-PRP titres. The cut off for all three pertussis Abs was 5 EL.U/ml. Antibodies against polio virus types 1, 2 and 3 were determined by a virus microneutralisation test adapted from the WHO guidelines. Titres were expressed in terms of the 50% inhibitory dose (ID_{50}). Values ≥8 times ID_{50} were considered to be protective. IgG Abs to MMR were assayed by ELISA (Enzygnost®, Behringwerke, Germany) using cut off limits of 150 mIU/ml for measles, 231 U/ml for mumps, and 4 IU/ml for rubella. Subjects with Ab titres above or equal to the cut-off value were considered seropositive. Seroprotection for anti-HBs was defined as an Ab titre ≥10 mIU/ml. An anti-PRP titre ≥0.15 mcg/ml was indicative of protection against Hib disease. Anti-tetanus and anti-diphtheria titres >0.1 IU/ml were considered seroprotective. Vaccine response for pertussis was defined as Ab titres above the assay cut off in initially seronegative subjects or by post-vaccination Ab titres ≥2 × pre-vaccination titres.

Subjects' parents/guardians recorded any pain, redness, swelling, fever, irritability, drowsiness or loss of appetite for three days after vaccination on a diary card. Pain was scored as: minor reaction to touch (grade 1); cries/protests to touch or limb movement (grade 2) or spontaneous pain (grade 3). General symptoms were assessed as: easily tolerated (grade 1); interfering with normal activity (grade 2); preventing normal activity (grade 3). Fever was graded: 38.0–38.5 °C (grade 1); 38.5–39.5 °C (grade 2); >39.5 °C (grade 3). In study 2, parents monitored the presence of rash/exanthema, parotid or salivary gland enlargement and signs of meningism (vomiting, neck stiffness or photophobia). Parents were also asked to record any additional symptoms occurring within 30 days of vaccination.

Primary endpoint was the proportion of previously seronegative subjects achieving anti-HAV Ab titres ≥33 mIU/ml and anti-HBs Ab titres ≥10 mIU/ml at month 7. No specific endpoints were defined for the immune response to DTPa-IPV/Hib and MMR vaccines but the data obtained in our study were compared to published reference values [4,6–8].

3. Results

3.1. Study population

Sixty subjects entered study 1 and received at least one dose of vaccine. Of these, 53 (32 males) could be evaluated for immunogenicity and constituted the per-protocol (PP) population. The mean age was 16.5 (range 15–18) months. Four subjects were found to be seropositive for hepatitis A/B and three did not comply with the vaccination schedule. Of the 57 subjects in study 2, the PP population comprised 37 subjects (17 males) with a mean age of 12.5 (range 11–15) months. The rest were either seropositive at entry or did not comply with the study schedules.

3.2. Immunogenicity

One month after the second dose of vaccine, all subjects were seroprotected against hepatitis B and were seropositive for anti-HAV Abs (Tables 1 and 2, Fig. 1). All subjects in study 1 were seroprotected against tetanus and diphthe-

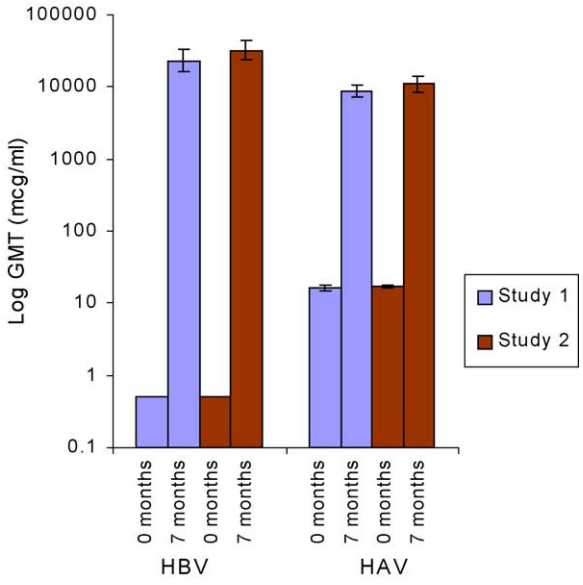

Fig. 1. Comparative anti-HAV and anti-HBV GMTs at 0 and 7 months (before first and following second vaccine dose) for study 1 (Hepatitis A/B plus DTPa-IPV/Hib) and study 2 (Hepatitis A/B plus MMR). Bars represent 95% confidence intervals for each GMT value plotted.

[1] Ambirix™ is a combined hepatitis A/B vaccine containing 720 EL.U of inactivated hepatitis A virus, 20 μg of recombinant hepatitis B antigen and 0.45 mg of aluminium as salt in a 1-ml dose.

Table 1
Comparison of seroprotection and seropositivity rates following administration of the combined hepatitis A/B and DTPa-IPV/Hib with reference values (study 1)

Antibody	Seroprotection[a] and seropositivity[b]					GMT	
	Month	*N*	%	95% CI	Reference value	Value	95% CI
Anti-HBs	0	53	0.0	0.0–6.7		0.5	0.5
	7	52	100.0	93.2–100.0	96.0–100[c]	23,092	16,133–33,053
Anti-HAV	0	53	0.0	6.7–16.5		17	14–18
	7	52	100.0	93.2–100.0	98.0–100[c]	8736	7299–10458
Anti-diphtheria	0	53	83.0	70.2–91.9		0.26	0.2–0.3
	1	53	100.0	93.3–100.0	98.2[d]	8	7–10
Anti-tetanus	0	53	100.0	93.3–100.0		0.60	0.5–0.7
	1	53	100.0	93.3–100.0	100[d]	18	15–21
Anti-PT	0	52	75.0	61.1–86.0		15	10.6–21.3
	1	51	98.0	89.6–100.0	100[d]	359	258–499
Anti-FHA	0	51	98.0	89.6–100.0		21	16–28
	1	51	100.0	93.0–100.0	100[d]	581	482–701
Anti-PRN	0	53	79.2	65.9–89.2		12	9–16
	1	53	100.0	93.3–100.0	100[d]	492	379–639
Anti-polio 1	0	53	96.2	87.0–99.5		29	22–39
	1	41	100.0	91.4–100.0	100[d]	1352	986–1854
Anti-polio 2	0	53	94.3	84.3–98.8		41	28–59
	1	38	100.0	90.7–100.0	100[d]	1217	923.6–1603.4
Anti-polio 3	0	52	57.7	43.2–71.3		15	11–22
	1	39	100.0	91.0–100.0	100[d]	1243	847–1824
Anti-PRP	0	51	41.2	27.6–55.8		0.1	0.1–0.2
	1	53	100.0	93.3–100.0	100[e]	10	7–16

N number of subjects tested.
[a] Seroprotection: anti-HBs >10 IU/ml; anti-diphtheria and anti-tetanus ≥0.1 IU/ml; anti-polio 1,2 and 3 ≥8 times ID_{50}; anti-PRP ≥0.15 mcg/ml.
[b] Seropositivity: anti-HAV ≥33 mIU/ml; anti-PT, anti-FHA and anti-PRN ≥5 El.U/ml arbitary value for anti-PRP titres ≥0.15 mcg/ml.
[c] 95% CI [24].
[d] Point estimate [23].
[e] Point estimate [8].

ria toxins, and all but one were seropositive for anti-PT Abs. All subjects were seroprotected against polio. One month after Hib vaccination, all subjects achieved titres >15 mcg/ml and 94.3% exceeded levels of 1 mcg/ml. All subjects became seropositive for anti-mumps, measles and rubella Abs (Table 2).

3.3. Safety and reactogenicity (Table 3)

Local symptoms were reported (Table 3) in 48% of cases who received the hepatitis A/B and MMR vaccines, and 18% who received the hepatitis A/B and DTPa-IPV/Hib vaccines. Rates for general symptoms were 46 and 32%, respectively.

Table 2
Comparison of seroprotection and seropositivity rates following administration of the combined hepatitis A/B and MMR with reference values (study 2)

Antibody	Seroprotection[a] and seropositivity[b]					GMT	
	Month	*N*	%	95% CI	Reference value		95% CI
Anti-HBs	0	36	0.0	0.0–9.7		0.5	0.5–0.5
	7	35	100.0	90.0–100	96.0–100[c]	32,540	23,685–44,705
Anti-HAV	0	37	2.7	0.1–14.2		17	16–18
	7	35	100.0	90.0–100	98.0–100[c]	11016	8511–14258
Anti-measles	0	37	0.0	0.0–9.5			
	1	26	100.0	86.8–100	99.6[d]	2746	2212–3411
Anti-mumps	0	37	0.0	0.0–9.5			
	1	26	100.0	86.8–100	97.0[d]	1307	1017–1680
Anti-rubella	0	37	0.0	0.0–9.5			
	1	26	100.0	86.8–100	100[d]	63	49–82

N number of subjects tested.
[a] Seroprotection: anti-HBs >10 IU/ml.
[b] Seropositivity: anti-HAV ≥33 mIU/ml; anti-mumps ≥231 U/ml, anti-measles ≥150 mIU/ml and anti-rubella ≥4 IU/ml.
[c] 95%CI [21].
[d] Point estimate [4].

Table 3
Summary of solicited local and general symptoms following administration of the combined hepatitis A/B and DTPa-IPV/Hib or MMR vaccines (per dose analysis)

		Study 1 (DTPa-IPV/Hib)		Study 2 (MMR)	
		DTP thigh ($N = 60$, %)	Hep thigh ($N = 120$, %)	MMR thigh ($N = 57$, %)	Hep thigh ($N = 109$, %)
Local symptoms					
Pain	All	16.7	13.8	22.8	24.8
	Grade 3	1.7	0.8	0.0	0.0
Redness	All	10.0	4.2	22.8	29.4
	Grade 3	0.0	0.8	0.0	0.0
Swelling	All	6.7	0.8	5.3	10.1
	Grade 3	0.0	0.0	0.0	0.0
General symptoms					
Drowsiness					
All			6.7[a]		21.1[b]
Grade 3			0.0[a]		0.9[b]
Fever					
All			13.3[a]		8.3[b]
Grade 3			0.0[a]		0.9[b]
Irritablility					
All			25.0[a]		30.3[b]
Grade 3			0.0[a]		0.9[b]
Loss of appetite					
All			11.7[a]		22.9[b]
Grade 3			0.0[a]		0.9[b]

N = number of doses for which the symptom sheet was completed.
[a] $N = 120$, %.
[b] $N = 109$, %.

The proportion of general solicited symptoms considered as being of probable or suspected relationship to vaccination was 82% in study 1 and 66% in study 2. No cases of parotid or salivary gland swelling occurred, and there were only two reports of rash, both of mild-to-moderate intensity and considered unrelated to the study vaccine. No unsolicited symptoms were attributed to vaccination in study 1 but three subjects developed symptoms (vomiting and two injection site reactions) that were suspected or probably related to vaccination in study 2. Six serious adverse events were reported in study 1, all considered unrelated to vaccination. Of five serious adverse events in study 2, only one was considered related to vaccination. The subject was hospitalized on day 1, with febrile convulsions (probably related to vaccination) and acute rhinopharyngitis (unlikely related to vaccination). The subject recovered and was discharged after 4 days.

4. Discussion

Our findings suggest that the immunogenicity of DTPa-IPV/Hib, MMR and hepatitis A/B vaccines is not reduced by co-administration. One month after vaccination, seroprotection or seroconversion was achieved in all subjects for all components except for one subject who did not respond to pertussis. Interestingly, anti-HBs titres were at least 2.5 times higher than those reported in children aged 1–6 years who received the hepatitis A/B vaccine alone, whereas the anti-HAV titres in our study were comparable to those previously published [8]. GMTs for anti-measles, mumps and rubella were similar to those reported in over 4700 children under 2 receiving the same MMR vaccine [3]. GMTs for DTPa-IPV/Hib compared favourably with a four-dose vaccination schedule given during the first year of life [6].

Solicited symptoms were reported slightly more frequently than in studies of the separate vaccines [3,4,9], except for DTPa-IPV/Hib [10]. Although it was not possible to evaluate the role of hepatitis A/B vaccine and co-administered vaccines in the incidence of general symptoms, the reactogenicity and safety of Ambirix™ was compared to that of the established schedule (Twinrix™ Junior) in another study conducted in young children aged 1–11 years [11], where both vaccines were shown to have a similar reactogenicity profile, with very few grade 3 symptoms. Hence, the high proportion of general symptoms related to vaccination observed in study 1 and 2 may, at least partly, be linked to co-administration of MMR and DTPa-IPV/Hib vaccines; in particular, in the latter case, our subjects were primed with a whole cell vaccine (DTPw) vaccine, which was shown to cause increased reactogenicity with subsequent doses in comparative studies with children primed with a DTPa vaccine [12]. However, the safety profile of all vaccines was within published limits, indicating mutual non-influence.

We conclude that combined hepatitis A/B vaccine can be co-administered with MMR or DTPa-IPV/Hib vaccines dur-

ing the second year of life without compromising immunogenicity or safety. Combined administration may improve compliance and will simplify the goal of providing protection against hepatitis A and B.

Acknowledgements

The authors express their sincere appreciation to the study group at GlaxoSmithKline Biologicals and the clinic staff who assisted in the study. The authors also thank all parents/guardians who allowed their children to participate in the study.

References

[1] André FE. Development and clinical application of new polyvalent combined paediatric vaccines. Vaccine 1999;17:1620–7.
[2] Halperin SA. Developing better paediatric vaccines. BioDrugs 1999;12(3):175–91.
[3] Usonis V, Bakasenas V, Kaufhold A, Chitour K, Clemens R. Reactogenicity and immunogenicity of a new live attenuated combined measles, mumps and rubella vaccine in children. Pediatr Infect Dis J 1999;18(1):42–8.
[4] Crovari P, Gabutti G, Giammanco G, Dentico P, Moiraghi AR, Ponzio F, et al., The Cooperative Group for the Study of MMR Vaccines. Reactogenicity and immunogenicity of a new combined Measles–Mumps–Rubella vaccine: results of a multicentre trial. Vaccine 2000;18(25):2796–803.
[5] World Health Organization. Informal consultation on quadrivalent diphtheria, tetanus, pertusissis, hepatitis B vaccine. Final Report. Geneva: WHO, 1992: 1–12.
[6] Dagan R, Igbaria K, Piglansky L, Melamed R, et al. Safety and immunogenicity of a combined pentavalent diphtheria, tetanus, acellular pertussis, inactivated poliovirus and *Haemophilis influenzae* type b-tetanus conjugate vaccine compared with a whole cell pertussis pentavalent vaccine. Pediatr Infect Dis J 1997;16(12):1113–21.
[7] Meriste S, Tamm E, Willems P, Lutsar I. Safety and immunogenicity of combined DTPa-IPV vaccine for primary and booster vaccination. Scand J Infect Dis 1999;31(6):587–91.
[8] Van der Wielen M, Van Damme P, Collard F. A two dose schedule for combined hepatitis A and hepatitis B vaccination in children aged 1–11 years. Pediatr Infect Dis J 2000;19(9):848–53.
[9] Usonis V, Bakasenas V, Chitour K, Clemens R. Comparative study of reactogenicity and immunogenicity of new and established measles, mumps and Rubella vaccines in healthy children. Infection 1998;26(4):222–6.
[10] Halperin SA, King JB, Mills E, Willems P. Safety and immunogenicity of *Haemophilus influenzae*–tetanus toxoid conjugate vaccine given separately or in combination with a three-component acellular pertussis vaccine combined with diphtheria and tetanus toxoids and inactivated poliovirus vaccine for the first four doses. Clin Infect Dis 1999;28(5):995–1001.
[11] Roberton D, Marshall H, Nolan TM, Sokal E, et al. Reactogenicity and immunogenicity profile of a two-dose combined hepatitis A and B vaccine in 1–11-year-old children. In: Proceedings of the 43rd Intersci Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 2003. p. 296.
[12] Bernstein HH, Rothstein EP, Reisinger KS, Blatter MM, et al. Comparison of a three-component acellular pertussis vaccine with a whole cell pertussis vaccine in 15-through 20-month-old infants. Pediatrics 1994;93(4):656–9.