0934-9723

Eur. J. Clin. Microbiol. Infect. Dis., 1996, 15: 927–932

Comparative Safety and Immunogenicity of an Acellular versus Whole-Cell Pertussis Component of Diphtheria-Tetanus-Pertussis Vaccines in Senegalese Infants

F. Simondon[1,2]*, A. Yam[1], J.Y. Gagnepain[1], S. Wassilak[3], B. Danve[4], M. Cadoz[4]

A diphtheria and tetanus toxoid two-component acellular pertussis vaccine (DTaP), consisting of 25 μg glutaraldehyde-detoxified pertussis toxin (PT) and 25 μg native filamentous hemagglutinin (FHA), was compared with diphtheria and tetanus toxoid whole-cell pertussis vaccine (DTwP) in a randomized, double-blind manner in 286 Senegalese infants inoculated at two, four, and six months of age. In infants receiving DTaP a significantly lower rate of local reactions, crying and fever was observed than in infants receiving DTwP. One month after the third dose, the geometric mean titres for FHA antibodies were higher in the DTaP group, whereas increases in PT antibody titres were higher in the DTwP group. More than 90% of the infants had a fourfold or more increase in antibodies to both PT and FHA with either vaccine. Diphtheria, tetanus, and polio antibody responses were also measured and found to be comparable between the two groups. The results of this pilot study support the implementation of a field trial to compare the protective efficacy of these vaccines against pertussis in the same setting.

The development of highly purified acellular pertussis vaccines has been driven by concern over adverse reactions associated with conventional vaccines and the need for long-term protection. Revaccination of older children and young adults is now being seriously considered but is only acceptable if better-tolerated vaccines are available. The efficacy of the acellular pertussis vaccines demonstrated in recent trials conducted in Europe favours their widespread use (1–4).

The same concerns are shared in developing countries (5, 6) considering the adoption of acellular pertussis vaccines. These considerations and suggestions that the immune response in African children may differ from that in Caucasian children (7), emphasize the need to compare acellular and whole-cell vaccines in the specific setting of developing countries.

The objectives of this study were to assess in Senegalese children the safety of a diphtheria-tetanus acellular pertussis vaccine (DTaP) compared with a diphtheria-tetanus whole-cell pertussis vaccine (DTwP), to study the immunogenicity of each component of the DTP vaccine, and to study the immunogenicity of the enhanced-potency inactivated polio vaccine routinely used in this region of Africa. This study preceded implementation of a field trial to compare the protective efficacy of these vaccines against pertussis in the same setting.

[1]Unité de Recherche sur les Maladies Infectieuses et Parasitaires, Institut Francais de Recherche Scientifique pour le Développement en Coopération (ORSTOM), Dakar, Senegal.
[2]Unité de Recherche sur les Maladies Infectieuses et Parasitaires, ORSTOM, BP 5045, 34032 Montpellier, France.
[3]Centers for Disease Control and Prevention, Atlanta, Georgia, USA.
[4]Pasteur-Mérieux Sérums et Vaccins, Lyon, France.

Materials and Methods

DT Acellular Pertussis Vaccine (DTaP). DTaP (Lot S2112, Pasteur Mérieux, France) consisted of 25 μg of glutaralde-

Fonds Documentaire ORSTOM
010011045
Fonds Documentaire ORSTOM
Cote : B*11045 Ex : 1

Table 1: Number of infants who were recruited for the trial and who were analysed for safety and immunogenicity.

	Dose 1				Dose 2				Dose 3			
	n	Percent female	Mean age (months)	Mean weight (kg)	n	Percent female	Mean age (months)	Mean weight (kg)	n	Percent female	Mean age (months)	Mean weight (kg)
Randomisation												
DTwP	145	52	3.1	5.4	123	53	5.1	6.4	107	51	7.1	6.9
DTaP	141	51	3.1	5.4	122	53	5.1	6.4	109	51	7.2	7.0
Safety analysis												
DTwP	118	55	3.0	5.5	87	53	5.0	6.5	80	55	7.0	7.1
DTaP	123	51	3.0	5.5	96	50	5.0	6.5	87	50	7.0	7.0
Immunogenicity analysis												
Pertussis toxin												
DTwP	39	56	2.9	5.4	39	56	4.8	6.4	39	56	6.9	7.0
DTaP	57	47	3.0	5.5	57	47	4.9	6.4	57	47	6.9	7.1
Filamentous haemagglutinin												
DTwP	28	54	2.9	5.6	28	54	4.8	6.5	28	54	6.9	7.1
DTaP	40	40	3.0	5.5	40	40	4.9	6.4	40	40	6.9	7.1

hyde-detoxified pertussis toxin (PT), 25 μg of native filamentous hemagglutinin (FHA), at least 30 IU of purified diphtheria toxoid, and 40 IU of purified tetanus toxoid per 0.5 ml dose adsorbed on aluminium hydroxide (aluminium content 0.29 to 0.61 mg per 0.5 ml dose).

DT Whole-Cell Pertussis Vaccine (DTwP). Conventional pertussis vaccine from a single lot (Pasteur Mérieux) contained at least 4 IU of heat-inactivated pertussis vaccine, in accordance with WHO and European Pharmacopeia requirements, at least 30 IU of purified diphtheria toxoid, and 60 IU of purified tetanus toxoid per 0.5 ml dose mixed in the presence of aluminium hydroxide (aluminium content 0.54 to 0.65 mg per 0.5 ml dose).

Polio Vaccine. The enhanced-potency inactivated polio vaccine (IPV) was produced by Pasteur Mérieux. Each 0.5 ml dose ready-to-use syringe contained 40, 8, and 32 antigen units of polio types 1, 2, and 3, respectively.

Study Population. The study was conducted in Niakhar, Senegal, a rural farming area 150 km from Dakar. The population of approximately 27,000 has been followed up since 1983 in demographic and health studies (8, 9).

Vaccinations. Vaccines were administered in monthly sessions organized at vaccination clinics. Eligible infants were summoned to the vaccination session, and those whose parents agreed were vaccinated. The infants received three injections of vaccine at 2 to 3, 4 to 5, and 6 to 7 months of age following this schedule: BCG, DTP1, and IPV1 (2–3 months); DTP2 and IPV2 (4–5 months); DTP3, IPV3, measles, and yellow fever (6–7 months). DTaP and DTwP were randomly assigned in a double-blind manner according to a computerized list by blocks of ten. Vaccines were injected intramuscularly, DTP in the right thigh and IPV in the left thigh.

Reaction Assessment. To assess local and systemic reactions, infants were visited at home by a physician within 48 to 72 hours of vaccination. Clinical examination was performed by one of three physicians after training, and data were recorded on a standardized form. The body temperature was measured, and the injection site examined for redness, induration or tenderness. A general clinical examination was performed, and the mother was questioned about signs of a moderate reaction, (such as anorexia, vomiting, restlessness, sleepiness, irritability or crying), or signs of a severe reaction, (such as persistent or high-pitched crying, seizure or hypotonia). Induration and redness was recorded only if 3 cm or more in diameter. All deaths were reported and assessed as part of the demographic registration system by the verbal autopsy method in which field workers interviewed parents in order to complete certificates; the likely cause of death was independently assessed retrospectively by two physicians.

Serological Assays. Serum samples were obtained by fingerprick before the first and third vaccinations and one month after the third vaccination. Micro-containers with serum separators were centrifuged at the field station and the serum stored at −20°C. All subsequent assays were performed at Pasteur Mérieux in France. Antibodies to PT were measured in duplicate by the Chinese hamster ovary (CHO) cell toxin-neutralization method (10, 11). Results were expressed as the reciprocal of the endpoint dilution, the lower limit of detection being set at a 1:2 dilution. IgG antibodies to FHA were measured in triplicate in an enzyme immunoassay (EIA) (12). Results were expressed in EIA units per milliliter by comparison with an internal reference serum. Tetanus and diphtheria antibody titres were determined by radioimmunoassay and expressed as international units per milliliter (IU/ml). Polio type I and III antibody titres were determined by a seroneutralisation test. For all antigens, seroconversion was defined as a fourfold increase above the pre-vaccination level. For diphtheria and tetanus, serum-levels of ≥ 0.05 IU/ml were considered to indicate protection. For polio types I and III, neutralizing antibody titres at a dilution of ≥ 1:5 were considered to indicate protection.

Statistical Analysis. Differences in reaction rates between the groups were tested by the two-tailed chi-square and two-tailed Fisher exact tests, when indicated. The chi-square test for trend was used to test for a trend in adverse reactions in the sequence of doses. Serological data calculations were performed on logarithmically transformed data, reporting the antilogarithm. Within groups, comparisons were made by the

Table 2: Local and systemic reactions following immunization with acellular (DTaP) or whole-cell (DTwP) pertussis vaccine.

Reaction	Percent of children with stated reaction								
	Dose 1			Dose 2			Dose 3		
	DTaP (n = 123)	DTwP (n = 118)	p	DTaP (n = 96)	DTwP (n = 87)	p	DTaP (n = 87)	DTwP (n = 80)	p
Local									
Redness	1	3	ns	0	3	ns	1	4	ns
Induration	3	15	<0.01	6	24	< 0.001	13	32	< 0.001
Tenderness	5	11	ns	3	8	ns	9	21	<0.01
Systemic									
Anorexia	1	5	ns	5	6	ns	5	4	ns
Vomiting	7	11	ns	12	10	ns	12	11	ns
Restlessness	10	17	ns	7	8	ns	4	6	ns
Irritability	18	24	ns	11	18	ns	9	12	ns
Sleepiness	11	16	ns	6	11	ns	8	7	ns
Crying	22	53	< 0.001	17	31	<0.01	17	27	<0.05
Unusual cry	3	3	ns	2	1	ns	2	2	ns
Fever > 38°	0	2	ns	0	2	ns	0	6	<0.01

ns, not significant

paired t-test. Inter-group comparisons were subjected to analysis of variance or the Kruskall-Wallis test, when indicated.

Results

Sample Size. A total of 291 infants were recruited into the study. Five of these children received the wrong vaccine at least once during the three-dose series and were excluded from the further analysis. Of the 286 infants who received the first dose and were retained for analysis (Table 1), 245 received the second dose and 216 received all three doses of the study vaccines. The vaccine groups were compared for factors such as age, gender, weight, height and village of origin, with no differences being found. A similar analysis was performed for the evaluation of safety for which more than 75% of the children were seen after each dose. Comparisons also revealed no differences between the groups in the immunogenicity results, in which only a sub-sample of the children were analysed because either parents refused to allow a blood sample to be collected or an insufficient blood volume was collected.

Clinical Reactions. As shown in Table 2, the frequency of observed adverse reactions for each dose in the DTaP group was generally equal to or lower than that in the DTwP group. Differences were significant for induration and crying after each dose, and for local tenderness and fever only after the third dose. A significant positive trend was observed for local reactions in the DTaP group for induration only ($p < 0.001$), and in the DTwP group for induration ($p < 0001$), tenderness ($p < 0.01$) and fever ($p < 0.05$). No severe reactions were detected at the physicians' visits. Six deaths occurred within two months after injection, four

Table 3: Geometric mean antibody titres after acellular and whole-cell pertussis vaccine.

	CHO cell toxin neutralization[a]	Antibody to FHA[b]
Acellular vaccine	n = 57	n = 40
prevaccine	5.1	0.45
post-dose 2	15.8[c]	12.0[e]
post-dose 3	46.7	38.0[d]
Whole-cell vaccine	n = 39	n = 28
prevaccine	4.9	0.61
post-dose 2	9.9	1.95
post-dose 3	88.1[e]	22.10

[a]Expressed in reciprocal dilutions.
[b]Expressed in EIA units per milliliter.
[c]$p < 0.05$, [d]$p < 0.01$, [e]$p < 0.001$ compared with the other vaccine group for the same number of doses.

Table 4: Geometric mean antibody titres (expressed as international units per milliliter) after diphtheria, tetanus, polio type I and III vaccine related to the type of pertussis vaccine co-administered.

	Diphtheria		Tetanus		Polio Type I		Polio Type III	
	n	GMT	n	GMT	n	GMT	n	GMT
Acellular vaccine								
Prevaccine	10	0.045	12	0.028	42	6.47	15	1.6[b]
Post-dose 2	10	0.184	13	0.699[a]	56	278.6	25	500.0
Post-dose 3	7	0.303	9	1.23	57	739.6	20	1503.1[b]
Whole-cell vaccine								
Prevaccine	9	0.021	11	0.033	39	5.32	15	5.26
Post-dose 2	11	0.282	12	2.39	41	205.6	29	283.1
Post-dose 3	7	0.296	6	2.48[a]	51	657.6	21	912.0

[a] $p < 0.01$
[b] $p < 0.05$

in the DTwP group and two in the DTaP group. According to the verbal autopsy the causes of death were as follows (with vaccine and dose number): unknown (10 days after DTwP1), malaria and pneumonia (14 days after DTwP2), diarrhea (41 days after DTwP3), diarrhea associated with protein energy malnutrition (57 days after DTwP3), meningitis (58 days after DTaP1), and diarrhea (10 days after DTaP2).

Immune Response. Pre-vaccine titres for FHA and PT were similar in the two groups. The DTaP vaccine was associated with significantly higher antibody titres to FHA after dose 2 and dose 3, and to PT after dose 2. In contrast, the mean PT-neutralizing antibody titre after dose 3 was significantly higher in the DTwP group (Table 3). With both vaccines, there was a significant increase in both FHA and PT antibody titres after dose 3 compared with dose 2. Seroconversion rates were almost identical in the two vaccine groups: 95% and 93% for PT in the DTaP and DTwP groups, respectively, and 95% and 93% for FHA.

Antibody titres for diphtheria, tetanus and polio types I and III are shown in Table 4. Diphtheria antibody titres were similar in the two vaccine groups. Protective levels were obtained in all subjects in both groups. Although the tetanus antibody titres were comparable before vaccination, rises in titres were significantly higher in the DTwP group; however, seroprotection rates against tetanus were 100% in both groups. Antibody titres for polio virus types I and III were similar in the two vaccine groups but titres for polio virus type III were significantly lower before vaccination in DTaP recipients than in DTwP recipients and higher after dose 3. Nevertheless, seroprotection rates were 100% in both groups after two doses. The seroconversion rates in infants who had pre-vaccination titres below the seroprotective level were 100% in both groups after two doses.

Discussion

The present study was performed in a different setting from two other immunogenicity and safety studies which used slightly different immunization regimens to compare the same acellular pertussis vaccine to other whole-cell pertussis vaccines (13, B. Auerbach et al.: Congress of the Society for Pediatric Research, Washington D.C., 1989, abstract). The results confirm the decreased reactogenicity of the acellular vaccine compared to the whole-cell vaccine. In the present study, an increased rate of crying and local induration was observed significantly more frequently after each dose in recipients of DTwP than of DTaP vaccine.

Six deaths occurred within two months of vaccination, four in the DTwP group and two in the DTaP group. As in most parts of sub-saharan Africa, there is very high infant mortality in the study area, and the number and causes of death are thus not unexpected. The verbal autopsy method of assessing the cause of death, developed for large population studies, lacks precision in determining the cause of individual deaths. No serious events associated with DTwP vaccination were observed. Studies with this new generation of vaccines in a larger population are needed to monitor for adverse effects that are less frequent or more severe.

All children had protective levels of antibody against diphtheria, tetanus and polio virus. Diphtheria and polio (types I and III) antibody titres

in the DTaP group were similar to or higher than those observed in the DTwP group, suggesting that the acellular pertussis vaccine does not interfere with the other antigens. Although the tetanus antibody titres were lower in the DTaP group than in the DTwP group, the response of every child was well above the protective level. An adjuvant effect of whole-cell pertussis vaccine on the tetanus and diphtheria response has been suggested but could be lacking with the acellular vaccine (14, 15).

Both vaccines produced a vigorous antibody response to PT and FHA. After the third dose, the acellular vaccine induced higher FHA antibody titres than the whole-cell vaccine, whereas the latter induced higher PT antibody titres. The same batch of acellular pertussis vaccine (lot S2112) was used in a large multicenter study comparing 13 acellular pertussis vaccines and two US whole-cell vaccines, allowing comparison of results between the two studies (16–18). The assays used in the two studies were slightly different and the values obtained are therefore not directly comparable. Nonetheless, the PT-neutralization test results were very similar: prevaccination and post-third dose geometric mean titres were 5.1 and 46.7, respectively, in this study, compared with 3.8 and 43 in the NIAID study (7). Our data do not suggest that there is a better response in black children per se, as suggested in the NIAID study (7).

Comparison of the FHA antibody titres is not possible since our assay was developed in 1989, prior to standardization. In the NIAID study, the present acellular pertussis vaccine induced PT and FHA antibody titres comparable with those induced by the other acellular pertussis vaccines tested, and for both antigens the titres were equal to or higher than those obtained with either of the US licensed whole-cell pertussis vaccines tested. The high PT antibody titres obtained in this study with the whole-cell pertussis vaccine confirm that it is appropriate as control vaccine in clinical trials in a developing country where it is routinely used.

Acknowledgments

We are grateful to the team of field workers in Niakhar, as well as to the nurses at the local dispensaries who participated actively in the collection of data. We also thank Dr. Marie Pierre Preziosi and Dr. Agnès Hoffenbach for helpful comments and Dr. Mark Fletcher for invaluable editorial assistance. Financial support was provided by Pasteur Mérieux Sérums et Vaccins (Lyon, France), and by the Unité de Recherche sur les Maladies Infectieuses et Parasitaires (ORSTOM, Dakar, Senegal).

References

1. Trollfors B, Taranger J, Lagergard T, Lind L, Sundh V, Zackrisson G, Lowe CU, Blackwelder W, Robbins JB: A placebo controlled trial of a pertussis toxoid vaccine. New England Journal of Medicine 1995, 333: 1045–1050.
2. Greco D, Salmoso S, Mastrantonio P, Giuliano M, Tozzi A, Anemona A, Ciofi Degli Atti M, Giammanco A, Panei P, Blackwelder W, Klein D, Wassilak S, and the Progetto Pertosse Working Group: A controlled trial of two acellular vaccines and one whole-cell vaccine against pertussis. New England Journal of Medicine 1996, 334: 341–348.
3. Gustafsson L, Hallander HO, Olin P, Reizenstein E, Storsaeter J: A controlled trial of a two-component acellular, a five-component acellular, and a whole-cell pertussis vaccine. New England Journal of Medicine 1996, 334: 349–355.
4. Schmitt HJ, von Konig CHW, Neiss A, Bogaerts H, Bock HL, Schulte-Wissermann H, Gahr M, Schult R, Folkens JU, Rauh W, Clemens R: Efficacy of acellular pertussis vaccine in early childhood after household exposure. Journal of the American Medical Association 1996, 275: 37–41.
5. Wright PF: Pertussis in developing countries: definition of the problem and prospects for control. Reviews of Infectious Diseases 1991, 13, Supplement 6: 528–534.
6. Mulholland K: Measles and pertussis in developing countries with good vaccine coverage. Lancet 1995, 345: 305–307.
7. Christy C, Pichichero ME, Reed GF, Decker MD, Anderson EL, Rennels MB, Englund JA, Edwards KM, Steinhoff MC: Effect of gender, race, and parental education on immunogenicity and reported reactogenicity of acellular and whole-cell pertussis vaccines. Pediatrics 1995, 96, Supplement 3: 584–587.
8. Aaby P, Samb B, Simondon F, Whittle H, Coll Seck AM, Knudsen K, Bennett J, Markowitz L, Rhodes P: Child mortality after high-titre measles vaccines in Senegal: the complete data set. Lancet 1991, 338: 1518.
9. Aaby P, Samb B, Simondon F, Knudsen K, Coll Seck AM, Bennett J, Whittle H: Divergent mortality for male and female recipients of low-titre and high-titre measles vaccines in rural Senegal. American Journal of Epidemiology 1993, 138: 746–755.
10. Hewlett EL, Sauer KT, Myers GA, Cowell JL, Guerrant RL: Induction of a novel morphological response in Chinese hamster ovary cells by pertussis toxin. Infection and Immunology 1983, 40: 1198–1203.
11. Granstrom M, Granstrom G, Gillenius P, Askelof P: Neutralization antibodies to pertussis toxin in whooping cough. Journal of Infectious Diseases 1985, 151: 646–649.

12. Manclark CR, Cowell JL: Pertussis. In: Germanier R (ed): Bacterial vaccines. Academic Press, New York, 1984, p. 69–106.
13. Edwards KM, Bradley RB, Decker MD, Palmer PS, Van Savage J, Taylor JC, Dupont W, Hager CC, Whright PJ: Evaluation of a new highly purified vaccine in infants and children. Journal of Infectious Diseases 1989, 160: 832–837.
14. Fleming DS, Greenberg L, Beith EM: The use of combined antigens in the immunization of infants. Canadian Medical Association Journal 1948, 59: 101–105.
15. Tarzaali A, Viens P, Quevillon M: Inhibition of the immune response to whooping cough and tetanus vaccines by malaria infection, and the effect of pertussis adjuvant. American Journal of Tropical Medicine and Hygiene 1977, 26: 520–524.
16. Edwards KM, Meade BD, Decker MD, Reed GF, Rennels MB, Steinhoff MC, Anderson EL, Englund JA, Pichichero ME, Deloria MA: Comparison of 13 acellular pertussis vaccines: overview and serologic responses. Pediatrics 1995, 96, Supplement 3: 548–557.
17. Steinhoff MC, Reed GF, Decker MD, Edwards KM, Englund JA, Pichichero ME, Rennels MB, Anderson EL, Deloria MA, Meade BD: A randomized comparison of reactogenicity and immunogenicity of two whole-cell pertussis vaccines. Pediatrics 1995, 96, Supplement 3: 567–570.
18. Edwards KM, Decker MD, Halsey NA, Koblin BA, Townsend T, Auerbach B, Karzon DT: Differences in antibody response to whole-cell pertussis vaccines. Pediatrics 1991, 88: 1019–1023.

European Journal of
Clinical Microbiology
& Infectious Diseases

Reprint

An International Journal on Pathogenesis, Diagnosis, Epidemiology,
Therapy and Prevention of Infectious Diseases