JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1993, p. 2439–2445
0095-1137/93/092439-07$02.00/0

Vol. 31, No. 9

Reactogenicity and Immunogenicity of a High-Titer Rhesus Rotavirus-Based Quadrivalent Rotavirus Vaccine

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Received 7 April 1993/Returned for modification 14 May 1993/Accepted 14 June 1993

We evaluated the reactogenicity and antigenicity of a quadrivalent rotavirus vaccine composed of serotype 3 rhesus rotavirus (RRV) and three single-gene-substitution reassortants of RRV and human strain D (D × RRV, serotype 1), DS1 (DS1 × RRV, serotype 2), or ST3 (ST3 × RRV, serotype 4) in a double-masked study with 302 infants in Caracas, Venezuela. Three doses of the quadrivalent vaccine composed of either 10^5 PFU (low titer) or 10^6 PFU (high titer) of each component were administered to 99 and 101 infants, respectively, at 4-week intervals starting at the second month of age; 102 infants received a placebo. Postvaccination reactions were monitored by home visits every other day during the week postvaccination. The vaccine was associated with the occurrence of mild, short-lived febrile episodes in 26 and 23% of the recipients after the first doses of high- or low-titer vaccine, respectively, in comparison with 13% of the infants receiving the placebo. Febrile reactions occurred less frequently in vaccinees after the second or third dose than after the initial dose. The vaccine was not significantly associated with diarrhea or any additional symptom or sign. Serum specimens obtained shortly before the first, 4 weeks after the first, and 4 weeks after the third dose of vaccine or placebo were tested by an immunoglobulin A enzyme-linked immunosorbent assay and by neutralization assays. Seroresponses occurred significantly more often after 3 doses than after a single dose of either vaccine. Immunoglobulin A responses were observed in 80 and 79% of the infants after 3 doses of high- or low-titer vaccine, respectively. Most of the infants tested developed a neutralization response to RRV after 3 doses of the high- (90%) or low-(88%) titer vaccine. Neutralization response rates to human rotavirus serotypes 1 to 4 after 3 doses were similar in both vaccine groups and ranged from 33 to 53%. Overall, 93 of 97 infants receiving the low-titer vaccine and 87 of 90 receiving the high-titer vaccine developed seroresponses, as detected by any of the assays employed. The study indicates that 3 doses of quadrivalent vaccine at a titer of 10^6 PFU of each component offered no advantage over the lower-titer preparation for use in efficacy trials.

Various approaches for development of a rotavirus vaccine have been suggested (2, 6). The most extensively evaluated strategy is based on the Jennerian approach of employing an animal rotavirus strain that was attenuated for humans and was capable of inducing immunity. Two bovine rotaviruses, the RIT 4237 and WC3 strains, have been shown to be attenuated in humans (4, 22). Several trials of these strains showed evidence of efficacy in some studies but not in others (1, 4, 5, 22). One of the factors responsible for this inconsistent efficacy is the serotypic difference between RIT 4237 or WC3 (which are serotype 6) and circulating human rotaviruses (the majority of which are serotypes 1 to 4). Another vaccine candidate, the serotype 3 rhesus rotavirus (RRV) vaccine MMU-18006, induced protection against rotavirus diarrhea in a study in Venezuela in which rotavirus VP7 serotype 3 strains predominated (9), but it failed to elicit heterologous efficacy against other serotypes in two of three studies in the United States (3, 15, 20). Because of the unpredictable distribution of rotavirus serotypes, an effective rotavirus vaccine should provide protection against diarrhea caused by each of the four epidemiologically important serotypes. Thus, four strains composed of the RRV and single-gene-substitution reassortants with VP7 1, 2, or 4 specificity were combined in a single quadrivalent vaccine (16, 17). Before embarking on efficacy trials of the quadrivalent vaccine candidate, we performed several phase I trials to test its reactogenicity and antigenicity (8, 18, 19). These studies have shown that in general, increasing the vaccine titer or the number of doses led to a higher take rate. The present study was undertaken to maximize the immunogenicity of the vaccine by administering 3 doses of the highest titer available at this time (4×10^6, or 1×10^6 PFU of each component) and comparing results with those obtained with the highest titer of quadrivalent vaccine previously tested (4×10^5 PFU).

MATERIALS AND METHODS

Quadrivalent vaccine. The quadrivalent rotavirus vaccine, developed at the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md., is composed of equal parts of serotype 3 RRV, a serotype 1 reassortant between RRV and human strain D (D × RRV), a serotype 2 reassortant between RRV and human strain DS1 (DS1 × RRV), and a serotype 4 reassortant between RRV and human strain ST3 (ST3 × RRV). Vaccines and placebo used in this study were prepared by Wyeth Ayerst Laboratories (Philadelphia, Pa.). The vaccine consisted of either 10^5 or 10^6 PFU of each of the four components grown in DBS-FRhL-2 cells; placebo consisted of uninfected tissue culture fluids. Lyophilized vac-

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TABLE 1. Vaccination schedule

Visit no.[a]	Age of infant (wk) at time of visit[b]	Serum specimen[c]	Vaccine group[d]		
			A (placebo)	B (quad [4 × 10^5])	C (QUAD [4 × 10^6])
1	0–4		OPV + BCG	OPV + BCG	OPV + BCG
2	8–10	S-1	Placebo	quad	QUAD
3	10–12		OPV + DPT	OPV + DPT	OPV + DPT
4	12–14	S-2	Placebo	quad	QUAD
5	14–16		OPV + DPT	OPV + DPT	OPV + DPT
6	16–18		Placebo	quad	QUAD
7	18–20		OPV + DPT	OPV + DPT	OPV + DPT
8	20–22	S-3			

[a] Visits to vaccination clinics.
[b] Visits were scheduled at 4-week intervals. A maximum interval of 4 weeks between visits 2 to 4 and 4 to 6 was allowed to accommodate for unpredicted delays.
[c] S-1, S-2, and S-3, serum specimens obtained before the first, 4 weeks after the first, and 4 weeks after the third vaccine doses, respectively.
[d] quad, 10^5 PFU of each component in quadrivalent vaccine (D × RRV, DS1 × RRV, RRV, and ST3 × RRV); QUAD, 10^6 PFU of each component in quadrivalent vaccine (D × RRV, DS1 × RRV, RRV, and ST3 × RRV).

cines and placebo were resuspended in 1 ml of sterile water just before administration. Vaccine titers were confirmed at the National Institutes of Health before initiation of the study.

Study site and subjects. The study was based at the Hospital Materno Infantil de Caricuao, an obstetric-pediatric hospital administered by the Venezuelan Ministry of Health that serves the region of Caricuao and adjacent neighborhoods in the south of Caracas, Venezuela. More than 400 infants are born monthly in this hospital. Recruitment took place by contacting mothers at about the time of delivery or when the infants were brought to the hospital to begin routine immunization with BCG and oral poliovirus vaccine (OPV) (within 4 weeks of birth). A written consent form approved by the Ethical Committee at the Instituto de Biomedicina in Caracas and the Clinical Research Subpanel at the National Institute of Allergy and Infectious Diseases was signed by all the participating mothers.

Only full-term, healthy infants weighing more than 2,500 g at birth were included in the study. The children were assigned to three groups according to a double-blind randomized code to receive 3 doses of one of the following: (i) placebo, (ii) quadrivalent vaccine at a titer of 10^5 PFU of each component, and (iii) quadrivalent vaccine at a titer of 10^6 PFU of each component. Ninety-nine infants received the high-titer vaccine, 101 received the low-titer vaccine, and 102 received placebo.

Vaccine administration. In order to neutralize stomach acid to prevent possible inactivation of the acid-labile vaccine strain, 30 ml of formula (Similac; Ross Laboratories) containing 400 mg of bicarbonate was administered before each vaccine dose. To avoid potential neutralization of vaccine virus by breast milk antibody, the mothers were asked not to feed their babies for at least 1 h before and 1 h after each vaccination.

Table 1 presents the vaccination schedule. At the initial visit, which took place during the first 4 weeks of life, all of the infants received OPV and BCG according to the Venezuelan Ministry of Health immunization schedule; these were administered by hospital staff. The first dose of quadrivalent vaccine or placebo was given at 8 to 10 weeks of age, the second dose was given 4 weeks later, and the third dose was given 4 weeks after the second. A maximum delay of 2 weeks was allowed for the second and third doses when the scheduled appointment was missed or when symptoms or signs of illness were present in the child. Routine OPV and diphtheria-pertussis-tetanus (DPT) immunizations were administered at 4-week intervals, 2 weeks following each dose of quadrivalent vaccine or placebo.

Monitoring postvaccination reactions. A physical examination was carried out at the time of each vaccine or placebo administration; "vaccination" was delayed when diarrhea, fever, or any symptoms or signs of illness other than upper respiratory complaints were present. Each child was visited at home by a physician every other day during the week following each of the three study vaccinations. The mothers recorded information related to the children's health every day and also maintained a daily record of the number and consistency of their children's stools during the week following each "vaccination." Rectal temperatures were also taken every morning and evening by the mothers during this period. The mothers were instructed to call or come to Hospital Materno Infantil de Caricuao in the event of any illness during the week after vaccination and at any time that their children developed diarrhea during the study.

Cumulative postvaccination follow-up data were periodically evaluated by a Clinical Monitoring Committee formed by two physicians and one statistician not participating directly in the study. The committee was also promptly informed of any unusual reactions or any hospitalizations during the week postvaccination. The committee had the authority to disclose the vaccination code for any child with severe symptoms and/or to stop the study if warranted.

Serology. Three serum samples (2 to 3 ml) were obtained by venous puncture of an antecubital vein at (i) the time of the first vaccination, (ii) 4 weeks after the first vaccine dose (i.e., at the time the children were brought for the second study vaccine dose), and (iii) 4 weeks after the third dose (Table 1). The sera were analyzed by an immunoglobulin A (IgA) enzyme-linked immunosorbent assay (ELISA) as described by Losonsky et al. (14) and by plaque reduction neutralization assays (13). RRV strain MMU 18006 (G3) was used as the antigen in the IgA ELISA. RRV and the human rotavirus strains Wa, DS1, P and ST3, belonging to serotypes G1, G2, G3, and G4, respectively, were employed in plaque reduction neutralization assays (24). In every serological assay carried out, the three serum specimens obtained from the same child were tested simultaneously.

Rotavirus detection. Stools collected from diarrheal episodes occurring during the study were tested for rotavirus by confirmatory ELISA as previously described (13); rotavirus-positive specimens were subgrouped and serotyped by conventional methods (21, 23). In addition to being tested for rotavirus detection, the stool specimens were tested by

TABLE 2. Clinical reactions observed during the week postvaccination in infants receiving quadrivalent rotavirus vaccine or placebo

Clinical manifestation(s)	No. (%) of infants								
	After the first dose of:			After the second dose of[a]:			After the third dose of[a]:		
	4×10^5 PFU	4×10^6 PFU	Placebo	4×10^5 PFU	4×10^6 PFU	Placebo	4×10^5 PFU	4×10^6 PFU	Placebo
Diarrhea only	6 (6)	8 (8)	8 (8)	2 (2)	1 (1)	6 (6)	3 (3)	5 (5)	1 (1)
Diarrhea and fever (≥38.1°C)	3 (3)	1 (1)	2 (2)	2 (2)	1 (1)	4 (4)	0	1 (1)	3 (3)
Any diarrhea	9 (9)	9 (9)	10 (10)	4 (4)	2[b] (2)	10 (11)	3 (3)	6 (7)	4 (4)
Fever only (≥38.1°C)	20[c] (20)	25[d] (25)	11 (11)	9 (9)	5 (5)	10 (11)	11 (11)	6 (7)	12 (13)
Any fever (≥38.1°C)	23[e] (23)	26[f] (26)	13 (13)	11 (12)	6 (7)	14 (15)	11 (11)	7 (8)	15 (16)
Any fever (>38.5°C)	6 (6)	9 (9)	4 (4)	2 (2)	3 (3)	5 (5)	5 (5)	5 (5)	10 (11)
Total no. of infants under surveillance	101	99	102	97	92	95	97	91	94

[a] The smaller number of infants tested after the second and third doses reflects infants who dropped out of the study.
[b] $P = 0.03$ compared with placebo group (the two-tailed Fisher exact test).
[c] $P = 0.08$ compared with placebo group (the two-tailed Fisher exact test).
[d] $P = 0.009$ compared with placebo group (the two-tailed Fisher exact test).
[e] $P = 0.06$ compared with placebo group (the two-tailed Fisher exact test).
[f] $P = 0.019$ compared with placebo group (the two-tailed Fisher exact test).

conventional bacteriological assays. Fresh microscopic examination of stools was carried out to detect parasites.

RESULTS

Vaccine reactogenicity. Table 2 summarizes the clinical findings observed during the week following the first, second, and third doses of vaccine or placebo. The frequencies of fever were similar in the two vaccine groups (high and low titer). Fever (38.1°C or higher) occurred significantly more often in infants receiving the first dose of the higher-titer vaccine preparation tested than in the placebo recipients and also tended to occur more often in those receiving the lower-titer vaccine than in those in the placebo group ($P = 0.08$, the Fisher exact test, two tails). Among high-titer-vaccine recipients, the frequency of fever after the first dose was significantly greater than that observed after the second or third dose ($P < 0.05$ for first dose versus second or third dose). In the low-titer-vaccine recipients, the frequency of fever tended to be lower after the second dose and was significantly lower after the third dose ($P < 0.05$, the Fisher exact test, two tails) than that after the first dose. Following the second and third doses, the frequency of fever in vaccinees was similar to that in placebo recipients.

Most of the febrile episodes observed were transient, lasting 1 day in 21 of the 26 high-first-dose vaccine recipients who developed fever, 17 of the 23 low-first-dose vaccine recipients, and 11 of the 13 first-dose placebo recipients with fever. Fever lasted 2 days in four infants receiving high-titer vaccine, five receiving low-titer vaccine, and two placebo recipients. The number of infants with febrile episodes with temperatures of more than 38.5°C was similar in the three study groups following each of the 3 doses (Table 2). Likewise, the average maximum temperatures observed were similar in the three groups after the first (38.5°C), second (38.6 to 38.8°C), or third (38.7 to 39°C) dose.

Diarrhea was defined as three or more liquid or semiliquid stools in 24 h. The frequencies of diarrhea after the first or third dose were comparable among the three groups (high titer, low titer, and placebo). However, diarrhea occurred significantly more often in the placebo group after the second dose (10 episodes) than in the group of infants receiving the higher-dose vaccine ($P = 0.03$, the Fisher exact test, two tails) and tended to be more frequent also in the placebo group than in infants receiving the low-titer vaccine (four infants) after the second dose.

Nine of the 302 infants studied required hospitalization during the approximately 1-week period following any of the vaccinations (Table 3). The three most severe cases, requiring hospitalization for 6 to 10 days, were associated with gastroenteritis and an admission diagnosis of probable meningitis which was ruled out later (child 606), pneumonia (child 185), and campylobacter gastroenteritis (child 536). Of the eight children admitted with diarrhea, two had received placebo, one had received low-titer vaccine, and five had received high-titer vaccine; rotavirus was detected by direct testing of the stool by ELISA for three of them (child 113, who received placebo, and children 451 and 436, who received high-titer vaccine). In two of the three cases, the rotavirus strains detected were subgroup 2, serotype G1, which circulated in the Caricuao area during the time of this study; the third rotavirus could not be subgrouped or typed. Nine diarrheal specimens from child 606 tested negative for rotavirus by direct ELISA of the stools; however, subgroup 1 rotavirus with an electropherotype similar to that of RRV was recovered after passage in tissue culture from two of the nine specimens. A stool specimen was not available from child 12, who was in the hospital for 6 h.

Clinical reports from this study were submitted at various times to the Clinical Monitoring Committee. At three meetings held by this committee in which partial follow-up data were examined, reasons to discontinue the study or reveal the vaccine code either for a particular child or for the entire group were not found.

Serological studies. The results of the serological studies are summarized in Table 4. IgA responses after the first vaccine dose were detected in 51% of the infants receiving low-titer and 50% of those receiving high-titer vaccine. Administration of the second and third doses resulted in an overall IgA seroresponse rate of 80% after low-titer or 79% after high-titer vaccine. Take rates after 3 doses of either vaccine were significantly greater than those after 1 dose.

Neutralization seroresponses to RRV were observed in 57 and 67% of the children after the first low- and first high-titer vaccine doses, respectively. After 3 doses, 88 and 90% of the infants in the low- and high-titer groups, respectively, had developed a neutralization response to RRV. These rates

TABLE 3. Summary of findings in children requiring hospital admission during the approximately 1-week period following administration of quadrivalent rotavirus vaccine or placebo

Child no.	Group[a] (dose[s])	Day admitted	Day(s) in hospital	Diagnosis and/or observation(s)[b]
113	Plac (3)	5	3	Gastroenteritis, RV+, SG 2, ST 1
185	Plac (3)	3	6	Pneumonia
536	Plac (1)	4	10	Gastroenteritis-dehydration, RV−, CA+
584	Low (1)	4	1	Gastroenteritis-dehydration, RV−, CR+
451	High (3)	5	3	Gastroenteritis, RV+, SG ?, ST ?
12	High (3)	6	<1	Gastroenteritis, not tested for RV
374	High (3)	8	3	Gastroenteritis-dehydration, RV−
436	High (1)	8	3	Gastroenteritis-dehydration, RV+, SG 2, ST 1
606	High (1)	2	7	Meningitis ?, gastroenteritis-dehydration, RV+, SG1[c]

[a] High, 4×10^6 PFU of quadrivalent rotavirus vaccine; low, 4×10^5 PFU of quadrivalent rotavirus vaccine; plac, placebo.
[b] RV, rotavirus; SG, rotavirus subgroup; ST, rotavirus serotype; CA, *Campylobacter* sp.; CR, *Cryptosporidium* sp.; +, positive; −, negative; ?, unknown.
[c] Initial clinical impression of meningitis at admission was later changed. Laboratory analysis of cerebrospinal fluid was normal. Rotavirus was detected in two of nine diarrheal stools from this infant tested only after amplification in tissue culture (MA 104 cell roller tubes).

were significantly greater than those detected after 1 dose for both vaccine groups (Table 4).

Neutralization responses to human rotavirus serotypes 1 to 4 were detected in only 7 to 20 and 3 to 19% of the infants after 1 dose of low- or high-titer vaccine, respectively; however, a significant increase in the response to each of the four serotypes was seen in both groups after 3 doses of vaccine (Table 4). A significant difference between the higher- and lower-titer-vaccine recipients was not observed in the responses to any of the four serotypes.

Seven (8%) and 23 (26%) of 90 placebo recipients developed an IgA response after 1 and 3 doses, respectively; shedding of rotavirus had been detected in 12 of these infants, and all of eight strains tested from these 12 infants were serotype G1, subgroup 2 (the only serotype circulating during the time of the study). Serum specimens randomly selected from among the placebo recipients who developed an IgA response were tested by neutralization assay; seroresponses to Wa and P were detected in 80 and 67% of these infants, respectively.

Occurrence of diarrhea during the study. Follow-up to detect diarrheal episodes during the study period was passive except for the week following each vaccine or placebo administration. A total of 148 diarrheal episodes were recorded during the study period (8 months in total); 58 of them occurred in the placebo group, 50 occurred among recipients of the low-titer vaccine, and 40 occurred among high-titer-vaccine recipients. The differences among the three groups are not statistically significant. Rotavirus was detected in 12 of the 58 diarrheal episodes in the placebo group, in 4 of the 50 diarrheal episodes in the low-titer-vaccine group, and in 5 of 40 diarrheal episodes in the high-titer-vaccine group (Table 5).

DISCUSSION

A panel of experts convened by the Institute of Medicine estimated that in developing countries, rotaviruses are responsible for more than 125 million cases of diarrhea, 7% of which are severe and cause more than 870,000 deaths per year (11). Therefore, there is a clear and urgent need to develop a rotavirus vaccine to prevent such morbidity and mortality. The Jennerian vaccination strategy was suggested when it was found that certain antigens are shared by human and animal rotavirus strains (12). One of these, the RRV strain MMU 18006, a serotype 3 rotavirus, failed to induce

TABLE 4. Serological responses to two different titers of quadrivalent vaccine[a]

Vaccine or placebo and no. of doses	No. of infants with IgA ELISA responses/no. tested (%)	No. of infants with neutralization responses to indicated virus (serotype)/no. of infants tested (%)[b]				
		Wa (1)	DS1 (2)	P (3)	ST3 (4)	RRV (3)
Placebo						
1	7/90 (8)	2/15 (13)	0/11 (0)	1/11 (9)	0/12 (12)	2/30 (7)
3	23/90 (26)	12/15 (80)	0/11 (0)	8/12 (67)	4/14 (29)	2/31 (6)
4×10^5 PFU						
1	49/97 (51)	6/30 (20)	2/30 (7)	2/28 (7)	5/30 (17)	53/93 (57)
3	78/97 (80)	15/29 (52)	10/30 (33)	11/29 (38)	16/30 (53)	85/97 (88)
P value[c]	<0.001	0.014	0.019	0.009	0.003	<0.001
4×10^6 PFU						
1	45/90 (50)	6/31 (19)	1/29 (3)	3/27 (11)	2/32 (6)	59/88 (67)
3	73/92 (79)	14/30 (47)	14/29 (48)	10/27 (37)	15/32 (47)	81/90 (90)
P value[c]	<0.001	0.03	<0.001	0.05	<0.001	<0.001

[a] Titers were 4×10^5 and 4×10^6 PFU.
[b] Sera from placebo recipients tested by neutralization assays were selected from infants who developed an IgA seroresponse. Sera from vaccinees tested by neutralization against human rotavirus serotypes 1 to 4 were randomly selected.
[c] Two-tailed Fisher exact test comparison of seroresponses to 1 versus 3 doses.

TABLE 5. Occurrence of rotavirus diarrhea following administration of any dose of quadrivalent vaccine or placebo[a]

Vaccine or placebo[b]	No. of children	No. of rotavirus episodes	Efficacy (%)	P value[c]
Placebo	92	12[d]		
Low dose	96	4	68	0.04
High dose	92	5[d]	58	0.08

[a] Children were monitored for an average of 4 months after the third dose of vaccine.
[b] Low dose, 4×10^5 PFU; high dose, 4×10^6 PFU.
[c] By the Fisher exact test, 2 tails.
[d] One of the 12 episodes in the placebo group and 2 of the 5 episodes in the high-dose group occurred during the week following the first dose.

heterotypic antibodies against human rotaviruses belonging to other serotypes (14). Thus, current vaccine strategies are aimed at developing vaccine candidates that consistently induce antibodies to each of the four epidemiologically important serotypes (1). The ability of rotaviruses to reassort during coinfection has made it possible to construct human X RRV reassortants with the VP7 neutralization specificity of human rotavirus serotype 1, 2, or 4 (20, 21).

We have conducted several studies of the RRV-based quadrivalent vaccine to evaluate its antigenicity and reactogenicity in Venezuelan infants before initiating field trials of efficacy. With these studies, we aimed at a vaccine formulation able to induce an arbitrary take rate of at least 50% against each of the four serotypes. In the first study, we tested vaccine doses of 0.25, 0.5, and 10^4 PFU of each of the four vaccine components (19). Although these doses were safe, their antigenicity was low, especially for serotypes 2 and 4, and therefore in a later trial the dose of these two serotypes was increased to 5×10^4 PFU, a regimen which also failed to achieve the desired results (8). In a subsequent study, 2 doses of quadrivalent vaccine were administered 1 month apart, a schedule which did not achieve our goal of 50% takes but indicated clearly that 2 doses of vaccine was more effective than 1 dose in inducing seroresponses (18). In further studies, a 2-dose schedule of 10^5 PFU of each component yielded seroresponses that achieved the goal of 50% for serotypes 1 and 3 and approached this goal for serotypes 2 and 4 (18).

In view of the increased antigenicity of the higher titer of vaccine and the additive effect of 2 doses, we designed the present study to test the maximal dose of vaccine available at this time, 10^6 PFU of each component, and compared it with 10^5 PFU, the highest dose in each component of a quadrivalent vaccine previously tested. In addition, the number of doses was increased to 3. Three doses of vaccine resulted in a significantly greater number of takes than 1 dose, as determined by IgA ELISA and neutralization assays. A recruitment effect was seen with both the high- and the low-titer vaccines. For instance, after 1 dose of low-titer vaccine, an IgA response was detected in 48 of 97 infants; 37 of the 49 infants (76%) who did not respond to the first dose developed an IgA seroresponse after the second or third dose (i.e., between sera obtained 4 weeks after the first and 4 weeks after the third doses). In 12 infants receiving the low-titer vaccine and 18 infants receiving the high-titer vaccine, IgA responses were detected after the first dose and again after the second and third doses (a booster effect).

Similar observations were made by analyzing neutralization responses to RRV. The second and third doses resulted in a recruitment effect: 27 of 40 low-titer recipients and 18 of 39 high-titer recipients who did not respond to the initial dose of vaccine developed a response after the second and third doses. Booster effects were detected in 14 and 22 of the infants receiving low- and high-titer vaccine, respectively.

Responses to human rotavirus serotypes 1 to 4 were detected in 33 to 53% of infants in the low-titer group and in 37 to 48% of high-titer recipients. It was noteworthy that 72 and 70% of the children in the low- and high-titer-vaccine groups, respectively, developed a response against at least one human rotavirus serotype and that 31% of low-titer and 30% of high-titer recipients developed responses to three or four serotypes after 3 vaccine doses.

The neutralization response rates to the human rotaviruses (33 to 53%) contrast with the high rates against RRV observed (88% of the low- and 90% of the high-titer recipients developed a response to RRV after 3 doses). This observation, noted also in previous studies (7), probably reflects the development of antibody to the outer capsid protein VP4, which is common to all four components of the quadrivalent vaccine and different from the VP4 of human rotaviruses.

The serological responses to 4×10^6 PFU of vaccine were not greater than those to 4×10^5 PFU after either 1 or 3 doses by any of the serological tests employed. Although our arbitrary goal of inducing a seroresponse to each serotype in at least 50% of the children tested was not achieved in the present study, the take rates observed did approach this figure. It was noteworthy that only 4 of 97 low-titer and 3 of 90 high-titer recipients tested failed to exhibit a demonstrable seroresponse by at least one of the tests employed.

A relatively high background of rotavirus infection occurred in the 3-month interval between the first and third serum samplings: 23 of 90 placebo recipients tested developed an IgA response. Many of these infections were silent, and therefore it is possible that some of the seroresponses observed in vaccinees were also due to natural infections.

The safety of the quadrivalent vaccine in this and in previous trials has been closely evaluated by home follow-up visits during the week after vaccine or placebo administration. In general, the quadrivalent vaccine has been associated with self-limited, low-grade febrile episodes in up to about one-third of children and is considered to be acceptably reactogenic but safe for oral administration to infants less than 6 months of age. In the present study, a febrile reaction occurred significantly more often after the first dose of the high-titer vaccine (26% of children in that group developed fever) than in the placebo group (13% of these infants developed fever). The lower-titer vaccine tended to cause excess febrile reactions (23%) compared with the placebo. No febrile reactions were associated with the second or third dose of vaccine. Diarrheal episodes were observed during the week postvaccination at the same frequency in the three groups of children after the first dose; most of them were mild, lasted 1 to 2 days, and consisted of three to four liquid or semiliquid stools per day.

Diarrhea requiring hospitalization occurred in eight infants during any of the approximately 1-week postvaccination periods. It is unlikely that the quadrivalent vaccine was the cause of diarrhea because (i) two of the cases occurred in the placebo group, (ii) two of the six cases among vaccines were not associated with rotavirus, and (iii) one case in a vaccinee was associated with a subgroup 2, serotype 1 wild-type strain (the four vaccine components are subgroup 1). For one patient (child 606), vaccine virus was detected by stool culture but not by direct ELISA in two of nine stool specimens tested (wild-type rotaviruses are easily detected

in the diarrheal stools of infected infants with diarrhea); however, the possibility that the vaccine was the cause of diarrhea in this infant cannot be completely excluded. Data to explain the origin of the diarrhea in the two other cases which occurred with the higher vaccine titer tested are not available. The significance of diarrhea in these cases is uncertain; proper caution toward such cases should be continuously exerted in future trials.

In a previous study in Sweden, 10^5-PFU dose of RRV vaccine resulted in fever in 79% and diarrhea in 42% of the children tested (10). This has not been observed in any of the studies carried out with this vaccine in Venezuela. A major difference that can explain this discrepant observation is the age at the time of vaccination. The infants studied in Venezuela were 8 to 10 weeks old, whereas those in Sweden were 4 to 12 months old. It is likely that maternal antibody (acquired transplacentally or from breast milk) plays a role in modulating reactions to the vaccine. Most infants in this study possessed high preexisting titers of neutralizing antibody against human rotavirus serotypes 1, 3, and 4 and against RRV.

Although this trial was not aimed at determining vaccine efficacy, the continuous contact of the field team with the participating families over the 8-month study period resulted in the detection of 21 rotavirus episodes in the study group. Most of the cases occurred at a time during the study when the incidence of rotavirus in the Caricuao area increased, judging by hospital admissions of infants and children not participating in the study. Vaccine codes were not revealed at any time to the field team, and therefore a preliminary analysis of vaccine efficacy was possible. Twelve of the 21 rotavirus diarrheal episodes detected occurred in the placebo group, 4 occurred in the group of children receiving the low vaccine titer, and 5 occurred in the group receiving the high titer, for efficacy rates of 68 and 58%, respectively (P = 0.04 and 0.08, the Fisher exact test, two tails).

Thus, from this study it appears that 3 doses of the 10^5-PFU titer quadrivalent vaccine should be the schedule of choice in vaccine studies.

ACKNOWLEDGMENTS

We thank the families of Caricuao who participated in this study for their collaboration. We thank Rosabel Gonzalez, Jordi Boher, Cidalia Urbina, Yesenia Rodriguez, Mayauri Mendez, and Johnna Sears for their valuable technical assistance. We also thank William Blackwelder, Genevieve Losonsky, and Nalini Singh, who formed the Clinical Monitoring Committee overseeing this study.

This study was supported by funds from the Agency for International Development (AID) Vaccine Program through a PASA agreement with the National Institute of Allergy and Infectious Diseases.

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