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SAFETY EVALUATION OF SPf66 MALARIA VACCINE IN BRAZIL

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The frequency and description of side effects secondary to the subcutaneous application of SPf66 malaria vaccine and placebo are reported for each dose of application in the participants of the vaccine efficacy trial in Brazil. Side effects evaluated two hours after each application were detected in 8.0%, 30.2% and 8.8%, for the 1st, 2nd and 3rd dose, respectively, in the SPf66 group, and in 7.0%, 8.5% and 2.9% in the placebo group. Local reactions such as mild inflammation, nodule and pain or erythema frequently accompanied by pruritus were the most common reactions detected in both groups (3.8%, 29.1% and 8.5% in the SPf66 group and 4.0%, 7.6% and 2.5% in the placebo group). Among vaccinees, local side effects after the 2nd dose were more frequent in females. Systemic side effects were expressed mainly through general symptoms referred by the participants and were most frequent after the 1st dose in both groups (4.3% in the SPf66 group and 3.0% in the placebo group). Muscle aches and fever were referred by few participants. No severe adverse reactions were detected for either dose of application or group.

Key-words: Malaria vaccine. Vaccine safety. SPf66 efficacy trial.

The synthetic malaria vaccine against the asexual forms of *Plasmodium falciparum* (SPf66) has recently being tested in populations of endemic areas of Colombia, Ecuador, Venezuela, Tanzania and Gambia[1,2,3,4,5,6,7,8,10]. These studies have provided evidence that the vaccine is safe for use in children as well as in adults. Vaccine application was responsible mainly for local minor effects, the most frequent of which were the induration of the site of application, pain, pruritus and erythema. Few cases of hypersensitivity were described. The present paper reports on the findings of the Brazilian trial regarding the safety of the vaccine.

Study population and procedures. The Brazilian trial consisted of a randomized, double-blinded, placebo-controlled, efficacy trial[9]. Eight hundred volunteers, male and female, aging 7 to 60 years, residing in the rural settlements of the Municipality of Costa Marques, Rondonia, and who fulfilled the selection criteria were randomly allocated to receive three doses of the vaccine or placebo. Written consent was obtained from all participants or tutors, in case of children .

The synthetic vaccine used in the trial was produced in the Instituto de Inmunologia, Universidad Nacional de Colombia by the research group of Dr. M.E. Patarroyo. It consisted of a sequence of protein fragments of 83, 55 and 35kDa derived from the merozoite and erithrocytic stages and a sequence of the tetrapeptide of the circumsporozoite protein (Asn-Ala-Asn-Pro) of *Plasmodium falciparum*. The peptide was reconstituted in 0.9% saline solution and absorbed onto aluminum hydroxide at a concentration of 4mg of synthetic protein in 2mg of aluminum hydroxide per ml[2]. Tetanus toxoid was used as placebo for the 1st dose and aluminum hydroxide for the 2nd and 3rd doses. Both preparations were bottled in clear glass recipients containing 10 doses each and coded

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with the letters *L* and *S* in Colombia. The preparations were sent "ready for use" with a member of the laboratory, who supervised their application during the first days of each programmed vaccination. No information is available for either the batch number or formulation lot of SPf66 vaccine preparations or the tetanus toxoid used in this trial.

Vaccination schedule was defined as the subcutaneous application of 0.5ml containing 2mg of the vaccine and 1mg of aluminum hydroxide, on days 0, 30 and 180[7]. To facilitate the detection of local reactions, the 1st and 3rd doses were applied on the deltoid region of the right arm and the 2nd on the left. A surveillance system for detecting any adverse reactions was set up during every planned vaccination. All participants were closely monitored during the first 30 minutes after vaccine application by the medical investigator in charge of vaccination. Emergency equipment and therapeutic procedures were readily available. Inquiries for possible symptoms and identification of local reactions were performed two hours after each application, as well as four weeks after the 1st dose and two weeks after the 2nd and 3rd doses. Participants were advised to contact the medical investigator in case of possible severe signs and symptoms secondary to vaccination.

The frequency, intensity, duration and description of any adverse effects were systematically recorded according to the following criteria: mild or moderate local inflammation (presence of edema, pain and erythema < 5mm or > 5mm, respectively), isolated erythema or pain, pruritus, nodule (induration), signs of hypersensitivity and general symptoms as described by each participant.

Statistical analysis. The frequencies of side effects were compared for the vaccine and placebo groups and for each dose application stratified by sex and age, and for the total study population. Corrected χ^2 tests or two-tailed Fisher's exact tests at a significance level of 0.05 were used to compare proportions under the null hypothesis of homogeneity (no differences in the proportion of individuals with side effects in the vaccine and placebo groups or between doses).

RESULTS

Side effects evaluated two hours after each application were detected in 8.0%, 30.2% and 8.8% of the participants of the SPf66 group for the 1st, 2nd and 3rd doses, respectively. In the placebo group they were detected in 7.0%, 8.5% and 2.9%. Local reactions at the site of application of the preparations were the most frequent side effects detected and/or referred by the participants at the three doses of either the vaccine or placebo preparations (Tables 1 and 2). Systemic reactions were more frequent during the 1st dose and consisted of symptoms referred by the participants with the exception of the blood pressure alterations. No severe adverse reactions were detected during the

Table 1 - Frequency of side effects observed two hours after the application of each vaccine and placebo dose.

	Dose								
	1st			2nd			3rd		
Side effect	SPf66 n = 400	T.T*. n = 400	p	SPf66 n = 361	$Al(OH)_3$† n = 354	p	SPf66 n = 283	$Al(OH)_3$ n = 279	p
Local (total)	15(3.8)	16(4.0)		105(29.1)	27(7.6)	< 0.0001	24(8.5)	7(2.5)	0.0035
isolated pain or erythema	8(2.0)	8(2.0)	0.8	5(1.4)	3(0.8)	0.7	3(1.1)	3(1.1)	
mild inflammation	2(0.5)	2(0.5)		85(23.5)	12(3.4)	< 0.0001	15(5.3)	2(0.7)	0.0034
moderate inflammation		1(0.2)							
nodule	5(1.3)	5(1.3)	0.7	15(4.1)	12(3.4)	0.8	2(0.7)	1(0.4)	
pain in arm							1(0.3)	1(0.4)	
paresthesia							2(0.7)		
subcutaneous emphysema							1(0.3)		
Systemic (total)	17(4.3)	12(3.0)	0.4	4(1.1)	3(0.8)		1(0.3)	1(0.4)	
headache	13(3.3)	10(2.5)	0.7	3(0.8)	2(0.6)		1(0.3)	1(0.4)	
blood pressure alterations	2(0.5)	1(0.2)							
unspecific gastric symptoms	2(0.5)	1(0.2)		1(0.3)					
muscle pain					1(0.3)				
Total	32(8.0)	28(7.0)	0.7	109(30.2)	30(8.5)	<0.0001	25(8.8)	8(2.9)	0.0047

*T.T. - Tetanus toxoid; †$Al(OH)_3$ - Aluminum hydroxide

Number (%) of participants with side effects.

Table 2 - Frequency of side effects referred by the participants 2 weeks after the application of each vaccine and placebo dose.

Side effect	Dose 1st			2nd			3rd		
	SPf66 n = 361	T.T*. n = 356	p	SPf66 n = 361	$Al(OH)_3$† n = 354	p	SPf66 n = 210	$AL(OH)_3$ n = 201	p
Local (total)	31(8.6)	57(16.0)	0.0035	122(33.8)	45(12.7)	< 0.0001	42(20.0)	24(11.9)	0.0366
isolated pain or erythema	9(2.5)	10(2.8)	0.9	5(1.4)	9(2.5)	0.4	12(5.7)	7(3.5)	0.39
mild inflammation	10(2.8)	10(2.8)	0.8	99(27.4)	23(6.5)	< 0.0001	16(7.6)	7(3.5)	0.11
moderate inflammation	1(0.3)	6(1.7)	0.07	1(0.3)	1(0.3)		2(0.9)	2(1.0)	
nodule	11(3.0)	31(8.7)	0.0021	17(4.7)	12(3.4)	0.5	9(4.3)	7(3.5)	0.9
pain in arm							2(0.9)	1(0.5)	
paresthesia							1(0.5)		
Systemic (total)	24(6.6)	12(3.4)	0.07	4(1.1)	3(0.8)		3(1.4)	3(1.5)	
headache	15(4.1)	10(2.8)	0.4	4(1.1)	2(0.6)	0.7	1(0.5)	1(0.5)	
unspecific gastric symptoms	2(0.5)						2(0.9)	2(1.0)	
muscle pain	5(1.4)	1(0.3)	0.2		1(0.3)				
fever	1(0.3)	1(0.3)							
dizziness	1(0.3)								
Total	55(15.2)	69(19.4)	0.2	126(34.9)	48(13.6)	<0.0001	45(21.4)	27(13.4)	0.0453

*T.T. - Tetanus toxoid; †$Al(OH)_3$ - Aluminum hydroxide

Number (%) of participants referring side effects.

application of any of the three doses on either group.

The most frequent local reactions detected in both vaccine and placebo groups after the three doses were mild inflammation, nodule and isolated pain or erythema, frequently accompanied by pruritus at the site of application (Table 1). These local reactions did not differ between groups after the 1st dose (3.8% in SPf66 and 4.0% in placebo), but increased significantly in vaccinees (29.1%; $p < 0.0001$) and slightly, in the placebo group (7.6%; $p = 0.047$) after the 2nd dose. Following the 3rd dose, the number of local side effects decreased significantly in both groups (8.5% for SPf66 and 2.5% for placebo; $p < 0.01$). Few participants referred pain that compromised the whole arm, paresthesia in the arm where the vaccine was applied, and one vaccinee had subcutaneous emphysema probably due to improper application technique.

Headache was the most frequent systemic reaction in both groups at each dose. Blood pressure alterations occurred only after the application of the 1st dose in 0.5% of the vaccinees and 0.2% of the placebo group. Other rare manifestations included unspecific gastric symptoms, muscle pains, fever and dizziness at very low frequencies.

Side effects were more frequent in females in both groups (Table 3). In the vaccinees, local reactions were significantly more frequent in females after the 2nd and 3rd doses, specially in participants over 15 years of age. In the placebo group, females had more systemic reactions after the 1st dose (Fisher; $p = 0.007$), while children showed more adverse reactions for the three doses.

Side effects persisted for a comparable period in both groups. Isolated pain and erythema generally disappeared within the first 24 hours. Inflammation generally persisted for 24 to 72 hours and nodules disappeared in 48 hours, but persisted in some cases for 15 days, as detected in the two weeks local evaluation after the 2nd dose. Muscle aches and fever, as referred by some participants (1.7% in those vaccinated), lasted a few days (1 to 5 days). No concomitant parasitemia was detected in these individuals. All other systemic side effects were transitory, lasting only a few minutes.

DISCUSSION

The vaccine was well tolerated by the participants, and most side effects were detected mainly at the site of application. Frequency of side effects was similar for SPf66 vaccine and tetanus toxoid with the exception of persisting nodules and moderate inflammation, which were more frequent in the latter. Mild inflammation at the site of application was considerably more frequent in the participants who received SPf66 than in those who received only aluminum hydroxide. Frequency of total side effects was greater for the first two doses than those reported in other trials.

Local side effects were more frequent after the 2nd dose, decreasing after the 3rd dose in

Table 3 - Frequency of observed side effects according to sex, age and dose of application.

Side effect/	Vaccine			Placebo		
age(yrs)	males	females	p	males	females	p
1st dose						
Local	7/247 (2.8)	8/153 (5.2)	0.34	8/245 (3.3)	8/155 (5.2)	0.50
15	4/76 (5.3)	2/65 (3.1)	0.69	5/86 (5.8)	5/66 (7.6)	0.75
> 15	3/171 (1.7)	6/88 (6.8)	0.06	3/159 (1.9)	3/89 (3.4)	0.67
Systemic	7/247 (2.8)	10/153 (6.5)	0.01	2/245 (0.8)	10/155 (6.4)	0.002
15	5/76 (6.6)	3/65 (4.6)	0.72	1/86 (1.2)	6/66 (9.1)	0.04
>15	2/171 (1.2)	7/88 (8.0)	0.008	1/159 (0.6)	4/89 (4.5)	0.06
Total	14/247 (5.7)	18/153 (11.8)	0.046	10/245 (4.1)	18/155 (11.6)	0.007
2nd dose						
Local	44/221 (19.9)	61/140 (43.6)	<0.0001	9/215 (4.2)	12/139 (8.6)	0.13
15	14/70 (20.0)	23/61 (37.7)	0.04	3/82 (3.6)	6/59 (10.2)	0.16
> 15	30/151 (19.9)	38/79 (48.1)	<0.0001	6/133 (7.5)	6/80 (7.5)	0.37
Systemic	1/221 (0.4)	3/140 (2.1)	0.30	0/215 (0.0)	3/139 (2.1)	
15	0/70 (0.0)	0/61 (0.0)		0/82 (0.0)	2/59 (3.4)	
> 15	1/151 (0.7)	3/79 (3.8)	0.12	0/133 (0.0)	1/80 (1.2)	
Total	45/221 (20.4)	64/140 (45.7)	<0.0001	9/215 (4.2)	15/139 (10.8)	0.03
3rd dose						
Local	7/173 (4.0)	17/110 (15.4)	0.0017	2/167 (1.2)	5/112 (4.5)	0.12
15	3/58 (5.2)	6/54 (11.1)	0.31	3/73 (4.1)	4/52 (7.7)	0.45
> 15	4/115 (3.5)	11/56 (19.6)	0.0013	0/94 (0.0)	1/60 (1.7)	
Systemic	0/173 (0.0)	1/110 (0.9)		1/167 (0.6)	0/112 (0.0)	
15	0/58 (0.0)	0/54 (0.0)		1/73 (1.1)	0/52 (0.0)	
> 15	0/115 (0.0)	1/56 (1.8)		0/94 (0.0)	0/60 (0.0)	
Total	7/173 (4.0)	18/110 (16.4)	0.0008	3/167 (1.8)	5/112 (4.5)	0.27

Proportion (%) of participants with observed side effects.

both groups as reported in Ecuador's trial[8]. Several local side effects not reported in other trials were detected, such as pain and paresthesia in the arm (0.3% and 0.7%, respectively). Nodules persisted for weeks in some cases and no contralateral local manifestations were detected as described in some trials[1,3]. The fact that local side effects caused by the repeated application of SPf66 were more frequent in females and were apparently enhanced by age is not clearly understood. Similar results were reported by Amador et al[3]. Noya et al reported that hypersensitivity type reactions were significantly higher in women[5].

Systemic reactions were mild and transitory except for the referred muscle aches and fever, that could last for days. Although no hypersensitivity reactions were detected, no conclusions can be drawn about their frequency because of the inadequacy of the sample size to detect these reactions.

The increased frequency of adverse reactions for both preparations in children and females might lead to differential losses to follow-up in field trials. Of the initial cohort of 800 participants, 714 received the 2nd dose and 572 the 3rd. A separate analysis of losses considering the frequencies of side effects according to the treatment group, age and sex indicated that the proportion of individuals lost to follow-up with secondary reactions was equal in both groups (χ^2, p = 0.4)[9].

SPf66 has not been associated with biochemical or autoimmune abnormalities, and the frequency of hypersensitivity reactions seems low as reported in previous trials[1,2,3,4,5,6,7,8,10]. However, the frequency of these types of reactions can only be determined in large field trials. Our conclusions are restricted to this particular trial in which SPf66 was responsible for minor local reactions.

RESUMO

A freqüência e descrição dos efeitos secundários à aplicação subcutânea da vacina antimalárica SPf66 e placebo, são notificadas para cada dose nos participantes do estudo da eficácia vacinal no Brasil. Efeitos colaterais avaliados duas horas após a aplicação dos preparados foram detectados em 8,0%, 30,2% e 8,8% para a 1ª, 2ª e 3ª doses, respectivamente, no grupo de vacinados; e em 7,0%, 8,5% e 2,9% no grupo que recebeu o placebo. Reações tais como inflamação leve, nódulo e dor freqüentemente acompanhadas de prurido, foram as reações locais mais freqüentes em ambos os

grupos (3,8%, 29,1% e 8,5% no grupo vacinado, e 4,0%, 7,6% e 2,5% no grupo placebo). No grupo que recebeu a vacina, as reações locais foram mais freqüentes em mulheres após a 2ª dose. Os efeitos colaterais sistêmicos basearam-se em sinais e sintomas referidos pelos participantes. Foram mais freqüentes após a aplicação da 1ª dose em ambos os grupos (4,3%, no grupo de vacinados e, 3,0%, no grupo placebo). Alguns participantes referiram mialgias e febre. Nenhum efeito colateral grave foi detectado em nenhuma dose de aplicação ou grupo.

Palavras-chaves: Vacina antimalárica. Segurança SPf66. Ensaio de campo SPf66.

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