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Combining antimalarial drugs and vaccine for malaria elimination campaigns: a randomized safety and immunogenicity trial of RTS,S/AS01 administered with dihydroartemisinin, piperaquine, and primaquine in healthy Thai adult volunteers

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ABSTRACT

Introduction: RTS,S/AS01 is currently the most advanced malaria vaccine but provides incomplete, short-term protection. It was developed for use within the expanded program on immunizations (EPI) for African children. Another use could be adding mass RTS,S/AS01 vaccination to the integrated malaria elimination strategy in the Greater Mekong Subregion (GMS), where multidrug-resistant *P.falciparum* strains have emerged and spread. Prior to evaluating RTS,S/AS01 in large-scale trials we assessed whether the vaccine, administered with and without antimalarial drugs, is safe and immunogenic in Asian populations.

Methods: An open-label, randomized, controlled phase 2 trial was conducted in healthy, adult Thai volunteers. Seven vaccine regimens with and without antimalarial drugs (dihydroartemisinin-piperaquine plus a single low dose primaquine) were assessed. Antibody titres against the *Pf*CSP full-length (NANP) 6, *Pf*CSP anti-C–term, *Pf*CSP full-length (N + C-Terminal) were measured by standard enzyme-linked immunosorbent assays. Liquid chromatography was used to measure piperaquine, primaquine and carboxy-primaquine concentrations.

Results: 193 volunteers were enrolled and 186 study participants completed the 6 months follow-up period. One month after the last vaccination all study participants had seroconverted to the *Pf*CSP (NANP)6, and the *Pf*CSP Full Length (N + C-Terminal). More than 90% had seroconverted to the *Pf*anti-C-Term CSP. There was no indication that drug concentrations were influenced by vaccine regimens or the antibody levels by the drug regimens. Adverse events were similarly distributed between the seven treatment groups. No serious adverse events attributable to the study interventions were detected.

Conclusion: This study found that RTS,S/AS01 with and without dihydroartemisinin-piperaquine plus a single low dose primaquine was safe and immunogenic in a healthy, adult Asian population.

Introduction

The most advanced malaria vaccine, RTS,S/AS01 has been developed to be used in conjunction with the expanded program on immunizations (EPI) in African children. It provides incomplete protection against uncomplicated and severe malaria over a limited time span. Phase 3 evaluation of RTS,S/AS01 showed a vaccine efficacy (VE) against malaria of 55.1% (95% confidence interval [CI], 50.5–59.3%) over 12 months after vaccination when delivered on a 0, 1, and 2-month schedule in children aged 5–17 months at first vaccination.[1,2] While this efficacy profile has the potential to provide significant health benefits to vaccinated children it is unlikely, on its own, to make a major impact on malaria transmission when implemented in this context. In the aforementioned Phase 3 trial, the vaccine was more protective in children

living in lower transmission settings (presumably more aligned with an elimination setting) than in higher transmission settings, perhaps due to lower forces of infection.[3] Furthermore, it has recently been shown that vaccine efficacy against controlled human malaria infection (CHMI) can be improved using alternative immunization schedules.[4] These observations have stimulated additional interest in the potential role of RTS,S/AS01 in accelerating *P. falciparum* parasite elimination in Asia. Mathematical models suggest that adding a temporarily effective vaccine to other basic malaria control measures could interrupt parasite transmission permanently if implemented at scale.[5]

To accelerate progress towards this ambitious goal, mass drug administrations (MDA) have been piloted in malaria hot-spots in addition to the conventional malaria control strategies; widespread use of long-lasting insecticide-treated

bed-nets and early diagnosis and effective treatment.[6] As with previous studies, the impact of a recent series of MDA conducted in the GMS with dihydroartemisinin-piperaquine (DP) waned over time.[7] The rebound in malaria months after MDA is a predictable consequence of the importation of malarial infections from surrounding areas where no intervention took place and also from residual infections in residents who did not participate in the MDA. A longer lasting or even permanent interruption of transmission could potentially be achieved by combined mass vaccination and drug administration campaigns.[5] The emergence and spread of multidrug-resistant *P.falciparum* strains in the Greater Mekong Subregion (GMS) have increased the urgency for regional elimination of malaria.[8,9]

While some studies have been conducted in African[10] and American adults[4,11] the vaccine has not been tested in Asia. As a necessary prelude to the evaluation of RTS,S/AS01 in large-scale trials in the GMS, we assessed in an open-label, randomized, controlled trial whether different dosing regimens of the vaccine, administered with and without the antimalarial drugs used for MDA, are safe and immunogenic in an Asian, adult population.

Results

Two hundred and sixty-seven participants were screened of whom 74 (28%) did not meet the inclusion criteria and were excluded. One hundred and ninety-three volunteers (including 3 replacements) were randomized into one of seven treatment groups (Table 1). Two participants were lost to follow up and one participant withdrew before completing the vaccination schedule (Figure 1).

Baseline characteristics

The baseline characteristics were well balanced across the groups (Table 2). Forty-five percent of the enrolled participants were male. The median age was 33.8 years, IQR (27.3–40.0) years and the median weight was 61.2 kg (range 53.2–71.2 kg).

Immunogenicity

One month after the last vaccination all study participants had seroconverted to the $PfCSP$ $(NANP)_6$ repeat region, and the $PfCSP$ full length (N + C-Terminal). More than 90% had seroconverted to the $PfCSP$ C-Term (Supplementary Table S1, S2, S3). Antibody levels against each of the three antigens peaked at month 2 ($PfCSP$ full-length; N + C-Terminal) or month 3 ($PfCSP$ NANP 6 repeat region, $PfCSP$ C – term) and then fell to a lower level by month 6 at which time participants in treatment groups 3 and 4 (3x standard dose with or without antimalarial drugs) had the highest antibody levels and participants in group 7E-SF+D, two doses only, had the

Table 1. Treatment groups.

Group	Vaccine*	Dose Month 0	Dose Month 1	Dose Month 2	Antimalarial‡	Group name†	Participants
1	RTS,S/AS01$_B$	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Fractional dose (0.1mL)		B-SSF	21
2	RTS,S/AS01$_E$	Double standard dose (1.0mL)	Double standard dose (1.0mL)	Double Fx dose (0.2mL)		EE-SSF	21
3	RTS,S/AS01$_E$	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Single standard dose (0.5mL)		E-SSS	30
4	RTS,S/AS01$_E$	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Single standard dose (0.5mL)	DHA-PIP+PQ	E-SSS+D	30
5	RTS,S/AS01$_E$	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Fractional dose (0.1mL)		E-SSF	30
6	RTS,S/AS01$_E$	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Fractional dose (0.1mL)	DHA-PIP+PQ	E-SSF+D	30
7	RTS,S/AS01$_E$	Single standard dose (0.5mL)		Fractional dose (0.1mL)	DHA-PIP+PQ	E-SF+D	31

* RTS,S/AS01$_B$ = adult dose, RTS,S/AS01$_E$ = paediatric dose‡DHA-PIP: Dihydroartemisinin-piperaquine; PQ: primaquine † B: RTS,S/AS01B; E: RTS,S/AS01E; EE: double dose of RTS,S/AS01E; S: standard dose; F: fractional dose; +D: DHA-PIP+PQ
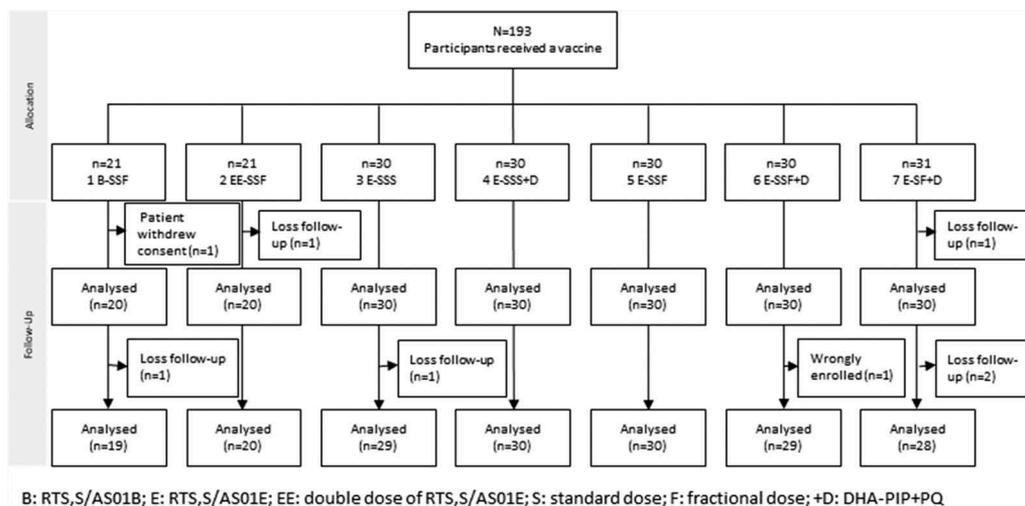

Figure 1. Study participant assembly (Consort chart).
B: RTS,S/AS01B; E: RTS,S/AS01E; EE: double dose of RTS,S/AS01E; S: standard dose; F: fractional dose; +D: DHA-PIP+PQ

Table 2. Baseline characteristics (M0 day 0 measurements) of the participants by vaccine group.
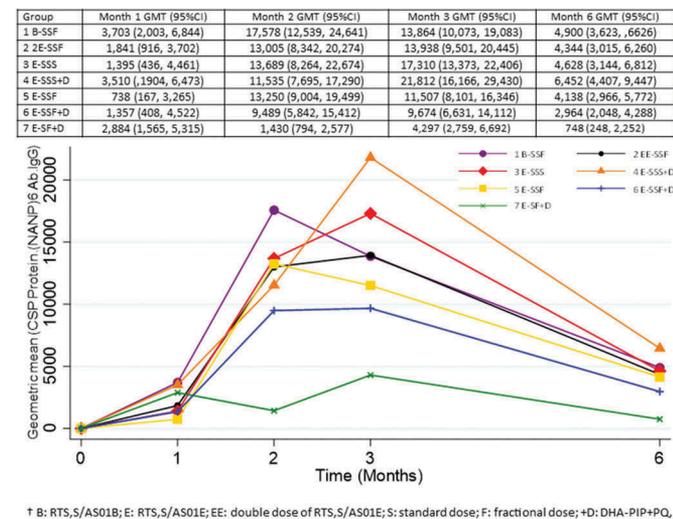
Group*	1 B-SSF	2 EE-SSF	3 E-SSS	4 E-SSS+D	5 E-SSF	6 E-SSF+D	7 E-SF+D	Total
n	20	20	30	30	30	30	30	190
Gender male n (%)	8 (40)	9 (45)	14 (46)	13 (43)	13 (43)	14 (46)	14 (46)	87 (45)
Age (years) median (IQR)	33.2 (24.8, 41.3)	30.9 (24.5, 41.9)	34.1 (28.7, 39.9)	36.3 (30.7, 42.0)	37.1 (32.1, 42.8)	32.4 (29, 39.5)	29.2 (26.8, 34.5)	33.8 (27.3, 40.0)
Weight (kg) median (IQR)	57.2 (46.2, 68.5)	60.9 (54.5, 69.0)	61.7 (52.5, 70.5)	61.0 (54.5, 70.3)	60.0 (53.2, 73.5)	64.2 (58.1, 75.6)	59.5 (54.0, 72.3)	61.2 (53.2, 71.2)
Number with fever n	0	0	0	0	0	0	0	0
Hb mean (sd)	13.4 (12.8, 14.5)	13.4 (13.3, 14.6)	13.4 (12.7, 14.8)	14 (12.4, 14.5)	13.6 (12.2, 14.5)	13.3 (12.5, 14.7)	13.1 (12.5, 13.4)	13.4(12.5, 14.5)
WBC median (IQR)	7.1 (5.9, 7.5)	7.0 (6.3, 9.1)	7.0 (6.4, 8.7)	6.6 (6.0, 7.7)	7.0 (5.6, 7.4)	7.0 (6.3, 8.2)	6.8 (6.0, 8.0)	6.9 (6.1, 8.0)
Platelets median (IQR)	312 (273, 345)	297 (263, 329)	268 (243, 322)	260 (226, 309)	284 (233, 354)	285 (250, 323)	267 (224, 309)	280 (243, 323)
Creatinine median (IQR)	0.7 (0.7, 0.9)	0.9 (0.7, 1.0)	0.8 (0.7, 1.0)	0.9 (0.7, 1.0)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)
AST median (IQR)	17 (16, 18)	18 (14.5, 20)	18 (16, 20)	23 (19, 28)	19 (17, 21)	20 (18, 26)	21 (19, 28)	19 (17, 23)
ALT median (IQR)	14 (12, 17)	14.5 (11, 20)	14.5 (11, 20)	18 (13, 26)	14 (10, 18)	16 (13, 24)	20 (16, 34)	16 (12, 21)
Temperature mean (sd)	36.1 (0.3)	36.2 (0.5)	36.1 (0.3)	36.1 (0.5)	36.0 (0.4)	36.0 (0.6)	36.1 (0.5)	36.1 (0.4)

* B: RTS,S/AS01B; E: RTS,S/AS01E; EE: double dose of RTS,S/AS01E; S: standard dose; F: fractional dose; +D: DHA-PIP+PQ

lowest antibody levels (Figure 2, Supplementary figures S1, S2). Vaccine regimens which included high vaccine doses (group 1B-SSF and 2EE-SSF) or regimens including fractional third doses (group 1B-SSF, 2EE-SSF, 5E-SSF, 6E-SSF+D) did not result in higher antibody levels than the standard pediatric three-dose regimen. Antibody avidity (*Pf*CSP C-Term and *Pf*CSP full length (N + C-Terminal)) reached over 50% by month 3 in all treatment groups and remained above 50% by month 6 (Figure 3 and Supplementary Figures S3, S4,). Avidity of antibodies against *Pf*CSP (NANP)$_6$ repeat region was below 50% in treatment groups 5E-SSF and 7E-SF+D at month 3 and fell below 40% in groups 3E-SSS and 7E-SF+D by month 6.

Pharmacokinetics

The median (range) of day 7 piperaquine concentrations during the first month was 32.8 ng/ml (11.4–115 ng/ml). The median trough concentration before the start of the second month of treatment was 6.1 ng/mL which accumulated by 48% to 9.0 ng/mL before the start of the third month of treatment. There were no significant differences between the piperaquine, primaquine or carboxy-primaquine concentrations at each sampling time

Group	Month 1 GMT (95%CI)	Month 2 GMT (95%CI)	Month 3 GMT (95%CI)	Month 6 GMT (95%CI)
1 B-SSF	3,703 (2,003, 6,844)	17,578 (12,539, 24,641)	13,864 (10,073, 19,083)	4,900 (3,623, ,6626)
2 EE-SSF	1,841 (916, 3,702)	13,005 (8,342, 20,274)	13,938 (9,501, 20,445)	4,344 (3,015, 6,260)
3 E-SSS	1,395 (436, 4,461)	13,689 (8,264, 22,674)	17,310 (13,373, 22,406)	4,628 (3,144, 6,812)
4 E-SSS+D	3,510 (,1904, 6,473)	11,535 (7,695, 17,290)	21,812 (16,166, 29,430)	6,452 (4,407, 9,447)
5 E-SSF	738 (167, 3,265)	13,250 (9,004, 19,499)	11,507 (8,101, 16,346)	4,138 (2,966, 5,772)
6 E-SSF+D	1,357 (408, 4,522)	9,489 (5,842, 15,412)	9,674 (6,631, 14,112)	2,964 (2,048, 4,288)
7 E-SF+D	2,884 (1,565, 5,315)	1,430 (794, 2,577)	4,297 (2,759, 6,692)	748 (248, 2,252)

† B: RTS,S/AS01B; E: RTS,S/AS01E; EE: double dose of RTS,S/AS01E; S: standard dose; F: fractional dose; +D: DHA-PIP+PQ,

Figure 2. Changes in Pf CSP NANP6 IgG levels over time.

point in study participants who received the standard or fractional vaccine doses (Figure 4).

The population pharmacokinetic model for piperaquine described the data well and suggested a relatively lower bioavailability of piperaquine compared to the prior study, i.e. a scaling factor of 0.327. The vaccine dose (i.e. full and fractional doses) did not have a significant impact on the pharmacokinetic parameters of piperaquine. Simulation-based diagnostics and standard goodness-of-fit plots showed an excellent predictive and descriptive performance of the model (Figure S5 and S6). Final pharmacokinetic parameter estimates are presented in Table S4.

Tolerability and safety

Ninety-nine percent study participants 190/193 reported one or more adverse events following vaccination. Seventy-five percent (1,219/1,630) of the adverse events were mild in character (Figure 5(a)). The most frequent complaint following vaccination was pain around the injection site (24%; 397/1,630) followed by fatigue 282/1630 (17%; Figure 5(c)). Individual study participants reported a median of five adverse events (Figure 5(b)). The number of vaccine-attributable mild, moderate and severe adverse events did not vary significantly between treatment groups (Table S6, S7, S8). The hematology and biochemistry test results stayed within the normal limits except for one study participant who was found to have elevated liver transaminases (ALT 263 U/l, AST 100 U/l) at month 3 which normalized spontaneously by month 6. One participant had thrombocytopenia (84,000/uL) on day 7 following the first round of vaccinations. The participant was diagnosed with dengue fever and recovered without further treatment. Of the 193 volunteers recruited in the study, three participants had a serious adverse event. One participant was diagnosed with acute pharyngitis, one with vestibular migraine and depression, and one with acute sinusitis. None of the serious adverse events were considered to be related to the study vaccine or drugs.

Discussion

This study found that the RTS,S/AS01 was safe and immunogenic in healthy Asian volunteers as has been reported in Caucasian and African populations.[12-15] Comparing the treatment groups
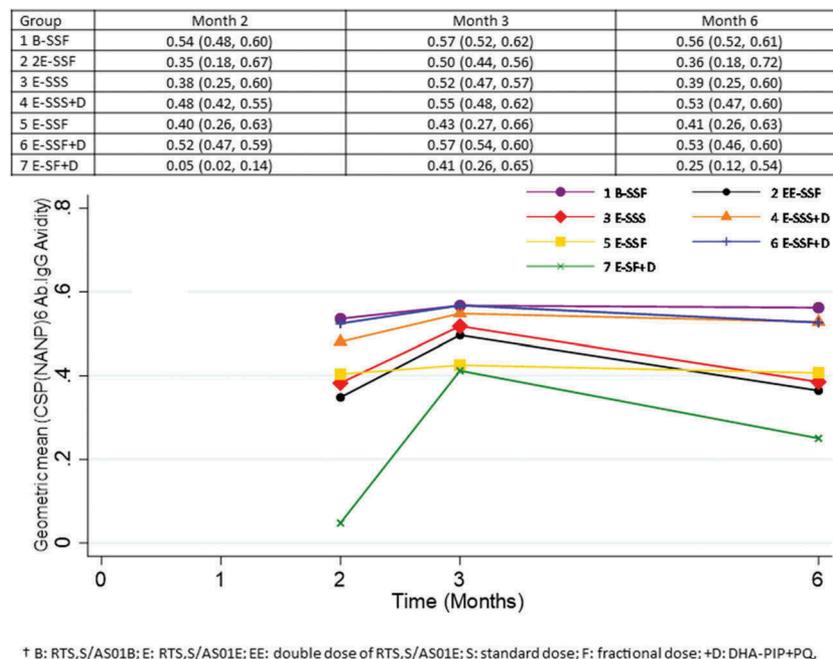
Group	Month 2	Month 3	Month 6
1 B-SSF	0.54 (0.48, 0.60)	0.57 (0.52, 0.62)	0.56 (0.52, 0.61)
2 2E-SSF	0.35 (0.18, 0.67)	0.50 (0.44, 0.56)	0.36 (0.18, 0.72)
3 E-SSS	0.38 (0.25, 0.60)	0.52 (0.47, 0.57)	0.39 (0.25, 0.60)
4 E-SSS+D	0.48 (0.42, 0.55)	0.55 (0.48, 0.62)	0.53 (0.47, 0.60)
5 E-SSF	0.40 (0.26, 0.63)	0.43 (0.27, 0.66)	0.41 (0.26, 0.63)
6 E-SSF+D	0.52 (0.47, 0.59)	0.57 (0.54, 0.60)	0.53 (0.46, 0.60)
7 E-SF+D	0.05 (0.02, 0.14)	0.41 (0.26, 0.65)	0.25 (0.12, 0.54)

† B: RTS,S/AS01B; E: RTS,S/AS01E; EE: double dose of RTS,S/AS01E; S: standard dose; F: fractional dose; +D: DHA-PIP+PQ.

Figure 3. Changes in Pf CSP NANP6 antibody avidity over time (%).

receiving vaccine with and without antimalarial drugs suggests that the antibody titers following vaccination with RTS,S/AS01 were not reduced by the co-administration of antimalarial drugs. This could have been an important operational concern when the vaccine is used in addition to MDA for the acceleration of malaria elimination. It has been observed that the immunogenicity of live, cell-culture rabies vaccines is reduced by co-administration with the antimalarial drug chloroquine which is structurally related to piperaquine and could have immunomodulatory effects that will negatively impact immunological responses to the vaccine.[16,17]

To explore which vaccine dosing schedule would be optimal we compared the immunogenicity of two vaccine doses and formulations, the pediatric dose, the adult dose, and a double pediatric dose. The three antigen and adjuvant doses resulted in comparable antibody titers. Importantly using a higher dose did not result in increased antibody titers or avidity. This finding suggests that the pediatric dose of RTS,S/AS01$_E$ can be used for immunization of populations irrespective of age in Asia and perhaps even globally. This finding is of importance for vaccine production which is currently geared to produce pediatric vaccine doses for a large pilot introduction in areas of sub-Saharan Africa.[18] Switching production to a different dose for adults would be a logistical challenge which the findings from the current study suggest is not necessary.

Recent work by Regules and co-workers in human malaria challenge studies found vaccine regimens consisting of two standard vaccine doses with a fractional third dose resulted in better protection against controlled human malaria infection compared with a regimen consisting of three standard doses despite the slightly lower ant-CS response.[4] In the current study in healthy volunteers, five regimens with a fractional third dose were compared with two regimens using a standard vaccine dose. The fractional dose regimens did not result in higher antibody titers than the three standard dose regimens.

The antibody titers measured in Thai volunteers were in the same range as observed in North American volunteers in the earlier study.[4]

It was hoped that the use of less resources requiring two-dose vaccine schedule would result in a similar immunogenicity as a three-dose regimen. By month 6 the antibody titers against all three antigens evaluated were lowest in the two-dose regimen. Based on antibody titers alone which may not be sufficient for recommendations in the absence of clinical efficacy data, further exploration of such a 2-dose regimen may not be warranted. Protection against *P.falciparum* infection in challenge studies indicated no discernible difference between groups of participants receiving two doses compared to three doses of RTS,S albeit with a slightly different adjuvant, AS02.[19]

Compared to the reference study[20] the absorption of piperaquine in the current study was lower resulting in a relative reduction in bioavailability by 67.3%. However, vaccine dosing (i.e. full and fractional vaccine doses) had no impact on the pharmacokinetic parameters of piperaquine. The co-administration of the vaccine with the antimalarial drugs is therefore unlikely to be the cause of the reduced bioavailability. The most likely explanation is the absence of fat-containing foods with the drug administration.[21] In the reference study on which the model was built a light meal was administered at the time of antimalarial dosing, while food intake was not monitored in the present study.[20] Intake of a high-fat meal has been seen to enhance the absorption of piperaquine while smaller amounts of fat have been shown not to have an effect.[22,23] The observed piperaquine levels were well above the threshold required to kill Plasmodium species in earlier studies, and the observed piperaquine concentrations are similar to what is seen in patients during acute treatment of uncomplicated malaria.[21]

Nearly all participants reported one or more adverse events. Some participants reported 14 and more AEs suggesting a highly variable threshold for reporting AEs is in this population. The
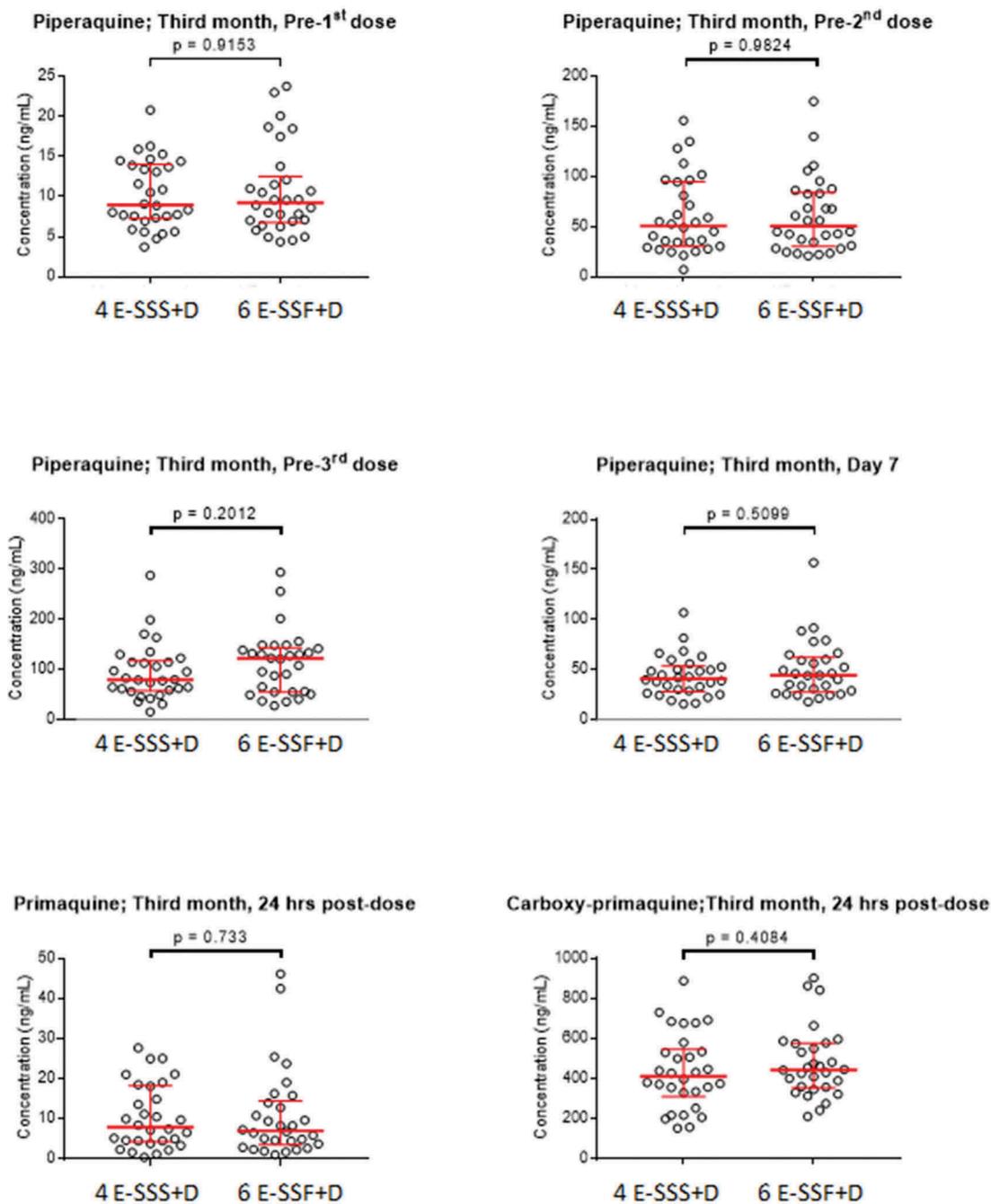
Figure 4. Comparison of concentration measurements when dihydroartemisinin-piperaquine and primaquine were given together with a normal RTS,S/AS01E vaccine dose (4 E-SSS+D) and when given with a fractional dose (6 E-SSF+D).

most frequently observed attributable adverse events were pain at the injection site and fatigue. Three serious adverse events were observed but none of them was considered to be related to the vaccinations. Hematological and biochemistry parameters were monitored with no attributable abnormalities. While the vaccine was found to be safe in this study, the perception of discomfort could be a deterrent for vaccine uptake in combined mass vaccination and drug administration campaigns. To assure adequate coverage of mass drug and vaccinations campaigns it will be essential to provide credible information to target communities regarding the relative risks and benefits of the campaigns.

The study had an excellent follow-up, 98% of enrolled participants completed the follow-up period suggesting that the reported discomfort did not lead to discontinuation in study participation. One hundred and ninety participants were evaluated, hence adverse events which occur at a frequency much smaller than 1/190 were likely to be missed. The absence of a control group receiving placebo prevents the attribution of adverse events to the vaccine or drugs is a limitation of the study. Inclusion of a control group receiving a placebo version of the vaccine and drugs so as to maintain blinding was considered not feasible with the available resources. The study was conducted in unexposed
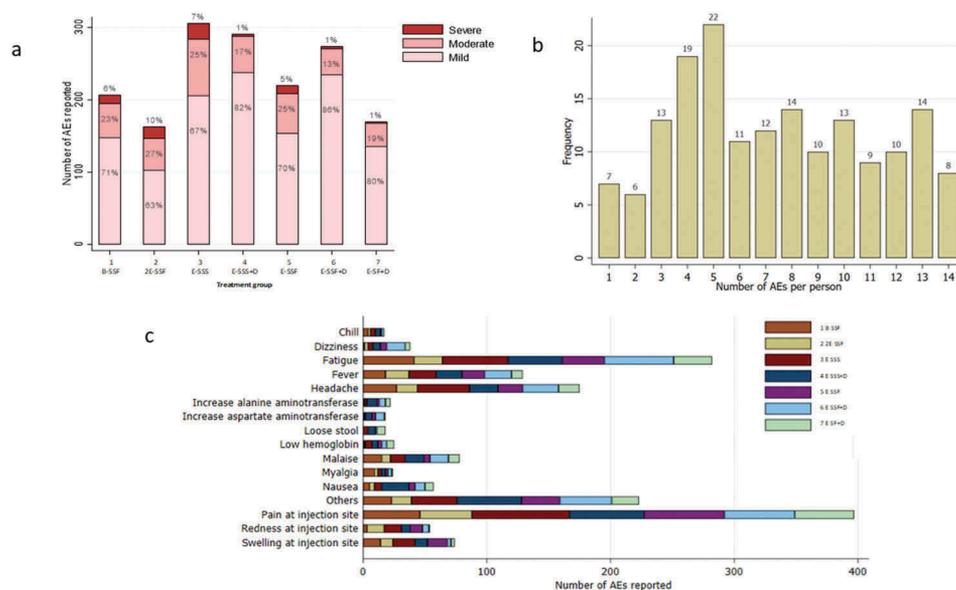
Figure 5. Safety and tolerability of RTS,S/AS01 with and without antimalarial drug administrations **a** Percentage of mild, moderate, severe adverse (AEs) in each treatment group **b** The number of AEs per person and the frequency of participants with a specific number of AE **c** Summary of AEs by frequency.

adult Thai volunteers, which leaves the possibility that safety and immunogenicity studies could be different in other Asian populations.

A sequence of interventions, universal access to early diagnosis and adequate treatment, vector control for example in the form of improved access to insecticide-impregnated bed nets, three-monthly rounds of combined mass vaccinations and drug administrations (MVDA), followed by screening and treatment of imported cases, could eliminate falciparum malaria from low transmission areas in the SE Asia over a period of 3 years.[5] The optimal schedule for MDA, three rounds of three doses of antimalarials matches the current schedule of three-monthly RTS,S/AS01 injections. Booster doses of RTS,S/AS01 and additional MDA rounds are probably necessary for the permanent interruption of falciparum malaria transmission, but their safety and effectiveness will need to be studied. The impact of MVDA will depend on the uptake of the intervention. In a recent series of MDA in Asia intense community engagement helped achieve MDA coverage above 80% which is thought to be the threshold for the interruption of malaria transmission.[24] The benefit of MVDA needs to be carefully communicated to target communities in order to reach adequate coverage. There is little reason to think that an intramuscular injection will reduce the uptake of the intervention substantially, on the contrary, it is possible that communities perceive injections more "powerful" than orally administered drugs and hence a desirable adjunct to drug administrations. The clinical development pathway of MVDA will require proof of individual protection in a randomized phase 3 trial in low transmission settings. Such a trial will require thousands of participants even when the planned endpoint is PCR-detected *P.falciparum* infection and not clinical malaria as was the case in earlier malaria vaccine trials. Once individual protection has been demonstrated in the target community, community-randomized trials will be needed to assess the impact of this approach on malaria transmission in residents

of all ages in high-risk areas. Assuming community-randomised trials demonstrate permanent interruption of transmission on a village level a wider, regional approach will be rolled out which in low-transmission settings is likely to be restricted to communities with evidence of ongoing malaria transmission. In conclusion, MVDA has the potential to interrupt malaria transmission permanently and could play a critical role in the speedy elimination of malaria from low transmission regions.

Methods

This was a phase 2, open-label, computer-randomized, controlled study in healthy, adult Thai volunteers conducted in the Healthy Volunteer Unit of Mahidol University in Bangkok, Thailand between 6 June 2017 and 20 February 2018. The following research questions were addressed.

(1) Is RTS,S/AS01 safe and immunogenic in a malaria-naïve, adult Asian population?

(2) Does the co-administration of RTS,S/AS01 with the antimalarial drugs used in MDA, dihydroartemisinin-piperaquine (DP) and single low dose primaquine (PQ) interfere either with the immunogenicity of the vaccine or the pharmacokinetics of the antimalarial drugs?

(3) Can currently manufactured dosages be deployed? RTS, S/AS01 has been manufactured in a pediatric dose (RTS,S/AS01$_E$) which contains half the antigen and adjuvant system components of the adult dose (RTS,S/AS01$_B$). Using this pediatric dose in adults would simplify vaccine procurement for mass vaccination campaigns but the immunogenicity of the pediatric and adult dose has not been compared previously.

(4) Could a fractional third dose be used? A vaccine regimen in which two standard doses are followed by

a fractional dose consisting of a fifth of a standard dose may be more efficacious than a regimen with three standard doses.[4]

(5) Would a two-dose regimen suffice?

To answer these questions a drug – vaccine interaction study was conducted. In addition, we compared the immunogenicity of vaccine regimens with a fifth of the standard third dose with regimens including standard doses only. We compared the tolerability, safety, and immunogenicity of two and three-dose regimens.

Study vaccines and antimalarials

The vaccines were manufactured by GlaxoSmithKline (Rixensart, Belgium). After reconstitution, 1 dose (0.5 ml) of the paediatric dose RTS,S/AS01$_E$ contains 25 micrograms (µg) of RTS,S and 25 µg of each of the components of the AS01E adjuvant system composed of Quillaja saponaria Molina, fraction 21 (QS-21) and 3-Odesacyl-4′- monophosphoryl lipid A (MPL). In the adult dose RTS,S/AS01$_B$ each component of the vaccine is doubled. After reconstitution, 1 dose (0.5 ml) contains 50 µg of each RTS,S, QS-21 and MPL. Dihydroartemisinin/piperaquine (DP) tablets (Sigma-Tau Industrie Farmaceutiche Riunite s.p.a., Pomezia, Italy) for adult patients contain 40 mg dihydroartemisinin and 320 mg piperaquine with a therapeutic dose range between 2 and 10 mg/kg/day dihydroartemisinin and 16–26 mg/kg/dose piperaquine. Participants in treatment groups 4 and 6 received the standard DP treatment on Days 0, 1, 2, on Month 0, 1, 2 and in group 7 on Month 0, 2 (Figure S7). In addition, each participant in groups 4, 6, and 7 received a single low dose of primaquine (0.25 mg base/kg; Thai Government Pharmaceutical Organisation, Bangkok, Thailand) on the first day of each vaccination (Day 0).

Sample size calculations

The sample size calculations were based on the primary objective of comparing the serologic response to RTS,S/AS01$_E$ vaccine co-administered with DP-PQ versus RTS,S/AS01$_E$ or RTS,S/AS01$_B$ vaccine given alone in Thai adults. A sample size of 30 participants per group gave 80% power at the 5% significance level to detect a difference in serologic response of 30% for groups 3 to 7. A higher than 30% in the difference in serologic response was anticipated in groups 1 and 2 compared to the other groups. For groups 1 and 2 (in which larger differences were anticipated) a sample size of 20 participants was thought to be sufficient to document immunogenicity. Whenever possible an attempt was made to replace study participants when a participant was withdrawn or chose to withdraw.

Study procedures

After a full explanation of study procedures healthy male or non-pregnant female volunteers, aged 18 to 55 years (inclusive), of Thai origin, without a history of malaria, who provided informed consent and were willing to adhere to the study requirements were recruited. Volunteers were screened before

enrolment for hematological or biochemical abnormalities, and for malaria and viral infections (HBsAg, HCV, HIV; Table S5).

Tolerability and safety assessments

Local injection site and general solicited adverse events (AEs) were monitored on days 2, 3, and 7 post-vaccination and graded as mild, moderate, or severe (grades 1, 2, or 3, respectively). All other AEs (unsolicited) were recorded over a 30-day period after each vaccination. Serious AEs (SAEs) were captured throughout the study period. All injection site AEs were considered causally related to vaccination; the causality of all other AEs was assessed by the investigator and these decisions were reviewed by the DSMB and the medical monitor. Hematological and biochemical tests for safety assessment were conducted at screening, on day 0 and 7 of the first vaccination (M0) and Month 3. Abnormal test results were followed until they resolved.

Immunogenicity assessments

The immunology assessments used in this trial were described in detail recently.[4] Briefly, antibody levels against PfCSP were measured by standard enzyme-linked immunosorbent assays (ELISAs), using PfCSP (NANP)$_6$ repeat region, PfCSP C – term, and PfCSP full-length (N + C-Terminal).[4,25] By using 4M urea as a chaotropic reagent, ELISA-based avidity assays were conducted to assess antibody binding to PfCSP (NANP)$_6$ repeat region, PfCSP C – term, and PfCSP full-length (N + C-Terminal).[4] All antibody assays were processed at WRAIR in Silver Spring, Maryland, USA.

Pharmacokinetics

Piperaquine concentrations (groups 4 and 6) were assessed during each vaccination round (Month 0, 1, and 2) on Day 0, 1, 2, and 7. Primaquine concentrations were assessed during each round 24 h after drug administration. The bioanalytical measurements were performed at the Department of Clinical Pharmacology, Mahidol Oxford Research Unit, Bangkok, Thailand using liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) detection. All assays were developed and validated according to US FDA regulations. The impact of the vaccine dose on the observed drug concentrations of piperaquine, primaquine, and carboxyprimaquine were investigated by comparing drug concentrations in group 4E-SSS+D (standard vaccine dose) and group 6E-SSF+D (one-fifth vaccine dose at month 3) during the third month of treatment. The pharmacokinetic comparison was made at each time point (pre-1st dose, pre-2nd dose, pre-3rd dose, and 7 days post-dose) using an unpaired Mann-Whitney U-test implemented in Graphpad Prism v7.02 (GraphPad Software, La Jolla California USA). Piperaquine concentration time data were analyzed using a population pharmacokinetic approach as implemented in the software NONMEM v 7.3 (Icon Development Solutions, Ellicott City, Maryland, USA).[26] To facilitate the modeling process and to diagnose developed models, Pirana v2.9.2, Perl-Speaks-NONMEM 4.6.0, and R v3.4.3 (the R Foundation for Statistical Computing, Vienna, Austria) with the package Xpose v4.6.1 were used to facilitate the modeling process and model diagnostics.[27-30] Due to the sparse nature of

the data, a frequentist prior model, based on a healthy volunteers study were implemented (supplementary materials).[31] Relative standard error was calculated using the Sampling Importance Resampling function in Perl-Speaks-NONMEM.[32]

Statistical analysis

All analyses were conducted according to a predefined Statistical Analysis Plan. The main strategy of analysis for the primary outcome analyses (immunogenicity) was carried out using the intention to treat (ITT) population including every participant who was randomized. A per-protocol (PP) analysis including only the study participants who completed the vaccinations and the follow-up schedule was also performed to assess the impact of protocol deviations. The safety outcomes were analyzed using the ITT approach. All participants who received at least one dose of vaccine were included in the safety analyses. Participants lost to follow-up before the completion of the follow-up period assessments were censored on the last day seen. Linear regression was performed on the log values of the protein titers and also on avidity (natural scale). Due to the large number of possible comparisons between vaccine regimens geometric mean titers (GMT) with 95% confidence intervals (95%CI) were reported. *P*-values less than 0.05 were considered statistically significant in this study. Statistical analyses were conducted using Stata 15 software (StataCorp LLC, College Station, Texas, USA).

Ethics review

The protocol was approved by the Mahidol Faculty Tropical Medicine (TMEC 16-097) and Oxford University's ethics review board (OxTREC ref 54-16) and the Western Institutional Review Board (WIRB # 20162633). The trial was undertaken in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines and good clinical practice (ICH GCP). Written informed consent was obtained from each participant before study procedures were initiated (Clinical Trials.gov identifier: NCT02992119)

Data Availability

The data that support the findings of this study can be made available following application to the MORU data sharing committee http://www.tropmedres.ac/data-sharing.

Disclosure of potential conflicts of interest

No potential conflict of interest was reported by the authors.

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