

EFFICACY AND TOLERABILITY OF ARTESUNATE PLUS SULFADOXINE-PYRIMETHAMINE AND SULFADOXINE-PYRIMETHAMINE ALONE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN PERU

WILMER MARQUINO, LAURA YLOQUIMICHE, YGOR HERMENEGILDO, ANA MARIA PALACIOS,
EDUARDO FALCONÍ, CÉSAR CABEZAS, NANCY ARRÓSPIDE, SONIA GUTIERREZ, AND TRENTON K. RUEBUSH II
Instituto Nacional de Salud, Lima, Peru; Dirección Sub-Regional de Salud Piura II, Ministerio de Salud, Sullana, Peru; Office of the
Director, National Center for Infectious Diseases, Centers for Disease Control and Prevention, U.S. Naval Medical Research Center
Detachment, Lima, Peru

Abstract. To assist the Peruvian Ministry of Health in modifying the malaria treatment policy for their north Pacific coastal region, we conducted an *in vivo* efficacy trial of sulfadoxine-pyrimethamine (SP) and SP plus artesunate (SP-AS) for the treatment for uncomplicated *Plasmodium falciparum* infections. A total of 197 patients were randomized to therapy with either SP (25 mg/kg of the sulfadoxine component in a single dose on day 0) or a combination of SP plus AS (4 mg/kg on days 0, 1, and 2) and were followed for 28 days for symptoms and recurrence of parasitemia. No statistically significant differences between the two groups were observed on enrollment with respect to age, sex, history of malaria, or geometric mean parasite density. A total of 185 subjects completed the 28-day follow-up. Of the 91 subjects treated with SP alone, two had recurrences of parasitemia on day 7 and one on day 21. Of the 94 subjects treated with SP-AS, one had a recurrence of parasitemia on day 21. Fever and asexual parasite density decreased significantly more rapidly and the proportion of patients with gametocytemia on days 3–28 was significantly lower in subjects treated with combination therapy than in those who received SP alone. No severe adverse drug reactions were observed; however, self-limited rash and pruritis were significantly more common and an exacerbation of nausea, vomiting, and abdominal pain were observed significantly more frequently among patients who had received SP-AS. These results have contributed to a National Malaria Control Program decision to change to SP-AS combination therapy as the first-line treatment for uncomplicated *P. falciparum* malaria in northern coastal Peru in November 2001, making Peru the first country in the Americas to recommend this combination therapy.

INTRODUCTION

Because of growing concerns about the development of resistance to antimalarial drugs when used alone, combination therapy is increasingly being regarded as the best strategy to prolong the useful therapeutic lifetime of these drugs.^{1–3} Combinations of artemisinin drugs with mefloquine have already proven to be highly efficacious in Southeast Asia and there is suggestive evidence that the use of this combination therapy has halted the steady increase in mefloquine resistance that had been seen when this mefloquine was used alone.⁴ Artemisinin and its derivatives produce more rapid resolution of fever and parasitemia than any other antimalarial agent due to their rapid reduction in parasite biomass.⁵ Furthermore, these drugs have the potential of reducing malaria transmission, at least in low transmission areas, by virtue of their activity against the gametocyte stage of the parasite.⁶ Other promising candidates for combination therapy with an artemisinin drug include the 4-aminoquinolines and sulfadoxine-pyrimethamine (SP), although experience with their use is much more limited.^{3,7–9}

Before 1999, chloroquine was the first-line treatment of uncomplicated *Plasmodium falciparum* infections on the north coast of Peru. In that year, 14-day *in vivo* efficacy tests at three sites showed that >50% of strains had RII/RIII resistance to chloroquine.¹⁰ In contrast, no RIII resistance to SP was observed and fewer than 5% of patients had RII resistance to this drug. Based on these findings, in June 1999 the Peruvian Ministry of Health made a change in first-line therapy for uncomplicated *P. falciparum* infections in this region from chloroquine to SP. However, because resistance to SP alone is expected to develop quite rapidly, the Ministry of Health made a commitment to implement combination

therapy with SP plus artesunate (SP-AS) as soon as a trial of its safety and efficacy could be carried out on the north coast of Peru.¹¹ This paper reports the results of that trial.

MATERIALS AND METHODS

Study site. The study was conducted at the Bellavista and Querecotillo Health Centers near the city of Sullana on the north coast of Peru during the peak malaria transmission season of 2000 (Figure 1). These two health facilities draw their patient population from the city's periurban and rural areas. The study was reviewed and approved by the Institutional Review Boards of the Instituto Nacional de Salud and the U.S. Navy.

Malaria transmission on the north coast of Peru is unstable with a peak between March and August. *Plasmodium vivax* is the predominant species; *P. falciparum* accounts for 10–20% of infections. All age groups are affected and the majority of infections are symptomatic, although severe malaria is quite uncommon. The principal vector is *Anopheles albimanus*.

Patients. Patients ≥ 5 years of age with suspected malaria attending the two health centers were screened for malaria parasitemia with thick blood smears. Those with *P. falciparum* mono-infections between 500 and 50,000 parasites/ μ L of blood and an axillary temperature $\geq 37.5^{\circ}\text{C}$ and/or a history of fever within the previous 48 hours were enrolled in the trial if they gave their informed consent. Subjects were excluded from the study if they had symptoms or signs of severe malaria, had another cause for their fever, had a history of allergy to the study drugs, or were pregnant or had a positive urine pregnancy test result.

Study design. Patients were assigned to receive SP monotherapy or combination therapy with SP-AS using a table of

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 2005		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE EFFICACY AND TOLERABILITY OF ARTESUNATE PLUS SULFADOXINE-PYRIMETHAMINE AND SULFADOXINE-PYRIMETHAMINE ALONE FOR THE TREATMENT OF UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN PERU				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Instituto Nacional de Salud, Lima, Peru; Dirección Sub-Regional de Salud Piura II, Ministerio de Salud, Sullana, Peru				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Naval Medical Research Center 503 Robert Grant Avenue Silver Spring, MD 20910-7500				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT SAR	18. NUMBER OF PAGES 5	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			



FIGURE 1. Study site in the city of Sullana on the north Pacific Coast of Peru.

random numbers. Since the mechanism of action and biochemical targets of sulfadoxine and pyrimethamine in the parasite are similar, this drug is not strictly considered combination therapy by the World Health Organization. Sulfadoxine-pyrimethamine (Fansidar®; Roche S.A., Basel, Switzerland) was administered in a single oral dose of 25 mg/kg of the sulfadoxine component on day 0. Patients randomized to SP-AS received a similar dose of SP plus AS (Plasmotrin®; Mepha, Ltd., Aesch-Basle, Switzerland) in a dose of 4 mg/kg/day on days 0, 1, and 2. All drug administration was supervised by study staff. Subjects were observed for vomiting for 30 minutes after ingesting the drugs; those who vomited the first dose during this period were re-treated with the same dose. Patients with axillary temperatures $\geq 37.5^{\circ}\text{C}$ were treated with paracetamol.

Patients were asked to return for follow-up histories of symptoms and possible adverse drug reactions, temperature measurements, and thick blood smears on days 1, 2, 3, 7, 14, 21, and 28. Patients who did not return were traced to their homes. The first 50 patients enrolled had venous blood samples taken for hemoglobin, red and white blood cell counts, differential counts, reticulocyte counts, and creatinine, blood urea nitrogen (BUN), bilirubin, and serum transaminase measurements on days 0, 7, and 28. Subjects with a recurrence of parasitemia after day 3 were treated with a supervised seven-day course of quinine plus tetracycline.

Laboratory methods. Thick blood smears were stained with Giemsa and the parasite density calculated by counting the number of asexual parasites per 300 white blood cells, assuming a mean white blood cell count of $6,000/\mu\text{L}$. Gametocyte density was estimated by counting the number of gametocytes per 500 white blood cells. All blood smears were examined by two independent microscopists. If there was a difference in species diagnosis or if the parasite density differed by $\geq 50\%$ between the two, a third microscopist would re-examine the smears for a final species diagnosis. The final parasite density

was the mean of the counts of the two initial microscopists or an average of the two closest counts. A total of 200 oil-immersion fields were examined before a blood smear was considered negative. Red blood cell, white blood cell and differential, and reticulocyte counts, as well as serum BUN, creatinine, bilirubin, aspartate aminotransferase, and alanine aminotransferase measurements were done manually.

Statistical analysis. The parasitologic response to therapy was classified according to the World Health Organization guidelines for *in vivo* efficacy trials¹² with minor modifications: RIII = a day 2 parasite density $\geq 100\%$ of day 0 or a day 3 parasite density $\geq 25\%$ of day 0; RII = a positive day 3 blood smear with a parasite density $< 25\%$ of day 0 and a positive day 7 blood smear; RI (early) = a negative day 3 blood smear with the reappearance of parasitemia between days 4 and 14 or a positive day 3 blood smear with a parasite density $< 25\%$ of day 0, a negative day 7 blood smear, and the reappearance of parasitemia between days 8 and 14 inclusive; RI (late) = a negative day 3 blood smear or a parasite density $< 25\%$ of days 0, 7, and 14 blood smears negative, and the reappearance of parasitemia between days 15 and 28; S (sensitive) = a negative day 3 blood smear or density $< 25\%$ of day 0 with negative blood smears between days 7 and 28.

The patient's clinical response was classified according to the Pan American Health Organization guidelines for *in vivo* antimalarial drug efficacy studies in the Americas.¹³ Early treatment failure (ETF) was defined as development of signs of severe malaria with parasitemia on days 1, 2, or 3; a day 2 parasite density $\geq 100\%$ of day 0; or a day 3 parasite density $\geq 25\%$ of Day 0. Late treatment failure (LTF) was the development of signs of severe malaria with parasitemia after day 3; clinical deterioration in the presence of parasitemia; or the reappearance of parasitemia between days 7 and 28. An adequate clinical response (ACR) was a patient who did not fulfill the criteria for ETF or LTF with negative blood smears on days 7, 14, 21, and 28.

Data were double-entered. Statistical analyses were carried out using SPSS software (SPSS, Inc., Chicago, IL). Dichotomous variables were compared with chi-square or Fisher's exact tests. The Shapiro-Wilk test was used to test for normality of continuous variables and the Student *t*-test or Mann-Whitney *U* test was used to compare means. Relative risk was used to evaluate incidence rates.

RESULTS

A total of 197 patients with uncomplicated *P. falciparum* malaria were enrolled in the trial. Sixty percent were male. Their mean $\pm$ SD age was 28.4 ± 14.6 years. On enrollment, 56% had an axillary temperature $\geq 37.5^{\circ}\text{C}$ and their geometric mean parasite density was $6,613/\mu\text{L}$. Twelve subjects were excluded from analysis, five treated with SP alone and seven with combination therapy. Reasons for exclusion included loss to follow-up ($n = 6$; one each on days 3, 7, and 14 and 3 on day 21), parasite densities less than 500 parasites/ μL on re-examination of their initial blood smears ($n = 2$), appearance of *P. vivax* malaria on a follow-up blood smear ($n = 1$), vomiting $\times 2$ of SP-AS ($n = 1$), voluntary withdrawal from the study ($n = 1$), or ingestion of trimethoprim-sulfamethoxazole within 24 hours of enrollment ($n = 1$).

The characteristics of the remaining patients are shown in

Table 1. No significant differences were noted between the two groups at the time of enrollment in terms of age, sex, mean geometric parasite density, hemoglobin level, or abnormalities in BUN, creatinine, or liver function test measurements.

Although all subjects responded well initially to therapy, none of those treated with SP-AS had fever on day 3 in comparison to nine (9.9%) treated with SP alone ($P = 0.001$). Asexual parasite density decreased significantly more rapidly in subjects treated with SP-AS than in those treated with SP alone (Figure 2). On day 3, 83 (91.2%) of the patients treated with SP had negative blood smears compared with 92 (97.8%) of those treated with SP-AS (relative risk = 1.07, 95% confidence interval = 1.00–1.15, $P = 0.05$); no difference in asexual parasite densities was noted on day 7.

On enrollment, no significant difference was observed in the proportion of patients in the two treatment groups who had gametocytes in their blood (3 or 3.3% with SP versus 6 or 6.4% with SP-AS). On days 3, 7, 14, 21, and 28, the proportion of subjects with gametocytemia was significantly lower with combination therapy than with SP alone (Figure 3). Among patients who had no gametocytes in their blood on enrollment, significantly fewer of those treated with SP-AS had gametocytemia on day 3 (8.6% versus 26.1%; $P = 0.003$), on day 7 (4.6% versus 75.0%; $P = 0.0001$), and on day 14 (4.6% versus 65.9%; $P = 0.0001$).

Three (3%) of the 91 subjects treated with SP alone had recurrences of parasitemia during the 28-day follow-up period: two on day 7 (RII resistance) and one on day 21 (late RI resistance) (Table 2). Only one of the 94 subjects treated with the combination of SP-AS had a recurrence of parasitemia on day 21 (late RI resistance). This subject had only one parasite seen on his blood smear but this was confirmed by two independent microscopists. Of the six subjects who were lost to follow-up, all cleared their parasitemia and none had a recurrence of parasitemia before they dropped out of the trial.

Four subjects, all of whom who had received combination therapy, developed a self-limited maculopapular rash within 48 hours of beginning therapy. One additional subject receiving combination therapy had pruritis without rash that resolved with antihistamine therapy. Four other patients receiving combination therapy had gastrointestinal symptoms: two complained of an increase in the severity of their nausea and vomiting, and two others complained of an increase in ab-

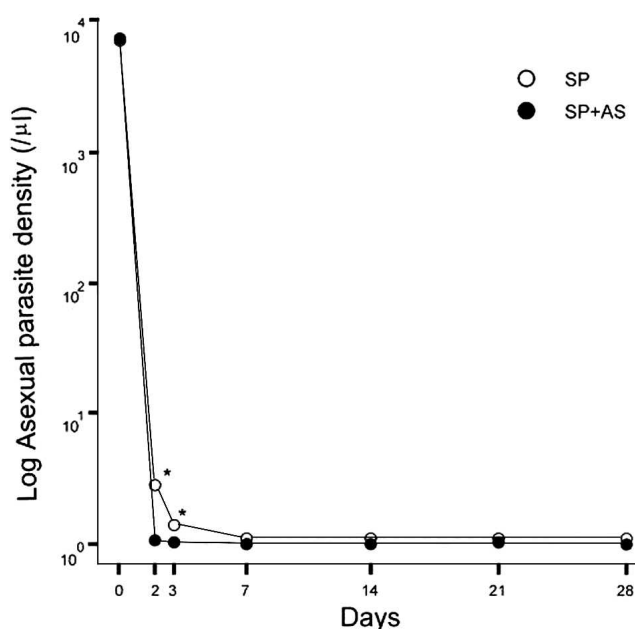


FIGURE 2. Geometric mean parasite density of patients treated with sulfadoxine-pyrimethamine (SP) monotherapy (-----) and sulfadoxine-pyrimethamine plus artesunate (SP+AS) combination therapy (——). Points marked with an asterisk indicate a P value ≤ 0.05 .

dominal pain when compared with their symptoms before therapy was initiated. Three of these patients received treatment of their symptoms. No significant differences were observed between subjects in the two treatment groups with respect to abnormal hematologic or blood biochemistry values on either day 7 or day 28.

DISCUSSION

This is the first trial of SP-AS combination therapy for *P. falciparum* in the Americas. Two studies of this combination have been reported from The Gambia, where resistance to SP

TABLE 1

Characteristics of patients enrolled in a sulfadoxine-pyrimethamine (SP) and SP-artesunate (SP-AS) *in vivo* drug efficacy study, north coast of Peru, 2000*

Characteristic	SP (n = 91)	SP-AS (n = 94)	P
Mean $\pm$ SD age (years)	28.9 $\pm$ 13.9	28.5 $\pm$ 14.6	NS
Sex (male)	65%	54%	NS
Axillary temperature $\geq 37.5^{\circ}\text{C}$ (day 0)	56%	55%	NS
Geometric mean parasite density (/μL) (day 0)	7,076	7,309	NS
Mean $\pm$ SD hemoglobin (g/dL) (day 0)	12.6 $\pm$ 1.3	12.3 $\pm$ 1.4	NS
Bilirubin > 1.1 mg/dL (day 0)	31%	36%	NS
AST > 37 U/L (day 0)	22%	27%	NS
Creatinine > 1.0 mg/dL (day 0)	19%	33%	NS

* NS = not significant; AST = aspartate aminotransferase.

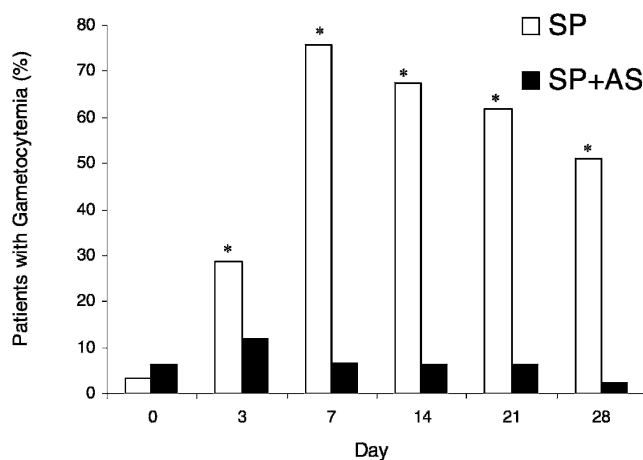


FIGURE 3. Proportion of patients with gametocytemia treated with sulfadoxine-pyrimethamine (SP) monotherapy and sulfadoxine-pyrimethamine plus artesunate (SP+AS) combination therapy. Columns marked with an asterisk indicate a P value ≤ 0.05 .

TABLE 2

Parasitologic and therapeutic response of strains of *Plasmodium falciparum* to sulfadoxine-pyrimethamine (SP) and SP-artesunate (SP-AS) on the North Coast of Peru, 2000

Drug regimen	n	Parasitologic response (%) [*]				Therapeutic response (%) [†]		
		RIII	RII	RI	S	ETF	LTF	ACR
SP	91	0	2	1	97	0	3	97
SP-AS	94	0	0	1	99	0	1	99

^{*} S, RI, RII, and RIII refer to resistance levels as defined in the Materials and Methods.
[†] ETF = early treatment failure; LTF = late treatment failure; ACR = adequate clinical response.

alone is uncommon.^{7,9} Both studies showed an efficacy of 100%. The 3% prevalence of *P. falciparum* strains resistant to SP monotherapy in the 28-day study reported here was slightly lower than that found in the studies done on the northern coast of Peru during 1999, where 4.5% of all patients showed RII/RIII resistance in a 14-day test.¹⁰ Only one subject treated with SP-AS had a recurrence of parasitemia. Perhaps because of the relatively high efficacy of SP in this area, we were not able to show a significant difference in the efficacy of the two treatment regimens. As in previous studies,⁷⁻⁹ we found that combination therapy with SP-AS reduced fever and asexual parasitemia significantly faster than SP alone. In addition, combination therapy reduced the proportion of patients with gametocytemia significantly faster than SP monotherapy.

Prospective clinical studies of more than 10,000 patients and the use of artemisinin and its derivatives in several million patients, including post-marketing surveillance of more than 4,600 patients in Thailand, has not shown any serious drug-related adverse effects.^{3,14,15} The most common adverse effects reported are nausea, abdominal pain, vomiting, and occasional diarrhea, symptoms that are also associated with malaria infections and that resolve with treatment. In this study, self-limited maculopapular rash and/or pruritis, probably associated with drug therapy, occurred significantly more frequently in the SP-AS treatment group than with SP monotherapy. While cutaneous adverse reactions are well known with sulfonamide drugs, they are not a common reported side effect of artemisinin and its derivatives.³ Four other subjects on combination therapy complained of an increase in the severity of their nausea, vomiting, and abdominal pain after therapy started. Adverse drug reactions were not reported in the two trials of SP-AS conducted in The Gambia.^{7,9}

Although *P. falciparum* resistance to SP is widespread and intense in the Amazon Basin^{16,17} (Stennies G, unpublished data), SP is still efficacious along the Pacific coast of Colombia and Peru.^{10,18} With the high levels of *P. falciparum* resistance to chloroquine on the north coast of Peru, SP might appear the logical first choice as replacement first-line therapy; however, given the presence of low levels of resistance to SP in the area and experiences from other countries, where SP resistance has increased rapidly when used as monotherapy,^{19,20} the Peruvian National Malaria Control Program decided to change to SP-AS as first-line treatment in this region to slow the selection of resistant strains.²¹ This new treatment policy was implemented in November 2001. With this change, Peru has become the first country in the Americas to recommend SP-AS combination therapy as part of its national malaria treatment policy.

As part of the implementation of SP-AS combination therapy in Peru, plans have been made for ongoing surveillance of SP efficacy at two-three sentinel sites on the north coast. Testing will be conducted at one site each year by local Ministry of Health staff supervised by a team from the Instituto Nacional de Salud. In addition, since Peru will be the first country in the Americas to use SP-AS combination therapy, the Ministry of Health plans to conduct surveillance for severe adverse drug reactions during the first 1–2 years of its use. Since all patients with *P. falciparum* malaria in Peru are routinely asked to return for follow-up blood smears 7 and 14 days after therapy is initiated to ensure that they are cured, surveillance for adverse drug reactions will be integrated into the existing follow-up system.

Received July 17, 2003. Accepted for publication January 22, 2004.

Acknowledgments: We thank Dr. José Luis Suárez, who acted as medical monitor for the study. We also thank Edith Guerrero, Enrique Javier Pacherez, and the staff of the Bellavista and Querecotillo Health Centers for their assistance with the enrollment, treatment, and follow-up of patients. The microscopic examinations were performed by Marcelino Mendoza and Angel Távara. Richard Vera carried out the hematologic and biochemical examinations. Statistical analyses were carried out by Christian Bautista.

Financial support: This study was supported by the U.S. Agency for International Development–Government of Peru Vigia Project, Addressing Threats of Emerging and Re-Emerging Infectious Diseases, (Activity 527-0391) and the U.S. Naval Medical Research and Development Command, National Naval Medical Center (Bethesda, MD) Work Unit No. 847705 82000 25GB B0016 GEIS Lima.

Disclaimer: The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or as reflecting the views of the Navy Department or the naval service at large. Authors' addresses: Wilmer Marquino, Laura Ylquimiche, Ygor Hermenegildo, Eduardo Falconí, César Cabezas, Nancy Arróspide, and Sonia Gutierrez, Instituto Nacional de Salud Lima, Peru. Ana Maria Palacios, Dirección Sub-Regional de Salud Piura II, Ministerio de Salud, Sullana, Peru. Trenton K. Ruebush II, Division of Parasitic Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway, Mailstop F-22, Atlanta, GA 30341, Fax: 770-488-4203, E-mail: tkr1@cdc.gov.

REFERENCES

- White NJ, 1998. Preventing antimalarial drug resistance through combinations. *Drug Resist Updates* 1: 3–9.
- White NJ, Nosten F, Looareesuwan S, Watkins WM, Marsh K, Snow RW, Kokwaro G, Ouma J, Hien TT, Molyneux ME, Taylor TE, Newbold CI, Ruebush TK II, Danis M, Greenwood BM, Anderson RM, Olliaro P, 1999. Averting a malaria disaster. *Lancet* 353: 1965–1967.
- WHO, 1998. *The Use of Artemisinin and Its Derivatives as Anti-Malarial Drugs: Report of a Joint CTD/DMP/TDR Informal Consultation*. Geneva: World Health Organization. WHO Document WHO/MAL/98.1086
- Nosten F, van Vugt M, Price R, Luxemburger C, Thway KL, Brockman A, McGready R, ter Kuile F, Looareesuwan S, White NJ, 2000. Effects of artesunate-mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western Thailand: a prospective study. *Lancet* 356: 297–302.
- White NJ, 1997. Assessment of the pharmacodynamic properties of antimalarial drugs *in vivo*. *Antimicrob Agents Chemother* 41: 1413–1422.
- Price RN, Nosten F, Luxemburger C, ter Kuile FO, Paiphun L, Chongsuphajaisiddhi T, White NJ, 1996. Effects of artemisinin derivatives on malaria transmissibility. *Lancet* 347: 1654–1658.
- Doherty F, Sadiq AD, Bayo L, Allouche A, Olliaro P, Milligan P, von Seidlein L, Pinder M, 1999. A randomized safety and

- tolerability trial of artesunate plus sulfadoxine-pyrimethamine versus sulfadoxine-pyrimethamine alone for the treatment of uncomplicated malaria in Gambian children. *Trans R Soc Trop Med Hyg* 93: 543–546.
8. von Seidlein L, Jawara M, Coleman R, Doherty T, Walraven G, Targett G, 2001. Parasitaemia and gametocytaemia after treatment with chloroquine, pyrimethamine/sulfadoxine, and pyrimethamine/sulfadoxine combined with artesunate in young Gambians with uncomplicated malaria. *Trop Med Int Health* 6: 92–98.
 9. von Seidlein L, Milligan P, Pinder M, Bojang K, Anyalebechi C, Gosling R, Coleman R, Ude JI, Sadiq A, Duraisingh M, Warhurst D, Allouche A, Targett A, McAdam K, Greenwood B, Walraven G, Olliaro P, Doherty T, 2000. Efficacy of artesunate plus pyrimethamine-sulphadoxine for uncomplicated malaria in Gambian children: a double-blind, randomised, controlled trial. *Lancet* 355: 352–357.
 10. Marquino W, MacArthur JR, Barat LM, Oblitas FE, Arrunátegui M, Garavito G, Chafloque ML, Pardavé B, Gutierrez S, Arróspide N, Carrillo C, Cabezas C, Ruebush TK II, 2003. Efficacy of chloroquine, sulfadoxine-pyrimethamine, and mefloquine for the treatment of uncomplicated *Plasmodium falciparum* malaria on the north coast of Peru. *Am J Trop Med Hyg* 68: 120–123.
 11. Ministerio de Salud, Peru, 1999. *Política Nacional de Medicamentos para el Control de la Malaria en el Perú*. Lima: MINSA.
 12. Bruce-Chwatt LJ, Black RH, Canfield CJ, Clyde DR, Peters W, 1986. *Chemotherapy of Malaria*. World Health Organization Monograph Series No. 27, Geneva: World Health Organization.
 13. Pan American Health Organization, 1998. *Evaluation of the Therapeutic Efficacy of Drugs for the Treatment of Uncomplicated Plasmodium falciparum Malaria in the Americas*. Washington, DC: Pan American Health Organization. OPS/HCP/HCT/113/98.
 14. Ministry of Public Health, Thailand, 1996. *Post-Registration Surveillance of the Artemisinin Derivatives Used Operationally in Thailand*. Nonthaburi, Thailand: Technical Division, Food and Drug Administration.
 15. Ribiero IR, Olliaro P, 1998. Safety of artemisinin and its derivatives: a review of published and unpublished clinical trials. *Med Trop (Mars)* 58: 50–53.
 16. Botero D, Restrepo M, Montoya A, 1985. Prospective double-blind trial of two different doses of mefloquine plus pyrimethamine-sulfadoxine compared with pyrimethamine-sulfadoxine alone in the treatment of falciparum malaria. *Bull World Health Organ* 3: 731–737.
 17. Reyes S, Osanai CH, Costa Passos AD, 1986. Resistência in vivo do *Plasmodium falciparum* as 4-aminoquinoleínas e á associação sulfadoxina-pirimetamina. II. Estudio de Manaus, Amazonas 1983-1984. *Rev Bras Mal Doencas Trop* 38: 37–44.
 18. Osorio LE, Giraldo LE, Grajales LF, Arriaga AL, Andrade AL, Ruebush TK, Barat LM, 1999. Assessment of the therapeutic response of *Plasmodium falciparum* to chloroquine and sulfadoxine-pyrimethamine in an area of low malaria transmission in Colombia. *Am J Trop Med Hyg* 61: 968–972.
 19. Nwanyanwu OC, Ziba C, Macheso A, Kazembe P, 2000. Efficacy of sulphadoxine-pyrimethamine for acute uncomplicated malaria due to *Plasmodium falciparum* in Malawian children under five years old. *Trop Med Int Health* 5: 355–358.
 20. White NJ, 1992. Antimalarial drug resistance: the pace quickens. *J Antimicrob Agents Chemother* 30: 571–585.
 21. Watkins WM, Mosobo M, 1993. Treatment of *Plasmodium falciparum* malaria with PSD: selective pressure for resistance is a function of long elimination half life. *Trans R Soc Trop Med Hyg* 87: 75–78.