1995

Treatment of Chloroquine-Resistant *Plasmodium vivax* with Chloroquine and Primaquine or Halofantrine

J. Kevin Baird
ALERTAsia Foundation, jkevinbaird@yahoo.com

Hasan Basri
US Naval Medical Research Unit 2, Jakarta, and Provincial Health Service, Jayapura, Irian Jaya, Indonesia

Budi Subianto
US Naval Medical Research Unit 2, Jakarta, and Provincial Health Service, Jayapura, Irian Jaya, Indonesia

David J. Fryauff
US Naval Medical Research Unit 2, Jakarta, and Provincial Health Service, Jayapura, Irian Jaya, Indonesia

Peter D. McElroy
US Naval Medical Research Unit 2, Jakarta, and Provincial Health Service, Jayapura, Irian Jaya, Indonesia

See next page for additional authors

Authors
J. Kevin Baird, Hasan Basri, Budi Subianto, David J. Fryauff, Peter D. McElroy, Budhi Leksana, Thomas L. Richie, Sofyan Masbar, F. Stephen Wignall, and Stehen L. Hoffman

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US Naval Medical Research Unit 2, Jakarta, and Provincial Health Service, Jayapura, Irian Jaya, Indonesia; Naval Medical Research Institute, Bethesda, Maryland

Optimal therapy for infection by chloroquine-resistant *Plasmodium vivax* has not been established. From 1992 to 1994 during three separate studies, 147 Javanese residents of Irian Jaya infected by *P. vivax* were treated with either chloroquine (25 mg of base/kg during 3 days or 10 mg of base/kg in one dose) plus primaquine (10 mg/kg during 28 days or 2.5 mg/kg during 3 days) ($n = 78$), chloroquine plus placebo ($n = 50$), or halofantrine (24 mg base/kg in 12 h; $n = 19$). There was no difference in tolerance to or side effects of any of the regimens. Within 14 days of starting therapy, therapeutic failure among these patients was 44% for chloroquine, 5% for chloroquine plus primaquine ($P < .001$), and 0 for halofantrine ($P < .001$). After 28 days, therapeutic failure was 78%, 15%, and 6%, respectively. Thus, chloroquine plus primaquine in combination and halofantrine alone are effective therapies for chloroquine-resistant *P. vivax*.

Resistance to chloroquine by *Plasmodium vivax* occurs in Southeast Asia and the southwest Pacific region [1, 2]. The infection develops despite standard chloroquine prophylaxis, and parasitemia persists or recurs within 14 days after initiation of standard chloroquine therapy. Alternative drugs for chloroquine-resistant *P. vivax* have not been evaluated. Resistance to chloroquine by *P. vivax* in the absence of specific therapies known to be effective threatens the health of the millions of people exposed to this infection. We investigated chloroquine plus primaquine in combination and halofantrine alone as therapies for chloroquine-resistant *P. vivax*.

Materials and Methods

Study sites and subjects. Between July 1992 and June 1994, studies were done at three villages in the Arso region of northeastern Irian Jaya, Indonesia, where *P. vivax* is endemic [3]. The villages were 7- to 18-month-old settlements of people from Java and other islands. Subjects were ≥6 years old with asexual

Received 26 September 1994; revised 7 February 1995.

Informed consent from patients or their parents or guardians was obtained from all subjects. A protocol describing the work was reviewed and approved by both American and Indonesian institutional committees for the protection of human subjects in medical research in accordance with US Navy regulation (SECNAVINST 3900.39B) governing the use of human subjects in medical research.

The views and opinions expressed herein are those of the authors and do not purport to reflect those of the US Navy.

Financial support: Naval Medical Research and Development Command (work unit nos. 623002A810.00101.HFX.1433 and 630624.HEX.2406.6.3a/1E).

Reprints or correspondence: LCDR J. K. Baird, US NAMRU #2–Jakarta, Box 3–American Embassy, APO AP 96520-8132 USA.

The Journal of Infectious Diseases 1995;171:1678–82

0022–1899/95/7106–0051$01.00

parasitemias of *P. vivax* demonstrable by light microscopy (>40 asexual parasites/μL of blood) of Giemsa-stained blood films read by experienced microscopists. A history of recent chloroquine consumption did not disqualify subjects. All subjects were screened for glucose-6-phosphate dehydrogenase deficiency using an NADPH spot test (Sigma, St. Louis).

Studies I and II. Two studies of identical design were done at two villages in 1992 and 1993. Patients infected with *P. vivax* received standard chloroquine (25 mg/kg in three doses over 48 h) and a placebo of primaquine (n = 27), standard chloroquine therapy plus 0.5 mg/kg primaquine base daily for 14 days followed by 0.5 mg/kg primaquine on alternate days for 13 days (n = 30), or 10 mg/kg chloroquine base in a single dose plus the regimen of primaquine above (n = 26). On day 0, all subjects began therapy with chloroquine (P.T. Bayer Indonesia, Jakarta; uncoated tablets, 150 mg of base). Receipt of daily primaquine (Sanofi-Winthrop, New York; sugar-coated tablets, 15 mg of base) or daily placebo (starch and amylose; Kimia Farma, Bandung, Indonesia) was randomized by blindly drawing a coded card. The code was unknown to subjects and to the research team.

A blood film for parasitologic examination and a 100-μL blot of blood on filter paper (no. 1; Whatman, Clifton, NJ) for assay of chloroquine were collected before administration of the first dose of chloroquine on day 0. A second 100-μL blot of capillary blood was collected on day 2, 4 h after administration of the last dose of chloroquine. Study subjects were examined on days 0, 1, 2, 4, 7, 11, 14, 18, 21, 25, and 28 or at any time that they again exhibited symptoms of malaria. Symptomatic patients (chills, fever, or malaise) with detectable parasitemia were given quinine therapy after obtaining a 100-μL blot of blood for measurement of blood chloroquine levels.

Study III. Based on results of studies I and II, a third study was done in the Arso region in 1994. Patients with *P. vivax* parasitemia were randomized to receive either standard chloroquine plus three doses of placebo during 48 h (n = 23), standard chloroquine plus three doses (two 1.0 mg/kg, one 0.5 mg/kg) of primaquine base at 24-h intervals (n = 22), or three doses of halofantrine (n = 19; SmithKline Beecham, UK; 500 mg of base, uncoated tablet) at 6-h intervals. Subjects were followed for 28 days and sampled as in studies I and II.

Assay of chloroquine in blood. Collection and analysis of whole blood specimens for chloroquine and desethylchloroquine was done as described [3] using high-performance liquid chromatography (Bio-Rad Laboratories, Richmond, CA).

Analysis of therapeutic outcome. Persistence or recurrence of *P. vivax* parasitemia within 28 days after starting therapy was considered a therapeutic failure. The cumulative incidence of therapeutic failure within groups and the ratio of incidence density (relative risk) among groups summarized outcomes. The significance of the relative risks and confidence intervals was obtained using Epistat 5.01b (CDC, Atlanta; WHO, Geneva). The significance of differences between means (geometric mean, log-transformed data) was estimated by the two-tailed Student's t test.

Subjects with intercurrent *Plasmodium falciparum* parasitemia remained in the analysis of the cohort up to the point of quinine therapy. In these instances, the product of 0.5 (assumed midinterval withdrawal) and the number of withdrawals per week was subtracted from the denominator used to calculate the cumulative incidence. The 14- or 28-day cumulative incidence of therapeutic failure was calculated using standard life table calculations of weekly intervals [4].

Results

Study subjects. Among chloroquine plus placebo, chloroquine (25 mg/kg) plus primaquine, and chloroquine (10 mg/kg) plus primaquine groups in studies I and II, subjects were predominantly male (23 vs. 7 female, 23 vs. 4, 25 vs. 1, respectively) and ranged in age from 6 to 51 years (means, 24, 21, and 23, respectively; P = .222). Geometric mean parasite counts were 650, 1307, and 1618 trophozoites/μL (P = .123), respectively. Eleven of the 83 subjects had mixed infections with *P. falciparum* and *P. vivax* at enrollment. Figure 1A shows the mean levels of chloroquine in blood at enrollment and on day 2 among groups.

Among chloroquine plus placebo, chloroquine (25 mg/kg) plus primaquine (2.5 mg/kg), and halofantrine groups in study III, subjects were predominantly male (17 vs. 5 female, 17 vs. 6, and 12 vs. 7, respectively) and ranged in age from 7 to 54 years (means, 26, 27, and 27, respectively; P = .377). Geometric mean parasite counts were 608, 555, and 367 trophozoites/μL (P = .1821), respectively. Five of the 64 subjects had mixed infections with *P. falciparum* and *P. vivax* at enrollment. Figure 1B shows the mean levels of chloroquine in blood at enrollment and on day 2 among groups.

Posttherapeutic parasitemias. All subjects in studies I and II receiving chloroquine plus primaquine cleared parasitemias within 5 days. Four patients receiving chloroquine plus placebo had asexual parasitemias that failed to clear by day 7.

All patients in study III cleared asexual parasitemias by day 4, and none reappeared by day 7. The first recurrent parasitemias appeared on day 11 in 2 patients who received chloroquine plus placebo.

Table 1 summarizes the cumulative incidence analysis of *P. vivax* therapeutic failure among subjects in the studies 14 and 28 days after the start of therapy. The relative risk of therapeutic failure among subjects taking chloroquine plus placebo was 8-fold higher than that among patients given chloroquine plus primaquine at both day 14 and day 28. When the data from the studies were combined, the 14-day crude failure rate, which excludes withdrawals from the analysis, was 44% among the 48 subjects given standard chloroquine therapy, 5% among the 74 subjects given primaquine, and 0 among the 19 subjects given halofantrine. The 28-day crude failure rate was 78% among the 45 subjects given standard chloroquine therapy, 15% among the 68 subjects given primaquine, and 6% among the 17 subjects given halofantrine.

Symptoms and signs after treatment. There was no indication that any treatment regimen was associated with more

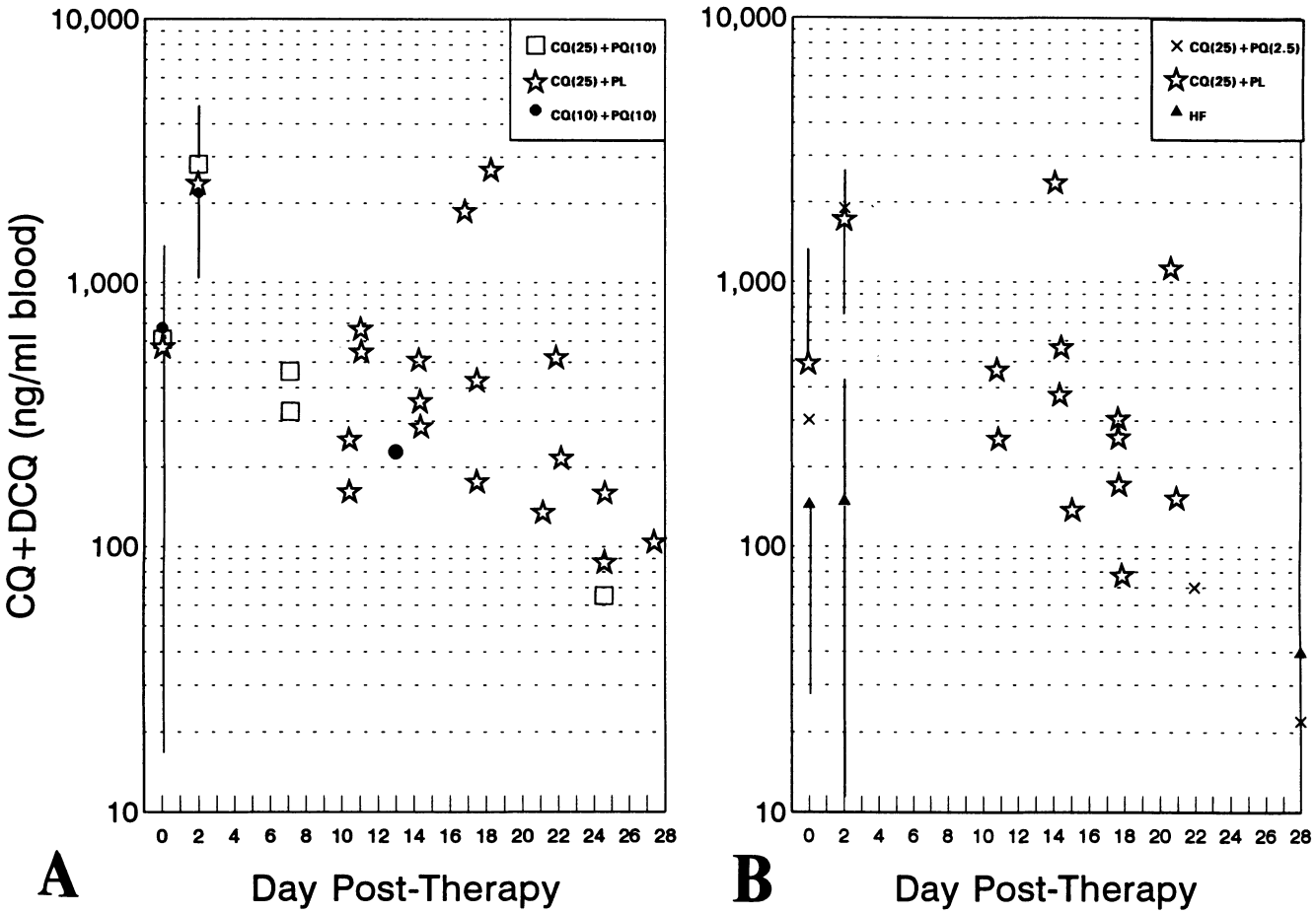

Figure 1. Measurements of chloroquine and desethylchloroquine among treatment groups in studies I and II (**A**) and III (**B**). Points at days 0 and 2 represent mean (±SD) chloroquine + desethylchloroquine level of groups (n = 17–22). All other points represent measurements of chloroquine + desethylchloroquine after onset of recurrent *P. vivax* parasitemia among individual subjects. CQ, chloroquine; PQ, primaquine; PL, placebo; HF, halofantrine. Numbers in parentheses refer to total doses (mg/kg).

side effects than any other regimen. Malaise, fever, chills, or headache persisted through the 48 h after starting therapy among 20% (6/30), 23% (6/26), and 22% (6/27) of subjects receiving chloroquine plus placebo, chloroquine (25 mg/kg) plus primaquine, and chloroquine (10 mg/kg) plus primaquine in studies I and II. There were also no differences in

Table 1. Treatment of chloroquine-resistant *P. vivax* with chloroquine, chloroquine plus primaquine, or halofantrine.

	14 days					28 days				
Study, treatment regimen (n)	No. withdrawing	No. with treatment failure	CIF (%)	RR (95% CI)	P*	No. withdrawing	No. with treatment failure	CIF (%)	RR (95% CI)	P*
Studies I and II										
CQ_{25} + PL (27)	2	14	53			4	20	80		
CQ_{25} + PQ_{10} (30)	2	2	7	8.38 (1.7–27.0)	.021	4	4	15	7.69 (4.5–14.0)	<.001
CQ_{10} + PQ_{10} (26)	1	2	8	7.63 (0.7–18.0)	.004	3	3	13	8.33 (4.5–17.0)	<.001
Study III										
CQ_{25} + PL (23)	0	7	31			1	15	69		
CQ_{25} + $PQ_{2.5}$ (22)	1	0	7	>7.0 —	.015	3	3	16	5.00 (2.9–9.0)	<.001
HF (19)	0	0	8	>7.0 —	.036	2	1	6	12.5 (5.3–33.0)	<.001

NOTE. CQ, chloroquine; PL, placebo; PQ, primaquine; HF, halofantrine; subscript numbers refer to total dose (mg/kg). Withdrawals were due to intercurrent parasitemia by *Plasmodium falciparum;* treatment failure, persistent or recurrent parasitemia by *P. vivax.* CIF, 14- or 28-day cumulative incidence of therapeutic failure. RR, risk of parasitemia in placebo group relative to PQ or HF groups (CI, confidence interval).

* Calculated by two-tailed Fisher's exact test.

relief of symptoms among the groups in study III (data not shown). Incidence density analysis revealed no differences among treatment regimens for overall symptoms during the remainder of the 28-day test ($P = .298$).

Chloroquine and desethylchloroquine blood levels. Figure 1 illustrates the sum of chloroquine and desethylchloroquine levels in whole blood before chloroquine therapy (day 0), 4 h after the last dose of supervised chloroquine therapy (day 2), and after the onset of recurrent parasitemia (days 7–28). At the time of enrollment, many subjects with *P. vivax* parasitemia had levels of chloroquine in their blood considered therapeutic (53% had >100 ng/mL chloroquine plus desethylchloroquine). Subjects had normal absorption of chloroquine (456–6257 ng/mL chloroquine plus desethylchloroquine at day 2; mean, 1932). Chloroquine levels before and after therapy were similar in the chloroquine plus primaquine and chloroquine plus placebo groups ($P = .578$). The day 2 mean level of chloroquine plus desethylchloroquine among subjects who received only 10 mg/kg chloroquine was indistinguishable from that among patients who received 25 mg/kg chloroquine plus primaquine or placebo ($P = .3422$). Most therapeutic failures occurred with >100 ng/mL chloroquine plus desethylchloroquine in whole blood. Ratios of chloroquine to desethylchloroquine (mean, 3.1) did not differ between primaquine and placebo on day 2 in studies I and II ($P = .450$) or III ($P = .558$).

Discussion

These studies demonstrate that the combination of 25 mg/kg chloroquine base and 2.5 mg/kg primaquine base administered in three divided doses during 48 h or 24 mg/kg halofantrine base administered in three divided doses during 12 h is effective treatment for chloroquine-resistant *P. vivax* malaria in Irian Jaya, Indonesia. The data also show that the chloroquine plus primaquine combination is as well-tolerated as chloroquine alone. The rate of therapeutic failure after treatment with a single dose of 10 mg/kg chloroquine base plus primaquine was similar to the resistance to 25 mg/kg chloroquine plus primaquine therapy. However, the levels of chloroquine on day 2 were similar between these groups, presumably because of the relatively high levels of chloroquine in the blood of most volunteers on day 0 due to self-medication. Therefore, we cannot conclude that a single dose of 10 mg/kg chloroquine in combination with primaquine would be adequate in persons with no chloroquine in their blood before therapy.

Primaquine at therapeutic doses has long been considered an exclusively tissue-schizonticidal drug, that is, eliminating infected hepatocytes without killing blood-stage asexual parasites [5]. After studies I and II, we considered the possibility that primaquine worked by preventing early relapse or reinfection but not by attacking infected erythrocytes. However, we believe that when primaquine is used with chloroquine against resistant *P. vivax*, the efficacy of this combination is due to activity directed against infected erythrocytes rather than infected hepatocytes. If the high therapeutic failure rate in the chloroquine plus placebo groups is linked to resistance to chloroquine by asexual blood forms of *P. vivax*, then superior efficacy of chloroquine plus primaquine against resistant blood-stage parasites would explain the low rate of therapeutic failure.

Several observations show that blood-stage resistance to chloroquine by *P. vivax* predominated among the patients in Irian Jaya. First, among 312 patients infected by *P. vivax* (most acquired naturally in tropical Asia and the South Pacific), none had a recurrent parasitemia before day 17 after initiating demonstrably effective quinine therapy [6–12]. Therefore, recurrent parasitemias within 14 days of starting chloroquine therapy, as among 44% of patients given chloroquine plus placebo, almost certainly represent recrudescence by *P. vivax*. Second, among 245 patients infected by *P. vivax* from the southwest Pacific during World War II and treated with chloroquine, none had recurrent parasitemia before day 30 after the start of therapy [6–12]. Therefore, recurrences within 30 days of starting chloroquine therapy constitute direct evidence of resistance; the 28-day cumulative incidence of recurrent parasitemia among the patients in Irian Jaya was 70% (crude failure rate of 78%, i.e., 35 failures among 45 complete tests). All except 3 recurrences of parasitemia in our studies occurred in the face of ordinarily suppressive levels of chloroquine in blood (figure 1).

Third, exposure of the study subjects to reinfection during our studies was much too low to account for the differences between the primaquine and placebo groups. In two studies, the incidence of *P. vivax* in the Arso region ranged from 0.6 to 1.25 infections/person-year [13]. Thus, among the 50 subjects in the chloroquine plus placebo groups (3.8 person-years of follow-up), 4 reinfections would be expected, whereas 35 recurrences appeared. In summary, relapse or reinfection could not account for the very large difference in therapeutic failure rates between the chloroquine plus placebo and chloroquine plus primaquine groups, both containing subjects infected with ~70% chloroquine-resistant *P. vivax*. The basis of that difference appears to be superior blood schizonticidal activity by chloroquine plus primaquine versus chloroquine plus placebo.

Combined chloroquine and primaquine therapy act additively or synergistically against chloroquine-resistant *P. vivax* infections. Studies of chloroquine and primaquine treatment of the chloroquine-resistant NS strain of *Plasmodium yoelii* in mice support the interpretation of synergy [14]. A clinical study of *P. vivax* in Thailand showed surprising blood schizonticidal activity of primaquine alone [15], but those patients could have had low levels of self-administered chloroquine in their blood. Determination of whether chloroquine and primaquine function additively or synergistically against chloroquine-resistant *P. vivax* and of how much activity pri-

maquine actually has on its own against chloroquine-resistant *P. vivax* must await further investigation.

Acknowledgments

We thank I. Wiady, S. Tirtokusumo, and Suradi, Awalludin, Ali, Iman, and Patriot for assistance in the conduct of the field studies and S. Harjosuwarno, B. Sandjaya in Jayapura and S. Gunawan in Jakarta (all Indonesian Ministry of Health) for providing essential help. M. James (Tulane University, New Orleans) guided J. K. B. through a Ph.D. program, much of the dissertation research for which appears here; D. Krogstad, M. Wiser, and F. Mather shared in this guidance.

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