CLINICAL LABORATORY PARAMETERS AMONG ADULT MALES DURING A PRIMAQUINE CHEMOPROPHYLAXIS TRIAL IN IRIAN JAYA, INDONESIA

David J. Fryauff[4], J. Kevin Baird[1], Allen L. Richards[1], Thomas L. Richie[1], Emiliana Tjitra[3], Eric Mouzin, Mochammad A. Sutamihardja[1], Sutanti Ratiwayanto[1], Hilda Hadiputranto[1], Ria P. Larasati[1], Nurani Pudjoprawoto[1], Budi Subianto[2], Bartolomeus Sandjaya[2], F. Stephen Wignall[1], and Stephen L. Hoffman[4]

ABSTRAK

PARAMETER LABORATORIUM KLINIK DARI PRIA DEWASA PADA WAKTU UJI COBA KEMOPROFILAKSIS PRIMAKUIN DI IRIAN JAYA, INDONESIA

Primakuin yang digunakan sebagai profilaksis malaria terbukti efektif dan diterima dengan baik oleh tubuh manusia yang normal terhadap aktivitas enzim 6 glukosa-6 fosfat dehidrogenase (G-6PD). Pemeriksaan laboratoris klinik adalah bagian dari uji coba secara acak dengan kontrol plasebo dalam rangka mengevaluasi penggunaan primakuin sebagai profilaksis pada penduduk transmigran yang tidak kebal di Irian Jaya.

Penelitian ini dilakukan terhadap 129 pria Jawa dewasa yang normal G-6PDnya. Pemeriksaan hematologi, fungsi hati dan ginjal, dan pemeriksaan limfosit dilakukan berulang kali selama waktu penelitian profilaksis dilakukan untuk menjamin keamanan dari sukarelawan tersebut dan mengawasi perubahan yang mungkin terjadi akibat obat profilaksis.

Seperti yang diperkirakan, pengguna primakuin tidak menunjukkan gejala peningkatan methemoglobin yang kembali dalam batas normal setelah 7 hari pemberian dosis terakhir. Pada akhir penelitian (12 bulan profilaksis) nilai hematologi, fungsi hati dan ginjal, dan nilai limfosit dari kelompok primakuin sebanding dengan kelompok plasebo, dan berada dalam batas nilai normal untuk orang Indonesia.

Hasil penelitian ini memberikan masukan adanya keluhan fisik yang sedikit dari sukarelawan pengguna profilaksis primakuin. Untuk membuktikan hasil penelitian ini dan mempersiapkan penggunaan secara umum primakuin untuk profilaksis malaria, perlu dilakukan uji coba lebih lanjut keamanan primakuin. Di Indonesia, primakuin tidak digunakan sebagai profilaksis dan laporan hasil penelitian ini hendaknya tidak ditafsirkan sebagai laporan keamanan dari primakuin.

---

1 U.S. Naval Medical Research Unit No. 2 Jakarta, Indonesia
2 Provincial Health Service, Jayapura, Irian Jaya, Indonesia
3 Communicable Diseases Research Centre, NIHRD, Jakarta, Indonesia
4 U.S. Naval Medical Research Institute, Bethesda, Maryland.

INTRODUCTION

Transmission of *Plasmodium falciparum* and *P. vivax* is intense throughout much of Irian Jaya, Indonesia.[1-4] Multi-drug resistant *P. falciparum*,[5-7] and chloroquine-resistant *P. vivax*[8,9] are documented from the Arso region of this province and seriously threaten the health and development of nonimmune transmigrant communities. Cross-resistance and high cost limit the use of many currently available antimalarial drugs for prophylaxis. New drug development is a slow, expensive process with unprofitable returns from the populations that could most benefit from malaria prevention. Against these obstacles we re-evaluated primaquine as an immediately available, low cost alternative to chloroquine for malaria chemoprophylaxis.

Primaquine has been the drug of choice for radical cure and terminal treatment of relapsing vivax malaria for nearly half a century. Primaquine is also gametocytocidal against all species of human malaria and its use has been advocated to reduce community transmission of falciparum malaria. Owing to its activity against the early liver stages of malaria parasites, primaquine has the potential to act as a causal prophylactic as well.[10,11] The Naval Medical Research Unit No. 2, in collaboration with the Infectious Diseases Research Center of the Indonesian Ministry of Health (Badan Penelitian dan Pengembangan Kesehatan, Departmen Kesehatan R.I.), and the Provincial Health Service, Jayapura, Irian Jaya, R.I. recently carried out chemoprophylaxis trials in Irian Jaya transmigration settlements that showed primaquine to be highly efficacious and well tolerated when used by non-pregnant individuals with normal glucose-6-phosphate dehydrogenase (G6PD) activity. The first of these trials took place in Arso X during December 1992 to April 1993 and compared an alternate day regimen of primaquine (0.5 mg base/kg) against weekly chloroquine for prevention of malaria in nonimmune Javanese transmigrants.[12] The relative efficacy of primaquine in this trial was 74% against *P. falciparum* and 90% against *P. vivax*. Compared with people using chloroquine, there were significantly fewer physical complaints among people in the primaquine group and no signs of toxicity during the period of drug use.

These encouraging results prompted a second, more detailed trial which sought to identify a more protective primaquine regimen and to evaluate the long-term tolerance of this drug. This second Irian Jaya trial randomized nonimmune adult male transmigrants of Arso XI to receive either daily primaquine, weekly chloroquine, or a daily placebo over a 12 month period. Chloroquine, the standard antimalarial provided to new transmigrants, was not significantly better than placebo in protecting against either falciparum or vivax malaria in this trial but daily primaquine provided >94% protection against falciparum and >90% protection against vivax malaria. Importantly, there was no difference between primaquine, placebo, and chloroquine in the occurrence of physical complaints and non-malarial illness.[13] This trial offered an additional opportunity to confirm the tolerance and safety of extended primaquine use and significant efforts were made to deter any side effects. This paper reviews and compares the results of repeated clinical laboratory testing that was conducted.

METHODS

Study site and subjects

The study took place in Arso XI, located 50 km south of Jayapura, Irian Jaya, during the period July 1994 and March 1995. After providing informed written consent, 129 adult male Javanese volunteers were tested to confirm normal G6PD activity, curatively treated for

malaria, and randomized to receive either weekly chloroquine (300 mg base 1 time weekly), daily primaquine (0.5 mg base/kg daily; 30 mg daily for men >55 kg) or a daily placebo over a 12 month period. The primaquine dosage in this trial was based on demonstrated safety and efficacy against the primaquine tolerant vivax malaria that occurs in the Indo-Pacific region.[14] Drug was always given after a meal and consumption was witnessed. At 20 and 40 weeks of prophylaxis, laboratory test results and volunteer health were reviewed by committees for protection of human subjects to determine and approve continuation of the prophylaxis trial. Continuation was also contingent upon informed written consent of all volunteers at both the 20 and 40 week decision points of prophylaxis. Physical complaints were recorded weekly by response to a list of questions read to each volunteer and by record of visits made to the on-site health clinic for any illness and injury. An exit questionaire was administered to all volunteers at the 52 week end point of prophylaxis. All medications and health needs were provided without cost. Volunteers were encouraged to self-report any illness and injury.

Hematology

Complete blood counts (hematocrit, hemoglobin, WBCs, granulocytes, lymphocytes + monocytes, platelets) were conducted on all volunteers on day 0 and at 1, 10, 20, 40, and 52 weeks of prophylaxis using automated CBC methodology (QBC Series Centrifugal Hematology System^{R}, Becton Dickinson, Sparks, MD). Methemoglobin testing by the Evelyn-Malloy spectrophotometric method[15] was performed on all subjects during week 50 of prophylaxis and 7 days after the final dose of drug.

Hepatic and renal function testing

A cross-sectional sample of volunteers (30%) was tested repeatedly for hepatic and renal function (urea, bilirubin, total protein, albumin, creatinine, alanine aminotransferase [ALAT], aspartate aminotranferase [ASAT], and alkaline phosphatase [ALP]) on day 0 and at 10, 20, 40, and 52 weeks of prophylaxis according to recommended spectrophotometric methods (Boehringer Mannheim GmbH Diagnostica, Mannheim, Federal Republic of Germany). Dipstick urinalysis testing (Chemstrip-8^{R}, Boehringer Mannheim Diagnostics, St. Louis, MO) was performed on all volunteers at the 52 week end point of prophylaxis.

Lymphocyte function and subset composition

A cross-sectional sample of volunteers (30%) was tested for lymphocyte function and subset composition at 10 and 52 weeks of prophylaxis. Lymphocyte function was assessed by measuring the in vitro proliferative response of peripheral blood mononuclear cells (PBMC) against a panel of mitogens (PHA, PWM, ConA) and antigens (PPD, tetanus toxoid) according to current standard methodology.[16] Lymphocyte subsets (total T cells, T helper, T effector, total B cells, NK cells, activated T cells) were enumerated by flow cytometry using Becton Dickinson Simultest^{R} methodology and a flourescent activated cell sorter (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Data analysis

Prophylaxis group test results were compared by one way analysis of variance and Bartlett's test for homogeneity of variance. Kruskal-Wallis non-parametric test was applied to data not normally distributed. Selected 2-sample comparisons used Student's t-test for normally distributed data or the Mann-Whitney U test for data not normally distributed. Two-tailed p values are reported.

RESULTS

Within group comparisons between sampling times (baseline, 1, 10, and 20 weeks prophylaxis), and within time comparisons between groups (placebo, primaquine, chloroquine) detected only minor hematologic changes (Figure 1). Platelet counts were depressed during the first week in which the quinine-doxycycline-primaquine radical cure was administered and hematocrit/ hemoglobin levels increased from baseline in all groups. Statistical comparison between primaquine and placebo groups for hematologic, renal, and hepatic test values at the week 52 endpoint of prophylaxis identified a statistically significant increase over placebo in the mean value of only urea (Table 1). Despite this difference, all values for urea in the primaquine group were within the normal range. Comparison between baseline and endpoint values within the primaquine group identified significant increases ($p<0.05$) over baseline in hemoglobin, % monocytes, serum protein, and ALP (Table 2). This within group comparison revealed significant ($p<0.002$) reductions from baseline in % granulocytes, ASAT, and ALAT, but these changes did not fall outside the normal range (Table 2), and baseline measures may have been abnormally skewed by malaria infections (38%) at the time of enrollment. Endpoint qualitative urinalysis did not yield results indicative of differences between placebo, primaquine, and chloroquine groups (data not shown).

Primaquine did induce asymptomatic methemoglobinemia. After 50 weeks of prophylaxis, the mean percent methemoglobin in the primaquine group (5.8%, SD 2.9%) was significantly higher ($p<0.001$) than in either chloroquine (0.7%, SD 0.4%), or placebo (1.2%, SD 0.7%) groups. Methemoglobin measures among primaquine users ranged from 1.4% to 13% (Figure 2). Seven days after the final prophylactic dose of primaquine, mean percent methemoglobin for this group had declined significantly ($p<0.0001$) to 2.4% (SD 1.2%, range 0-4.5%). Methemoglobin level decreased in 27 of 30 subjects and the percent decline from week 50 values averaged 51% (range 21-100%) for the group (Figure 2).

Laboratory assay of lymphocyte function and subset composition were intentionally delayed until week 10 of prophylaxis to achieve a more valid detection of prophylaxis-induced changes and to avoid possible confounding effects caused by malaria infection and radical curative therapy. Lymphocyte function in prophylaxis groups, as measured by proliferative response to mitogen (ConA, PHA, PWM) and antigen (PPD) stimulants is presented as geometric mean stimulation indices (GMSI) in Tables 3 and 4. Statistical comparison of GMSI values revealed no significant differences between primaquine, placebo, or chloroquine groups after 10 weeks of prophylaxis (Table 3). Lymphocyte function at the week 52 endpoint of prophylaxis, as measured by proliferative response to mitogen (PHA) and antigen (PPD, Tetanus toxoid) stimulants was also not significantly different among the three groups (Table 4). Lymphocyte subset composition (total T cells, total B cells, T helper, T effector, T helper:T effector ratio, NK cells, and activated T cells) was comparable among placebo, primaquine, and chloroquine groups at both the 10 and 52 week sampling points. Notably, the subset composition in each of the prophylaxis groups fell within a normal ethnic range derived from a larger (n=68) sample population of healthy Javanese-Sundanese males.

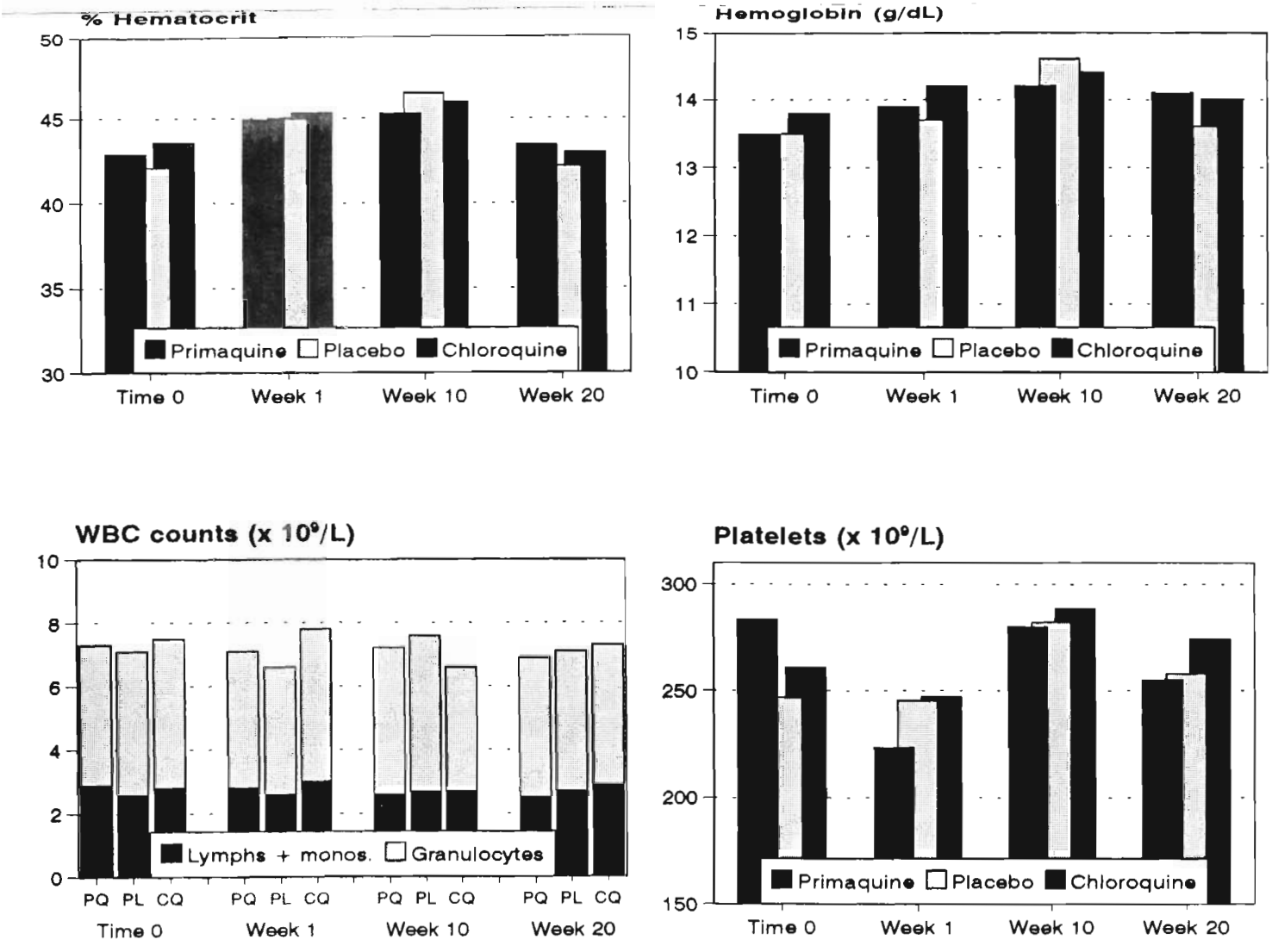

Figure 1. Hematology (CBC): Comparison between prophylaxis groups and sampling times.

Table 1. Clinical laboratory test values for placebo and primaquine groups at the 52 week endpoint pf prophylaxis.

Characteristic	Placebo* (n=22)	Primaquine* (n=29)	Normal Range	P value**
Hemoglobin (g/dL)	14.6 (1.1)	14.7 (1.0)	14-18	0.72
White blood cells (x 10^9/L)	6.6 (1.7)	7.3 (2.2)	4.3-10.0	0.23
Granulocytes (%)	50.9 (10.9)	52.2 (11.9)	42-77	0.71
Lymphocytes + Monocytes (%)	49.1 (10.9)	47.8 (11.9)	40-50	0.71
Platelets (x 10^9/L)	305.0 (84.4)	291.5 (100.4)	140-400	0.63
Urea (mg/dL)	26.4 (5.4)	30.6 (7.7)	10-50	0.03
Bilirubin (mg/dL)	0.2 (0.1)	0.3 (0.1)	0.2-1.0	0.36
Total protein (g/dL)	8.3 (0.6)	8.2 (0.5)	5.3-8.7	0.32
Albumin (mg/dL)	5.2 (0.5)	5.3 (0.8)	3.3-5.8	0.44
Creatinine (mg/dL)	1.1 (0.1)	1.1 (0.2)	0.6-1.2	0.79
ALAT (U/L)	18.7 (17.4)	21.5 (17.9)	<30	0.58
ASAT (U/L)	24.7 (25.9)	33.6 (34.5)	<40	0.31
ALP (U/L)	144.8 (38.1)	161.5 (39.3)	60-207	0.13

* Values given as mean (SD)

** Unpaired comparison by t-test or Mann-Whitney U-test.

Table 2. Clinical laboratory test values for daily primaquine group: Baseline vs. week 52 of prophylaxis.

Characteristic	Baseline* (n=27)	Endpoint* (n=29)	P value**	Normal range
Hemoglobin (g/dL)	13.5 (1.8)	14.7 (1.0)	0.0002	14 - 18
White blood cells (x 10^9/L)	7.2 (2.1)	7.3 (2.2)	nsd	4.3 - 10.0
Granulocytes (%)	61.2 (10.3)	52.2 (11.2)	0.002	42 - 77
Lymphocytes + Monocytes (%)	40.2 (13.9)	47.8 (11.8)	0.025	40 - 50
Platelets (x 10^9/L)	283.5 (77.0)	291.5 (100.4)	nsd	140 - 400
Urea (mg/dL)	26.4 (10.4)	30.6 (7.7)	nsd	10 - 50
Bilirubin (mg/dL)	0.3 (0.1)	0.3 (0.1)	nsd	0.2 - 1.0
Total protein (g/dL)	7.6 (0.9)	8.2 (0.5)	0.004	5.3 - 8.7
Albumin (mg/dL)	5.0 (0.9)	5.3 (0.8)	nsd	3.3 - 5.8
Creatinine (mg/dL)	1.1 (0.2)	1.1 (0.2)	nsd	0.6 - 1.2
ALP (U/L)	139.5 (39.6)	161.5 (39.3)	0.042	60 - 207
ASAT (U/L)	41.7 (15.2)	33.6 (34.5)	<0.0005	< 40
ALAT (U/L)	32.4 (12.8)	21.5 (17.9)	0.0003	< 30

* Values given as mean (SD)

** Unpaired comparison by t-test or Mann-Whitney U-test; nsd if $P > 0.05$..

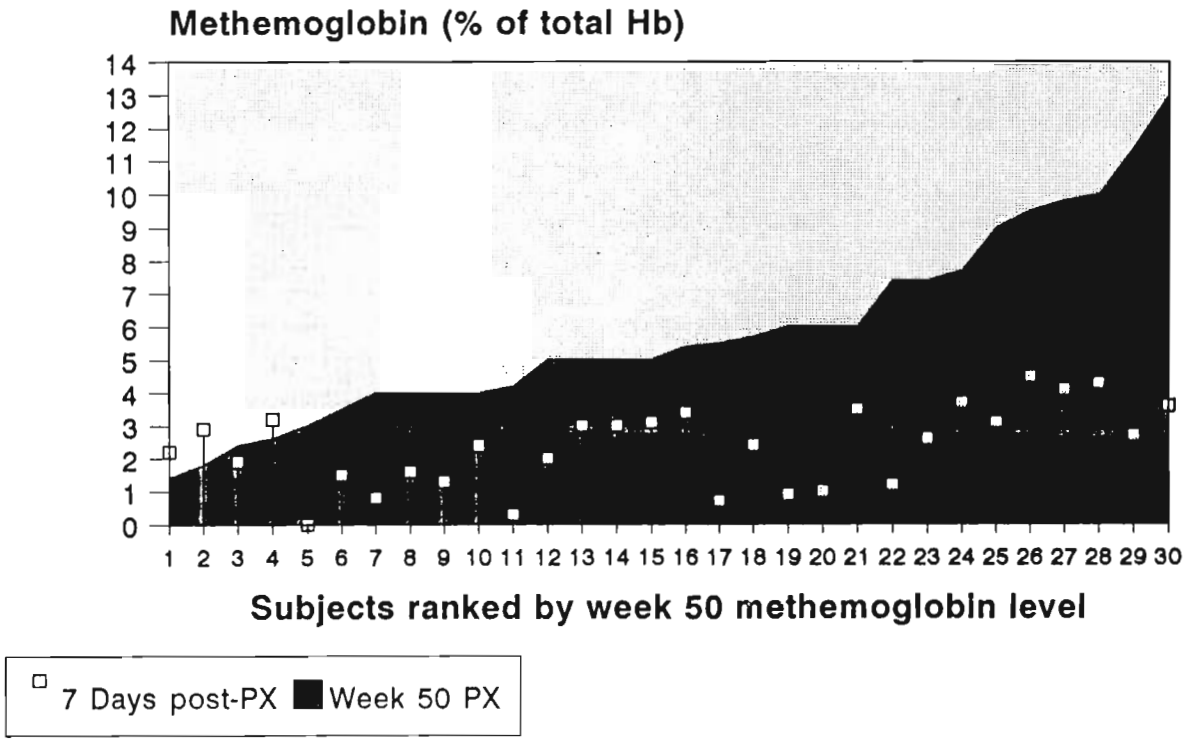

Figure 2. Paired comparison of methemoglobin levels in primaquine users (N=30) during week 50 prophylaxis and 7 days after ending prophylaxis.

Table 3. Lymphocyte function* among test groups after 10 weeks of chemoprophylaxis.

Description	Placebo (n=17)	Primaquine (n=15)	Chloroquine (n=14)
PWM			
1:400	21.4 ± 29.4	29.5 ± 30.2	39.7 ± 55.4
1:1200	24.5 ± 31.7	29.0 ± 25.2	37.3 ± 45.4
1:3600	25.1 ± 31.4	29.8 ± 24.3	32.0 ± 37.2
PHA			
10 ug/ml	4.6 ± 6.6	7.0 ± 5.3	5.4 ± 5.3
3.3 ug/ml	4.5 ± 6.4	4.9 ± 4.6	5.1 ± 5.6
1.1 ug/ml	1.4 ± 1.8	1.3 ± 1.3	1.3 ± 0.6
ConA			
12.5 ug/ml	13.4 ± 21.4	14.7 ± 14.7	11.8 ± 14.0
1.25 ug/ml	8.9 ± 16.8	10.0 ± 10.9	4.9 ± 5.6
PPD			
10 ug/ml	10.5 ± 15.2	7.6 ± 6.8	8.9 ± 12.4
3.3 ug/ml	8.3 ± 12.6	5.5 ± 4.7	4.5 ± 7.7
1.1 ug/ml	5.8 ± 9.4	3.3 ± 2.9	3.6 ± 5.9

* Geometric Mean Stimulation Index (cpm stimulated/cpm control) ± SD.

Table 4. Lymphocyte function* among test groups at the 52 week endpoint of chemoprophylaxis.

Description	Placebo (n=21)	Primaquine (n=30)	Chloroquin (n=21)
PHA			
5 ug/ml	32.2 ± 2.3	29.0 ± 2.9	18.0 ± 6.5
2.5 ug/ml	9.2 ± 7.1	14.4 ± 3.7	13.6 ± 2.9
PPD			
10 ug/ml	5.5 ± 3.3	7.3 ± 2.6	5.6 ± 3.6
5 ug/ml	4.5 ± 5.3	7.2 ± 2.3	5.4 ± 3.0
Tetanus			
10 ug/ml	1.3 ± 1.9	2.2 ± 2.6	1.9 ± 2.4
1 ug/ml	0.9 ± 1.7	1.3 ± 2.4	0.9 ± 2.5
0.1 ug/ml	1.3 ± 1.7	1.8 ± 2.5**	1.0 ± 2.3

* Geometric Mean Stimulation Index (cpm stimulated/cpm control) ± SD

** Significantly different ($p<0.05$).

Table 5. Lymphocyte subset composition* among test groups after 10 weeks of chemoprophylaxis.

Description	Placebo (n=17)	Primaquine (n=15)	Chloroquine (n=14)	Normal Range** (Indonesian)
CD3 (Total T cell)	65.1 ± 9.0	71.1 ± 7.6	69.4 ± 11.5	61.0 ± 10.3
CD19 (B cells)	13.9 ± 4.3	12.3 ± 3.7	13.3 ± 4.2	13.2 ± 5.0
CD4 (T helper/inducer)	34.5 ± 6.1	33.4 ± 5.2	33.2 ± 7.2	30.2 ± 7.4
CD8 (T suppressor/cytotoxic)	38.9 ± 5.7	37.3 ± 6.1	39.3 ± 8.8	43.0 ± 8.0
CD4:CD8 ratio	0.91 ± 0.22	0.94 ± 0.23	0.88 ± 0.24	0.75 ± 0.33
CD3/CD16+CD56 (NK cells)	21.2 ± 8.7	17.3 ± 8.9	23.7 ± 15.4	26.6 ± 11.2
CD3/Anti-HLA-DR (Activated T cell)	16.2 ± 8.7	14.9 ± 7.6	21.4 ± 10.2	21.0 ± 6.4

* Mean percentages ± SD
** Healthy male Javanese-Sundanese (n=68).

Table 6. Lymphocyte subset composition* among test groups at 52 week endpoint of chemoprophylaxis.

Description	Placebo (n=15)	Primaquine (n=27)	Chloroquine (n=9)	Normal Range** (Indonesian)
CD3 (Total T cell)	70.2 ± 8.1	69.8 ± 9.1	68.6 ± 7.2	61.0 ± 10.3
CD19 (B cells)	14.7 ± 5.8	12.8 ± 4.0	13.4 ± 5.6	13.2 ± 5.0
CD4 (T helper/inducer)	36.2 ± 8.7	36.6 ± 6.0	35.4 ± 4.8	30.2 ± 7.4
CD8 (T suppressor/cytotoxic)	38.9 ± 8.8	39.8 ± 7.9	41.8 ± 5.3	43.0 ± 8.0
CD4:CD8 ratio	1.04 ± 0.51	0.99 ± 0.29	0.91 ± 0.22	0.75 ± 0.33
CD3/CD16+CD56 (NK cells)	22.2 ± 11.3	26.3 ± 14.1	23.6 ± 14.8	26.6 ± 11.2
CD3/Anti-HLA-DR (Activated T cell)	20.5 ± 12.7	22.6 ± 7.4	19.0 ± 4.6	21.0 ± 6.4

* Mean percentages ± SD
** Healthy male Javanese-Sundanese (n=68).

DISCUSSION

Although primaquine has been in use for nearly 50 years, its protective efficacy and tolerance as a primary prophylactic against falciparum and vivax malaria has only recently been evaluated under natural conditions of exposure.[12,17] In these trials, primaquine was shown to be an effective, well-tolerated prophylactic with the advantage over other currently recommended antimalarial chemoprophylactics of low cost and immediate availability. Unlike most antimalarials which target the blood stages of malaria, primaquine is active against the primary liver stages and is probably not required after leaving the area of exposure.[17] Furthermore, the rapid metabolism of primaquine, combined with its double action against both tissue schizont and gametocyte stages, would seemingly give malaria parasites very little chance for selection of resistance genotypes.

There are a number of factors that must be realized and controlled for to ensure safe, efficacious use of primaquine for malaria prophylaxis: 1) Rapid metabolism of the drug requires that it be used on a daily or alternate day schedule, 2) Primaquine must only be used by individuals with proven, normal G6PD activity, 3) Primaquine has not yet been tested and proven safe for use during pregnancy, 4) It is necessary that each dose be taken with food to reduce the chances of stomach upset, and 5) Primaquine users should be aware of or monitored for signs and symptoms of methemoglobinemia.[10,11,18,19] Immune suppression, a side effect of many antimalarial drugs,[10,20] was also considered to be a potential drawback of primaquine.[21]

The toxicology of primaquine in animals and man has been studied in depth and a large body of accumulated safety data supported our prophylaxis study.[17,22,23] Extended daily and alternate day use of primaquine has previously been shown to have no effect on hematologic, hepatic, and renal parameters that were measured in G6PD normal volunteers. Nevertheless, hemolytic reactions, hepatotoxicity, and methemoglobinemia have been manifestations of primaquine intoxication and were the primary focus of our testing program. The levels of asymptomatic methemoglobinemia detected after 12 months of primaquine use and the consistently normal measures obtained for hematologic, hepatic and renal function throughout the study period are signal findings that lend support for the safety and acceptability of this drug. It is notable that methemoglobin levels we detected during the twelfth month of primaquine prophylaxis were not significantly different from those reported for subjects given the usual 14 day treatment of primaquine at half the dosage we employed.[18]

The negative results we obtained in repeated tests of lymphocyte function and subset composition were not anticipated but are clearly ideal. Primaquine is credited with distortions of T cell populations and potential immune suppression.[10,11,20-23] This nonacute toxicity has not been well characterized and has not been a major concern given the current modality and low frequency of primaquine use, but over an extended period of prophylactic use, such a subtle, chronic effect on lymphocytes, if present, could have profound effects. Our negative results provide important new in vivo measures of long-term prophylaxis effect that document the immunologic safety of primaquine. Because we saw an unexpected trend toward primaquine-induced stimulation of lymphocyte response, this line of investigation is continuing with studies aimed at determining the interaction of primaquine prophylaxis and vaccination.

In summary, the collective clinical laboratory results of hematologic, hepatic, renal, and immune response testing herein reported provide compelling preliminary support for the safety of primaquine as a daily chemoprophylactic drug against malaria. Although cheap and available, primaquine is not approved or recommended for prophylactic use in Indonesia. This research report must not be construed as a definitive statement of primaquine safety or an official viewpoint of acceptance. It is important that further safety testing be conducted to verify the results we have obtained and to precede licensure of the drug for general use.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the help of many officials of the Indonesian Ministry of Health that assisted this research effort. Special thanks are extended to Dr. Slamet Harjosuwarno in Jayapura, and Drs. Suriadi Gunawan, Sri Oemiyati, Hariyani Marwoto, and P.R. Arbani in Jakarta. We also thank Drs. Hendra Widjaya, Ating Solihin, Dennis Shanks, and Ronald Anthony for valuable field assistance and to the supporting staff of NAMRU-2 parasitology, tropical medicine, fiscal, and supply departments. Financial support for this study was from the U.S. Naval Medical Research and Development Command work unit numbers 620828, 6281453E033 and 6281453UO52. This work was conducted in accordance with U.S. Navy and Republic of Indonesia regulations governing the protection of human subjects in medical research. American and Indonesian committees for the protection of human subjects reviewed and approved the procedures followed in this research. The views of the authors expressed herein do not purport to reflect those of the U.S. Navy, The U.S. Department of Defense, or the Indonesian Ministry of Health.

REFERENCES

1. Metselaar D. (1956). Spleens and holoendemic malaria in West New Guinea. *Bull World Health Organ* **15**: 635-649.

2. Anthony RL, Bangs MJ, Hamzah N, Basri H, Purnomo, Subianto B (1992). Heightened transmission of stable malaria in an isolated population in the highlands of Irian Jaya, Indonesia. *Am J Trop Med Hyg* **47**: 346-356.

3. Lee VH, Atmosoedjono S, Aep S, Swaine CD, (1980). Vector studies and epidemiology of malaria in Irian Jaya, Indonesia. *SE Asian J Trop Med Publ Hlth* **11**: 341-347.

4. Jones TR, Baird JK, Basri H, Purnomo, Danudirgo EW. (1991). Prevalence of malaria in native and transmigrant populations. Effects of age and history of exposure. *Trop Geogr Med* **43**: 1-6.

5. Baird JK, Basri H, Jones TR, Purnomo, Bangs MJ, Ritonga A. (1991). Resistance to antimalarials by Plasmodium falciparum in Arso PIR, Irian Jaya, Indonesia. *Am J Trop Med Hyg* **44**: 640-644.

6. Clyde DF, McCarthy VC, Miller RM, Hornick RB. (1976). Chloroquine-resistant falciparum malaria from Irian Jaya (Indonesian Guinea). *J Trop Med Hyg* **79**: 38-41

7. Hoffman SL, Rustama D, Dimpudus AJ, Punjabi NH, Campbell JR, Oetomo HS, Marwoto HA, Harun S, Sukri N, Heizmann P, Laughlin LW. (1985). RII and RIII type resistance of *Plasodium falciparum* to combination of mefloquine and sulfadoxine/pyrimethamine in Indonesia. *Lancet* ii.1039-1040.

8. Baird JK, Basri H, Purnomo, Bangs MJ, Patchen LC, Hoffman SL. (1991) Resistance to chloroquine by *Plasmodium vivax* in Irian Jaya, Indonesia. *Am J Trop Med Hyg* **44**: 547-552.

9. Murphy GS, Basri H, Purnomo, Anderson EM, Bangs MJ, Mount DL, Gorden J, Lal AA, Purwokusumo AR, Harjosuwarno S, Sorensen K, Hoffman SL. (1993). Vivax malaria resistant to treatment and prophylaxis with chloroquine. *Lancet* **341**: 96-100.

10. Carson PE. 8-aminoquinolines. (1984). In Peters W, Richards WHG, eds., Antimalarial Drugs II. Handbook of Experimental Pharmacology, Volume 68. Berlin: Springer-Verlag, pp 83-121.

11. Peters W. (1987). Chemotherapy and drug resistance. In: malaria, Vol. 2, 2nd. ed. London. Academic Press.

12. Baird JK, Fryauff DJ, Basri H, Bangs M et al. (1995). Primaquine for prophylaxis against malaria among nonimmune transmigrants in Irian Jaya, Indonesia. *Am J Trop Med Hyg* **52**: 479-484.

13. Fryauff DJ, Baird JK, Basri H, Sumawinata IW, Ohrt CK, et al. (1994). A double blinded placebo-controlled evaluation of primaquine for causal prophylaxis against drug-resistant malaria in Indonesia. 43rd Ann. Meeting of the American Society of Tropical Medicine and Hygiene, Cincinnati, OH.

14. Clyde DF, McCarthy VC. (1977). Radical cure of Chesson strain vivax in man by 7, not 14 days of treatment with primaquine. *Am J Trop Med Hyg* **26**: 562-563.

15. Beutler E. (1983) Carboxyhemoglobin, methemoglobin, and sulfhemoglobin determinations. In: Williams WJ, Beutler E, Erslev AJ, Lichtman MA, eds., Hematology, 3rd. ed. McGraw-Hill. pp 1632-34.

16. Fletcher MA, N Klimas, R Morgan & G Gjerset. (1992). Lymphocyte Proliferation. In: Rose NR, de Macario EC, Fahey JL, Friedman H, Penn GM, eds., Manual of Clinical Laboratory Immunology, 4th ed. American Society for Microbiology, Washington, DC. pp 213-219.

17 Weiss WR, Oloo AJ, Johnson A, Koech D, Hoffman SL. (1995). Daily primaquine is an effective prophylaxis against falciparum malaria in Kenya: Comparison with mefloquine, doxycycline, and chloroquine/proguanil. *J Inf Dis* (In Press).

18. Fletcher KA, Evans-Price DA, Giles HM, Greaves J, Bunnag D, Harinasuta T (1981). Studies on the pharmacokinetics of primaquine. *Bull Wld Hlth Org* **59**: 407-12.

19. Clyde DF. (1981). Clinical problems associated with the use of primaquine as a tissue schizonticidal and gametocytocidal drug *Bull Wld Hlth Org* **59**: 391-95.

20. Salmeron G, Lipsky PE. (1983). Immunosuppressive potential of antimalarials *Am J Med* **75**: 19-24.

21 Thong YH, Ferrante A, Rowan-Kelly B. (1978). Primaquine inhibits mitogen-induced human lymphocyte proliferative responses. *Trans R Soc Trop Med Hyg* 72: 537-539.

22. UNDP/World Bank/WHO (1984). Primaquine: Pharmacokinetics, metabolism, toxicity and activity. Wernsdorfer WH, Trigg PI, eds. John Wiley & Sons, NY

23. Greval RS. (1981) Pharmacology of 8-aminoquinolines. *Bull Wld Hlth Org* 59: 397-406.