Randomised placebo-controlled trial of primaquine for prophylaxis of falciparum and vivax malaria

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Summary

Drug resistance has made malaria prevention difficult and the new agents are too expensive for widespread use. Primaquine, an established drug for treatment, is potentially useful for prevention. Malaria prophylaxis with primaquine was evaluated in Irian Jaya during one year in Javanese men who were not deficient in glucose-6-phosphate dehydrogenase (G-6-PD). 126 volunteers were randomised to receive 0·5 mg/kg primaquine base or placebo daily (double-blinded), or 300 mg chloroquine base weekly (open).

The protective efficacy of primaquine relative to placebo was 94·5% (95% confidence interval 57–99) for *Plasmodium falciparum* and 90·4% (95% CI 58–98) for *P vivax*. Attack rates for either parasite did not differ significantly between the chloroquine and placebo groups. Incidence density of physical complaints not associated with parasitaemia was low (17–18 complaints/person-year) and was about the same in all groups except for cough, which was increased in the primaquine group. Complete blood counts were normal and no evidence of hepatic or renal dysfunction was found with primaquine. However, at 50 weeks the primaquine group had a mean methaemoglobin of 5·8% (range 1·4–13%), which declined by half within 7 days of ending prophylaxis.

When used daily for one year by men with normal G-6-PD activity, primaquine was well tolerated and effective for prevention of malaria.

Lancet 1995; **346:** 1190–1193

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Introduction

Chemoprophylaxis is the primary form of malaria prevention for travellers, and it may reduce mortality among children in malarious regions.[1] When chloroquine and proguanil were effective, chemoprophylaxis was simple; but drug resistance has made malaria prevention difficult even for travellers who can afford drugs such as mefloquine.[2] These drugs are too expensive for the great majority of people exposed to infection. Antimalarial drug development is slow and yields initially expensive products.[3] We therefore reconsidered a widely used drug, primaquine, for malaria chemoprophylaxis.[4]

Primaquine is a synthetic 8-aminoquinoline used to eliminate the liver stages of *Plasmodium vivax* and *P ovale*.[5,6] Early clinical trials established the potential of primaquine for prophylaxis against vivax and falciparum malaria.[7–9] However, the requirement for daily dosing, the side-effects, and the efficacy of chloroquine and proguanil apparently deterred further development of primaquine for chemoprophylaxis.[10,11] If primaquine was effective for prophylaxis, travellers would not have to take it for 4–6 weeks after leaving a malarious area, as they do with currently recommended chemoprophylactic agents, because the prophylactic activity of primaquine is primarily against infected hepatocytes rather than erythrocytes.[4–7]

We recently showed that an alternate-day regimen of 0·5 mg/kg primaquine was better tolerated and more effective than chloroquine in preventing malaria during 16 weeks.[12] To identify a more protective regimen and to evaluate long-term tolerance, we conducted a one-year, randomised, placebo-controlled trial of daily primaquine for malaria prevention.

Methods

Study site

The study took place between July, 1993, and August, 1994, in Arso XI, a new community of Javanese families living in three hundred uniform wood plank houses within a 5 sq km area cleared of lowland rain forest in northeastern Irian Jaya, Indonesia. Before arrival in January, 1993, most residents had never been exposed to malaria. The incidence of malaria on Java has been roughly 1 case per 10 000 person-years since 1965.[13] The prevalence of malaria in Arso XI rose from 0% to 41% during the first 120 days of residence. Bednet and chloroquine prophylaxis was mandatory during the first 90 of those 120 days.

Volunteers and radical cure

This project was conducted in accordance with US Navy and Republic of Indonesia regulations governing the protection of volunteers in medical research. American and Indonesian committees reviewed and approved the procedure.

From estimated attack rates of 25% and 2% for each species of malaria among volunteers taking placebo and primaquine, respectively,[12] a sample size of 42 per group was calculated as necessary to demonstrate a significant difference (alpha=0·05, two-tailed, beta=0·20). 131 Javanese males more than 15 years old with no chronic illness or history of drug reaction were asked

to volunteer. After informed consent and clinical history taking, laboratory tests were done to exclude those with haematocrit <30% or qualitative glucose-6-phosphate dehydrogenase (G-6-PD) deficiency (NADPH spot test, Sigma Diagnostics, St Louis, MO). All eligible participants received radical cure with a combination of doxycycline (100 mg twice daily for 10 days), quinine sulphate (600 mg thrice daily for 4 days), and primaquine (0·5 mg/kg base daily for 14 days).

Chemoprophylaxis and follow-up

Before radical cure, volunteers were randomised by lottery to receive either 0·5 mg/kg primaquine base daily, primaquine placebo daily, or 300 mg chloroquine base once a week. The primaquine and placebo arms were conducted in double-blind fashion to allow accurate assessment of side-effects and of protective efficacy. An unblinded chloroquine group was included because this is the standard of care for this area. Prophylaxis began the day after the last dose of primaquine for radical cure. Each volunteer taking primaquine or placebo was visited every day for the duration of the study. All medications were given after morning meals and drug consumption was witnessed by a member of the research team. Malaria smears were made weekly or on any occasion of symptoms compatible with malaria. Giemsa-stained thick and thin blood smears were examined by microscopists blinded to primaquine/placebo assignments. A blood film was judged negative by standard oil immersion microscopy (1000×) if asexual parasites were absent in 300 ocular fields. Parasite density (count/μL) was estimated from the count/200 white blood cells in the thick film, on the assumption of a leucocyte count of 8000/μL.[14] Individuals with slide-proven malaria received quinine sulphate (10 mg/kg thrice daily for 7 days).

Tolerance and side-effects

Physical complaints were recorded weekly by individual responses to a standard questionnaire. Volunteers were asked to take no medications except those provided, and to report to the health clinic with any illness. Health clinic visits, symptoms, and signs were recorded without knowledge of drug assignments. An exit questionnaire was administered at the 52-week endpoint of prophylaxis.

Laboratory testing

Complete blood counts (CBC) were performed on day 0, on days 3–5 of radical cure, and at 10, 20, 40, and 52 weeks of prophylaxis. Total protein, albumin, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin, and urea were assayed on day 0 and at 10, 20, 40, and 52 weeks of prophylaxis. Dipstick urine tests and methaemoglobin assays (Evelyn-Malloy spectrophotometric method[15]) were done on all volunteers during week 50 of prophylaxis. In the primaquine group, methaemoglobin was remeasured 7 days after the final dose.

Data analysis

The cumulative incidence of infection by *P falciparum* and *P vivax* at weekly intervals was calculated by the standard life table method.[16] Comparative efficacy, relative risk of malaria, and physical complaints were assessed by incidence density analysis. To allow endpoint comparisons between groups for laboratory test values and responses to the exit questionnaire, volunteers who developed malaria remained under routine observation even after quinine treatment. All resumed prophylaxis according to the original study group, but any subsequent parasitaemias and symptoms were excluded from the analysis.

Statistical analyses were by EpiStat version 5.01b (CDC, Atlanta, 1991). Significance was determined by Fisher's exact test or by chi-square test with Yates' correction. Prophylaxis group characteristics and laboratory test results were compared by one-way analysis of variance and Bartlett's test for homogeneity of variance. The Kruskal-Wallis non-parametric test was applied to data not normally distributed. Selected two-sample comparisons were done with Student's t-test for normally distributed data or the Mann-Whitney U test for data not normally distributed. Two-tailed p values and 95% confidence intervals (CI) are reported.

Results

Enrolment, compliance, and withdrawals

Of the 131 candidates initially screened, 2 were excluded because of G-6-PD deficiency and 2 because of chronic illness. Radical cure was completed by 126 of 127. The three groups were well matched for mean age (primaquine 30, placebo 31, chloroquine 31 yr), weight (49, 51, and 51 kg), and malaria prevalence (37%, 24%, and 26%). No volunteer had had malaria upon arrival in Arso six months before, but at enrolment 29% were parasitaemic.

Witnessed compliance with prophylaxis was >98% in all three groups. Drug distribution records identified 35 missed doses and 146 unwitnessed doses among the primaquine group (13 391 doses scheduled). Among placebo recipients, 54 doses were missed and 72 were not witnessed. 23 men elected to withdraw—9 in the primaquine group (3 noncompliers), 6 in the chloroquine group (1 noncomplier), and 8 in the placebo group (2 noncompliers). In the three groups these individuals contributed 205, 109, and 212 person-weeks of observation.

Attack rates

P falciparum—The 52-week cumulative incidences of *P falciparum* prophylaxis failure in the primaquine, chloroquine, and placebo groups were 2·6%, 29·7%, and 35·8% (figure 1). The first cases of *P falciparum* were identified during weeks 16, 4, and 5, respectively. Relative to placebo, primaquine efficacy against *P falciparum* was 94·5% (95% CI 57–99) (table 1). Relative to placebo, chloroquine efficacy against *P falciparum* was 33% (95% CI −58 to 72, p=0·50). Geometric mean densities of *P falciparum* were 654 and 452 asexual parasites/μL (p=0·69) in the chloroquine and placebo groups. The single volunteer in the primaquine group who came down with falciparum malaria had 160 asexual parasites/μL with symptoms but no fever. *P falciparum* parasitaemias were accompanied by axillary temperatures >37°C in 8 of 9 chloroquine failures and 8 of 12 placebo cases. Symptoms consistent with malaria illness were reported in 8 of 9 chloroquine failures and in 10 of 12 placebo cases. In the chloroquine group, all those with *P falciparum* had blood chloroquine and desethylchloroquine in excess of the minimum effective concentration (mean 524 ng/mL, range 272–988 ng/mL).

P vivax—The 52-week cumulative incidences of *P vivax* in the primaquine, chloroquine, and placebo

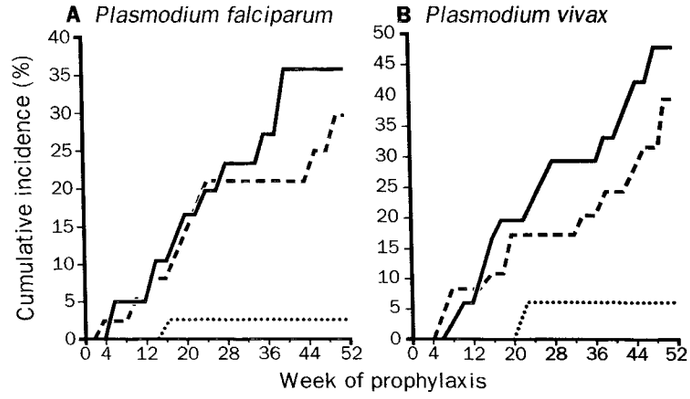

Figure 1: **Cumulative incidence of infection by *P falciparum* (A) and *P vivax* (B)**

Primaquine group (.....), chloroquine group (– – – –), placebo group (———).

	Primaquine	Placebo	IDR	Efficacy (95% CI)*	p
Sample	43	42			
Person-week	1916	1282			
Incident cases					
P falciparum	1	12	18	95% (57–99)	0·0004
P vivax	2	14	11	90% (58–98)	0·0003
Both species	3	26	13	93% (74–98)	<0·0001

IDR=incidence density ratio (placebo incidence/primaquine incidence).
*Efficacy of primaquine relative to placebo=1-[(primaquine incidence)-(placebo incidence)]×100.

Table 1: **Primaquine versus placebo**

groups were 5·4%, 39·6%, and 47·7% (figure 2). The first cases of *P vivax* occurred at weeks 22, 5, and 7, respectively. Relative to placebo, primaquine efficacy against *P vivax* was 90·4% (95% CI 58–98) (table 1). Relative to placebo, chloroquine efficacy against *P vivax* was only 16·5% (95% CI −77 to 61, p=0·79). Geometric mean densities of *P vivax* were 1093 and 437 asexual parasites/μL (p=0·19) in the chloroquine and placebo groups, respectively. *P vivax* parasitaemias were accompanied by axillary temperatures >37°C in 9 of 12 chloroquine failures and 7 of 14 placebo cases. Symptoms consistent with malaria illness were reported in all 12 chloroquine failures and in 12 of 14 placebo cases. The 2 primaquine failures against vivax malaria had 120 and 2120 asexual parasites/μL with symptoms but no fever. All *P vivax* parasitaemias in chloroquine recipients occurred with blood chloroquine and desethylchloroquine in excess of minimum effective levels (mean 474 ng/mL concentrations, range 264–1049 ng/mL).

P falciparum and *P vivax*—The 52-week cumulative incidence of both plasmodia was 7·8%, 55%, and 66·1% in the primaquine, chloroquine, and placebo groups. Relative to placebo, primaquine efficacy was 92·3% (95% CI 74–98) (table 1). The risk of malaria in the placebo group was 10–18 relative to the primaquine group (table 1). Relative to the chloroquine group, the risk of malaria with placebo was just 1·2 to 1·5 (p=0·43).

Tolerance

Physical complaints in the absence of malaria were infrequent in all groups (table 2). Volunteers receiving placebo had significantly fewer complaints than those

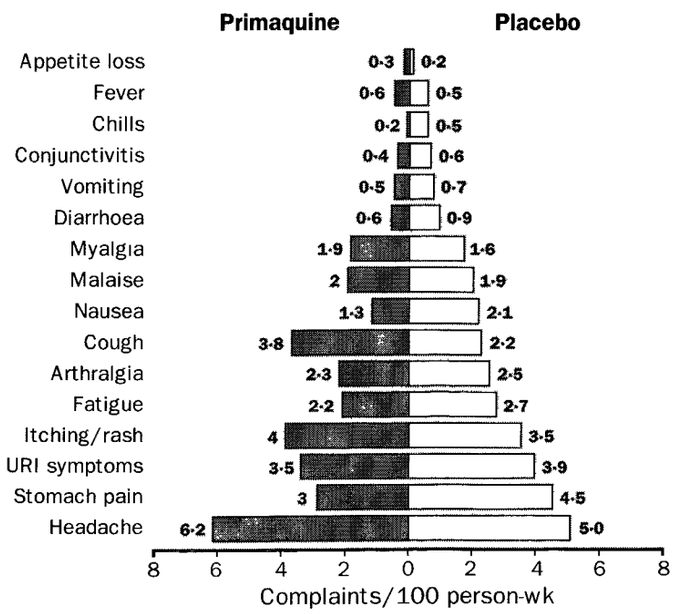

Figure 2: **Paired graphs illustrating the incidence density of specific complaints**
URI=upper respiratory infection. Only the incidence density of cough differed significantly between the two groups (p=0·03)).

	Primaquine	Chloroquine	Placebo
Sample	43	40	38
Person-week	1913	1377	1087
ID complaints	4·7*	6·2	3·6*
ID symptoms or signs	17·2	17·7	17·7
ID clinic visits	9·9	9·9	10·9

*p=0·01.

Table 2: **Incidence density (ID) of complaints, symptoms, and signs, and clinical visits not associated with malaria**

	Placebo No (SD)	Primaquine No (SD)	p*
Sample size	22	29	
Haemoglobin	14·6 (1·1)	14·7 (1·0)	0·72
WBC	6·6 (1·7)	7·3 (2·2)	0·23
Granulocytes (%)	50·9 (11)	52·2 (12)	0·71
Lymphocytes and monocytes (%)	49·1 (11)	47·8 (12)	0·71
Platelets (×10³/L)	305 (84)	292 (100)	0·63
Urea (mmol/L)	4·4 (0·9)	5·1 (1·3)	0·03
Bilirubin (μmol/L)	3·9 (2)	4·4 (1·9)	0·36
Total protein (g/L)	83 (6)	82 (5)	0·32
Albumin (g/L)	52 (5)	53 (8)	0·44
Creatinine (μmol/L)	97 (9)	97 (18)	0·79
ALAT (U/L)	18·7 (17)	21·5 (18)	0·58
ASAT (U/L)	24·7 (26)	33·6 (35)	0·31
Alkaline phosphatase (U/L)	145 (38)	162 (39)	0·13

ALAT/ASAT=alanine/aspartate aminotransferases.
*Unpaired comparison by t-test or Mann-Whitney U-test.

Table 3: **Laboratory test values at end of prophylaxis**

taking primaquine or chloroquine, but there were no differences between groups in number of visits made to the health clinic or number of complaints registered during these visits. Men in each group made fewer verbal affirmations of complaint by weekly interview in their homes (3–6 per person-year) than actual presentations (10–11 per person-year) to the health clinic. Figure 3 shows incidence density of physical complaints among primaquine versus placebo users. In responses to the exit questionnaire there was no association between primaquine use and cough (p=0·64), shortness of breath (p=0·28), or fatigue (p=0·64); 6 of 22 men who took primaquine on an empty stomach reported gastrointestinal discomfort.

Laboratory tests

Blood samples analysed at the end of the study provided no indication that use of primaquine daily for one year had any negative impact on complete blood count or renal or hepatic function (table 3). After 50 weeks the mean methaemoglobin value in the primaquine group (5·8 SD 2·9%) was significantly higher (p<0·001) than in either the chloroquine (0·7 SD 0·4%) or placebo group (1·2 SD 0·7%) (figure 4). Methaemoglobin values among primaquine users ranged from 1·4% to 13%, but clinical signs of methaemoglobinaemia were never seen. There was no correlation ($r=-0·04$) between complaints of

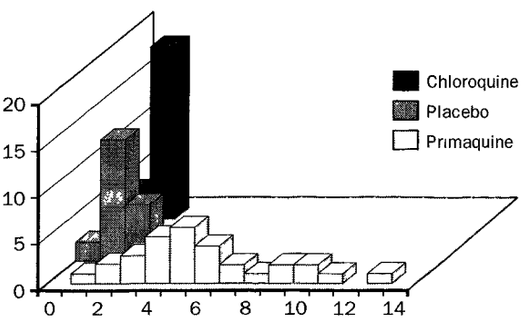

Figure 3: **Graphs representing number of volunteers within defined ranges of methaemoglobin (%) at week 50 of prophylaxis**

cough and methaemoglobin values. 7 days after the final dose of primaquine the mean methaemoglobin among primaquine users had declined to 2·4% (SD 1·2%, range 0–4·5%) ($p<0·0001$) The methaemoglobin had decreased in 27 of 30 volunteers and the decline averaged 51% (range 21–100%).

Discussion

In this study, daily 0·5 mg/kg primaquine base offered highly efficacious and well tolerated prophylaxis against malaria to G-6-PD normal men recently exposed to malaria infection in Irian Jaya. As previously reported from this region,[17,18] chloroquine has little or no protective efficacy against *P falciparum* or *P vivax*.

The prophylactic efficacy of primaquine is comparable with that of doxycycline or mefloquine in Irian Jaya (Ohrt C, personal communication). Against *P falciparum* in Kenya, daily primaquine for 10 weeks was as efficacious as mefloquine or doxycycline, and equally well tolerated.[19] Relative to chloroquine, primaquine was 74% more effective against *P falciparum* with alternate-day dosage[12] and >90% more effective with daily use. There was no significant difference between alternate-day and daily regimens in the risk of *P vivax* infection (89% *vs* 90%).[12] Overall, the daily regimen gave superior protection and was equally well-tolerated.

We cannot explain why daily primaquine did not provide absolute protection in this trial. However, 100% efficiency is seldom observed in field-based trials of chemoprophylaxis. Factors such as inadequate drug absorption, rapid or altered drug metabolism, unreported missed doses, emesis before drug absorption, and variability of *Plasmodium* strain sensitivity usually explain failures with an otherwise effective regimen.

Physical complaints, discomfort, and illness not associated with parasitaemia were low among men using daily primaquine. This may be because we gave primaquine after meals; high doses of primaquine (up to 240 mg base daily) given with food do not cause gastrointestinal distress.[20] The results of blood counts and laboratory tests for renal and hepatic function showed no evidence of toxicity with daily primaquine use.

Primaquine-induced methaemoglobinaemia was anticipated and the volunteers were closely monitored for consequent symptoms and signs. Despite the extreme physical demands at this agrarian frontier settlement, and a high frequency of habitual cigarette use (26/32 primaquine users), no clinical indications of respiratory stress were seen in primaquine users. None had greater than 13% methaemoglobin (up to 25% methaemoglobin is said to be tolerated well and without cyanosis[10]). A long-lived primaquine metabolite may be responsible for the mild methaemoglobinaemia that occurs with a standard 14-day regimen.[21] However, after 50 weeks of daily primaquine use, the concentration of methaemoglobin in our primaquine group resembled ($p=0·23$) that reported by Fletcher et al[22] (respectively, mean 8·5 g/L, range 2–19 *vs* mean 7·5 g/L, range 2–16 g/L) after a standard 14-day course of 0·25 mg primaquine base per day .

We conclude that primaquine provides well-tolerated and effective prophylaxis against *P falciparum* and *P vivax* in males who have had little exposure to malaria. Primaquine offers a potential advantage over currently used chemoprophylactic agents because its activity is almost certainly against infected hepatocytes. This means that, unlike all currently recommended agents, it would probably not have to be taken after departure from a malarious area. A 14-day primaquine regimen for terminal treatment of *P vivax* has been used safely by millions of people over the past 50 years, so this agent is likely to provide safe and effective short-term prophylaxis of malaria in G-6-PD normal, non-pregnant, visitors to malarious areas. Whether primaquine can be safely stopped after departure from a malarious area requires investigation.

We gratefully acknowledge the help of many officials of the Indonesian Ministry of Health in this research effort. Special thanks are extended to Dr Slamet Harjosuwarno in Jayapura, and Dr Suriadi Gunawan, Dr Emiliana Tjitra, Dr Harijani Marwoto, Dr Sri Oemijati, and Dr Arbini R Poerwokoesumo in Jakarta. We also thank Dr Hendra Widjaja, Dr Ating Solihin, Dr Dennis Shanks, and Dr Ronald Anthony for valuable field assistance and the supporting staff of NAMRU-2 parasitology, tropical medicine, fiscal, and supply departments. Financial support for this study was from the Naval Medical Research and Development Command work unit numbers 620828, 6281453E033, 6281453UO52, and 63002A00101HFX.

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