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Therapeutic and Transmission-Blocking Efficacy of Dihydroartemisinin/Piperaquine and Chloroquine against *Plasmodium vivax* Malaria, Cambodia

Jean Popovici, Amelie Vantaux, Lyse Primault, Reingsey Samreth, Eak Por Piv, Sophalai Bin, Saorin Kim, Dysoley Lek, David Serre, Didier Menard

We assessed the efficacy of standard 3-day courses of chloroquine and dihydroartemisinin/piperaquine against *Plasmodium vivax* malaria. Compared with chloroquine, dihydroartemisinin/piperaquine was faster in clearing asexual *P. vivax* parasites and blocking human-to-mosquito transmission. This drug combination was also more effective in preventing potential recurrences for ≥ 2 months.

Plasmodium vivax is the most widespread human malaria parasite. Almost 2.5 billion persons are at risk for infection in >90 countries (1,2). Since the 1950s–1960s, Southeast Asia has been the cradle of emergence and spread of *P. falciparum* antimalarial drug resistance, a major obstacle for malaria control. Over the past decade, control efforts in Cambodia have led to an impressive decrease in malaria burden, with a slower decrease of *P. vivax* than for *P. falciparum* (3).

P. vivax resistance to chloroquine has emerged more recently; the first cases were observed in 2009 in Rattanakiri Province in northeastern Cambodia (17.4% treatment failures after 28 days of follow-up), which led to withdrawal of chloroquine and use of dihydroartemisinin/piperaquine (DHA/PPQ) as first-line therapy for uncomplicated *P. vivax* malaria in 2012 (4). We assessed the efficacy of standard 3-day courses of chloroquine and DHA/PPQ for treating *P. vivax* malaria, preventing recurrences, and blocking human-to-mosquito transmission.

The Study

We conducted an open-label, randomized, control trial in June–December 2014 in Rattanakiri Province, Cambodia. Febrile patients or patients with a history of fever in the

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previous 48 h who sought treatment in health facilities and had positive results by rapid diagnostic test (CareStart Malaria HRP2/pLDH Pf/PAN Combo; Access Bio, Inc., Somerset, NJ, USA) for non-*P. falciparum* malaria were offered participation in the study. Pregnant or lactating women and patients with signs of severe malaria, other known illnesses, or inability to provide informed consent were excluded. Patients with *P. vivax* mono-infection confirmed by PCR were eligible for the study (5).

At enrollment, after we obtained written informed consent, patients were randomized to receive supervised standard 3-day courses of DHA/PPQ (Duo-Cotecxin; Zhejiang Holley Nanhu Pharmaceutical Co., Ltd., Jiaxing, China) or chloroquine (Nivaquine; Sanofi-Aventis, Paris, France). For each participant, medical histories were obtained and clinical and biological examinations performed. We followed up with patients according to an extended World Health Organization protocol on days 1, 2, 3, 5, and 7 and then weekly until day 63. At each visit, we performed clinical examinations and obtained an axillary temperature and a capillary blood sample.

Malaria parasites were detected by microscopy (Giemsa-stained blood films) and PCR as described (5). Chloroquine resistance was ruled out for patients if no parasites were detected by microscopy on day 28 or, in case of recurrence, if the chloroquine blood concentration on the day of recurrence did not exceed >100 ng/mL (6,7). We measured chloroquine blood concentrations by using liquid chromatography–tandem mass spectrometry for 50- μ L samples of whole blood.

During January–March 2016, we conducted an additional study at the same site to evaluate the infectivity of *P. vivax* from blood of symptomatic patients to *Anopheles dirus* mosquito vectors; we tested pretreatment and posttreatment blood samples by using membrane feeding assays without serum replacement (8). Any febrile patients seeking antimalarial treatment with similar inclusion/exclusion criteria described previously were enrolled in this study.

After we obtained written informed consent, we fed batches of 50 *An. dirus* mosquitoes with blood collected from these patients on 3 occasions: 1) before the first dose of DHA/PPQ or chloroquine, 2) on the same day at 9:00 PM (i.e., 2–11 h after treatment), and 3) at 24 h posttreatment for patients treated with chloroquine. We performed statistical analyses by using GraphPad Prism 5 (GraphPad, San Diego,

CA, USA) and R software (9). Both studies were approved by the Cambodian National Ethic Committee (038 NECHR, 2/24/2014 and 475 NECHR, 12/28/2015).

For the drug comparison study, we enrolled 50 patients (25 in each study arm); in each arm, 5 patients were lost to follow-up during days 2–35. A total of 40 patients (20 in each

Table 1. Baseline characteristics of *Plasmodium vivax*-infected patients in a clinical drug trial and a human-to-mosquito transmission study, Cambodia*

Characteristic	Chloroquine	DHA/PPQ	p value
Clinical drug trial study, June–December 2014			
No. patients followed up until day 63 (% Male)	20 (80)	20 (80)	1.00†
Patient age, y	26.5 (18.5–35)	28.5 (21.5–46)	0.11‡
Patient weight, kg	56.0 (50–59)	51.0 (49.5–53)	0.14‡
Parasites/μL of blood	5,000 (1,850–8,350)	8,900 (3,500–17,500)	0.051‡
Gametocytes/μL of blood	108 (58–200)	245 (105–745)	0.12‡
Proportion with G6PD deficiency by spot test and PCR§	2/20 (Viangchan variant)	1/20 (Canton variant)	1.00†
Leukocytes, ×10 ⁹ cells/L	7.7 (6.2–9.2)	6.9 (5.2–8.6)	0.38‡
Erythrocytes, ×10 ¹² cells/L	5.01 (4.53–5.26)	5.10 (4.69–5.65)	0.42‡
Hemoglobin, g/dL	11.2 (10.3–13.8)	12.3 (11.4–13.1)	0.43‡
Hematocrit, %	37 (34–43)	40 (37–43)	0.49‡
Human-to-mosquito transmission study, January–March 2016¶			
No. (%) male patients	9 (100)	10 (70)	0.21†
Patient age, y	13.0 (12.7–38.5)	24.5 (19.0–29.0)	0.68‡
Patient weight, kg	37.0 (28.7–52.2)	53.5 (42.0–60.0)	0.09‡
Parasites/μL of blood	4,565 (3,462–6,184)	9,069 (6,833–11,591)	0.01‡
Gametocytes/μL of blood	221 (74.2–381.5)	1,915 (693–2,729)	0.001‡
Proportion of infectious patients before treatment; feeding assay before first dose of treatment#	8/9 (89)	9/10 (90)	1.00†
Proportion of infected mosquitoes before treatment; feeding assay before first dose of treatment	69.6 (26.2–84.9)	72.9 (29.75–92.7)	0.71‡
Average no. oocysts in infected mosquitoes before treatment; feeding assay before first dose of treatment	12.2 (2.4–29.8)	12.2 (2.4–40.6)	0.74‡

*Values are median (IQR) or no. positive/no. tested (%) unless otherwise indicated. Bold indicates statistical significance (p<0.05). DHA/PPQ, dihydroartemisinin/piperazine; G6PD, glucose-6-phosphate dehydrogenase; IQR, interquartile range.

†By Fisher exact test.

‡By Mann-Whitney U test.

§For details, see Khim et al. (10).

¶Mosquitoes were 6–8 days old and were allowed to feed for 20 min. Feeding was conducted at the same place immediately after obtaining blood from patients. Dissections were performed 6 days after the blood meal. Midguts were dissected in 1% mercurochrome stain, and the presence and number of oocysts were determined by microscopy (×20 magnification).

#Patients were defined as being infectious when ≥1 mosquito became infected with oocysts.

Table 2. *Plasmodium vivax* clearance among infected patients, time to malaria recurrence, and vector transmission results, by allocated antimalarial drug treatment, Cambodia*

Characteristic	Chloroquine	DHA/PPQ	p value
Clinical drug trial study, June–December 2014			
Proportion of patients parasitemic at day 1 by microscopy	17/20 (85)	6/20 (30)	0.001†
Parasites/μL of blood at day 1	180 (80–600)	0 (0–57)	0.0002‡
Parasite reduction ratio at day 1	96.2 (68.5–98.6)	100 (99.4–100)	0.0002‡
Proportion of patients parasitemic at day 2 by microscopy	5/20 (25)	0/20 (0)	0.047†
Parasites/μL of blood at day 2	0 (0–15)	0	0.03‡
Parasite reduction ratio at day 2	100 (99.8–100)	100	0.03‡
Proportion of patients parasitemic at day 3 by microscopy	0/20 (0)	0/20 (0)	1.00†
Proportion of patients with recurrence detected by PCR	12/20 (60)	4/20 (20)	0.02†
Time to recurrence, d	49 (42–49)	56 (52.5–56)	0.04‡
Human-to-mosquito transmission study, January–March 2016			
Proportion of infectious patients after first dose of treatment; feeding assay at 9:00 PM	8/9 (89)	1/10 (10)	0.001†
Proportion of infected mosquitoes after first dose of treatment; feeding assay at 9:00 PM	60.7 (23.7–78.6)	0	0.004‡
Average no. of oocysts in infected mosquitoes after first dose of treatment; feeding assay at 9:00 PM	9.9 (4.1–25.7)	356.4	0.22‡
Parasite transmissibility reduction ratio (%) at 9:00 PM	19 (–13.8 to 62.7)	100	0.003‡
Proportion of infectious patient 24 h after first dose of chloroquine	2/9 (22)	ND	ND
Average no. of oocysts in infected mosquitoes after first dose of treatment for 2 infectious patients; feeding assay 24 h after first dose of chloroquine	1.3 and 2.4	ND	ND
Proportion of infected mosquitoes 24 h after first dose of chloroquine	0 (0–4.4)	ND	ND

*Values are no. positive/no. tested (%) or median (IQR) unless otherwise indicated. Bold indicates statistical significance (p<0.05). DHA/PPQ, dihydroartemisinin/piperazine; IQR, interquartile range; ND, no data.

†By Fisher exact test.

‡By Mann-Whitney U test.

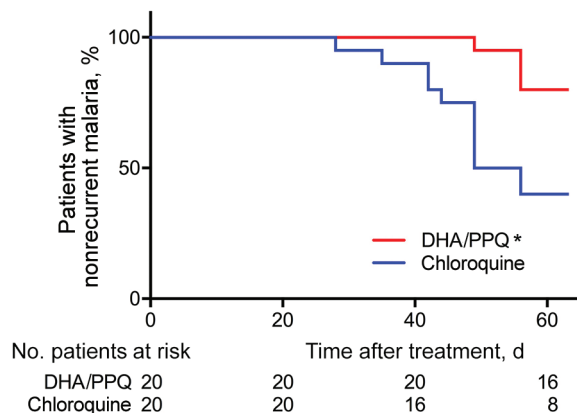


Figure 1. Cumulative proportion of patients with nonrecurrent *Plasmodium vivax* malaria given a 3-day course of DHA/PPQ and chloroquine detected by PCR within 63 days of follow-up, Cambodia. * $p < 0.01$, by log-rank test during Kaplan-Meier survival analysis. DHA/PPQ, dihydroartemisinin/piperazine.

study arm) were followed up until day 63. Baseline patient characteristics were similar for both patient groups (Table 1). We did not observe any adverse events or early clinical failures. The proportion of patients still parasitemic on days 1 and 2 (detected by microscopy) was lower for the DHA/PPQ-treated group than for the chloroquine-treated group (Table 2). Medians of the parasite reduction ratio recorded on

days 1 and 2 were higher for the DHA/PPQ-treated patient group (Table 2). All patients, regardless of their treatment, were microscopically parasite free at day 3.

Within 2 months of follow up, there were fewer patients with a recurrence (detected by PCR) in the DHA/PPQ-treated group than in the chloroquine-treated group (odds ratio 0.17, 95% CI 0.05–0.66; $p < 0.05$, by log-rank test, $p < 0.01$ by Kaplan-Meier survival analysis) (Table 2; Figure 1). Median time to recurrence after treatments was also delayed in patients given DHA/PPQ (56 days) compared with those patients given chloroquine (49 days) (Table 2). No recurrence occurred before day 28 in either study arm, which is suggestive of relapse or reinfection, rather than recrudescence of drug-resistant parasites (6,7). In the chloroquine-treated group, 12/20 patients with recurrence had a chloroquine blood concentration < 100 ng/mL on the day of recurrence (chloroquine + desethyl chloroquine: median 55.6 ng/mL, interquartile range 40.0–61.7 ng/mL); these results excluded likely chloroquine resistance (6,7).

For the mosquito-to-human transmission study, we enrolled 19 patients (9 given chloroquine and 10 given DHA/PPQ). Baseline patient characteristics were similar in both patient groups, except for day 0 parasitemia and gametocytemia, which were higher for the DHA/PPQ-treated group (Table 1). The proportion of infectious blood from *P. vivax*-infected patients and the median proportion of infected mosquitoes fed on blood collected before the

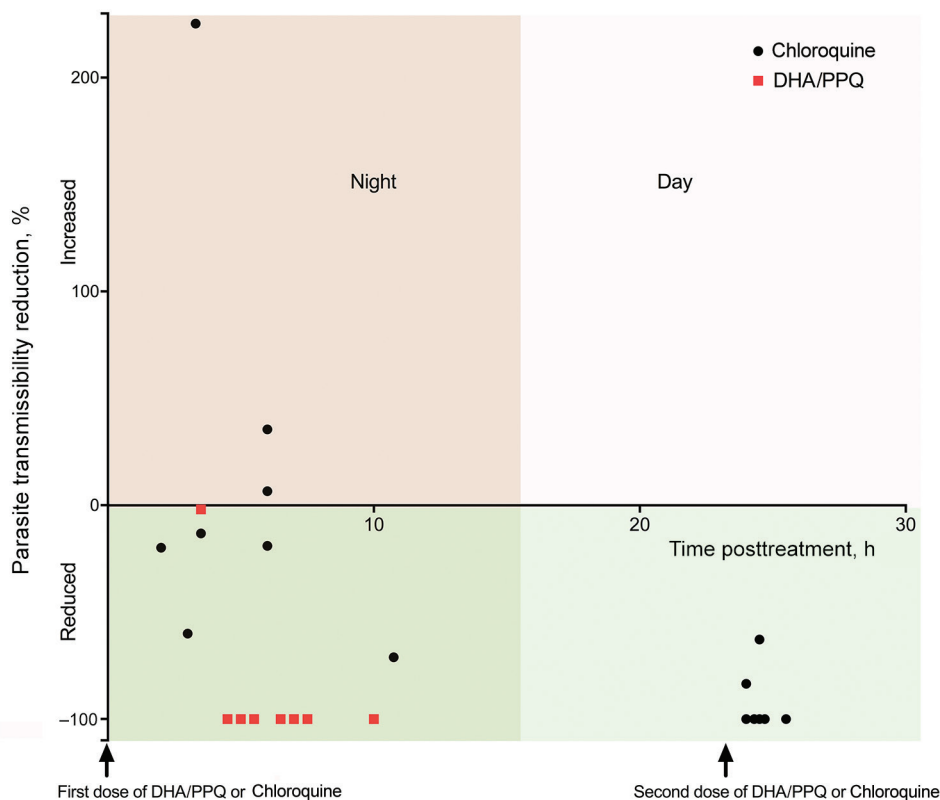


Figure 2. Transmission-blocking efficacy of allocated antimalarial drug treatment (chloroquine and DHA/PPQ) on human-to-mosquito transmission of *Plasmodium vivax*, January–March 2016, Cambodia. Each dot represents the parasite transmissibility reduction ratio (i.e., $100 - [\text{proportion of infected mosquitoes fed with blood samples collected at 9:00 PM after the first dose of treatment} \times 100 / \text{proportion of infected mosquitoes fed with blood samples collected at patient enrollment before the first dose of treatment}]$). Only data of infectious patients at enrollment are shown (17/19 patients; Table 1). For the chloroquine-treated patient group, a second mosquito blood feeding was performed 24 h after the first dose. DHA/PPQ, dihydroartemisinin/piperazine.

first dose of DHA/PPQ or chloroquine were similar for both patient groups (Table 1). Despite an initial higher day 0 gametocytemia for the DHA/PPQ-treated group, the proportions of infectious *P. vivax* blood collected at 9:00 PM after the first dose of DHA/PPQ or chloroquine and the median proportion of infected mosquitoes were lower for the DHA/PPQ-treated group than for the chloroquine-treated group (Table 2). Overall, DHA/PPQ acted faster than chloroquine in decreasing over time the proportion of infectious patients (generalized linear mixed model time for drug interaction, $\chi^2_1 = 113.1$, $p < 0.0001$) (Figure 2). For the group given chloroquine, 2 (22%) of 9 blood samples were still infectious 24 hours after the first dose (Table 2).

Conclusions

We confirm that DHA/PPQ acts faster (≤ 48 h) than chloroquine (≈ 72 h) in eliminating sexual and asexual *P. vivax* parasites and that DHA/PPQ provides an excellent post-exposure prophylaxis against potential recurrences for ≥ 2 months (11). This benefit relies on the combination of artemisinin derivatives (DHA), which are fast-acting drugs capable of eliminating any *P. vivax* blood stages, and a long-lasting partner drug (PPQ), which has a long terminal elimination half-life and is highly effective in preventing *P. vivax* recurrence for up to 56 days. Although the number of patients enrolled was small, we demonstrated that DHA/PPQ also acts faster (< 5 h) than chloroquine in killing *P. vivax* sexual stages and thus prevents the risk for transmission of parasites to the mosquito vector the night after uptake of the first dose. This rapid clearance of gametocytes is a major benefit of DHA/PPQ in comparison with chloroquine, given that *P. vivax* gametocytes appear early in the course of disease and must be eliminated as soon as possible to limit risk of transmission (12,13).

In summary, our findings support the recommendation of DHA/PPQ as first-line treatment for *P. falciparum* and *P. vivax* uncomplicated malaria in regions to which these species are co-endemic. These findings apply to areas in which chloroquine is still effective and no *P. falciparum* resistance to PPQ has been observed.

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About the Author

Dr. Popovici is a research scientist at the Institut Pasteur, Phnom Penh, Cambodia. His primary research interests are a better

understanding of *P. vivax* malaria and providing more adapted control solutions to policy makers.

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