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CLINICAL THERAPEUTICS

Efficacy and Safety of Pyronaridine-Artesunate plus Single-Dose Primaquine for the Treatment of Malaria in Western Cambodia

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ABSTRACT This single-arm trial ($n = 104$) in western Cambodia showed high efficacy for 3-day treatment with pyronaridine-artesunate plus single-dose primaquine in *Plasmodium falciparum* malaria. Day 42 PCR-adjusted adequate clinical and parasitological response (ACPR) was 98.3% (58/59) (95% confidence interval [CI], 90.9 to 100.0) in Trapeng Chau in Kampong Speu and 100% (41/41) (95% CI, 91.4 to 100) in Veal Veng in Pursat; 80.6% (83/103) of the patients had *P. falciparum* with drug resistance molecular markers. For *Plasmodium vivax* malaria, pyronaridine-artesunate day 28 ACPR was 98.3% (59/60) (95% CI, 91.1 to 100) and 100% (60/60) (95% CI, 94.0 to 100), respectively. (This study is registered in the Australian New Zealand Clinical Trials Registry [ANZCTR] under reference no. ACTRN12618001999224.)

KEYWORDS Cambodia, *Plasmodium falciparum*, *Plasmodium vivax*, antimalarial agents, artemisinin, drug resistance, malaria, pyronaridine-artesunate

Multidrug-resistant *Plasmodium falciparum* malaria is highly prevalent in western Cambodia, and therapeutic options for the treatment of uncomplicated malaria are increasingly limited (1–5). New therapeutic options are needed to support malaria elimination in the region (6). Pyronaridine-artesunate is an artemisinin-based combination therapy for the treatment of uncomplicated *P. falciparum* and *Plasmodium vivax* malaria. Previous studies with pyronaridine-artesunate in *P. falciparum* malaria indicated >95% efficacy in eastern Cambodia (7), while efficacy was lower in western Cambodia (Pursat/Pailin) (87.2%; 95% confidence interval [CI], 79.7 to 92.6) (8). The current study evaluated the efficacy and safety of pyronaridine-artesunate plus single-dose primaquine in uncomplicated *P. falciparum* malaria in two areas of known *P. falciparum* multidrug resistance in western Cambodia. This study follows the same methodology as a previous study in eastern Cambodia (7), except that pyronaridine-artesunate efficacy and safety in *P. vivax* malaria was also investigated.

Patients with microscopically confirmed malaria were recruited between September and December 2018 from health centers in western Cambodia (Trapeng Chau in Kampong Speu and Veal Veng in Pursat). Eligible patients were ≥7 years old, weighed ≥20 kg, had fever/history of fever, and had *P. falciparum* or *P. vivax* monoinfection (500 to 250,000 sexual parasites μl^{-1}). Exclusion criteria were pregnancy, severe or complicated malaria, malnutrition, concomitant disease, known kidney or liver disease, hypersensitivity, and contraindication to the study drugs. All patients or their guardians

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TABLE 1 Baseline characteristics and population disposition

Characteristic	Results for:			
	P. falciparum from:		*P. vivax* from:	
	Trapeng Chau	Veal Veng	Trapeng Chau	Veal Veng
Male/female (*n*)	60/2	36/6	58/2	59/1
Mean age (SD) (yr [range])	26.5 (10.4) (7–56)	30.7 (13.7) (9–60)	21.9 (7.9) (6–53)	25.2 (10.8) (8–55)
Mean weight (SD) (kg [range])	53.2 (10.8) (20–75)	51.8 (10.7) (20–73)	51.5 (11.0) (20–77)	52.9 (12.5) (22–82)
Geometric mean parasitemia (μl^{-1} blood [range])	20,363 (1,530–251,752)	12,875 (892–107,936)	6,801 (1,164–42,435)	6,829 (1,794–21,367)
Safety population	62	42	60	60
PCR-adjusted per-protocol population	59	41	60	60
Withdrawn	0	0	0	0
Lost to follow-up	2	0	0	0
PCR adjustment	1	1	Not applicable	Not applicable

provided written informed consent plus assent from patients aged <18 years. Ethical approval was granted by the National Ethics Committee at the National Institute of Public Health, Phnom Penh, and the Ethics Review Committee of the World Health Organization Regional Office for the Western Pacific.

The study was conducted using the standard World Health Organization protocol for the assessment of antimalarial efficacy (9). Study procedures, evaluations, and analyses were consistent with a previously reported study conducted in eastern Cambodia (7). Patients received oral pyronaridine-artesunate (Shin Poong Pharmaceutical Co., Ansan, South Korea) once daily for 3 days, dosed according to body weight, plus single-dose primaquine 15 mg on day 0 for *P. falciparum* cases (7, 10). The primary endpoint was adequate clinical and parasitological response (ACPR), defined as no parasitemia without previous treatment failure (9). The primary outcome was evaluated for *P. falciparum* isolates at day 42 and for *P. vivax* isolates at day 28. For *P. falciparum* isolates, ACPR was adjusted for reinfection with PCR genotyping using published methods (11). Data were analyzed by using a WHO Excel spreadsheet. To determine the prevalence of *P. falciparum* molecular markers to artemisinin, piperaquine, and mefloquine, the *Kelch13* (*K13*) gene was sequenced, and gene copy numbers for *plasmepsin 2* (*Pfpm2*) and *multidrug resistance 1* (*Pfmdr1*) were determined using published methods (12, 13). The threshold for genic amplification was defined as >1.5 copies.

The study population included 104 patients with *P. falciparum* and 120 with *P. vivax* malaria; baseline data were similar across the two sites, although *P. falciparum* parasitemia was higher in Trapeng Chau (Table 1). For *P. falciparum* isolates, day 42 PCR-adjusted ACPR was 98.3% (95% CI, 88.8 to 99.8) in Trapeng Chau and 100% in Veal Veng (Kaplan-Meier analysis). In a per-protocol analysis, the proportion of patients with day 42 PCR-adjusted ACPR was 98.3% (58/59) (95% CI, 90.9 to 100.0) in Trapeng Chau and 100% (41/41) (95% CI, 91.4 to 100) in Veal Veng.

Previous data from Pursat indicated a day 42 ACPR of 89.8% (95% CI, 78.8 to 95.3%) (Kaplan-Meier analysis) (8). Efficacy in the current study was higher in Veal Veng (Pursat) (100%), but this difference was not statistically significant ($P = 0.4$). The single recrudescence at Trapeng Chau occurred in a 7-year-old child at day 35 consecutively with an infection by an artemisinin- and piperaquine-resistant strain (*K13* C580Y, *Pfpm2* amplification). Only two patients (both from Trapeng Chau) had *P. falciparum* gametocytes at day 0, which is an unusually low baseline gametocyte prevalence (1.9% [2/104]), with rates of ~10% previously reported from Cambodia (14, 15). However, stringent quality control for microscopy at these experienced sentinel sites supports these findings. No further gametocytes were detected throughout the study in any patient, indicating the high efficacy of primaquine in suppressing transmission.

The day 3 parasite positivity rate was 41.7% (25/60) (95% CI, 29.1 to 55.1) in Trapeng Chau and 28.6% (12/42) (95% CI, 15.7 to 44.6) in Veal Veng. This is consistent with a higher prevalence of *K13* molecular markers associated with

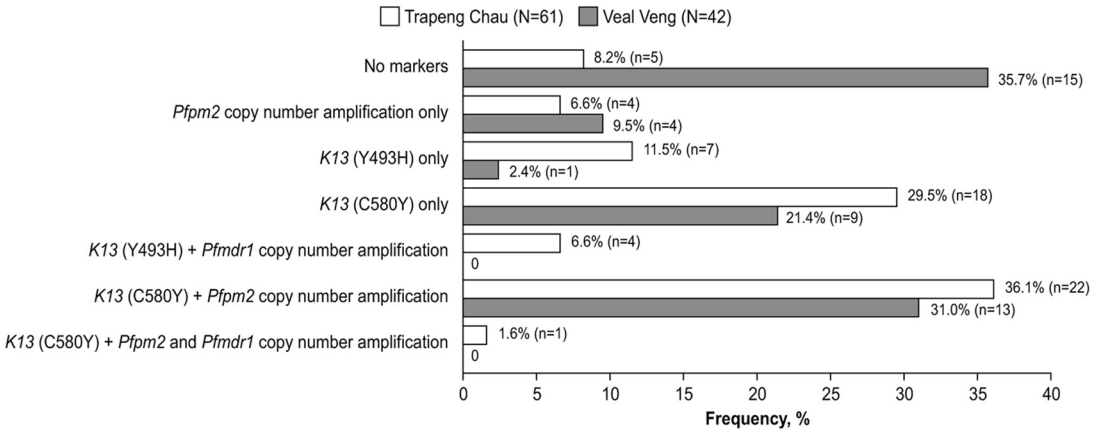

FIG 1 Prevalence of molecular markers associated with resistance to artemisinin (*K13*), piperaquine (*Pfpm2*), and mefloquine (*Pfmdr1*). Note that data were incomplete for one patient from Trapeng Chau.

artemisinin resistance in Trapeng Chau (85.2% [52/61]) versus Veal Veng (54.8% [23/42]) (Fig. 1). The prevalence of multiresistant strains, with *K13* mutations and *Pfpm2* and/or *Pfmdr1* copy number amplification, was similar in Trapeng Chau (44.3% [27/61]) and Veal Veng (40.5% [17/42]) (Fig. 1). A triple mutant, with molecular markers associated with artemisinin, piperaquine, and mefloquine resistance, was identified in Trapeng Chau, as was previously noted in northeastern Cambodia (Preah Vihear province) (16).

For *P. vivax* malaria, day 28 ACPR was 98.3% (59/60) (95% CI, 91.1 to 100) in Trapeng Chau and 100% (60/60) (95% CI, 94.0 to 100) in Veal Veng. The day 3 parasite positivity rate was 0% (0/60) in Trapeng Chau and 1.7% (1/60) in Veal Veng.

Adverse events were consistent with symptoms of malaria and generally declined in frequency during treatment (Table 2). Notably, there were no cases of dark urine in *P. falciparum* cases, consistent with recent data indicating the safety of single-dose primaquine administration (17). There were no serious adverse events or deaths.

Pyronaridine-artesunate had >98% efficacy in two sentinel sites in western Cambodia in both *P. falciparum* and *P. vivax* malaria with no adverse events of concern. This is in the context of a high prevalence of molecular markers for artemisinin resistance (72.8% [75/103]) in *P. falciparum* isolates, which was associated with markers for piperaquine and/or mefloquine resistance in 42.7% (44/103)

TABLE 2 Frequency of adverse events of any cause by study day

	Frequency (%) in:					
	P. falciparum (*n* = 104) on:			*P. vivax* (*n* = 120) on:		
Adverse event	Day 0	Day 1	Day 2	Day 0	Day 1	Day 2
Fever	99.0	56.3	8.8	100	49.2	0.8
Chills	80.8	28.2	2.9	85.8	37.5	0.8
Headache	94.2	58.3	44.1	90.8	53.3	29.2
Nausea	18.3	9.7	3.9	14.2	7.5	0.8
Vomiting	15.4	9.7	1.0	9.2	2.5	0.8
Body pain	84.6	51.5	29.4	75.0	40	10
Fatigue	71.2	34.0	15.7	67.5	30	10.8
Vertigo	31.7	23.3	6.9	23.3	15.8	1.7
Confusion	7.7	4.9	0	1.7	0	0
Insomnia	25.0	6.8	2.0	20.8	3.3	1.7
Deafness	15.4	5.8	0	0	0.8	0
Diarrhea	10.6	7.8	2.0	3.3	1.7	0
Abdominal pain	19.2	13.6	2.9	13.3	11.7	0
Rash	0	0	0	0	0.8	0
Palpitations	1.9	3.9	1.0	0.8	0.8	0
Tachycardia	1.0	0	0	0	0	0

of the isolates. Pyronaridine-artesunate is a suitable option for the treatment of malaria in Kampong Speu and Pursat, with continuing efficacy surveillance at these sites.

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R.L., H.C., and R.H. designed the study. D.M.B. and M.D.B. entered the data and validated microscopy. R.L., M.D.B., P.R., and B.W. analyzed and interpreted the data. M.M.K., N.K., and B.W. conducted the laboratory work (therapeutic efficacy, PCR adjustment, and analysis of molecular markers). All authors critically reviewed the paper and approved the final version of the paper for submission.

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