

Pyronaridine for Treatment of *Plasmodium ovale* and *Plasmodium malariae* Infections

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The clinical efficacy of oral pyronaridine was assessed in 22 symptomatic Cameroonian patients infected with *Plasmodium ovale* or *Plasmodium malariae*. All patients were cured on or before day 4. In vitro drug assays confirmed the sensitivity of *P. ovale* and *P. malariae* isolates to chloroquine and pyronaridine.

Among the four human malaria parasites, drug resistance occurs mainly in *Plasmodium falciparum*. For this reason, as well as the large number of *P. falciparum*-infected patients around the world, most clinical trials of new candidate antimalarial drugs have been conducted against this potentially fatal malaria infection. Drug-resistant *Plasmodium ovale* and *Plasmodium malariae* parasites have not yet been documented. However, recent reports on chloroquine-resistant *Plasmodium vivax* in several geographic regions seem to suggest the potential of malaria parasites belonging to different species to develop drug resistance (1, 8). This observation, together with the fact that some patients present mixed infections, highlights the importance of clinical assessment of new antimalarial compounds against all four human malaria parasites.

Pyronaridine is a new antimalarial drug developed in China in 1970 (7). Preliminary clinical studies conducted in China have demonstrated its safety and efficacy against both *P. falciparum* and *P. vivax* (3, 4). The clinical efficacy of oral pyronaridine against chloroquine-resistant *P. falciparum* and its good tolerance were confirmed in two recent studies conducted in Thailand and Cameroon (5, 9). The efficacy of pyronaridine for treating *P. ovale* and *P. malariae* infections has not been evaluated in previous studies. Although clinical evaluation of drug therapy for *P. vivax* and *P. ovale* is complicated by the fact that these malaria species are subject to recrudescence independently of blood schizontocidal actions, the following criteria may accurately reflect therapeutic efficacy: rapid clearance of parasites from peripheral circulation, disappearance of signs and symptoms associated with malaria infections, and negative blood smears for at least 14 days after treatment. With these criteria in mind, we have evaluated the efficacy of pyronaridine to treat *P. ovale*- or *P. malariae*-infected patients.

Symptomatic Cameroonian patients presenting spontaneously at the Nlongkak Catholic Missionary Dispensary in Yaoundé, Cameroon, with *P. ovale* or *P. malariae* parasites were enrolled in the study after their informed consent was obtained. The following inclusion criteria were used: monoinfection with either *P. ovale* or *P. malariae*, parasite density of >2,000 asexual parasites/ μ l, and presence of fever at the time of consultation or within 24 h preceding consultation. Patients who were pregnant or who presented mixed infections or a

positive Saker-Solomons urine test for antimalarial drugs were excluded (6). The study was approved by the Cameroonian national ethics committee.

Enteric-coated pyronaridine tablets (total dose, 32 mg/kg of body weight; 16 mg/kg on day 0 in two divided doses and 8 mg/kg on days 1 and 2) were administered under supervision. Eight patients (age range, 7 to 38 years) with *P. ovale* and 14 patients (age range, 8 to 45 years) with *P. malariae* were recruited and monitored on days 1, 2, 3, 4, 7, and 14 on an outpatient basis, as recommended by the World Health Organization (WHO) (12).

In addition to the in vivo test described above, in vitro drug assays were performed against chloroquine and/or pyronaridine in 8 and 10 isolates of *P. ovale* and *P. malariae*, respectively. Blood samples with less than 5,000 asexual parasites/ μ l were not used for in vitro studies. A modified drug sensitivity assay was adopted from the techniques described by Basco and Le Bras (2) and Tan-ariya and Pasuralertsakul (11). Briefly, infected erythrocytes were washed twice in RPMI 1640 and suspended in a 3:1 (vol/vol) mixture of RPMI 1640 and Waymouth media (2.5% hematocrit). The culture media were supplemented with 10% human serum and buffered with 25 mM HEPES and 25 mM NaHCO₃. The parasites were incubated in 5% CO₂ at 37°C in the presence of eight twofold dilutions of chloroquine (12.5 to 1,600 nM) or pyronaridine (0.5 to 64 nM) for 48 h (72 h for *P. malariae*). Parasite growth was assessed by tritium-labeled hypoxanthine incorporation. The 50% inhibitory concentration (IC₅₀), defined as 50% inhibition of hypoxanthine incorporation compared with drug-free wells, was calculated by nonlinear regression curve fitting.

One patient from each group (PO-3, PM-14) did not complete the 14-day follow-up (Table 1). Parasite and fever clearance was obtained on or before day 4 and day 2, respectively, for both of these patients, but data pertaining to these two patients were excluded from further analysis. The remaining 20 patients responded favorably to pyronaridine treatment, with negative blood smears on or before day 4 and on subsequent follow-up on days 7 and 14. Compared with Cameroonian patients infected with *P. falciparum* (mean parasite clearance time, 76.8 h; mean fever clearance time, 33.5 h), the mean parasite clearance time in the *P. ovale*- and *P. malariae*-infected patients treated with pyronaridine was shorter (58.3 and 60.9 h, respectively), while the mean fever clearance time was longer (44.6 and 49.8 h, respectively) (9). All patients in this study were afebrile on or before day 4. According to the

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TABLE 1. Clinical efficacy and in vitro activity of chloroquine and pyronaridine against *P. ovale* and *P. malariae* isolates

Isolate ^a	Patient age (yr)	Parasitemia (no. of parasites/ μ l)	Treatment	Clearance time (h)		IC ₅₀ (nM) ^b	
				Fever	Parasite	Chloroquine	Pyronaridine
PO-1	23	9,110	Pyronaridine	72	48	—	—
PO-2	35	20,960	Pyronaridine	48	72	—	—
PO-3 ^c	15	20,160	Pyronaridine	48	48	37.3	7.87
PO-4	18	8,580	Pyronaridine	24	48	—	—
PO-5	7	40,680	Pyronaridine	72	72	32.7	3.49
PO-6	38	9,000	Pyronaridine	24	48	8.9	1.61
PO-7	13	2,250	Pyronaridine	48	48	ND	ND
PO-8	13	2,292	Pyronaridine	24	72	ND	ND
PO-9	9	13,680	Chloroquine	24	48	34.7	4.29
PO-10	7	6,656	Chloroquine	24	72	43.5	4.21
PM-1	21	7,800	Pyronaridine	72	72	—	—
PM-2	25	35,680	Pyronaridine	96	48	—	—
PM-3	22	15,000	Pyronaridine	48	72	40.5	4.24
PM-4	20	8,740	Pyronaridine	24	48	—	—
PM-5	30	26,760	Pyronaridine	72	96	—	—
PM-6	15	6,820	Pyronaridine	48	72	—	—
PM-7	22	2,280	Pyronaridine	24	48	ND	ND
PM-8	8	2,296	Pyronaridine	48	72	ND	ND
PM-9	45	6,000	Pyronaridine	72	48	83.2	11.1
PM-10	33	2,376	Pyronaridine	48	72	ND	ND
PM-11	9	3,350	Pyronaridine	24	24	ND	ND
PM-12	10	15,540	Pyronaridine	24	72	—	—
PM-13	28	2,472	Pyronaridine	48	48	ND	ND
PM-14 ^c	15	28,770	Pyronaridine	48	96	ND	5.37
PM-15	55	2,392	Chloroquine	24	48	ND	ND
PM-16	6	15,960	Chloroquine	24	72	76.2	2.62

^a *P. ovale* (PO) and *P. malariae* (PM) isolates in Yaoundé, Cameroon.

^b ND, not determined; —, uninterpretable results.

^c Did not complete study; data does not include follow-up on days 7 or 14.

revised WHO classification of clinical and parasitological response to antimalarial treatment, all patients in this study were cured (12). Two *P. ovale*-infected patients and ten *P. malariae*-infected patients had gametocytes on day 0. No gametocyte was seen in blood smears taken on day 3, suggesting gametocytocidal action of pyronaridine. Nine and three of 20 patients complained of mild gastrointestinal disturbances (abdominal pain, diarrhea) and pruritus after pyronaridine therapy was initiated, respectively. These minor side effects resolved spontaneously before day 7.

Of the eight in vitro assays performed against the *P. ovale* isolates, five gave interpretable results. The range of IC₅₀ values for chloroquine (8.9 to 43.5 nM) and pyronaridine (1.61 to 7.87 nM) obtained in these *P. ovale* isolates lies within the same range obtained in *P. falciparum* isolates in our previous studies (9, 10). Similar results were obtained in 4 of 10 *P. malariae* isolates that gave interpretable results. The in vitro assay using *P. ovale* parasites had a higher success rate than that with *P. malariae*. The metabolic needs for short-term in vitro parasite propagation have been defined for *P. vivax* and *P. ovale* (2). Since similar studies that aim to define the particular metabolic needs for in vitro culture of *P. malariae* have not been conducted, the relatively poor success rate with *P. malariae* may be due to suboptimal in vitro culture conditions. Nevertheless, the similar level of IC₅₀ values obtained in *P. ovale* and *P. malariae*, compared with *P. falciparum*, together with the favorable clinical outcome in patients treated with pyronaridine, indicates that all three human malaria parasites tested so far in our study site are sensitive to pyronaridine and that *P. ovale* and *P. malariae* are sensitive in vitro to chloroquine.

While a larger number of patients would be required for us

to draw a more definite conclusion, the results of the present study suggest the high efficacy of pyronaridine for treating *P. ovale* and *P. malariae* infections in symptomatic patients. Using the same protocol, we determined the clinical and parasitological response to chloroquine (25 mg/kg in three divided oral doses: 10 mg/kg on day 0, 10 mg/kg on day 1, and 5 mg/kg on day 2) in two *P. ovale*-infected patients and two *P. malariae*-infected patients. All four patients had negative smears on or before day 3, and subsequent smears on days 7 and 14 were also negative. Further studies are needed to compare the efficacy of chloroquine and pyronaridine for treating *P. ovale* and *P. malariae* infections.

Previous studies have shown the efficacy of pyronaridine for treating *P. falciparum* and *P. vivax* malaria (3, 4, 5, 9). The present study extends previous observations and provides evidence that the drug is also useful for treating *P. ovale* and *P. malariae* infections. Our study does not by any means constitute a recommendation of the use of pyronaridine instead of chloroquine to treat infections other than those due to *P. falciparum*. However, since *P. ovale* and *P. malariae* are not uncommon in central and west Africa and may occur in mixed infections with *P. falciparum*, our study suggests that, in case of diagnostic doubt or presumptive treatment of acute, uncomplicated malaria infections, pyronaridine may be an effective alternative oral treatment for the three human malaria species that are present in this part of sub-Saharan Africa.

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