

## An *in vivo* Test to Assess Mefloquine 25 mg/kg for the Treatment of Uncomplicated Falciparum Malaria in Rondônia, Brazil

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**Drug-resistant *Plasmodium falciparum* is undermining malaria control efforts worldwide. In Brazil, mefloquine (MQ) at a dose of 15 mg/kg body weight is used to treat *P. falciparum*. At this dose, MQ resistance developed rapidly in Thailand. Use of a higher MQ dose may retard the development of resistance. We treated 50 patients aged one to 67 years who had acute, uncomplicated *P. falciparum* malaria using MQ 25 mg/kg. There were no serious adverse events. Two patients complained of dizziness and insomnia. Assessing evaluable patients, the day 42 cure rate was 40/42 [95.2% (95% confidence interval 83.8 to 99.4%)]. Mefloquine was efficacious and well tolerated in this small cohort from the state of Rondônia.**

**Key Words:** Malaria, mefloquine, Amazon, Rondônia, therapeutics.

Malaria is one of the most serious global health problems of our time. In Brazil, almost all malaria is transmitted in the Amazon region. In 2002, the total number of reported malaria cases was approximately 430,000, and the annual cumulative incidence was 18.8 cases per 1000 population in Amazonia [1]. *Plasmodium vivax* is the dominant species, accounting for 79% of the malaria burden. *Plasmodium falciparum* resistant to chloroquine and sulfadoxine/pyrimethamine is well established [2,3]. Resistance to mefloquine (MQ) is emerging, and there are *in vitro* data of reduced quinine sensitivity [4,5]. In one trial of 51 MQ treated children, three had RIII parasitological resistance [6]. By contrast, MQ cured 93 of 94 adults; but there was *in vitro* evidence of reduced MQ sensitivity in the one case of recrudescence infection [7]. Quinine-antibiotic combinations remain important for treating resistant falciparum malaria, but poor compliance with a seven-day regimen is common (LMA Camargo, unpublished observations).

The evolution of mefloquine resistance has been well documented from western Thailand, where there is low transmission of *P. falciparum* and *P. vivax*, in an epidemiologically similar setting to parts of Amazonia. Within five years, low dose mefloquine (15 mg/kg) had an efficacy of 60%. Increasing the dose of MQ to 25 mg/kg restored this to 90%, but cure rates declined rapidly thereafter [8]. The addition of three days of oral artesunate resulted in consistently high (>95%) cure rates against *P. falciparum*.

It has been argued that malaria drug policy, whereby drugs are introduced sequentially, should be changed. The use of artemisinin-based combinations is now advocated by the WHO

as a means of preserving the life span of antimalarial drugs [9]. They should ideally be introduced before there is significant resistance to the partner drug, so that both drugs can protect each other against resistance [10]. Furthermore, introducing mefloquine at 25 rather than 15 mg/kg may also contribute to retarding the development of resistance, because this reduces the probability of exposing malaria parasites to sub-therapeutic MQ concentrations [11]. This effect is enhanced greatly by adding an artemisinin derivative (AD), because the AD will kill most of the parasites rapidly, and leave the small remaining number to be killed by high MQ concentrations [10]. As a first step to the possible joint use of artesunate and mefloquine in Rondônia, we assessed the efficacy and tolerability of mefloquine 25 mg/kg for treating acute, uncomplicated falciparum malaria.

### Material and methods

#### Study design, site and patients

This was a 42-day therapeutic efficacy assessment of high dose mefloquine in 50 patients of all ages. The study took place from March to August 2000 in Porto Velho and Monte Negro in the north and central part of Rondônia, a state of the Brazilian Amazon (Figure 1). The study was approved by the University of São Paulo Ethics Committee.

Over the past 20 years, Rondônia has seen a substantial influx of migrant workers from south and southeastern Brazil. Much of the tropical rain forest was cleared to make way for economic activities that consist mostly of cattle raising, lumbering, coffee growing, and milk production. There are also areas of tin and gem mining. The principal public health problems in Rondônia are malaria, cutaneous leishmaniasis, leprosy, and viral hepatitis [12]. The mean annual temperature is 28.6°C. There are two seasons. The rainy season extends from November to April. Malaria transmission in Rondônia is low in global terms but is relatively high for Brazil; the annual cumulative incidence is 49 cases per 1,000 [1]. Malaria is also

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transmitted in the towns of Monte Negro and Porto Velho. The *P. vivax* to *P. falciparum* rate is 4:1. Malaria affects patients of all ages. Most of the patients who are seen in the clinics have symptomatic malaria.

#### Conduct of the *in vivo* test

Febrile patients presenting to the clinics were approached by the research teams and briefed about the study. A blood slide was taken routinely as part of the fever work up. If *P. falciparum* malaria was confirmed, and informed, voluntary consent was obtained in writing, patients were enrolled in the study if they met all of the following inclusion criteria: (i) acute, uncomplicated *P. falciparum* malaria, (ii) no symptoms or signs of severe malaria [13], (iii) age >11 months, (iv) not pregnant or breast feeding, (v) no mefloquine contraindications: allergy, psychiatric disease, epilepsy, (vi) no antimalarial drug use within the previous 14 days, and (vii) no known, coexisting cardiac, renal or hepatic disease. Enrolled patients received supervised mefloquine (Larium<sup>®</sup>, Hoffman-La Roche, Switzerland). A single dose of 15 mg/kg of body weight was given on the first day, and 10 mg/kg on the second day. If the patient vomited within one hour, a repeat dose was given. All patients were admitted to hospital for the first three days; thereafter, they were seen as outpatients on days 7, 14, 28, 35, and 42. At these visits, patients were assessed clinically, blood was taken for routine hematology and biochemistry, and Giemsa-stained thick films prepared and read. Asexual and sexual parasites were counted and the number per mL recorded, assuming a total white cell count of 8,000.

#### End points and data management

The efficacy end point was parasite clearance by day 42. A treatment failure was defined as (i) the development of severe malaria, (ii) failure to clear asexual parasites by day 7, (iii) reappearance of asexual falciparum parasites following initial clearance, (iv) a mefloquine-induced adverse event necessitating MQ withdrawal, and (v) the use of another drug with antimalarial activity. Treatment failures were treated with artemether intramuscularly (3.2 mg/kg on the first day, 1.6 mg/kg/d for days 2-5) combined with clindamycin (15 mg/kg bd x 7d). There were no facilities available to distinguish between resistant or new infections during follow up. All data were recorded onto standard case record forms, double entered, validated, and analyzed using Epi Info 6.04b.

#### Results

Fifty patients were enrolled, including 10 from Porto Velho, and 40 from Monte Negro. All patients lived in or around Monte Negro or Porto Velho. Baseline characteristics are shown in Table 1. Most patients were young males. No patient reported prior antimalarial drug use. One patient had *P. vivax* parasites detected on day 2 and was withdrawn from the study. Seven patients were lost to follow up.

Mefloquine was generally well tolerated. There were no serious adverse events. Two patients reported dizziness 12 to 24 hours after the second dose of MQ, one of whom also reported insomnia. All symptoms resolved after 48 to 72 hours. Within 48 hours, all patients were afebrile. By 72 hours, most (35/37 = 94.6%) of the patients had cleared their asexual parasites (data not shown). During follow up, there were two cases of recurrent parasitaemia. One was a 31-year-old female who vomited her first dose of mefloquine 20 minutes post ingestion. However, she did not advise the medical team and was not redosed. By day 28, she developed asymptomatic parasitaemia of 320 asexual forms/ $\mu$ L. The other case was a nine-year-old male who received the full drug regimen. On day 35, asymptomatic parasitaemia (960 asexual parasites/ $\mu$ L) was detected.

Excluding those lost to follow up and the one case of vivax malaria, 40 [95.2% (95% CI 83.8–99.4)] of 42 patients were cured by day 42. Gametocyte carriage peaked by day 3 and fell thereafter (Figure 2). The mean hemoglobin (n=31 paired samples) rose significantly by day 42 compared to day 0: 12.19 vs. 13.25 g/dL [ $\Delta$ =1.06 (95% CI 0.55–1.57),  $P$ =0.0002]. Liver enzymes were unremarkable at enrolment and follow up.

#### Discussion

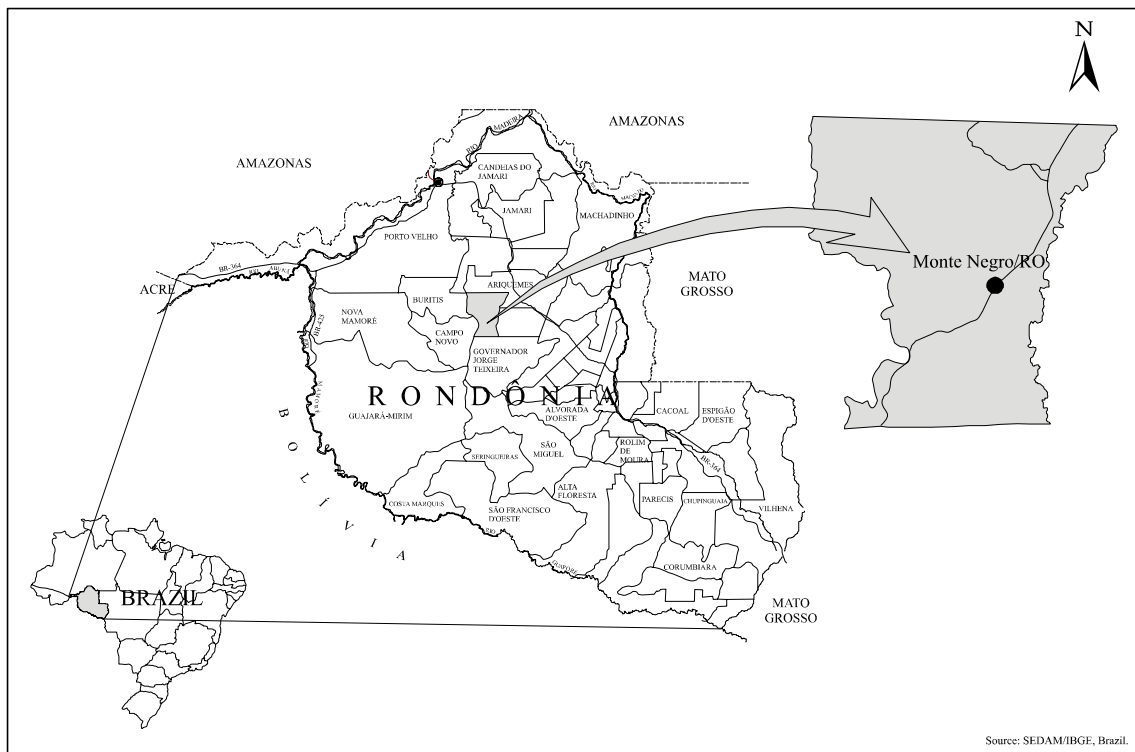
This small *in vivo* test study has shown that high dose mefloquine, dosed 24 hours apart, was well tolerated and efficacious in this small cohort of children and adults from Rondônia, an area of drug resistant *P. falciparum*. Mefloquine rapidly cleared asexual parasites, but this favorable pharmacodynamic effect did not result in cure in two patients; although in one case the patient did not take the full drug regimen and in the other we cannot definitely exclude a new infection. We extended the follow-up time to 42 days because mefloquine has a long half-life of 14–21 days in malaria patients. Prematurely stopping at 28 days would have missed one of our two cases of recurrent parasitaemia, and reminds us of the need to have adequate follow up to detect early resistance.

The use of 25 mg/kg of mefloquine rather than 15 mg/kg was based upon the experience from the Thai-Burmese border, where resistance to MQ developed rapidly, necessitating an increase in dose to 25 mg/kg. Malaria transmission in western Thailand is low but this results in greater drug pressure because patients of all ages have symptomatic malaria and seek treatment [14]. Whether there is a clinical advantage for individual patients to use the higher dose MQ over the lower dose is questionable. When there is a high degree of drug sensitivity to a given drug, the pharmacodynamic and clinical effects are similar. We did not attempt to answer this question, as this was a one-arm *in vivo* test. Most of our patients recovered quickly and cleared their parasites by 48 hours. From a public health perspective, the question that needs to be answered is whether using a higher dose of MQ will have a greater impact on slowing down the development of

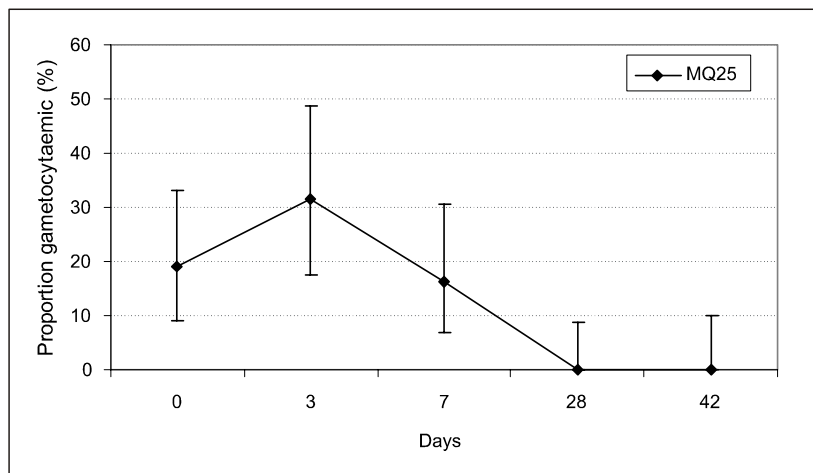
**Table 1.** Characteristics of the enrolled patients (n=50) at baseline

Characteristic	
Gender: male:female ratio	34:1
Mean age (SD) in years	25:9 (18.2)
Mean (SD) body weight in kg	48.31 (26.13)
Mean (SD) temperature	37.54 (1.24)
Proportion with splenomegaly	10/50 (20%)
Median (range) parasite count/ $\mu$ L	8,000 (120-79.140)
Proportion of gametocyte carriers on Day 0	9/50 (18%)

**Figure 1.** Map of Brazil and of Rondônia state.



**Figure 2.** *Plasmodium falciparum* gametocyte carriage in a cohort of Brazilian adults and children treated with mefloquine.



resistance. The greatest factor in promoting resistance is inadequate treatment of malaria infections, which increases the risk of the *de novo* development of resistance [15]. Therefore, it may be better to treat malaria infections with higher drug doses, provided they are well tolerated, rather than increasing the drug dose when resistance has already developed. The experience from Thailand with *P. falciparum* suggests that a strategy of increasing drug dosage in the face of resistance is, at best, an interim measure [8]. The use of an artemisinin derivative (AD) may further retard the development of resistance because the AD will kill most of the parasites and leave a maximum of 10,000 parasites to be killed by high concentrations of the companion drug [16]. The systematic use of artesunate and mefloquine in the low transmission areas of Thailand not only produced consistently high cure rates but also reduced falciparum transmission [8]. Encouraged by the findings of our *in vivo* study, we are planning to test artesunate combined with mefloquine in Rondônia on a larger scale.

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