

Investigation of the *in vitro* Gender-Specific Partitioning of Mefloquine in Malarial Infected Red Blood Cells and Plasma

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Abstract. The investigation of gender-specific partitioning of the antimalarial drug mefloquine to cellular and fluid blood compartments was performed using blood collected from a female and male healthy subject that were infected with *Plasmodium falciparum* PCM2 clone and spiked with mefloquine (0.25, 1, and 5 μM). Mefloquine concentrations in red cells of both female and male subjects were significantly higher than plasma, which suggests an intensive uptake by red cells. This was supported by a high ratio of mefloquine concentrations in the parasitized and non-parasitized red cells of about 4-fold. Gender-specific partitioning of mefloquine in parasitized blood was seen only in plasma where significantly higher concentrations were observed in female compared with male plasma. Down-adjusting the therapeutic dose of mefloquine in female patients with malaria is not advisable because mefloquine concentrations in the target cellular compartment are similar in both genders.

INTRODUCTION

Malaria remains one of the major global public health problems. The most recent *World Malaria Report* in 2009 revealed an estimated 2.37 billion people at risk, 225 million confirmed cases, and 781,000 deaths, of which 91% occurred in Africa.¹ *Plasmodium falciparum* is the most virulent and widespread infectious species of malaria parasite in tropical and subtropical countries caused by the resistance of the parasite to most available antimalarial drugs. In a race to combat increasing multidrug resistance in *P. falciparum*, artemisinin-based combination therapies have thus been recommended as first-line treatment of uncomplicated *P. falciparum* in all malaria-endemic countries to improve efficacy and delay development and selection of drug-resistant parasites.² In Thailand, a 3-day artesunate-mefloquine combination (600 mg artesunate and 1,250 mg mefloquine given as two split doses of 750 and 500 mg) has been introduced as first-line treatment of multidrug resistance *P. falciparum* all over the country since 2008.³

As the use of mefloquine increases, reports of its adverse effects are also accumulating particularly gastrointestinal and central nervous system adverse reactions.^{4–10} Mefloquine inhibits acetylcholinesterase and butyrylcholinesterase enzymes, a possible explanation for these adverse reactions when the drug is given at higher doses.¹¹ Recent studies have provided evidence for gender-specific difference in plasma or serum mefloquine concentrations and the frequency and severity of adverse drug reactions in patients after treatment with mefloquine.^{12–18} This leads to the proposal for down-adjusting prophylactic and/or therapeutic dose guidelines for female patients. Higher plasma or serum mefloquine levels observed in females may at least, in part, be attributable to difference from males in the partition and distribution of mefloquine in cellular and fluid blood compartments. This is of significant relevance to therapeutic and prophylactic outcome because the antimalarial effect of mefloquine has been shown to be

through its accumulation in the parasite food vacuole within host red cells where it competes with the complexing protein for heme binding.¹⁹ The aim of this study was therefore, to investigate the gender-specific differences in partitioning of mefloquine to various cellular (red blood cells, white blood cells, and platelets) and fluid (whole blood, plasma, and serum) blood compartments *in vitro*.

MATERIALS AND METHODS

Blood sample collection. Blood samples (300–350 mL each) used in the culture model were collected from one healthy female and one healthy male donor with type O blood group, into citrate-phosphate-dextrose sterile bags. All were tested for human immunodeficiency virus and hepatitis B antibodies. Approval of the study protocol was obtained from the Ethics Committee of the Ministry of Public Health of Thailand. Written informed consents were obtained from all subjects. Red blood cells and plasma were separated through centrifugation at $2,000 \times g$ for 10 min and transferred to a sterile 250 mL culture flask and stored at 4°C for up to 4 weeks.

Parasite culture. Mefloquine-resistant *Plasmodium falciparum* PCM2 clone isolated from a patient with acute uncomplicated falciparum malaria from Tak Province in 2003 was used in the study. The parasite was cultured according to the method of Trager and Jensen²⁰ with modifications. Cultivation was carried out according to the standard aseptic technique in an Envair class II laminar flow safety cabinet (Envair Limited, Lancashire, England). Unless otherwise stated by the manufacturers, all containers (e.g., culture flasks and centrifuge tubes) were presterilized disposable plastics. Glassware was autoclaved at 121°C, 15 atm for at least 15 min before use. All of the test standard drug solutions were sterilized before use by filtering through a 0.2 μm acrylic filter (Gelman Sciences Inc., Northampton, U.K.).

Mefloquine partition study. Highly synchronous ring stage parasite was used in the experiment.²¹ Gender-specific partitioning of mefloquine into the parasitized red blood cells and plasma was investigated in samples collected from one female and one male subjects. The non-parasitized control sample was also included using a blood sample obtained from the same female subject. Aliquots of parasitized red cells

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(10% parasitemia, 2% hematocrit in RPMI medium supplemented with human O serum, 200 μ L each) and plasma (200 μ L each) from each individual donor were resuspended in a 1.5 mL cryotube. Cell suspension was spiked with 0.25 μ M mefloquine (10 μ L in 50% ethanol), mixed thoroughly and incubated at 37.5°C for 30, 60, 120, and 240 minutes. Experiment was performed in triplicate. Plasma was separated through centrifugation at 2,000 \times g for 5 minutes. The same procedures were repeated but with mefloquine spiked at the concentrations of 1 and 5 μ M. Mefloquine concentrations used in the experiment (5, 1, and 0.25 μ M) were selected based on the average whole blood mefloquine concentrations at 14 (peak) and 168 (Day 7: distribution phase) hours and Day 42 (elimination phase) after the administration of the first dose, respectively.⁴ Mefloquine concentrations in red cells and plasma of each sample were determined using high performance liquid chromatography (HPLC) according to the method of Karbwang and others,²² with limit of quantification of 1 ng/mL. The concentrations of mefloquine in whole blood (red blood cells + plasma), red blood cells, and plasma were calculated based on the average values of hematocrit in female (40.8%) and male (45.2%) Thai population.

RESULTS

Mefloquine concentrations were measurable in both female and male parasitized red cells spiked with different concentrations of mefloquine (5, 1, and 0.25 μ M) at all time points (30, 60, 120, and 240 min). For plasma, the concentrations were detectable in almost all cases except in a sample collected from one male, which was spiked with 0.25 μ M mefloquine. The calculated concentrations of mefloquine in red cells, plasma, and whole blood for various time points at each spiked concentration were similar in blood from both male and female subjects. Mean (SD) of the ratio of mefloquine concentrations in parasitized and non-parasitized red cells of blood from the female subject was 4.23 (0.38). Mean (SD) calculated whole blood concentrations of mefloquine in females versus males parasitized blood spiked with 5, 1, and 0.5 μ M mefloquine were 3.61 (0.17) versus 3.51 (0.26), 0.90 (0.02) versus 0.78 (0.09), and 0.29 (0.03) versus 0.15 (0.05) μ M, respectively. Mean (SD) calculated plasma concentrations were 1.72 (0.32) versus 1.08 (0.12), 0.34 (0.01) versus 0.21 (0.01), and 0.12 (0.02) versus 0 (0) μ M, respectively (corresponding to 713.4 [132.7] versus 447.9 [49.7], 141.0 [4.1] versus 498.0 [8.2] versus 0 [0] ng/mL, respectively). Mean (SD) calculated concentrations in red blood cells were 2.38 (0.12) versus 2.43 (0.15), 0.56 (0.02) versus 0.57 (0.08), and 0.16 (0.02) 0.19 (0.09) μ M, respectively (corresponding to 987.1 [49.8] versus 1007.9 [62.2], 232.2 [8.3] versus 236.4 [33.1] versus 633.2 [8.2] ng/mL, respectively). The calculated mefloquine concentrations in red blood cells of both female and male subjects were significantly higher than those in the plasma (Figure 1). Gender-specific partitioning of mefloquine in parasitized blood was seen only in plasma where significantly higher concentrations (P values = 0.05, 0.01, and 0.01 at 5, 1, and 0.25 μ M, respectively) were observed in female compared with male sample (Figure 2).

DISCUSSION

The study was the first investigation of gender-specific *in vitro* partitioning of the antimalarial drug mefloquine in

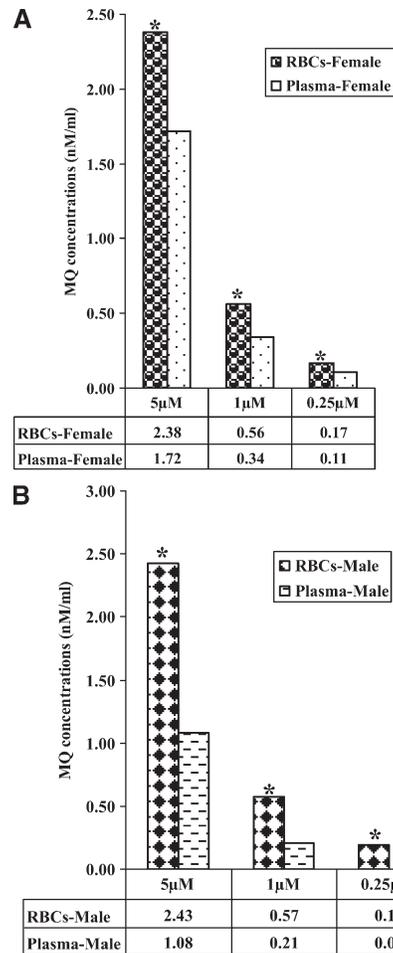


FIGURE 1. Mean concentrations of mefloquine (nM/mL) in the red blood cells and plasma measured after 30, 60, 120, and 240 min of spiking infected blood (*Plasmodium falciparum* parasitemia 10%) from (A) female and (B) male subjects with 5, 1, and 0.25 μ M mefloquine solution (* = significant difference between group, $P < 0.05$ for all concentrations).

various cellular and fluid blood compartments. The results provide some evidence of gender-specific partitioning of mefloquine in parasitized blood where plasma mefloquine concentration was shown to be significantly higher in females compared with males. However, this observation was not supported by the results of the clinical study in Thai patients with acute uncomplicated *P. falciparum* malaria following the administration of 1,250 mg mefloquine as a 2-day artesunate-mefloquine combination regimen.²³ Mefloquine concentrations in all cellular components/fluids (whole blood, red cells, white cells, platelets, plasma, and serum) in both female and male patients were found to be similar. There appeared to be no relationship between the occurrence, frequency, and severity of drug-related adverse reactions and gender-specific partitioning of mefloquine to cellular and fluid blood compartments when Thai female and male patients with acute uncomplicated falciparum malaria were given a therapeutic course of mefloquine. It was noted however that considering the relatively lower body weight in females, mefloquine concentrations on a *per weight* basis in females are even lower than males. A previous investigation in both healthy subjects and patients with falciparum malaria also showed similar

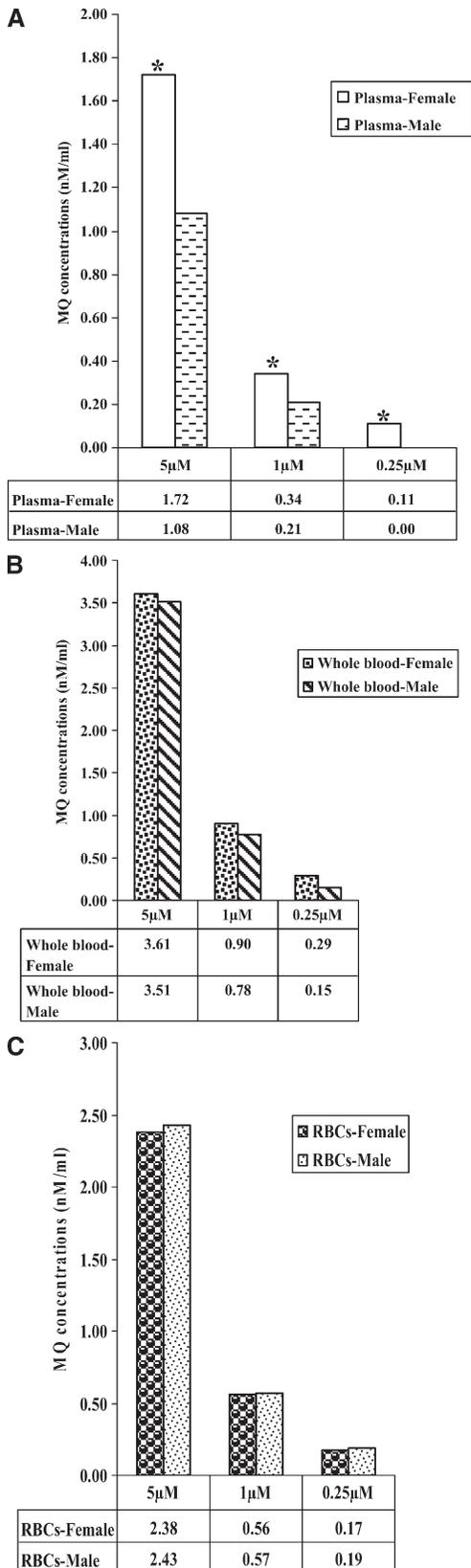


FIGURE 2. Mean concentrations of mefloquine (nM/mL) in the (A) plasma, (B) whole blood and (C) red blood cells measured after 30, 60, 120, and 240 min of spiking infected blood (*Plasmodium falciparum* parasitemia 10%) from female and male subjects with 5, 1, and 0.25 μ M mefloquine solution (* = significant difference between group, $P = 0.005, 0.01$ and 0.01 at 5, 1 and 0.25 μ M, respectively).

plasma concentration-time profiles of mefloquine and lack of correlation with severe adverse drug reactions.¹² However, gender-specific pharmacokinetics of mefloquine and relationship with frequency and severity of adverse drug reactions was reported in healthy Caucasian subjects, where females had a significantly higher mefloquine concentrations than males after therapeutic and prophylactic doses of mefloquine.¹⁷ These inconsistent findings could be explained by the concurrent contributions of several factors in various studies apart from the contribution of gender-specific distribution and pharmacokinetics of mefloquine. These include a difference in mefloquine formulations used, ethnicity of study participants, and most importantly, the influence of malaria disease itself. As mefloquine hydrochloride is readily absorbed in the gastrointestinal tract, leading to an immediate onset of gastrointestinal adverse reactions, it is produced in a number of different formulations that are geared towards relatively slow drug absorption. Considerable differences in the bioavailability were reported for various mefloquine formulations.²⁴⁻²⁵ Pharmacokinetic differences were also reported among individuals of different ethnic backgrounds regarding both systemic clearance and apparent volume of distribution.⁴ A comparison of overall means derived from pharmacokinetic investigations in patients with falciparum malaria and healthy volunteers suggests that the terminal elimination half-life and apparent volume of distribution of mefloquine are reduced in malarial infection.²⁶ Karbwang and others²⁷ proposed that enterohepatic recirculation might be augmented during malarial infection. Palmer and others²⁶ suggested that the fever and parasitemia associated with severe infection affects the distribution of mefloquine to various tissues and body fluids.

Mefloquine is a lipophilic drug with high affinity to plasma protein binding (98%) and cell membranes⁴; the drug is extensively distributed throughout the body with apparent volume of distribution ranging from 13 to 40 l/kg body weight. Relatively lower body weight and smaller apparent volume of distribution of mefloquine in female compared with male subjects are likely to be the major explanation for gender-specific partitioning of mefloquine to various tissues and blood components. The observation of no significant difference in the concentrations in all blood components (red blood cells, white blood cells and platelets) of female and male patients with *P. falciparum* infection reported in our previous study,²³ and red cell concentrations in parasitized blood (*in vitro*) observed in this study could be caused by its extensive plasma protein binding. The *in vitro* investigation showed a significantly higher partitioning of mefloquine in plasma of female parasitized blood spiked with various concentrations of mefloquine compared with male plasma. Furthermore, red cell concentrations of mefloquine in the parasitized blood of both female and male subjects were found to be significantly higher than plasma, suggesting an intensive uptake of mefloquine by the parasitized red cells. As compared with non-parasitized red blood cells, the selective uptake by parasitized red cells was on average four times higher. This feature was markedly observed at all spiked mefloquine concentrations, which suggests that selective uptake would occur at the concentrations commonly observed under chemoprophylaxis and chemotherapy, thus specifically targeting infected red cells. The rationale for using relatively high parasitemia (10%) in the experiment was to maximize the uptake of mefloquine into the infected red cells. However, this could have also affected the actual level of

mefloquine uptake into the infected red cells. Mefloquine has been reported to accumulate in *P. falciparum*-infected human red cells with relatively constant red cells to plasma concentration ratio of about 1–2.²⁶ It also has a high affinity for binding to red cell membranes^{28,29} and the concentrations in red cell membranes could be 60-fold of the bathing medium and buffer saline.³⁰

Mefloquine is certainly one of the best antimalarial drugs for treatment and prophylaxis of malaria. Although its effectiveness for both purposes is undisputed except for problems related to drug resistance; in particular, endemic regions, occurrence of adverse drug reactions pose a problem in the clinical use of the drug. The most commonly reported adverse reactions are related to gastrointestinal problem, but the main concern of its clinical use is neuropsychiatric effect.^{6,7,9,10,31} Women appear to be more susceptible than men.^{13–16,18} This concern leads to the proposal of down-adjusting prophylactic/therapeutic dose guidelines for women.¹⁷ The significant effect of gender on tolerability, in term of adverse reaction resolution time and symptom grading may be caused by gender-related pharmacokinetic differences. In a comparative study,⁷ most of the patients with disabling neuropsychiatric adverse effect caused by mefloquine were women. Definitive conclusion on neuropsychiatric adverse effects after therapeutic doses of mefloquine is difficult because malaria itself often causes nervous system symptoms. Mefloquine, given orally in divided doses up to 1,500 mg or in 250 mg doses every week for 1 year, is generally well tolerated. In the previous study, no adverse reaction was found after drug administration.²³ Similarly, in a previous study using combination therapy with mefloquine-artesunate, severe vomiting was found to be less common with combination regimens.³² The usage of a split dosage prevents the occurrence of early vomiting within 1 hour after drug intake.³³ Based on the results of current observation together with that from our previous study in patients with malaria, down-adjusting the therapeutic dose of mefloquine in female patients with malaria may in fact not be advisable.

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