Sensitivity to artemisinin, mefloquine and quinine of Plasmodium falciparum in northwestern Thailand

Felix Hüttinger¹, Wichai Satimai², Gunther Wernsdorfer³, Ursula Wiedermann¹, Kanungnit Congpuong², Walther H. Wernsdorfer¹

¹Centre for Pathophysiology, Infectiology and Immunology, Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria
²Directorate of Vector-Borne Disease Control, Ministry of Public Health, Nonthaburi, Thailand
³Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Sensitivity to artemisinin, mefloquine and quinine of Plasmodium falciparum was assessed in a study in an area with severe drug resistance. The study was conducted in northwestern Thailand, where resistance to mefloquine and quinine has been observed. The mean effective concentrations (IC₅₀ and IC₉₀) for artemisinin, mefloquine, and quinine were determined in vitro, using a method to measure drug-dependent inhibition of Plasmodium falciparum, particularly at the schizont stage. Artemisinin showed high efficacy, with an IC₅₀ of 0.0081 and an IC₉₀ of 2.5040 μM. Mefloquine had an IC₅₀ of 0.2155 μM and an IC₉₀ of 2.5040 μM. Quinine showed an IC₅₀ of 0.1260 μM and an IC₉₀ of 2.5040 μM. These findings indicate the need for further research on the resistance mechanisms and the development of new antimalarial strategies.

Key words: Plasmodium falciparum, drug sensitivity, artemisinin, mefloquine, quinine, Thailand.

Introduction

The history of drug resistance in Thailand started in 1959 with the increased occurrence of chloroquine resistant Plasmodium falciparum. In 1973 chloroquine was replaced by the combination of sulphadoxine/pyrimethamine, both members of the antifolate group. In less than ten years P. falciparum developed strong resistance to sulphadoxine/pyrimethamine, necessitating alternative treatment with a combination of quinine and tetracycline. This multidose regimen was associated with problems of tolerability and compliance. It was replaced by mefloquine in 1985, a 4-quinolinemethanol derivative structurally related to quinine, developed at the Walter Reed Army Institute of Research for the treatment of falciparum malaria in areas with resistance to chloroquine and antifols. Mefloquine is a blood schizontocidal drug and active against all human-pathogenic plasmodia. It has the longest half life of the currently used antimalarial drugs – 20 days in average – and is for that reason widely used in prophylaxis. On the other hand, the long half life makes mefloquine also prone for resistance especially in areas with intensive malaria transmission. The advent of mefloquine resistance in Thailand may have been also influenced by the intensive use of the chemically related drug quinine, just before the introduction of mefloquine [1, 2]. These factors may have contributed to the relatively early occurrence of mefloquine resistant cases in 1990. However, combination treatment with mefloquine and Na-argesunate was found to be still highly effective. Artemisinin and its derivates act against all intraerythrocytic stages of the malaria parasite, quickly reducing parasitaemia below detectable levels, but failing to eliminate them totally. Monotherapy or short-term treatment often results in the relapse of the disease, which makes the development of resistance against mefloquine in areas with intensive malaria transmission inevitable. This suggests a further rise of resistance of local P. falciparum, which is alarming especially for artemisinin and quinine.
in recrudescence [3]. These recrudescences are not caused by true resistance, but due to the short half life of the artemisinins [4]. Therefore derivates of artemisinin are always given in combination with drugs with a long half life, like mefloquine or lumefantrine for example.

In 1995 the combination treatment Na-artesunate and mefloquine was introduced in all areas affected by mefloquine resistance, i.e. the eastern and western border areas of Thailand. This combination showed high efficiency until 2008. Meanwhile artemisinins have become established antimalarial agents and artemisinin-based combination (ACT) therapies are now recommended by the World Health Organization (WHO) as first-line treatment of uncomplicated falciparum malaria in all areas in which malaria is endemic [5–7]. Unfortunately, there have been recently signs that the efficacy of artemisinin-based combination therapy and artesunate monotherapy has declined in western Cambodia and the Thai–Cambodian border [8–11]. Although the use of both drugs, artemisinins and mefloquine, has been strictly controlled in Thailand and neither mefloquine nor artesunate are available in the private sector on the Thai side of the border, there have been recent reports of a moderate increase in resistance to the ACT on the north-western border of Thailand [12]. A spread of artemisinin resistance would have a disastrous effect on the efficacy of malaria control [13]. These developments highlight the role of surveillance and containment of drug resistance as an important element of successful malaria control.

The objective of this study was the assessment of drug sensitivity to artemisinin, mefloquine and quinine at the border region of Thailand and Myanmar.

Materials and methods

The study was conducted in the framework of the monitoring of the drug sensitivity of Plasmodium falciparum, a programme of the Ministry of Public Health of Thailand.

Study site

The study took place at the Malaria Clinic of Mae Sot, Tak Province, in the border region of Thailand and Myanmar. It was conducted during the rainy season months of June and July 2009, coinciding with the usual peak of malaria transmission. Although the local malaria incidence in this area is below an API (Annual Parasite Incidence) of 1‰, it is known for its high incidence of drug-resistant P. falciparum strains [14].

The study included 50 patients. All of them had clinically manifest, non-severe falciparum malaria and microscopically confirmed monoinfections with P. falciparum. Patients with recent malaria treatment were excluded. So were patients with a parasitaemia over 150,000/μl and under 1000/μl. About 76% of the participants declared, that they have acquired their infections in Myanmar, although nationality-wise the distribution was equal between Thailand and Myanmar. Only five patients included in this study were female.

In vitro tests

The test procedure was based on the standard World Health Organisation in vitro technique for the assessment of the response of P. falciparum to antimalarial drugs [14]. The test system measures the inhibition of schizont maturation (SMI) dependent on the drug concentration. The tests were conducted with artemisinin, mefloquine and quinine. After finger-prick, 200 μl blood were collected in sterile, heparinised capillary tubes and mixed with complete RPMI-1640 culture medium. Aliquots of 50 μl of the blood-medium-mixture (BMM) were transferred to the scheduled wells of a 96-well microtiter test plate. All plates were pre-dosed with seven increasing drug concentrations of artemisinin (laboratory standard Academy of Military Medical Sciences) 3–3000 nM, mefloquine hydrochloride (BASE, LJ 14 268/175) 80–80,000 nM related to blood, or quinine hydrochloride (Sigma, Q-1125) 20–20,000 nM. The plates were incubated for 24 h in a candle box at 37.5°C. After incubation, the cultures were harvested and thick films were prepared from the sediment of each well. The slides were stained with a Giemsa solution at pH 6.9. For the microscopic analysis the number of schizonts per 200 asexual parasites was counted in each thick film. The test was considered valid with a growth of ≥10% schizonts in the control well. For the calculation of the regression parameters and the inhibitory concentrations [16].

Results

Artemisinin

In total, 43 isolates were successfully tested for the in vitro susceptibility to artemisinin. The mean cut-off concentration of schizont maturation for artemisinin was ≥2.9286 μM. The mean cut-off concentration is a specific drug concentration where no more schizont maturation is observed in 200 asexual parasites. It corresponds to the minimum inhibitory concentration (MIC). Eight isolates showed growth even at the highest drug concentration at 3000 nM. The IC₉₀, IC₉₉, and IC₉₉₉ values were calculated for every individual test isolate. For the calculation of the log-probit regressions the full range of IC₅₀ has been used. As compared to earlier years, the summary regression yielded a flatter slope ($S = 8.9733$; Fig. 1, Table 2). The diagram shows a comparison of the SMI for artemisinin as measured in