การหาและเฝ้าระวังความไวของยาคลอโรควิน ในการรักษาผู้ป่วยมาลาเรียชนิดไวแวกซ์ ที่ไม่มีอาการรุนแรง ที่จังหวัดแม่ฮ่องสอน

In vivo sensitivity monitoring of chloroquine for the treatment of uncomplicated vivax malaria in Mae Hong Son province Thailand

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Abstract

Background & objectives: Chloroquine (CQ), followed by 14-day primaquine, is a recommended regimen for the treatment of *Plasmodium vivax* infection in Thailand. CQ resistant *P. vivax* (CRPv) has not yet challenged the efficacy of the drug. The present study was conducted to assess the current response of *P. vivax* to CQ alone in Mae Hong Son province.

Methods: A 28-day *in vivo* therapeutic efficacy study was conducted from June 2009 to December 2010 in 2 sentinel sites. Recurrence of parasitaemia and the clinical condition of patients were assessed on each visit during follow-up. The drug levels in recurrent patients' blood were measured using HPLC. Data were analyzed using the WHO 2008 program for the analysis of *in vivo* tests.

Results: Of the total 56 patients included in the study, 49 completed the 28-days follow-up, while 7 cases were excluded. Adequate Clinical and Parasitological Response: ACPC in Mae Hong Son were 100 (49/49), respectively.

Conclusion: Although *in vivo* sensitivity monitoring of chloroquine for the treatment of uncomplicated vivax malaria in Mae Hong Son province (border province of north-western border with Myanmar during 2009–2010 ACPR in Mae Hong Son is 100 % but the resistance of *P. vivax* to chloroquine is emerging in Kanchanaburi province, near the border of Myanmar. Chloroquine remains the first-line drug for *P. vivax* infections in Thailand; regular monitoring is needed to detect further development of parasite resistance in this area. The monitoring is also needed in the other areas of the country where malaria endemic to estimate the level of burden across the country.

Keywords: Chloroquine resistance, malaria, parasitaemia, *P. vivax*, *Northern* Thailand

บทคัดย่อ

หลักการเหตุผลและวัตถุประสงค์: ประเทศไทยได้กำหนดแนวทางการรักษาเชื้อมาลาเรียชนิดไวแวกซ์ด้วยยาคลอโรควิน Chloroquine (CQ) และไพรมาควิน ต่อเนื่อง 14 วัน ประเด็นเชื้อไวแวกส์ดื้อยาคลอโรควิน (การตรวจพบเชื้อซ้ำหลังการรักษาครบกำหนด) เป็นความท้าทายที่จะต้องคำนึงถึงประสิทธิภาพของยา จึงกำหนดวัตถุประสงค์ของการศึกษาคือต้องการศึกษาว่าเชื้อมาลาเรียชนิดไวแวกส์ตอบสนองต่อยา CQ ในจังหวัดแม่ฮ่องสอนอย่างไร โดยศึกษาประสิทธิภาพยาต่อเชื้อในคน ตั้งแต่ เดือนมิถุนายน พ.ศ. 2552 ถึงเดือนธันวาคม พ.ศ. 2553 ใน 2 พื้นที่ของจังหวัด คือ อำเภอสบเมย และอำเภอเมือง วิธีการคือติดตามเจาะหาเชื้อมาลาเรียและสอบถามอาการของผู้ป่วยแต่ละราย วัดระดับยาในเลือดผู้ป่วยด้วยวิธีใช้ HPLC วิเคราะห์ข้อมูลโดยใช้โปรแกรมการวิเคราะห์การทดสอบเชื้อในคนขององค์การอนามัยโลก ผู้ป่วยที่ศึกษามีจำนวน 56 คน และตัดออกจากการศึกษา 7 คนเนื่องจากติดตามตัวไม่ได้ จึงมีจำนวน 49 คนที่สามารถติดตามและศึกษาต่อเนื่องจนครบ 28 วัน ผลการศึกษาพบว่า ทั้ง 49 รายมีการรักษาหายขาด (ACPR) 100 % แม้ว่าประสิทธิภาพการเฝ้าระวังเชื้อมาลาเรียชนิดไวแวกซ์ต่อยาคลอโรคควินในผู้ป่วยที่ไม่มีอาการแทรกซ้อนในจังหวัดแม่ฮ่องสอน ซึ่งเป็นจังหวัดด้านชายแดนพม่าภาคเหนือด้านตะวันตก จะมีประสิทธิภาพ 100 % ก็ตาม แต่มีรายงานพบเชื้อมาลาเรียไวแวกส์ดื้อยาในจังหวัดกาญจนบุรี ซึ่งเป็นจังหวัดอยู่ในภาคตะวันตกติดชายแดนพม่า และปัจจุบันประเทศไทยยังคงใช้คลอโรควินเป็นยาอันดับแรกในการรักษาเชื้อมาลาเรียไวแวกส์ ดังนั้นจึงมีความจำเป็นที่จะต้องมีการเฝ้าระวังเชื้อมาลาเรียดื้อยาในพื้นที่นี้ รวมทั้งในพื้นที่อื่นของประเทศโดยเฉพาะพื้นที่ที่มีการระบาดของมาลาเรียหรือพื้นที่ที่มีการเดินทางข้ามไปมาระหว่างประเทศ

Introduction

In Thailand, malaria transmission is seasonal and unstable, causing frequent epidemics. The two species, *Plasmodium falciparum* and *P. vivax* are the two species that most commonly cause malaria in Thailand. The proportion of *P. vivax* to *P. falciparum* in Thailand increases from 49.3% in 2007 to 58.2% in 2010[1]. Resistance to antimalarial drugs in *P. falciparum* is well-recorded in Thailand but is not well-known for P.vivax. However, relapsing and chloroquine-resistant *P. vivax* CRPv have been emerging in different parts of the world[3]. The present study is part of the routine monitoring for efficacy of antimalarial drugs against *P. falciparum* and *P. vivax* in the country. The study was conducted in Mae Hong Son province:during 2009 and 2010.

Materials &Methods

This study was registered with the Australia-NewZealand Clinical Trials Registry (ANZCTR) No: ACTRN

12610000554066. The study was conducted at two sentinel sites situated in Maung

and Sob Moey districts in Mae Hong Son province (north-western border with Myanmar) during June—December 2010. Malaria patients were both Thai and non-Thai. The majority of patients were male adults and had agricultural and forest-related occupations. The climate is typically tropical with rainy season extending from May/June to November. The annual parasite incidence (API) in 2010 for malaria was 8.34 per 1,000 population in Mae Hong Son.[8]

Patients

Clinically suspected patients seeking medication at malaria clinics in the study sites were examined for the presence of *P. vivax* infection on thick blood film preparations. Among the screened patients, those who fulfilled the inclusion criteria set by WHO[13] were recruited for the 28-day *in vivo* study. Inclusion criteria were: uncomplicated *P. vivax* mono-infection with parasitaemia between 250 and 100,000 parasites/µl of blood; age >6 months; axillary temperature >37.5°C or a history of fever in the past 24 h; informed consent from patient or parent/guardian (in the case of children); and ability to attend the stipulated follow-up visits. Exclusion criteria were: inability to drink or feed; repeated vomiting, convulsions during the present illness; inability to sit or stand up; presence of a severe disease; presence of severe malnutrition; pregnancy and any febrile diseases other than malaria.

Treatment and follow-up

The in vivo tests were performed according to the WHO guidelines13. The patients were treated with a 25 mg/kg chloroquine, administered for 3 consecutive days (15 mg/kg loading dose divided into 3 meals on Day 0 and 5 mg/kg daily on Day 1 and Day 2). Successive monitoring of the parasitological and clinical responses overtime was conducted for 28 days. The in vivo testing was conducted with the purpose of determining the parasite clearance time (PCT), defined as the time from the start of chloroquine treatment until blood films became negative. All doses were administered under direct observation. Physiological complains were recorded at the time of each visit. Subjects were checked for vomiting for 30 min after ingestion of the drug; those who vomited were re-treated with an identical dose provided that the subject vomited the entire ingested drug. Subjects who vomited twice were dropped from the study. The study participants were advised not to take other drugs, except for patients with axillary temperature > 37.5°C who were treated with paracetamol. Patients were asked to return for follow-up on Days 1, 2, 3, 7, 14, 21 and 28, and on any occasion of malaria like illness, for clinical examination including recording of temperature. Thick blood smears were prepared at all follow-up visits. 100 µl of blood sample was collected on filter paper using heparinized capillary tube from lancet pricked finger on Days 0, 7, 28

and any day when the patients had recurrent parasitaemia for measurement of whole blood chloroquine (CQ) and desethyl chloroquine (DCQ) concentrations. Patients who did not come for follow-up were traced to their homes. After completion of the follow-up, all patients were given 15 mg primaquine daily for 14 consecutive days. Those who failed to respond to CQ were retreated with 25 mg/kg chloroquine plus primaquine according to the national treatment guidelines.

Parasite identification

Parasites were identified by microscopic examination of morphology using thick blood smears taken at enrolment (Day 0). Subsequently, blood slides were taken at following visits (scheduled and non-scheduled). Smears were stained with Giemsa (3%, pH 7.2) for 45 min, and thick films were examined for malaria parasites under oil immersion. *Plasmodium vivax* asexual stages were counted against 200 white blood cells (WBCs) or 500 WBCs, if the number of asexual parasites was below 10 per 200 WBC, assuming the mean total WBC count of 6000/µl for the study population. WHO[13] recommended that white blood cell density of normal subject was typically 6000–8000/µl. However, WBC counts in malaria patients were lower than those in normal subjects. The median values for WBC counts of Thai malaria patients were in the range of 5900–7100/µl[5]. Gametocytes were counted, based on the same mean WBC count of 6000/µl. Experienced laboratory technicians working in the malaria clinic were assigned to examine each blood smear. All the slides were re-examined by an expert microscopist at the Reference Laboratory of the Bureau of Vector Borne Disease.

Endpoints

Treatment efficacy was determined based on the WHO classification of treatment outcome[13] as follows: 1) early treatment failure (ETF); 2) late clinical failure (LCF); 3) late parasitological failure (LPF); and 4) adequate clinical and parasitological response (ACPR). Recurrence denoted clinical and parasitological recurrence of malaria after the initial clearance of parasite from circulation. In the per protocol analysis, the proportion of treatment failures was calculated by dividing the number of subjects with recurrent parasitaemia by the total number of subjects who either suffered recurrent parasitaemia or completed the full 28-days follow-up period. In the Kaplan-Meier analysis, subjects were censored from the point at which they were either lost to follow-up or showed infection by *P. falciparum*. Subjects were considered to have cleared parasitaemia if there were at least two sequential negative smears. The day on which the first such negative smear was observed was defined as the day of clearance. This study was done in out-patient malaria clinics. Thick blood smear was examined daily on Day 0, 1,2, 3 and during the follow-up visit on Day 7, 14, 21 and 28. Daily parasite counting limited the possibility

to calculate the true PCT. Because smears were not taken on days 4–6, subjects with a reported clearance on Day 7 may actually have cleared their parasitaemia on any day between 4 and 7. When failure occurred in the presence of blood chloroquine concentrations (CQ + DCQ) > 100 ng/ml, the reappeared parasite should be considered resistant to CQ irrespective of its genotype (relapse, recrudescence or reinfection)[6]. Chloroquine (CQ) and desethylchloroquine (DCQ) levels in whole blood were assayed using solid-phase extraction and high-performance liquid chromatography (HPLC) equipped with UV detector[7]. The mobile phase was 5:95 acetonitrile/100 mM phosphate buffer, with a flow rate of 1 ml/min through a CN column. The UV detection was set at 342 nm. The dried blood spots were cut into small pieces and CQ and DCQ were extracted using solid-phase extraction. Eluted fractions were evaporated to dryness at 70°C under a stream of air, then reconstituted in 100 µl mobile phase and 50 µl of sample was injected into LC-system.The assays were linear over the ranges of 30–14,600 ng/ml for chloroquine and desethylchloroquine.

Data analysis

Data collected from in vivo therapeutic efficacy study were double entered and analyzed using WHO program for the in vivo therapeutic efficacy study. Kaplan-Meier survival probability analysis was used to evaluate the treatment outcome of the studied participants during follow-up period. Parasite clearance time and gametocyte clearance time of parasites from two sentinel sites were compared using Log Rank (Mantel-Cox) in the Kaplan-Meier analysis. In non-normally distributed data, the median was used to measure the central tendency. In all analysis, p-values of < 0.05 were considered significant.

Ethical clearance

The study was reviewed and approved by the Ethical Review Committee of the Department of Disease Control, Ministry of Public Health, Thailand. Written informed consent was obtained from each patient or guardian parents in cases when the study subjects were younger than18 yr. Written informed accent was also obtained from each patient between 15 and 17 yr.

Results

Characteristics of the study population

A total of 56 patients who met all the inclusion criteria were enrolled at two sentinel sites. Characteristics of the study population are shown in Table 1.

Table 1. Demographic and clinical characteristics of the study participants

Characteristics at day ofadmission (Day 0)	Mae Hong Son
Sample size	56
Median age (yr, range)	30.5 (12–66)
Male (%)	45 (80.4 %)
Female (%)	11 (19.6 %)
Mean body temperature	38.9°C
(95% CI)	(38.7–39.2)
Febrile–axillary Temp. > 37.5°C (%)	55.(98.2)
Geometric mean parasite	3879
(per µl/95% CI)	(2762–5446)

The majority of patients were males (80.4%) and females (19.6%). The median age was 30.5 yr (range = 12–66yr), but was statistically different at the two sentinel sites (p = 0.001). The highest median age was in Mae Hong Son (30.5). The proportions of male and female subjects in the two sentinel sites were not statistically different (p >0.05). About 80% were males in Mae Hong Son. Of the total 56 cases included in the study, 7 cases (12.1%) were excluded from the analysis: one had *P. falciparum* on in Day 7 and was re-treated with artesunate mefloquine combination; 7 cases were lost to follow-up on Day 3 (1 case), Day 7 (4 cases) and Day 28 (2 cases). A total of 49 cases completed the study and were included in per protocol analysis. The geometric mean (GM) of parasite density on the Day of enrolment was 3879 parasites/µl (95% CI = 2762–5446). Patients in Mae Hong Son had the GM parasite density (3,879) which was not statistically significant (p=0.001). Most of the studied participants, 98.2% (n = 55), were febrile, axillary temperature >37.5°C on the day of enrolment. The percentage declined to 37.3, 16.5, and 4.3% on Days 1, 2, and 3, respectively. All remained to have febrile on follow-up. The mean body temperature on the Day of enrolment was 38.9°C (95% CI = 38.7–39.2) (Table 1).

Treatment response

Mean parasite clearance time (MPCT) analyzed by Kaplan-Meier analysis was 2 days. Mean gametosite clearance time (MGCT) was 2.2 days. Clearance time in Mae Hong Son was not significant (p <0.0001), 71.4 % of vivax malaria patients in this study were presented with gametocytaemia. The geometric mean gametocytaemia on the enrolment day was 114 gametocytes/µl blood (95% CI = 87–149) (Table 2).

Table 2. Presence and geometric mean of *P. vivax* gametocytes on Day of enrolment in Mae Hong Son province

No. samples	presence of gametocytes on Day 0 (%)	GM gametocyte/µl
56	**40 (71.4)**	**114 (95% CI, 87–149)**

Among patients who completed follow-up and were treated with CQ under supervision at the therapeutic dose, 5 of 201 had recurrent parasitaemia within 28 days of following-up (1, 1, and 3 on Days 14, 21, and 28, respectively). Three patients cleared parasites on Day 1 while the other two cleared on Day 3. According to the WHO criteria, all were classified as LPF. The 28-day cure rate or ACPR according to per protocol analysis was 97.5% (196/201). ACPR in Mae Hong Son, were 100% (49/49), respectively Table 3 shows the treatment outcome (per protocol analysis) and Table4 shows the life table estimation of interval risk and cumulative risk of recurrent parasitaemia.

Table 3. Treatment outcome (per protocol analysis).

Sentinel site	No	ETF	LTF	LPF	ACPR
MaeHongSon	49	0	0	0	49 (100%)

Table 4. Life table estimation of cumulative incidence (risk) of recurrent parasitaemia after chloroquine therapy of vivax malaria in Mae Hong Son province of Thailand in 2009 and 2010.

Days	No. of subjects remaining at risk (N)	No. of subjects withdrawn due to any reason (w)	No. of cases of therapeutic failure (i)	Interval risk (IR)	Cumulative risk (CR)
Day 0	56	56	0	0	0
Day 1	56	55	0	0	0
Day 2	56	55	0	0	0
Day 3	55	55	0	0	0
Day 7	52	55	0	0	0
Day 14	52	55	0	0	0
Day 21	52	55	0	0	0

Days	No. of subjects remaining at risk (N)	No. of subjects withdrawn due to any reason (w)	No. of cases of therapeutic failure (i)	Interval risk (IR)	Cumulative risk (CR)
Day 28	49	55	0	0	0

Note: Interval risk was calculated as follows: i = [N – (w/2)]-1, where N is the number of subjects remaining at risk, i is the number of cases of therapeutic failure, and w is the number of subjects withdrawn for any reason. The cumulative incidence of therapeutic failure (CRn) was 0%.

Safety and tolerability

No serious adverse events (SAE) were reported during the study. Many adverse events (AE) such as headache, muscle pain and anorexia were most likely related to the underlying malaria disease. These symptoms disappeared by Day 2 or 3 after the treatment.

Discussion

Infections with CQ-sensitive *P. vivax* were routinely cured with as little as 0.3 g of CQ base, even though 1.5 g has been the recommended therapy since 1946. The clinical failure of standard therapy therefore represents infection with an organism with a high degree of resistance. A persistent or recurrent parasitemia within 14 days of the start of treatment probably represents recrudescence by a highly resistant strain of *P. vivax*[16]. Chloroquine, the first antimalarial drug in Thailand, was used to treat both uncomplicated falciparum and vivax malaria since 1945. *Plasmodium falciparum* has developed resistance to various antimalarial drugs, resulting in several changes of drug policy, i.e. sulfadoxine-pyrimethamine (in 1973), mefloquine-sulfadoxine-pyrimethamine (1983), and mefloquine alone (1991). Current first line treatment is a combination of artesunate-mefloquine since 1995. Treatment of *P. vivax* by chloroquine, on the contrary, has remained effective[5, 10, 15]. However, there was a recent report of chloroquine resistant *P. vivax* in a pregnant woman in Tak province of Thailand[11]. Measurement of drug levels from finger-prick blood dropped onto the filter papers results in lower drug levels compared to the method measuring from venous whole blood[4, 8]. This is in part due to the lower yield of the extraction method and the dilution of blood by the interstitial fluid during blood collection from finger prick. Blood collection on filter paper is feasible in areas where laboratory facilities are inadequate. However, drug concentration measurement from blood collected on filter paper should be standardized. The minimum effective concentration (MEC) of CQ+DCQ against *P. vivax* is 90–100 ng/ml of whole blood[1]. Because of the wide use of finger

prick blood collected on filter paper, the MEC should be set for the measurement of CQ+DCQ from filter paper.

This study was done in two sentinel sites located innorth-western border with Myanmar. It is well-known that *P.falciparum* malaria has different sensitivity to various antimalarial drugs[14]. In Thailand, patients infected with *P. vivax* treated in the Ministry of Public Health facilities receive both CQ and PQ (usually 0.25 mg/kg/day for 14 days). This treatment regimen may have played a role in suppressing the appearance and/or extension of CQ-resistant parasites in the region.

Conclusion

Although *In vivo* sensitivity monitoring of chloroquine for the treatment of uncomplicated vivax malaria in Mae Hong Son province (bordered province of north-western border with Myanmar) during 2009–2010 is 100 %, *P. vivax* resistant to chloroquine is emerging in Kanchanaburi, a province, near the border of Myanmar. Chloroquine remains the first-line drug for *P. vivax* infections in Thailand; regular monitoring is needed to detect further developmentof parasite resistance in this area. The monitoring is also needed in the other areas of the country where malaria is endemic to estimate the level of burden across the country and should be awared of the impact of The ASEAN Economic Community (AEC) and the goal of regional economic integration by 2015 may be the mobile population.

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