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Efficacy of oral single dose therapy with artemisinin–naphthoquine phosphate in uncomplicated falciparum malaria

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ABSTRACT

All artemisinin-based combination therapies (ACTs), recommended by the World Health Organization, are 3-day regimens. A considerable level of non-compliance on ACTs has been reported from some countries. The study aimed to assess the therapeutic efficacy of single dose treatment with new generation ACT containing artemisinin plus naphthoquine. An oral single dose of eight tablets (400 mg of naphthoquine + 1000 mg artemisinin) of the combination drug was administered to adult uncomplicated falciparum malaria patients. Observations of fever, parasite clearance and reappearance, and other clinical manifestations were made on Days 0, 1, 2, 3, 7, 14, 21 and 28. Fifty-three adult falciparum positive cases, with fever or history of fever within the previous 24 h, were included in the final evaluation of the study. Mean fever clearance time, parasite clearance time were 18.2 ± 8.6 h and 34.6 ± 14.3 h, respectively. Adequate clinical and parasitological response was achieved in 52 cases, the rate being 98.1% (95% CI, 91.1–99.9). One patient was classified as late parasitological failure because of the reappearance of falciparum parasite on Day 14. The drug was well tolerated and no adverse reactions were detected in the patients. Since it is a single dose therapy, health workers can administer the drug as directly observed treatment.

1. Introduction

The treatment policy on falciparum malaria in all countries experiencing resistance to monotherapies should be artemisinin-based combination therapies (ACTs). The four ACTs recommended by the World Health Organization are artemether–lumefantrine, artesunate–mefloquine, artesunate–amodiaquine and artesunate plus sulfadoxine–pyrimethamine. The treatment courses of all currently recommended ACTs are 3-day regimens. Patients have to take a large number of tablets per course with a minimum of 15 tablets to a maximum of 24 tablets for adults (WHO, 2006). The number of tablets taken per day and the dosing schedule may influence the adherence. Artemether–lumefantrine is found to be 10% definitely or probably non-adherent in Uganda (Fogg et al., 2004) and 40.9% certainly or probably non-adherent in Southern Sudan (Depoortere et al., 2004a). With regard to artesunate plus sulfadoxine–pyrimethamine, it is 60.6% certainly or probably non-adherent in Zambia (Depoortere et al., 2004b) and 10.4% and 25% non-adherent at 24 h and 48 h respectively in Tanzania (Kachur et al., 2004). Taking drugs in incomplete dosages, including non-adherence, is a likely factor for the development of drug resistance leading to a reduction in drug efficacy.

ACTs are shown to be very effective in the treatment of uncomplicated *Plasmodium falciparum* malaria in the region of sub-Saharan Africa (Ogbonna and Uneke, 2008). However, efficacy of ACTs, particularly artesunate–mefloquine, is declining in the South-east Asia Region. The efficacy of artesunate–mefloquine against falciparum malaria showed 85.7% at 28-day follow-up in 2002 and 79.3% at 42-day follow-up in 2004 at Palin on the Cambodian side (Denis et al., 2006a) and 78.6% at 28-day follow-up with a 2-day regimen in 2003 at Trat on the Thailand side of the Cambodia–Thailand border (Vijaykadga et al., 2006). Development of new ACT regimens which have the properties of good efficacy as well as good compliance is essential.

The single dose therapy is usually better than the 3-day regimen for ideal compliance. The Academy of Military Medical Science of China developed an oral single dose therapy of artemisinin and naphthoquine phosphate [4-(7-chloro-4-aminoquinoline)-2-*tert*-butylaminomethyl-5,6,7,8-4hydro-1-naphthol diphosphate] combination. Naphthoquine is absorbed rapidly and completely following oral administration, and reaches peak plasma concentration 2–4 h after administration. The elimination half-life is 41–57 h. It is excreted mainly from urine. It is widely distributed, being highest in the liver, kidneys and lungs. The blood cells' concentration

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is higher than the blood plasma. The synergistic index of naphthoquine phosphate and artemisinin is more than 4 in the test against chloroquine-sensitive strain of *P. berghei* in mice and more than 8 in chloroquine-resistant strain (Wang et al., 2004).

A study conducted in China on 320 patients with falciparum malaria showed that the artemisinin–naphthoquine combination gave rapid parasite and fever clearance with high efficacy (Wang et al., 2004). This finding in China needs to be supported by another study in different epidemiological settings. Therefore, we conducted this study to assess the therapeutic efficacy of artemisinin–naphthoquine combination in one of the malaria endemic townships in Upper Myanmar.

2. Material and methods

2.1. Participants

The study was conducted from June to September 2007 in an area with moderate malaria transmission. Screening of patients was done at Wet-Won Village of Pyin-Oo-Lwin Township in Mandalay Division. Clinically suspected malaria cases were initially tested for *P. falciparum* with Rapid Diagnostic Test kit (RDT) (Paracheck P.f.; Orchid Biomedical Systems, Verna, Goa, India). Microscopic examination of blood smears from the RDT positive patients was done for determination of parasite density and confirmation of mono-infection. Patients aged between 15 and 55 years, *P. falciparum* mono-infection, parasite density in the range of 1000–100,000 per μl, axillary temperature ≥37.5 °C or history of fever during the previous 24 h, being able to return for the follow-up visits were eligible to participate in the study.

Patients with presence of one or more of the general danger signs or any sign of severe or complicated malaria, presence of mixed species infection, severe malnutrition, febrile conditions caused by diseases other than malaria, any type of severe disease, contraindications related to the antimalarial drugs used, especially history of allergy and pregnancy in females, were excluded from the study. Patients who fulfilled the inclusion criteria were then asked to join in the study. Written informed consent was obtained from each and every participant. All participants were admitted to temporary in-patient unit of Department of Medical Research (Upper Myanmar) for 3 days and were requested to come back for follow-up on respective days.

2.2. Ethical approval

Pharmacodynamics studies, toxicity tests and clinical efficacy trials of this combination were conducted in China (Wang et al., 2004). Since the above studies confirmed that the combination was effective, safe and well tolerated, this study was approved by the Institutional Ethical Committee of the Department of Medical Research (Upper Myanmar).

2.3. Sample size

Sample size was determined according to WHO protocol based on the population proportions of clinical failures of 5% in a previous study, with a confidence interval of 95% and a precision of 10%. Although the calculated sample size was only 18, WHO has recommended a minimum sample size of 50 patients in antimalarial drug efficacy trials (WHO, 2003). A total of 55 patients participated in this study.

2.4. Intervention

The test drug was manufactured and supplied by Kunming Pharmaceutical Corporation (KPC), China. Each tablet contained artemisinin 125 mg and naphthoquine 50 mg (equal to naphthoquine phosphate 78.3 mg). A single dose of eight tablets (400 mg of naphthoquine + 1000 mg of artemisinin) was given orally with water under supervision on Day 0. Baseline data such as age, sex and occupation, were collected on Day 0. Liver function tests were done on Days 0 and 14. Blood smear collection, microscopic examination of blood slides and recording of vital signs, temperature, signs and symptoms of adverse drug reactions were carried out on Days 0–3, 7, 14, 21 and 28. In this study, we followed the protocol of "Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria" developed by WHO (WHO, 2003).

2.5. Laboratory methods

Thick and thin blood smears were taken, stained with Giemsa's stain and examined under 100 × oil immersions for detection of parasite, species identification and parasite count, 12 hourly in the first 3 days and once a day on subsequent scheduled days. Parasite density was calculated by counting the number of asexual parasites against 200 white blood cells (WBC) in the thick blood film using a hand counter, up to a maximum of 500 parasites. Parasite density, expressed as the number of asexual parasites per micro-litre, was calculated by dividing the number of asexual parasites by the number of WBCs counted and then multiplying by an average count of 8000 leukocytes per micro-litre. The blood slides were recorded as negative when the examination of 200 thick-film fields did not show the presence of asexual parasites. Three consecutive negative findings were defined as a negative result and clearance of parasite. All the blood slides were examined blindly by two separate technicians; a trained technician from DMR (Upper Myanmar) and an experienced technician from the Malaria Control Programme of Department of Health.

2.6. Classification of therapeutic outcome

The therapeutic outcomes were classified according to the WHO classification for low to moderate transmission areas, which included three categories for treatment failure (early treatment failure, late clinical failure, and late parasitological failure) and one for treatment success (adequate clinical and parasitological response) (WHO, 2003).

2.7. Statistical analysis

Data entry and analysis were done using Microsoft Excel based Efficacy Calculation Software, developed jointly by the Centres for Disease Control and Prevention (CDC) and WHO and Epi-info 6 software. Frequencies, means and proportions of baseline data were calculated by using Epi-info 6 software. Proportions of treatment success and treatment failure were calculated by using Microsoft Excel based Efficacy Calculation Software.

3. Results

3.1. Characteristics of the patients

Of the 55 enrolled patients, two patients showed appearance of *P. vivax* on Day 28 and were excluded leaving only 53 patients for final analysis. The mean age of the patients was 26.7 years (range 15–54). Males accounted for 67.9% (36/53) of the participants and females 32.1% (17/53). The average body weight was 47.8 ± 4.3 kg. More than half (56.6%) of the participants were forest related workers. About 80% (42/53) of the patients gave a previous history of malaria. The average body temperature at the time of admission was 38.8 ± 0.6 °C. The average duration of illness at the time of

admission was 3.2 ± 1.0 days. The mean parasite count on Day 0 was 17,556 (95% CI, 13,427–21,685) per micro-litre with the range from minimum 2400 to maximum 56,000.

3.2. Safety and tolerability

Slight dizziness was reported by two female patients on Day 1, relieved by oral glucose. No other adverse reactions were observed in the remaining 51 patients. Liver function tests showed no significant abnormal changes between Days 0 and 14.

3.3. Efficacy

None of the patients vomited within the first hour after drug intake. The mean fever clearance time was 18.2 ± 8.6 h and that for parasite clearance was 34.6 ± 14.3 h. The 28-day adequate clinical and parasitological response (ACPR) rate was 98.1% (52/53) (95% CI, 91.1–99.9). One patient was classified as late parasitological failure because of reappearance of falciparum parasites on Day 14.

4. Discussion

Artemisinin has rapid onset of schizontocidal action. It is quickly absorbed through the intestine after oral administration, the peak plasma concentration occurring around 3 h. The half-life of artemisinin is very short (1 h approximately) (WHO, 2006). Taken alone, a long therapy course is essential (Li et al., 1994) otherwise the recrudescence rate is high (Alin et al., 1996). Naphthoquine phosphate has a long half-life and the dosage is small. The treatment success rate is high and the recrudescence rate is relatively low. It has properties that, when used in combination with artemisinin, make it more efficient. Tests on *P. knowlesi* in monkey conducted in China revealed that the appropriate proportion of naphthoquine and artemisinin was 1:2.5, and the appropriate regimen was only one dose (Wang et al., 2004).

Only one dose of artemisinin derivatives may provide less protection from the resistance of its long acting partner drug. On the other hand, one possible disadvantage of 3-day regimens of ACTs is poor compliance which can lead to early development of drug resistance. Therefore, both regimens have their own potential limitations.

The length of the follow-up in the therapeutic efficacy test is based on the time for complete clearance of drug from the body. Naphthoquine clearance will take 10.2–14.4 days, which is six times its elimination half-life (WHO, 2003). In order to cover the actual treatment failure rate of napthoquine, it is assumed that 28 days is enough for post-treatment follow-up.

Poor compliance and low efficacy of some recommended ACTs have been encountered in some areas of the world. In one study in Kenya, a few health workers suggested that a shorter duration of Artemether–lumefantrine with fewer tablets were likely to improve patients' compliance to the treatment regimen (Wasunna et al., 2008). According to data from the national malaria control programme of Myanmar, the 28-day cure rate of a 3-day regimen of artesunate–mefloquine combination at Kawthaung, Thai–Myanmar border, was only 88% in 2003. Artemether–lumefantrine is the currently recommended ACT in Myanmar. It is best taken with fatty food (Hutagalung et al., 2005). One study conducted in Cambodia showed a poor result of 71.1% cure rate at 28-day follow-up in patients taking Artemether–lumefantrine without fatty food (Denis et al., 2006b).

Trials have been conducted to discover new ACTs with regimen of shorter duration. Single day regimens of artesunate plus sulfamethoxypyrazine–pyrimethamine demonstrated high efficacy, with a cure rate of 92.3% in eastern Sudan (Adam et al., 2006) and 99% in Ivory Coast (Penali and Jansen, 2008). In both studies, participants were followed up for 28 days. Our study with an oral single dose artemisinin–naphthoquine combination showed 98.1% efficacy at 28-day follow-up. This rate was consistent with a finding from China which demonstrated a 28-day cure rate of 97.5% with the same drug and same dose (Wang et al., 2004).

Studies indicate that efficacy of antimalarial drugs is enhanced in partially immune individuals (Cravo et al., 2001; Dorsey et al., 2000). The majority of the participants in our study were forest related workers who frequently went with or without their families into the forests where they contracted malaria. Since 80% of them gave previous history of malaria attacks, our finding would represent mostly the partially immune population. The single dose treatment with artemisinin–naphthoquine combination may not yield a similarly high cure rate in non-immune subjects. However, all the remaining 20% (11/53) with no history of malaria attack before, who were assumed as non-immunes, showed no recrudescence up to Day 28. But the number was too small to give a conclusive remark about efficacy of the drug in non-immune patients.

The present findings in Myanmar, as well as previous results from China, have confirmed the high efficacy and safety of the artemisinin–naphthoquine combination. With the single dose therapy, all *P. falciparum* positive cases can be treated as directly observed treatment, immediately after confirmation of diagnosis. In favour of operational aspect including compliance, this combination may become the preferable regimen in the treatment of uncomplicated falciparum malaria in future.

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