AAC Accepts, published online ahead of print on 14 June 2010
Antimicrob. Agents Chemother. doi:10.1128/AAC.01187-09

New fixed dose artesunate/mefloquine for treating multidrug resistant *Plasmodium falciparum* in adults – a comparative phase IIb safety and pharmacokinetic study with standard dose non-fixed artesunate plus mefloquine.

Running title: fixed dose artesunate/mefloquine for falciparum malaria

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ABSTRACT

A new fixed dose artesunate (AS)/mefloquine (MQ) was assessed in adults, hospitalized for 28 days, with uncomplicated, drug resistant falciparum malaria. Patients (n=25/arm) were treated with: (i) two fixed dose tablets (AS/MQ arm: AS 100 mg/MQ 200 mg/tablet) daily for 3 days (D0, 1, 2) or (ii) non fixed AS (AS+MQ arm: 4 mg/kg/d x 3d) + MQ (15 mg/kg D1, and 10 mg/kg D2), dosed by weight. Clinical, laboratory, ECG adverse events (AEs), efficacy, pharmacokinetic parameters were assessed over 28 days. Both regimens were well tolerated. No AEs were drug related. Two serious AEs, malaria induced hypotension occurring in the AS/MQ arm, necessitated rescue treatment. There were no significant changes in hematology, biochemistry, PR and QRS intervals. For all patients, mean Fridericia corrected QT intervals were significantly ($p \leq 0.0027$) prolonged on D3 (407 ms) and D7 (399 ms) vs. D0 (389 ms) in parallel with significant ($p \leq 0.0003$) falls in heart rates [67 (D3), 73 (D7), 83 (D0) beats /minute]. Fixed-non fixed formulations were bioequivalent for MQ but not for AS and DHA. One AS/MQ patient developed a new infection on D28; his D28 plasma MQ concentration was 503.8 ng/mL.

Fixed dose AS/MQ was well tolerated, had broadly similar PK profiles to non fixed AS+MQ and is a suitable replacement.

KEY WORDS

Artesunate, mefloquine, pharmacokinetics, malaria

INTRODUCTION

Multidrug resistant *Plasmodium falciparum* on the Thai Burmese border has been treated with artesunate (AS) and mefloquine (MQ) since 1994, seven years before the WHO recommendation to use artemisinin based combinations (ACTs) as first line treatment for acute uncomplicated falciparum malaria (17, 36). AS+MQ, dosed at 12 and 25 mg/kg over three and two days, respectively, has achieved sustained cure rates of 95%, reduced *P. falciparum* transmission, and reversed the progression of the median *in vitro*, mefloquine 50% inhibitory concentration (50 IC_{50}) (23). More recent data still show high cure rates despite a slowing of the parasite clearance time compared to earlier studies (8).

The adequacy of the artesunate dose was supported by a dose ranging and pharmacodynamic (PD) study in falciparum infected patients from the Thai Burmese border which found that 2 mg/kg of oral artesunate was the minimum dose that resulted in maximal parasite killing, the minimum parasiticidal concentration (MPC) (4). Allowing for the interindividual variation of AS absorption (7), 4 mg/kg was recommended. This dose has been adopted widely for other AS based combinations(3). Artesunate is tolerated remarkably well. (25, 31) Serious toxicity is limited to acute anaphylaxis that occurs at an estimated 1 in 2,833.(15)

Mefloquine is associated with nausea, vomiting and dizziness. When used as a stat dose of 25 mg/kg, high rates of early (≤1 hour) vomiting occurred, especially in children < 7 and adults > 50 years old. (29, 34) Vomiting was reduced by 43% by giving MQ as 15 and 10 mg/kg 24 hours later and by two and three fold in those who received an artemisinin derivative on the first day of treatment followed by MQ25 ≥ the second day of treatment. (19, 34) The 15-10 mg/kg split dose alone also increased the mean area under the concentration-time curve ($AUC_{0-\infty}$) by ~50% to

51,020 ng·d/mL compared to MQ25 alone (34,106 ng·d/mL) and a 20% increase was seen when MQ was combined with artesunate: 24,343 (MQ15-10) vs. 20,292 (MQ25) ng·d/mL (28). Similar AUC results were found in artesunate treated children who received MQ25 on D0 (21,196 ng·d/mL) or delayed to D1 (28,196 ng·d/mL).(24) An increased AUC translates into more time MQ concentrations are above the MPC and MIC (minimum inhibitory concentration) which are important PK parameters for parasite killing.

Serious, dose related, acute psychiatric side effects occurred in 1/2,089 (15 mg/kg) and 1/1,217 (25 mg/kg) (18). Rates of sinus bradycardia and sinus arrhythmia were similar between MQ and other antimalarials and are probably due to fever resolution (9, 14). The cardiotoxicity (QT prolongation) of halofantrine is enhanced when halofantrine is used to treat mefloquine failures (16, 22).

A new, easy to use, fixed dose combination of AS/MQ has been developed and registered. It has four aged based dosing regimens. The MQ dose has been split into 8 mg/kg daily for three days. AS/MQ underwent a phase III field trial in patients older than six months in Thailand (5). It was well tolerated, resulted in a lower rate of early vomiting compared to the conventional non-fixed AS+MQ regimen and achieved a Day 63, PCR corrected cure rate of almost 92%, a similar rate to the non-fixed combination. A population PK model of non-fixed MQ, delivered as 8 mg/kg daily for three days in 50 patients, resulted in a mean, whole blood Cmax of 2,202 ng/mL and an $AUC_{0-\infty}$ of 31,395 ng·d/mL, a 40% increase compared to a previous population PK model of 24,343 ng·d/mL of AS plus MQ15-10 mg/kg (6, 27).

As part of the development of AS/MQ, a phase IIb study was conducted focusing on tolerability, ECG changes, and the PK profiles of the two different formulations and dosing regimens. The study results are reported here.

MATERIALS AND METHODS

Study design & site

This was a randomized, open label trial comparing the safety, PK and efficacy of fixed dose AS/MQ compared to the currently used, non-fixed AS and MQ. It took place from December 2004 to July 2005 at the Hospital for Tropical Diseases, Bangkok, and received ethical approved from the Faculty of Tropical Medicine, Mahidol University, and the WHO. The trial was registered (http://www.controlled-trials.com/mrct/trial/228997/DNDi). A sample size of 25 patients per arm was considered sufficient for an intensive PK study.

Study conduct

Study entry criteria were: (i) age 18 to 65 years, (ii) body weight ≥ 40kg, (iii) microscopically confirmed, monoinfection of *P. falciparum* (parasitemia ≥ 2/200 WBC), (iv) history of fever or a documented fever (axillary temperature ≥ 37.5°C), and (v) written informed consent given. Exclusion criteria were: (i) Pregnancy (all women of child bearing age underwent a urine pregnancy test) or lactating women, (ii) *P. falciparum* parasitemia ≥ 4% red blood cells (≥ 175,000/uL), (iii) clinical and/or laboratory features of severe malaria (1), (iv) other significant illnesses e.g. liver disease, renal disease, (v) ingestion of mefloquine within the previous 60 days, (vi) contraindications to mefloquine - history of convulsions and/or psychiatric illnesses, (vii) known hypersensitivity to artemisinins or mefloquine, and (viii) splenectomy.

A randomization list was computer generated. Sealed envelopes in a locked cabinet contained the drug regimen and were opened only after informed consent was obtained. All study drugs were administered by study nurses.

AS MQ dosing regimens

One fixed dose tablet (Far-Manguinhos, Rio de Janeiro, Brazil, batch number 070.008, expiry date April 2006) contained 100 mg of AS and 200 mg of MQ base. The dose was two fixed dose tablets/day for three days (total dose = 600 mg AS, 1200 mg MQ).

The non fixed AS tablets contained 50 mg of AS (Arsumax®, Sanofi Aventis, France, batch number 031201, expiry date December 2005) and were dosed at 4 mg/kg/d on Days (D) 0, 1 and 2 The non fixed mefloquine tablets contained 250 mg of mefloquine base (Roche, Basel, Switzerland, batch number B1100, expiry date March 2006) and were dosed at 15 (D1) and 10 (D2) mg/kg, rounding to the nearest ¼ tablet (21). Drugs were administered 12 hourly with ~200 mL of water and independent of meals. Patients were observed for one hour for vomiting. A full or half dose was readministered if vomiting occurred within 30 minutes and 60 minutes, respectively. Patients were rescued with parenteral artesunate.

Study evaluations

Patients were hospitalized for 28 days but short home leave was allowed. Clinical evaluations (symptoms, vital signs, clinical examinations) were done on D0, 1, 2, 3, 7, 14, 21, 28. Giemsa stained thick and thin blood films were examined at baseline, six hourly for the first three days and on D7, D14, D21 and D28. Asexual parasites were reported as the number/µL. Patients with recurrent parasitemia were classed as new or recrudescent infections by analyzing by PCR and comparing three polymorphic genetic markers: merozoite surface proteins 1 and 2 (MSP-1 and

MSP-2) and the glutamate rich protein (GLURP). A new infection was diagnosed if any of the genotypes of the three genes were different, based on their electrophoretic patterns. (30)

Standard 12 lead ECGs were done at baseline, D3 and D7.

Blood sampling

Full blood count and liver function tests were performed at baseline, D7 and D28. Urine dipstick examinations were done on D0, D7, 14, 21, and 28. PK samples (5 mL heparinized tube) for AS and MQ were taken at baseline, 0.25h, 0.5h, 0.75h, 1h, 1.5h, 2h, 3, 4h, 6h, 8h, 12h, 24h, D3, D7, D14, D21 and D28, centrifuged immediately and the plasma stored at -70^{0}C until analyzed at the Universiti Sains Malaysia. Plasma samples were analyzed within 6 months of collection. Mefloquine (MQ), artesunate (AS) and its metabolite dihydroartemisinin (DHA) were simultaneously extracted by solid phase extraction (SPE) and measured separately by reversed phase high pressure liquid chromatography (RPHPLC) with ultraviolet detection for MQ (Lower Limit of Quantification LLOQ = 25ng/0.5mL) and electrochemical detector operating in the reductive mode for AS and DHA. (13) The lower limit of quantification of mefloquine was 25 ng/0.5 mL with an accuracy of 98.9% and a coefficient of variation of 8%. The method was found to be accurate with an average deviation of <5.2% from the true value. The within-day and day-to-day precision for mefloquine were less than 4%. The extraction recoveries of artesunate, dihydroartemisinin and QHS were above 86% with a coefficient of variation ≤13%. The lower limit of quantification for artesunate was 10 ng/0.5 mL with an accuracy of 109.9% and a coefficient of variation of 7.2%. The lower limit of quantification for dihydroartemisinin was 10 ng/0.5 mL with an accuracy of 99.1% and a coefficient of variation of 5.9%. The within-day and day-to-day precision for both artesunate and dihydroartemisinin were less than 8%.

The pharmacokinetic parameters C_{max}, T_{max} and AUC_{0-t} were determined using model independent formulae (26). The C_{max} and T_{max} were obtained from a visual inspection of the

plasma concentration versus time curve. The AUC_{0-t} was calculated using the linear trapezoidal rule and the $AUC_{0-\infty}$ was calculated by the formula $AUC_{0-t} + Ct / \lambda z$ where Ct is the concentration at the last quantifiable time. From the terminal log-linear (disposition) phase, a first order elimination rate constant (λz) was estimated by linear regression and the terminal half-life value (T½) was estimated using the equation $T½ = \ln 2 / \lambda z$. AS, DHA and total DHA (AS converted stoichiometrically to DHA + measured DHA) concentrations are presented.

An adverse event (AE) was defined as either the development of a new symptom, sign, clinical syndrome, laboratory result or their exacerbation that was reported or detected after study drug administration (11). AEs were graded 1-4 using the Common Toxicity Criteria (CTC, National Cancer Institute, NIH, USA 1999). The relationship of the adverse event to study drugs was determined as unrelated, unlikely, possibly, probably or very probably related. A serious AE was an AE with at least one of the following characteristics: (i) life threatening (ii) resulted in death, (iii) caused residual significant disability, or (iv) resulted in a prolongation of a patient's hospital stay. This study had an independent Drug Safety and Monitoring Board.

An early treatment failure was defined as: (i) the development of severe malaria within the first 72 hours, (ii) a D2 parasite count > D0 parasite count, (iii) a D3:D0 parasite count ≥ 25% and (iv) parasitemia on Day 3 with fever. A late treatment failure was: (i) an initial clearing of parasites by Day 7 followed by a recurrence of parasitemia with the same genotype as baseline, or (iii) parasitemia on D4 to D7 with fever. The parasite clearance time (PCT) was defined as the time taken for malaria slides to become and remain parasite negative for at least two consecutive slides. The fever clearance time (FCT) was the time taken to become afebrile (axillary temperature < 37.5ºC) for at least 24 hours within the first seven days. (2)

The study end points were: (i) the occurrence of adverse events, (ii) pharmacokinetic parameters (AS, DHA, MQ), (iii) the PCR adjusted D28 parasitological failure rate, (iv) PCT and FCT, (v) the proportions of patients without parasitemia on D1, D2, and D3, (vi) gametocyte carriage, (vii) the fractional change (D28-D0) in the hemoglobin (HB) concentrations.

Continuous parameters were compared by paired and unpaired 't' tests or their non parametric tests, as indicated. Categorical data are presented as the number of patients and the percentage of patients (%) and analyzed using the Cochran-Mantel-Haenszel test or Fisher's exact test, as appropriate.

Data were recorded on case record forms by the research team. Double data entry (Clintrial 4.3), cleaning and analysis using SAS System (Version 8.02, SAS® Institute, Cary, North Carolina, USA), to a prospectively defined analytical plan, were done by a clinical research organization.

RESULTS

Patient characteristics

Of the 55 screened patients, 50 were recruited into the study. Patient disposition is presented in Figure 1. Most were Burmese males (Table 1). Reported symptoms were of mild or moderate intensity and consisted of headache [n=46 (92.0%)], weakness [n=45 (90.0%)], myalgia [n=39 (78.0%)], chills/rigors [n=37 (74.0%)], anorexia [n=34 (68.0%)], dizziness [n=31 (62.0%)], nausea [n=24 (48.0%)], vomiting [n=20 (40.0%)], abdominal pain [n=10 (20.0%)], insomnia [n=7 (14.0%)], palpitations [n=7 (14.0%)], coughing [n=6 (12.0%)] and diarrhea n=6 (12.0%)]. Two patients were lost to follow up (both arms) and there were two early and one late (D28) treatment failures in the fixed group.

The total doses of AS and MQ received by the fixed group ranged from 9.6 to 15 (median 12) mg/kg/d and 19.5 to 30 (median 24) mg/kg/d, respectively; corresponding doses for the non-fixed group were 9.3 to 15.9 (median 12) and 20.1 to 32.7 (median 25.2).

Adverse events and tolerability

Both drug regimens were well tolerated. Over 28 days, a total of 181 clinical or laboratory AEs (irrespective of their causality) were reported or detected in 24 (96.0%, AS/MQ) and 25 (100%, AS+MQ) patients (Table 2). Two AEs were serious AEs (AS/MQ arm) that were classed as CTC Grade 3 hypotension due to the development of severe malaria; both patients were treated with intravenous (iv) artesunate, iv fluids, iv antibiotics and dopamine and recovered. Of the remaining 22 AS/MQ patients, 21 had mild (CTC grade 1) AE/s and 1 had a moderate (grade 2) AE; all AS+MQ patients had mild AEs. No AEs were considered drug related. There was no vomiting within one hour of drug administration. Two patients from each arm reported mild, late vomiting within the first three days.

Hematological changes over time

Compared to Day 0, the mean hemoglobin (Hb) fell significantly on Day 7 to: (i) 11.42 [Δ= -1.25 ± 1.13, p <0.0001) for AS/MQ and (ii) 11.25 g/dL ([Δ= -0.86 ± 1.12, p=0.0001) for AS+MQ. By Day 28, the mean Hb values increased modestly to 13.05 and 12.8 g/dL, respectively, and were not significantly different compared to Day 0 (p=0.24 and p=0.20, respectively).

The mean total white cell count increased at each time interval over time and by Day 28, the mean total white cell counts were 8.65 for AS/MQ and 7.5 $x10^9$/L for AS+MQ, representing mean increases over baseline of 2.82 (p<0.0001) and 2.41 $x10^9$/L (p=0.0002), respectively. There was some fluctuation in the absolute neutrophil counts over time. One patient with a baseline

neutrophil count of 2170/μL developed a Grade 3 neutropenia of 970/μL on Day 21 which resolved by Day 28 (2930/μL). Compared to Day 0, the median platelet counts rose significantly by Day 28 to 238.5 $x10^9$/μL for AS/MQ ($p<0.001$), and 245 $x10^9$/μL for AS+MQ, ($p<0.001$). Three patients had mild and one had moderate Day 28 thrombocytopenia.

Biochemistry

The mean, Day 0 total bilirubin values in both arms were mildly raised 1.36 (AS/MQ) and 1.32 (AS+MQ) mg/dL; they normalized by Day 7 (0.58 & 0.63 mg/dL, respectively), and remained stable to Day 28 (0.6 & 0.63 mg/dL, respectively). The corresponding (D0, D7, D28) AST, ALT and creatinine values were: (i) AS/MQ: (a) AST: 24.7, 24.2, 21.2 IU/L, (b) ALT: 20.9, 35.8, 26.1 IU/L, (c) creatinine: 0.87, 0.78, 0.76 mg/dL, and (ii) AS+MQ: (a) AST 32.2, 31.5, 24.5 IU/L, (b) ALT: 30, 51.9, 27 IU/L and (c) creatinine: 0.84, 0.78, 0.76 mg/dL. Shift tables showed that most patients had biochemical values within the normal range at baseline and study end (data not shown). By D28, a total of five and two patients with normal baseline liver enzymes had raised Day 28 ALT and AST values, respectively. The highest ALT values were 67 IU/L (D0 value 3 IU/L) in the AS+MQ arm (Grade 1 CTC AE) and 109 IU/L (D0 value 32 IU/L) in the AS/MQ arm, a CTC grade 2 (>2.5 x ULN) AE. The AST values, 47 (AS/MQ) & 66 (AS+MQ) IU/L were classed as CTC grade 1 AEs; their respective Day 0 values were 18 and 20 IU/L.

ECG findings

A total of 145 ECGs were analyzed (D0=50, D3=47, D7=48). ECG interval and heart rate data were very similar between the fixed and non-fixed combination arms (data not shown) and have been combined. There were no significant changes in the mean PR and QRS intervals over time (Table 3). The Fridericia corrected QT (QTcF) interval was increased significantly on Day 3 and Day 7 compared to baseline in parallel with a significant decline in the mean temperature and

heart rates (Table 3). Using a backward stepwise manual modeling only the heart rate was a significant variable (data not shown).

Pharmacokinetic findings

The interindividual variations in absorption (i.e. C_{max} variation) were similar (Table 4) for MQ [3.2 fold (fix) *vs*. 2.5 fold (non-fixed)] and AS [24.9 fold (fix) *vs*. 29.4 fold (non-fixed)]. However, the C_{max} variations for DHA, reflecting absorption and metabolism, differed: 13.1 fold (fix) *vs*. 21.5 fold (non-fixed)]. The coefficients of variation (CV%) for the all PK parameters were higher for the fixed dose group except for the AS C_{max} and DHA AUC_{0-t}. The clearance for the fixed and non fixed MQ formulations were similar 0.4 (+ 0.2) and 0.4 (+0.1) ml/min/kg. Based on the 90% confidence intervals of the ratios (fixed:non fixed) of the geometric least squares means of the Cmax and AUC values, the formulations were not bioequivalent (i.e. outside the limits of 80 to 125%) for AS and DHA but were for MQ (Table 4). The Tmax values for AS, DHA and MQ were not significantly different between the two arms. A more systematic comparison of the bioavailability and disposition of the two products will be published separately.

Efficacy

The D28 parasitological failure rates were 2/24 (8.3%) for AS/MQ and 0/24 (0%) for AS+MQ (p=0.49). Two AS/MQ patients (A & B) developed ETF and one developed a new infection on return from a home visit. Patient A had a D0 parasitemia of 3680/μL and received 4.8 (AS) and 9.7 (MQ) mg/kg. Four hours post dose, her blood pressure fell from 90/50 to 80/50. A repeat blood film showed an increased parasitemia, 7,360/μL, so she was rescued. Her AS PK values were: (i) Cmax 1319.9 ng/mL, (ii) Tmax 45 min, and (iii) AUC_{0-t} 407.4 ng·h/mL; corresponding

DHA values were 1501.6 ng/mL, 4h, and 2752.23 ng·h/mL and those for total DHA were 1501.64 ng/mL, 4h, and 3059.34 ng·h /mL. These values confirm adequate absorption.

Patient B had a D0 parasitemia of 170,800/μL and blood pressure of 100/60. She received 4.3 (AS) and 8.7 (MQ) mg/kg. She reported palpitations and mild dyspnea soon after treatment that became worse some six hours later. She was treated with oxygen and frusemide and transferred to the ICU. Two blood films showed declining parasitemia: 161,040/μL (~8 h post admission) and 9760/μL (~17 h post admission). The following day, she was persistently hypotensive (92/55 supine, 81/48 sitting) and was rescued. Her AS PK data were: (i) Cmax 319.7 ng/mL, (ii) Tmax 1 h, and (iii) AUC_{0-t} 674.3 ng·h/mL; the corresponding values for DHA were 1,283.1 ng/mL, 4 h, and 3,792.7 ng·h/mL and for total DHA were 1301.32 ng/mL, 3h and 2840.97 ng·h/mL. These values confirm adequate absorption.

For patient C. His D0 parasite count was 20,960/μL, fell to 110/μL (24 h) and cleared by 60 h after treatment: 3.7 (AS) and 7.4 (MQ) mg/kg/d. He became and remained afebrile after 48 hours. He went home on D21 and on D28 was afebrile and asymptomatic but had a falciparum parasitemia of 10,060/μL. Paired parasite genotyping results were: (i) MSP-1 genes 471 D0 & D28, (ii) MSP2 D0 682 and 743 (indicating a double infection), D28 616 & 665, (iii) GLURP 814 (D0) & 900 (D28), indicating a new infection. His MQ PK parameters were: (i) Cmax 1,835.8, (ii) AUC_{0-t} 16,416.8 ng·d/mL (AUC0-∞ not available), (iii) D28 MQ 503.81 ng/mL. The AS and DHA parameters were: (i) AS Cmax 149.3 ng/mL and AUC_{0-t} 96.22 ng·h/mL and (ii) DHA 489.50 ng/mL and 1329.76 ng·h /mL and (iii) total DHA 887.30 ng/mL and 1408.17 ng·h /mL, respectively. All PK parameters were below the mean values for the AS/MQ group.

The proportions of patients with parasites or fever on Days 1, 2 and 3 were similar between the two arms (data not shown). Combing the two arms, these proportions for parasites were 36/48 (75%), 13/48 (27.1%) and 3/48 (6.3%), respectively, and for fever were 37/48 (77.1%), 13/48 (27.1%) and 5/48 (10.4%). For both arms, gametocyte carriage declined over time; no patients carried gametocytes by Days 14 (AS/MQ) and 21 (AS+MQ).

DISCUSSION

This small study has shown the new AS/MQ fixed dose combination was well tolerated, with no drug related clinical AEs, and did not produce any clinically significant ECG changes.

AS/MQ was well tolerated but the small sample size precluded detecting the rare, well known and potentially serious neuropsychiatric side effects of mefloquine.(31) There were two, malaria related, serious AEs, hypotension, which necessitated rescue treatment. Over the course of the study, at least one AE was detected in virtually all patients but none were considered drug related. Hematological findings were unremarkable. A small number of patients had D28 thrombocytopenia, probably related to continuing bone marrow recovery.(32) The high rate of post treatment eosinophilia was related to resolution of malaria.(10) Biochemical parameters were generally normal during the study and there was a downward trend in the total bilirubin and liver enzymes, consistent with disease resolution. There were a few patients with mildly abnormal liver enzymes at Day 28 but these are of doubtful clinical significance.

The ECG findings in these predominantly fit young men were unremarkable and consistent with the good cardiac safety record of mefloquine.(34) (35) The QTcF interval increase was best

explained by the falling heart rate consequent to disease resolution (detailed results will be reported elsewhere).

The fixed dose AS/MQ combination has been developed using two well known antimalarial drugs and choosing target doses that are known to be effective: 4 mg/kg/d for AS and 8 mg/kg/d for MQ. Patients in the fixed arm received 3.2 to 5 mg/kg/d of AS and 6.5 to 10 mg/kg/d of MQ. The MQ PK parameters were similar. The fixed group had lower mean AS & DHA exposures but this did not result in lower mean parasite clearance times, compared to the non fixed group.

Our fixed and non fixed AS and DHA PK data are similar to those of others regarding the short Tmax and rapid half life values (20) (33) but our interindividual fold differences in absorption and AUC were higher. The interindividual fold differences reported by Teja-Isavadharn *et al* were 3.2 for the DHA Cmax and 3.6 DHA AUC_{0-12}, respectively. (33) The interindividual variations for MQ Cmax [3.2 (F), 2.5 (nonF)] and $AUC_{0-\infty}$ [6 (F), 3.7 (nonF)] were tight and similar to those of Price *et al*. (3.7 & 3, respectively).(7, 24) Our mean fixed MQ $AUC_{0-\infty}$ (≡ 47,749 ng·d/mL, n=13) was higher than that reported in a study of non-fixed MQ dosed at 8 mg/kg/d x 3d (31,395 ng·d/mL, n=50). (6) The mean non fixed MQ $AUC_{0-\infty}$ (≡ 45,643 ng·d/mL) was higher in 25 children (28,654 ng·d/mL) but lower in 12 adults (63,590 ng·d/mL), using the same commercial formulation.(12) (24) More rapid MQ clearance [0.87 L/kg/d (≡ 0.6mL/min/kg) *vs*. our 0.4 mL/min/kg] in the children is likely to explain their lower $AUC_{0-\infty}$.

This study was not powered to compare efficacy and the follow up extended to only 28 rather than the standard 63 days. Two AS/MQ patients, with good AS and DHA absorption, developed early treatment failure due to hypotension, a sign consistent with severe malaria. One patient had a rising parasite count 4 hours post AS whilst the other had a declining parasite count. Patients with uncomplicated malaria may deteriorate because of the evolution of their disease and despite

commencing highly effective ACT treatment; this was the probable cause in our patients. The AS/MQ patient with a D28 new infection had a D28 MQ concentration of just over 500 ng/mL, a value that is associated with resistant *P. falciparum* parasites from Thailand.(27, 28)

To conclude, fixed dose AS/MQ was well tolerated and had a broadly similar PK profiles to the non-fixed combination. These data support the use of this AS/MQ fixed dose combination for treating drug resistant falciparum malaria. Continuing assessment of the safety and effectiveness of AS/MQ should be done in large field trials.

ATHOURS' CONTRIBUTIONS

Protocol development – WT, SK, SL, JRK

Study execution – SK, SL, PW, WP, US, WL, NT, KC

Study coordination and supervision – WT, JRK

Pharmacokinetic measurements, analyses & interpretation – SR, VN, MV, PO

PK ECG analyses – MV, WT

First draft of the paper – WT

Critical review and input of manuscript – PO, MV, SK, JRK, VN.

ACKNOWLEDGEMENTS

We thank the patients and the ward nurses for their help with study execution. We are grateful to the DSMB members, Drs. N. Day, R. Price, S. Awasti, A. Babiker, and I. Ribeiro.

DEDICATION

We dedicate this work and publication to the late Professor S. Looareesuwan. He was a well liked colleague who played a significant part in the AS MQ development project.

CONFLICT OF INTEREST

None of the authors have a conflict of interest.

SOURCES OF FUNDING

Financial support for this study came from the INCO-DEV Programme ("Confirming the International Role of Community Research for Development"), European Commission. Proposal No ICA4-2001-10193.

DISCLAIMER

PO is a staff member of the WHO. MV is a staff member of the CRP-Santé. The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of the WHO and the CRP-Santé.

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Table 1. Patient characteristics at baseline. All continuous data are reported as mean (range) unless stated.

Variable	**AS/MQ fixed n=25**	**AS+MQ non-fixed n=25**
Age in years	26.6 (17-50)	28.9 (16-45)
Weight in kg*	50 (40-62)	51.0 (40-65)
Male:Female N (%)	19 (76):6 (24)	22 (88):3 (12)
Burmese	13	14
Karen	7	8
Mon	4	3
Thai	1	0
Temperature* ^{0}C	38.5 (37.3-40)	38.1 (37-40) ^{0}C
Pulse beats*/minute	90 (72-118)	90 (76-124)
Supine blood pressure* mmHg	110 (90-130)/70 (60-90)	110 (90-130)/70 (60-80)
Palpable spleen N (%)	0	0
Palpable liver N (%)	3 (12)	4 (16)
Asexual parasitemia* /μL	30,816 (69-170,800)	28,231 (20-140,280)
Gametocyte carriage N (%)	3 (12.5)	6 (25)
Hb g/dL	12.58 (10.3-17)	12.05 (8.7-15.5)
Total white cell count x10^9/L	5.72 (1.5-9.2)	5.04 (2-10.1)
Neutrophils x10^9/L	3.67 (0.7-6.9)	3.15 (1.1-6.1)
Lymphocytes x10^9/L	1.45 (0.5-3.4)	1.34 (0.4-4.0)
Eosinophils x10^9/L	0.13 (0-0.4)	0.15 (0-0.7)

Monocytes x10^9/L	0.39 (0.1-0.8)	0.34 (0.1-0.8)
Platelet count x10^9/L*	85 (19-334)	99 (28-239)
Creatinine mg/dL	0.87 (0.5-1.2)	0.84 (0.4-1.3)
AST IU/L	24.7 (11-44)	32.2 (12-111)
ALT IU/L	20.9 (9-48)	30 (3-113)
Total bilirubin mg/dL	1.36 (0.5-2.5)	1.32 (0.4-2.8)
Dose of AS mg/kg*	4 (3.2-5)	4
Dose of MQ mg/kg*	8 (6.5-10)	15 & 10

* median (range)

Table 2. The main clinical adverse events reported by patients and the main laboratory adverse events detected at any time after the first dose of fixed or non fixed artesunate mefloquine until the end of follow up at 28 days. Patients may have had more than one adverse event.

	AS/MQ fixed **n=24**	**AS+MQ non fixed** **n=25**
Gastrointestinal		
Anorexia	3 (12.5)	0 (0.0)
Nausea	2 (8.3)	2 (8.0)
Vomiting	2 (8.3)	2 (8.0)
Flatulence	1 (4.2)	1 (4.0)
Abdominal pain	4 (16.7)	2 (8)
Diarrhea	1 (4.2)	1 (4.0)
Constipation	0 (0.0)	2 (8.0)
Respiratory		
Nasal congestion	2 (8.3)	0 (0.0)
URTI	6 (25.0)	3 (12.0)
Cough	2 (8.3)	1 (4.0)
Chest pain	1 (4.2)	0 (0.0)
Palpitations	1 (4.2)	0 (0.0)
Musculoskeletal		
Myalgia	1 (4.2)	1 (4.0)
Neck pain	0 (0.0)	1 (4.0)
Pain in extremity	0 (0.0)	2 (8.0)

Central nervous system		
Weakness	0 (0.0)	2 (8.0)
Dizziness	0 (0.0)	4 (16.0)
Headache	3 (12.5)	3 (12.0)
Insomnia	4 (16.7)	1 (4.0)
Vertigo	0 (0.0)	1 (4.0)
Skin		
Itching	1 (4.2)	2 (8.0)
Rash	1 (4.2)	1 (4.0)
General symptoms		
Fever	4 (16.7)	2 (8.0)
Dehydration	0 (0.0)	1 (4.0)
Epistaxis	1 (4.2)	0 (0.0)
Infections/infestations		
Abscess	0 (0.0)	1 (4.0)
Hordeolum	1 (4.2)	0 (0.0)
Gut helminths	15 (62.6)	6 (24.0)
Tuberculosis	1 (4.2)	0 (0.0)
Laboratory findings		
Anemia	5 (20.8)	2 (8.0)
Eosinophilia	20 (83.3)	21 (84.0)
ALT/AST increased	2 (8.3)	4 (16.0)
Hypoglycemia*	0 (0.0)	1 (4.0)

Microscopic hematuria	3 (12.5)	4 (16.0)
Proteinuria	8 (33.3)	3 (2.0)

* Plasma glucose 66 mg/dL, lower limit of normal for laboratory 75 mg/dL.

Table 3. The ECG intervals, the temperature and heart rate data in all patients combined who were treated for uncomplicated falciparum malaria with either the fixed or non-fixed dose combinations of artesunate and mefloquine.

	Day 0	Day 3	Day 7	D3-D0	D7-D0
Temperature ^{0}C	38.4 (37-40)	37.1 (36.5-37.7)	37.1 (36.5-39)	<0.000	<0.000
Heart rate /min	83 (59-112)	67 (44-84)	73 (59-101)	<0.000	0.0003
[MQ] ng/mL	0	3095	1721	0.6908	0.1473
PR ms	148 (122-232)	148 (111-190)	145 (101-182)	0.97	0.23
QRS* ms	91 (75-118)	94 (63–135)	91 (73-111)	0.055	0.67
QTcF ms	389 (344-434)	407 (319-504)	399 (357-443)	<0.000	0.0027

* median (range), QRS was not normally distributed. QRS comparisons used the Sign test.

Table 4. The main disposition parameters of mefloquine, artesunate and dihydroartemisinin and total DHA equivalents when given as either fixed or non-fixed products for the treatment of uncomplicated falciparum malaria. The Tmax for total DHA was measured after the first dose and the Tmax of MQ after the full course was given. Parameters are expressed as means ± standard deviations. Ranges and patient numbers are given in parentheses. Geometric least squares mean ratios are: (i) MQ Cmax 100.8% (90% CIs 84.4-120.3), AUC_{0-t} 100.9% (82.4-123.6), $AUC0_{-\infty}$ 100.8% (81.2;125.1), (ii) AS Cmax 55% (36-83), AUC_{0-t} 74% (45-121), and (iii) DHA: Cmax 58% (42-82), AUC_{0-t} 75% (57-102).

Treatment	Drug	Cmax (ng/mL)	Tmax (h)	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	T½ (h)
Fixed	MQ	3279 ± 1252 (n=20) (1809 - 5796)	72.0 ± 19.1 (n=20) (48 - 120)	837064 ± 378271 (n=20) (376173 - 1671400)	1145977 ± 678719 (n=13) (519164 - 3103075)	285.6 ± 128.2 (n=13)
	CV%	38.2	26.5	45.2	59.2	44.9
	DHA	1234 ± 857 (n=20) (306.2 - 4019.2)	1.99 ± 1.12 (n=20) (0.75 - 4)	3027 ± 2491 (n=20) (745 - 12661)	3138 ± 2491 (n=14) (16 - 272)	1.1 ± 0.5 (n=14)
	CV%	69.4	56.3	82.3	79.4	45.5
	AS	255 ± 175 (n=19) (50 - 1248)	0.833 ± 0.70 (n=19) (0.5 - 1.5)	310 ± 142 (n=19) (61 - 1075)	DNS*	DNS*
	CV%	68.6	84.0	45.8		
Non fixed	MQ	3239 ± 734 (n=23) (1817 - 4583)	70.9 ± 13.5 (n=23) (48.0 - 120.0)	814365 ± 232116 (n=23) (425319 - 1362127)	1095421 ± 370167 (n=19) (571397 - 2127627)	321.7 ± 113.7 (n=19)
	CV%	22.7	19.0	28.5	33.8	35.3
	DHA	2043 ± 949 (n=23) (192 - 4119)	1.4 ± 0.71 (n=23) (0.25 – 3)	3633 ± 1367 (n=23) (518 - 6245)	3745 ± 1371 (n=18) (26 - 333)	0.8 ± 0.2 (n=18)
	CV%	46.4	50.7	37.6	36.6	25
	AS	451 ± 440 (n=23)	0.925 ± 0.563 (n=23)	419 ± 670 (n=21)	DNS*	DNS*

	(75 - 2206)	(0.25 – 2)	(57 - 1124)
CV%	97.6	60.9	159.9

* DNS=Data not shown because of small patient numbers.

Figure 1. Trial profile.

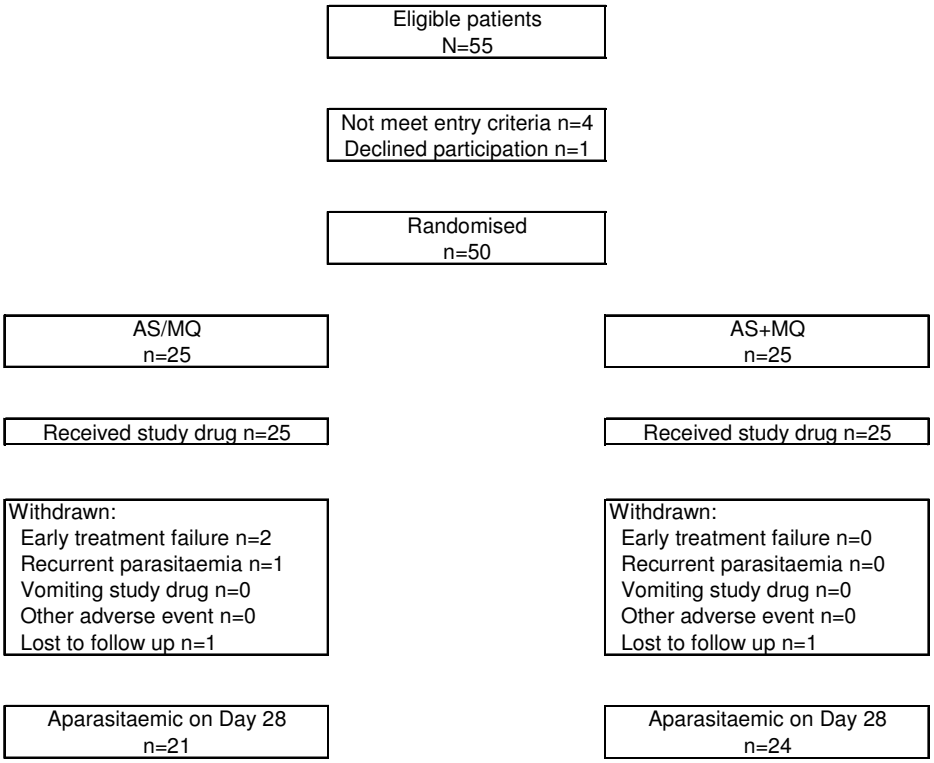

Figure 2. The mean (standard errors) plasma concentrations artesunate and dihydroartemisinin (DHA) expressed as the total DHA equivalents (AS + DHA) over time for patients with uncomplicated falciparum malaria who were treated with fixed and non fixed combinations of artesunate and mefloquine.

Figure 3. The mean (standard errors) plasma mefloquine concentrations over time for patients with uncomplicated falciparum malaria who were treated with fixed and non fixed combinations of artesunate and mefloquine.