Journal of Bioequivalence & Bioavailability - Open Access

Research Article
OPEN ACCESS Freely available online
doi:10.4172/jbb.1000032

JBB/Vol.2 Issue 3

Pharmacokinetics and Comparative Bioavailability of Artesunate and Mefloquine Administered Separately or as a Fixed Combination Product to Healthy Volunteers and Patients with Uncomplicated *Plasmodium falciparum* Malaria

Piero Olliaro[1]*, Surash Ramanathan[2], Michel Vaillant[3], Stephanie E Reuter[4], Allan M Evans[4], Srivicha Krudsood[5], Sornchai Looareesuwan[5§], Jean-René Kiechel[6], Walter RJ Taylor[1,7] and Visweswaran Navaratnam[2]

[1]*UNICEF/UNDP/World Bank/WHO Special Programme for Research & Training in Tropical Diseases (TDR), Geneva, SWITZERLAND*
[2]*Centre for Drug Research, Universiti Sains Malaysia, Penang, MALAYSIA*
[3]*Clinical Epidemiology and Public Health Unit, Centre for Health Studies, CRP-Santé, LUXEMBOURG*
[4]*School of Pharmacy & Medical Sciences, University of South Australia, Adelaide, AUSTRALIA*
[5]*Hospital for Tropical Disease, Bangkok, THAILAND*
[6]*Drugs for Neglected Diseases initiative (DNDi), Geneva, SWITZERLAND*
[7]*Division of International and Humanitarian Medicine, Geneva, SWITZERLAND*
[§]*Deceased*

Abstract

Purpose: The current World Health Organization recommendation for the treatment of uncomplicated *Plasmodium falciparum* malaria is with artemisinin-based combination therapy. Artesunate and mefloquine combination therapy has achieved consistently high efficacy rates and reduced malaria morbidity; however, the current standard treatment regimen is complex and may be difficult to comply with outside of a research setting. Consequently, an artesunate-mefloquine fixed dose oral co-formulation has been developed and is now registered in Brazil. This study was conducted in order to assess the pharmacokinetics and comparative bioavailabilities of artesunate and mefloquine administered as separate products and the new co-formulated product.

Methods: The pharmacokinetics of artesunate, dihydroartemisinin, the artesunate metabolite and predominant species and mefloquine were assessed in a single-dose, randomised, crossover design study in healthy volunteers and in a multiple-dose, randomised, parallel group study in patients with uncomplicated falciparum malaria.

Results: For artesunate/dihydroartemisinin the lower bound of the 90% confidence intervals for the comparison between co-formulated and separate products extended below the 80% bioequivalence limit; area under the curve and C_{max} values were 15-25% and 25-40% lower than those observed after administration of the separate products. The two formulations were bioequivalent in terms of mefloquine pharmacokinetics in uncomplicated falciparum malaria patients; the 90% confidence intervals for dose-normalised area under the curve (AUC_{last} and AUC_{inf}) and maximum observed concentration (C_{max}) were within the 80 - 125% limits. In contrast, mefloquine area under the curve and C_{max} values were 15% and 30% lower for the co-formulated products compared to the separate products in healthy volunteers.

Conclusions: These differences in the exposure to artesunate, dihydroartemisinin and mefloquine are unlikely to be of clinical relevance based on *in vitro* and clinical data. However, the results of this study do emphasise the importance of evaluating the bioavailability and bioequivalence of new formulations, particularly in specific patient groups.

Keywords: Artesunate; Dihydroartemisinin; Mefloquine; Pharmacokinetics; Malaria

Introduction

Malaria is a leading cause of mortality and morbidity in developing areas of the world. Every year, malaria is responsible for an estimated 250 million infections and results in approximately 1 million deaths, predominantly in children under 5 years of age (World Health Organization, 2008).

Due to the increasing resistance to traditional anti-malarials in falciparum malaria endemic areas, the current World Health Organization (WHO) recommendation for the treatment of uncomplicated falciparum malaria is with artemisinin-based combination therapy (ACT) (World Health Organization, 2006). These treatments generally combine rapid acting artemisinin or one of its derivatives (i.e., artesunate, artemether, dihydroartemisinin) with another more slowly eliminated anti-malarial (e.g. amodiaquine, mefloquine, piperaquine) (World Health Organization, 2006). The principle of artemisinin-based combination therapies is that the two schizontocidal drugs have independent modes of action, thereby increasing the therapeutic efficacy and delaying the development of resistance to the individual components of the combination treatment (World Health Organization, 2006). Artesunate and mefloquine combination treatments have been in use for several years, particularly on the Thai-Myanmar border. In this area of multi-drug resistance, artesunate and mefloquine combination therapy has achieved consistently high efficacy rates and reduced malaria morbidity (Nosten et al., 1991).

***Corresponding author:** Piero L Olliaro, MD, PhD, UNICEF/UNDP/World Bank/WHO Special Programme on Research & Training in Tropical Diseases (TDR), World Health Organization, Avenue Appia 20, CH-1211 Geneva 27, SWITZERLAND, Tel: +41 22 791 3734; Fax: + 41 22 791 4774; Email: olliarop@who.int

Received March 21, 2010; **Accepted** May 12, 2010; **Published** May 12, 2010

Citation: Olliaro P, Ramanathan S, Vaillant M, Reuter SE, Evans , et al. (2010) Pharmacokinetics and Comparative Bioavailability of Artesunate and Mefloquine Administered Separately or as a Fixed Combination Product to Healthy Volunteers and Patients with Uncomplicated *Plasmodium falciparum* Malaria. J Bioequiv Availab 2: 059-066. doi:10.4172/jbb.1000032

Artesunate is rapidly absorbed and hydrolysed to its pharmacologically active metabolite, dihydroartemisinin (Navaratnam et al., 2000). Conversion of artesunate to dihydroartemisinin occurs chemically within the gastrointestinal tract and is further catalysed by tissue and blood esterases (Navaratnam et al., 2000). Whilst both artesunate and dihydroartemisinin share the critical peroxide bridge necessary for anti-malarial effect, due to artesunate's rapid conversion, it is thought that the anti-malarial efficacy of artesunate is primarily dependent on adequate systemic exposure to dihydroartemisinin. The *in vitro*-determined minimum inhibitory concentration (MIC) against *P. falciparum* is 0.1 - 2.0 μg/L for both compounds (de Vries and Dien, 1996).

Mefloquine is structurally related to quinine and is active against the erythrocytic stages of *P. falciparum* (Palmer et al., 1993). Based on *in vivo* and *in vitro* data of multi-drug resistant *P. falciparum*, pharmacokinetic-pharmacodynamic modelling suggests that the MIC of mefloquine is 500 μg/L and the minimum parasiticidal concentration (MPC; i.e., the minimum concentration to provide maximum parasite killing) is 600 μg/L (Simpson et al., 1999).

Artesunate and mefloquine combination therapy is generally available as separate 50 mg artesunate and 250 mg mefloquine tablets, individually packaged or co-blistered. Several treatment regimens have been examined with the optimal regimen, which has been adopted as standard, being 4 mg/kg artesunate administered daily for 3 days and a total mefloquine dose of 25 mg/kg (Simpson et al., 1999; World Health Organization, 2006). Mefloquine doses of 15 mg/kg and 25 mg/kg have been evaluated, and whilst the higher dose has been shown to be associated with poor absorption and drug-induced vomiting especially in young children and older adults, the lower dose has been associated with inferior efficacy and is therefore not recommended (Luxemburger et al., 1998; World Health Organization, 2006). Common practice, in order to improve both tolerability and absorption, is to administer 25 mg/kg mefloquine as a split dose with an overall treatment regimen of: 4 mg/kg artesunate alone without mefloquine on Day 1; 4 mg/kg artesunate + 15 mg/kg mefloquine on Day 2, and; 4 mg/kg artesunate + 10 mg/kg mefloquine on Day 3 (Price et al., 1999; World Health Organization, 2006). Pharmacokinetic-pharmacodynamic modelling has demonstrated that with this split dose treatment regimen, whole blood mefloquine concentrations would remain above the MPC of 600 μg/L for approximately 20 days as opposed to 6 days for a single dose of 15 mg/kg mefloquine (Simpson et al., 1999). This prediction is supported by the findings of other studies (Price et al., 1999; Ramharter et al., 2007).

Although this treatment regimen for artesunate plus mefloquine has been proven to be efficacious, the regimen may be difficult to comply with outside of a research setting. Consequently, with the aim of simplifying the standard treatment regimen and improving patient adherence, an artesunate - mefloquine fixed dose oral co-formulation has been developed. The formulation consists of artesunate and mefloquine in a dose ratio of 1:2, with the intention of administration of daily doses of 4 mg/kg artesunate and 8 mg/kg mefloquine for 3 days, corresponding to total doses of 12 mg/kg and 24 mg/kg respectively, equivalent to the current total dose recommendations. This new fixed dose regimen has been shown to be as effective and tolerated as the standard regimen in a large field trial in Thailand (Ashley et al., 2006). The fixed dose co-formulation has been developed through a collaborative project coordinated by the Drugs for Neglected Diseases initiative (DNDi) and FarManguinhos, a publicly-owned technical/scientific unit of the Foundation Oswald Cruz (Fiocruz). The co-formulated product has been approved by the Brazilian regulatory agency and is used by the Brazilian malaria control programme.

In order to assess the pharmacokinetics and comparative bioavailabilities of artesunate and mefloquine administered as separate products and the developed fixed dose co-formulated product, two studies were conducted: a randomised, crossover design study in healthy volunteers and a randomised, parallel group study in patients with uncomplicated falciparum malaria.

Methods

The clinical studies were conducted at the Hospital for Tropical Disease, Mahidol University (Bangkok, Thailand) and were reviewed and approved by the institutional Human Research Ethics Committee. Participants were fully informed of the study procedures and provided written informed consent prior to study initiation. The studies were conducted in accordance with the study protocols, the Declaration of Helsinki and the principles of Good Clinical Practice.

The Fixed formulation was administered as tablets containing 100 mg artesunate and 200 mg mefloquine, manufactured by FarManguinhos (Rio de Janeiro, Brazil; Batch Numbers 070008 & 069002). The Non-Fixed treatment comprised of artesunate administered as 50 mg Arsumax® tablets manufactured by Guilin Pharmaceutical (Guangxi, China; Batch Number 031201) and mefloquine administered as 250 mg tablets manufactured by Roche (Basel, Switzerland; Batch Number B1100).

Healthy volunteer study

The pharmacokinetics of artesunate and mefloquine administered as the Fixed and Non-Fixed formulations were assessed in 24 healthy, adult male and non-pregnant female volunteers. The study was conducted according to the randomised, cross-over design with a 90 day washout period between administrations of the study treatments. This clinical trial was registered as http://www.controlledtrials.com/mrct/trial/673005/DNDi.

Volunteers were screened and were required to meet the following eligibility criteria prior to treatment administration: age 18-50 years; negative pregnancy test for female volunteers; non-smoker (<10 cigarettes/day); no significant abnormal findings in the medical history or examination (including ECG, haematology, liver and renal function tests); no history of alcohol or drug abuse; negative malaria thick smear; no history of travel to or residency in a malarial area in the preceding 8 weeks; no history of anti-malarial medication ingestion within the preceding three months; no other medications (including over the counter products) within the preceding week; no known allergy to the study treatment; no concurrent clinical trial participation; adequate venous access; provision of written informed consent.

Participants were administered a single oral dose of artesunate and mefloquine as the Fixed formulation (200 mg artesunate/400 mg mefloquine) or the Non-Fixed formulation (200 mg artesunate + 500 mg mefloquine). Blood samples were collected for analysis of plasma artesunate and dihydroartemisinin concentrations prior to dosing (0 hr) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 12 hours after treatment administration. Blood samples were collected for analysis of plasma mefloquine concentrations prior to dosing (0hr) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 120, 168, 336, 504, 672, 1008, 1344, 1680 and 2160 hours after treatment administration.

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doi:10.4172/jbb.1000032
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Uncomplicated falciparum malaria patient study

The pharmacokinetics of artesunate and mefloquine administered as the Fixed and Non-Fixed formulations were assessed in 50 male and non-pregnant female patients with slide-proven uncomplicated *P. falciparum* malaria. The study was conducted according to a randomised, parallel design with patients randomly allocated to receive multiple dosing with the Fixed or Non-Fixed formulation, according to a 1:1 ratio. This clinical trial was registered as http://www.controlled-trials.com/mrct/trial/590967/DNDi.

Patients were screened and were required to meet the following eligibility criteria prior to treatment administration: age 18-65 years; not pregnant or lactating for female patients; minimum weight of 40 kg; microscopically confirmed mono-infection of *P. falciparum*; history or presence of fever; *P. falciparum* asexual stage parasitaemia <4% red blood cells; absence of clinical features of severe malaria and other significant illnesses or signs; no history of convulsions and/or psychiatric illnesses; no history of splenectomy; normal baseline ECG reading; no history of mefloquine ingestion within the preceding two months; no known contraindications or hypersensitivity to the study treatment; provision of written informed consent.

Participants administered the Fixed formulation received two co-formulated tablets (200 mg artesunate/400 mg mefloquine) each day for three days. Blood samples were collected for analysis of plasma artesunate and dihydroartemisinin concentrations prior to dosing (0hr) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours after treatment administration on the first day of dosing (Day 1). Blood samples were collected for analysis of plasma mefloquine concentrations prior to dosing (0hr) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 120, 168, 336, 504 and 672 hours after treatment administration on Day 1. The blood samples scheduled for collection at the same time as dosing on Day 2 (24hr) and Day 3 (48hr) were collected prior to treatment administration.

Participants allocated to the Non-Fixed formulation received daily administration of 4 mg/kg artesunate for three days as well as 15 mg/kg mefloquine on the second day of artesunate dosing and 10 mg/kg mefloquine on the third day of artesunate dosing. Blood samples were collected for analysis of plasma artesunate and dihydroartemisinin concentrations prior to dosing (0hr) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours after treatment administration on the first day of dosing (Day 1). Blood samples were collected for analysis of plasma mefloquine concentrations prior to dosing on Day 2 (24hr) and 48, 72, 120, 168, 336, 504 and 672 hours after treatment administration on Day 1. The blood samples scheduled for collection at the same time as dosing on Day 2 (24hr) and Day 3 (48hr) were collected prior to treatment administration.

Analytical methods

Plasma samples were analysed for artesunate, dihydroartemisinin and mefloquine concentrations by Universiti Sains Malaysia (Penang, Malaysia) (Lai et al., 2007).

The analysis of artesunate and dihydroartemisinin was conducted using BAS 200A HPLC-EC systems operating at reductive mode. Chromatographic separation of artesunate, dihydroartemisinin and the internal standard (artemisinin, QHS) was obtained at room temperature (25°C) on a Hypersil C4 column, 250 x 4.6 mm ID, and 5 μm particle size (Thermo Hypersil-Keystone, Bellefonte, USA). The mobile phase consisted of acetonitrile and 0.05 M acetic acid (42:58% v/v) adjusted to pH 5.2 with 0.1 M NaOH. The flow rate was 1.5 mL/min. Detection was performed in the reductive mode at -1000 mV in an oxygen free environment. The HPLC-EC (reductive) system produced a linear for the concentration of artesunate and dihydroartemisinin in the range of 10 ng/0.5 mL - 800 ng/0.5 mL. 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 analysis of mefloquine was conducted using Waters 2487 (Dual Absorbance Detector) HPLC-UV systems coupled to Waters 515 HPLC pump. The analysis of mefloquine as internal standard was carried out at room temperature (25°C) using a Inertsil C8-3 column, 150 x 4.6 mm ID, 5 μm particle size (GL Sciences Inc, Tokyo, Japan). The mobile phase consisted of acetonitrile, methanol and 0.05 M KH_2PO_4 (80:500:420% v/v) adjusted to pH 3.9 with orthophosphoric acid. The flow rate was 1.00 mL/min. Detection was performed in the UV mode at 285 mm. The extraction recoveries of mefloquine were all above 80% with a coefficient of variation less than 8%. The HPLC-UV system produced a linear response for the concentration of mefloquine in the range of 10 ng/0.5 mL - 800 ng/0.5 mL. The lower limit of quantification of mefloquine was 10 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%.

Pharmacokinetic and statistical analysis

Plasma analyte concentrations were utilised for calculation of pharmacokinetic parameters using a standard model-independent approach. Area under the plasma concentration time curve from time zero to the last quantifiable concentration (AUC_{last}) was calculated using the linear-trapezoidal method. Area under the plasma concentration time profile from zero extrapolated to infinite time (AUC_{inf}) was calculated as AUC_{last} + last quantifiable concentration / terminal rate constant. The maximum observed concentration (C_{max}) and the time of the maximum observed concentration (T_{max}) were taken directly from the data without interpolation.

A linear mixed-effects analysis of variance (ANOVA) model was used to analyse dose-normalised (where appropriate), Ln-transformed AUC_{last}, AUC_{inf} and C_{max} parameters. The model incorporated factors for treatment, period, sequence and subject nested in sequence for the healthy volunteer cross-over study and treatment and subject for the uncomplicated falciparum malaria patient parallel study. The residual error (error mean square) was used to construct the 90% confidence intervals for the ratio of the Non-Fixed and Fixed formulation means. In constructing these 90% confidence intervals, the Non-Fixed formulation was used as the reference treatment.

A non-parametric Wilcoxon Signed Ranks test was used to assess treatment differences for paired T_{max} data obtained in the healthy volunteer study. A Mann-Whitney U test was used to assess treatment differences for unpaired T_{max} data obtained in the uncomplicated falciparum malaria patient study.

Bioequivalence was concluded if the 90% confidence intervals were within the regulatory limits of 80 - 125%. Significance was set at an α-level of 0.05.

WinNonlin Professional, Version 5.3 (Pharsight Corporation, Mountain View, CA, USA) was used for pharmacokinetic and parametric statistical analyses. SPSS for Windows, Version 16.0 (SPSS Inc, Chicago, IL, USA) was used for non-parametric statistical analysis.

Results

Healthy volunteer study

Twenty-four healthy volunteers completed the study. Participant demographic information is summarised in Table 1.

Plasma concentration-time profiles for artesunate, dihydroartemisinin and mefloquine are displayed in Figure 1, Figure 2 and Figure 3 respectively. Pharmacokinetic parameters are summarised in Table 2 and the ratio of Fixed/Non-Fixed data and associated 90% confidence intervals for the pharmacokinetic parameters are summarised in Figure 4.

The results indicate substantially lower artesunate and dihydroartemisinin concentrations after administration of the Fixed formulation compared to the Non-Fixed formulation (Figure 1 and Figure 2). Bioequivalence analysis of artesunate pharmacokinetic data indicated that the two formulations were not bioequivalent with significantly lower AUC_{last} ($p=0.0479$) and C_{max} ($p=0.0006$) values for the Fixed formulation and the 90% confidence intervals extending below the 80 - 125% limits (Figure 4). Due to limited data AUC_{inf} could not be calculated for artesunate. Similarly, the two formulations were not bioequivalent with respect to dihydroartemisinin pharmacokinetics with significantly lower C_{max} values ($p=0.0455$) and the 90% confidence intervals for AUC_{last}, AUC_{inf} and C_{max} outside of the 80 - 125% limits (Figure 4).

Statistical analysis of dose-normalised mefloquine data indicated that there were significant differences between the Fixed and Non-Fixed formulations for AUC_{last} ($p=0.0023$), AUC_{inf} ($p=0.0276$) and C_{max} ($p<0.0001$). In addition, the 90% confidence intervals for dose-normalised data were not within the 80 - 125% limits (Figure 4), indicating that the formulations were not bioequivalent with respect to both the rate and extent of mefloquine absorption. A significant period effect was found for AUC_{last}, AUC_{inf} and C_{max} ($p<0.02$) with area under the curve and maximum concentration values approximately 20% higher in Period 2, irrespective of treatment sequence.

Parameter	Healthy Volunteers	Uncomplicated Falciparum Malaria Patients	
		Fixed Formulation	Non-Fixed Formulation
Count	24	21	24
Gender	4 Male / 20 Female	17 Male / 4 Female	21 Male / 3 Female
Ethnicity	24 Thai	13 Burmese / 4 Karen / 3 Mon / 1 Thai	12 Burmese / 8 Karen / 3 Mon
Age (years)	35.0 ± 7.17	27.3 ± 10.5	28.9 ± 10.4
Weight (kg)	55.9 ± 10.4	50.2 ± 6.33	50.6 ± 6.49
Height (cm)	158 ± 7.98	161 ± 5.74	160 ± 7.31

Table 1: Participant demographic information. Data expressed as mean ± standard deviation.

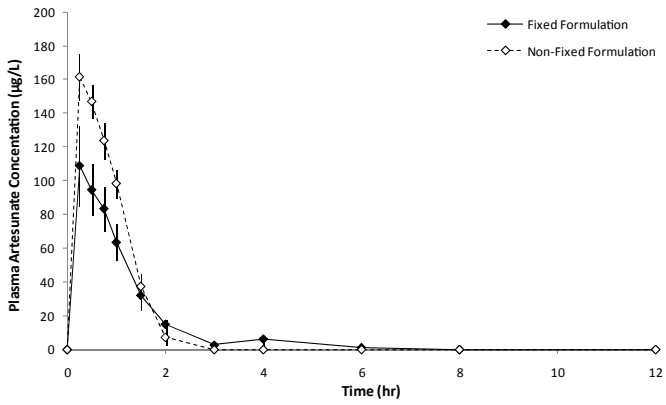

Figure 1: Plasma artesunate concentration-time profiles after administration of single oral doses of artesunate and mefloquine given as a Fixed formulation (200 mg artesunate/400 mg mefloquine) and a Non-Fixed formulation (200 mg artesunate + 500 mg mefloquine) to healthy volunteers (n=24). Data expressed as mean ± standard error of the mean.

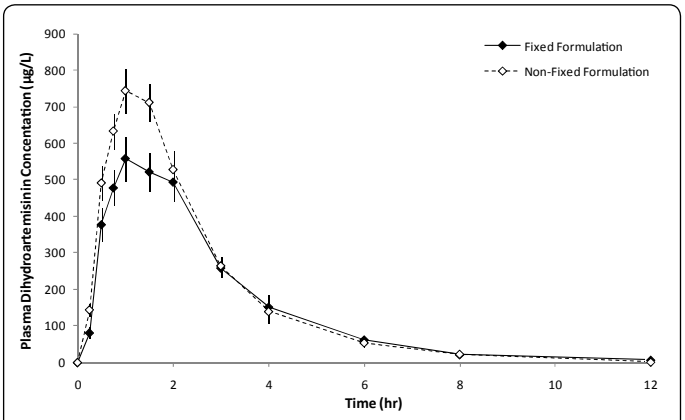

Figure 2: Plasma dihydroartemisinin concentration-time profiles after administration of single oral doses of artesunate and mefloquine given as a Fixed formulation (200 mg artesunate/400 mg mefloquine) or a Non-Fixed formulation (200 mg artesunate + 500 mg mefloquine) to healthy volunteers (n=24). Data expressed as mean ± standard error of the mean.

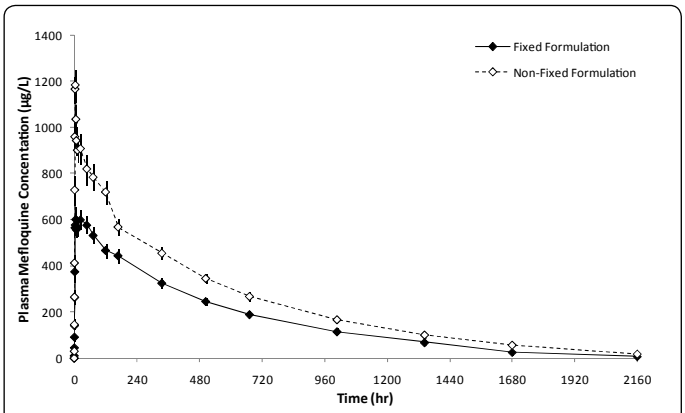

Figure 3: Plasma mefloquine concentration-time profiles after administration of single oral doses of artesunate and mefloquine given as a Fixed formulation (200 mg artesunate/400 mg mefloquine) or a Non-Fixed formulation (200 mg artesunate + 500 mg mefloquine) to healthy volunteers (n=24). Data expressed as mean ± standard error of the mean.

Uncomplicated falciparum malaria patient study

Forty four patients with confirmed *P. falciparum* malaria completed the study, of whom 21 were administered the Fixed formulation and 23 were administered the Non-Fixed formulation. Participant demographic information is summarised in Table 1.

Plasma concentration-time profiles for artesunate, dihydroartemisinin and mefloquine are displayed in Figure 5, Figure 6 and Figure 7 respectively. Pharmacokinetic parameters are summarised in Table 3 and the ratio of Fixed/Non-Fixed data and associated 90% confidence intervals for the pharmacokinetic parameters are summarised in Figure 8.

The results reveal significantly lower dose-normalised maximum artesunate and dihydroartemisinin concentrations ($p=0.0192$ and

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doi:10.4172/jbb.1000032

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Parameter	Non-Fixed Formulation	Fixed Formulation
Artesunate		
AUC_{last} (µg.hr/L)	149 ± 70.8	125 ± 88.6 *
C_{max} (µg/L)	206 ± 96.7	133 ± 59.3 *
T_{max} (hr)	0.510 ± 0.281 (0.500)	0.594 ± 0.793 (0.250)
Dihydroartemisinin		
AUC_{last} (µg.hr/L)	1947 ± 621.9	1711 ± 717.3
AUC_{inf} (µg.hr/L)	2039 ± 639.9	1811 ± 725.4
C_{max} (µg/L)	902 ± 351	694 ± 283 *
T_{max} (hr)	1.06 ± 0.418 (1.00)	1.46 ± 0.827 (1.25)
Mefloquine		
AUC_{last} (µg.hr/L)	481403 ± 137378	321836 ± 114473
AUC_{inf} (µg.hr/L)	544350 ± 153692	385899 ± 124168
C_{max} (µg/L)	1288 ± 319	715 ± 217
T_{max} (hr)	7.10 ± 7.82 (3.98)	37.0 ± 68.2 (15.0) *
Mefloquine, Dose-Normalised to 400 mg Mefloquine		
Dose-Normalised AUC_{last} (µg.hr/L)	385122 ± 109902	321836 ± 114473 *
Dose-Normalised AUC_{inf} (µg.hr/L)	435480 ± 122954	385899 ± 124168 *
Dose-Normalised C_{max} (µg/L)	1031 ± 256	715 ± 217 *

*p < 0.05 compared to Non-Fixed formulation data; statistical analysis not conducted on non-dose-normalised data.

Table 2: Derived artesunate, dihydroartemisinin and mefloquine pharmacokinetic parameters for healthy volunteers administered artesunate and mefloquine as a Fixed formulation (200 mg artesunate/400 mg mefloquine) and a Non-Fixed formulation (200 mg artesunate + 500 mg mefloquine) (n=24). Data expressed as mean ± standard deviation (median).

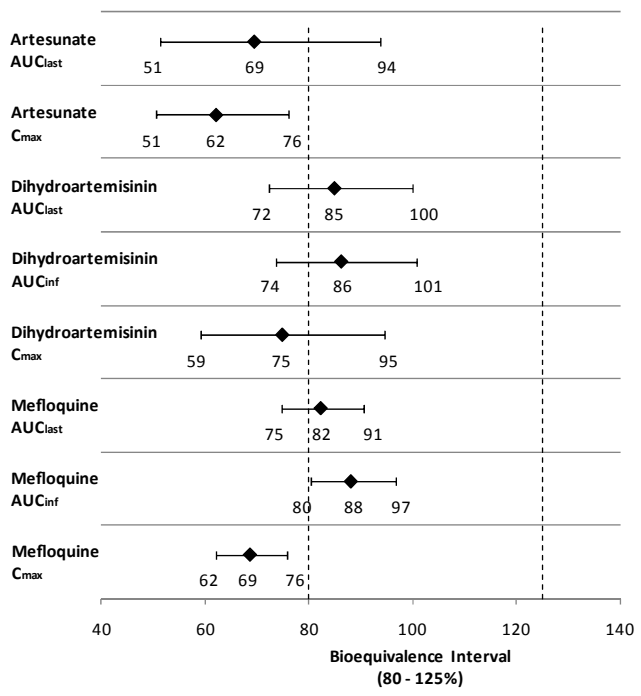

Figure 4: 90% confidence intervals for artesunate, dihydroartemisinin and dose-normalised mefloquine pharmacokinetic parameters obtained after single oral doses of artesunate and mefloquine given as a Fixed formulation (200 mg artesunate/400 mg mefloquine) or a Non-Fixed formulation (200 mg artesunate + 500 mg mefloquine) to healthy volunteers. Data expressed as 90% confidence intervals around the ratio (% Fixed/Non-Fixed) of geometric least square means.

p=0.0113 respectively) after administration of a single oral dose of the Fixed formulation compared to the Non-Fixed formulation (Figure 5 and Figure 6). There were no significant differences between the treatments with respect to area under the curve values for artesunate and dihydroartemisinin. Bioequivalence analysis of dose-normalised artesunate and dihydroartemisinin pharmacokinetic data indicated that the two formulations were not bioequivalent with the 90% confidence intervals below the 80 - 125% limits in all cases (Figure 8).

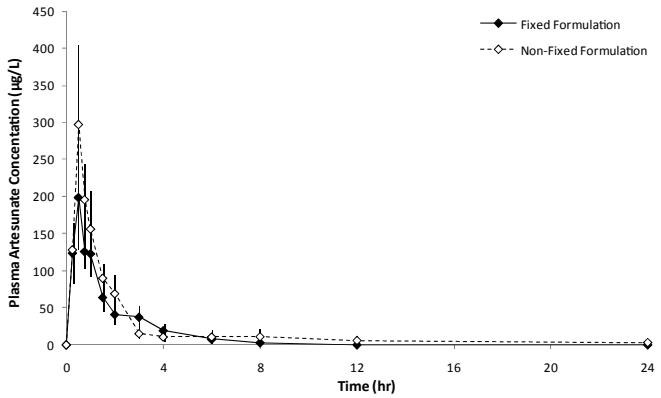

Figure 5: Plasma artesunate concentration-time profiles after administration of single oral doses of artesunate and mefloquine given as a Fixed formulation (200 mg artesunate/400 mg mefloquine) or a Non-Fixed formulation (4 mg/kg artesunate + 0 mg/kg mefloquine) to uncomplicated falciparum malaria patients (Fixed: n=18; Non-Fixed: n=23). Data expressed as mean ± standard error of the mean.

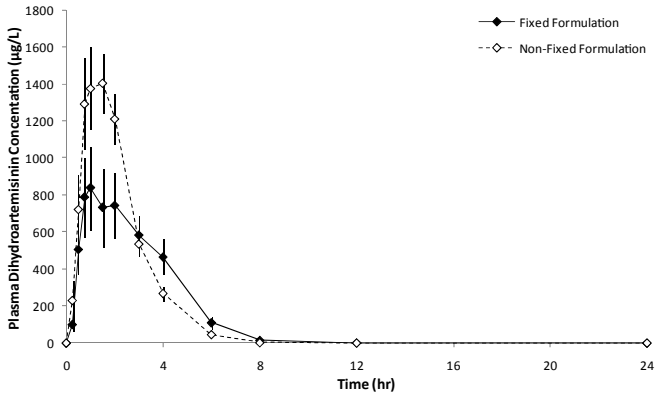

Figure 6: Plasma dihydroartemisinin concentration-time profiles after administration of single oral doses of artesunate and mefloquine given as a Fixed formulation (200 mg artesunate/400 mg mefloquine) or a Non-Fixed formulation (4 mg/kg artesunate + 0 mg/kg mefloquine) to uncomplicated falciparum malaria patients (Fixed: n=19; Non-Fixed: n=23). Data expressed as mean ± standard error of the mean.

Statistical analysis of dose-normalised mefloquine data indicated that there were no significant differences between the Fixed and Non-Fixed formulations for AUC_{last}, AUC_{inf} and C_{max}. In addition, the 90% confidence intervals for dose-normalised AUC_{last} and C_{max} data were within the 80 - 125% limits (Figure 8), indicating that the formulations were bioequivalent with respect to both the rate and extent of absorption. The 90% confidence interval for dose-normalised AUC_{inf} extended slightly beyond the upper bioequivalence limit.

Discussion

The fixed dose co-formulated product was not bioequivalent to the separately administered products for artesunate and dihydroartemisinin pharmacokinetics in both healthy volunteers and uncomplicated falciparum malaria patients. Administration of the Fixed formulation resulted in 15 - 25% lower AUC_{last} and AUC_{inf} values and 25 - 40% lower C_{max} values for dihydroartemisinin than those observed after administration of the separate products. This reduction in the exposure to dihydroartemisinin with the fixed dose co-formulated product is most likely reflective of a less complete dissolution of the Fixed formulation for the clinical batch used (data not shown). Whilst decreased exposure may also be indicative of a reduction in the conversion of artesunate to dihydroartemisinin, the ratio of artesunate to dihydroartemisinin concentrations is the same for the two study treatments, in both the healthy volunteer and patient populations, and thus it can be concluded that the conversion of artesunate to dihydroartemisinin has not changed. Alternatively, lower dihydroartemisinin concentrations may also be indicative of a higher rate of elimination of dihydroartemisinin, perhaps due to the presence of co-administered mefloquine; however, artesunate was administered both with and without mefloquine in the patient study and the half-lives of dihydroartemisinin in both cases were similar (data not presented) thereby suggesting that the elimination of dihydroartemisinin remains unchanged.

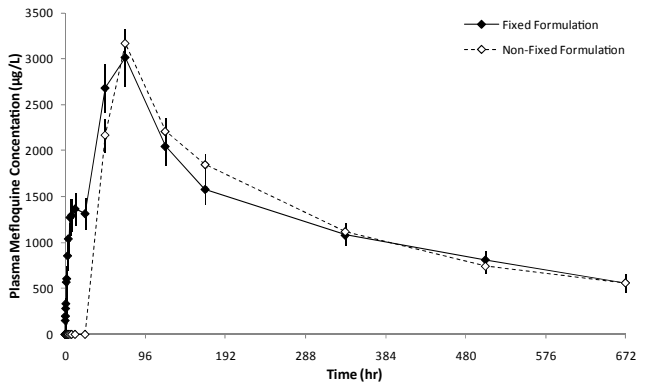

Figure 7: Plasma mefloquine concentration-time profiles after administration of multiple oral doses of artesunate and mefloquine given as a Fixed formulation (200 mg artesunate/400 mg mefloquine daily for 3 days) or a Non-Fixed formulation (4 mg/kg artesunate + 0 mg/kg mefloquine on Day 1; 4 mg/kg artesunate + 15 mg/kg mefloquine on Day 2; 4 mg/kg artesunate + 10 mg/kg mefloquine on Day 3) to uncomplicated falciparum malaria patients (Fixed: n=20; Non-Fixed: n=23). Data expressed as mean ± standard error of the mean.

The study treatments were shown to be bioequivalent with respect to mefloquine pharmacokinetics in uncomplicated falciparum malaria patients but not in healthy volunteers. This may be reflective of the observed differences in the disposition of anti-malarial drugs in healthy volunteers and malaria patients combined with the added complexity of the differing dosing regimens. Whilst the experimental design of these clinical studies did not permit a direct comparison of non-compartmental parameters between healthy volunteers and uncomplicated falciparum malaria patients, we are currently utilising a population pharmacokinetic approach to interrogate these differences. Nonetheless, the observed difference in the disposition of mefloquine in the two study populations highlights the importance of assessing bioequivalence in specific patient groups.

Parameter	Non-Fixed Formulation	Fixed Formulation
Artesunate		
AUC_{last} (µg.hr/L)	419 ± 670	310 ± 324
C_{max} (µg/L)	451 ± 440	255 ± 272
T_{max} (hr)	0.925 ± 0.563 (0.750)	0.833 ± 0.702 (0.500)
Artesunate, Dose-Normalised to 200 mg Artesunate		
Dose-Normalised AUC_{last} (µg.hr/L)	430 ± 707	310 ± 324
Dose-Normalised C_{max} (µg/L)	448 ± 381	255 ± 272 *
Dihydroartemisinin		
AUC_{last} (µg.hr/L)	3633 ± 1367	3027 ± 2491
AUC_{inf} (µg.hr/L)	3745 ± 1371	3138 ± 2491
C_{max} (µg/L)	2043 ± 949	1234 ± 857
T_{max} (hr)	1.41 ± 0.714 (1.50)	1.99 ± 1.12 (2.00)
Dihydroartemisinin, Dose-Normalised to 200 mg Artesunate		
Dose-Normalised AUC_{last} (µg.hr/L)	3695 ± 1424	3027 ± 2491
Dose-Normalised AUC_{inf} (µg.hr/L)	3812 ± 1443	3138 ± 2491
Dose-Normalised C_{max} (µg/L)	2059 ± 941	1234 ± 857 *
Mefloquine		
AUC_{last} (µg.hr/L)	782607 ± 224234	810835 ± 364880
AUC_{inf} (µg.hr/L)	1062533 ± 365937	1149417 ± 758992
C_{max} (µg/L)	3239 ± 734	3279 ± 1252
T_{max} (hr)	70.9 ± 13.5 (72.0)	71.3 ± 17.3 (72.0)
Mefloquine, Dose-Normalised to 400 mg Mefloquine		
Dose-Normalised AUC_{last} (µg.hr/L)	763147 ± 232259	810835 ± 364880
Dose-Normalised AUC_{inf} (µg.hr/L)	1029264 ± 351032	1149417 ± 758992
Dose-Normalised C_{max} (µg/L)	3184 ± 934	3279 ± 1252

*$p < 0.05$ compared to Non-Fixed formulation data; statistical analysis not conducted on non-dose-normalised data.

Table 3: Derived artesunate, dihydroartemisinin and mefloquine pharmacokinetic parameters for uncomplicated falciparum malaria patients administered artesunate and mefloquine as a Fixed formulation (200 mg artesunate/400 mg mefloquine daily for 3 days) or a Non-Fixed formulation (4 mg/kg artesunate + 0 mg/kg mefloquine on Day 1; 4 mg/kg artesunate + 15 mg/kg mefloquine on Day 2; 4 mg/kg artesunate + 10 mg/kg mefloquine on Day 3) (Fixed: n=20; Non-Fixed: n=23). Data expressed as mean ± standard deviation (median).

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OPEN ACCESS Freely available online
doi:10.4172/jbb.1000032
JBB/Vol.2 Issue 3

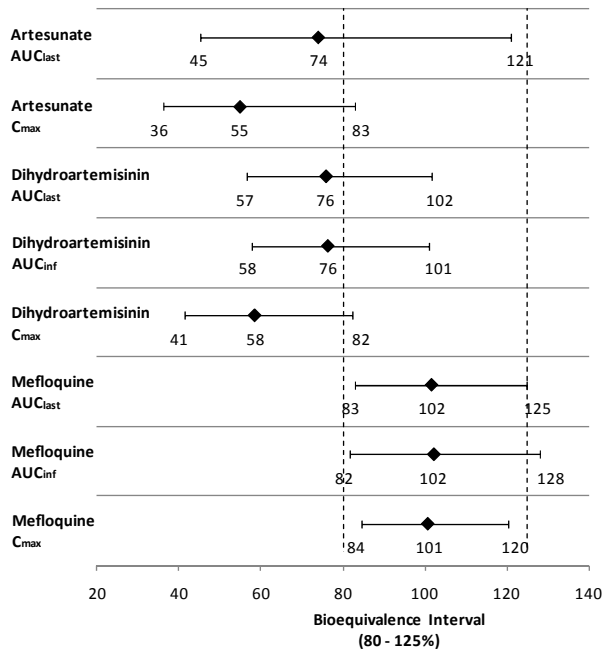

Figure 8: 90% confidence intervals for dose-normalised artesunate and dihydroartemisinin pharmacokinetic parameters obtained after single oral doses of artesunate and mefloquine given as a Fixed formulation (200 mg artesunate/400 mg mefloquine) or a Non-fixed formulation (4 mg/kg artesunate + 0 mg/kg mefloquine) to uncomplicated falciparum malaria patients and dose-normalised mefloquine pharmacokinetic parameters obtained after multiple oral doses of artesunate and mefloquine given as a Fixed formulation (200 mg artesunate/400 mg mefloquine daily for 3 days) or a Non-Fixed formulation (4 mg/kg artesunate + 0 mg/kg mefloquine on Day 1; 4 mg/kg artesunate + 15 mg/kg mefloquine on Day 2; 4 mg/kg artesunate + 10 mg/kg mefloquine on Day 3) to uncomplicated falciparum malaria patients. Data expressed as 90% confidence intervals around the ratio (% Fixed/Non-Fixed) of geometric least square means.

Interestingly, a significant period effect was observed for mefloquine in the healthy volunteer study with a 1.2-fold increase in AUC_{last}, AUC_{inf} and C_{max} values after treatment administration in the second study period. Apparent total systemic clearance and volume of distribution were approximately 15% lower in Period 2; half-life was unchanged (data not presented). Whilst, due to the longer half-life of mefloquine, a carry-over effect is likely, this is estimated to account for only a 1% increase in values and does not explain the substantial increase in the rate and extent of absorption observed in the second treatment period. The underlying cause of this is unknown and warrants further investigation.

The clinical relevance of the differences in the exposure of these anti-malarial treatments is uncertain. For artesunate and mefloquine combination therapy, the efficacy is dependent upon the initial reduction in parasite biomass by artesunate and dihydroartemisinin and the killing of residual parasites by mefloquine. Due to their very short half-lives, the maximum plasma concentration of artesunate and dihydroartemisinin is probably more important than the extent of absorption; for the longer acting mefloquine, the time that mefloquine concentrations remain above the MPC and MIC, hence extent of absorption is important.

The plasma levels of artesunate and dihydroartemisinin observed in both healthy volunteers and malaria patients in this study are many-fold higher than that required for schizontocidal activity. Mean C_{max} values for dihydroartemisinin observed in patients were 1234 µg/L and 2043 µg/L for the Fixed and Non-Fixed formulations, respectively. This is compared to the *in vitro* MIC values against *P. falciparum* of 0.1 - 2.0µg/L for dihydroartemisinin (de Vries and Dien, 1996). Artemisinin resistance is now reported on the Thai-Cambodian border and the IC_{50} values of the strains collected from this area for dihydroartemisinin are 2.3 µg/L (IQR 1.1 - 3.2 µg/L) (Dondorp et al., 2009). However, as the primary objective of this study was to assess bioequivalence of the two study treatments and therefore did not allow for measurement of artesunate and dihydroartemisinin concentrations after the second and third dose of artesunate in patients, no firm conclusions can be made.

Mean mefloquine concentrations in malaria patients remained above the MIC of 500 µg/L throughout the assessment period, with mean plasma concentrations at the last study time-point (672 hours = 28 days) approximately 560 µg/L for both study treatments. This is in keeping with the findings of other studies (Price et al., 1999; Ramharter et al., 2007; Simpson et al., 1999).

Both the separate products and the developed fixed dose co-formulated product have been shown to be efficacious in curing malaria in this small-scale study (Krudsood et al., submitted) as well as in a larger clinical trial (Ashley et al., 2006). Additional studies are currently being conducted (Myanmar NCT00902811, India ISRCTN70618692).

Conclusion

This study has demonstration that the developed fixed dose co-formulated artesunate and mefloquine product is equivalent to the current standard treatment regimen in terms of mefloquine pharmacokinetics in malaria patients but not in healthy volunteers nor in terms of artesunate and dihydroartemisinin concentrations in either study population. While the plasma concentrations of artesunate, dihydroartemisinin and mefloquine were adequate to achieve effective anti-malarial activity, the results of this study underscore the importance of conducting bioequivalence evaluations of new anti-malarial products.

Acknowledgements

The authors would like to thank the participating subjects, patients, the nurses and administrative staff at the Hospital for Tropical Diseases.

The Drug for Neglected Diseases initiative (DNDi) supported the conduct of the clinical studies and pharmacokinetic analysis. Data analyses were conducted independently. The work of Piero Olliaro (UNICEF/UNDP/World Bank/WHO Special Programme for Research & Training in Tropical Diseases) as well as Stephanie E Reuter and Allan M Evans (University of South Australia) were supported by the respective institutions.

Dedication

We dedicate this work to the late Professor Sornchai Looareesuwan, the Clinical Principal Investigator for both studies.

Disclaimer

Piero Olliaro is a staff member of the WHO and Walter RJ Taylor was a member at the time of the conduct of the study; Michel Vaillant 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 or the CRP-Santé.

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