**Mr Mark Reid** 30 August 2018
**Supplementary Submission**

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Ms Lyn Beverley
Committee Secretary
Senate Foreign Affairs, Defence and Trade references Committee
PO Box 6100 parliament House
Canberra ACT 2600

**Re Senate Inquiry – Use of the Quinoline anti-malarial drugs Mefloquine and Tafenoquine in the Australian Defence Force – Mr Brian McCarthy's statement that Mark Reid mislead the Committee on the 30th August 2018**

Mr Brian McCarthy made a statement to the Committee on the 30th August 2018 during his oral testimony that I additionally conducted mefloquine vs. doxycycline studies in East Timor and had deliberately misled the Committee by not disclosing this information. I asked the Committee Secretary to strike this statement from the Hansard record, but he recommended I make a submission instead.

I would like to offer "alternative facts" to the Committee for their consideration.

Michael Patrick Reid was born on the 3rd June 1976 at 2103 hours.

Lieutenant (Dr) Michael Patrick Reid (Retired) served 20 years in the Australian Army and the Royal Australian Navy as a medic and medical doctor. He deployed on six occasions to Bougainville, East Timor and the International Coalition Against Terrorism (ICAT) (Maritime support for the Afghan Mission).

Mark George Reid was born on the 3rd June 1976 at 2111 hours, 8 minutes after Michael Patrick Reid.

Captain Mark George Reid (Retired) served 12 years in the Australian Army in the Royal Australian Infantry Corp and the Royal Australian Army Medical Corp. I also deployed on two occasions to East Timor.

I therefore describe the relatively common situation, where two siblings happen to share an initial and served in the same Army unit before Lieutenant Reid transferred to the Royal Australian Navy.

Our birth certificates clearly identify each of us as immigrants to Australia. In case this is raised as a point of dispute regarding eligibility to serve in the ADF, I confirm that both Michael Patrick Reid and Mark George Reid are Australian Citizens and were naturalised prior to enlisting in the Australian Army at 18 years of age.

**Mefloquine assignments for the Australian Army:**

Lieutenant Michael Patrick Reid was involved in the mefloquine studies and is a co-author on the following publication:

- Charles BG, Blomgren A, Nasveld PE, Kitchener SJ, Jensen A, Gregory RM, Robertson B, Harris IE, Reid MP, Edstein MD. Population pharmacokinetics of mefloquine in military personnel for prophylaxis against malaria infection during field deployment. Eur J Clin Pharmacol 2007; 63(3): 271-8 (**Attachment 1**).

Michael Patrick Reid is also acknowledged in the following publication as "Private Michael Reid" while still an enlisted soldier in the Australian Army.

- Kitchener SJ, Nasveld PE, Gregory RM and Edstein MD. Mefloquine and doxycycline malaria prophylaxis in Australian soldiers in East Timor. Med J Australia 2005; 182(4): 168-171 (**Attachment 2**)

**Tafenoquine assignments for the Australian Army:**

I was never involved in mefloquine vs. doxycycline studies for the Australian Army. In my written and oral testimony, I declared that I conducted one malaria assignment for the Australian Army on the orders of my Commanding Officer, Lieutenant Colonel Michael Edstein and this was the study coordinator role for Study 033 "Randomized, double-blind study of the safety, tolerability and efficacy of tafenoquine versus mefloquine for malaria in prophylaxis in nonimmune subjects".

My declaration therefore stands as an accurate (but alternative fact) that disagrees with Mr Brian McCarthy's statement. Peer-reviewed medical publications published by the ADF where I am named are as follows:

- Nasveld PE, Edstein MD, Reid M, Brennan L, Harris IE, Kitchener SJ, Leggat PA, Pickford P, Kerr C, Ohrt C, Prescott W; Tafenoquine Study Team. Randomized, double-blind study of the safety, tolerability, and efficacy of tafenoquine versus mefloquine for malaria prophylaxis in nonimmune subjects. Antimicrob Agents Chemother 2010; 54(2): 792-8 (**Attachment 3**).
- Charles BG, Miller AK, Nasveld PE, Reid MG, Harris IE, Edstein MD. Population pharmacokinetics of tafenoquine during malaria prophylaxis in healthy subjects. Antimicrob Agents Chemother 2007; 51(8): 2709-15 (**Attachment 4**).

Lieutenant Michael Patrick Reid is also acknowledged as a Tafenoquine Study Team member as "Michael Reid" in the publication Nasveld-2010.

## Attachment 1: Charles-2007a

Eur J Clin Pharmacol (2007) 63:271 278
DOI 10.1007/s00228 006 0247 3

PHARMACOKINETICS AND DISPOSITION

# Population pharmacokinetics of mefloquine in military personnel for prophylaxis against malaria infection during field deployment

**B. G. Charles · A. Blomgren · P. E. Nasveld · S. J. Kitchener · A. Jensen · R. M. Gregory · B. Robertson · I. E. Harris · M. P. Reid · M. D. Edstein**

Received: 9 October 2006 / Accepted: 29 November 2006 / Published online: 11 January 2007


**Abstract**
*Objective* The purpose of this study was to determine the population pharmacokinetics of mefloquine in healthy military personnel during prophylaxis for malaria infections.
*Methods* The subjects were 1,111 Australian soldiers participating in two studies: a randomised double-blinded study (group A, 161 subjects) and an open-label study (group B, 950 subjects). Following a loading dose (250 mg mefloquine base daily, 3 days), subjects received an oral weekly maintenance dose of 250 mg over 6 months. Blood was collected after the last split loading dose then at weeks 4, 8 and 16 for group A, and at weeks 13 and 26 for group B. Plasma mefloquine concentrations were measured by high-performance liquid chromatography (HPLC). Pharmacokinetic modelling was performed using NONMEM.
*Results* A two-compartment model with inter-occasion variability (IOV) for clearance satisfactorily described the pharmacokinetics. Typical values were clearance (CL/F, 2.09 l/h), central volume of distribution (V1/F, 528 l), absorption rate constant (KA, 0.24 h $^{1)}$, inter-compartmental clearance (Q/F, 12.5 l/h), peripheral volume of distribution (V2/F, 483 l) and elimination half-life ($t_{1/2}$, 14.0 days). Weight had a positive influence on central volume but was insufficient to warrant dosage adjustments. The inter-individual variability (coefficient of variation, CV%) for CL/F and V1/F was 24.4% and 29.6%, respectively. The IOV for CL/F was 17.8%. The proportional residual error (CV%) for groups A and B was 11.5% and 19.5%, respectively, and the additive error standard deviation (SD) was 57 ng/ml and 149 ng/ml, respectively.
*Conclusion* The typical parameter values were comparable with those estimated in much smaller cohorts of healthy subjects and in malaria patients treated with single-dose mefloquine. The lower unexplained variability in the blinded study suggested these subjects may have been more compliant in taking their medication than soldiers in the open-label study.

**Keywords** Mefloquine · Population pharmacokinetics · NONMEM · Malaria prophylaxis · Healthy subjects

B. G. Charles (✉) · A. Blomgren
School of Pharmacy, Steele Building,
The University of Queensland,
Brisbane 4072 QLD, Australia
e mail: B.Charles@pharmacy.uq.edu.au

P. E. Nasveld · S. J. Kitchener · A. Jensen · R. M. Gregory · B. Robertson · I. E. Harris · M. P. Reid · M. D. Edstein
Australian Army Malaria Institute,
Brisbane, QLD, Australia

## Introduction

Mefloquine, a quinoline methanol structurally related to quinine, has been extensively used for more than 20 years for malaria prophylaxis. Of the three drugs, doxycycline, atovaquone/proguanil and mefloquine, that are currently recommended for malaria prophylaxis by the World Health Organization (WHO) [1] and the US Centers for Disease Control and Prevention, [2], mefloquine is the only one that is administered weekly, the others having to be taken daily. Weekly chemoprophylaxis has been favoured by both civilian and military populations due to the likelihood of better compliance and, therefore, potentially increased effectiveness compared with a daily regimen [3, 4]. The effectiveness, safety and tolerability of mefloquine for

malaria prophylaxis have been established in different populations across various study designs, such as cohort or randomised control studies [3–12].

Mefloquine has a long terminal half-life of about 2 weeks, partly because it is highly lipophilic and extensively distributed into tissues [13]. Much of the earlier published pharmacokinetic data for mefloquine were obtained in small, single-dose studies in healthy subjects and in patients with acute falciparum malaria [13–18]. Despite the extensive clinical use of mefloquine for malaria prophylaxis, to our knowledge, there are no pharmacokinetic data on mefloquine from very-large-scale studies involving long-term prophylaxis in soldiers in military operations. Here we report the results of the first population pharmacokinetic study of mefloquine in a large cohort of healthy soldiers who received oral mefloquine prophylactically for 6 months while deployed in field operations in the tropics.

## Methods

### Subjects, study design and drug administration

Study subjects were 1,111 Australian soldiers on a 6-monthly deployment for peace-keeping duties to East Timor. Three contingents (1st, 2nd, 3rd) of soldiers participated from October 2000 to April 2002. There were 161 subjects (seven females) from the 1st contingent (designated as group A for this analysis) who were participating in a phase III randomised, double-blind trial to compare the effectiveness, tolerability and safety of tafenoquine and mefloquine for malaria prophylaxis [22], the clinical findings of which will be published elsewhere. There were 950 subjects (one female) from the 2nd and 3rd contingents (designated as group B) who were involved in an open-label study to evaluate the tolerability of mefloquine [23]. All subjects were determined to be healthy by a complete medical history and physical examination to satisfy the medical requirements for military deployment and were willing and able to give written informed consent and comply with the study protocol. Women were excluded if they were pregnant, lactating or unwilling/unable to comply with recognised contraceptive methods during deployment, and for a period of 30 days after cessation of the drug. A subject was excluded if there was a history of allergy or intolerance to mefloquine or a history of drug or alcohol abuse.

After an oral loading dose of mefloquine (Lariam; Roche, Switzerland) equivalent to 250 mg mefloquine base per tablet administered daily for 3 days in group A and 250 mg mefloquine delivered every second day on three occasions in group B, all subjects subsequently received an oral weekly maintenance dose of mefloquine (250 mg) during their 6 months of deployment. For group A subjects who were participating in the double-blind trial, each received their dose as an opaque Swedish Orange hard gelatine capsule (size 1, Capsugel) containing the Lariam (250 mg base) tablet at loading and for maintenance dosing during the deployment. Dosage administration was observed and recorded for each subject.

### Ethics

The double-blind trial was approved by the US Army Human Use Research Review Board and the Australian Defence Human Research Ethics Committee (ADHREC). The open-label study was approved by ADHREC. Informed consent was obtained in writing from all subjects prior to their participation.

### Blood sampling

Venous blood samples (7 ml) were collected into ethylenediamine tetraacetic acid EDTA (haematology) tubes after the last loading dose and at weeks 4, 8 and 16 for group A and weeks 13 and 26 for group B. A total of 2,141 plasma samples were obtained. Most subjects in group A (155/161) had four blood samples collected, with three samples from each of the remaining six subjects. In group B, two samples were collected from 553/950 subjects, with one sample per subject obtained from the remainder. Subjects' characteristics, sampling and observational data are displayed in Table 1.

The samples were transported on ice to the field laboratory within 3 h of collection. The plasma was separated (1,200 *g*, 15 min), stored in liquid nitrogen or at −25 °C for no longer than 4 weeks then air freighted on dry ice to the Australian Army Malaria Institute (Brisbane, QLD, Australia) for storage at −80°C pending analysis.

### Mefloquine analysis

Plasma mefloquine concentrations were measured by high-performance liquid chromatography (HPLC) [24, 25], with some minor modifications. Briefly, plasma work-up involved protein precipitation and centrifugation. Chromatograms of the injected supernatant fluid were developed on a Symmetry C18 cartridge (150×3.9 mm; Waters; Milford, MA, USA), with a mobile phase consisting of 1-octanesulfonic acid (PIC B-8) low ultra-violet (UV) reagent (5 mM) in acetonitrile:methanol:water (5:65:30 v/v/v), which was delivered isocratically at 0.6 ml/min and monitored at 222 nm. Retention times for the internal standard [WR184806; 2,8-bis(trifluoromethyl)-4-[1-hydroxy-3-(N-tertbutylamino)-propyl]quinoline] and meflo-

**Table 1** Characteristics of study subjects and observational data

| Characteristics | |
|---|---|
| Number of subjects | |
| Group A (1st contingent) | 154 male, 7 female |
| Group B (2nd, 3rd contingents) | 949 male, 1 female |
| Age (years)[a] | |
| Group A | 26 (18 51) |
| Group B | 26 (18 55) |
| Pooled | 26 (18 55) |
| Weight (kg)[a] | |
| Group A | 81 (53 135) |
| Group B | 82 (57 121) |
| Pooled | 82 (53 135) |
| Plasma samples per subject [b] | |
| Group A | 3 (3 4) |
| Group B | 1 (1 2) |
| Pooled | (1 4) |
| Mefloquine concentration (ng/ml) [a] | |
| Group A | 762 (248 1914) |
| Group B | 785 (62 2549) |
| Pooled | 778 (62 2549) |
| Pooled (trough concentrations)[c] | 547 (108 1914) |
| Post dose sample times (h) [a] | |
| Group A (1st contingent): | |
| Post third loading dose (week 1) | 5.4 (0.9 167.9) |
| Post maintenance dose (week 4) | 76.0 (0.0 173.4) |
| Post maintenance dose (week 8) | 65.3 (0.2 170.7) |
| Post maintenance dose (week 16) | 79.4 (0.1 173.8) |
| Group B | |
| 2nd contingent: | |
| Post maintenance dose (week 13) | 39.8 (0.0 176.3) |
| Post maintenance dose (week 26) | 78.6 (1.0 170.4) |
| 3rd contingent: | |
| Post maintenance dose (week 13) | 75.4 (0.2 174.7) |
| Post maintenance dose (week 26) | 88.9 (1.2 168.2) |

[a] Mean (range)
[b] Median (range)
[c] Samples drawn within 5% of scheduled 168 h (weekly) post dose sampling time

quine were 6.6 and 9.5 min, respectively. The limit of quantification (LOQ) of mefloquine from 0.2 ml of plasma was set at 100 ng/ml. The inter-day assay coefficients of variation (CV%) for the measurement of mefloquine at 200, 500 and 2,000 ng/ml were 6.2%, 4.4% and 3.3% ($n$=41), respectively. The inaccuracy of the method at 100 ng/ml and 500 ng/ml was 10% and 2%, respectively.

## Pharmacokinetic modelling and statistical methods

Population pharmacokinetic modelling was performed using NONMEM (version 5, level 1.1; Globomax LLC, Hanover, MD, USA), with a G77 compiler and Wings for NONMEM (WFN; http://wfn.sourceforge.net/). One- and two-compartment models with first-order absorption and elimination were fitted to the plasma-concentration-time data using first-order conditional estimation (FOCE) with interaction. A model for lag time was screened to account for any time delay between administration and the beginning of drug absorption.

In formulating the fixed effects (structural) model, an initial analysis was conducted by estimating the base model parameters without the inclusion of factors (covariates) that could modify the pharmacokinetic values. Potential factors centred about the average population value for that factor were screened by individual addition to the base model for each pharmacokinetic parameter. A reduction in the objective function value (OFV) of at least 6.6 was considered to be statistically significant ($P$<0.01). All significant factors were included in the full model, which was then tested for masking by backwards elimination in which the value of each factor in turn was set to 0, and the model was re-run; a factor was eliminated from the model if the OFV value did not increase by at least 6.6 ($X^2_{1,0.01}$). The final structural model took the form of the following general equation:

$$P = \theta_1 + \theta_2 \cdot \left(\mathrm{Fac}_1 - \mathrm{Fac}_{1,\mathrm{A}}\right) + \ldots\ldots + \theta_\mathrm{N} \cdot \left(\mathrm{Fac}_\mathrm{M} - \mathrm{Fac}_{\mathrm{M,A}}\right)$$

where $P$ is the typical population value of a pharmacokinetic parameter, and $\theta_1$ is the typical value of $P$ for subjects having average population values of $\mathrm{Fac}_{1,\mathrm{A}}$ .... $\mathrm{Fac}_{\mathrm{M,A}}$ for factors $\mathrm{Fac}_1$ .... $\mathrm{F}_\mathrm{M}$, respectively. Estimated parameters $\theta_2$ .... $\theta_\mathrm{N}$ modify the value of $P$ above or below the typical value.

For the variance (random effects) model, the inter-individual variability (IIV) about $P$ was modelled as follows:

$$P_{ij} = P \cdot \exp\left(\eta_{\mathrm{Pi}} + \kappa_{\mathrm{Pij}}\right)$$

where, $\mathrm{P}_{\mathrm{ij}}$ represents the true but unknown value of P in the $\mathrm{i}^{\mathrm{th}}$ individual on the $\mathrm{j}^{\mathrm{th}}$ occasion, $\eta_{\mathrm{Pi}}$ and $\kappa_{\mathrm{Pij}}$ are random variables distributed normally with means of 0 and variances of $\omega_P^2$ and $\pi_P^2$, respectively. The IIV and the inter-occasion variability (IOV) of $\omega_\mathrm{P}$ and $\pi_\mathrm{P}$, respectively, are approximately equal to a CV (expressed as a fraction). An "occasion" was defined as a group of sequential dosing records terminated by at least one observation event (concentration), and the variances were considered to be sampled from the same distribution. The covariance ($\omega^2_{P1,P2}$) between the variances ($\omega^2_{P1}$, $\omega^2_{P2}$) of parameters P1 and P2 was simultaneously estimated, with the correlation coefficient ($r$) being calculated as:

$$r = \omega^2_{P1,P2} \div \left[\omega^2_{P1} \cdot \omega^2_{P2}\right]^{0.5}$$

The residual unexplained variability (RUV) representing the variance between the observed plasma concentrations and those predicted by the model were estimated using a combined proportional-additive error model:

$$C_{ij} = C_{ij,PRED} \cdot \left(1 + \varepsilon_{1,ij}\right) + \varepsilon_{2,ij}$$

where $C_{ij}$ is the $i^{th}$ observed plasma concentration in the $j^{th}$ individual, $C_{ij}=C_{ij,PRED}$ is the corresponding concentration predicted by the pharmacokinetic model, and $\varepsilon_{1,ij}$ and $\varepsilon_{2,ij}$ are randomly distributed variables each with a mean value of 0 and variances of $\sigma_1^2$ and $\sigma_2^2$, respectively, conveniently expressed as the CV% ($\sigma_1$) and the standard deviation ($\sigma_2$).

### Model evaluation

A visual predictive check (VPC) [35] was performed on the final model via an ActivePerl script (version 5.8.4; http://www.activestate.com) in which 1,000 concentration-time profiles were simulated from the model to obtain the 5%, 50% (population median) and 95% percentiles for the predicted concentrations onto which the raw concentration-time data were superimposed. Standard diagnostic plots of weighted residuals (WRES) versus population model-predicted concentration and post-dose sampling time were examined, together with the values of the parameters and their imprecision [relative standard deviation (RSD%)], estimated by expressing the asymptotic standard error of estimation as a percentage of the parameter value.

## Results

### Model building

Of a total of 2,141 samples, two from different subjects (62 ng/ml, 92 ng/ml) were below the LOQ of 100 ng/ml set a priori by the analytical chemists. Accordingly, data were remodelled using methods M1, M5 and M6, which represent some of the different ways for dealing with population data below the LOQ [34]: M1, both concentrations were discarded; M5, both concentrations were set to LOQ/2 (50 ng/ml); M6, the highest concentration (92 ng/ml), was set to LOQ/2 and the other (62 ng/ml) was discarded. There was no observable difference in the results among these three methods or when both data points were included; therefore, all the data were included in developing the population model.

A two-compartment model (ADVAN4) with first-order absorption from the gut and elimination from the central compartment fitted the data best. The first order-rate constants were re-parameterised (TRANS4) to estimate clearance (CL/F), central compartment volume (V1/F), peripheral volume of distribution (V2/F), inter-compartmental clearance (Q/F) and absorption rate constant (KA). A sister model (ADVAN4 TRANS3) also was evaluated but was discarded because the OFV was 44 points greater, and the steady-state volume of distribution (VSS/F) could not be estimated. Data also supported a single compartment model (ADVAN2 TRANS2), but the fit was inferior as judged by the residual error magnitude, the Akaike Information Criterion (AIC) and inspection of residuals. Addition of a lag-time parameter (ALAG1) for delayed absorption from the gut into blood was not supported. While the inclusion of weight decreased the IIV about V1/F by 7%, neither weight nor age significantly reduced the OFV when screened separately for influence on the fixed-effects parameters. Allometric scaling on body size was not successful, presumably because of the reasonably uniform distribution of weights in the study population. Gender was not considered as a potential covariate, as only eight of the 1,111 subjects were females.

For the variance model, the IIV (CV%) about CL/F and V1/F was 24.5% and 29.6%, respectively. Modelling the IIV about KA, Q/F and V2/F was not supported by the data. The only covariance model supported was that between CL/F and V1/F, in which an $r$ value of 0.9 indicated marked within-subject correlation of these parameters, presumably via body size and oral bioavailability (F). The addition of IOV to the CL/F variance model reduced the variability from 28.2% to 24.2%. However, while a full covariance block resulted in a small reduction in the OFV, this was not considered further, as $r$ values of ~1 between combinations of parameters raised suspicions that the variance(s) about 1 or more of the blocked parameters was being explained by the other(s). A combined additive + proportional RUV was superior compared to when these models were evaluated separately, shown by lower OFV values and, for the additive model, implausible values for Q/F and V2/F. Using indicator variables, the RUV model was partitioned between group A and group B subjects separately, which improved the fit seen by lower OFV values and lower IIV and IOV about CL/F.

### Final population model

Structural and variance model parameter values and the derived parameters from the final model are summarised in Table 2. Typical values (RSD%) for CL/F, V1/F, KA, Q/F and V2/F were 2.09 l/h (1.2%), 528 l (12.0%), 0.24 $h^{-1}$ (18.1%), 12.5 l/h (25.4%) and 483 l (14.2%), respectively. When normalised to individual body weight, the average CL/F and V1/F values were 0.025 l/h per kilogram and 12.3 l/kg, respectively. The population mean elimination half-life of mefloquine was estimated to be 14 days. Steady-state volume of distribution (VSS/F), derived from the sum of V1/F and V2/F, was 1,011 (l). The IIV expressed

**Table 2** Population pharmacokinetic parameters of mefloquine based on the final model

| Parameter (units) | Description | Value (RSD%)[a] |
|---|---|---|
| Structural model | | |
| KA ($h^{-1}$) | Absorption rate constant | 0.24 (18.1) |
| CL/F (l/h) | Clearance | 2.09 (1.2) |
| Q/F (l/h) | Inter compartmental clearance | 12.5 (25.4) |
| V1/F (l) | Central volume | 528 (12.0) |
| V2/F (l) | Peripheral volume | 483 (14.2) |
| Variance model | | |
| $\omega_{CL/F}$ (CV%)[b] | Inter individual variability in CL/F | 24.4 (10.6) |
| $\omega_{V1/F}$ (CV%)[b] | Inter individual variability in V1/F | 29.6 (29.1) |
| $\pi_{CL/F}$ (CV%)[b] | Inter occasion variability in CL/F | 17.8 (27.8) |
| $\sigma_{1,A}$ (ng/ml)[c] | Additive residual error (group A) | 56.7 (86.9) |
| $\sigma_{1,B}$ (ng/ml)[c] | Additive residual error (group B) | 149.3 (21.5) |
| $\sigma_{2,A}$ (CV%)[b] | Proportional residual error (group A) | 11.5 (56.0) |
| $\sigma_{2,B}$ (CV%)[b] | Proportional residual error (group B) | 19.5 (33.3) |
| Derived parameters | | |
| $t_{1/2}$ (days) | Elimination half life | 14.0 |
| VSS/F (l) | Steady state volume | 1,011 |

[a] Relative standard deviation = Standard error of estimation . 100% ÷ Value
[b] Coefficient of variation
[c] Standard deviation

as CV% (RSD% of estimation) for CL/F, V1/F and the IOV for CL/F were 24.4% (10.6%), 29.6 % (29.1%) and 17.8 (27.8%), respectively. The additive (SD) and proportional (CV%) components of the RUV model were, respectively, 56.7 ng/ml and 11.5% (group A) and 149 ng/ml and 19.5% (group B).

## Model evaluation

Assessment of the model performance was based on a number of criteria; the population typical values were estimated with good precision and were plausible when compared with published values from smaller, non-population studies. The variance model parameters likewise were plausible, with satisfactory precision, although the precision associated with estimating the additive and proportional components of the RUV model was greater for group B subjects, perhaps because there were far fewer subjects in group A (161) than in group B (950). Weighted residuals (WRES) versus population-model-predicted values (Fig. 1a) and elapsed time post-dose (Fig. 1b) showed most of the data symmetrically distributed about and within 3 U of the null ordinate, indicating a good fit of model to the data. The VPC showed that the raw post-dose concentration data mirrored the concentrations simulated from the final model parameters, as seen in Fig. 1c. The structural model was deemed to be satisfactory, as the

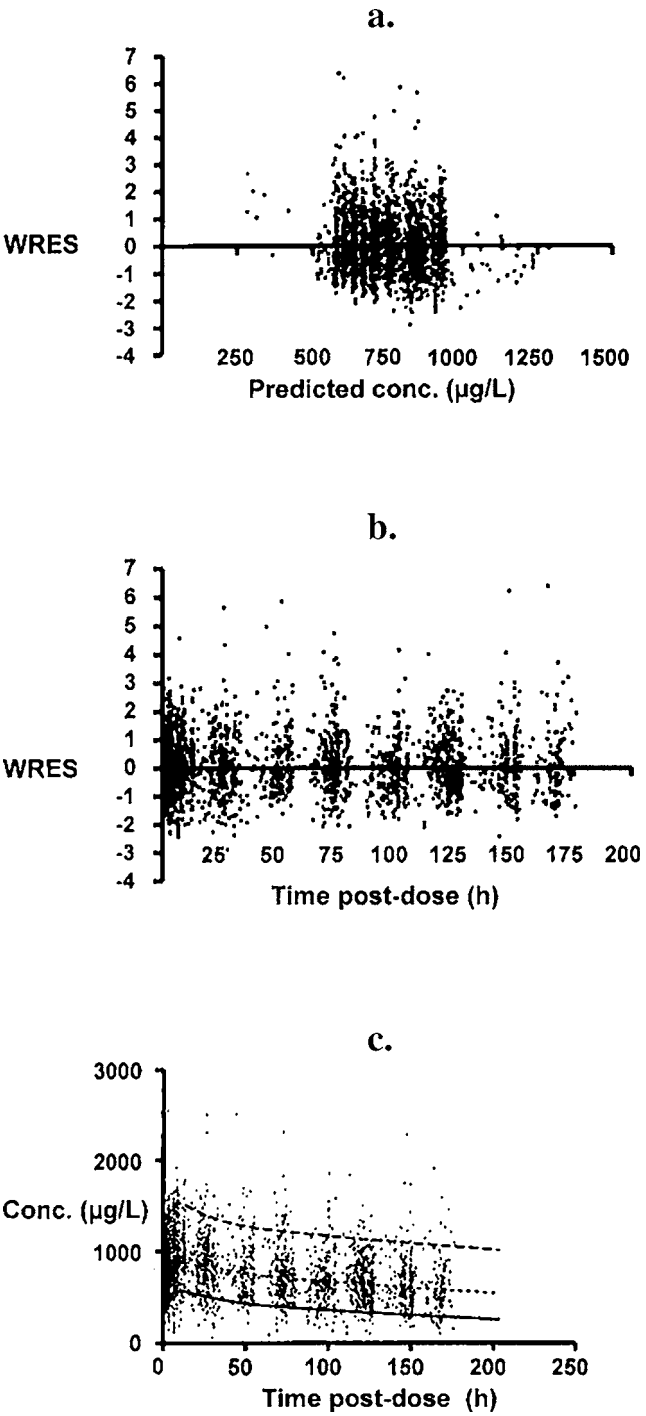


**Fig. 1** Diagnostic plots of; **a** Weighted residual (WRES) versus population model predicted concentration; **b** WRES versus post dose time; **c** VPC of plasma concentration versus post dose time. The population predicted profile (50th percentile) is shown by the central profile (*dotted line*), bounded by an upper profile (*dashed line*) and lower profile (*solid line*) representing, respectively, the 95th and 5th percentile prediction intervals estimated from 1,000 simulated concentrations at sampling times up to 174 h post dose using the final population model

raw data points were symmetrically distributed about the 50th percentile, and the variance model was satisfactory, as 11% (10% expected) of the raw data lay outside the 5th and 95th percentile profiles.

## Discussion

Mefloquine has been a popular prophylactic agent due to its effectiveness against chloroquine-resistant *Plasmodium falciparum* and the convenience of weekly dosing compared with alternatives such as doxycycline. This has major advantages in terms of compliance for military personnel in field deployment. A number of mefloquine pharmacokinetic studies have been published, but most used a two-stage analysis involving extensive blood sampling in each individual [13–18]. Besides the inconvenience and increased infection risk of multiple sampling in the tropics, the pharmacokinetic variability in the two-stage approach can be inflated because the uncertainty associated with fitting the model parameters to the data is ignored. A representative sample of these include analyses using single-compartment models [18, 26, 27], multi-compartment models [13–15, 17, 28] or non-compartmental approaches [16–18, 24]. Simpson et al. [26] used a population model to describe the kinetics after a single or split dose to 257 patients with acute falciparum malaria. Presently, a two-compartment model with first-order absorption and elimination was found to best characterise the plasma mefloquine concentration data. While this study had by far the largest number of subjects of any mefloquine pharmacokinetic study yet undertaken, the data were sparse, with only 1–4 samples per subject drawn over a lengthy period in the field. The mean CL/F and V1/F values of 0.025 l/h per kilogram and 12.3 l/kg, respectively, were comparable to published studies in healthy subjects and malaria patients [13–18, 26–28]. A relatively small CL/F and large V1/F was consistent with the long derived elimination half-life of 14 days. In four traditional (two-stage) pharmacokinetic analyses in healthy Caucasian subjects, mean CL/F values ranged from 0.022 to 0.029 l/h per kilogram, V/F was 8.6–15.5 l/kg and the elimination half-lives were 13.9–20.1 days following a single oral dose of mefloquine [13–15, 17].

Interestingly, Pennie et al. [19] reported CL/F and V/F values in 15 Canadian tourists taking mefloquine weekly over 3 months, which were, respectively, 1.85-fold and 1.5-fold less than presently estimated values, but the half-lives were comparable. As in the present study, Lariam 250 mg had been administered to mainly healthy adult Caucasian subjects, so this discordance is difficult to explain. Because plasma concentrations were markedly higher in the Pennie et al. study than reported here and in other studies [5, 20, 21], a possible explanation was that the systemic bioavailability was higher. While Pennie et al. did not state whether their subjects were fed or fasted, it is unlikely that food had any major effect on mefloquine bioavailability [29, 30].

The present study subjects constituted a reasonably homogeneous group; thus, the final model did not justify the inclusion of weight as a predictor of any clearance or volume parameter, in contrast to two previous population studies [26, 27]. The small influence of weight on V1/F would be insufficient to require individualising the loading dose regimen, and, in any case, would be impracticable, as Lariam is currently available in Australia only as a 250-mg cross-scored tablet.

In accord with other studies [5, 19, 21, 31, 32], there was a considerable amount of inter-individual variability in the steady-state concentrations, reflected in an IIV of 24–30% in CL/F and V1/F. However, the IOV on CL/F was reasonably low (17%), indicating that within individual subjects, the steady-state concentrations could be maintained within a reasonably narrow band over the 6-month deployment period. While an additive RUV model is often sufficient over a limited range of observations, a combined additive plus proportional model was presently used because the plasma concentrations ranged from 62 to 2,549 ng/ml. There was approximately a two-fold larger RUV in both the additive and proportional components of the RUV for group A data compared with group B data. Since the drug assay, tablet brand and dose were common to both studies, it was possible that such a difference reflected the fact that the subjects in the open-label study (group B) were less stringently supervised than the blinded study subjects (group A) and that variation in dosing times may have contributed to the increased amount of unexplained error.

To minimise malaria infection, effective steady-state mefloquine concentrations should be achieved within a 5–14 day pre-patent period for *P. falciparum* malaria [5]. Blood mefloquine concentrations of 500–600 ng/ml are considered to be protective against *P. falciparum* malaria in Africa [6, 33]. Presently, the average concentration exceeded this target at 778 ng/ml and was achieved within 3 days by using a 3-day split-loading dose regimen. The mean steady-state trough mefloquine concentration was 547 ng/ml, which was comparable with other published studies [5, 20, 21]. Only one subject (group B) contracted malaria in the malaria-endemic area, but this was most likely a result of compliance difficulties after changing from mefloquine to doxycycline.

In conclusion, the population pharmacokinetics of mefloquine were studied using sparse data from 1,111 Australian soldiers deployed in the field who were on weekly mefloquine for malaria prophylaxis. As the study subjects were predominately healthy male Caucasians, the

results may not be directly applicable to the wider population, including children and the elderly and patients with malaria, as a number of pathophysiological changes may affect mefloquine absorption and distribution [26].

**Acknowledgments** The authors thank the Australian military personnel who participated as the subjects in this study and the co ordinational and monitoring support of Squadron Leader Karen Leshinkas, Captain Kym Ward, Captain John Cunningham, Captain Damien Wood and Captain Tracy Carthew. We are grateful to Lieutenant Colonel William Prescott [US Army Medical Materiel Development Activity (USAMMDA)] and Philip Pickford [Glaxo SmithKline (GSK)] for being the project managers for the Phase III clinical study. The technical contribution of Mr. Tom Travers and Sergeant Hamish Barbour to the mefloquine HPLC analysis is gratefully acknowledged. We also thank the military staff from the Australian Army Malaria Institute who assisted with the blood collections and processing, and Mr. Tom Travers and Sergeants Hamish Barbour, Kerry Rowcliffe and Christine Atkins for data entry. Financial support for the study was provided by USAMMDA, GSK and the Australian Defence Force. The opinions expressed in this paper are those of the authors and do not necessarily reflect those of the Australian Defence Health Service or any extant Australian Defence Force policy. The experimental work complies with the current laws of Australia. The work reported herein forms part of a dissertation by Ms. Anna Blomgren to Uppsala University, Sweden, for the MSc degree (Chemical Engineering).

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**Attachment 2: Kitchener-2005**

# Mefloquine and doxycycline malaria prophylaxis in Australian soldiers in East Timor

Scott J Kitchener, Peter E Nasveld, Robin M Gregory and Michael D Edstein

## ABSTRACT

**Objectives:** To describe the tolerability of mefloquine in Australian soldiers for malaria prophylaxis, including a comparison with doxycycline.

**Design:** Open-label, prospective study and cross-sectional questionnaire and interview.

**Setting and participants:** Two contingents of Australian soldiers, each deployed to East Timor for peacekeeping duties over a 6-month period (April 2001–October 2001 and October 2001–May 2002).

**Outcome measures:** Withdrawals during the study; adverse events relating to mefloquine prophylaxis; willingness to use mefloquine again on deployment.

**Results:** Of 1157 soldiers starting on mefloquine, 75 (6.5%) withdrew because of adverse responses to the drug. There were three serious adverse events of a neuropsychiatric nature, possibly relating to mefloquine. Fifty-seven per cent of soldiers using mefloquine prophylaxis reported at least one adverse event, compared with 56% using doxycycline. The most commonly reported adverse effects of both drugs were sleep disturbance, headache, tiredness and nausea. Of the 968 soldiers still taking mefloquine at the end of their deployments, 94% indicated they would use mefloquine again. Of 388 soldiers taking doxycycline prophylaxis who were deployed with the first mefloquine study contingent, 89% indicated they would use doxycycline again.

**Conclusions:** Mefloquine was generally well tolerated by Australian soldiers and should continue to be used for those intolerant of doxycycline.

MJA 2005; 182: 168–171

A limited number of drugs are available for malaria protection, particularly for long-term prophylaxis. In 2001, doxycycline, mefloquine and Malarone (a combined preparation of atovaquone and proguanil) were recommended by the World Health Organization and the US Centers for Disease Control and Prevention for non-immune travellers in malarious areas where chloroquine-resistant *Plasmodium falciparum* malaria is prevalent.[1,2] Like travellers, military personnel must also take chemoprophylaxis in malarious areas, to minimise non-battle casualties.

In 1999, the Australian Defence Force (ADF) participated in an international peacekeeping operation in East Timor. During the first 5 months of the operation, 64 soldiers presented with malaria in-country.[3] Most soldiers had been prescribed daily doxycycline (100 mg) for prophylaxis, and these cases are believed to have resulted from poor compliance. These findings provided the stimulus to look at other chemoprophylactic options for soldiers in East Timor. During 2000–2001, a double-blind trial comparing weekly tafenoquine and mefloquine was conducted in Australian soldiers in East Timor.[4] Mefloquine was found to be well tolerated and accepted by the soldiers, and, as a result, there were requests for wider use of mefloquine from subsequent military units and soldiers being deployed to East Timor.

There are limited data on the tolerability of mefloquine for long-term prophylaxis in military personnel. Short-term studies (ranging from 2 to 5 months) in British, Dutch, Indonesian, Italian and US soldiers have shown weekly mefloquine to be safe and well tolerated.[5-9] To expand on our previous study in East Timor,[4] we monitored the tolerability of mefloquine in a larger number of Australian soldiers under peacekeeping conditions during two 6-month periods. We report here our preliminary findings.

## METHODS

The study was carried out in two contingents of Australian soldiers, each deployed for 6 months on peacekeeping duties on the border between East Timor and Indonesia. The first contingent was deployed from April 2001 to October 2001 and the second from October 2001 to May 2002. Before enrolling, the soldiers received briefings in Australia regarding vector-borne diseases, personal protection measures, and information on the use of mefloquine and the nature of the study. Those choosing to enrol in the study signed an "information and consent" form. They were advised in the form and verbally that enrolment was voluntary and that they could withdraw from the trial at any time. Common, uncommon and rare side effects associated with mefloquine use (detailed in the manufacturer's product insert) were presented during enrolment and were listed in the information and consent form. Soldiers choosing not to enrol in the mefloquine study received doxycycline.

Soldiers meeting the inclusion criteria of fitness for deployment and providing informed consent were administered a loading dose of one 250 mg tablet of mefloquine (Lariam, Roche, Switzerland) given every other day on three occasions, followed by regular weekly doses of one 250 mg tablet.

After 6 months' deployment, the trial participants completed a health questionnaire followed by a structured interview, conducted by a clinical investigator, about adverse events. At the end of the first contingent's deployment, all soldiers being medically processed for return to Australia were invited to complete the health questionnaire

FOR EDITORIAL COMMENT, SEE PAGE 148. SEE ALSO PAGES 164, 181 AND 186.

**Centre for Military and Veterans' Health, Mayne Medical School, Herston, QLD.**
Scott J Kitchener, MB BS, DrPH, FAFPHM, Associate Professor.
**Army Malaria Institute, Enoggera, QLD.**
Peter E Nasveld, MB BS, BMedSci(Hons), FACTM, Senior Research Physician; Robin M Gregory, BAppSc, MBus, Scientific Officer; Michael D Edstein, MSc, PhD, Pharmacologist.
Reprints will not be available from the authors.
Correspondence: Associate Professor Scott J Kitchener, Centre for Military and Veterans' Health, Mayne Medical School, Herston Road, Herston, QLD 4006. s.kitchener@uq.edu.au

— including soldiers who had withdrawn from the mefloquine study, those who had used mefloquine but were not participants in the study, and those who had used doxycycline only.

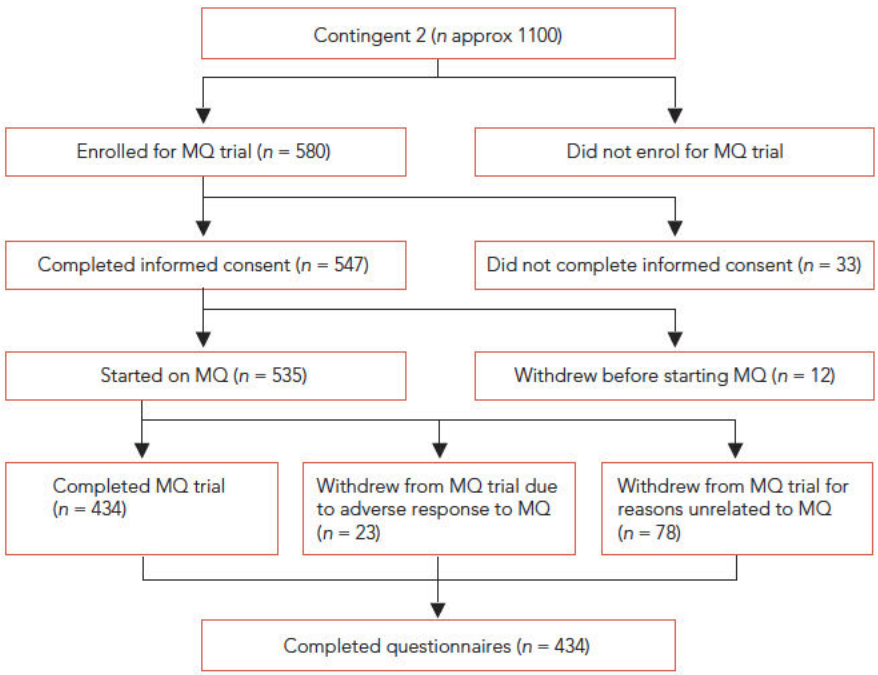
Participants were asked to grade the severity of any adverse events as (i) mild (not affecting daily activities), (ii) moderate (causing some interference with daily activities), or (iii) severe (preventing completion of daily duties). The adverse events were classified into body systems: gastrointestinal (including nausea, vomiting, diarrhoea and abdominal pain); constitutional (including headache, tiredness and malaise); neuropsychiatric (including sleep disturbance, anxiety, irritation, depression, hallucinations, confusion and balance problems); dermatological (including rash, skin disorders and dermatitis); and musculoskeletal (including muscle and joint pain). A "serious adverse event" was defined as "an untoward medical occurrence resulting in death, causing a threat to life, requiring or prolonging hospitalisation, or resulting in significant disability or incapacitation". The principal investigator, in consultation with the clinical investigators, assessed serious adverse responses on a four-point scale for causality ("not related", "unlikely", "possible" or "probable").

Our study was approved by the Australian Defence Human Research Ethics Committee.

## RESULTS

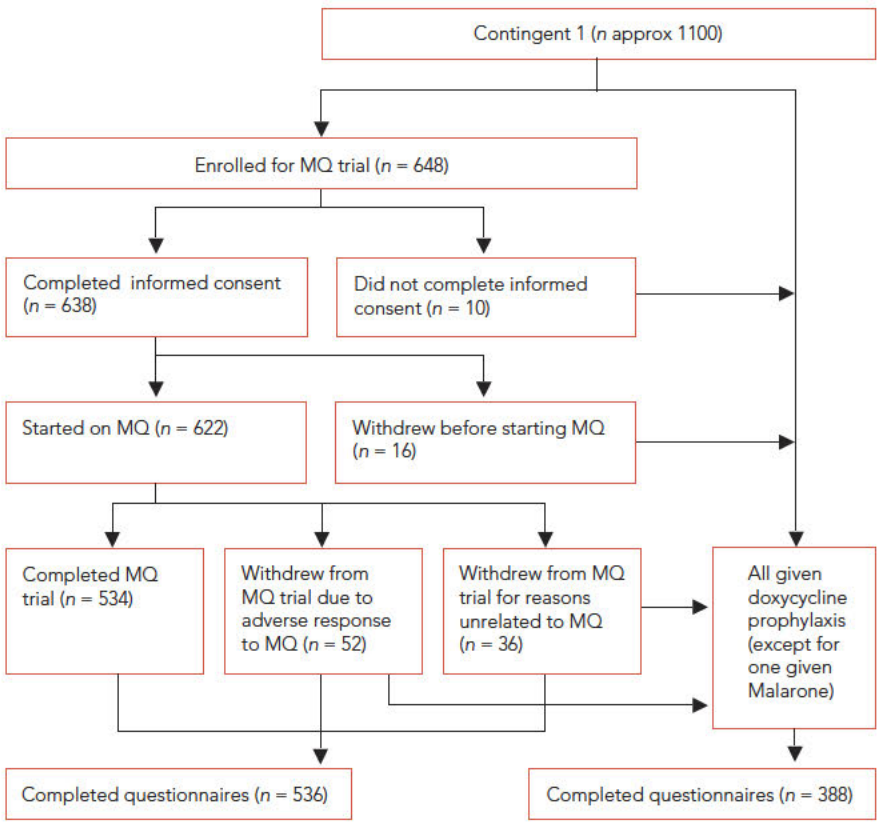
Of the 1228 soldiers who enrolled in the trial (648 in the first contingent and 580 in the second contingent), 1185 provided informed consent, of whom 1157 (1155 men, 2 women) started to take mefloquine (Box 1 and Box 2). Those who enrolled but did not start mefloquine prophylaxis either were not deployed for other reasons or chose not to continue after enrolment. Of the 1157 soldiers who started on mefloquine, 16.3% (189/1157) did not complete their deployment on mefloquine, and 6.5% (75/1157) had adverse responses to the drug. The withdrawal rate from mefloquine prophylaxis due to adverse effects of the drug was higher in the first contingent than in the second (8.4% v 4.3%). The body systems affected, as reported by soldiers who withdrew from mefloquine prophylaxis, are listed in Box 3. All soldiers who stopped taking mefloquine were given doxycycline instead (or Malarone, if they were doxycycline intolerant). Out of the first contingent, 388 soldiers were questioned about the tolerability of doxycycline prophylaxis during their deployment.

**1 Participants in mefloquine trial in first contingent, Apr 2001–Oct 2001**




MQ = mefloquine.

**2 Participants in mefloquine trial in second contingent, Oct 2001–May 2002**




MQ = mefloquine.

**3 Adverse events, by body system, reported among Australian soldiers who withdrew from the mefloquine trial due to adverse effects of the drug***

| Body system | First contingent withdrawals (*n* = 52) | Second contingent withdrawals (*n* = 23) |
|---|---|---|
| Gastrointestinal | 6 | 4 |
| Constitutional | 9 | 9 |
| Neuropsychiatric | 42 | 20 |
| Dermatological | 3 | 3 |
| Musculoskeletal | 2 | 0 |

* Some participants reported more than one reason for withdrawing.

## Serious adverse events

There were nine serious adverse events in the mefloquine arm of the study (four in the first contingent and five in the second), all occurring in men. Three of these men were withdrawn from the study because of neuropsychiatric symptoms possibly associated with mefloquine use. The first soldier had auditory hallucinations, which, on psychological assessment, were consistent with his undisclosed history of auditory hallucinations preceding mefloquine use and the episode in East Timor. The second soldier experienced heat illness while on patrol, with symptoms of nausea, dizziness and abdominal discomfort. He was observed to have a generalised seizure. However, he was later found to have an undisclosed history of epilepsy. He recovered with rehydration and was returned to Australia. The third soldier experienced depression, episodic anxiety, mild paranoia, short-term memory loss and suicidal ideation. Although he was taken off mefloquine and placed on doxycycline, his mental state continued to deteriorate. He was psychologically evaluated and returned to Australia.

## Malaria incidence

During the trial period, only one soldier developed malaria while in East Timor. He had started on mefloquine but became infected with falciparum malaria after he had changed to doxycycline and had difficulty complying with the daily regimen.

Despite primaquine post-exposure prophylaxis, eight soldiers who were taking mefloquine presented with a primary episode of vivax malaria after returning to Australia.

## Responses to health questionnaire

At the conclusion of the first contingent's deployment, 924 soldiers received health questionnaires, including 536 who had taken mefloquine chemoprophylaxis and 388 who had taken doxycycline. Of this group, 57% of soldiers reported one or more adverse events during their use of mefloquine compared with 56% of soldiers using doxycycline. Sleep disturbance, headache, tiredness and nausea were the most commonly reported adverse events (Box 4). A detailed report of adverse events, including data on the second contingent, will be published elsewhere.

Of the 968 soldiers still taking mefloquine at the end of their 6-month deployment, 96% and 92% from the first and second contingents, respectively, indicated that they would take mefloquine on their next deployment to a malarious area. Of the 388 soldiers in the first contingent who were questioned after using doxycycline, 89% indicated they would use it again on deployment.

## DISCUSSION

In our study, the most common adverse events relating to malaria prophylaxis with either drug were sleep disturbance, headache, tiredness and nausea. Apart from mild sleep disturbance, which was more common in soldiers taking mefloquine, and mild tiredness, which was more commonly associated with doxycycline, the incidence of these adverse events was similar for both drugs.

Among the 1157 Australian soldiers taking mefloquine in our study, the 6.5% withdrawal rate from the drug due to adverse events was higher than among Italian soldiers on peacekeeping duties in Africa (0.9% of 1386),[8] British soldiers exercising in Kenya (3.4% of 183)[5] and US Marines stationed in Hawaii, USA (4.9% of 202).[9] The emotional and environmental pressures of peacekeeping operations in East Timor may have contributed to the higher withdrawal rate in the Australian soldiers compared with other military experiences with mefloquine.

In previous studies of mefloquine in military volunteers, no serious neuropsychiatric reactions were observed.[5-9] We observed three serious adverse events of this nature that were possibly associated with mefloquine use. This is a higher incidence than the 1 in 6000 to 1 in 10 600 reported in travellers.[1] However, two of the three soldiers involved had undisclosed pre-existing conditions that are contraindications for mefloquine use.

When monitoring the tolerability of a drug under military operational conditions, there is a need to account for the physiological and psychological stress associated with such activities that may confound the relationship between drug intake and adverse events. Thus, the tolerability of antimalarial drugs as assessed in these Australian soldiers may not be directly comparable to circumstances of recreational travel. Nevertheless, the withdrawal rate from mefloquine in our study was comparable to that reported in civilian travellers (range, 5%–6.5%).[10,11]

Furthermore, the cohorts in our study were not randomly allocated and therefore comparisons made may be biased. Our results are also subject to the limitations of self-report and memory.

**4 Adverse events reported by Australian soldiers in the first contingent* after taking mefloquine (*n* = 536) or doxycycline (*n* = 388) for malaria prophylaxis in East Timor, Apr–Oct 2001**

| | Mild degree | | Moderate degree | | Severe degree | |
|---|---|---|---|---|---|---|
| | Mefloquine | Doxycycline | Mefloquine | Doxycycline | Mefloquine | Doxycycline |
| Sleep disturbance | 128 (24%) | 53 (14%) | 33 (6%) | 28 (7%) | 2 (< 1%) | 2 (< 1%) |
| Headache | 53 (10%) | 51 (13%) | 17 (3%) | 14 (4%) | 1 (< 1%) | 4 (1%) |
| Tiredness | 72 (13%) | 78 (20%) | 20 (4%) | 16 (4%) | 0 | 1 (< 1%) |
| Nausea | 86 (16%) | 63 (16%) | 22 (4%) | 20 (5%) | 4 (1%) | 0 |

* Some participants reported more than one adverse event.

Despite the possibility of side effects associated with mefloquine (and other antimalarial drugs), this drug protects against potentially life-threatening infections that may also jeopardise the success of military operations. Malaria is endemic in East Timor, and, at the time of the first contingent's deployment, the prevalence of malaria among East Timorese in the area of deployment was as high as 35%.[12] The fact that a soldier not complying with doxycycline use developed malaria in East Timor, as did a small proportion of soldiers after returning to Australia, suggests that the participants were exposed to malaria infections in East Timor and that mefloquine was effective as a suppressive agent against blood stages of both falciparum and vivax malaria.

In conclusion, our study has been one of the largest tolerability trials of mefloquine and doxycycline conducted in military personnel. While the comparison must be interpreted cautiously in light of possible selection bias, it may be concluded that these drugs were generally well tolerated. As Australian soldiers will continue to exercise in and be deployed to malaria-endemic areas, there is a continuing need to seek out effective and well tolerated antimalarial drugs and to maintain alternative chemoprophylactic options. Enhanced surveillance of alternative antimalarial drugs under operational conditions will ensure that the most appropriate chemoprophylaxis is available for the ADF.

## ACKNOWLEDGEMENTS

The authors would like to thank Private Michael Reid, Lieutenant Kieran McCarthy, Captain Anne Jensen, Captain Leith Baird, Captain Tracey Carthew, Captain John Cunningham, Captain Damien Woods, Squadron Leader Karen Leshinskas, Major Brett Robertson and Lieutenant Colonel Bob Cooper for either their medical or technical support towards the study. The opinions expressed are those of the authors and do not necessarily reflect those of the Defence Health Service or any extant ADF policy. The study was wholly conducted using the resources and personnel of the ADF.

## COMPETING INTERESTS

The authors were full-time employees of the Australian Army Malaria Institute at the time this research was carried out and have no other conflict of interest to declare. The Australian Defence Human Research Ethics Committee, in accordance with National Health and Medical Research Council guidelines, requires all clinical research to be submitted for peer review.

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*(Received 10 Nov 2004, accepted 21 Dec 2004)* ❑

**Attachment 3: Nasveld-2010**

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2010, p. 792–798 Vol. 54, No. 2
0066-4804/10/$12.00 doi:10.1128/AAC.00354-09


# Randomized, Double-Blind Study of the Safety, Tolerability, and Efficacy of Tafenoquine versus Mefloquine for Malaria Prophylaxis in Nonimmune Subjects[▽]

Peter E. Nasveld,[1,5]* Michael D. Edstein,[1] Mark Reid,[1] Leonard Brennan,[1,5] Ivor E. Harris,[1] Scott J. Kitchener,[1,5] Peter A. Leggat,[1] Philip Pickford,[2] Caron Kerr,[2] Colin Ohrt,[3] William Prescott,[4] and the Tafenoquine Study Team†

*Australian Army Malaria Institute, Brisbane, Queensland, Australia[1]; GlaxoSmithKline Research & Development Limited, Harlow, Essex, United Kingdom[2]; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Silver Spring, Maryland[3]; U.S. Army Medical Materiel Development Activity, Frederick, Maryland[4]; and Centre for Military and Veterans' Health, University of Queensland, Herston, Queensland, Australia[5]*

Received 16 March 2009/Returned for modification 3 June 2009/Accepted 3 August 2009

**This study represents the first phase III trial of the safety, tolerability, and effectiveness of tafenoquine for malaria prophylaxis. In a randomized (3:1), double-blinded study, Australian soldiers received weekly malaria prophylaxis with 200 mg tafenoquine (492 subjects) or 250 mg mefloquine (162 subjects) for 6 months on a peacekeeping deployment to East Timor. After returning to Australia, tafenoquine-receiving subjects received a placebo and mefloquine-receiving subjects received 30 mg primaquine daily for 14 days. There were no clinically significant differences between hematological and biochemical parameters of the treatment groups. Treatment-related adverse events for the two groups were similar (tafenoquine, 13.4%; mefloquine, 11.7%). Three subjects on tafenoquine (0.6%) and none on mefloquine discontinued prophylaxis because of possible drug-related adverse events. No diagnoses of malaria occurred for either group during deployment, but 4 cases (0.9%) and 1 case (0.7%) of *Plasmodium vivax* infection occurred among the tafenoquine and mefloquine groups, respectively, up to 20 weeks after discontinuation of medication. In a subset of subjects recruited for detailed safety assessments, treatment-related mild vortex keratopathy was detected in 93% (69 of 74) of tafenoquine subjects but none of the 21 mefloquine subjects. The vortex keratopathy was not associated with any effect on visual acuity and was fully resolved in all subjects by 1 year. Tafenoquine appears to be safe and well tolerated as malaria prophylaxis. Although the volunteers' precise exposure to malaria could not be proven in this study, tafenoquine appears to be a highly efficacious drug for malaria prophylaxis.**

The continuing spread of multidrug-resistant *Plasmodium* species and concerns about adverse effects associated with antimalarial drugs has made the prevention of malaria problematic for nonimmune subjects, such as tourists and soldiers who travel to malaria endemic areas. No antimalarial drug is completely effective in preventing malaria (10); however, an ideal prophylactic drug would be highly effective against all malaria-inducing species, very well tolerated, and taken infrequently to enhance compliance (21). Currently, mefloquine, doxycycline, and atovaquone-proguanil are recommended for malaria prophylaxis (5, 23). These drugs are highly effective in preventing malaria but have shortcomings that limit their effectiveness, such as adverse effects, expense, and the difficulty of monitoring daily compliance within deployed military populations. Furthermore, none of these recommended drugs prevents the development and relapse of *Plasmodium vivax* and *P. ovale* dormant liver stages (hypnozoites).

Tafenoquine, a long-acting 8-aminoquinoline, is currently being codeveloped by GlaxoSmithKline (GSK) Research & Development Limited and the Walter Reed Army Institute of Research as a replacement for primaquine and for the prevention of malaria. Like primaquine, tafenoquine produces hemolysis in glucose-6-phosphate dehydrogenase (G6PD)-deficient recipients (21). Tafenoquine acts on all stages of the malaria parasite, with the potential to protect against all species of malaria parasites. Previous studies with a challenge model (4) and of indigenous populations in areas in which malaria is endemic have shown that tafenoquine was highly efficacious in preventing *P. falciparum* malaria and well tolerated (9, 13, 21). Tafenoquine was also shown to be efficacious in preventing both *P. falciparum* and *P. vivax* malaria for up to 6 months in Thai soldiers (22).

This first phase III study of tafenoquine for malaria prophylaxis was a randomized, double-blind, active controlled study carried out with healthy Australian soldiers deployed to East Timor as part of a United Nations (UN) peacekeeping mission. The primary study objective was to compare the safety and tolerability of tafenoquine with those of mefloquine in malaria prophylaxis for 6 months. A subset of 98 subjects underwent extra safety assessments to investigate the possible effects of phospholipidosis, methemoglobin, and cardiac safety. Since a

* Corresponding author. Mailing address: Centre for Military and Veterans' Health, Mayne Medical School, University of Queensland, Herston, QLD, Australia 4006. Phone: 61-7-33464876. Fax: 61-7-33464878. E-mail: P.Nasveld@uq.edu.au.

† For a list of Tafenoquine Study Team members, see the Acknowledgments.

▽ Published ahead of print on 7 December 2009.

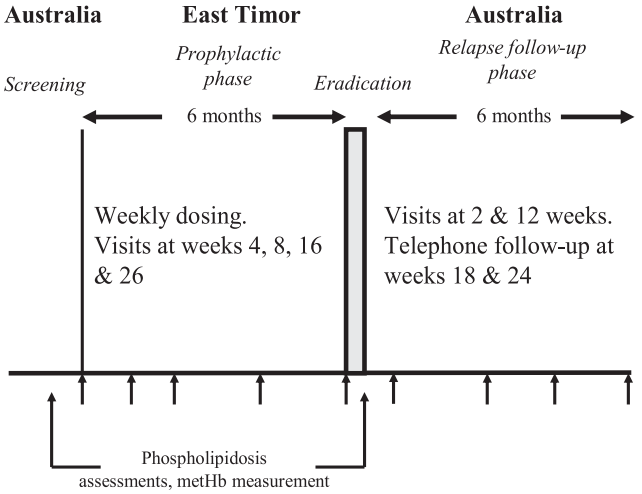


FIG. 1. Drug administration and safety analysis schedule for tafenoquine and mefloquine. metHb, methemoglobin.

placebo arm to document exposure was not possible, the key secondary objective was to assess the efficacy of tafenoquine in preventing *P. falciparum* and *P. vivax* malaria during and following deployment.

(This study was presented in part at the 51st Annual Meeting of the American Society of Tropical Medicine and Hygiene, Denver, CO, November 2002.)

## MATERIALS AND METHODS

**Study site and subjects.** The subjects were Australian soldiers deployed on UN peacekeeping duties to East Timor from October 2000 to April 2001. The soldiers were deployed to the Bobonaro District, on the western border of East Timor. The study included male and female subjects who were between 18 and 55 years of age, judged to be healthy by a medical history and physical examination with normal hematological and biochemical values, G6PD normal, and willing and able to give written informed consent and comply with the study protocol. Females were excluded if they were pregnant, lactating, or unwilling/unable to comply with recognized contraceptive methods. Subjects with a history of psychiatric disorders and/or seizures were also excluded. All subjects gave written informed consent, and the study protocol was approved by the Australian Defence Human Research Ethics Committee (ADHREC protocol no. 216/00) and the U.S. Army Human Subject Research Review Board.

**Study design and drug administration.** This comparative, randomized, double-blind, active controlled study had 4 phases: screening, loading, prophylactic phase, and relapse follow-up (Fig. 1). Following a loading-dose regimen of 200 mg tafenoquine or 250 mg mefloquine daily for 3 consecutive days, the subjects then received an oral weekly maintenance dose of 200 mg tafenoquine or 250 mg mefloquine for 26 ± 4 weeks, respectively. Subjects were directed to take their study medication at the same time each week with food (breakfast/dinner) to enhance drug bioavailability. Upon their return to Australia, subjects commenced a hypnozoite eradication regimen, receiving primaquine 15 mg twice a day (for the mefloquine group) or matched placebo twice a day (for the tafenoquine group) for 14 days. Drug compliance was observed and recorded for each subject by using medication logs.

**Randomization.** A coding memo block randomization system (block size = 8) to provide a 3:1 ratio of tafenoquine-receiving subjects to mefloquine-receiving subjects was used to assign the subjects to a treatment group. Study drugs were prepackaged and prelabeled with a unique study number.

**Drug sources.** Tafenoquine was supplied by GlaxoSmithKline in an opaque, hard gelatin capsule (Capsugel), each containing a 200-mg tafenoquine base. Placebo tafenoquine capsules were of identical appearance. Mefloquine (Lariam; 250-mg base tablet) was obtained from Hoffman-La Roche, and primaquine (15-mg base tablet) was supplied by GlaxoSmithKline. The matched placebos for mefloquine and primaquine were identical in external appearance to active capsules. All medication was provided in blinded individual foil blister packs and stored between 15°C to 30°C.

**Safety and tolerability.** Assessment of adverse events and sample collection for hematological and blood chemistry parameters were carried out at the loading stage and then at weeks 4, 8, 16, and 26 during the prophylactic phase and at weeks 2 and 12 during the relapse follow-up phase. Adverse event monitoring was supplemented by review of subjects' medical records. For a subset of 98 subjects (77 on tafenoquine and 21 on mefloquine), more-detailed safety assessments were performed. These subjects were assessed for phospholipidosis and its effects (by ophthalmic assessments, lung function tests, and electron microscopy of peripheral blood lymphocytes) and methemoglobin assessment and an electrocardiogram were performed (to assess QT interval) at screening and at the end of the prophylactic phase. Following the identification of corneal deposits at the end of this study, a wider range of ophthalmic assessments was included at follow-up.

Disclosure of adverse events was elicited by the investigator asking the subject the nonleading question, "Do you feel differently in any way since starting the new treatment?" A study physician assessed the level of relationship of any adverse event on the basis of the subject's response and any temporal association and/or known adverse responses to the drug. The physician graded the severity of adverse events as mild (not affecting daily activities), moderate (with some interference in daily activities), and severe (when daily duties could not be completed). A causal relationship to the study drug was judged by the physician to be not related, unlikely, suspected, or probable.

**Efficacy assessment.** Thick and thin blood smears were collected from all subjects at screening, at weeks 4, 8, 16, and 26 during the prophylactic phase, and at weeks 2 and 12 during the relapse follow-up phase or if symptoms suggestive of malaria developed. Telephone interviews with all subjects were carried out at weeks 18 and 24 during the relapse follow-up phase to determine their general health status. The Giemsa stain-treated blood smears were each read twice for malaria parasites by blinded microscopists at 2 separate institutions. A blood slide was considered negative if an examination of 200 oil immersion thick fields (magnification, ×1,000) showed no parasites. Any discrepant findings were to have been read by a third blinded expert microscopist and were to be used to define a prophylaxis failure if symptoms consistent with malaria were present.

**Statistical analysis.** With at least 450 subjects on tafenoquine and 150 subjects on mefloquine, the study had 94% power to detect a 10% difference in failure rates, assuming an underlying failure rate of 10% in each treatment group (15). Safety and tolerability analyses were performed on data from all subjects who took at least one dose of prophylactic study medication (tafenoquine or mefloquine). Hematological/blood chemistry values for the two groups were compared by a paired Student's *t* test, and 95% confidence intervals (CIs) were calculated. The efficacy analysis was performed for the per-protocol population, which was defined as the subjects who met the inclusion criteria, were protocol compliant, and completed the prophylactic and relapse follow-up phases. Proportions were examined by using a $\chi^2$ test with Yates' correction or by Fisher's exact test. No adjustment was made for multiple testing.

## RESULTS

**Subject population.** In total, 663 subjects were screened, and of these, 9 subjects failed the inclusion criteria. Of the remaining eligible subjects, 492 subjects were randomized to receive tafenoquine, and 162 subjects were randomized to receive mefloquine. Thirty-nine subjects (30 [6.1%] of the 492 tafenoquine subjects and 9 [5.6%] of the 162 mefloquine subjects) violated the protocol or did not complete the study, due to adverse events or other withdrawal reasons (Fig. 2). There were no marked differences between the groups in the proportions of subjects with protocol violations or withdrawals from the study (data not shown). The treatment groups were well balanced with respect to baseline demographic characteristics and history of malaria (Table 1), with the majority of subjects being white, male, and <35 years of age.

**Compliance.** As a result of observed therapy, compliance was high in both treatment groups (100% for the loading dose, 99% for the weekly regimens, and 96% for the follow-up antihypnozoite regimen).

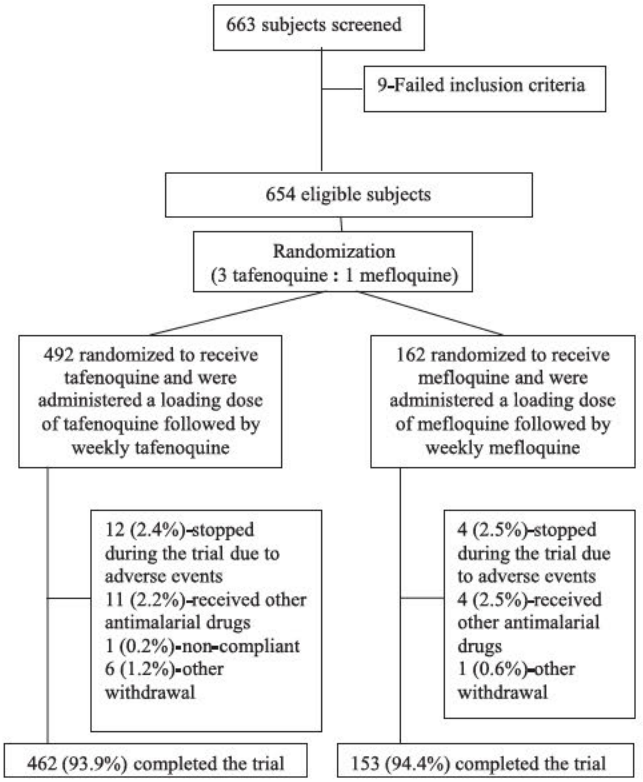


FIG. 2. Flow diagram of subject accountability during the study.

**Routine laboratory tests.** For most laboratory variables, the proportion of subjects with results that fell outside an extended normal range during the prophylactic phase was <5% (data not shown). In addition, the proportions of subjects with clinically significant changes from baseline values were similar across the treatment groups for most laboratory parameters. The parameters that were exceptions were hematocrit, bilirubin, and creatinine.

Decreases in hematocrits were seen in both subjects on tafenoquine and subjects on mefloquine, with up to 98 (20%) of the 492 tafenoquine subjects having a 15% decrease from the baseline at any one visit, compared to 23 (14.4%) of the 162 mefloquine subjects. However, only 2 subjects, both on tafenoquine, had a clinically significant hematocrit value (<85% of the lower limit of normal range) during the study. A higher proportion of tafenoquine subjects was reported to have an increase in bilirubin (>2 μmol/liter from the baseline) at any one visit during the study (10% of tafenoquine subjects versus 3.2% of mefloquine subjects). Of these, only 13 (2.6%) tafenoquine subjects and 1 (0.6%) mefloquine subject had a clinically significant bilirubin value (>150% of the upper limit of normal range) at some point during the study. Serum creatinine increases (>125% baseline value) were seen in both the tafenoquine and mefloquine groups, with an increase in serum creatinine in up to 19% of tafenoquine subjects at any one visit versus 10% of mefloquine subjects. At the follow-up, 6 to 8% of subjects in both groups had creatinine values that were still 25% above the baseline; however, few subjects had values outside the normal range, and none of these values was considered clinically significant.

TABLE 1. Baseline demographic characteristics and previous malarial histories of subjects on tafenoquine and mefloquine for malaria prophylaxis

| Characteristic | Value for subjects who received: | |
|---|---|---|
| | Tafenoquine ($n$ = 492) | Mefloquine ($n$ = 162) |
| No. (%) of subjects | | |
| Gender | | |
| Male | 478 (97.2) | 154 (95.1) |
| Female | 14 (2.8) | 8 (4.9) |
| Age (yr) | | |
| 18–25 | 286 (58.1) | 97 (59.9) |
| 26–35 | 178 (36.2) | 48 (29.6) |
| 36–45 | 27 (5.5) | 16 (9.9) |
| 46–55 | 1 (0.2) | 1 (0.6) |
| Race | | |
| White | 484 (98.4) | 160 (98.8) |
| Aboriginal/Torres Strait Islander | 4 (0.8) | 1 (0.6) |
| Other | 4 (0.8) | 1 (0.6) |
| Previous history of malaria | 15 (3.0) | 4 (2.5) |
| Having malaria attacks in 6 mo prior to deployment | 9 (1.8) | 1 (0.6) |
| Age | | |
| Mean (SD) | 25.4 (5.3) | 26.0 (6.5) |
| Range | 18–47 | 18–51 |
| Weight (kg) | | |
| Mean (SD) | 80.9 (11.9) | 81.3 (12.2) |
| Range | 50–135 | 53–135 |
| Height (cm) | | |
| Mean (SD) | 177.8 (7.0) | 177.1 (6.7) |
| Range | 155–198 | 157–192 |

**Safety evaluation subgroup.** The ophthalmic assessments in the subgroup of subjects on tafenoquine and mefloquine are summarized in Table 2. At the end of prophylaxis, vortex keratopathy (corneal deposits) was found in 69 (93.2%) of 74

TABLE 2. Ophthalmic assessments of a subgroup of subjects on tafenoquine or mefloquine

| Activity | Screening | Posttreatment assessment |
|---|---|---|
| Visual field tests | Amsler grid | Amsler grid<br>Humphrey perimetry |
| Visual acuity | Snellen chart | Snellen chart |
| Color vision | Ishihara test | Ishihara test<br>Standard pseudoisochromatic plates part 2<br>Farnsworth-Munsell 100 hue test |
| Physical examination | Fundoscopy<br>Corneal examination | Fundoscopy<br>Corneal examination<br>Digital retinal photography<br>Digital corneal photography<br>Fundus fluorescein angiogram[a] |

[a] Small number of subjects with possible retinal findings only.

TABLE 3. Adverse events occurring in >5% of subjects on tafenoquine or mefloquine (prophylactic phase)[a]

| Adverse event | No. (%) of subjects by AE severity and treatment group | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mild | | Moderate | | Severe | | Total | |
| | Tafenoquine | Mefloquine | Tafenoquine | Mefloquine | Tafenoquine | Mefloquine | Tafenoquine | Mefloquine |
| At least one AE | 431 (88) | 140 (86) | 194 (39) | 46 (28) | 18 (4) | 3 (2) | 454 (92) | 143 (88) |
| Gastrointestinal | | | | | | | | |
| Gastroenteritis | 109 (22) | 36 (22) | 80 (16) | 17 (11) | 6 (1) | 0 | 182 (37) | 51 (32) |
| Diarrhea | 77 (16) | 28 (17) | 0 | 2 (1) | 1 (<1) | 0 | 77 (16) | 30 (19) |
| Nausea | 27 (6) | 13 (8) | 1 (<1) | 0 | 0 | 0 | 28 (6) | 13 (8) |
| Abdominal pain | 19 (4) | 11 (7) | 5 (1) | 3 (2) | 1 (<1) | 0 | 24 (5) | 13 (8) |
| Vomiting | 19 (4) | 8 (5) | 2 (<1) | 1 (<1) | 0 | 0 | 21 (4) | 8 (5) |
| Musculoskeletal | | | | | | | | |
| Injury | 149 (30) | 46 (28) | 45 (9) | 4 (3) | 3 (<1) | 2 (1) | 178 (36) | 49 (30) |
| Back pain | 65 (13) | 24 (15) | 12 (2) | 2 (1) | 0 | 0 | 74 (15) | 26 (16) |
| Arthralgia | 52 (11) | 17 (11) | 9 (2) | 1 (<1) | 0 | 0 | 55 (11) | 18 (11) |
| Respiratory | | | | | | | | |
| URTI | 97 (20) | 30 (19) | 6 (1) | 2 (1) | 0 | 0 | 101 (21) | 32 (20) |
| Pharyngitis | 24 (5) | 2 (1) | 2 (<1) | 1 (<1) | 0 | 0 | 25 (5) | 3 (2) |
| Dermatological | | | | | | | | |
| Rash | 70 (14) | 20 (12) | 1 (<1) | 1 (<1) | 0 | 0 | 70 (14) | 21 (13) |
| Fungal dermatitis | 43 (9) | 8 (5) | 1 (<1) | 0 | 0 | 0 | 44 (9) | 8 (5) |
| Headache (constitutional AE) | 59 (12) | 18 (11) | 2 (<1) | 2 (1) | 0 | 0 | 61 (12) | 20 (12) |
| Viral infection | 23 (5) | 7 (4) | 16 (3) | 6 (4) | 1 (<1) | 0 | 39 (8) | 13 (8) |

[a] In total, there were 492 tafenoquine subjects and 162 mefloquine subjects. AE, adverse event; URTI, upper respiratory tract infection.

tafenoquine subjects but was absent in the 21 mefloquine subjects (Table 2). These changes were not associated with any visual disturbances and there were no differences between the groups in visual acuity, Amsler grid score, or Ishihara (color vision) score. All subjects with vortex keratopathy were followed up until resolution, with the incidence reducing to 39% at 3 months and 10% at 6 months; there was complete resolution by all subjects by 1 year. Based on the initial findings, fundoscopic examinations were carried out on 86 subjects at the 3-month postprophylaxis follow-up. Abnormalities (e.g., granularity/pigmentation of retinal pigment epithelium or hard drusen) were noted for 27 (39.1%) of 69 tafenoquine subjects and 4 (23.5%) of 17 mefloquine subjects. Retinal fluoroscein angiograms were performed on 14 tafenoquine subjects and 1 mefloquine subject for whom possible retinal findings had been observed. Of these, 4 (28.6%) tafenoquine subjects and 1 (100%) mefloquine subject were considered possibly abnormal. However, review by an expert ophthalmology review board concluded that the retinal findings may well have been normal variations and that there was no evidence to support drug-related visual disturbances. It should be noted that fundoscopic examination of the retina at follow-up was not blinded, because the examination was carried out with the knowledge that corneal deposits were present and no baseline data were available for comparison.

In addition to undergoing phospholipidosis assessments, the safety subgroup also underwent methemoglobin assessment and electrocardiograms for assessment of QT interval. Mean methemoglobin levels increased by 1.8% in the tafenoquine group and by 0.1% in the mefloquine group at the end of prophylaxis, but by week 12 of follow-up, the increase in methemoglobin had resolved. In the tafenoquine group, there was a small reduction in the mean QT interval (difference of −4.5 ms; 95% CI, −9.7 to 0.7 ms), whereas a small increase in the interval was seen in the mefloquine group (difference of 1.6 ms; 95% CI, −12.1 to 15.4 ms) at the end of prophylaxis. There were no subjects for which there was a clinically dangerous prolongation of the QT interval. None of the safety findings impacted participants' well-being or was considered clinically significant.

**Tolerability.** During the prophylactic phase, 454 (91.9%) of 492 tafenoquine subjects and 143 (88.3%) of 162 mefloquine subjects reported at least one adverse event. The most common adverse events (occurring in >5% of subjects) are summarized in Table 3. There was no significant difference between the 2 treatment groups in the number or type of adverse events, with the most common events being gastroenteritis and injury, which occurred in >30% of subjects in both treatment groups. The majority of adverse events were mild or moderate in severity. In total, there were 21 severe adverse events (18 [4%] tafenoquine subjects and 3 [2%] mefloquine subjects). The most common severe events were gastroenteritis (6 [1.2%] tafenoquine subjects and 0 mefloquine subjects) and injury (3 [0.6%] tafenoquine subjects and 2 [1.2%] mefloquine subjects). During the relapse follow-up phase, 203 (41.3%) tafenoquine/placebo subjects and 53 (33.9%) mefloquine/primaquine subjects reported adverse events; however, there was no notable difference between the treatment groups in the incidence or nature of events.

In total, 64 (13.0%) tafenoquine subjects and 23 (14.2%)

TABLE 4. Neuropsychiatric events in subjects on tafeoquine or mefloquine (prophylactic phase)[a]

| Adverse event | No. (%) of subjects by AE severity and treatment group | | | | | |
|---|---|---|---|---|---|---|
| | Mild | | Moderate | | Total | |
| | Tafenoquine | Mefloquine | Tafenoquine | Mefloquine | Tafenoquine | Mefloquine |
| Vertigo | 22 (5) | 7 (4) | 0 | 1 (<1) | 22 (5) | 8 (5) |
| Somnolence | 12 (2) | 6 (4) | 0 | 0 | 12 (2) | 6 (4) |
| Abnormal dreams | 7 (1) | 2 (1) | 0 | 0 | 7 (1) | 2 (1) |
| Dizziness | 5 (1) | 2 (1) | 0 | 0 | 5 (1) | 2 (1) |
| Insomnia | 4 (<1) | 3 (2) | 1 (<1) | 0 | 5 (1) | 3 (2) |
| Abnormal coordination | 2 (<1) | 1 (<1) | 0 | 0 | 2 (<1) | 1 (<1) |
| Anxiety | 2 (<1) | 0 | 0 | 0 | 2 (<1) | 0 |
| Agitation | 2 (<1) | 0 | 0 | 0 | 2 (<1) | 0 |
| Euphoria | 2 (<1) | 0 | 0 | 0 | 2 (<1) | 0 |
| Tremor | 2 (<1) | 0 | 0 | 0 | 2 (<1) | 0 |
| Depression | 0 | 0 | 1 (<1) | 1 (<1) | 1 (<1) | 1 (<1) |
| Paroniria | 1 (<1) | 0 | 0 | 0 | 1 (<1) | 0 |
| Amnesia | 1 (<1) | 0 | 0 | 0 | 1 (<1) | 0 |

[a] In total, there were 492 tafenoquine subjects and 162 mefloquine subjects. There were no severe adverse events (AEs) of this type.

mefloquine subjects reported neuropsychiatric adverse events, the most common being vertigo, dizziness and various sleep disorders (Table 4). There was no significant difference between the treatment groups in the incidence and type of neuropsychiatric events, and all were reported as mild or moderate.

Fifteen subjects withdrew from the study as a result of adverse events (12 [2.4%] tafenoquine subjects and 3 [1.9%] mefloquine subjects). Four tafenoquine subjects sustained injuries requiring evacuation from the study area, while 2 experienced arthralgia (1 subject on each drug). Three tafenoquine subjects withdrew for possible treatment-related adverse events, namely, abdominal pain (severe), depression (moderate), and hyperesthesia (moderate). The incidences of severe adverse events in the 2 groups were comparable (18 [3.7%] tafenoquine subjects and 5 [3.1%] mefloquine subjects).

In total, during the prophylactic phase, 66 (13.4%) tafenoquine subjects and 19 (11.7%) mefloquine subjects had adverse events with a suspected/probable relationship to treatment (Table 5). There were no significant differences between the treatment groups in the incidence or nature of treatment-related adverse events during the prophylactic phase. Only 1 subject on tafenoquine reported a severe adverse event (diarrhea and abdominal pain) suspected to be related to treatment.

TABLE 5. Table of adverse events attributed as related to study drug during prophylactic phase in the safety population[a]

| Adverse event | No. (%) of patients in treatment group | |
|---|---|---|
| | Tafenoquine ($n$ = 492) | Mefloquine ($n$ = 162) |
| At least one AE | 66 (13.4) | 19 (11.7) |
| Nausea | 14 (2.8) | 4 (2.5) |
| Vertigo | 10 (2.0) | 2 (1.2) |
| Diarrhea | 9 (1.8) | 3 (1.9) |
| Abdominal pain | 7 (1.4) | 2 (1.2) |
| Abnormal dreaming | 6 (1.2) | 1 (0.6) |
| Somnolence | 6 (1.2) | 1 (0.6) |
| Headache | 3 (0.6) | 2 (1.2) |
| Insomnia | 3 (0.6) | 2 (1.2) |

[a] Events occurring in >1% of subjects are shown. AE, adverse event.

**Efficacy.** No symptomatic malarial infections occurred during the prophylactic phase in either treatment group. Smears collected from symptomatic subjects and during routine screening for malaria diagnosis were all negative. There were 4 cases (0.9%) of malarial infection in the tafenoquine group and a single case (0.7%) in the mefloquine group during the relapse follow-up phase (95% CI, −1.32 to 1.74; $P$ = 1.0). All cases corresponded to *P. vivax* infection, which occurred between 16 and 20 weeks following the return from East Timor.

## DISCUSSION

This phase III study describes the safety and tolerability of tafenoquine administered for malaria prevention in a nonimmune population of predominately young Caucasian males. Both tafenoquine and mefloquine were well tolerated. There were no clinically significant differences between hematological and blood chemistry results for the 2 treatment groups.

Assessment for phospholipidosis and its effects in a subgroup of 98 subjects showed at the end of the prophylactic phase a high incidence (93.2%) of mild vortex keratopathy (corneal deposits) in the tafenoquine group. Based on these findings, an independent expert ophthalmology board was asked to review the data. It concluded that the corneal changes were benign, fully reversible, and similar to those seen with several other drugs, including chloroquine, for which it is not considered to be a contraindication for continuous use (1). It also advised us that vision had not been impaired in any subject. A lack of baseline retinal photography data meant that the relevance of retinal findings could not be ascertained, but they reflected normal variability. Further assessment of the eye changes observed with tafenoquine will need to be undertaken to determine with certainty the overall significance of the observed changes and to clarify the retinal issues raised during the review.

As would be expected in a long-term study, the incidence of adverse events was high, with 92% of tafenoquine subjects and 88% of mefloquine subjects reporting one or more adverse events during the 6 months of prophylaxis. The majority of these events was mild or moderate in severity, and the events

were typical of the type of events expected in a population of soldiers on active duty (e.g., injury or gastroenteritis). The number of withdrawals from the study was low for a long-term study, also reflecting the nature of the study population. There were no significant differences in the occurrence of treatment-related adverse events, including gastrointestinal and neuropsychiatric disturbances between the 2 treatment groups.

Limited comparative data on the tolerability of tafenoquine used for prophylaxis are available. In adult black Kenyans, the incidences of adverse events for subjects on placebo and on weekly 200 mg tafenoquine for 13 weeks were similar (21). Relative to our findings, the study of the Kenyans reported a higher incidence of headache (24% versus 12.4%) but lower incidences of diarrhea (7% versus 15.7%) and rashes (4% versus 14.2%) with the same maintenance dose. However, such comparisons are difficult to make when the subject populations differ so markedly in ethnicity, nutritional status, culture, employment, and tolerance to medication.

Mefloquine was well tolerated by the Australian soldiers, which is in accordance with the results of other randomized, double-blind studies of military populations (2, 6, 17). No soldiers on mefloquine withdrew from the study due to treatment-related adverse events, and no more than 2% of the soldiers on either tafenoquine or mefloquine experienced drug-associated neuropsychiatric disturbances. Severe neuropsychiatric adverse events in European travelers on mefloquine have been reported (18, 20), but such events were not observed in the present study. Neuropsychiatric adverse events related to mefloquine use are reported to be more common in females (20), and the somewhat atypical distribution of participants in this study should be considered when generalizing these findings.

Without a placebo control, the exposure to malaria experienced by the Australian soldiers could not be directly estimated. As an indication of the malaria exposure that the soldiers probably encountered, 2 malaria prevalence surveys were conducted (January 2001 and April 2001) in 7 East Timorese villages (about 200 residents in each village), all within 1 km of where the soldiers were located (3). The surveys showed that malaria was present in 6 of the 7 locations, with point prevalence rates ranging from 0 to 35.3% (*P. falciparum*, 0 to 14.4%; *P. vivax*, 0 to 16%). In addition to this evidence, several studies have confirmed a high incidence of malaria in East Timor (8, 11–12, 14, 19). While these studies are not conclusive proof that subjects in the present study were exposed to malaria, it is highly likely that the soldiers were exposed to both *P. falciparum* and *P. vivax* malaria. Because no prophylactic failures occurred during the treatment phase in East Timor, both treatments appeared to be effective in suppressing malaria infections. During the 6-month relapse follow-up period, 4 (0.9%) subjects on tafenoquine/placebo and 1 (0.7%) subject on mefloquine/primaquine developed *P. vivax* infections. These findings indicate that tafenoquine and primaquine are equally effective in preventing *P. vivax* relapse when primaquine compliance is monitored and confirm the results of previous studies in Papua New Guinea (16) and East Timor (7). Although the relapse rates for primaquine and tafenoquine appear to be similar, tafenoquine offers a major advantage in that there is no need to take additional medication after leaving the endemic area if tafenoquine is used for prophylaxis.

In summary, tafenoquine at 200 mg weekly is safe and well tolerated in nonimmune Caucasian subjects following 6 months of prophylaxis. Although mild vortex keratopathy was seen in the subjects on tafenoquine, this was benign and fully reversible. The most frequently recorded treatment-related adverse events for both tafenoquine and mefloquine were gastrointestinal disturbances, and these tended to be mild or moderate. Both treatments fully suppressed malarial infections during prophylaxis, and less than 1% of subjects developed postexposure malaria after either completion of tafenoquine prophylaxis or primaquine treatment. Tafenoquine is an effective alternative weekly antimalarial that can be used without the need for further medication after leaving an endemic area.

## ACKNOWLEDGMENTS

Tafenoquine Study Team members included Karl Rieckmann, Bob Cooper, Stephen Frances, Michael Reid, Alyson Auliff, Bruce Russell, Stephen McLeod-Robertson, John Staley, Kerryn Rowcliffe, John Ross, and Brian Potter from the Australian Army Malaria Institute, Keith Barker and Dominic Galvin from GlaxoSmithKline Research & Development Limited, and Ann Aultman from the U.S. Army Medical Materiel Development Activity.

We thank John Calagari, Stephen Ferndale, Damien Wood, and officers and soldiers of the 1st Battalion Group, Royal Australian Regiment, East Timor, who participated in the study for their support and cooperation. We are grateful to G. Dennis Shanks and Bob Cooper for commenting on the manuscript.

Financial support was from the U.S. Army Medical Materiel Development Activity, GlaxoSmithKline Research & Development Limited, and the Australian Defence Force.

P.P. and C.K. are employees of GlaxoSmithKline Research & Development Limited. For all other authors, there are no conflicts.

The opinions expressed are ours and do not necessarily reflect those of the Joint Health Command, Australian Defence Force, the U.S. Army, or any extant defense force policy.

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**Attachment 4: Charles-2007b**

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2007, p. 2709–2715 Vol. 51, No. 8
0066-4804/07/$08.00+0 doi:10.1128/AAC.01183-06


# Population Pharmacokinetics of Tafenoquine during Malaria Prophylaxis in Healthy Subjects[▽]

Bruce G. Charles,[1]* Ann K. Miller,[2] Peter E. Nasveld,[3] Mark G. Reid,[3]
Ivor E. Harris,[3] and Michael D. Edstein[3]

*School of Pharmacy, The University of Queensland, Brisbane, Queensland, Australia[1]; GlaxoSmithKline, Clinical Pharmacokinetics/Modeling and Simulation, King of Prussia, Pennsylvania[2]; and Australian Army Malaria Institute, Brisbane, Queensland, Australia[3]*

Received 21 September 2006/Returned for modification 19 February 2007/Accepted 8 May 2007

**The population pharmacokinetics of tafenoquine were studied in Australian soldiers taking tafenoquine for malarial prophylaxis. The subjects (476 males and 14 females) received a loading dose of 200 mg tafenoquine base daily for 3 days, followed by a weekly dose of 200 mg tafenoquine for 6 months. Blood samples were collected from each subject after the last loading dose and then at weeks 4, 8, and 16. Plasma tafenoquine concentrations were determined by liquid chromatography-tandem mass spectrometry. Population modeling was performed with NONMEM, using a one-compartment model. Typical values of the first-order absorption rate constant ($K_a$), clearance (CL/*F*), and volume of distribution (*V*/*F*) were 0.243 $h^{-1}$, 0.056 liters/h/kg, and 23.7 liters/kg, respectively. The intersubject variability (coefficient of variation) in CL/*F* and *V*/*F* was 18% and 22%, respectively. The interoccasion variability in CL/*F* was 18%, and the mean elimination half-life was 12.7 days. A positive linear association between weight and both CL/*F* and *V*/*F* was found, but this had insufficient impact to warrant dosage adjustments. Model robustness was assessed by a nonparametric bootstrap (200 samples). A degenerate visual predictive check indicated that the raw data mirrored the postdose concentration-time profiles simulated ($n$ = 1,000) from the final model. Individual pharmacokinetic estimates for tafenoquine did not predict the prophylactic outcome with the drug for four subjects who relapsed with *Plasmodium vivax* malaria, as they had similar pharmacokinetics to those who were free of malaria infection. No obvious pattern existed between the plasma tafenoquine concentration and the pharmacokinetic parameter values for subjects with and without drug-associated moderate or severe adverse events. This validated population pharmacokinetic model satisfactorily describes the disposition and variability of tafenoquine used for long-term malaria prophylaxis in a large cohort of soldiers on military deployment.**

Tafenoquine, a synthetic analog of primaquine, is a new 8-aminoquinoline antimalarial drug being codeveloped by GlaxoSmithKline Pharmaceuticals and the Walter Reed Army Institute of Research (1). Clinical trials have shown tafenoquine to be an effective antimalarial agent that has been generally well tolerated, with transient gastrointestinal discomfort being the most commonly reported adverse event (8, 10, 11, 13, 15). To date, it has been evaluated in more than 2,000 subjects in six phase II clinical studies. Since tafenoquine acts on all malaria stages, it has potential in the chemoprophylaxis of malaria, in radical cure/relapse prevention of *Plasmodium vivax* infections, and as a transmission-blocking agent (gametocytocidal activity).

The pharmacokinetics of tafenoquine in humans have been derived from studies after oral administration, as no parenteral formulation exists. Tafenoquine is slowly absorbed following oral administration, with maximum plasma concentrations observed at about 12-h postdose in fasted subjects (1). Plasma tafenoquine concentration-time data have been described by a one-compartment model with first-order absorption and elimination (1, 2). The elimination half-life of tafenoquine is about 2 weeks. It is extensively distributed to tissues, with a large volume of distribution and a low clearance, but data on the metabolism of tafenoquine in humans are limited. Although animal studies have shown that absorbed tafenoquine secreted via the bile is found predominantly in the form of metabolites, which accounted for the majority of the drug-related material eliminated in the urine and feces, unchanged tafenoquine was the only drug-related component detected in human plasma by high-performance liquid chromatography–mass spectrometry (HPLC-MS) and HPLC with fluorescence detection (GlaxoSmithKline Pharmaceuticals, unpublished data).

Tafenoquine is highly effective in preventing malaria infections following a weekly dose of either 200 mg or 400 mg for 13 weeks (13) or 400 mg monthly for 6 months (15). In developing the dosage regimen for malaria prophylaxis, a phase III study was conducted to assess the safety, tolerability, and effectiveness of tafenoquine in Australian soldiers deployed for 6 months on peacekeeping duties to an area where malaria is endemic. The full clinical results of that study will be published elsewhere. The soldiers were on a weekly regimen of 200 mg of tafenoquine, and blood samples were collected on four occasions for drug analysis. No malaria infections occurred during the prophylactic phase, but four soldiers were diagnosed with *P. vivax* infection after returning to Australia.

The primary aim of the present study was to use these data to develop a population pharmacokinetic model for tafenoquine and to estimate the disposition of this drug in the target population of soldiers on military deployment. Secondary aims

* Corresponding author. Mailing address: The University of Queensland, Brisbane, QLD, Australia. Phone: 61 7 336 53194. Fax: 61 7 336 51688. E-mail: B.Charles@pharmacy.uq.edu.au.
▽ Published ahead of print on 21 May 2007.

were to determine whether individual pharmacokinetic estimates for tafenoquine would predict prophylactic outcomes and to investigate if there was any relationship between tafenoquine concentrations and drug-associated adverse events.

## MATERIALS AND METHODS

**Study design and subjects.** The clinical trial was designed as a prospective, randomized, double-blind comparative study of the safety, tolerability, and effectiveness of tafenoquine and mefloquine in Australian soldiers on weekly malaria prophylaxis. The subjects were deployed on peacekeeping duties to East Timor for 6 months. They all were judged to be healthy by a complete medical history, physical examination, and normal hematological and biochemical values. They had to be glucose-6-phosphate dehydrogenase normal and willing and able to give written informed consent and comply with the study protocol. Females were excluded if they were pregnant, lactating, or unwilling/unable to comply with recognized contraceptive methods. The study protocol received prior written approval by the Australian Defence Human Research Ethics Committee and the U.S. Army Human Subject Research Review Board.

**Tafenoquine dosing regimen.** Following a loading dose regimen of 200 mg tafenoquine base daily for three consecutive days, the subjects then received an oral weekly maintenance dose of 200 mg tafenoquine over approximately 6 months. An opaque Swedish Orange size 1 hard gelatin capsule (Capsugel) containing tafenoquine at 200 mg (pure free base) was used as the dosage form. Subjects were directed to take their tafenoquine with food (breakfast or dinner) at the same time each week. Dosage administration was observed and recorded for each subject.

**Pharmacokinetic sampling.** The sampling design was guided by the results from a previous smaller study of Thai soldiers (2) and also by logistical issues of the field operations. Blood samples were collected at prerandomized times after the last loading dose and then at prerandomized times at weeks 4, 8, and 16. Samples were collected on predetermined days after dosing on each of the assessment weeks. The predetermined days included day 1 (early postdose; absorption phase), days 3 and 5 (72 to 120 h postdose), and day 7 (predose; trough phase). For example, on week 4, one group of soldiers (about 125 subjects) was bled on day 1, one group was bled on day 3, one group was bled on day 5, and one group was bled on day 7. Thereafter, the groups of soldiers were bled in a cyclical fashion such that at the end of the study each group had been bled on at least one occasion on days 1, 3, 5, and 7. However, the sample for day 2 of the study (1 to 12 h; post-final loading dose) was collected from the study subjects.

Blood (7 ml) was drawn by venipuncture into EDTA tubes and transported on ice bricks to the field laboratory within 3 h of collection. Whole-blood samples were centrifuged at ~1,200 × *g* for 15 min (Sigma, Quantum, Australia), and plasmas were separated and stored in liquid nitrogen (<4 weeks) and then air freighted on dry ice to Quintiles Limited (Edinburgh, United Kingdom) for storage at −70°C until analysis. Tafenoquine was stable under these handling and storage conditions.

**Measurement of tafenoquine.** Plasma tafenoquine concentrations were determined using a validated HPLC method with a triple-quadrupole mass spectrometer. Briefly, plasma (0.05 ml) was spiked with [$^2H_4{}^{15}N$]tafenoquine as a stable-isotope-labeled internal standard, and the protein was precipitated with methanol, followed by centrifugation and then injection of 4 μl of the supernatant fluid onto a reversed-phase HPLC column (4-μm-diameter particles; Genesis $C_{18}$ column; 30 mm × 2.1-mm internal diameter) held at 40°C. The mobile phase was methanol-1 mM ammonium acetate buffer, pH 2.5 (70:30 [vol/vol]), pumped at 1 ml/min and split approximately 1 to 4 into the TurboIonSpray interface of a PE-Sciex API 3000 LC/MS/MS system (Applied Biosystems) operated in positive-ion multiple-reaction monitoring mode. A chromatographic cycle time of 1.3 min was used, with the peaks being eluted at 0.4 min. The multiple-reaction monitoring transitions monitored were 464 to 379 *m/z* for tafenoquine and 469 to 379 *m/z* for stable-isotope-labeled tafenoquine. Linear responses in analyte/internal standard peak area ratios were observed for tafenoquine concentrations ranging from 5 to 500 ng/ml, using a weighted ($1/C^2$) linear regression. Results of a three-run validation gave an intra-assay imprecision (coefficient of variation [CV%]) of <5.8% and an interassay imprecision of <7.3%, with an inaccuracy of 1.5 to 4.4%. The lower limit of quantification of the method was 5 ng/ml.

**Population pharmacokinetic modeling.** The population pharmacokinetics of tafenoquine were determined in double precision by using NONMEM (version 5, level 1.1; Globomax LLC, Hanover, MD) in conjunction with a G77 compiler. A one-compartment model with first-order absorption and elimination was fitted to the data, using first-order conditional estimation with interaction. An initial analysis was conducted by permitting NONMEM to estimate the base model parameters (i.e., no covariates). The influence of mean-centered continuous variables, i.e., age, current weight, and estimated creatinine clearance ($CL_{CR}$ [by the Cockcroft-Gault method]), and the categorical variables, i.e., sex or evidence of phospholipidosis, was assessed by adding these to the base model in turn and noting the change in the objective function value (OFV). The inclusion of a covariate improved the fit of the data to the model if there was a decrease in the OFV. The difference between a pair of OFV values when a covariate was included (full model) and then excluded (reduced model) was tested for significance ($\alpha = 0.01$), using the chi-square statistic with 1 degree of freedom ($\chi^2_{1,0.01} = 6.6$).

The interindividual variability (IIV) was modeled, assuming a log-normal distribution, as follows:

$$CL/F_{ij} = CL/F \cdot e^{(\eta_{i,CL/F} + K_{j,CL/F})}$$

$$V/F_{ij} = V/F \cdot e^{(\eta_{i,V/F} + K_{j,V/F})}$$

$$K_{a_{ij}} = K_a \cdot e^{(\eta_{i,K_a} + K_{j,K_a})}$$

where $CL/F_{jj}$, $V/F_{ij}$, and $K_{aij}$ represent the true but unknown values of the parameters for the *i*th subject on the *j*th occasion about the typical respective population values $CL/F$, $V/F$, and $K_a$. The parameters $\eta_{i,CL/F}$, $\eta_{i,V/F}$, and $\eta_{i,K_a}$ are random variables distributed with means of 0 and respective variances of $\omega^2_{CL/F}$, $\omega^2_{V/F}$, and $\omega^2_{K_a}$. *K* (kappa) is a random variable representing the variability of a given pharmacokinetic parameter value on different occasions, with an occasion being defined a priori as a dose or sequential doses followed by at least one observation (in this study, there were typically four occasions). The interoccasion variability (IOV) was assumed to be sampled from a normal distribution having a mean of 0 and a variance of $\pi^2$. In modeling the IOV, it was assumed that the variances of each parameter were sampled from the same distribution. The residual unexplained variability (RUV) among observed plasma tafenoquine concentrations and those predicted by the final population model were estimated by a combined proportional plus additive error model, as follows: $C_{ij} = C_{pred,ij}(1 + \varepsilon_{1,ij}) + \varepsilon_{2,ij}$, where $C_{ij}$ is the *i*th observed concentration in the *j*th subject, $C_{pred,ij}$ is the plasma tafenoquine concentration predicted by the pharmacokinetic model, and $\varepsilon_{1,ij}$ and $\varepsilon_{2,ij}$ are randomly distributed variables having mean values of 0 and variances of $\sigma_1{}^2$ and $\sigma_2{}^2$, respectively.

**Model assessment.** The final model was assessed by an inspection of standard diagnostic plots of observed concentration versus population model predicted concentration and separate plots of weighted residual versus model-predicted concentration, elapsed time, subject identification, and screened covariates (3). A degenerate visual predictive check was performed by simulating from the final model 1,000 concentrations at each of 44 sampling times of up to 200 h postdose, at week 1 (after the third loading dose), and then at weeks 4, 8, and 16 during maintenance dosing. The 50th percentile concentration (as an estimator of the population-predicted concentration) and the 5th and 95th percentile concentrations were processed by ActivePerl (v.5.8.4; ActiveState) and then plotted against elapsed time for each of the above four sampling windows. Observed tafenoquine concentrations were superimposed on the plots. Model robustness was assessed by a nonparametric bootstrap, with replacement, of 200 NONMEM runs of the final model, comparing the bootstrapped median parameter values and the percentile bootstrap 90% confidence intervals (4, 5) with the respective values estimated in the final model.

**Adverse events, severity rating, and association with drug.** As part of the clinical phase III trial, adverse events were elicited by an investigator asking the subject a nonleading question, such as "Do you feel differently in any way since starting the new treatment?" A physician assessed the level of relationship of any adverse event on the basis of the subject's response and any temporal association and/or known adverse responses to the drug. The physician graded the severity of adverse events as follows: mild, not affecting daily activities; moderate, causing some interference with daily activities; severe, daily duties could not be completed. Attribution or relationship to tafenoquine was judged by the physician to be not related, unlikely to be related, suspected (reasonable probability) to be related, or probably related.

## RESULTS

**Population characteristics.** The study population consisted of 476 males and 14 females, with a mean (± standard deviation [SD]) age of 25.4 ± 5.3 years (range, 18 to 47 years) and

TABLE 1. Development of structural model for pharmacokinetics of tafenoquine

| Model | Parameterization[d] | ΔOFV[a] |
|---|---|---|
| 1 | $CL/F = \theta_1$; $V/F = \theta_2$; $K_a = \theta_3$ | |
| 2 | $CL/F = \theta_1 \cdot (1 + \theta_4 \cdot \text{age}/25.4)$; $V/F = \theta_2$; $K_a = \theta_3$ | −2 |
| 3 | $CL/F = \theta_1$; $V/F = \theta_2 \cdot (1 + \theta_4 \cdot \text{age}/25.4)$; $K_a = \theta_3$ | −9[b] |
| 4 | $CL/F = \theta_1 \cdot (1 + \theta_4 \cdot CL_{CR}/121)$; $V/F = \theta_2$; $K_a = \theta_3$ | −4 |
| 5 | $CL/F = \theta_1 \cdot \text{PHOS} + \theta_4 \cdot (1 - \text{PHOS})$; $V/F = \theta_2$; $K_a = \theta_3$ | 0 |
| 6 | $CL/F = \theta_1$; $V/F = \theta_2 \cdot \text{PHOS} + \theta_4 \cdot (1 - \text{PHOS})$; $K_a = \theta_3$ | −1[b] |
| 7 | $CL/F = \theta_1 \cdot \text{sex} + \theta_4 \cdot (1 - \text{sex})$; $V/F = \theta_2$; $K_a = \theta_3$ | −3[b] |
| 8 | $CL/F = \theta_1$; $V/F = \theta_2 \cdot \text{sex} + \theta_4 \cdot (1 - \text{sex})$; $K_a = \theta_3$ | −12 |
| 9[c] | $CL/F = \theta_1 \cdot (1 + \theta_4 \cdot \text{WT}/80.9)$; $V/F = \theta_2 \cdot (1 + \theta_5 \cdot \text{WT}/80.9)$; $K_a = \theta_3$ | −39 |
| 10 | $CL/F = \theta_1 \cdot (\text{WT}/70)^{0.75}$; $V/F = \theta_2 \cdot (\text{WT}/70)^{1.0}$; $K_a = \theta_3$ | +37[b] |

[a] ΔOFV, change in OFV from that of model 1 (OFV = 22,177).
[b] Rounding errors occurred during fitting.
[c] Final model.
[d] WT/80.9, body weight (kg) centered on average weight (80.9 kg); age/25.4, age (years) centered on average age (25.4 years); $CL_{CR}/121$, $CL_{CR}$ (ml/min) centered on average $CL_{CR}$ (121 ml/min); PHOS, phospholipidosis (tested in 77 subjects; 1 = phospholipidosis present, 0 = phospholipidosis not present); sex, male = 0 and female = 1.

a mean (± SD) weight of 80.9 ± 11.9 kg (range, 50 to 135 kg). All but eight were of Caucasian background. Of the 490 subjects, 2 subjects provided one blood sample, 3 subjects provided two blood samples, 23 subjects provided three blood samples, and the remaining 462 subjects provided four blood samples, giving a total of 1,925 plasma concentration-time points available for the pharmacokinetic analyses.

**Population pharmacokinetic modeling.** Summary results of the population model-building process are shown in Table 1. The data did not support the inclusion of an absorption lag time in any model. Neither age nor $CL_{CR}$ on $CL/F$ significantly improved the fit, nor did sex or phospholipidosis as indicator variables. Both age and sex effects on $V/F$ produced small but significant decreases in the OFV, of 9 and 12, respectively. Use of an allometric size model scaled to 70 kg for $CL/F$ (power, 0.75) and $V/F$ (power, 1.0) was not supported (OFV = +37). Inclusion of centered linear weight on both $CL/F$ and $V/F$ significantly decreased the OFV, from 22,177 to 22,138. This model predicted that a 1-kg change in weight from the population average value of 80.9 kg would give a commensurate change of 0.0167 liters/h (0.38%) in $CL/F$ and a change of 9.7 liters (0.51%) in $V/F$. The linear, positive influence of weight on both $CL/F$ and $V/F$ is shown in Fig. 1a and b, respectively.

Modeling the covariance between $\omega^2_{CL/F}$ and $\omega^2_{V/F}$ reduced the OFV from 22,265 to 22,248 compared with the corresponding model when $\omega^2_{CL/F}$ and $\omega^2_{V/F}$ were assumed to be independent. Inclusion of the IOV for $CL/F$ reduced the OFV further, to 22,177. However, while the addition of IOV to $V/F$ further reduced the OFV, the value for $\omega^2_{V/F}$ was suspiciously low and the correlation coefficient ($r$) calculated from the diagonal and off-diagonal elements of the variance matrix [$r = \omega^2_{CL/F,V/F}/(\omega^2_{CL/F} \cdot \omega^2_{CL/F})^{0.5}$] was ~1, indicating an inappropriate variance model. The RUV was best modeled by using a combined proportional and additive model, as seen by an increase in the OFV and by numerical difficulties when the additive and proportional models were used separately.

Parameter values for the final population model and the bootstrap validation are shown in Table 2. The estimated time ($T_{max}$) for peak concentration to occur after a dose was 21.4 ± 8.57 h, calculated from each subject's conditional estimates of $K_a$ and $K_e$ by the standard formula $T_{max} = \ln(K_a/K_e)/(K_a - K_e)$ for a one-compartment extravascular model. The observed mean (± SD) peak tafenoquine concentration measured in samples drawn within 5% of the time of the estimated mean population $T_{max}$ (21.4 h) for 42 subjects at weeks 4, 8, and 16 was 321 ± 63 ng/ml. The observed mean (± SD) trough tafenoquine concentration drawn within 5% of the target 168-h-postdose sampling time for 162 subjects at weeks 4, 8, and 16 was 221 ± 57 ng/ml. The typical population $CL/F$ and $V/F$

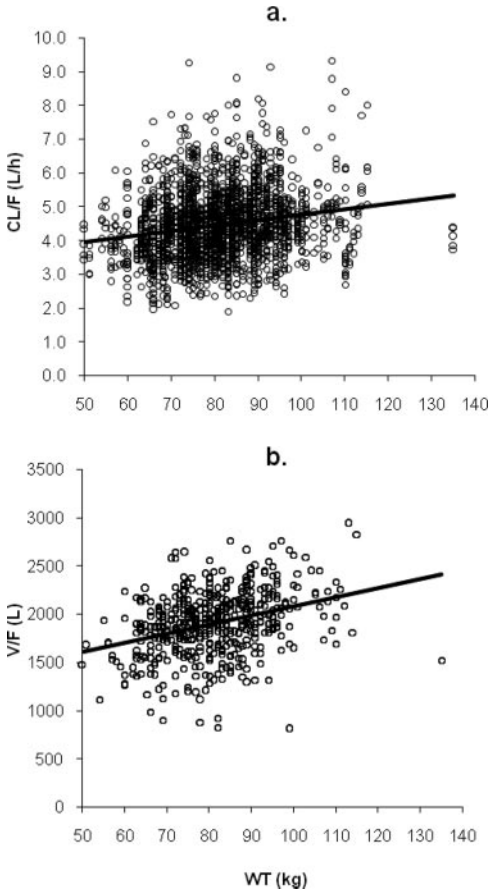


FIG. 1. Relationship of body weight (WT) to individual estimates of (a) $CL/F$ and (b) $V/F$ for tafenoquine.

TABLE 2. Comparison of parameter estimates for the population model with the results of 200 bootstrapped runs

| Parameter and model | Final model value | Bootstrap value ($n$ = 200) (median [90% CI])[b] |
|---|---|---|
| Structural model[a] | | |
| CL/$F$ ($\theta_1$; liters/h) | 3.02 | 3.01 (2.42–3.52) |
| $V/F$ ($\theta_2$; liters) | 1,110 | 1,110 (874–1,382) |
| $K_a$ ($\theta_3$; $h^{-1}$) | 0.243 | 0.245 (0.212–0.280) |
| Weight centered on CL/$F$[c] | 0.448 | 0.447 (0.249–0.816) |
| Weight centered on V/$F$[d] | 0.713 | 0.713 (0.371–1.20) |
| Variance model | | |
| $IIV_{CL/F}$ (CV%) | 18 | 18 (16–20) |
| $IIV_{V/F}$ (CV%) | 22 | 22 (20–25) |
| $IIV_{K_a}$ (CV%) | 76 | 75 (64–85) |
| $IOV_{CL/F}$ (CV%) | 18 | 18 (16–20) |
| RUV (CV%) | 5.9 | 5.9 (4.7–7.4) |
| RUV (ng/ml) | 22.9 | 23.1 (18.7–26.3) |

[a] $CL/F = \theta_1 \cdot (1 + \theta_4 \cdot WT/80.9)$; $V/F = \theta_2 \cdot (1 + \theta_5 \cdot WT/80.9)$; $K_a = \theta_3$.
[b] Percentile bootstrap 90% confidence interval (5th to 95th percentiles).
[c] Linear coefficient ($\theta_4$) for weight centered on CL/$F$.
[d] Linear coefficient ($\theta_5$) for weight centered on $V/F$.

values for all subjects, with a mean weight of 80.9 kg, were 4.37 liters/h and 1,901 liters, respectively. The typical value of $K_a$ over all subjects was 0.243 $h^{-1}$. The IIV about CL/$F$, $V/F$, and $K_a$ was 18%, 22%, and 76%, respectively. The IOV for CL/$F$ was 18%. Mean values per kg for CL/$F$ and $V/F$ calculated from conditional estimates for each subject were 0.056 ± 0.013 liters/h/kg and 23.7 ± 4.5 liters/kg, respectively. The elimination half-life ($t_{1/2}$), derived from the expression $t_{1/2} = (0.693 \cdot V/F)/(CL/F)$ with individual estimates of CL/$F$ and $V/F$, was 12.7 ± 3.0 days.

Routine diagnostic weighted residuals versus population model-predicted values (data not shown) were symmetrically distributed and were mostly within about 3 units of the null ordinate, indicating a good fit of the model to the data. Plots of weighted residuals versus both subject identification and time (data not shown) were distributed symmetrically in a band with no obvious trend and were mostly within approximately 3 units of the null ordinate, indicating that no time-related factor affected the data and that no subject's data contributed to any marked deviation from the model. The bootstrapped median parameter values very closely agreed with the respective values from the final population model (Table 2). The degenerate visual predictive check showed the observed data to be symmetrically distributed about the 50th percentile profile, with approximately 10% of the data distributed outside the 5th- to 95th-percentile boundaries (Fig. 2a, b, c, and d).

**Individual pharmacokinetics of tafenoquine in subjects with malaria and with drug-associated adverse events.** The four subjects who had a relapse after returning to Australia had a mean (± SD) CL/$F$ of 0.060 ± 0.014 liters/h/kg, a $V/F$ of 23.2 ± 8.0 liters/kg, and a $t_{1/2}$ of 11.1 ± 2.3 days, calculated from conditional parameter estimates for each individual.

One or more adverse events with a suspected/probable relationship to tafenoquine were reported by 73 subjects. These were ranked as mild in 67 subjects (91.8%), moderate in 5 subjects (6.8%), and severe in 1 subject (1.4%) and encompassed the following: nausea, abdominal pain, flatulence, vomiting, vertigo, agitation, amnesia, headache, eye abnormality, reflux, dreaming abnormality, insomnia, somnolence, diarrhea, hyperesthesia, tremor, paranoia, headache, anorexia, depression, coordination abnormality, appetite increase, and thirst. Tafenoquine was not withdrawn in any of the 67 mild cases, but it was withdrawn for three subjects who reported either moderate hyperesthesia, abdominal pain, or depression. Assessment for phospholipidosis was carried out in a subgroup of 77 subjects because tafenoquine has cationic amphiphilic characteristics and, therefore, the potential to cause phospholipid accumulation. Table 3 shows adverse events reported in the five moderate cases and one severe case where tafenoquine was suspected to cause the discomfort, together with individual estimates of the pharmacokinetic responses for these subjects. All moderate adverse events were experienced 1 to 24 days after the initiation of tafenoquine, while the single subject with

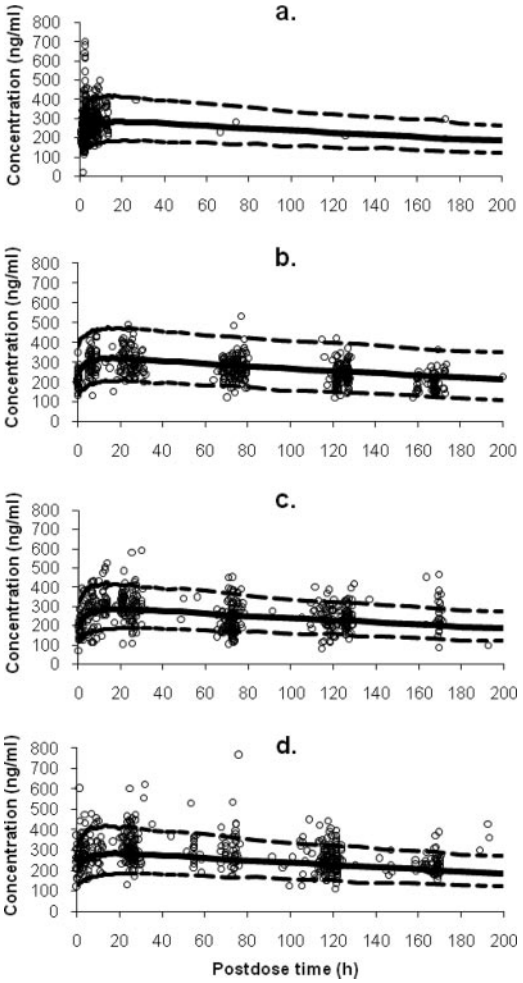


FIG. 2. Degenerate visual predictive check of the final population model for tafenoquine. Plots are shown for plasma tafenoquine concentration versus postdose time in sampling windows of (a) week 1 (post-loading dose), (b) week 4, (c) week 8, and (d) week 16. The population-predicted profile (50th percentile) is shown by the solid line, and the 90% prediction intervals estimated from 1,000 simulated concentrations over 200 h (postdose) are encompassed by the broken lines in each plot.

TABLE 3. Tafenoquine pharmacokinetic data for six subjects reporting at least one adverse effect classified as severe ($n = 1$) or moderate ($n = 5$)

| Adverse event | Treatment duration (days)[a] | Cumulative dose (mg)[b] | Dosing stopped | $C_{last}$ (ng/ml)[c] | CL/*F* (liters/h/kg) | *V*/*F* (liters/kg) | $t_{½}$ (days) |
|---|---|---|---|---|---|---|---|
| Severe event | | | | | | | |
| Diarrhea and/or abdominal pain | 2 | 400 | No | * | 0.059 | 24.4 | 12.0 |
| Moderate events | | | | | | | |
| Insomnia | 1 | 200 | No | * | 0.059 | 23.2 | 11.3 |
| Hyperesthesia | 12 | 800 | Yes | 283 | 0.046 | 20.7 | 13.1 |
| Abdominal pain | 20 | 1,000 | Yes | 253 | 0.053 | 27.8 | 15.1 |
| Depression | 24 | 1,000 | Yes | 275 | 0.061 | 25.1 | 12.0 |
| Vomiting and/or nausea | 3 | 600 | No | 315 | 0.077 | 26.1 | 9.8 |

[a] Number of days from starting dosing until adverse event reported.
[b] Total amount of drug taken before adverse event reported.
[c] Last plasma tafenoquine concentration before adverse event reported. *, adverse event was reported before first plasma sample was drawn.

severe effects reported diarrhea and abdominal pain 2 days after commencing tafenoquine treatment.

## DISCUSSION

This study of the population pharmacokinetics of tafenoquine in 490 Australian soldiers is the largest undertaken by far with this promising new oral antimalarial agent. Previously, a two-stage dose-ranging pharmacokinetic study was performed with 48 healthy adult males (Caucasian [$n = 20$], African-American [$n = 12$], and Hispanic [$n = 16$]) (1), while a subsequent population pharmacokinetic study was reported for 104 Thai soldiers on a monthly prophylactic regimen of tafenoquine (2). The present findings confirm the knowledge of tafenoquine disposition in humans and considerably extend the pharmacokinetic data to a large population of healthy, Caucasian military personnel deployed in field operations.

The apparent *V*/*F* was similar to that reported by Edstein et al. (2), but the systemic CL/*F* was greater (4.37 liters/h versus 3.20 liters/h). The derived typical elimination $t_{1/2}$ of 12.7 days was slightly shorter than the 14 to 16 days reported previously, which may partly reflect the fact that the last samples were drawn at only up to 1 week postdose and therefore the presumed "terminal" phase may have included some components of a distribution phase, but not substantial enough to be supported by a two-compartment model. The mean values for CL/*F* and *V*/*F* obtained by Brueckner et al. (1) for fasted subjects of similar average weight to that from this study were 5.7 liters/h and 2,558 liters, respectively, which are 30% to 35% higher than the present typical values. However, in the current study, the subjects took tafenoquine with food, which reportedly can increase the bioavailability (*F*) by up to one-third (R. P. Brueckner, personal communication), which brings the respective CL/*F* and *V*/*F* values into closer agreement when corrected for *F*. While the extent of tafenoquine absorption may be greater, food could also slow the rate of drug absorption, as evidenced by the typical $K_a$ of 0.243 $h^{-1}$, compared with 0.391 $h^{-1}$ and 0.694 $h^{-1}$ reported by Brueckner et al. (1) and Edstein et al. (2), respectively. As a result, the average $T_{max}$ of 21.4 h was greater than the 8.6 h to 13.8 h reported previously (1, 2), which as well as the influence of food, may reflect continuous absorption along the intestinal tract, perhaps due in part to microprecipitation and redissolution of tafenoquine, which is only slightly water soluble (1). Unpublished data on file (GlaxoSmithKline) for healthy volunteers showed mean (CV%) $T_{max}$ values of 18.6 h (84%) and 26.3 h (126%) under fasted conditions and when administered with a standard high-fat meal, respectively, indicating that the $T_{max}$ and its variability were increased by food. Nonetheless, it should be remembered that $T_{max}$ is a model-dependent parameter in that the true value is likely to be overestimated when a one-compartment model is used compared with that for a two-compartment model. In agreement with previous reports (1, 2), there was marked IIV in the $T_{max}$, reflecting the considerable variability in both $K_a$ and $K_e$, with the latter being estimated from conditional estimates of *V*/*F* and CL/*F* for each subject.

The variability in CL/*F* and *V*/*F* was not excessive, at 18% to 22%, most likely reflecting the uniformity of the military subjects. The variance model supported estimation of the IOV in CL/*F* but not that in *V*/*F* or $K_a$. While Edstein et al. (2) used a proportional (exponential) model for RUV, presently a combined additive-proportional RUV model was supported, which is the preferred model wherever possible, especially where the range of concentration data is as wide as in this study. There was a positive linear association between weight and both CL/*F* and *V*/*F*, but attempts to model these parameters using an allometric size model scaled to 70 kg were not supported by the data, most likely because of the reasonably narrow range of body weights. Although heavier subjects tended to have a slightly greater CL/*F* and *V*/*F*, this would not have any major implications for changes in the way that tafenoquine would be prescribed, at least on the basis of the pharmacokinetic data alone. Using the present steady-state plasma tafenoquine concentrations as the appropriate clinical target, a 20-kg change in weight would require changes in the loading dose and maintenance dose of only about 10% and 7.5%, respectively. Unpublished data (GlaxoSmithKline) indicated that a considerable fraction of a tafenoquine dose may be excreted unchanged, while the clinical data from the trial of which the present study was a part showed that mean serum creatinine concentrations increased 12.1 mmol/liter from baseline until the end of the prophylaxis. However, estimated creatinine clearance explained an insignificant amount of the variability

about CL/*F*. Age explained a small yet significant amount of the variability in both *V*/*F* and CL/*F* but was positively correlated with weight and thus was not considered further.

In assessing performance, model robustness was evaluated via a nonparametric bootstrap, which indicated that randomly selected combinations of data gave very similar results to those obtained with the original data set. In addition, a degenerate visual predictive check showed that the raw data obtained after the third split loading dose and at week 4, 8, and 16 during maintenance dosing mirrored the corresponding profiles obtained from simulations using point estimates of the final model parameter values. This convenient approach has been shown elsewhere (16) to give a good approximation of the full posterior predictive check, in which the simulations are performed using posterior distributions of the parameter values (6), which are difficult to calculate from the NONMEM output. The predictive check showed, firstly, that the structural model was satisfactory by the symmetrical distribution of the raw data about the 50th percentile profile and, secondly, that the variance model was appropriate, with about 10% of the raw data lying outside the 5th and 95th percentiles.

The prophylactic efficacy of tafenoquine is determined by its ability to prevent parasitemia from developing, which is associated with the susceptibility of malaria parasites to tafenoquine concentrations achieved in the target population. Tafenoquine has both causal prophylactic activity against the hepatic stages of the parasite and suppressive activity, which eradicates the erythrocytic stages of the parasite (1). In the present study, no subject developed parasitemia during the 6 months of prophylaxis, but four had a relapse of *P. vivax* infection after returning to Australia. In contrast, one subject in a population of 104 Thai soldiers on 400 mg tafenoquine monthly for 6 months developed vivax malaria during prophylaxis (15). At the time of diagnosis, the Thai soldier had a plasma tafenoquine concentration of 40 ng/ml, which was >5-fold lower than the mean steady-state trough tafenoquine concentration of 221 ng/ml presently recorded. Six Australian soldiers had tafenoquine concentrations of <100 ng/ml at either week 4, 8, or 16. Of those, only one subject had consistently lower tafenoquine concentrations (<120 ng/ml) on the three occasions sampled and therefore may have had a reduced margin of suppressive protection against malaria infection. The Thai soldier who developed parasitemia also had consistently lower tafenoquine concentrations during the prophylactic phase (15). Unlike the Thai soldier, the four Australian soldiers who relapsed had comparable tafenoquine concentrations to subjects who did not have a recurrence of malaria. Although the number of subjects who relapsed was small, the individual estimates of the pharmacokinetic responses for these subjects did not provide a prediction or correlation with tafenoquine's prophylactic efficacy.

There was no apparent correlation between either the pharmacokinetic parameter values predicted for individual subjects or the last tafenoquine concentration measured in subjects reporting moderate or severe adverse events. These findings suggested that plasma tafenoquine concentrations are not the primary predictor of tafenoquine tolerability. This lack of an association between plasma drug concentrations and adverse events has also been seen with another antimalarial agent, mefloquine, which shares similar pharmacokinetic properties with tafenoquine (12) in that both are lipophilic, are slowly absorbed from the gastrointestinal tract, are extensively bound to tissues, and have elimination $t_{1/2}$ values of about 2 weeks (1, 2, 9, 14).

In conclusion, the pharmacokinetic properties of tafenoquine determined in this study support a weekly dosing regimen for prolonged periods. Although body weight influenced CL/*F* and *V*/*F*, it was not considered to have sufficient impact to warrant changing the maintenance or loading dose for any individual from such a population. Nonetheless, dose changes may be warranted for other patients who are markedly overweight or underweight compared with this homogenous group of soldiers. Any dosing requirements for markedly overweight subjects may need special consideration, as reviewed recently (7). Tafenoquine was generally well tolerated. Individual pharmacokinetic parameter estimates for subjects with malaria did not predict prophylactic outcomes, and plasma concentrations at steady state did not appear to be related to the occurrence of adverse events. Since this population was a homogenous group of healthy Australian soldiers of predominantly Caucasian background, additional pharmacokinetic studies may be required for other populations.

## ACKNOWLEDGMENTS

We thank all the Australian soldiers who participated in the phase III study for providing blood samples, the Australian Army Malaria Institute personnel who conducted the clinical trial, and Kym Ward for giving coordination and monitoring support. We are grateful to William Prescott (U.S. Army Medical Materiel Development Activity [USAMMDA]) and Philip Pickford (GlaxoSmithKline) for being the project managers. We acknowledge the support of Thomas Travers in data assessment. We also thank Jürgen Bulitta (IBMP, Nürnberg, Germany) for assistance with the ActivePerl script used for the visual predictive checks.

Financial support for the study was provided by USAMMDA, GlaxoSmithKline, and the Australian Defence Force.

The opinions expressed are those of the authors and do not necessarily reflect those of the Australian Defence Health Service or any extant Australian Defence Force policy.

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