Linking Murine and Human Plasmodium falciparum Challenge Models in a Translational Path for Antimalarial Drug Development

James S. McCarthy, a,b Louise Marquart, a Silvana Sekulski, a Katharine Trenholme, a,b Suzanne Elliott, c Paul Griffin, a,b,c,d Rebecca Rockett, b,b Peter O’Rourke, b Theo Sloots, b,e Iñigo Angulo-Barturen, f Santiago Ferrer, f María Belén Jiménez-Díaz, f María-Santos Martínez, f Rob Hooft van Huijssduijnen, g Stephan Duparc, b Didier Leroy, g Timothy N. C. Wells, g Mark Baker, g* Jörg J. Möhrle* b

QIMR Berghofer Medical Research Institute, Brisbane, Australia; a University of Queensland, Brisbane, Australia; b QPharm Pty. Ltd., Brisbane, Australia; c Mater Health Services, Brisbane, Australia; d Queensland Paediatric Infectious Diseases (QPID), Herston, Australia; e QPharm Pty. Ltd., Brisbane, Australia; f GlaxoSmithKline, Tres Cantos Drug Development Campus, Diseases of the Developing World, Tres Cantos, Spain; g Medicines for Malaria Venture, Geneva, Switzerland

Effective progression of candidate antimalarials is dependent on optimal dosing in clinical studies, which is determined by a sound understanding of pharmacokinetics and pharmacodynamics (PK/PD). Recently, two important translational models for antimalarials have been developed: the NOD/SCID/IL2Rγ−/− (NSG) model, whereby mice are engrafted with noninfected and Plasmodium falciparum-infected human erythrocytes, and the induced blood-stage malaria (IBSM) model in human volunteers. The antimalarial mefloquine was used to directly measure the PK/PD in both models, which were compared to previously published trial data for malaria patients. The clinical part was a single-center, controlled study using a blood-stage Plasmodium falciparum challenge inoculum in volunteers to characterize the effectiveness of mefloquine against early malaria. The study was conducted in three cohorts (n = 8 each) using different doses of mefloquine. The characteristic delay in onset of action of about 24 h was seen in both NSG and IBSM systems. In vivo 50% inhibitory concentrations (IC50s) were estimated at 2.0 μg/ml and 1.8 μg/ml in the NSG and IBSM models, respectively, aligning with 1.8 μg/ml reported previously for patients. In the IBSM model, the parasite reduction ratios were 157 and 195 for the 10- and 15-mg/kg doses, within the range of previously published clinical data for patients but significantly lower than observed in the mouse model. Linking mouse and human challenge models to clinical trial data can accelerate the accrual of critical data on antimalarial drug activity. Such data can guide large clinical trials required for development of urgently needed novel antimalarial combinations. (This trial was registered at the Australian New Zealand Clinical Trials Registry [http://anzctr.org.au] under registration number ACTRN12612000323820.)

The cumulative success rate from candidate selection to registration for the development of anti-infectives is below 5% (benchmark data from the Centres for Medicines Research [2008 to 2011] as cited in reference 1). There are multiple reasons for this low success rate, but a recent survey cites “uncertainties related to dose selection” as the top cause for unsuccessful FDA applications (2). One of the key challenges in moving drugs from animal studies into the clinic, where there is very limited opportunity for testing different regimens, is to provide reasonable prior estimates to inform the design of clinical studies that will have optimal safety and efficacy in patients.

Major technical improvements have been made in the past few years in preclinical development of antimalarial drugs. These improvements have included improved rodent models (3–6) that better replicate human malaria, more comprehensive pharmacometric modeling (7, 8), and better design of proof-of-concept clinical trials (9, 10). Drawbacks of the older “standard” mouse model for malaria include the use of a rodent parasite, Plasmodium berghei, with different growth kinetics and drug response from those of the major human pathogen Plasmodium falciparum and the fact that the parasite resides within murine, instead of human, erythrocytes. Moreover, the older standard preclinical assay known as Peter’s test (11) is not commensurable with clinical studies because its design only allows assessing how parasite growth is delayed by drug treatment and not the parasite killing or clearance under therapeutic treatment (12). The development of the NOD SCID IL-2Rγ−/− (NSG) immunodeficient rodent model of malaria (P. falciparum humanized mouse model, described in reference 13) represents an improved standard for preclinical in vivo studies of blood-stage antimalarial drugs. In this model, NSG mice engrafted with human erythrocytes are susceptible to infection by the human pathogen P. falciparum, which allows a precise analysis of the parasitological response to different exposure levels of drugs in vivo (14). Importantly, the experimental design used in preclinical studies allows production of estimates for all pharmacodynamic (PD) data of interest obtained in clinical trials assessing both parasite clearance and recrudescence. A reliably predictive transition from mice to humans remains crucial and must overcome considerable differences between mu...

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Address correspondence to Jörg J. Möhrle, moehrlej@mmv.org.

* Present address: Mark Baker, Novartis, Nyon, Switzerland.

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rine and human pharmacokinetics (PK) and their overall response to malaria (15). One approach to obtaining more rapid proof of concept in humans is the controlled human malaria infection protocol. Volunteers can be infected following the bite of infected mosquitoes (reviewed in reference 10) or by intravenous (i.v.) injection with cryopreserved sporozoites (16). Such sporozoite-induced malaria is particularly well suited to test vaccines or drugs that act prior to the development of blood-stage malaria, i.e., directly against sporozoites or targeting infected hepatocytes. More recently, an experimentally induced blood-stage malaria (IBSM) model has been developed as a system to test antimalarial drugs with activity against the blood stages of the parasite (9, 17), using parasite-infected human erythrocytes. By closely monitoring parasite multiplication using quantitative PCR (qPCR) (18), rich pharmacodynamic data can be obtained over an effective dynamic range of >1,000-fold at levels of parasitemia several orders of magnitude below those causing clinical malaria. Moreover, time to recrudescence (for up to 4 weeks) can be measured.

To investigate the potential of the combined use of these two models, we evaluated a well-established and metabolically stable malaria drug, mefloquine (19), in both and compared our findings with earlier observations regarding mefloquine in more traditional phase II clinical trials with malaria patients.

MATERIALS AND METHODS

**Induced blood-stage infection and mefloquine treatment in NSG mice.** In vivo therapeutic efficacy against *P. falciparum* was assessed in a 4-day parasite-normalized standard assay (PNSA) previously described (12). NOD SCID IL-2Rγ−/−/− (NSG) mice (Charles River, France) were injected daily with 1 ml of human erythrocytes (50% hematocrit) (20) and approximately 10 days later infected i.v. with 2.0 × 10^7 erythrocytes infected with non-synchronized *P. falciparum* strain 3D70087/N9 obtained from the Plasmodium strain had been generated at GlaxoSmithKline (GSK), Tres Cantos, Spain (20). Parasitemia was measured every 24 h from days 3 to 7 after infection.

Four in vivo studies were performed in the *P. falciparum* humanized mouse model: (i) per os (p.o.) multidose administration once a day for 4 days at 0.2, 1, 3, 10, and 30 mg/kg of body weight (*n* = 3 mice per dose level); (ii) multidose administration p.o. once a day for 4 days at 1, 2.5, 5, 10, 20, 30, 40, 50, 60, and 70 mg/kg (*n* = 1 mouse per dose level); (iii) bolus i.v. single administration at 1 mg/kg in both infected (*P. falciparum*) and non-infected humanized mice (*n* = 3 mice per group); and (iv) single administration p.o. at 20 mg/kg of infected (*P. falciparum*) and non-infected humanized mice (*n* = 3 mice per group). Data from the three experiments were used to model exposure kinetics.

Mefloquine levels in whole blood were measured in serial samples of peripheral blood (25 μl) from every mouse in the in vivo studies. The samples were taken at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156 h. Plasma was separated and samples collected predose and at the following times after dosing: 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, and 144 h. Plasma was separated and frozen at ≤−70°C within 30 min. It was found that mefloquine standards in plasma could be stored for 10 days at 4°C with no change in measured concentration (data not shown). Mefloquine was assayed in 200-μl plasma samples following acetonitrile extraction as described previously (20). For PK assessment, all observations below the lower limit of quantitation were reported as <0.25 ng/ml.

**Statistical methods.** (i) **PK Modeling.** One- and two-compartment PK models were evaluated, with and without a lag in first-order absorption (see supplemental material for details). A one-compartment model had been used previously to model mefloquine exposure (23). A two-compartment model provided the best fit for the NSG mice in our study, with two-compartment PK clearly demonstrated after i.v. administration (see Fig. S2 and S3 in the supplemental material). This model provided a robust characterization of the observed PK data. There was no evidence of an effect of inoculation with parasites on the mefloquine PK (data not shown).

Concentration-versus-time data were analyzed by noncompartment analysis methods using Phoenix software (v.6.3; Pharsight). Additional statistical analysis and modeling were performed with GraphPad Prism (v.5.01; GraphPad Software Inc., San Diego, CA) and NONMEM software (v.7.2; ICON Development Solutions, MD).

(ii) **PRR calculations.** Parasite reduction ratio (PRR) estimates were calculated as previously described (24). In brief, the decay rate (slope coefficient from the log linear decay regression) for each individual was calculated, and then the weighted average slope estimate and corresponding standard error were calculated using an inverse-variance method. All parasitemia values were log_10-transformed, and the mean of the log_10-transformed parasitemia was implemented as a summary parasitemia value per time point per subject. For the historical data sets for which aggregated parasitemia data were available, PRR estimates were calculated using the mean parasitemia for each study and dose of interest over time (hours since treatment).
where \( k_G \) is a first-order parasite growth constant, \( k_D \) is a first-order parasite clearance constant, \( C \) is the drug concentration in the effect compartment, \( IC_{50} \) is the drug concentration corresponding to when parasite clearance is half maximal, and \( H \) is the Hill coefficient, determining the steepness of the drug concentration-parasite clearance relationship.

**Ethical considerations.** The clinical protocol and associated documents were reviewed and approved by the Queensland Institute of Medical Research Berghofer Human Research Ethics Committee. The trial was registered at anzctr.org.au (registration number ACTRN12612000323820). All the animal experiments performed at GSK were approved by the DDW Ethical Committee on Animal Research, performed at the DDW Laboratory Animal Science facilities accredited by AAALAC, and conducted in accordance with European Directive 86/609/EEC and the GSK policy on the care, welfare, and treatment of animals (34). The human biological samples used at GSK were sourced ethically, and their research use was in accord with the terms of the informed consent.

**RESULTS**

**PK/PD of mefloquine in the P. falciparum-infected NOD SCID mouse model.** Mefloquine exposure (Fig. 1A) was dose dependent, with a half-life significantly longer than the 23-h duration of observation. Parasitemia increased in a log linear manner in infected control animals (see Fig. S1 in the supplemental material), with an average \( \log_{10} \) growth rate of 0.013 h\(^{-1}\) (coefficient of variation [CV], 10%). In mefloquine-treated animals (Fig. 1B and C), a dose-dependent response with a lag in onset of effect was observed. When the PK (see Fig. S2) data were analyzed simultaneously with the parasite clearance data (see Fig. S3) using a nonlinear mixed-effects approach, the maximum \( \log_{10} \) parasite reduction ratio (log\(_{10}\) PRR\(_{Max}\)) was calculated as 3.3 (see Tables 1 and 2 for all PK/PD parameters).

**Testing of mefloquine in the IBSM clinical study.** Twenty-two volunteers (11 male and 21 Caucasian) were enrolled in a clinical trial to evaluate the PK/PD relationship of mefloquine administered in three doses (5 mg/kg \([n = 6]\), 10 mg/kg \([n = 8]\), and 15 mg/kg \([n = 8]\)). The average age of volunteers was 25 (±4) years, with a weight of 72 (±11) kg, a height of 175 (±11) cm, and body mass index (BMI) of 23 (±2). The only serious adverse event (recurrent tonsillitis) was considered unrelated to the clinical trial treatment. The mild and moderate adverse events were either main-

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**FIG 1** Mefloquine exposure and parasitemia in *P. falciparum*-infected NOD SCID IL-2R\(^{-/-}\) (NSG) mice. (A) Mefloquine concentrations in blood observed over time for various oral dosings. The lines represent the PK/PD model predictions (see the text). B but with dosing at 0.2, 1, 3, 10, and 30 mg/kg (\(n = 3\)). RESULTS for the 0.2-, 1-, and 3-mg/kg dosings overlap. The observed data points were overlaid graphically on the data points in Fig. S2 in the supplemental material for other model parameters.

**TABLE 1** Mefloquine PK key parameters in NOD SCID IL-2R\(^{-/-}\) mice

| Parameter | Estimate | Relative SE | 95% confidence interval |
|-----------|----------|-------------|------------------------|
| Theta     |          |             |                        |
| Ka        | 0.34/h   | 26          | 0.17–0.51              |
| V         | 2,810 ml/kg | 37         | 772–4,580              |
| CL        | 162 ml/h/kg | 14         | 117–207                |
| Vz        | 5,110 ml/h/kg | 15        | 3,640–6,580            |
| Q         | 2,730 ml/h/kg | 2.8       | 2,580–2,880            |
| F         | 0.56%    | 16          | 0.39–0.73              |
| Eta       |          |             |                        |
| Ka        | 0.28     | 39          |                        |
| V         | 0.61     | 50          |                        |
| CL        | 0.065    | 31          |                        |
| Sigma     |          |             |                        |
| PK        | 0.0319   |             |                        |

*CL, clearance; F, extent of absorption or bioavailability; Ka, absorption rate constant; Q, intercompartmental clearance; V, central volume; Vz, peripheral volume. See supplemental material for other model parameters.
laria related or mefloquine related (n = 52 and n = 22, respectively; see Table S2 in the supplemental material). These events were comparable to those seen earlier in the IBSM studies or reported following the use of mefloquine. Among the 52 adverse events attributed to malaria, all were judged to be of mild to moderate severity, and they were generally of a transient nature. The most common adverse events attributable to malaria were fever symptoms (n = 6) and mild leukopenia (n = 7). Of the adverse events attributable to mefloquine, all except two (lucid dreams and nausea) occurred in the highest-dose cohort (15 mg/kg). Of note, three of the five subjects who experienced dizziness reported a significant duration of symptoms (4, 12, and 20 days). Four subjects reported nausea and/or vomiting following receipt of the highest dose of mefloquine, and two reported insomnia, with one of these two reporting that this lasted for 6 days.

PK/PD of mefloquine in induced blood-stage malaria in human volunteers. Orally administered mefloquine in IBSM displayed exponential kinetics (Fig. 2), which were approximately linear (see Table S1). The noncompartmental PK parameters confirmed the increase with dose to be supraproportional (see Fig. S4). A one-compartment with first-order absorption, lag time, and dose-dependent volume of distribution was determined to most accurately describe the data, as shown in Fig. 2, in which the modeled data are graphically overlaid. The half-life of mefloquine derived from the PK model fit was 200 h (8.3 days).

A readily apparent dose–response relationship was observed at the three dose levels of mefloquine (Fig. 3). At the lowest dose, 5 mg/kg, the drug stalled parasite growth but did not effect clearance, and rescue treatment with arteether-lumefantrine was required after 60 h because subjects began to become symptomatic. At the intermediate dose of 10 mg/kg, after an initial lag time of approximately 24 to 36 h (during which a surge in parasitemia was observed), a transient reduction in parasitemia was observed until approximately 96 h, when parasite growth began again and rescue treatment with arteether-lumefantrine was required. The 15-mg/kg dose of mefloquine was administered as a split dose of 10 mg/kg followed 4 h later by 5 mg/kg in an effort to reduce side effects. Given mefloquine’s quick absorption and long half-life (Fig. 2), this split dose does not affect further PK/PD modeling. At this dose level, the lag time (before parasitemia begins to drop), as judged from visual inspection of the data, was shorter (approximately 12 to 24 h). This was followed by clearance of parasitemia, with no recrudescence observed.

Rates of clearance of parasitemia, as measured by the dose-specific PRRmax and log10 PRRmax were 157 (95% confidence interval [CI], 130 to 189) and 2.20 (95% CI, 2.11 to 2.28) for the 10-mg cohort and 195 (95% CI, 155 to 246) and 2.29 (95% CI, 2.19 to 2.39) for the 15-mg cohort. For the 10-mg/kg administration, the MIC of mefloquine was reached between 96 and 108 h. The geometric mean MIC was 784 ng/ml (CV = 18%). This is equivalent to an IC50 of 667 ng/ml, or 1.8 μM, comparable to the value we found in mice (768 ng/ml, or 2 μM).

TABLE 2 Mefloquine PD key parameters in NOD SCID IL-2Ry−/− mice

| Parameter | Estimate (95% confidence interval) | Derived parameter | Estimate (95% confidence interval) |
|-----------|-----------------------------------|-------------------|-----------------------------------|
| Theta     |                                   | Growth rate       | 0.65/48 h (0.59–0.71) |
|           |                                   | Death rate        | 3.94/48 h (2.2–5.7) |
| Dmax      | 0.89 (95% CI, 1.11–2.07)           | Log10 PRRas       | 3.29 (95% CI, 2.19–2.39) |
| k         | 0.00892/h (95% CI, 0.00036–0.014)  |                   |                                   |
| IC50      | 0.768 μg/ml (95% CI, 0.64–0.89)    |                   |                                   |
| H         | 1.81 (95% CI, 0.94–2.68)           |                   |                                   |
| BL        | 0.376% parasitemia (95% CI, 0.369–0.383) |
| Eta       |                                   |                   |                                   |
| G         | 0.15 (95% CI, 40)                  |                   |                                   |
| BL        | 0.046 (95% CI, 26)                 |                   |                                   |
| Sigma PD  | 0.0364 (95% CI, 27 to 224)         |                   |                                   |

Note: All parameters are expressed as relative standard errors (SEs) and 95% confidence intervals (CIs).  

PD of mefloquine in published clinical trial data. We identified two earlier clinical trial studies in which serial quantitative parasite count data were reported following administration of mefloquine monotherapy. Using the published data (Fig. 3), we calculated the PRR. In the first study, mefloquine was administered as a single oral dose of 500, 750, or 1,000 mg in a phase II trial undertaken with 147 adult male patients with acute, uncomplicated falciparum malaria admitted to the Hospital for Tropical Diseases, Bangkok, Thailand (27). In the second, 50 male Zambian patients with symptomatic falciparum malaria were treated with a single 1,000-mg dose of mefloquine (28) and chloroquine (Fig. 3). In the first study, at doses of 500 mg, 750 mg, and 1,000 mg, the respective PRRs were 802 (95% CI, 76 to 8,505), 1,031 (95% CI, 195 to 5,458), and 5,610 (95% CI, 119 to 265,238). In the second study, in which 1,000 mg of mefloquine was administered, the PRR was calculated as 77.5 (95% CI, 27 to 224).

Correlating the SCID/Hu mouse and the human challenge models. For mefloquine, blood exposure can be compared directly between humans and rodents because levels of plasma (protein) binding are roughly equivalent, measured at 95 to 96% in humans and ~97% in rodents (29). The estimate of IC50 derived in NSG mice infected with P. falciparum, 768 ng/ml, closely matches that estimated by modeling in infected humans (665 ng/ml [23]).

DISCUSSION

We found that mefloquine-induced parasite clearance kinetics observed in the IBSM matched those reported in earlier clinical studies (27,28), despite an approximately 10,000-fold difference in parasite load. Further, comparison of clearance kinetics be-
between the volunteers in our study and malaria patients in the two previously published studies showed that the reduction in parasitemia followed similar gradients for similar doses of mefloquine, with the same lag time (Fig. 3). This delay in onset of activity cannot be explained by a delay in reaching an exposure sufficient to exert a parasiticidal effect (Fig. 1A), suggesting that this lag is mechanism related. Although the antimalarial mode of action of mefloquine is not well understood, it has been proposed to be mediated by inhibition of heme polymerization. Thus, the lag may reflect the time required for nonpolymerized heme to accumulate to toxic levels, or for this toxicity to exert its effect. Alternatively, mefloquine could affect parasites only at a specific stage of their asexual reproduction cycle. The fact that we saw a similar lag for synchronized parasites in the challenge model and in clinical patients suggests that it is the time required for the toxic free heme to accumulate and exert its toxic effect rather than a life cycle-stage-specific effect.

Mefloquine exposure in volunteers (Fig. 1A) was consistent with earlier findings (19). The key parameters from this PK model and observed data correspond well with those reported previously for mefloquine in different human settings (30). Overall, a good correspondence was observed for the in vivo IC50 measured in the mouse and human challenge models and in clinical patients. However, PRRs differ substantially between the models and also between published clinical studies, suggesting that the IC50s may be more useful in predicting optimal dosing of new drugs. Moreover, other factors, including parasite biology and immunity (31, 32), have a significant effect on rates of clearance. From this perspective, the performance of antimalarials in the IBSS model offers some advantages, including the facts that immunity cannot confound drug clearance and that a range of candidate antimalarials can be compared under similar circumstances.

In vitro evaluation of drug efficacy in P. falciparum strains is relatively straightforward. However, an understanding of the in vivo interplay between a drug’s changing exposure over time and the associated parasite growth/killing dynamics is critical in designing clinical studies needed for antimalarial drug development. Selection of an inappropriate dose in phase II or phase III studies may significantly prolong development or, worse, result in discontinuation of development of a drug with good potential. Antimalarials, especially those providing fast clearance of blood-stage parasites (Medicines for Malaria Venture target candidate profile 1 [1, 33]), are intended for use in large numbers of patients, many of whom may be vulnerable due to additional disease, malnutrition, young age, and/or variable immune status. This makes it even more important to optimize safe and effective dosing.

While falciparum malaria models involving both NSG mice and human volunteers have been developed and refined recently, a gap to date has been a formal assessment of these models’ validity in a coherent pathway for drug discovery using a clinically well-established antimalarial as a benchmark. In this study, we have evaluated mefloquine both in infected NSG mice and in volun-
teers, fitted the key parameters in a PD model, and found an excellent correlation between the variables that describe each in vivo system. It remains to be seen if the correlations that we have found between the in vivo model and in volunteers also hold for drugs with a different mechanism of action, with exposure characteristics different from those of mefloquine, and that produce significantly different exposures in rodents and humans. But with anti-malarials typically targeting either the parasite directly or components in the human infected erythrocyte compartment, it would seem that the two models that we have evaluated in this study have all the critical elements in place for confidently guiding clinical development of molecules currently progressing through the anti-malarial pipeline.

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