The effects of body size and gender on the population pharmacokinetics of artesunate and its active metabolite dihydroartemisinin in pediatric malaria patients

Carrie A. Morris\textsuperscript{1}, Beesan Tan\textsuperscript{2}, Stephan Duparc\textsuperscript{3}, Isabelle Borghini-Fuhrer\textsuperscript{3}, Donald Jung\textsuperscript{4}, Chang-Sik Shin\textsuperscript{5}, Lawrence Fleckenstein\textsuperscript{1}\#

Running Title: Population pharmacokinetics of artesunate and DHA

1 College of Pharmacy, University of Iowa, Iowa City, Iowa, USA

2 Clinical Pharmacology, Pfizer Inc, Cambridge, Massachusetts, USA

3 Medicines for Malaria Venture, Geneva, Switzerland

4 Pharmaceutical Research Services, Cupertino, California, USA

5 Shin Poong Pharmaceuticals, Seoul, Republic of Korea

#Corresponding author:
Lawrence Fleckenstein, College of Pharmacy, University of Iowa, 115 South Grand Avenue, Iowa City, Iowa, USA, 52242, Tel: 319-335-8804, Fax: 319-353-5646, E-mail: l-fleckenstein@uiowa.edu
ABSTRACT

Despite the important role of the antimalarial artesunate, and its active metabolite dihydroartemisinin (DHA), in malaria treatment efforts, there are limited data on the pharmacokinetics of these agents in pediatric patients. This study evaluated the effects of body size and gender on the pharmacokinetics of artesunate/DHA using data from pediatric and adult malaria patients. Nonlinear mixed effects modeling was used to obtain a base model consisting of first-order artesunate absorption and one-compartment models for artesunate and for DHA. Various methods of incorporating body size descriptors on clearance and volume parameters were tested. An allometric scaling model for weight and a linear body surface area (BSA) model were deemed optimal. The apparent clearance and volume of distribution of DHA obtained with the allometric scaling model, and normalized to a 38 kg patient, were 63.5 L/h and 65.1 L, respectively. Estimates for the linear BSA model were similar. The 95% confidence intervals for the estimated gender effects on clearance and volume parameters for artesunate fell outside the pre-defined no relevant clinical effect interval of 0.75 – 1.25. However, the effect of gender on apparent DHA clearance was almost entirely contained within this interval, suggesting a lack of influence of gender on this parameter. Overall, the pharmacokinetics of artesunate and DHA following oral artesunate can be described for pediatric patients using either an allometric scaling or linear BSA model. Both models predict that, for a given mg/kg artesunate dose, younger children are expected to have lower DHA exposure than older children or adults.

Keywords: artesunate; dihydroartemisinin; population pharmacokinetics; antimalarial
INTRODUCTION

The World Health Organization (WHO) estimates that malaria infection was responsible for approximately 660,000 deaths in 2010. Young children bear a devastating extent of the global mortality burden associated with malaria, with approximately 86% of the deaths occurring among children under five years of age (1). Malaria is caused by protozoa of the genus *Plasmodium*; the species *P. falciparum* and *P. vivax* are most commonly responsible for infections, with *P. falciparum* causing the vast majority of fatal infections. Derivatives of the endoperoxide antimalarial artemisinin efficiently effect profound reductions in parasite counts, and are the cornerstone of the global treatment approach for acute malaria infection. Intravenous administration of artesunate is endorsed by WHO for treatment of severe malaria, with oral artemisinin-based combination therapies (ACTs) recommended for treatment of uncomplicated malaria. ACTs couple artemisinin derivatives, which are rapidly eliminated from the body, with more slowly eliminated partner drugs. These partner drugs eradicate residual parasites and guard against emergence of parasites with reduced artemisinin sensitivity (2).

Artesunate, a hemisuccinate ester of its active metabolite, dihydroartemisinin (DHA), is the most water soluble of the artemisinin derivatives. Following absorption, artesunate is rapidly converted to DHA by hepatic and plasma esterases. DHA, in turn, undergoes glucuronide conjugation mediated by UGT2B7 and UGT1A9. DHA is considered the principal source of the antimalarial activity associated with oral artesunate treatment, largely due to the comparatively lower exposure to artesunate than to DHA observed following oral artesunate administration (3).

Given the therapeutic prominence of the artemisinin derivatives, and the particular vulnerability of the pediatric population to malaria-related mortality, any differences between children and adults in the disposition of artemisinins should be thoroughly characterized.
However, as noted in two recent reviews, numerous gaps exist in our understanding of artemisinin derivative pharmacokinetics among pediatric patients (4, 5). The analysis that follows represents an attempt to describe the population pharmacokinetics of the artemisinin derivative artesunate, and its active metabolite DHA, in falciparum and vivax malaria patients participating in Phase II and III trials for the novel ACT pyronaridine tetraphosphate/artesunate (PA); the primary focus of the analysis was the description of artesunate and DHA pharmacokinetics in pediatric patients.

As body size exerts a substantial influence on pediatric pharmacokinetics, a particular emphasis of this analysis was the evaluation of methods for describing the relationship between body size descriptors and clearance and volume parameters. The methods investigated included linear, estimated exponent, and allometric scaling models for body weight, as well as linear and estimated exponent models for body surface area (BSA) and lean body mass. A further purpose of this analysis was to estimate the magnitude of covariate effects of potential clinical interest using a full covariate model approach. The final aim was to assess the sensitivity of the estimated parameter-body size relationships and covariate-parameter relationships to inclusion or exclusion of adult data from the modeling dataset.

METHODS

Data

Two datasets were utilized for modeling. The first, termed the full dataset, included data from both pediatric and adult participants in the five PA trials, which included one Phase II and four Phase III studies. The second dataset, termed the pediatric dataset, was a subset of the full dataset; it contained data only for patients less than 12 years of age, the ICH age category cutoff between children and adolescents (6).
A summary of patient demographics for included clinical trials is given in Table 1. In all five trials, patients were administered PA once daily for three days without regard to food intake. In the Phase II study (Study 1), plasma samples were collected prior to the first dose of PA, and at 0.25, 0.5, 1, 1.5, 2.5, 4, 8, 12 hours following that dose; patients were administered 2, 3, or 4 mg/kg/day artesunate (7). In the Phase III studies, one sample was drawn during a 0.25 to 12 hour window following either the first or second PA dose, and a second sample was drawn during that same window following the third dose. During Phase III studies, patients received artesunate doses ranging from 2.3 to 4.6 mg/kg/day. A granule formulation of PA was administered to approximately one fourth of the patients in the Phase II study and all of the patients in one of the Phase III studies, while all other patients were given a tablet formulation (8). Written informed consent, in accordance with local practice, was obtained for participants in the studies, and approval for each study was granted by local Ethics Committees. For Study 5, a pediatric study, written informed consent was provided by a parent or guardian, with patient assent sought where possible.

Sample handling

Collected samples were processed as follows: blood was collected in tubes containing potassium oxalate/sodium fluoride for the separation of plasma drawn at times specified. The samples were centrifuged within 15 minutes of collection. Plasma was removed from cells and transferred into two approximately equal volume aliquots in screw cap cryovials immediately after the centrifugation. The plasma samples were immediately frozen at or below -80°C in a laboratory freezer. They were later shipped separately via air express on dry ice to the Clinical
Pharmacokinetics Laboratory at the College of Pharmacy, the University of Iowa. All samples were stored at -80°C until drug analysis was performed.

Artesunate and dihydroartemisinin assay

Plasma concentrations of artesunate and DHA were quantified using the method described by Naik et al (9). Briefly, plasma concentrations of artesunate and DHA were quantified by LC-MS. Chromatographic analysis was carried out on a Shimadzu Model 2010 liquid chromatograph and mass spectrometer (Shimadzu, Columbia, MD, USA) using a LC-10AD Solvent Delivery system. The injection was made with a Shimadzu SIL-10AD automatic injector. The analysis was carried out using Synergi Max-RP 80A HPLC column, 75 mm × 4.6 mm, 4 μ (Phenomenex, Torrance, CA, USA) using a guard column (Phenomenex) with C-12 max-RP cartridges. The lower limit of quantification was 1 ng/mL for both artesunate and DHA. The coefficients of variation for intra-day precision and inter-day precision were less than 15% for AS and DHA.

Base model

Prior to model building, artesunate and DHA data were converted from ng/mL to nmol/L values using the compounds' respective molecular weights. A visual exploratory data analysis was undertaken to examine the basic structure of the concentration-time data and to identify outliers. Due to the predominance of sparse sampling data, the more extensive full dataset was primarily used for base model development; however, models which successfully converged with plausible estimates were implemented with the pediatric dataset to assess for reasonably similar model fit. Population pharmacokinetic modeling was performed using NONMEM 7.2 (10) implemented on a Windows XP operating system with a G95 Fortran compiler. Model
development and evaluation was facilitated through use of Perl speaks NONMEM 3.5.3 (PsN) (11) and Pirana 2.6.0 (12).

For all models assessed in this analysis, inter-individual variability (IIV) was modeled as following a log-normal distribution for all parameters:

\[ P_i = P_{\text{pop}} \times e^{\eta_i} \]

where \( P_i \) is the estimated parameter value for individual \( i \), \( P_{\text{pop}} \) represents the typical population estimate for the parameter, and \( \eta_i \) is the deviation of \( P_i \) from \( P_{\text{pop}} \). The term \( \eta \) is assumed to be normally distributed with a mean of zero and a variance of \( \omega^2 \). For all models considered during base model development, both full and diagonal IIV variance-covariance matrices were evaluated. Models with some, but not all, covariance terms fixed to zero were assessed as deemed appropriate based on parameter estimates from full matrix results.

Data were first modeled using a simultaneously implemented parent-metabolite model with first-order artesunate absorption, and a one-compartment, first-order elimination model for both artesunate and DHA. The concentration data were natural log transformed and an additive model for log-transformed data was applied:

\[ \ln(C_{ij}) = \ln(C_{\text{pred},ij}) + \varepsilon_{ij} \]

where \( C_{ij} \) and \( C_{\text{pred},ij} \) represent the j-th observed and model predicted analyte concentrations, respectively, for individual \( i \). The term \( \varepsilon_{ij} \) denotes the residual random error for individual \( i \) and observation \( j \), with \( \varepsilon \) assumed to be normally distributed with a mean of zero and a variance of \( \sigma^2 \) in the population. Models with single and distinct \( \varepsilon \) distributions for the two analytes were assessed during early model building; for distinct distributions, the covariance term was either estimated or fixed to zero. Due to model stability problems encountered with alternative models, distinct \( \varepsilon \) values, with covariance fixed to zero, were utilized for all but early model building.
Estimation methods utilized during initial base model development included first-order conditional estimation (FOCE), importance sampling expectation maximization, and Laplacian estimation. Attempts were made to account for concentrations below the lower limit of quantification of the assay through implementation of the M2 and the M3 methods as described by Beal (13).

Alternative models were tested to improve upon the initial base model including the addition of a second DHA compartment, as well as evaluation of multiple alternative absorption models including zero-order absorption, transit compartment absorption, mixed zero-order/first-order absorption, and parallel first-order absorption. Additionally, a model with $\eta$ on artesunate bioavailability (and with a diagonal $\omega$ matrix and population bioavailability ($F_1$) set to 1) was also assessed. Finally, based on research suggesting differences in artemisinin derivative pharmacokinetics between the most acute phase of infection and the convalescence phase (14), a model with $F_1$ fixed to 1 for the first day of treatment, and estimated for days 2 and 3, was also evaluated.

Model selection was guided by the following factors: plausibility and precision of parameter estimates, goodness-of-fit plots, magnitude of residual variability, sensitivity of the model to initial estimates, minimum objective function value (MOFV), equal to minus twice the log likelihood function, and Akaike Information Criterion, equal to MOFV plus two times the number of parameters. Data for goodness-of-fit plots were stratified by age (<5 years, 5 through 11 years, 12 through 18 years, older than 18 years) to identify if a given base model was associated with unique goodness-of-fit features in a particular age group. Finally, given that DHA is considered principally responsible for antimalarial activity following oral artesunate
administration, concerns regarding appropriate fit of DHA data took precedence over parallel concerns regarding artesunate data.

Covariate modeling: Body size descriptor

Following base model development, relationships between various body size descriptors and the clearance and volume parameters of artesunate and DHA were modeled. These descriptors included total body weight (WT), body surface area (BSA), and two estimators of lean body mass (LBM1 and LBM2). The formulas for these descriptors are given in Table 2.

Obtaining estimates of lean body mass was complicated by the lack of LBM formulas applicable across the entire age range in this dataset. Therefore, formulas were applied in a piecewise fashion according to age. To compute LBM1, formulas by Janmahasatian et al. (15), developed with adult subjects, were applied to patients at least 18 years of age, whereas formulas by Foster et al. (16), developed with children and adolescents, were applied to patients older than five but younger than eighteen years of age. This formula requires that each child’s body mass index (BMI) z-score for age and gender be obtained. For purposes of this analysis, BMI z-scores corresponding to the CDC growth charts were computed using a SAS macro available from the CDC (http://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm). As the Foster et al. formula was not developed with very young children, patients five years of age and younger had LBM1 set to equal total body weight.

The second lean body mass estimation approach followed the method proposed by Peters et al. (17). This method is based on the underlying assumption that the relationship between extracellular fluid volume (ECV) and lean body mass is similar between adults and children. Peters et al. apply a proportionality constant linking ECV and lean body mass in adults to a
pediatric ECV estimation formula described by Bird et al. (18). This yields a lean body mass
formula applied in the present analysis to patients less than 50 kg, a cutoff identified by Peters et
al. For patients weighing 50 kg or more, a lean body mass formula derived by Boer (19), and
utilized in the Peters et al. analysis, was applied.

Artesunate and DHA clearance and volume parameters were modeled with the various
body size parameters utilizing the following relationship:

\[ P = \theta_1 \times \left( \frac{\text{SIZE}}{\text{SIZE}_{\text{median}}} \right)^{\theta_2} \]

where \( P \) is the typical value of a clearance or volume parameter, \( \text{SIZE} \) is the value of a given
body size descriptor for an individual, \( \text{SIZE}_{\text{median}} \) is the median value of the descriptor in the full
dataset, and \( \theta_1 \) is the typical value of that parameter for a person with \( \text{SIZE} \) equal to \( \text{SIZE}_{\text{median}} \).
The value of \( \theta_2 \) was estimated as a free parameter or fixed to a set value. All estimated models
are described in Table 3. For all body size descriptors, \( \theta_2 \) was estimated on all clearance and
volume parameters for one model and set to one for an alternative, linear model. For weight, an
additional allometric scaling model was tested with the exponent on body size set to 0.75 for
clearance terms and 1.0 for volume terms. For purposes of model harmony, a single type of
body size descriptor was used on all clearance and volume parameters in any given model. All
models were evaluated using both the full and pediatric datasets.

The models incorporating body size descriptors as described above were evaluated per
multiple criteria in order to identify a model to be carried forward for subsequent analyses.
These criteria included the following: developmental and physiologic plausibility of the
estimated parameter/body size descriptor relationships, overall model goodness-of-fit and
estimate precision, goodness-of-fit equivalency across age strata, sensitivity of parameter
estimates to inclusion of adolescent and adult data, and model complexity (i.e. number of
Once a model incorporating a body size descriptor was selected, additional covariate relationships of potential clinical interest were investigated in accordance with a full covariate model approach (20). These relationships included the effects of formulation on the absorption rate constant (Ka) and the effect of gender, after accounting for body size, on all artesunate and DHA clearance and volume parameters. The effects of the granule formulation on Ka were modeled as follows:

\[
P_{\text{popKa}} = \theta_1 \times (\theta_2)^{\text{FORM}}
\]

where PopKa is the typical value of Ka in the population, FORM is an indicator variable equal to 0 if patients were administered the tablet formulation and 1 if patients were administered the granule formulation, \(\theta_1\) is the typical value of Ka for patients administered the tablet formulation, and \(\theta_2\) is the multiplicative factor describing the increase or decrease in Ka associated with administration of the granule formulation.

The effects of gender on clearance and volume parameters were similarly modeled:

\[
P = \theta_1 \times \left( \frac{\text{SIZE}}{\text{SIZE}_{\text{median}}} \right)^{\theta_2} \times (\theta_3)^{\text{SEX}}
\]

where SEX is an indicator variable equal to 1 for males and 0 for females and \(\theta_3\) is a multiplicative factor describing the effect of male gender on the clearance or volume parameter.

In order to obtain estimates for the magnitude of the covariate effects in the population, PsN was used to generate 500 bootstrap datasets from the full dataset, with stratification by sampling type (extensive vs. sparse) and formulation. The final full covariate model was fitted to these datasets. This process was then repeated using the pediatric dataset. The distributions for the covariate effect estimates were then plotted using the metrumrg R package (21).
Predictive checks

After the full covariate models for both the full and pediatric datasets were obtained, PsN was used to perform numerical predictive checks (NPC) and visual predictive checks (VPC) for the analytes. For both NPC and VPC, one thousand virtual observations at each sampling time point were simulated using the final parameter estimates. Due to the variability in administered dose, prediction-correction was employed for VPC. NPC and VPC results were stratified per the following age categories: five years and younger, six years through eleven years, twelve years through eighteen years, and greater than eighteen years. The latter two categories were not applicable to the pediatric dataset evaluation. VPC results were visualized using Xpose 4.4.0 (22). Categorical predictive checks for both artesunate and DHA were also implemented using PsN and Xpose. In this evaluation, the 95% confidence interval for the proportion of concentrations below the lower limit of quantification for a given analyte was calculated from simulations and compared to the observed proportion.

Covariate modeling: Exploring trends

After development of a full covariate model, conditional weighted residuals (CWRES) for artesunate and DHA and individual $\eta$ estimates for parameters were plotted against various remaining covariates in the datasets. These included the following: age, baseline parasite count, baseline clinical laboratory findings (hemoglobin, hematocrit, red blood cell count), baseline aspartate aminotransferases (AST) greater than $1.5 \times$ upper limit of normal (ULN), and baseline alanine aminotransferases (ALT) greater than $1.5 \times$ ULN. Covariates displaying potential and plausible relationships with parameters were tested for statistical significance using forward
addition ($\alpha=0.05$) followed by backward elimination ($\alpha=0.01$).

RESULTS

Data

Data arising from 631 uncomplicated falciparum or vivax malaria patients were included in the full dataset, with the 274 patients under 12 years of age also being included in the pediatric dataset. The full dataset contains data from 8 patients younger than two years, 266 patients 2 years through 11 years, 103 patients 12 years through 18 years, and 254 patients older than 18 years. A total of 1490 observations were available for artesunate and DHA in the full dataset, with 613 artesunate (41.2%) and 54 DHA (3.6%) concentrations below the lower limit of quantification. For the pediatric dataset, 786 observations were available, with 303 artesunate (38.6%) and 26 DHA (3.3%) concentrations below the lower limit of quantification.

Base model

The structural model ultimately selected included first-order artesunate absorption and a one-compartment model with first-order elimination for both artesunate and DHA. Importance sampling expectation maximization was the estimation method ultimately selected for modeling. Complete conversion of artesunate to DHA was assumed (23). The absorption rate constant for artesunate ($K_a$), as well as the apparent clearance and volume of distribution for artesunate ($CL/F$, $V_2/F$) and DHA ($CLM/F$, $V_3/F$), were estimated. An IIV structure with covariance terms between all clearance and volume parameters was selected. Such a structure acknowledges that non-negligible between subject differences in artesunate bioavailability likely exist within the population. Discussion of alternative models evaluated during model development is provided in...
Covariate modeling: Body size descriptor

Since DHA is considered of greater clinical relevance than artesunate, the impact of incorporating various body size descriptors on DHA goodness-of-fit and DHA parameter estimates represented the principal focus of the body size descriptor modeling. Supplemental Tables S1 and S2 contain the point estimates and NONMEM derived relative standard errors (%RSE) for CLM/F and V3/F related parameters, respectively. These point estimates were utilized to estimate the population predicted CLM/F and V3/F values for each individual in the dataset. As artemisinin derivative doses are typically expressed on a mg/kg basis, the population predicted apparent clearance and volume of distribution values were converted to L/hr/kg and L/kg units, respectively. Plots of weight-adjusted DHA CLM/F and V3/F population predicted values are given in Figure 1 and 2, respectively, for models including weight or BSA.

Goodness-of-fit plots did not suggest superiority of any particular body size model, regardless of whether or not stratification by age was applied; therefore, this criterion did not inform model selection. Examination of the results from the models with estimated exponents revealed that the predicted CLM/F vs. age and V3/F vs. age curves for the pediatric dataset models tended to differ, in some instances substantially, from their counterparts estimated with the full dataset. Any interpretation of these observed differences is complicated by the imprecision associated with the estimates for the parameters in the pediatric dataset derived versions of these models. That is, due to the uncertainty in the parameter estimates, drawing conclusions based on the distinctions between the curves for the full and pediatric datasets would be inadvisable. Given this challenge, and the necessity of estimating four additional parameters,
these models were not carried forward for use in further analyses.

The linear weight model was estimated with fairly good precision. However, the pediatric and full datasets yielded fairly different predicted CLM/F and V3/F values. The linear LBM1 model displays dramatic fluctuations across age, presumably due to the piecewise application of formulas used in LBM1 calculation. From a developmental plausibility perspective, such abrupt fluctuations are undesirable. In contrast, the linear LBM2 model did not display such fluctuations. Unfortunately, the parameter estimates for the full dataset version of this linear LBM2 model displayed extremely poor precision, which supported rejection of this model.

The allometric scaling model and the linear BSA model were both estimated with adequate precision and displayed a relative insensitivity to inclusion or exclusion of adolescent and adult data. In actuality, the predicted CLM/F values, as shown in Figure 1, were extremely similar between the two models, regardless of the dataset employed. Ultimately, the allometric scaling and linear BSA models were deemed to be the best body size descriptor models, as judged per the pre-specified criteria, from among the various models assessed. Given the similarities between the two models in the evaluation criteria, the most justifiable choice was to carry forward both models into further stages of modeling.

Full covariate model

The full covariate model included gender on all clearance and volume parameters and formulation on Ka. However, only unrealistically high estimates for the increase in Ka associated with the granule formulation could be obtained. Ultimately, the granule Ka was fixed to 10 h$^{-1}$ to reflect the apparently quite rapid absorption of the formulation, with the tablet Ka left
to be freely estimated. Further discussion of this formulation effect is provided in Supplemental Materials.

The parameter estimates for the full covariate models with this modified Ka coding, and with the gender effects estimated on the clearance and volume parameters, are given in Table 4 for the allometric scaling and the linear BSA models, with analogous parameter estimates from the full population models given in Table S3. IIV covariance estimates for the various models are given in Table S4. The 95% confidence intervals for the effects, determined through 500 bootstrap runs, are plotted for CLM/F and V3/F in Figures 3 and 4, respectively, with plots for the artesunate parameters provided in Figure S1 and S2. Plots of DHA CWRES vs. time after dose and population predicted DHA vs. observed DHA concentrations for the pediatric linear BSA model are given in Figure 5. The analogous plots for the allometric scaling model (not shown) were essentially identical.

**Predictive checks**

The full covariate models were used to perform VPCs stratified by age. The DHA VPCs for the full population linear BSA model are given in Figure 6. DHA VPCs for remaining models were quite similar. The percentages of concentrations below the 5th percentile and above the 95th percentile of the simulated concentrations are given in Table S5. Although these results do not indicate a clear difference in predictive ability for the two body size models, the stratification does indicate that predictive ability is superior for patients 18 years of age and younger. This is an acceptable result given that the target population to be described by the analysis is pediatric patients.

Figures S3 and S4 display the categorical VPC results for artesunate and DHA,
respectively, for the full population linear BSA model; alternative models yielded similar results.

Per this evaluation, the proportion of artesunate concentrations below the lower limit of quantification is underpredicted by the model. However, for DHA, the simulated and actual proportions are well aligned, suggesting that failure to incorporate concentrations below the lower limit of quantification into the modeling did not bias predictions for DHA, the analyte of primary interest.

**Covariate modeling: Exploring trends**

The plots of DHA CWRES vs. age for patients 12 years of age and younger are given in Figure 7. There appears to be a slight trend towards underestimation of DHA concentrations for very young patients. For artesunate, this trend was also apparent (plots not shown). Plots of estimated $\eta$ values vs. age indicated a trend towards negative $\eta$ values for multiple parameters (CL/F, CLM/F, and V3/F) for patients four years of age and younger; that is, these plots suggested that the patients may have lower actual parameter values than predicted by the model. Given that this trend appears to be acting across multiple parameters, a likely explanation may be that the patients are displaying higher bioavailability than accounted for by the model. To investigate this possibility, F1 was set to 1 for patients older than four years, with relative bioavailability estimated for patients four years and younger. This did result in statistically significant differences ($p<0.01$) in all models and datasets.

Major potential confounding factors related to this finding must be considered, however. For example, among patients four years of age and younger, 77% of the patients were administered granules, as compared to 26% of patients ages 5 – 11. Considering only the pediatric dataset, for patients four years and younger, 91% of the artesunate and 86% of the DHA
concentrations used in modeling were obtained on day 1, with the remainder obtained on day 3. In contrast, for patients between 5 – 11 years, 71% of the artesunate and 63 % of the DHA concentrations were obtained on day 1. Therefore, the modeled increase in bioavailability in this age group could reflect a bioavailability effect of acute illness or of the granule formulation. Unfortunately, neither of these effects was previously successfully modeled. To avoid spuriously ascribing an effect to age which in actuality likely might have stemmed from other causes, full evaluation of models including this effect was not pursued and the effect was not included in the final models.

DISCUSSION

The intent of this analysis was to describe the population pharmacokinetics of artesunate and DHA in pediatric patients utilizing data from 631 pediatric, adolescent, and adult uncomplicated malaria patients participating in Phase II and III clinical trials for the combination agent pyronaridine tetraphosphate/artesunate. To this end, a parent-metabolite base model with first-order artesunate absorption and a one-compartment model with first-order elimination for both artesunate and DHA was developed. Various methods for incorporating body size descriptors on clearance and volume parameters were assessed, and two highly similar models, a linear BSA model and an allometric scaling model, were ultimately selected as the optimal body size models. Building upon these two models, the effects of gender on the clearance and volume parameters were evaluated using a full covariate model approach; although the covariate effect estimates were sufficiently imprecise to preclude drawing any definitive conclusions, the findings could be considered tentatively consistent with the lack of a clinically relevant effect of
gender on DHA apparent clearance. Finally, in this analysis, it was found that modeling with a dataset including adolescent and adult data allowed for increased precision in estimation of parameters without introducing any meaningful bias in the point estimates for those parameters.

Physiologic basis for models

In this analysis, the clearances of artesunate and DHA were described using relationships with either weight or BSA, but not with patient age. The choice to not incorporate the covariate of age *a priori* with body size, as well as to not assess for an age effect in the full covariate model, essentially rested on two assumptions. The first assumption was that across the studied patient population, the hepatic clearances of artesunate and DHA would be dependent on the rate of hepatic blood flow rather than intrinsic clearance. The second assumption was that the developmental changes in hepatic blood flow could be satisfactorily accounted for using clearance-body size descriptor relationships. Additionally, it should be noted that no claim is being made that these assumptions would, or would not, be applicable to patients under two years of age. As there were only eight such patients in the dataset, all with sparse sampling data, no attempt was made in the analysis to derive and justify pharmacokinetic findings appropriate for this age group.

Evidence for the assumption that the analyte clearances will display hepatic blood flow-limited kinetics among patients as young as two years of age can be found in a study by Nealon et al. (24). This study included assessment of artesunate and DHA pharmacokinetics following intravenous (IV) administration of artesunate to children with severe malaria. The two subgroups of children in the study had median ages of 36 and 21 months. Pooled pharmacokinetic findings from the two patient subgroups indicate median artesunate and DHA
clearance values of 46 mL/kg/min and 25 mL/kg/min, respectively, with substantial individual
variability being associated with both estimates. As a point of reference, the clearance of
indocyanine green (25), a probe substrate for hepatic blood flow, had a mean clearance of 15.6
mL/kg/min (SD: 7.3 mL/kg/min) among patients younger than 10 years of age. Allowing for the
observed variability in both the IV artesunate pharmacokinetic findings and hepatic blood flow
estimates, it appears that artesunate and DHA clearances are not limited by intrinsic clearance
even in children as young as 21 months.

Further evidence that intrinsic clearance does not limit artesunate and DHA clearances
even in young children can be obtained from *in vivo* and *in vitro* findings for agents with
analogous metabolic profiles. With regard to artesunate, pediatric pharmacokinetic findings for
agents undergoing esterase mediated hydrolysis, such as oseltamivir and remifentanil, are
indicative of efficient esterase activity for patients at, and even prior to, one year of age (26, 27).
With regard to DHA, various *in vivo* studies with morphine, a probe substrate for UGT2B7, have
indicated achievement of adult UGT2B7 activity well prior to two years of age (28). Similar
conclusions were reached following an *in vitro* investigation of epirubicin glucuronidation by
UGT2B7 in pediatric and adult liver microsomes (29).

The pediatric IV artesunate results, coupled with the findings related to the ontogeny of
the individual metabolizing enzymes, provide support for the assumption that artesunate and
DHA hepatic clearance will be limited by hepatic blood flow among patients at least two years of
age. Granting this assumption, then clearly a body size descriptor-clearance relationship
appropriately modeling changes in hepatic blood flow would account for the developmental
pattern of artesunate and DHA clearances. Hepatic blood flow is proportional to liver volume;
liver volume, expressed per kg of total body weight, is higher in younger children than older
children (30). Therefore, for agents with clearance dependent on hepatic blood flow, pediatric clearance values, expressed per kg of total body weight, decline as children mature. Liver volume, when normalized to BSA, but not to total body weight, is constant over the pediatric age range (30, 31). In actuality, a general nonlinear trend between age and per kg clearance in pediatric subjects is approximated by both the linear BSA model and the allometric scaling models, and both models have been extensively employed in pediatric pharmacokinetic analyses. The potential clinical implications of this nonlinear trend in per kg clearance do merit attention. For example, with the allometric scaling model, given patients administered equivalent mg/kg doses, a typical 10 kg patient’s expected DHA exposure (AUC) would be approximately one-quarter and one-third lower, respectively, than that of a 35 kg or a 60 kg patient. Put another way, given a 60 kg patient administered 3 mg/kg/day artemesunate, a 10 kg patient would need to receive, on average, 4.5 mg/kg/day to attain similar exposure. Of course, such estimates of exposure differences reflect expected population values; within a given pair of individuals, exposure differences are far less predictable.

**Covariate Model: Gender Effects**

For the full covariate model, the effect intervals for all of the gender-parameter relationships crossed 1.0, indicating a lack of statistically significant effects. No interval, regardless of parameter, model, or dataset employed, had a bootstrap 95% confidence interval contained entirely within the 0.75 – 1.25 interval for a clinically irrelevant effect. Essentially, the results indicated that insufficient information was available to conclusively judge any effect as clinically relevant or irrelevant. It is worth noting, however, that for CLM/F, the large bulk of the effect estimate distribution for each model fell within the 0.75 – 1.25 no effect region. This
would appear to offer some evidence for the lack of a clinically relevant gender effect on CLM/F. For strictly predictive purposes, more parsimonious models without the gender effects would be justifiable.

The standard interval of 0.75 – 1.25 was used to indicate a clinically relevant effect of a covariate on a parameter. This interval was adopted because, at the time of this analysis, a clear relationship between concentrations and clinical efficacy remains largely undefined for the artemisinin derivatives (32). However, were such a relationship to be determined, the full covariate modeling in the present analysis could be reinterpreted. For example, if a threshold DHA AUC for efficacy were known, the CLM/F gender effect results could be used to estimate the probability that being male would result in failure to meet that target. More generally, additional pharmacodynamic information could be used to adjust the 0.75 – 1.25 default limits to artesunate-specific values.

Pharmacokinetic Comparisons

One of the studies (Study 1) included in the present analysis was previously analyzed using non-compartmental methods (7). The mean apparent DHA clearance from the non-compartmental analysis, approximated from subgroup means, was 2.4 L/kg/hr. For the average weight of 18.5 kg, the full population and pediatric allometric scaling models yield a prediction of 2.1 L/kg/hr. Given that this value reflects a population prediction for an average weight, this model predicted value is adequately similar to the non-compartmental findings.

The population pharmacokinetics of oral artesunate in pediatric patients has previously been examined by Stepniewska et al, who studied artesunate pharmacokinetics following oral artesunate administration to uncomplicated falciparum malaria patients in Burkina Faso between...
Artesunate and DHA pharmacokinetic data were obtained from 70 children who received artesunate and amodiaquine, with samples taken once in the first dosing period and once in the third dosing period. Pharmacokinetic modeling was conducted using DHA concentrations, as well as total antimalarial activity. The DHA concentration data were fit to a one-compartment model. The authors estimated a DHA apparent clearance of 0.636 L/hr/kg for the first dosing period with a substantial increase of 0.760 L/hr/kg associated with the third dosing period, yielding a day 3 apparent clearance of approximately 1.4 L/hr/kg.

Considering a patient with a weight of 13 kg, an approximate average weight for patients in the Stepniewska et al study, the population predicted CLM/F would be 2.2 L/hr/kg for a female per the allometric scaling model (pediatric or full dataset) and 2.4 L/hr/kg for a male. Clearly, the estimated apparent clearance in the Stepniewska et al study, particularly for day 1, is substantially lower than the model estimated apparent clearance from the present analysis. The difference is unlikely to be due in any large part to the failure of the model to account for a simple effect of acute infection on day 1 pharmacokinetics. After all, as previously described, Study 1 non-compartmental results, which were derived entirely from day 1 samples, are consistent with model estimated clearance predictions. However, a more complex disease effect, dependent on the severity of infection and the age of the patients, could perhaps be operating. The median parasite count for patients in the pediatric dataset in the present analysis was 10,341, whereas the median parasite counts for the two cohorts of the Stepniewski et al study were 29,000 and 30,000. In a recent analysis of artesunate pharmacokinetics in pregnant women, it was observed that women who were moderately unwell displayed significantly higher combined exposure to artesunate and DHA than women who were mildly unwell (14). This dynamic could account for some of the discrepancy observed for day 1 clearance estimates.
Furthermore, the median age in the two cohorts of the Stepniewski et al study was 3.1 and 2.7 years, compared to 7 years in the pediatric dataset of the present analysis. It is not inconceivable that pediatric patients might experience a more dramatic physiologic response to acute illness than older patients, resulting in a more pronounced disease effect on artesunate and DHA pharmacokinetics. Further investigation would be required to characterize such a potential interaction effect between age and acute illness.

Inclusion of adult data

Throughout this analysis, models were evaluated using two datasets, one with the full age range of patients, and another including patients only younger than 12 years of age. The intention of this parallel modeling approach was to allow for utilization of additional data, which could bolster model stability and estimate precision, while simultaneously checking for possible estimate bias in covariate-parameter relationships introduced through inclusion of data from non-pediatric patients. Indeed, the full dataset models did allow for more precise estimation of gender effects, as well as multiple other parameters, than their pediatric counterparts. However, the point estimates of the final models for essentially all of the parameters were quite similar, suggesting that bias was not introduced through inclusion of the adolescent and adult data. Further discussion of this aspect of the analysis is provided in Supplemental Materials.

Conclusions

Overall, the results of the present analysis indicate that the pharmacokinetics of artesunate and DHA following oral artesunate administration can be described for pediatric patients using either an allometric scaling or linear BSA model, a finding consistent with the
likely tight relationship between hepatic blood flow and artesunate/DHA clearance. Furthermore, the analysis demonstrated that when utilizing these body size models, adolescent and adult pharmacokinetic data could be included in the modeling dataset to enhance parameter estimate precision. Limitations of the dataset used in this analysis include the relatively mild infection experienced by a majority of the patients and the minimal number of patients below two years of age. Both the allometric scaling and linear BSA models predict that, for the same mg/kg artesunate dose, younger children are expected to have lower DHA exposure than older children or adults. The extent to which this pattern can be extrapolated to children younger than two years of age is dependent on the relative influences of hepatic blood flow and metabolizing enzyme maturation, as well as any interaction between age and an acute disease effect. Further investigation clearly is required, and should be undertaken, to elucidate these various dynamics. Given the high risk of malaria-related mortality experienced by young children, there is clearly a significant need for such investigation.

ACKNOWLEDGEMENTS

Funding for this study was provided by Medicines for Malaria Ventures and Shin Poong Pharmaceuticals.

REFERENCES


Table 1. A summary of demographic and covariate data. Values given as median (interquartile range) unless otherwise specified.

<table>
<thead>
<tr>
<th>Study 1 (Phase II)</th>
<th>Study 2 (Phase III)</th>
<th>Study 3 (Phase III)</th>
<th>Study 4 (Phase III)</th>
<th>Study 5 (Phase III)</th>
<th>All studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artesunate mg/kg dose</td>
<td>3.4 (2.8, 3.9)</td>
<td>3.3 (3.0, 3.6)</td>
<td>3.3 (2.9, 3.9)</td>
<td>3.4 (3.0, 3.7)</td>
<td>3.0 (2.6, 3.3)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>5 (4, 6)</td>
<td>24 (19, 35)</td>
<td>11 (8, 17)</td>
<td>19 (14, 34)</td>
<td>5 (3, 7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>16 (14, 20)</td>
<td>50 (44, 56)</td>
<td>30 (25, 48)</td>
<td>47 (35, 53)</td>
<td>17 (14, 20)</td>
</tr>
<tr>
<td>Gender (% Male)</td>
<td>51</td>
<td>77</td>
<td>46</td>
<td>61</td>
<td>48</td>
</tr>
<tr>
<td>Parasite count (per µL)</td>
<td>6,304 (2,051, 14,928)</td>
<td>12,838 (5,843, 31,168)</td>
<td>12,607 (3,363, 29,408)</td>
<td>10,275 (3,757, 15,692)</td>
<td>10,074 (1,994, 44,068)</td>
</tr>
<tr>
<td>Patients administered granules (N)</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>Patients &lt;12 years (N)</td>
<td>56</td>
<td>23</td>
<td>104</td>
<td>4</td>
<td>87</td>
</tr>
</tbody>
</table>
Table 2. Formulas for body size descriptors used in modeling. In formulas, weight is in kg, height in cm, and age in years. LBM1 and LBM2 were constrained to not exceed total body weight. BMIz are z-scores for subjects’ body mass index values.

<table>
<thead>
<tr>
<th>Body size descriptor</th>
<th>Age range</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA: Body surface area (m²)</td>
<td>All ages</td>
<td>(Weight^{0.5378})*(Height^{0.3964})*0.024265</td>
</tr>
<tr>
<td>LBM1: Lean body mass method 1 (kg)</td>
<td>Age ≤ 5</td>
<td>Total body weight</td>
</tr>
<tr>
<td></td>
<td>5&lt;Age&lt;18</td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ln(LBM) = -2.8990 + 0.8064<em>ln(Height) + 0.5674</em>ln(Weight) + 0.0000185<em>Weight – 0.0153</em>(BMIz)^2 + 0.0132*Age</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ln(LBM) = -3.8345 + 0.954<em>ln(Height) + 0.6515</em>ln(Weight) – 0.0102*(BMIz)^2</td>
</tr>
<tr>
<td></td>
<td>Age ≥ 18</td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(9.27 x 10^3 x Weight) / (6.68 x10^3 + 216*BMI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(9.27 x 10^3 x Weight) / (8.78 x10^3 + 244*BMI)</td>
</tr>
<tr>
<td>LBM2: Lean body mass method 2 (kg)</td>
<td>Weight &lt; 50 kg</td>
<td>3.8*(0.0215 xWeight^{0.6409} x Height^{0.7236})</td>
</tr>
<tr>
<td></td>
<td>Weight ≥ 50 kg</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.407<em>Weight + 0.267</em>Height – 19.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.252<em>Weight + 0.473</em>Height – 48.3</td>
</tr>
</tbody>
</table>
Table 3. Body size models estimated using both full and pediatric datasets.

<table>
<thead>
<tr>
<th></th>
<th>Apparent clearance: CL/F &amp; CLM/F</th>
<th>Apparent volume of distribution: V2/F &amp; V3/F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear Weight</td>
<td>$\theta_1 \times \left(\frac{WT}{38}\right)$</td>
<td>$\theta_3 \times \left(\frac{WT}{38}\right)$</td>
</tr>
<tr>
<td>Allometric Scaling</td>
<td>$\theta_1 \times \left(\frac{WT}{38}\right)^{0.75}$</td>
<td>$\theta_3 \times \left(\frac{WT}{38}\right)$</td>
</tr>
<tr>
<td>Estimated Weight</td>
<td>$\theta_1 \times \left(\frac{WT}{38}\right)^{\theta_2}$</td>
<td>$\theta_3 \times \left(\frac{WT}{38}\right)^{\theta_4}$</td>
</tr>
<tr>
<td>Linear BSA</td>
<td>$\theta_1 \times \left(\frac{BSA}{1.23}\right)$</td>
<td>$\theta_3 \times \left(\frac{BSA}{1.23}\right)$</td>
</tr>
<tr>
<td>Estimated BSA</td>
<td>$\theta_1 \times \left(\frac{BSA}{1.23}\right)^{\theta_2}$</td>
<td>$\theta_3 \times \left(\frac{BSA}{1.23}\right)^{\theta_4}$</td>
</tr>
<tr>
<td>Linear LBM1</td>
<td>$\theta_1 \times \left(\frac{LBM1}{28}\right)$</td>
<td>$\theta_3 \times \left(\frac{LBM1}{28}\right)$</td>
</tr>
<tr>
<td>Estimated LBM1</td>
<td>$\theta_1 \times \left(\frac{LBM1}{28}\right)^{\theta_2}$</td>
<td>$\theta_3 \times \left(\frac{LBM1}{28}\right)^{\theta_4}$</td>
</tr>
<tr>
<td>Linear LBM2</td>
<td>$\theta_1 \times \left(\frac{LBM2}{31}\right)$</td>
<td>$\theta_3 \times \left(\frac{LBM2}{31}\right)$</td>
</tr>
<tr>
<td>Estimated LBM2</td>
<td>$\theta_1 \times \left(\frac{LBM2}{31}\right)^{\theta_2}$</td>
<td>$\theta_3 \times \left(\frac{LBM2}{31}\right)^{\theta_4}$</td>
</tr>
</tbody>
</table>
Table 4. A summary of the results obtained from the Allometric Scaling model and Linear BSA model as implemented with the pediatric dataset. RSE: Relative standard error.

<table>
<thead>
<tr>
<th>Parameter ( ^a )</th>
<th>Allometric Scaling</th>
<th>Linear BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model estimate (Bootstrap %RSE)</td>
<td>Bootstrap 95% CI</td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>923 (9.41%)</td>
<td>765, 1101</td>
</tr>
<tr>
<td>V2/F (L)</td>
<td>1130 (16.6%)</td>
<td>794, 1538</td>
</tr>
<tr>
<td>CLM/F (L/h)</td>
<td>65.1 (8.54%)</td>
<td>55.2, 77.5</td>
</tr>
<tr>
<td>V3F (L/hr)</td>
<td>79.1 (12.1%)</td>
<td>61.6, 101</td>
</tr>
<tr>
<td>Ka (hr(^{-1}))</td>
<td>2.46 (20.7%)</td>
<td>1.92, 3.92</td>
</tr>
<tr>
<td>Gender on CL/F</td>
<td>1.07 (12.0%)</td>
<td>0.826, 1.34</td>
</tr>
<tr>
<td>Gender on V2/F</td>
<td>1.06 (22.9%)</td>
<td>0.738, 1.72</td>
</tr>
<tr>
<td>Gender on CLM/F</td>
<td>1.05 (10.6%)</td>
<td>0.861, 1.29</td>
</tr>
<tr>
<td>Gender on V3/F</td>
<td>0.891 (17.6%)</td>
<td>0.600, 1.21</td>
</tr>
<tr>
<td>IIV-CL/F</td>
<td>0.279 (22.4%)</td>
<td>0.165, 0.412</td>
</tr>
<tr>
<td>IIV-V2/F</td>
<td>0.830 (21.1%)</td>
<td>0.499, 1.19</td>
</tr>
<tr>
<td>IIV - CLM/F</td>
<td>0.248 (25.7%)</td>
<td>0.147, 0.408</td>
</tr>
<tr>
<td>IIV-V3/F</td>
<td>0.414 (29.7%)</td>
<td>0.192, 0.643</td>
</tr>
<tr>
<td>IIV- Ka</td>
<td>0.987 (50.8%)</td>
<td>0.548, 2.55</td>
</tr>
<tr>
<td>Residual variability (( \sigma^2 )) for AS</td>
<td>0.586 (29.2%)</td>
<td>0.510, 1.19</td>
</tr>
<tr>
<td>Residual variability (( \sigma^2 )) for DHA</td>
<td>0.876 (15.5%)</td>
<td>0.575, 1.13</td>
</tr>
</tbody>
</table>

\( ^a \) CL/F, V2/F, and Ka are artesunate apparent clearance, apparent volume of distribution, and absorption rate constant, respectively. CLM/F and V3/F are DHA apparent clearance and apparent volume of distribution, respectively.
FIGURE LEGENDS

Figure 1. Best fit lines for model predicted typical DHA weight-normalized apparent clearance (CLM/F).

Figure 2. Best fit lines for model predicted typical DHA weight-normalized apparent volume of distribution (V3/F).

Figure 3. Covariate effect plots for gender on CLM/F. Distributions correspond to the 2.5th to 97.5th percentile for gender effects obtained from the bootstrap results from each model.

Figure 4. Covariate effect plots for gender on V3/F. Distributions correspond to the 2.5th to 97.5th percentile for gender effects obtained from the bootstrap results from each model.

Figure 5: Goodness-of-fit plots for DHA in the full covariate model. The solid lines are lines of identity. The broken lines are smoothing lines. Both plots represent the pediatric Linear BSA model. CWRES: Conditional Weighted Residuals.

Figure 6: VPC plots for DHA stratified by age for the full population linear BSA model. The open circles represent the observed concentrations, the solid line represents the median of the observed data, the dashed lines represent the 5th and 95th percentiles for the observed data, and the shaded areas represent the 95% confidence intervals surrounding the simulation derived prediction intervals (5th, 50th, and 95th percentiles) obtained from the simulations.

Figure 7. Plots of DHA Conditional Weighted Residuals (CWRES) vs. age for pediatric Linear BSA and Allometric Scaling models.