

A Bayesian Approach for Population Pharmacokinetic Modeling of Dihydroartemisinin

Bayesian Statistics (22S:138) – Project Final Report

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ABSTRACT

Dihydroartemisinin (DHA) is an active metabolite of artesunate (ARTS) and is highly effective in the treatment of malaria, a life threatening parasitic disease. ARTS causes rapid reduction in parasitemia and fever in patients with falciparum malaria and is associated with a radical cure rate of (> 90%) if administered for a period of 5-7 days.

A phase I single oral dose escalation study in healthy volunteers was conducted to characterize the pharmacokinetics and to assess safety and tolerability of ARTS and DHA. The study was a randomized, double-blind placebo-controlled, staggered, parallel design to study ARTS doses from 2 to 5 mg/kg in 36 healthy Korean subjects. Plasma samples for pharmacokinetic assessment were obtained at regular time intervals and analyzed using a previously validated LC-MS method. Non-compartmental method of analysis was used for the determination of pharmacokinetic parameters using WinNonlin.

Since ARTS is rapidly and almost completely converted to its active metabolite DHA, in this present study we are interested only in characterizing the pharmacokinetics of DHA by a Bayesian approach with PKBugs/WinBUGS using data from this completed phase I trial. Permission to use the dataset was obtained from Dr. L. Fleckenstein, the director of the Clinical Pharmacokinetics Lab, College of Pharmacy.

In conclusion, using a Bayesian approach with a one-compartment structural model with first order input and elimination, the estimates for Cl/F , V/F , t_{lag} , k_e and k_a for DHA were 130.06 L/hr, 195.98 L, 8.34 minutes, 0.664/hr and 1.271/hr respectively.

INTRODUCTION

Malaria

Malaria is among the top 10 killer diseases in the world. It accounts for 1.5 to 2.7 million deaths worldwide, 90% of which occur in tropical Sahara, where malaria is the leading cause of mortality in children under five years of age. Outside Africa, some two-thirds of the remaining cases occur in just three countries; Brazil, India and Sri Lanka. However, malaria exists in some 100 countries, which are visited by more than 125 million international travelers every year¹. In 2005, it is reported that over 10,000 international travelers were infected with malaria while visiting countries where the disease is endemic. Due to under-reporting, the exact figure may be as high as 30,000². Therefore, malaria is not a problem confined to African countries anymore, but a real world health issue.

Malaria is a life threatening parasitic disease transmitted by anopheles mosquitoes. Human malaria is caused by four different species of the protozoan parasite *Plasmodium*: *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. The most severe form of the disease is caused by *P. falciparum*, in which variable clinical features include fever, chills, headache, muscular aching and weakness, vomiting, cough, diarrhea and abdominal pain; other symptoms related to organ failure may supervene, such as: acute renal failure, generalized convulsions, circulatory collapse, followed by coma and death³.

The main factor contributing to the increasing malaria mortality and morbidity is the widespread resistance of *P. falciparum* to conventional antimalarial drugs, such as chloroquine, sulfadoxine–pyrimethamine (SP) and amodiaquine. Therefore, WHO recommends the use of combination therapies, preferably those containing artemisinin derivatives, in countries experiencing resistance to conventional monotherapies⁴. Artesunate-pyronaridine combination is one of the antimalarial drugs under development to combat the issue of resistance.

Artemisinin, artesunate and dihydroartemisinin

Artemisinin (Qinghaosu) is a naturally occurring sesquiterpene lactone containing an endoperoxide group extracted from the leaves of Chinese herb *Artemisia annua*. Studies in China, Vietnam and Thailand have shown that artemisinin and its derivatives quickly reduce parasitemia in patient with acute falciparum malaria and induce fast resolution of symptoms without any toxicity^{5,6}. Parasitic and fever clearance times have been shown to be shorter than those observed with other classical antimalarials. Various derivatives of artemisinin with improved pharmacological properties have been synthesized because artemisinin is poorly soluble in either water or oil. These include sparingly water soluble artesunate (ARTS) and the lipophilic alkyl ethers: arteether and artemether.

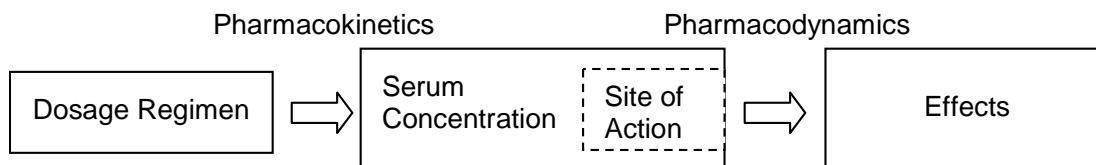
Artesunate (ARTS) is a water-soluble hemisuccinate ester of dihydroartemisinin (DHA) and is highly effective in the treatment of malaria. ARTS is one of the semi synthetic derivative of artemisinin developed for clinical use, and has advantage over the naturally occurring product in that it can be administered parenterally, an important factor for use in patient with severe falciparum malaria. ARTS is rapidly converted to its active metabolite DHA *in vivo* which is responsible for the antimalarial action. Hence, it is regarded

as a prodrug of DHA. Substantial conversion into DHA takes place in the intestine and liver prior to reaching the systemic circulation.

Pharmacokinetic concepts

Pharmacokinetics (PK), sometimes referred to as what the body does to a drug, is the study of the time course of the drug concentrations within a biological system.

Concentrations over time are determined by the rate and extent of the processes of absorption, distribution, metabolism, and excretion (ADME). Pharmacokinetics is often studied in conjunction with pharmacodynamics which describe as what a drug does to the body.



A standard approach to describing PK behavior is to represent the body by a series of compartments. Under a specified route of administration of drug, the compartmental representation leads to a system of differential equations in terms of parameters related to the ADME processes to describe mathematically the instantaneous rates of change of the amounts or concentrations of agent residing in each compartment based on assumptions on how the drug moves within and among the compartments. The solution of the system provides a formal mathematical description of the concentrations in the compartments at any time as a function of the parameters.

The concentrations of the drug in blood over time are dictated by some pharmacokinetic parameters, such as clearance, volume of distribution, elimination rate constant, absorption rate constant and what not, depending on the model chosen. These parameters all have meaningful interpretations, although they do not correspond precisely to specific physiological phenomena. Given the values of these parameters for a specific individual, one can determine the concentration of drug at any given time t following a dose of any magnitude D . It is this feature that enables clinical pharmacists to predict the time course of concentrations under different dosing regimens and thereby formulate regimens that achieve concentrations in a desired range. It should be noted that the empirical PK models, e.g. one compartmental model, are deterministic mathematical models for the PK behavior of only a single individual.

In 1972, the concepts of population pharmacokinetics were introduced. Population pharmacokinetics is the study of the sources and correlates of variability in drug concentrations among individuals. The population model defines at least two levels of hierarchy. At the first level, pharmacokinetic observations in an individual (such as concentrations of drug in plasma) are viewed as arising from an individual probability model, whose mean is given by a pharmacokinetic model (e.g., a biexponential model) quantified by individual-specific parameters, which may vary according to the value of

individual-specific time varying covariates. The variance of individual pharmacokinetic observations (intrasubject variance) is also modeled using additional individual-specific pharmacokinetic parameters. The population model employs certain inferential approaches, which focus on providing estimates of some or all of the components of variability, along with estimates of the mean parameters. At the second level, the individual parameters are regarded as random variables and the probability distribution of these (often the mean and variance, i.e., intersubject variance) is modeled as a function of individual-specific covariates. These models, their parameter values, and the use of study designs and data analysis methods designed to elucidate population pharmacokinetic models and their parameter values, are what is meant by population pharmacokinetics.

METHODS

Study design and data set

The data used in this project was obtained from a completed phase I single-dose escalation study to assess the pharmacokinetics and tolerability of ARTS and DHA in healthy Korean volunteers. A single oral ascending dose, randomized, double-blind, placebo-controlled, staggered, parallel study design was used to study doses from 2 to 5 mg/kg in 36 healthy Korean subjects. Subjects were screened depending on the criteria specified for the study. As subjects were enrolled in the study, they were assigned unique consecutive number by computer generated randomized schedule. Subjects were enrolled in cohorts of 9 for each dose level (2, 3, 4, and 5 mg/kg) and were randomly allocated to either the ARTS or matching placebos such that there were 7 active and 2 placebo subjects per cohort.

Before dosing, the subjects were fasted from approximately midnight the previous evening until 4 hours after receiving the test drug the following day. Each subject received a single oral dose of ARTS or placebo and thirteen samples were collected over a period of 12 hours at the following times: 0, 0.33, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 4, 5, 8 and 12 hours. Plasma concentration of ARTS and DHA were determined using a validated liquid chromatography and mass spectrometric method. Pharmacokinetic analysis was also carried out using WinNonlin and non-compartmental method of analysis was used for the determination of pharmacokinetic parameters.

For this project, we modeled only the DHA data. Historically, ARTS was not detected in the blood in up to 50% of cases after oral administration and thus only DHA was modeled. This was attributed to poor sample processing and storage of samples after collection and difficulty in finding a reliable and sensitive method for the determination of ARTS and DHA simultaneously. Even though we managed to measure the concentrations of ARTS in all subjects, we modeled only the data for DHA for the purpose of this project, for the following reasons:

1. DHA is the active metabolite of ARTS which has the anti-malarial activity. Thus, we are interested primarily in characterizing the pharmacokinetic behavior of DHA.
2. The concentration-time profile for ARTS in these subjects showed large inter-subject variability. Also, the elimination half-life of ARTS is so short that only very few

measurements were obtained for each of these subjects. Therefore, modeling the ARTS data might not yield meaningful results.

Figure 1 shows the mean plasma concentration-time profile for DHA following administration of 2-5mg/kg of ARTS in 28 healthy Korean volunteers. Figure 2 shows the plasma concentration-time profile for DHA in log scale for all the 28 subjects.

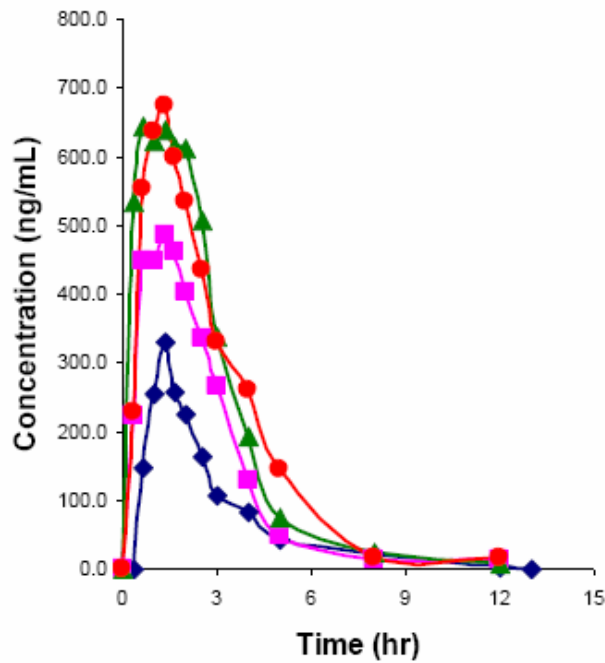


Figure 1: Mean plasma concentration-time profile for DHA following administration of 2-5mg/kg of ARTS in 28 healthy Korean volunteers (7 for each dose level excluding two placebos). Data are presented as mean value. (♦ – 2mg, ■ – 3 mg, ● – 4mg, ▲ – 5 mg/kg of dose)

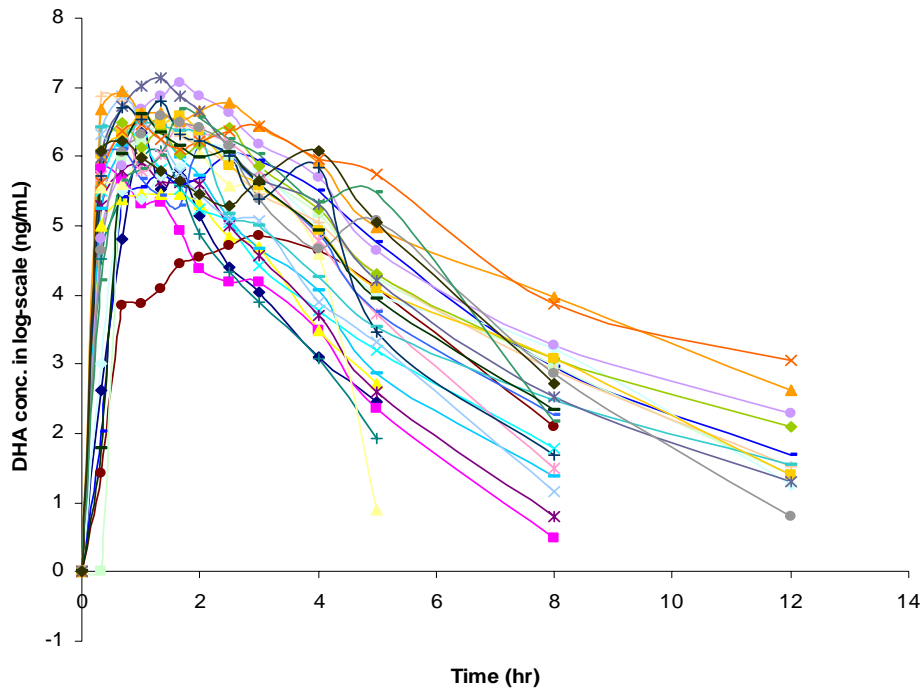


Figure 2: Plasma concentration-time profile for DHA in log scale for all the 28 subjects.

Data analysis

The data were analyzed using PKBugs (version 1.1)/WinBUGS (version 1.3). PKBugs, an add-on interface to WinBUGS was used to construct an object-oriented internal representation of the model that is compatible with WinBUGS. Then WinBUGS can be used, in the normal way, to conduct the remainder of the analysis.

A three stage hierarchical model (shown below) was used to analyze the data^{7,8}. In the following notation, bold font indicates a vector or matrix and “ \sim ” is used to indicate “distributed as.”

Stage 1: Model for the data

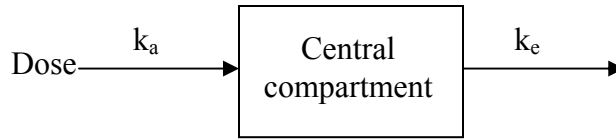
$$y_{ij} = f(\boldsymbol{\theta}_i, x_{ij}) + \varepsilon_{ij}$$

$$\varepsilon_{ij} \sim N(0, \tau), \sigma^2 = \tau^{-1}$$

where y_{ij} denotes the j th observation for the i th patient, $f(\boldsymbol{\theta}_i, x_{ij})$ is the expected value of the data from the model, $\boldsymbol{\theta}_i$ is a vector of individual pharmacokinetic parameter values for the i th individual, x_{ij} is a sampling time, ε_{ij} is the residual difference between the expected value and the observed value, and N represents a normal distribution with (in

this case) zero mean and variance σ^2 . The normal distribution is parameterized as mean and precision τ (the inverse of variance).

We used one compartmental model with first order absorption and first order elimination to describe DHA pharmacokinetics. The schematic diagram of the one compartmental model is shown below:



The expression for DHA concentration at time t in the central (blood) compartment is given by the following equation:

$$C(t) = \frac{k_a FD}{V(k_a - k_e)} \{ \exp(-k_e(t - t_{lag})) - \exp(-k_a(t - t_{lag})) \}, \quad k_e = \frac{Cl}{V}$$

Where:

$C(t)$ is the concentration of DHA in central compartment at a given time, t ;

D is the dose of ARTS administered;

F is the bioavailability of DHA or the fraction of DHA absorbed into the systemic circulation;

k_a is the first order input rate constant for DHA;

k_e is the first order elimination rate constant for DHA from the central compartment;

t_{lag} is the delay in time before DHA first appears in the central compartment;

V is the volume of distribution, defined as the apparent space which DHA distributes into;

Cl is the drug clearance defined as the volume of blood or plasma that is totally cleared of DHA per unit time.

In PKBugs, the model parameterizes $\log(Cl/F)$, $\log(V/F)$, $\log(\text{lag-time})$, $\log(k_a^*)$;

where $k_a^* = k_a - k_e$

Stage 2: Model for the variability between subjects

$$\theta_i \sim N_p(\boldsymbol{\theta}, \boldsymbol{\Omega}^{-1})$$

where $\boldsymbol{\theta}$ is a vector of mean population pharmacokinetic parameters and $\boldsymbol{\Omega}$ is the variance–covariance matrix of between subject random variability. N_p represents a p -dimensional multivariate normal distribution where p is the number of parameters.

Stage 3: Model for the Priors

$$\tau \sim Ga(a, b)$$

$$\boldsymbol{\theta} \sim N_p(\boldsymbol{\mu}.\text{mean}, \boldsymbol{\Sigma}^{-1})$$

$$\boldsymbol{\Omega} \sim Wi_p(\mathbf{R}, \rho)$$

where Ga denotes the gamma distribution with parameters a and b , $\boldsymbol{\mu}.\text{mean}$ is a vector of prior population mean values of the parameters and $\boldsymbol{\Sigma}^{-1}$ is the precision matrix that describes the informativeness of the prior distribution of $\boldsymbol{\theta}$. Wi represents a Wishart distribution with parameters \mathbf{R} and ρ . \mathbf{R} is the scaled prior value of the variance covariance matrix of between-subject random variability and ρ is the degrees of freedom of the Wishart distribution, the larger the value of ρ the more informative the prior \mathbf{R} . \mathbf{R} is provided automatically by PKBugs. Figure 3 shows the graphical representation of the three-stage hierarchical model used. Note that we did not incorporate any covariates in this analysis, thus theta.mean is equal to mu.

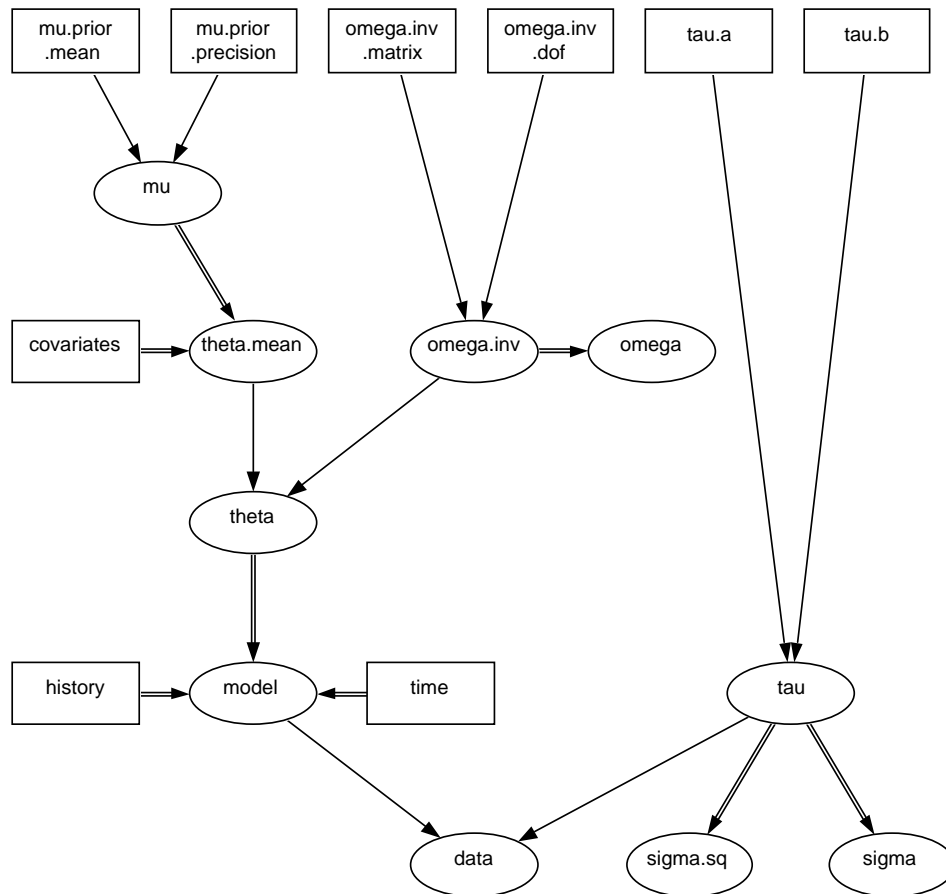


Figure 3: Graphical representation of the three-stage hierarchical model.

RESULTS

3 Markov chains of 100,000 iterations for each chain were generated. Figures 4, 5, and 6 show the history plots, Gelman Rubin plots and autocorrelation plots for the PK parameters $\log(CI/F)$ as $\mu[1]$, $\log(V/F)$ as $\mu[2]$, $\log(\text{lag-time})$ as $\mu[3]$ and $\log(k_a^*)$ as $\mu[4]$ respectively.

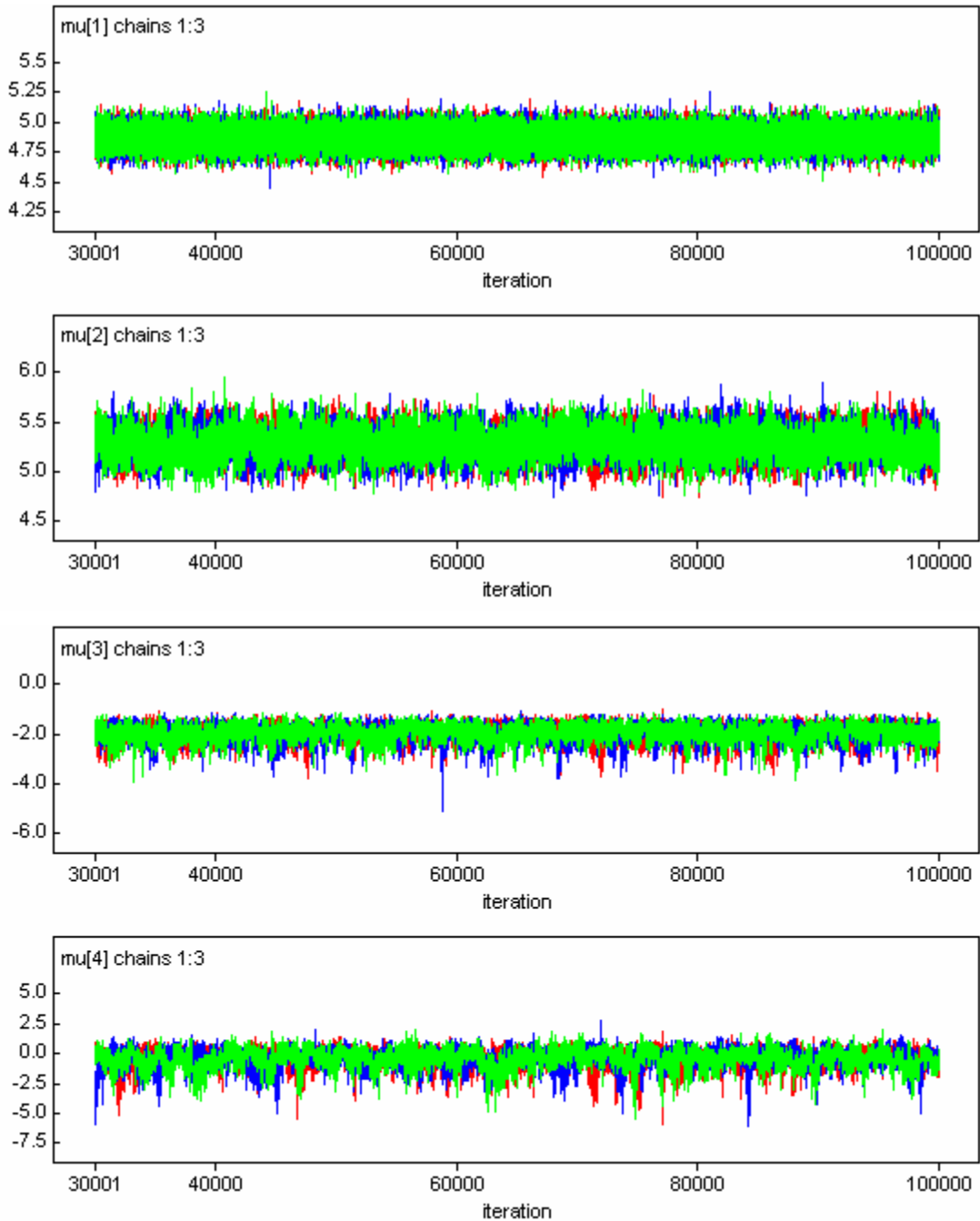


Figure 4: History plots for the PK parameters.

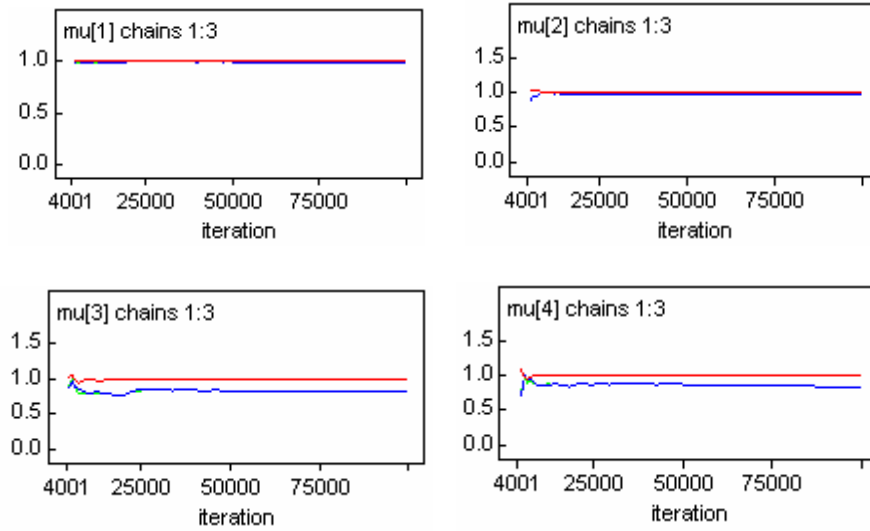


Figure 5: Gelman Rubin plots for the PK parameters.

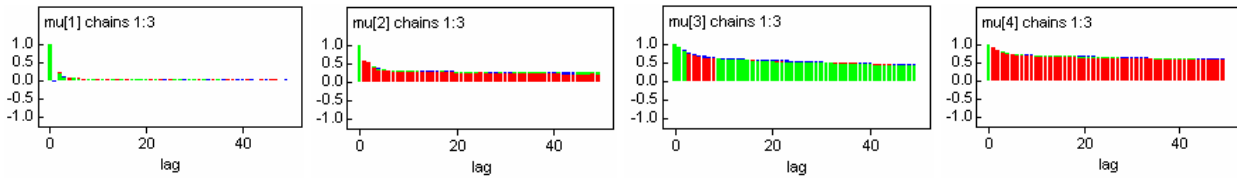


Figure 6: Autocorrelation plots for the PK parameters.

From the history plots, all the four parameters of interest seemed to achieve satisfactory convergence. After analyzing the Gelman Rubin statistic generated from the Gelman Rubin plots for all the parameters, we decided to discard the first 30,000 iterations as burn-ins. The autocorrelation for $\mu[1]$ was minimal, the autocorrelation for $\mu[2]$ was not as good, while the autocorrelation for $\mu[3]$ and $\mu[4]$ were very high. The high autocorrelation for $\mu[3]$ and $\mu[4]$ might be due to the fact that we had relatively poor prior knowledge about the two parameters. To compensate for the high autocorrelation seen, we decided to run a large number of iterations to reduce the MC errors. Table 1 summarizes the statistics for the μ 's, ω and σ .

Table 1: Statistical output

Node	mean	sd	MC error	2.50%	median	97.50%	start	sample
mu[1]	4.868	0.0719	4.00E-04	4.728	4.868	5.012	30001	210000
mu[2]	5.278	0.122	0.002082	5.039	5.279	5.518	30001	210000
mu[3]	-1.976	0.3176	0.007016	-2.725	-1.933	-1.478	30001	210000
mu[4]	-0.4986	0.7385	0.01935	-2.289	-0.3801	0.6193	30001	210000
omega[1,1]	0.1194	0.03989	2.34E-04	0.06272	0.1125	0.2164	30001	210000
omega[1,2]	0.005555	0.04486	3.78E-04	-0.08593	0.006022	0.09508	30001	210000
omega[1,3]	0.0552	0.08881	8.93E-04	-0.09825	0.0462	0.2574	30001	210000
omega[1,4]	-0.09256	0.1898	0.002395	-0.5183	-0.07693	0.2491	30001	210000
omega[2,1]	0.005555	0.04486	3.78E-04	-0.08593	0.006022	0.09508	30001	210000
omega[2,2]	0.2791	0.09681	0.001123	0.1427	0.2619	0.5141	30001	210000
omega[2,3]	-0.321	0.1916	0.003741	-0.7934	-0.2847	-0.05627	30001	210000
omega[2,4]	0.7439	0.4645	0.01027	0.1688	0.6398	1.938	30001	210000
omega[3,1]	0.0552	0.08881	8.93E-04	-0.09825	0.0462	0.2574	30001	210000
omega[3,2]	-0.321	0.1916	0.003741	-0.7934	-0.2847	-0.05627	30001	210000
omega[3,3]	0.968	0.685	0.01604	0.2396	0.782	2.793	30001	210000
omega[3,4]	-1.573	1.021	0.0247	-4.251	-1.319	-0.3764	30001	210000
omega[4,1]	-0.09256	0.1898	0.002395	-0.5183	-0.07693	0.2491	30001	210000
omega[4,2]	0.7439	0.4645	0.01027	0.1688	0.6398	1.938	30001	210000
omega[4,3]	-1.573	1.021	0.0247	-4.251	-1.319	-0.3764	30001	210000
omega[4,4]	4.233	3.642	0.0927	0.8941	3.178	13.99	30001	210000
sigma	78.69	3.653	0.02555	71.92	78.55	86.26	30001	210000

Pharmacokinetic parameters calculation

1. $\log (Cl/F) = \mu[1] = 4.868$
 $Cl/F = 130.06 \text{ L/hr}$

2. $\log (V/F) = \mu[2] = 5.278$
 $V/F = 195.98 \text{ L}$

3. $\log (t_{lag}) = \mu[3] = -1.976$
 $t_{lag} = 0.139 \text{ hr} = 8.34 \text{ minutes}$

4. $\log (k_a^*) = \mu[4] = -0.4986$
 $k_a^* = 0.607/\text{hr}$

5. $k_e = Cl/V = 130.06/195.98 = 0.664/\text{hr}$

6. $k_a = k_a^* + k_e = 1.271/\text{hr}$

DISCUSSION

Table 2 shows the comparison of the PK parameters estimated in this analysis using Bayesian approach to the estimation obtained by non-compartmental analysis using WinNonlin for the same dataset.

Table 2: Comparison of the PK parameters using Bayesian approach and non-compartmental approach.

PK parameters	Bayesian approach	Non-compartmental approach
Cl/F (L/hr)	130.06	137.86
V/F (L)	195.98	248.46
k_e (1/hr)	0.664	0.593

From Table 2, the estimates for Cl/F and k_e were very similar using the two different approaches, while the V/F estimation was lower using Bayesian approach. t_{lag} and k_a were not estimated using non-compartmental approach.

The posterior means for all omegas were small except for omega [4,4]. This suggests that the between-subject variances or covariances between the mu's were small except for the variance of mu[4] which is k_a^* . The estimate for the posterior mean of sigma was very large, suggesting that high variability exists for residual error.

CONCLUSION

Using a Bayesian approach with a one-compartment structural model with first order input and elimination, the estimates for Cl/F, V/F, t_{lag} , k_e and k_a for DHA were 130.06 L/hr, 195.98 L, 8.34 minutes, 0.664/hr and 1.271/hr respectively.

APPENDIX: WinBUGS CODE

```

model {
  for (i in 1:n.ind) {
    for (j in off.data[i]:(off.data[i + 1] - 1)) {
      data[j] ~ dnorm(model[j], tau)(lower[j], upper[j])
      model[j] <- pk.model(1, theta[i, 1:p], time[j], hist[off.hist[i]:(off.hist[i + 1] - 1), 1:n.col], pos[j])
    }
    theta[i, 1:p] ~ dnorm(theta.mean[i, 1:p], omega.inv[1:p, 1:p])
    theta.mean[i, 1] <- mu[1]
    theta.mean[i, 2] <- mu[2]
    theta.mean[i, 3] <- mu[3]
    theta.mean[i, 4] <- mu[4]
  }
  tau ~ dgamma(tau.a, tau.b)
  sigma <- 1 / sqrt(tau)
  mu[1:q] ~ dnorm(mu.prior.mean[1:q], mu.prior.precision[1:q, 1:q])
  omega.inv[1:p, 1:p] ~ dwish(omega.inv.matrix[1:p, 1:p], omega.inv.dof)
  for (i in 1:p) {
    for (j in 1:p) {
      omega[i, j] <- inverse(omega.inv[1:p, 1:p], i, j)
    }
  }
}

list(
  n.ind = 28, n.col = 12,
  p = 4, q = 4,

  hist = structure(
    .Data = c(
      0.0, 0.0, 4.0, 1.0, 1.3E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.3E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.1E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.0E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.3E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.0E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.2E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.65E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.95E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.5E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.5E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.95E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.95E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 1.8E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 2.6E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 2.2E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 2.4E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 2.8E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 2.8E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 2.6E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 2.4E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 2.5E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 3.25E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 2.5E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 3.25E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 2.75E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 3.25E+5, 0.0, 0.0, NA, NA, NA, NA, NA,
      0.0, 0.0, 4.0, 1.0, 3.0E+5, 0.0, 0.0, NA, NA, NA, NA, NA),
    .Dim = c(28, 12)),

  data = c(
    13.8, 122.1, 564.5, 252.0, 337.4, 173.0,
    80.8, 57.1, 22.4, 11.6, NA, NA,
    332.2, 287.2, 201.9, 206.6, 138.9, 80.4,
    65.8, 65.3, 32.52, 10.6, 1.6, NA,
    149.6, 219.6, 236.4, 236.0, 232.0, 205.0,
    124.8, 108.5, 33.0, 15.1, NA, NA,
    244.6, 550.1, 502.6, 332.8, 253.5, 187.5,

```

154.9,	83.5,	43.3,	24.4,	5.9,	NA,
198.1,	327.8,	367.9,	281.4,	275.2,	269.9,
147.4,	96.3,	40.3,	13.5,	2.2,	NA,
4.2,	46.6,	48.5,	59.6,	86.0,	95.1,
111.1,	128.1,	103.7,	61.3,	8.1,	NA,
92.1,	227.1,	398.7,	441.1,	261.2,	132.3,
75.9,	48.9,	21.7,	6.8,	NA,	NA,
7.6,	221.1,	259.6,	311.3,	268.1,	295.2,
421.9,	375.7,	247.8,	117.2,	19.3,	5.4,
190.8,	472.3,	730.7,	568.6,	400.2,	301.8,
161.4,	107.1,	58.4,	17.4,	4.0,	NA,
20.4,	243.5,	217.5,	307.7,	347.8,	478.2,
449.0,	332.5,	145.4,	55.8,	25.3,	3.5,
NA,	396.0,	500.9,	662.5,	646.0,	524.4,
388.8,	297.1,	174.3,	71.2,	20.0,	4.0,
254.9,	271.1,	427.0,	613.0,	605.6,	422.5,
262.5,	265.6,	99.0,	2.4,	10.8,	557.0,
964.7,	667.8,	519.2,	318.5,	232.2,	163.0,
159.1,	49.5,	27.9,	3.2,	NA,	301.7,
568.9,	330.6,	412.3,	647.2,	571.6,	494.9,
326.7,	119.7,	41.2,	4.4,	NA,	121.8,
353.4,	789.0,	966.4,	1156.0,	958.3,	760.6,
479.6,	295.9,	104.1,	26.4,	9.8,	957.4,
904.8,	665.7,	649.6,	542.6,	466.3,	358.8,
231.9,	154.7,	74.2,	18.3,	4.5,	396.7,
442.7,	293.2,	229.5,	197.9,	469.5,	408.9,
273.4,	132.3,	42.6,	9.6,	NA,	614.3,
553.1,	657.8,	627.0,	578.6,	558.3,	176.2,
146.7,	69.9,	34.4,	11.8,	4.6,	430.9,
664.6,	453.2,	591.1,	421.2,	480.6,	616.3,
353.9,	190.6,	73.8,	21.9,	8.2,	413.0,
552.8,	743.5,	648.6,	728.2,	579.4,	350.0,
264.0,	137.0,	59.8,	21.9,	4.1,	799.9,
1038.3,	766.2,	767.2,	680.4,	780.3,	879.8,
621.3,	366.7,	145.1,	52.6,	13.7,	279.9,
588.8,	640.8,	516.4,	450.1,	509.6,	587.8,
622.8,	386.8,	310.1,	47.9,	21.2,	395.1,
832.6,	1107.8,	1267.3,	970.3,	785.4,	445.2,
293.3,	203.9,	66.5,	12.6,	3.7,	103.6,
431.6,	556.3,	726.2,	663.6,	608.2,	465.9,
218.0,	107.3,	157.8,	17.6,	2.2,	306.3,
806.7,	691.8,	897.2,	561.9,	508.8,	402.2,
216.9,	343.1,	31.9,	5.4,	NA,	67.1,
282.2,	333.2,	410.3,	797.7,	700.0,	521.2,
414.7,	205.4,	238.3,	8.8,	59.8,	5.9,
417.8,	735.1,	564.3,	468.6,	396.6,	425.3,
272.3,	139.1,	52.0,	10.5,	NA,	436.1,
507.9,	393.1,	330.9,	285.2,	233.0,	197.9,
280.9,	435.8,	156.3,	15.2,	NA),	

off.hist = c(
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,
13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24,
25, 26, 27, 28, 29),

off.data = c(
1, 13, 25, 37, 49, 61, 73, 85, 97, 109, 121, 133,
144, 156, 168, 180, 192, 204, 216, 228, 240, 252, 264, 276,
288, 300, 312, 324, 336),

lower = c(
-1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10,
-1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10,
-1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10,
-1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10,
-1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10,
-1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10,
-1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10, -1.0E+10,


```

4.605170186, 4.605170186, 0.0, 0.0,
4.605170186, 4.605170186, 0.0, 0.0,
4.605170186, 4.605170186, 0.0, 0.0,
4.605170186, 4.605170186, 0.0, 0.0,
4.605170186, 4.605170186, 0.0, 0.0,
4.605170186, 4.605170186, 0.0, 0.0,
4.605170186, 4.605170186, 0.0, 0.0,
4.605170186, 4.605170186, 0.0, 0.0,
4.605170186, 4.605170186, 0.0, 0.0,
4.605170186, 4.605170186, 0.0, 0.0,
4.605170186, 4.605170186, 0.0, 0.0),
.Dim = c(28, 4)),
tau = 0.1,
mu = c(
-10, -10, -10, -10),
omega.inv = structure(
.Data = c(
20, 0.0, 0.0, 0.0,
0.0, 20, 0.0, 0.0,
0.0, 0.0, 20, 0.0,
0.0, 0.0, 0.0, 20),
.Dim = c(4, 4))
)

```

REFERENCES

1. WHO. http://www.searo.who.int/en/Section10/Section21/Section334_4008.htm.
2. WHO. http://whqlibdoc.who.int/publications/2005/9241580364_chap7.pdf
3. Suh KN, Kain KC, Keystone JS, 2004. "Malaria." *Canadian Medical Association Journal* 170(11):1693-702
4. *Antimalarial drug combination therapy*: Report of WHO technical consultation, 4-5 April 2001. Geneva, World Health Organization (WHO/CDS/RBM/2001.35).
5. Ashton M, Nguyen DS, 1998 "Artemisinin kinetics and dynamics during oral and rectal treatment of uncomplicated malaria." *Clinical Pharmacology and Therapeutics* 63(4): 482-93
6. Hassan Alin M, Ashton M, 1996. "Multiple dose pharmacokinetics of oral artemisinin and comparison of its efficacy with that of oral artesunate in falciparum malaria patients." *Transaction of Royal Society of Tropical Medicine and Hygiene* 90(1): 61-5
7. Spiegelhalter D, Thomas A, Best N, 2000. "WinBUGS Version 1.3 User Manual." Vol Version 1.3. London: Imperial College.
8. Duffull SB, Kirkpatrick CMJ, Green B, Holford NHG, 2004. "Analysis of population pharmacokinetic data using NONMEM and WinBUGS." *Journal of Biopharmaceutical Statistics* 15(1):53 - 73