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Pharmacokinetic-Pharmacodynamic Model for the Effect of L-Arginine on Endothelial Function in Patients with Moderately Severe Falciparum Malaria

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Impaired organ perfusion in severe falciparum malaria arises from microvascular sequestration of parasitized cells and endothelial dysfunction. Endothelial dysfunction in malaria is secondary to impaired nitric oxide (NO) bioavailability, in part due to decreased plasma concentrations of L-arginine, the substrate for endothelial cell NO synthase. We quantified the time course of the effects of adjunctive L-arginine treatment on endothelial function in 73 patients with moderately severe falciparum malaria derived from previous studies. Three groups of 10 different patients received 3 g, 6 g, or 12 g of L-arginine as a half-hour infusion. The remaining 43 received saline placebo. A pharmacokinetic-pharmacodynamic (PKPD) model was developed to describe the time course of changes in exhaled NO concentrations and reactive hyperemia-peripheral arterial tonometry (RH-PAT) index values describing endothelial function and then used to explore optimal dosing regimens for L-arginine. A PK model describing arginine concentrations in patients with moderately severe malaria was extended with two pharmacodynamic biomeasures, the intermediary biochemical step (NO production) and endothelial function (RH-PAT index). A linear model described the relationship between arginine concentrations and exhaled NO. NO concentrations were linearly related to RH-PAT index. Simulations of dosing schedules using this PKPD model predicted that the time within therapeutic range would increase with increasing arginine dose. However, simulations demonstrated that regimens of continuous infusion over longer periods would prolong the time within the therapeutic range even more. The optimal dosing regimen for L-arginine is likely to be administration schedule dependent. Further studies are necessary to characterize the effects of such continuous infusions of L-arginine on NO and microvascular reactivity in severe malaria.

The majority of severe malaria cases and deaths are caused by *Plasmodium falciparum* (1, 2). While mortality has been reduced with wider use of the rapidly parasiticidal drug artesunate (3), and with improved supportive care, mortality from severe falciparum malaria remains 8 to 15% (4–7). Adjunctive therapies targeting the underlying pathogenic processes may reduce mortality further (8).

Impairment of organ perfusion arising from microvascular sequestration of parasitized red cells and endothelial dysfunction is central to the pathophysiology of severe falciparum malaria (9–12). Both adults and children with severe falciparum malaria have impaired bioavailability of nitric oxide (NO) (11, 13) and reduced plasma concentrations of L-arginine (11, 14), a semiessential amino acid and substrate for endothelial cell NO synthase. Impaired NO bioavailability is associated with endothelial activation (11), impaired endothelial function (11), impaired perfusion, and mortality (15) in severe malaria. Agents that increase NO bioavailability, such as L-arginine, inhaled NO, or NO donors, have been proposed as potential adjunctive treatments to improve endothelial function and microvascular perfusion in severe malaria. For patients with moderately severe malaria, comparable hypoargininemia has been described (11), although endothelial dysfunction is not as profound as in patients with severe malaria (16).

In a previous study with adults with moderately severe falciparum malaria, we described the natural time course of the recovery of plasma L-arginine concentrations and endothelial function (16). In addition, a nonrandomized trial evaluated the changes in L-arginine concentration by intervention with ascending-dose arginine infusions (17). We found that endothelial dysfunction in moderately severe malaria (5) is L-arginine reversible and that adjunctive L-arginine treatment was safe (18) and able to improve NO bioavailability (11). Although artesunate is very rapidly parasiticidal (7), it has not been able to reduce the case fatality in the first 48 h in adult severe malaria. It is possible, within the first 2 days when the arginine and NO concentrations are significantly reduced, that administration of L-arginine might be beneficial in improving microvascular function and organ perfusion in these patients.

In the current study, this existing pharmacokinetic (PK) model of L-arginine (17) was expanded to include two important pharmacodynamic (PD) biomeasures, exhaled NO concentrations and

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TABLE 1 Patient characteristics

Characteristic	L-Arginine infusion group	Saline infusion group	All subjects
No. of patients	30	43	73
No. (%) of males	20 (67)	28 (65)	48 (66)
No. (%) of Papuans	24 (80)	39 (90)	63 (86)
Weight (kg)[a]	57.5 (43–77)	57 (42–73)	57 (42–77)
Age (yrs)[a]	24.5 (18–54)	28 (18–56)	27 (18–56)

[a] Values for this parameter are medians, with ranges in parentheses.

the reactive hyperemia-peripheral arterial tonometry (RH-PAT) index, a validated measure of endothelial function (19, 20). The aim of this study was to quantify the time course of the effects of L-arginine adjunctive therapy on measures of endothelial function in patients with moderately severe malaria and to explore doses and dosing schedules of adjunctive L-arginine that would increase RH-PAT significantly and thus guide dosing regimens for intervention studies in severe malaria.

MATERIALS AND METHODS

Data. Patient data were available from two previous studies and are described elsewhere (11, 16, 17). Briefly, these studies included an observational study that addressed the natural history of arginine recovery involving 48 patients with moderately severe falciparum malaria (16) and a nonrandomized trial with another 30 patients (17). Overall, 78 adult patients with moderately severe falciparum malaria were included. They were patients at the Mitra Masyarakat Hospital in Timika, Papua, Indonesia. Thirty patients were included in an intervention arm receiving L-arginine, and 48 patients were in an observational group receiving a similar volume of saline to study the natural history of arginine recovery. Patient characteristics for both groups were not significantly different (Table 1). In the intervention arm, 3 g, 6 g, or 12 g of L-arginine was administered as a half-hour infusion to three groups of 10 patients. Plasma arginine concentrations were measured before and at the end of the infusion and at approximately 5, 20, 30, and 60 min and 2, 4, 8, 24, and 48 h after arginine administration. In addition, exhaled NO concentrations were measured before and approximately 0.5, 1, 1.5, 2, 3, 5, 20, and 48 h postdose. After the treatment, patients were monitored for 72 h or until well enough to be discharged. During this follow-up, the RH-PAT index (19, 20) was determined at approximately 1, 3, 20, and 48 h postdose and before discharge. Patients without pharmacodynamic data (i.e., NO and RH-PAT) were excluded from the pharmacokinetic-pharmacodynamic (PKPD) analysis, leaving 73 patients available for analysis.

Assays for arginine, nitric oxide, and RH-PAT index. (i) Determination of plasma concentrations of L-arginine. Venous blood was collected from patients and within 30 min after collection, plasma was separated by centrifugation and stored at −70°C until analysis. Amino acids were extracted from a plasma sample of 50 μl and mixed with an internal standard (50 μl of norleucine) and 200 μl of cold ethanol. Deproteinized plasma was derivatized with AccQFluor reagent (Waters Corp., Milford, MA), and amino acids were measured by high-performance liquid chromatography (Shimadzu, Kyoto, Japan) as described by Wang et al. (21). The assay was validated, and the linear range was quantified at 12.01 to 384.24 μM. The limit of detection and limit of quantification were 3.71 and 11.24 μM, respectively.

(ii) Determination of nitric oxide concentration in breath. Breath samples were analyzed with an NO analyzer (NIOX; Aerocrine AB, Solna, Sweden) using American Thoracic Society guidelines (22). The exhaled NO concentration (in parts per billion) was measured at a flow rate of 250 ml per second (11).

(iii) Determination of RH-PAT index. Reactive hyperemia-peripheral arterial tonometry (RH-PAT; Itamar Medical, Caesarea, Israel) is a validated noninvasive NO-dependent measure method of measuring endothelial function (19, 20). After a 20-min equilibration time, RH was induced by a 5-min occlusion of the arm. Changes in pulse wave amplitude before and after occlusion of the arm were measured by finger probes. The RH-PAT index was calculated by dividing the PAT scores during RH by the initial PAT scores (19).

Model development. (i) Population (PK) model. Data were analyzed using the nonlinear mixed effect modeling software NONMEM version 7.2 and Wings for NONMEM (WFN). The first-order conditional estimation (FOCE) method with interaction was used to fit models to the data. Yeo et al. previously developed a two-compartment PK model describing L-arginine concentrations over time in patients with moderate severe malaria. This model accounted for endogenous L-arginine concentrations (17). It was built on data for 78 patients, with 73 arising from this data set. Parameters have been reestimated with the new data set, and different residual error models were evaluated. This evaluation was based on goodness-of-fit criteria such as measurement of objective function (equivalent to minus twice the log likelihood), parameter estimates, between-subject variability, and graphical diagnostics, including goodness-of-fit plots and individual predictions overlaid with the data.

(ii) PKPD model: NO. The PK model was used to drive a PD model for nitric oxide (NO). A combined model was developed in NONMEM to link the population PK model with the exhaled nitric oxide concentrations in both patient groups (saline and L-arginine group). The PD model was fitted via the sequential population PK parameters and data (PPPD) method by conditioning the PK parameters (23). NO models were based on arginine as a precursor. Initially, given the very short half-life of NO (only seconds [24]), models that incorporated an immediate link between arginine and NO concentration were considered. Three assumptions were required to relate exhaled NO concentrations to plasma arginine concentrations. (i) The equilibrium for intracellular and extracellular arginine is reached faster than arginine clearance. Under this assumption, the cellular steady state is reached rapidly and arginine disposition is the rate-limiting step, rather than the arginine transport into the cell. Therefore, plasma arginine concentrations will proportionally represent the intracellular arginine concentrations and can be used directly. (ii) The equilibrium for NO in exhaled air and airway and alveolar epithelial cells is reached faster than NO clearance from the lungs. Therefore, the NO concentration in exhaled air is proportional to the concentration in airway and alveolar epithelial cells, and exhaled concentrations indirectly represent epithelial NO concentrations. (iii) Lung epithelial cell NO concentrations parallel endothelial cell NO concentrations. Deviation from these assumptions would be seen if there is a delay in the time to maximum (T_{max}) plasma arginine concentration versus the T_{max} exhaled NO.

(iii) PKPD model: endothelial function. The population PKPD model was then expanded to incorporate RH-PAT index, an NO-dependent measure of endothelial function (19). Initially, RH-PAT was directly linked to plasma arginine concentration. Finally, models were considered that were based on the likely pathophysiology of malaria that links L-arginine administration via the intermediary biochemical steps (NO production) to endothelial function (RH-PAT index). The links between RH-PAT and NO were tested to be related in a linear, log linear, or a maximum-effect (E_{max}) model.

Model evaluation. Models were evaluated by goodness-of-fit plots and visual predictive checks (VPCs) (25, 26). Graphics were created in MATLAB (MathWorks; version 7.13, release R2011b) and the Xpose4 package in R (R platform, version 3.0.3). For VPCs, the 10th, 50th (median), and 90th percentiles of the observed and simulated NO concentrations and RH-PAT index values were plotted against time after the study began. In addition, the 95% confidence intervals around the simulated percentiles were constructed.

Dose simulations. The final PKPD model was used to describe and predict the expected beneficial effect of L-arginine for the treatment of malaria. Deterministic simulations were performed using MATLAB to identify suitable dosing regimens for L-arginine resulting in increased exhaled NO concentrations and improved measurements of the RH-PAT

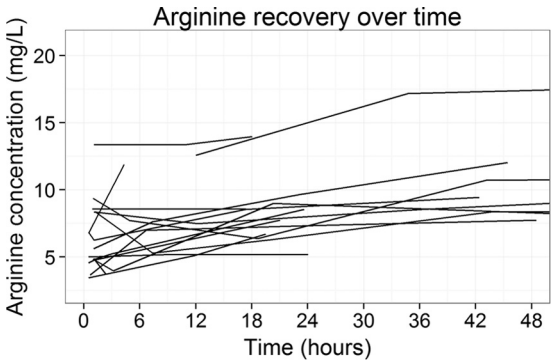

FIG 1 Arginine recovery over time during 2 days of antimalarial treatment. Arginine concentrations from subjects in the control arm ($n = 43$) are plotted against time after start of treatment.

index. Improvement in RH-PAT (a measure of endothelial function) was used as a surrogate endpoint for clinically relevant improvement in organ perfusion in comparing different dosages and dosing schedules to determine the best dosing regimen. The therapeutic range, a range of RH-PAT measurements believed to represent an improvement in overall microvascular function and organ perfusion, was defined as an RH-PAT index above the population mean found after 48 h of antimalarial treatment in the control arm in those patients that recovered completely. This value occurs at a time when there has been significant clinical and microvascular recovery (16). The percentage of time in the therapeutic range was calculated for each dose regimen to find the optimal dosing schedule.

RESULTS

Data. A total of 73 patients were enrolled, 43 in the observational group and 30 in the group receiving L-arginine. For the observational group, the natural time course of the recovery of L-arginine concentrations during malaria treatment is shown in Fig. 1. In the intervention arm, arginine concentrations are the sum of predicted endogenous arginine and infused arginine. In Fig. 2a to d, arginine concentrations are shown over time and an increase in concentration is seen over the observation period in the placebo group. For the same patients, exhaled NO concentrations are shown in Fig. 2e to h. Baseline concentrations of NO are highly variable. In the group receiving the highest dose of arginine, an increase in NO concentrations was seen just after time zero (Fig. 2h). For each patient, the RH-PAT index was determined at multiple time points (Fig. 2i to l), with increasing RH-PAT values indicating recovery of endothelial function. Again, high variability was seen, but with evidence of higher peak values of RH-PAT with the higher dose groups.

Population (PK) model. Yeo et al. described arginine in a two-compartment model with arginine baseline concentrations and intravenous dosing (17). This model was tested with the modified

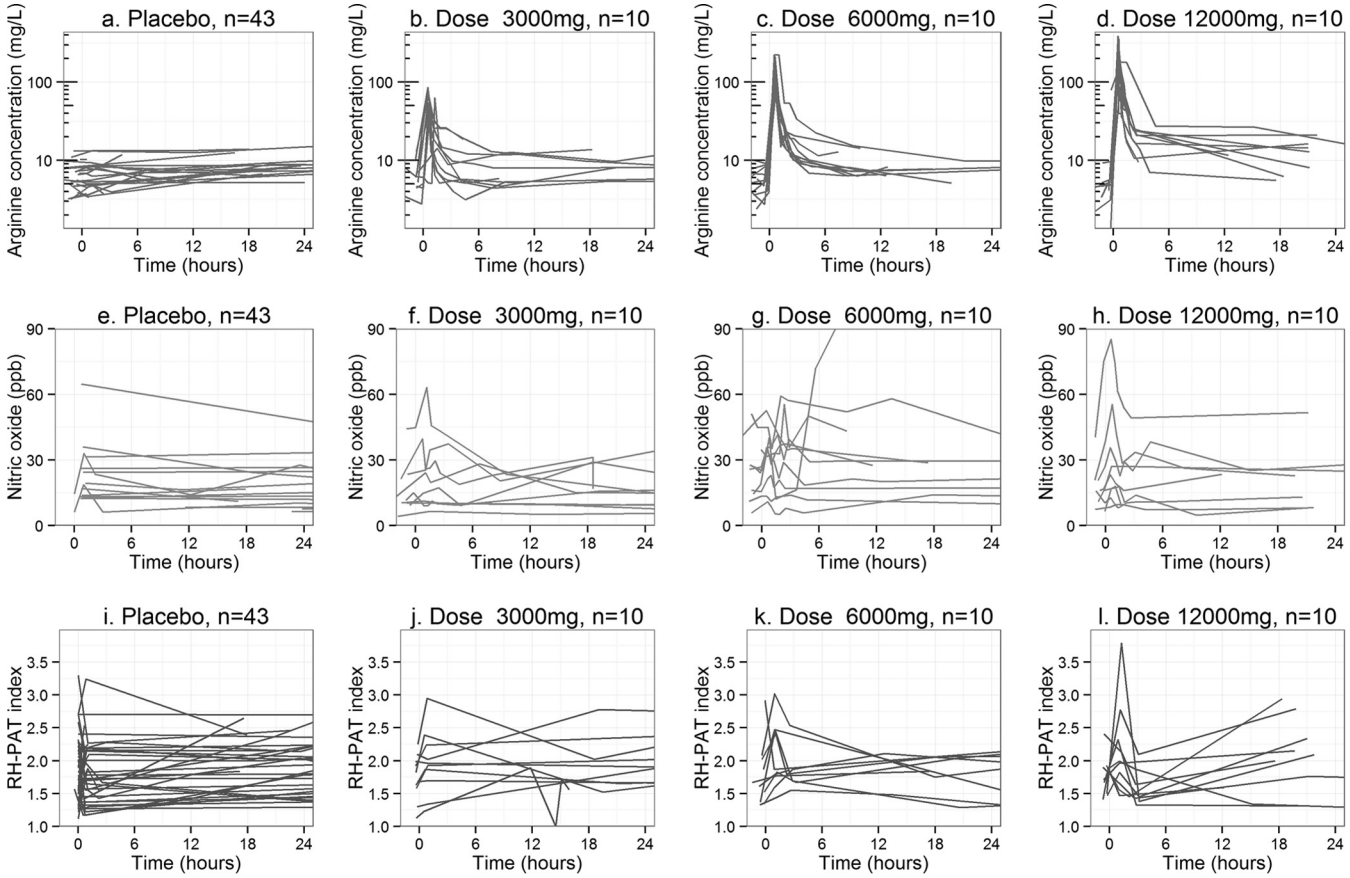

FIG 2 Arginine concentrations (a to d), exhaled nitric oxide concentrations (e to h), and RH-PAT index values (i to l) against time for subjects in different dose groups: those receiving no arginine (a, e, and i), a dose of 3 g (b, f, and j), a dose of 6 g (c, g, and k), or a dose of 12 g (d, h, and l). The dose or saline was administered at time zero. Arginine concentrations are plotted on a semilog scale. Nitric oxide was measured by breath analysis. RH-PAT index values are the results from measurements with an EndoPAT device.

TABLE 2 Parameter estimates for the population PK model

Parameter	Value	SE (% RSE)[l]	Bootstrap[m]
Exogenous L-arginine			
$CL_i = CL \times (WT_i/60)^{k1} \times f$			
CL (liters/h)[a]	30.3	15.4	28.9
k1 (allometric constant for CL)	2.47	21.9	2.45
f (fractional effect of Papuan ethnicity on CL)	1.9	16.9	2.1
$V1_i = V1 \times (WT_i/60)^{k2}$			
V1 (liters)[b]	26.6	12	26.4
k2 (allometric constant for V1)	0.757	70.4	0.69
V2 (liters)[c]	80.1	27.5	90.3
Q (liters/h)[d]	22	18.6	22.8
ωCL (% CV)[e]	33.9	14.2	31.0
ωV1 (% CV)[f]	19.5	54.5	24.6
Endogenous L-arginine			
Arg_0 (mg/liter)[g]	6.07	5.7	6.03
θ_{t1} (mg/liter/h)[h]	0.0365	50.4	0.0533
θ_{t2} (mg/liter/h^2)[i]	−0.0000348	53.4	−0.0000871
ωArg_0 (% CV)[j]	44.5	17.8	43.1
Residual error			
σ_{add} (mg/liter)[k]	≈0	FIX	0
σ_{prop} (% CV)[k]	28.6	20.5	27.0

[a] CL, clearance; CL_i, clearance of the *i*th subject, WT_i, body weight of the *i*th subject.
[b] V1, volume of distribution in the central compartment.
[c] V2, volume of distribution in the peripheral compartment.
[d] Q, intercompartmental clearance.
[e] ωCL, between-subject variance for clearance in the central compartment (percent coefficient of variation [CV]).
[f] ωV1, between-subject variance for volume of distribution in the central compartment (percent CV).
[g] Arg_0, population mean of initial baseline arginine concentration.
[h] θ_{t1}, first-order coefficient of the polynomial to describe arginine recovery after *P. falciparum* infection.
[i] θ_{t2}, second-order coefficient of the polynomial to describe arginine recovery after infection.
[j] ωBL_{Arg}, between-subject variance for arginine baseline concentration over time.
[k] σ_{add} and σ_{prop}, standard deviation of the additive and proportional components of the residual error, respectively.
[l] RSE, relative standard error (%).
[m] Bootstrap was performed with 1,000 samples.

data set, in which 5 people were excluded due to no PD measurements. The structural model was found to be stable and parameters were reestimated (Table 2). An empirical second-order polynomial was used to describe natural recovery of arginine as per the previously published model (17). The model improved significantly (drop in objective function value of 28 points; $P < 0.05$) when variability was used to describe the differences between individuals, rather than baseline alone (as per reference 17). The model was then defined as per equation 1:

$$BL_{Arg}(t) = (Arg_0 + \theta_{t1} \quad t + \theta_{t2} \quad t^2) \exp \quad (\eta_1) \qquad (1)$$

where $BL_{Arg}(t)$ is the baseline value of arginine concentration at time *t*, *t* was indexed to a time set at 2 days prior to presentation (approximately the start of symptoms), Arg_0 is the average baseline value of arginine in the population (at time zero), and θ_{t1} and θ_{t2} are the coefficients in the polynomial linking arginine recovery to time. The between-subject differences (η_1) were described by a log normal distribution with variance (ω). A visual predictive check (VPC) was performed to evaluate the model (Fig. 3).

PD model for nitric oxide. The final population PK model was linked with the exhaled NO concentrations in both patient groups (saline and L-arginine groups). The best model was a linear model (equation 2):

$$NO_{i,j} = (BL_{NO,i} + SL_{NO} \times (E[Arg_{i,j}] - BL_{Arg,i})) \times \exp(\varepsilon 1_{i,j}) + \varepsilon 2_{i,j} \qquad (2)$$

where $NO_{i,j}$ is the *j*th predicted exhaled nitric oxide concentration for the *i*th subject, $BL_{NO,i}$ the *i*th patient's baseline value of NO (at time zero), SL_{NO} the slope to describe the linear relationship between arginine concentrations and NO concentrations, $E[Arg_{i,j}]$ the expectation of plasma arginine concentration for the *j*th observation of the *i*th subject, and $BL_{Arg,i}$ the estimated baseline arginine concentration for the *i*th subject. The model includes between-subject variability for $Arg_{i,j}$ and $BL_{NO,i}$, which describe the variability of the individual parameters around the population mean values. Also, a combined (proportional [$\varepsilon 1_{i,j}$] and additive[$\varepsilon 2_{i,j}$]) error model is included to account for the residual unexplained variability (RUV) in NO. Using this model, the decrease in objective function value was significant ($P < 0.05$), and to evaluate the model, a visual predictive check was performed (Fig. 3).

PD model for endothelial function. The population PKPD

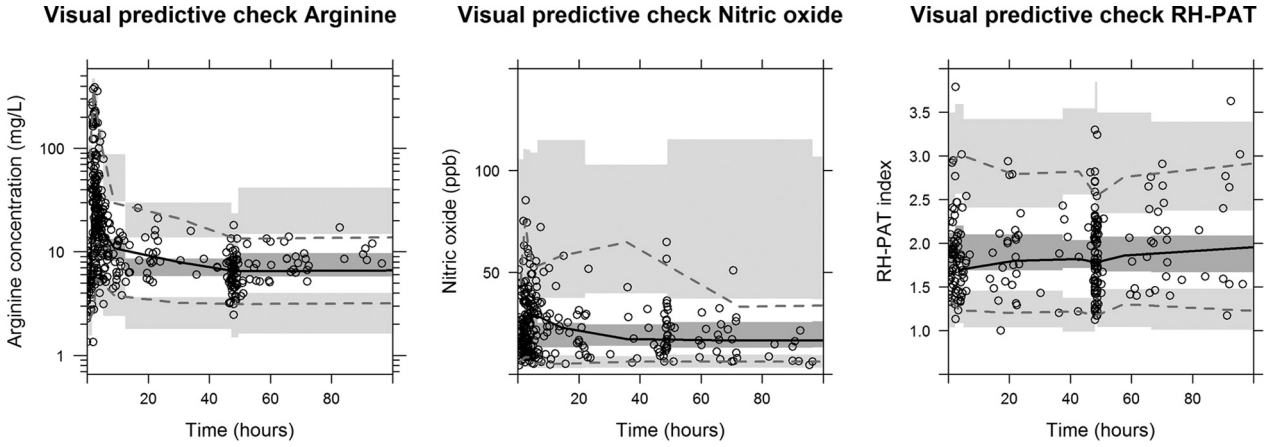

FIG 3 Visual predictive checks of arginine concentrations (left), nitric oxide concentrations (middle), and RH-PAT (right) over time. Observed data (circles) and the median (solid line) and 10th and 90th percentiles (dashed lines) for simulated data with 95% confidence intervals (shaded areas) are shown.

TABLE 3 PD parameter estimates for final PKPD model

Parameter	Value	SE (% RSE)[i]	Bootstrap[j]
PD model, nitric oxide			
BL_{NO} (ppb)[a]	18.2	7.5	18.2
SL_{NO}[b]	0.0243	56.8	0.0281
ωBL_{NO} (% CV)[c]	59.1	8.7	58.8
Residual error (NO)			
σ_{add} (ppb)[d]	≈0	FIX	≈0
σ_{prop} (% CV)[d]	35.5	22.7	35.4
PD model, RH-PAT			
BL_{EF}[e]	1.86	1.9	1.89
SL_{EF}[f]	0.145	73.1	0.190
ωRH-PAT(0) (% CV)[g]	12.5	12.4	12.2
Residual error (RH-PAT)			
σ_{add}[h]	≈0	FIX	≈0
σ_{prop} (% CV)[h]	21.3	10.0	21.3

[a] BL_{NO}, baseline nitric oxide concentration prior to treatment.
[b] SL_{NO}, slope to describe linear relation arginine plasma concentration with NO.
[c] ωBL_{NO}, between-subject variance for baseline nitric oxide concentration.
[d] σ_{add} and σ_{prop}, standard deviation of the additive and proportional components of the residual error for nitric oxide, respectively.
[e] BL_{EF}, baseline measurement of RH-PAT index prior to treatment.
[f] SL_{EF}, slope to describe linear relation NO with RH-PAT.
[g] ωBL_{EF}, between-subject variance for baseline RH-PAT measurement.
[h] σ_{add} and σ_{prop}, standard deviation of the additive and proportional components of the residual error for RH-PAT, respectively.
[i] RSE, relative standard error (%).
[j] Bootstrap was performed with 1,000 samples.

model was expanded to incorporate the RH-PAT index, a marker for endothelial function. RH-PAT was directly linked to plasma arginine concentration, but this model was not stable. Linking the RH-PAT data to NO concentration improved the stability and predictive performance of the model. The decrease in the objective function values for both a linear and an E_{max} model were significant ($P < 0.05$). However, parameter estimates were more plausible in the linear model than in the E_{max} model, and the linear model (equation 3) was more stable:

$$EF_{i,j} = (BL_{EF,i} + SL_{EF} \times (E[NO_{i,j}] - BL_{NO,i})) \times \exp(\varepsilon 3_{i,j}) + \varepsilon 4_{i,j} \quad (3)$$

where $EF_{i,j}$ (endothelial function) is the predicted value of the RH-PAT index describing endothelial function for the jth observation of the ith subject, $BL_{EF,i}$ the ith patients' initial value of RH-PAT (at time zero), SL_{EF} the slope to describe the linear relation between NO and RH-PAT, $E[NO_{i,j}]$ the expected value of exhaled NO concentration (from equation 2, evaluated at $\varepsilon_1 = \varepsilon_2 = 0$), and $BL_{NO,i}$ the estimated baseline value of NO (at time zero). The model includes between-subject variability for $NO_{i,j}$ and $BL_{EF,i}$. Also, a combined (proportional and additive) error model is included to account for the RUV in RH-PAT ($\varepsilon 3_{i,j}$ and $\varepsilon 4_{i,j}$, respectively). Parameter estimates of the complete PD model are shown in Table 3. Furthermore, another VPC was performed to evaluate the model (Fig. 3).

Dosing simulations. The final PKPD model, linking L-arginine administration via NO production to endothelial function, was used to perform deterministic dose simulations. The lower limit of the therapeutic range was defined as the mean of RH-PAT index found in the patients in the saline group after 48 h of antimalarial treatment (1.87), and there was no upper limit to the range other than that provided by normal hyperemia. The 48-h time point was chosen because by this time there is significant microvascular and clinical recovery (16). A standard virtual subject (with a weight of 60 kg and of Papuan descent) was dosed with one of the following dosage regimens: (i) 3, 6, 12, or 30 g over 0.5 h or (ii) 12 g by continuous infusion over 0.5, 4, 12, 24, and 48 h. Each regimen was assessed for the percentage of time in the therapeutic range (above the base value of RH-PAT of 1.87). Increasing doses led to increased time percentage, with 14.8, 25.6, 36.2, and 50.5% for the 3-, 6-, 12-, and 30-g doses over 30 min, respectively (Fig. 4). With increasing infusion duration, the percentages of time that therapeutic RH-PAT values occurred over the 48-h period after start of the infusion were 36.2, 40.5, 51.8, 71.5, and

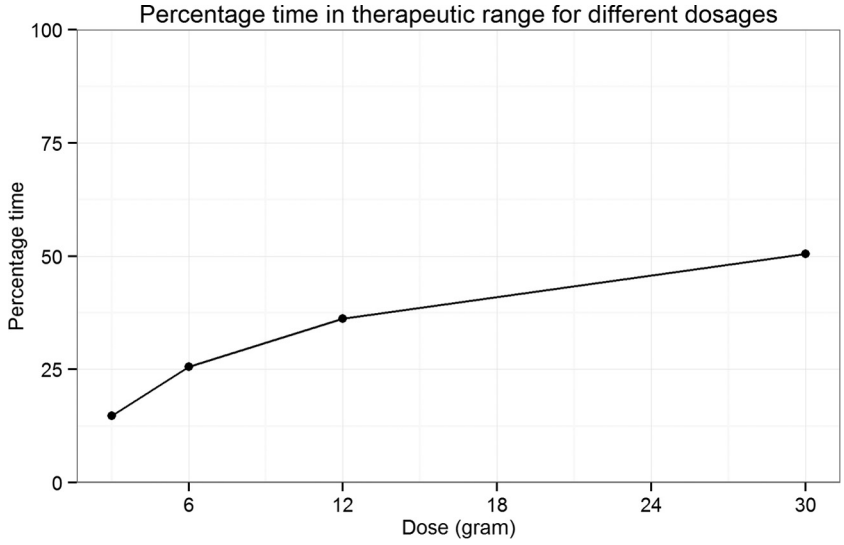

FIG 4 Percentage of time above the therapeutic target for different dosages. The therapeutic target was defined as an RH-PAT score of 1.87. Each dose was administered as a 0.5-h infusion.

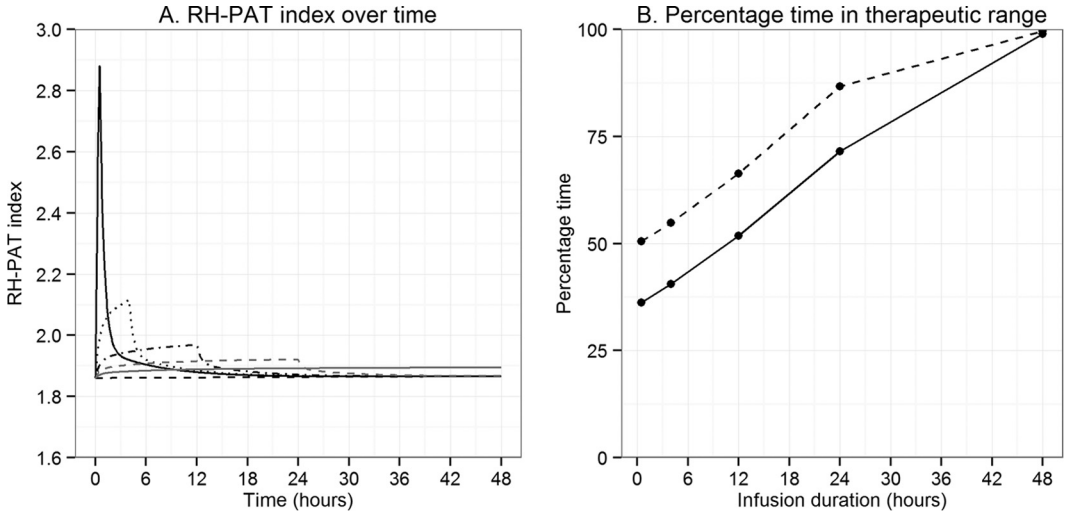

FIG 5 Increasing infusion durations for a 12-g dose of arginine. (A) The black dashed line represents no arginine dose, the black solid line a 0.5-h infusion, the dotted line a 4-h infusion, the dashed dotted line a 12-h infusion, the gray dashed line a 24-h infusion, and the gray solid line a 48-h infusion. (B) Percentage of time above the therapeutic target for different dosing schedules for a 12-g dose of arginine (solid line) and a 30-g dose of arginine (dashed line).

98.9% for infusion durations of 0.5, 4, 12, 24, and 48 h of a 12-g dose, respectively, and 50.5, 54.9, 66.3, 86.7, and 99.6% for a 30-g dose (Fig. 5).

DISCUSSION

Previous work (27, 28) has formulated a link between L-arginine administration and endothelial function. In their work, Bode-Böger et al. describe a strong correlation between plasma arginine concentrations and its vascular effects in healthy human subjects (28). This is the first work to quantify the time course of the relationship, using a PKPD model between L-arginine administration to endothelial function in acute clinical illness. L-Arginine is a known substrate for endothelial cell nitric oxide synthase, and intravenous administration of arginine has been shown to improve endothelial function in moderately severe falciparum malaria (11). In this study, data from 73 patients with malaria from previous projects (11, 16, 17) were used to develop a PKPD model that links L-arginine administration via NO production as the intermediary biochemical step to endothelial function (RH-PAT index). Visual predictive checks indicated that the final model was able to describe arginine concentrations in plasma, NO concentrations in breath, and RH-PAT measurements adequately.

Yeo et al. described the pharmacokinetics of a single-dose L-arginine infusion in adult patients with moderately severe malaria in a two-compartment model with a second-order polynomial for the natural time course of the recovery of L-arginine (17). Since the polynomial function is an empirical description of the change in arginine baseline concentrations over time, it should not be used to predict arginine concentrations that occur >48 h postadmission. This earlier study found that exogenous L-arginine had a shorter half-life in patients with malaria than in healthy individuals. Arginine metabolism is known to be compartmentalized within the body, which causes the plasma arginine concentration to be only indirectly in balance with intracellular arginine metabolism (29). Within endothelial cells, the arginine concentration is high enough to saturate endothelial NO synthase, but when exogenous arginine is introduced, NO production still increases, the so-called "arginine paradox" (30). Therefore, in patients with decreased NO production, such as patients with falciparum malaria, administration of arginine could be expected to increase NO production instantly.

The endothelial NO production *in vivo* is dependent on the intracellular movement of extracellular L-arginine by cationic amino acid transporters (CAT-1 and CAT-2) (31). When patients received exogenous arginine, the exhaled NO concentrations increased without any apparent delay. Our assumption that the equilibrium for intracellular and extracellular arginine is rapid was therefore supported by the data. Under this assumption, the cellular steady state is reached rapidly and arginine disposition is rate-limiting, rather than the arginine transport into the cell. Therefore, plasma arginine concentrations will proportionally represent the arginine concentrations available for NO production and can be used directly. The model was used to perform deterministic dose simulations to study the effect of adjunctive arginine treatment on the RH-PAT index. RH-PAT index, a measure of endothelial function, was used as a surrogate measure of organ perfusion and the therapeutic range was used as biomarker for the improvement in organ perfusion. The percent time achieving therapeutic response increased with increasing arginine dose, but simulations demonstrated that regimens of continuous infusion over longer periods might prolong the therapeutic response even more. Because of the short half-life of NO, elevated NO concentrations would be maintained only for the duration of increased arginine concentrations. Therefore, reversal of endothelial dysfunction in patients with falciparum malaria would be expected only for the duration that arginine concentrations remain elevated. We expect, therefore, that in administering L-arginine to the eventual target group, patients with severe malaria, continuous infusions of L-arginine will also be required. The safety of 8-h infusion of 12 g of L-arginine has now been demonstrated in a small pilot study with adults with severe malaria (32); however, the volume of distribution and

clearance of L-arginine appeared to be greater in severe malaria than in moderately severe malaria. Trials of larger doses than used in this pilot study will be needed, again as a continuous infusion. Randomized clinical trials of continuous infusions of higher doses of L-arginine in severe malaria are in progress (ACTRN12612000571875).

Since the variability in NO concentrations and RH-PAT is very high, it may be hard to predict the concentration effects of L-arginine for each individual patient, and further research is needed to determine potential causes of variability among patients. Now that variability has been quantified, it is possible that patient characteristics or other biomarkers influencing NO bioavailability and severity of microvascular dysfunction (33, 34) could potentially be used to individualize the dose.

The study had several limitations. We note that exhaled NO may not reflect the time course of endothelial NO exposure and has not been useful as a marker of vascular endothelial function in some settings (35–37). In this work, we assumed that exhaled NO was a marker of epithelial NO production and that this would parallel the time course of endothelial exposure. While this assumption has not been specifically tested, the model does not provide evidence against this measure of the time course of NO exposure. It remains clear that the relationship between plasma L-arginine concentrations is highly variable. In addition, although it is estimated that at least 50% of reactive hyperemia peripheral arterial tonometry (RH-PAT [19]) response is dependent on endothelial NO production (11, 38), other mechanisms for endothelial dysfunction could not be excluded. While there have been recent concerns regarding the suitability of RH-PAT to assess endothelial function in early-phase clinical pharmacology studies (39), its utility in acute systemic inflammation has been demonstrated (11, 40). To evaluate endothelial function, we assumed that RH-PAT is a good indicator of vascular reactivity for which there is good evidence (19).

In conclusion, the time course of effects of L-arginine adjunctive therapy on NO production and endothelial function in patients with moderately severe falciparum malaria was quantified. The developed PKPD model was used for simulations of dosing schedules and predicted that the percentage of time of normalized RH-PAT might increase with increased infusion duration, indicating strong schedule dependence. Further work is necessary to characterize the effects of these regimens on NO and vascular reactivity in patients with severe malaria.

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