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The effect of sickle cell genotype on the pharmacokinetic properties of artemether-lumefantrine in Tanzanian children

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ABSTRACT

Since there are inconsistent data relating to the effect of haemoglobinopathies on disposition of artemisinin antimalarial combination therapy, and none in sickle cell trait (SCT) or sickle cell disease (SCD), the aim of this study was to characterize the pharmacokinetic properties of artemether-lumefantrine (ARM-LUM) in children with SCD/SCT. Thirty-eight Tanzanian children aged 5–10 years with normal (haemoglobin AA; n = 12), heterozygous (haemoglobin AS; n = 14) or homozygous (haemoglobin SS; n = 12) sickle genotypes received six ARM-LUM doses (1.7 mg/kg plus 10 mg/kg, respectively) over 3 days. Sparse venous and mixed-capillary dried blood spot (DBS) samples were taken over 42 days. Plasma and DBS ARM and LUM, and their active metabolites dihydroartemisinin (DHA) and desbutyl-lumefantrine (DBL), were assayed using validated liquid chromatography-mass spectrometry. Multi-compartmental pharmacokinetic models were developed using a population approach. Plasma but not DBS concentrations of ARM/DHA were assessable. The majority (85%) of the 15 measurable values were within 95% prediction intervals from a published population pharmacokinetic ARM/DHA model in Papua New Guinean children of similar age without SCD/SCT who had uncomplicated malaria, and there was no clear sickle genotype clustering. Plasma (n = 38) and corrected DBS (n = 222) LUM concentrations were analysed using a two-compartment model. The median [inter-quartile range] LUM $AUC_{0-\infty}$ was 607,296 [426,480–860,773] μg.h/L, within the range in published studies involving different populations, age-groups and malaria status. DBS and plasma DBL concentrations correlated poorly and were not modelled. These data support use of the conventional ARM-LUM treatment regimen for uncomplicated malaria in children with SCT/SCD.

1. Introduction

Genetically determined variations in the structure and function of haemoglobin underlie differences in malaria susceptibility and severity across human populations (Taylor et al., 2012). These haemoglobinopathies, which include sickle cell disease (SCD) and the thalassaemias, may afford protection against malaria, but the infection can still have adverse clinical consequences. In the case of SCD in children, falciparum malaria can result in hospitalisation and even death (Ambe et al., 2001; Makani et al., 2010; McAuley et al., 2010). Ensuring optimal antimalarial therapy remains a priority in this situation.

The World Health Organisation (WHO) recommends artemisinin combination therapy (ACT) as first line treatment for children with uncomplicated falciparum malaria (World Health Organisation, 2021).

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Among available ACTs, artemether-lumefantrine (ARM-LUM) is the most commonly prescribed in Sub-Saharan Africa (Ndeffo Mbah et al., 2015) . In Tanzania, ARM-LUM has been first-line treatment for malaria for 15 years (Michael and Mkunde, 2017). Although there are some data suggesting that ARM-LUM is safe and effective in African children with SCD (Adjei et al., 2014), there are few studies of the effects of abnormal hemoglobins on the disposition and efficacy of antimalarial drugs, and the data are inconsistent (Sugiarto et al., 2018). For example, one study found a marked difference in the pharmacokinetics of artesunate in Thai adults with versus without alpha-thalassaemia (Ittarat et al., 1998) but there was no such effect in Papua New Guinean (PNG) children with uncomplicated malaria (Karunajeewa et al., 2004). Similarly, there are *in vitro* data showing that haemoglobinopathies such as alpha-thalassaemia may reduce the efficacy of the artemisinins (Kamchonwongpaisan et al., 1994), but *in vivo* studies show that drugs such as artesunate clear parasites promptly in children with this mutation (Karunajeewa et al., 2004).

In the case of sickle cell trait (SCT; haemoglobin AS genotype) and SCD (haemoglobin SS genotype), there are no published studies of the pharmacokinetic properties of artemisinin drugs as part of ACT. However, available data suggest that African children with SCD developing falciparum malaria despite the relative protection associated with their haemoglobinopathy (Eridani, 2011) have delayed initial parasite clearance after ACT (Adjei et al., 2014; Ndounga et al., 2015). This may represent depressed reticuloendothelial (splenic) function (Pearson et al., 1979) but it could also result from effects of SCD on artemisinin pharmacokinetics (Sugiarto et al., 2018). There are few data on the disposition of longer half-life ACT partner drugs in either SCD or SCT, but SCD has been associated with reduced desethylamodiaquine exposure in children with acute malaria treated with artesunate-amodiaquine (Adjei et al., 2019). This finding is consistent with the accelerated clearance of morphine, suggesting the need for higher doses in the management of SCD vaso-occlusive crises (Darbari et al., 2011). There are no studies of the disposition of longer half-life ACT partner drug lumefantrine in either SCD or SCT.

Conventional intensive venous blood sampling schedules for antimalarial drug levels are inappropriate in parasitemic, anaemic and crisis-prone children with SCD. The measurement of drug concentrations in small volume dried blood spots (DBS) rather than plasma samples represents an alternative ethical approach (Taneja et al., 2013), and sparse sampling adequate for population pharmacokinetic modelling would further reduce the blood volume required. The data in this way collected could inform antimalarial prescribing in vulnerable children with SCD or SCT who present with malaria. Since malaria increases the already significant risk of hospitalisation and death in SCT and SCD (Komba et al., 2009; Makani et al., 2010; McAuley et al., 2010), optimised ACT treatment regimens should be a priority in these groups.

The aim of the present study was to characterize the pharmacokinetic properties of ARM, LUM and their respective active metabolites dihydroartemisinin (DHA) and desbutyl-lumefantrine (DBL) in Tanzanian children with SCT and SCD compared to children with normal haemoglobin (AA genotype). A secondary aim was to develop a modified ARM-LUM treatment regimen if the population pharmacokinetic properties in the children with SCT and/or SCD suggested that this was warranted.

2. Materials and methods

2.1. Study site, participant sample and approvals

The present study was conducted at Muhimbili University of Health and Allied Sciences (MUHAS) within the national referral hospital in Dar es Salaam, Tanzania between July and November 2017. Recent estimates suggest that the prevalence of SCT and SCD in Tanzania are around 20% and 1.3%, respectively (Ambrose et al., 2018, 2020). Forty children aged 5–10 years with approximately equal numbers with confirmed haemoglobin AA (normal), AS (SCT) or SS (SCD) genotypes were recruited from outpatient clinics. This sample size was sufficient to show clinically relevant changes in key pharmacokinetic parameters and allowed for an average 30% attrition rate in paediatric clinical trials (Zimmerman et al., 2019). Using the Monte-Carlo Mapped Power method with Perl speaks NONMEM (PsN) and published population PK parameters (Karunajeewa et al., 2004; Salman et al., 2011; Sugiarto et al., 2022), a $\geq$20% change per sickle gene in the volume of distribution of LUM relative to bioavailability (V_d/F) at 80% power and $\alpha = 0.05$ would require $\geq$9 participants/group, and $\geq$10 participants/group would be required for a $\geq$15% change per sickle gene in LUM relative clearance (CL/F).

Inclusion criteria included no known allergy or sensitivity to ARM-LUM, no treatment with an ACT within the previous 28 days, no significant acute illness (including malaria) or other co-morbidity, and willingness to complete allocated sampling. Exclusion criteria included symptomatic anemia (dyspnea and/or postural hypotension), haemoglobin concentration <60 g/L, and evidence of an emerging crisis. Written informed consent for all children was obtained from their parents. The study was approved by the MUHAS Research and Ethics Committee in Tanzania (2017-05-25/AEC/Vol. XII/66) and Human Ethics Research Committee at The University of Western Australia (RA/, 4/1/8920).

2.2. Clinical methods

Participants were recruited from outpatient clinics at the Muhimbili Sickle Cell Centre at MUHAS. In these clinics, children attend 3 monthly for haemoglobin measurement, provision of folic acid 5 mg tablets for daily administration, parenteral penicillin prophylaxis for children under the age of 6 years, and, if indicated, pneumoccocal vaccination as under the national immunisation schedule. Each participant's sociodemographic data and medical history were recorded on standard forms, and a physical examination was performed by a medical officer (SRS). A baseline haemoglobin concentration was measured, a blood film for malaria microscopy was prepared, and a DBS was taken for drug assay. A 3 mL venous baseline blood sample was also taken for subsequent drug assay of separated plasma. Each child was given ARM-LUM (Coartem®, Novartis Pharma, Switzerland) at a dose of 1.7 and 10 mg/kg respectively to the nearest whole tablet at 0, 12, 24, 36, 48 and 60 h. All doses were administered by the parents with at least 50 mL of cow's milk (equivalent to 2 g of fat) as recommended by the manufacturer to ensure adequate drug absorption. Parents were asked to report if any dose was refused or if vomiting occurred. Any child vomiting a dose within 30 min of administration was to be retreated. The exact dates and times of each dose and DBS/blood sample were recorded.

After the baseline assessment, six DBS samples were taken from each participant from randomly selected time points in the following sparse sampling schedule: 4, 8, 12, 24, 36, 40, 48, 60, 64, 68 and 72 h, and Days 4, 5, 7, 14 and 28. This schedule was developed to reflect the disposition of LUM in children (Karunajeewa et al., 2004; Salman et al., 2011), but approximately 40% of the samples were within 8 h of the prior dose and were therefore potentially suitable for ARM/DHA analysis. The exact dates and times of each sample were recorded. For collection of DBS at each time point, 5 blood spots were collected from a finger prick onto a single filter paper (Whatman 903™ protein saver card, GE Healthcare Australia Pty Ltd, Parramatta, NSW, Australia). Each DBS was air dried at room temperature, placed into a gas impermeable plastic bag with desiccant. A 3 mL venous blood sample was taken into a VACUETTE™ 4 mL lithium heparin tube (Greiner Bio-One GmbH, Kremsmünster, Austria) for drug assay at one randomly selected time point in parallel with a DBS sample to allow validation of DBS versus plasma drug concentrations. All venous samples were centrifuged promptly and the separated plasma also stored at −80 °C together with the DBS samples. Clinical assessment, including a symptom questionnaire, blood film and point-of-care haemoglobin, was repeated according to the individual

DBS sampling schedule.

2.3. Drug assay methods

Previously validated methods were used for assay of ARM, DHA, LUM and DBL in plasma and DBS (Sugiarto et al., 2022) which were performed at Curtin University in Western Australia. A triple quadrupole mass spectrometer with UHPLC pump (LCMS/MS-8060 Shimadzu, Kyoto, Japan) was used for analysis of ARM and DHA, with artesunate as internal standard. Quantitation was performed in DUIS (APCI$^+$ and ESI$^+$) mode by multiple reaction monitoring. Chromatographic separation was performed on a Synergy MAX-RP column (50 × 2.0 mm, 2.5 μm; Phenomenex, Lane Cove West, Australia). Stock solutions (1 mg/mL) of ARM, DHA and ARS were prepared in methanol, serial dilutions made, and isovolumetric standards spiked in blood to create, after extraction, a plasma calibration curve. DBS were also prepared as for those collected in the field using blood spiked in an appropriate standard series with 50 μL aliquots spotted on a Whatman 903 TM protein saver card. For DBS, the extraction method was based on modified validated methods (Blessborn et al., 2013; Sugiarto et al., 2022). Calibration curves (20–1000 μg/L) were constructed for plasma and DBS. The intra-day and inter-day variability for ARM in DBS were <14.0% and those for DHA in DBS were <12.7%. The intra-day and inter-day variability for ARM in plasma were <11.9% and those for DHA in plasma were <12.9%. Chromatographic data (peak area ratio of ARM, DHA and ARS) were processed using LAB Solution (Version 5.56, Shimadzu, Japan). The lower limit of quantification (LOQ) of ARM and DHA in DBS and plasma were 20 μg/L and 5 μg/L, respectively. The lower limit of detection (LOD) of ARM and DHA in DBS and plasma were 10 μg/L and 2 μg/L, respectively.

Quantification of LUM and DBL in extracted plasma (0.5 mL) and DBS was by validated ultra-high-performance liquid chromatography-tandem mass spectrometry assay (Wong et al., 2011) with modifications (Blessborn et al., 2013; Sugiarto et al., 2022) using LUM-d-$_{18}$ and DBL-d-$_9$ as internal standards. Quantitation was performed in ESI $^+$ mode. Chromatographic separation was performed on a Waters Aquity T3 UPLC C18 column (50 × 2.1 mm, 1.7 μm; Waters Corp, Wexford, Ireland). Calibration curves ranged from 1 to 20,000 μg/L. The intra-day and inter-day variability for LUM in DBS was <9.0% and for DBL in DBS <10.7%. The equivalent figures for LUM in plasma were <9.4% and for DBL in plasma <9.9%. The LOQ and LOD of LUM and DBL in DBS were both 2.5 μg/L and 1 μg/L, respectively. The LOQ and LOD of LUM and DBL in plasma were both 2.0 μg/L and 0.6 μg/L, respectively.

2.4. Population pharmacokinetic analysis

There were limited valid DBS data available for paired comparison with plasma concentrations (n = 4 for ARM, n = 1 for DHA) and so the DBS data could not be included in the ARM/DHA pharmacokinetic model. There were 15 plasma concentrations within 8 h after a prior dose when ARM/DHA concentrations would be measurable. Initial modelling utilizing these concentrations was unable to generate valid comparisons between haemoglobin genotype groups, the primary variable of interest, with these data and so no formal population pharmacokinetic model was developed. Instead, and given that there is no evidence of significant racial or ethnic differences in the disposition of ACT component drugs (Sugiarto et al., 2017), a numerical predictive check with 1000 replicates was performed using a published pharmacokinetic model in children from Papua New Guinea who were of similar age to those in the present study (Salman et al., 2011). Additionally, as a qualitative visual assessment, 500 datasets were simulated using this model with the 5th, 50th and 95th simulated percentiles compared to the present observed values.

In the case of LUM, there was a significant correlation between DBS and plasma samples (r^2 = 0.723; $P < 0.001$), although the slope was significantly lower than 1 (0.231) indicating that the DBS concentrations were only about one quarter those in plasma samples. There was no significant influence of sickle genotype or haemoglobin on this relationship. There was poor correlation between DBS and plasma samples for DBL (r^2 = 0.485). Although DBL has potent antimalarial activity (Wong et al., 2011), its plasma concentrations are substantially lower and they are much less predictive of recrudescence of falciparum malaria than those of LUM (Kloprogge et al., 2015). Given the limited clinical relevance of these concentrations and concerns regarding their validity in the present study, they were not included in the population pharmacokinetic modelling. Comparisons of DBS and plasma data for LUM and DBL are included in Fig. 1.

Log$_e$ concentration-time datasets for lumefantrine were analysed by nonlinear mixed effects modelling using NONMEM (v 7.2.0, ICON Development Solutions, Ellicott City, MD, US) with an Intel Visual FORTRAN 10.0 compiler. Both plasma and DBS concentration were included with a parameter that corrected the DBS concentrations to those in plasma by multiplying by 4.33 based on a single population value for the ratio between the two given only a single paired sample was taken from each patient. The first order conditional estimate with interaction (FOCE with INTER) estimation method was used. The minimum value of the objective function (OFV) and visual predictive checks were used to choose suitable models during the model-building process. A significance level of $P < 0.01$ was set for comparison of nested models. Allometric scaling for size was employed *a priori*, with an exponential of 1 for volume (V) and ¾ for clearance (CL) terms (Anderson and Holford, 2009). Residual variability (RV) was estimated as additive error for the log-transformed data with separate terms for plasma and DBS data.

Initial modelling was carried using standard one-, two- and three-compartment models (ADVAN 2, 4 and 12, respectively) provided within NONMEM paired with first order absorption. Once a suitable base model was selected, inter-individual variability (IIV), as well as correlations between IIV terms, were evaluated for each suitable parameter and included where supported by the data. Inter-occasion variability (IOV) was also assessed for bioavailability of the multiple doses, with a median value set at 1. IIV and IOV was exponentially modelled for all parameters. Relationships between model parameters and covariates were assessed through inspection of scatterplots and boxplots of individual parameters vs covariate, and subsequently evaluated within NONMEM. The principal covariate was SC genotype, specifically AA, AS and SS. Other covariates assessed included gender, age, haemoglobin at enrolment, and mean upper arm circumference. Selection of a covariate relationship required a significance of $P < 0.01$, as well as a reduction in IIV and biological plausibility.

For model evaluation, plots of observed vs individual- and population-predicted values, and time vs WRES, were first assessed. A bootstrap using Perl-speaks-NONMEM (PsN) with 1000 samples was performed, and the parameters derived from this analysis summarised as median and 2.5th and 97.5th percentiles (95% empirical CI) to facilitate evaluation of model parameter estimates. In addition, prediction corrected visual predictive checks (pcVPCs) and numerical predictive checks (NPCs) were performed with 1000 datasets simulated from the final models. These were stratified according to sickle genotype, haemoglobin concentration and sample type (plasma versus DBS), the latter stratification to assess any potential bias introduced through the use of DBS samples for modelling. The observed 5th, 50th, and 95th percentiles were plotted with their respective simulated 90% CIs to assess the predictive performance of the model and to evaluate any major bias.

3. Results

3.1. Participant characteristics and clinical course

Of the 40 children recruited, the parents of two children withdrew them on Day 2 after only a baseline blood sample had been taken. Neither child had experienced any adverse effects during treatment. The characteristics of the remaining 38 children are summarised in Table 1.

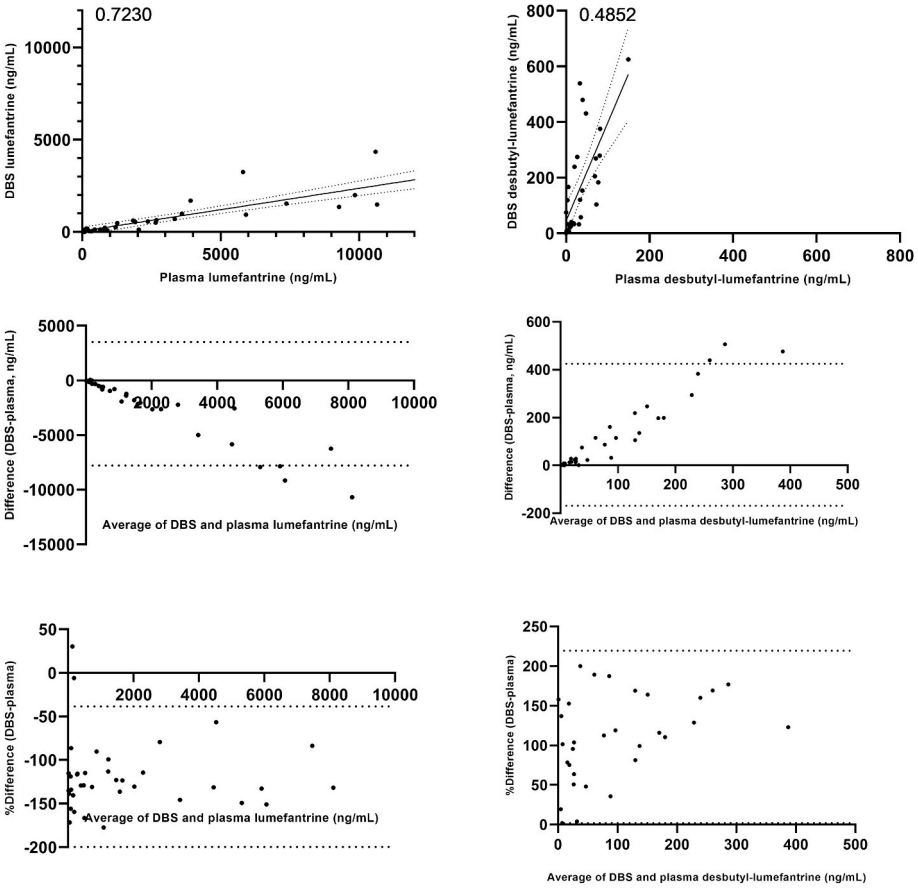

Fig. 1. Comparisons of paired DBS and plasma concentrations for lumefantrine and desbutyl-lumefantrine. These include linear correlation and Bland-Altman plots for both absolute (ng/mL) and percentage difference. Correlation co-efficients are given in the top two graphs.

Table 1
Baseline characteristics of children with haemoglobin AA, SS and AS genotypes. Data are presented as mean (SD) [range] or number (%). MUAC = mid-upper arm circumference, BMI = body mass index.

Variable	All	AA (n = 12)	AS (n = 14)	SS (n = 12)	*P*-value
Age (years)	7.2 (1.7) [4.75–10]	6.9 (1.7) [4.75–10]	7.2 (1.6) [5–10]	7.4 (1.8) [4.92–9.75]	0.75
Male (n (%))	19 (50.0%)	5 (41.7)	7 (50.0)	7 (58.3)	0.72
Height (cm)	117.6 (11.4) [97.4–145.9]	113.7 (10.0) [97.4–125]	121.3 (13.5) [99.9–145.9]	117.3 (9.3) [101.7–128.9]	0.24
Weight (kg)	21.2 (5.4) [13.6–42.4]	19.4 (3.3) [14.2–25.4]	23.2 (7.6) [13.6–42.4]	20.5 (3.3) [20.3–27.5]	0.18
MUAC (cm)	17.0 (1.8) [14.0–22.7]	16.8 (1.5) [15–20]	17.9 (2.2) [15.6–22.7]	16.3 (1.1)[a] [14.0–18.0]	0.045
BMI (kg/m^2)	15.1 (1.6) [12–19.9]	15.0 (1.4) [13–17.6]	15.4 (2.0) [12–19.9]	14.8 (1.0) [13.4–16.7]	0.59
Haemoglobin (g/dL)	10.7 (2.2) [6.3–14.1]	11.6 (1.6) [7.2–13.4]	12.3 (0.9) [10.7–14.1]	8.0 (0.9)** [6.3–9.6]	<0.001

[a] $P = 0.045$ vs AS adjusted for multiple comparisons using the Bonferroni correction; **$P < 0.001$ vs AS.

The 12 children in the SS group had lower mid-upper arm circumference compared with the 14 in the AS group and they also had significantly lower haemoglobin concentrations than the other two groups. Only one child, in the SS group, was slide positive for *Plasmodium falciparum* malaria on review of a blood smear taken at enrolment, but he was asymptomatic and had a low parasite density (80/μL).

The six doses ARM-LUM were well tolerated, although two participants vomited >2 h after one of the doses and were not retreated. Three children in the SS group (25%) were hospitalised with crises during follow-up, on Day 2, Day 3 and Day 7, respectively. The first child experienced severe limb pain requiring morphine but was able to take study medication and was discharged two days later. The second developed severe anaemia (haemoglobin concentration 51 g/L) and generalised weakness, and was discharged two days later after blood transfusion. The third presented with abdominal pain, anorexia and lethargy, was intravenously rehydrated and given morphine as analgaesia, and discharged after two days. These hospitalisations were assessed as unrelated to study medication. The single child who was slide positive for malaria at recruitment was negative by Day 2 and did not have clinical or blood slide evidence of recrudescence during follow-up. One other child in the SS group had an isolated positive blood film for low level *P. falciparum* parasitemia on Day 7 (density 120/μL) but was slide negative on subsequent serial blood films.

3.2. Pharmacokinetic modelling

For ARM/DHA, the NPC using simulated data based on the previously published population pharmacokinetic model (Salman et al., 2011) showed that 85% of the plasma concentrations were within each 90% prediction interval for ARM and DHA (2.5% above and 12.5% below; see Fig. 2).

For LUM, there were 226 DBS and 38 plasma concentrations from the 38 patients that were included in the pharmacokinetic analysis. Less than 2% (n = 4) of the DBS concentrations and none of the plasma

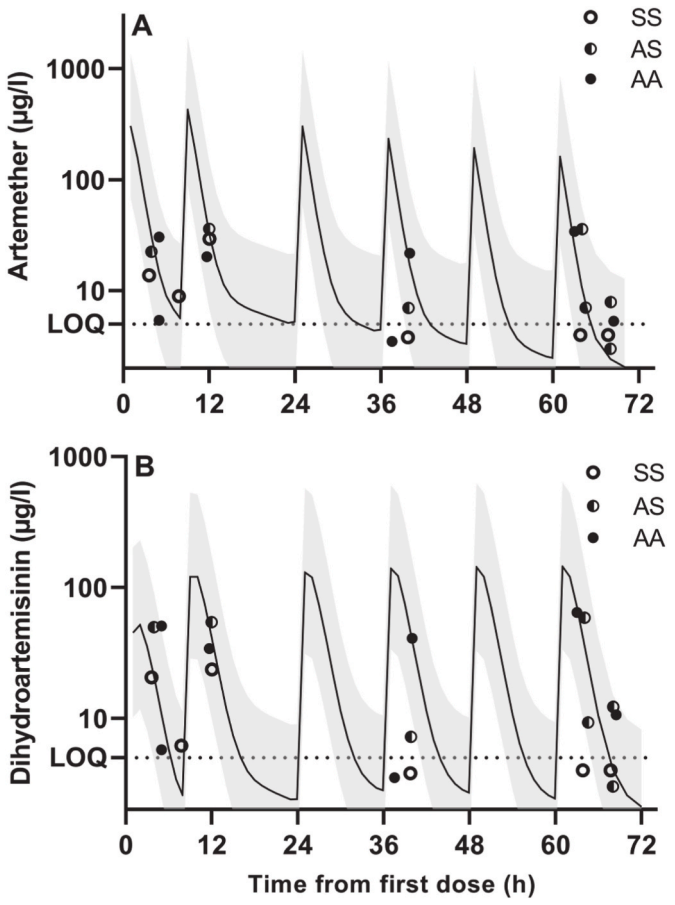

Fig. 2. Simulated 5th, 50th and 95th intervals plotted against observed data for artemether (A) and dihydroartemisinin (B). Observed limit of quantification data is randomly scattered below this limit to separate out points. Data points are separated with respect to genotype; AA - filled circles, AS – half filled circles and SS – empty circles.

concentrations were below the limit of quantification and therefore these (n = 4) were censored from the analysis. For the structural model, a two-compartment model outperformed a single compartment with no benefit with the addition of third compartment. Therefore, the structural model parameters were absorption rate (k_a), central volume of distribution (V/F), clearance (CL/F), peripheral volume of distribution (V_P/F) and inter-compartmental clearance (Q/F). IIV was estimable for clearance (CL/F) and bioavailability (F), with IOV also estimated for the latter. The ratio between DBS and plasma samples in the model was estimated as 0.233, corresponding to the value obtained independently through simple linear regression. There was no significant effect of SC genotype on any of the pharmacokinetic parameters when this covariate was examined within the population pharmacokinetic model. No other significant covariate relationships were present.

The final model parameter estimates, and the bootstrap results are summarised in Table 2. Bias was less than 5% for all fixed and random model parameters. Fig. 3 show goodness-of-fit plots, with no bias evident, and Fig. 4 the pcVPC plots. The actual 5th, 50th and 95th percentiles of observed data fell within their respective 95% CI demonstrating suitable predictive performance of the model. Stratified VPCs and NPCs did not suggest bias from sickle genotype status, haemoglobin concentration, or sample type (data not shown). Secondary LUM pharmacokinetic parameters for the participants in the study, stratified according to sickle genotype, are presented in Table 3. While there was trend for higher median AUC extrapolated from baseline to infinity ($AUC_{0-\infty}$) from AA to AS and SS genotypes, this was not significant ($P > 0.1$), consistent with the lack of an effect of haemoglobin

Table 2
Final population pharmacokinetic estimates and bootstrap results for lumefantrine in children in Tanzania with or without sickle trait or disease.

Parameter	Mean	RSE%	Bootstrap median [95% CI]
Objective Function Value	59.992		47.582 [-25.800 – 109.338]
Structural model parameters:			
CL/F (liters/h/70 kg)	6.68	14	6.65 [5.13–8.76]
V_C/F (liters/70 kg)	354	18	345 [238–492]
Q/F (liters/h/70 kg)	1.53	22	1.53 [1.03–2.57]
V_P/F (liters/70 kg)	490	19	487 [337–734]
k_a	0.392	18	0.387 [0.225–0.636]
Ratio (DBS:plasma)	0.233	9	0.229 [0.193–0.277]
Variable model parameters [shrinkage %]:			
IIV in CL/F	24.2 [29]	22	23.2 [9.5–35.3]
IIV in F	67.5 [13]	15	65.0 [42.1–84.9]
IOV in F	68.3	22	68.3 [27.4–94.2
RV (plasma)	41.8 [18]	16	39.8 [24.5–53.3]
RV (DBS)	49.0 [18]	11	48.2 [38.4–59.7]

k_a (absorption rate), CL/F (clearance), V_C/F (central volume of distribution), Q/F (inter-compartmental clearance), V_P/F (peripheral volume of distribution), IIV (inter-individual variability), IOV (inter-occasion variability) and RV (residual variability). Variability parameters are presented as.$100\% \times \sqrt{\text{variability estimate}}$

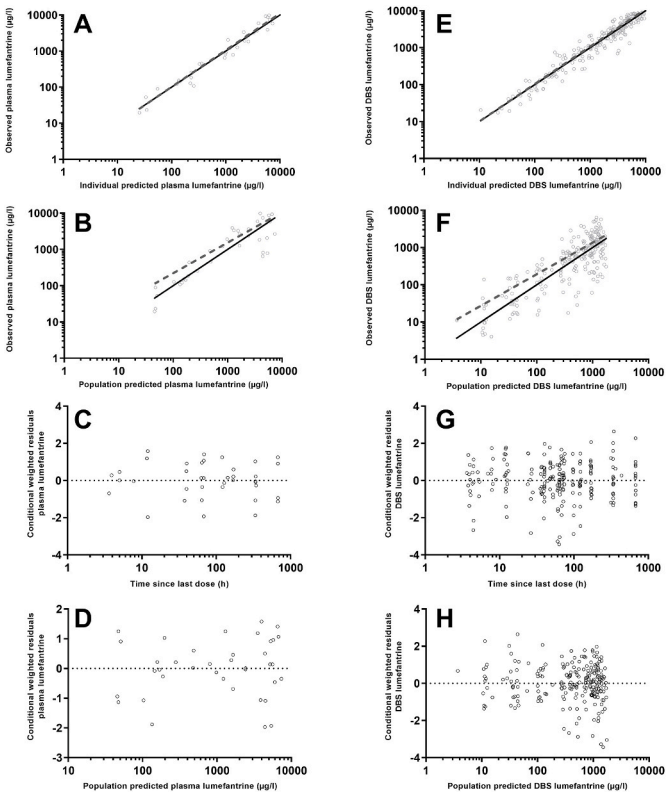

Fig. 3. Goodness-of-fit plots for lumefantrine in plasma (A–D) and dried blood spots (E–H), including observed concentrations against population (A, E) and individual predicted concentrations (B, F), and conditional weighted residuals against time from last dose (C, G) and population predicted concentrations (D, H).

genotype in the population pharmacokinetic model. The overall $AUC_{0-\infty}$ was within the range of a variety of published studies involving different populations, age groups and malaria status (see Table 4).

4. Discussion

This is the first study to examine the pharmacokinetic properties of

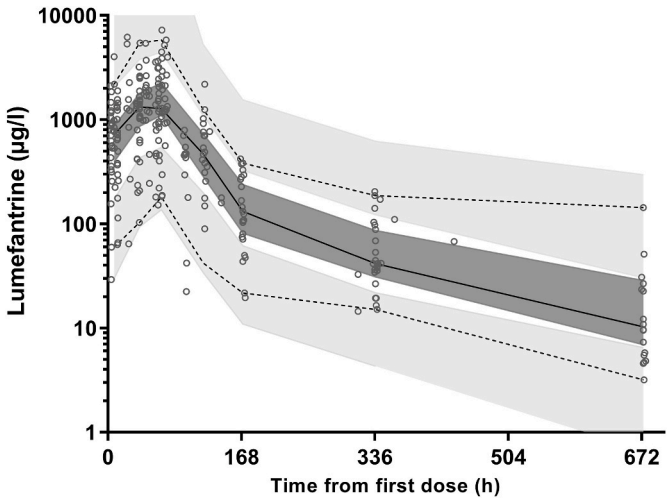

Fig. 4. Prediction corrected visual predictive check (pcVPC) for lumefantrine in plasma and dried blood spots with observed (black) and simulated (grey) 50th (solid line), 5th and 95th centiles (dotted lines) with overlying data points (°).

ARM-LUM in children with SCT and SCD. The results provide reassurance that conventional dose regimens for this ACT are appropriate in this vulnerable patient group. Although equivalent DBS data could not be used in analysis, available plasma ARM and DHA concentrations were consistent with those reported previously for paediatric patients from PNG with falciparum malaria treated with the same ARM-LUM regimen (Salman et al., 2011). In addition, the LUM population pharmacokinetic model, which utilised DBS as well as plasma concentration data, generated parameters including total drug exposure from $AUC_{0-\infty}$ that were comparable to those in other studies. We found no significant effect of haemoglobin genotype on the disposition of ARM, DHA or LUM.

Studies of the disposition of antimalarial drugs in children with SCD or SCT and malaria present ethical challenges. The recommended maximum blood volume that can be drawn safely in paediatric studies is limited to ≤8 mL/kg over 8 weeks (≤10% of total blood volume) (Howie, 2011). In addition, children with SCD can have marked pre-existing anemia which can worsen significantly during a crisis (Kane and Nagalli, 2022), a potentially life-threatening complication which can result from malaria infection itself (Makani et al., 2010). Because of these considerations, the present pragmatic study design included use of DBS as the main blood sampling modality, and a sparse sampling schedule limiting participant discomfort but sufficient for valid pharmacokinetic modelling. At the time the study was approved, a DBS assay for ARM-LUM and their active metabolites seemed feasible based on *ex vivo* experiments. However, due to a range of unanticipated technical and infrastructure issues, there was a delay of more than 12 months before the DBS samples could be analysed, and optimal conditions for their storage and transportation between Africa and Australia proved difficult to achieve. The apparent lack of validity of ARM, DHA and DBL DBS assays suggests that there are critical steps between fingerprick and mass spectrometry that influence the concentrations of these analytes, as we have since found in other studies using the same approach (Sugiarto et al., 2022).

The present study was primarily designed and powered based on LUM pharmacokinetic characteristics, in part because artemisinin compounds are known to have *ex vivo* stability issues with implications for their measurement in biological samples (Huang et al., 2013). While we have previously reported close agreement between DBS and plasma LUM concentrations in a study of Malaysian adults with knowlesi malaria (Sugiarto et al., 2022), the present DBS concentrations were lower than in reference plasma samples. This suggests degradation, including to DBL (Wong et al., 2011), during transportation and storage which was substantially longer and more difficult in the present study than in our previous Malaysian study (Sugiarto et al., 2022). Consistent with this, DBS DBL concentrations were around five times those in plasma. Fortunately, comparisons with reference plasma concentrations provided relatively robust evidence that this was uniform across all the samples collected with no bias by time of sampling or LUM concentration. In addition, there was no effect of sample type identified in the LUM pharmacokinetic modelling, further justifying our decision to include LUM concentrations from both DBS and plasma samples.

The final population pharmacokinetic parameters for LUM in the present study were comparable to those found previously in similar studies in children. The population apparent clearance reported in a sample of PNG children with uncomplicated malaria was 7.29 L/h/70 kg (Salman et al., 2011), close to the value of 6.68 L/h/70 kg obtained in the present study. The apparent central volume of distribution was lower in the PNG study at 227 L/70 kg compared to the 354 L/70 kg in our participants. While the two studies included children of similar age, there were clinical and analytical differences between them including malaria status and the use of a more complex 3-compartment pharmacokinetic model in the PNG study. A study of Ugandan children reported a higher apparent clearance and central volume of distribution, specifically 10.7 L/h/70 kg and 691 L/70 kg scaled from the reported/8.43 kg (Tchaparian et al., 2016). This difference may reflect the younger age of the Tanzanian participants (6–24 months), especially since the population pharmacokinetic model included increasing bioavailability with age that would result in lower apparent clearance and volume of distribution relative to bioavailability (CL/F and V/F, respectively). All three studies reported a similar and high inter-occasion variability in bioavailability of 59.4%–68.3% (Salman et al., 2011; Tchaparian et al., 2016) as reflected in the wide AUC distributions seen within studies.

The present LUM pharmacokinetic parameters are also comparable to those in a meta-analysis which included studies of both adults and children (Kloprogge et al., 2018). After adjustment for different weights used for allometric scaling (42 kg vs 70 kg) and the saturation model for dose (in mg/kg) effect on bioavailability, the value for CL/F in the meta-analysis is approximately 5.5 L/h/70 kg, aligning with the value obtained in the present study. Hietala et al. studied 50 Tanzanian children aged 1–10 years and reported a clearance equivalent to 5.39 L/h for a 70 kg patient (Hietala et al., 2010), also similar to the present result. Comparisons with this latter study, while most relevant in terms of population demographics, should be made with some caution given the abbreviated sampling duration of only 72 h after the start of treatment and the use of a single compartmental model (Hietala et al., 2010).

Overall exposure (AUC) for LUM, which is correlated with treatment

Table 3
Secondary pharmacokinetic parameters of lumefantrine for each sickle genotype and whole cohort. Data presented as median [interquartile range].

Parameter	Sickle genotype			Whole cohort
	AA	AS	SS	
$t_{½\alpha}$ (h)[a]	19.5 [17.7–21.7]	20.2 [19.3–23.2]	21.6 [21.0–22.4]	20.8 [19.3–22.5]
$t_{½\beta}$ (h)[a]	197 [193–205]	209 [199–213]	207 [204–211]	205 [198–211]
$AUC_{0-\infty}$ (µg.h/L)[b]	421,246 [357,668–918,809]	582,751 [475,759–728,029]	688,784 [497,159–1,127,755]	607,296 [426,480–860,773]

[a] $t_{½\alpha}$ is the distribution half-life, and $t_{½\beta}$ is the terminal elimination.
[b] Represents the $AUC_{0-\infty}$ for all six doses together.

Table 4
Summary of studies reporting area under the plasma concentration-time curve (AUC) for lumefantrine.

Sample	Study	$AUC_{0-\infty}$ (μg.h/liter)[a]
Healthy adults	German 2002 (German et al., 2009)	456,000
	Lefevre et al., 2002 (Lefevre et al., 2002)	383,000
	Bindschedler et al., 2000 (Bindschedler et al., 2000)	1,242,000–2,730,000[b]
	Olafuyi et al., 2017 (Olafuyi et al., 2017)	$AUC_{0\text{-last}}$ 195,000
Non-pregnant adults with malaria	Ashley et al., 2007 (Ashley et al., 2007)	432,000
	Lefevre and Thomsen, 1999 (Lefevre and Thomsen, 1999)	925,000–955,000
	Ezzet et al., 2000 (Ezzet et al., 2000)	356,000
	Hatz et al., 2008 (Hatz et al., 2008)	273,000–335,000
	Kloprogge et al., 2013 (Kloprogge et al., 2013)	630,000
Pregnant women with malaria	McGready et al., 2006 (McGready et al., 2006)	$AUC_{60-\infty}$ 252,000
	Tarning et al., 2009 (Tarning et al., 2009)	472,000
	Kloprogge et al., 2013 (Kloprogge et al., 2013)	570,000
	Kloprogge et al., 2015 (Kloprogge et al., 2015)	552,000
	Lohi Das 2018 (Lohy Das et al., 2018)	641,000
Pregnant women without malaria	Hughes et al., 2020 (Hughes et al., 2020)	287,000
Children with malaria	Hietala et al., 2010 (Hietala et al., 2010)	$AUC_{60-\infty}$ 210,000
	Abdulla et al., 2008 (Abdulla et al., 2008)	574,000–636,000 [c]
	Salman et al., 2011 (Salman et al., 2011)	$AUC_{60-\infty}$ 257,000 $AUC_{0-\infty}$ 459,980
	Mwesigwa et al., 2010 (Mwesigwa et al., 2010)	210,040
Young children with malaria	Tchaparian et al., 2016 (Tchaparian et al., 2016)	347,800
HIV-infected adults without malaria	Byakika-Kibwika et al., 2012 (Byakika-Kibwika et al., 2012)	281,550 [b]
	Kredo et al., 2016 (Kredo et al., 2016)	445,000
HIV-malaria-infected adults	Maganda et al., 2015 (Maganda et al., 2015)	784,830
Children with sickle cell disease	Current publication	607,296

[a] AUC was either a median, geometric mean or mean and was reported to ∞ unless otherwise stated.
[b] As subjects in Bindschedler et al. (2000) and Byakika-Kibwika et al. (2012) only received a single dose, the reported AUC has been multiplied by six.
[c] This study used a pooled approach from single observations in each subject to calculate AUC.

outcome, was within the range previously reported across a range of studies as summarised in Table 4. While the use of DBS sampling versus plasma concentrations in the other studies might bias the present AUC estimate, the inclusion of plasma samples in the present pharmacokinetic modelling along with reports that mixed capillary and venous LUM concentrations correlate with a 1:1 ratio (Huang et al., 2018) suggest that this was not significant. The non-significant trend to a higher AUC from AA to AS and SS genotypes might become significant with a larger sample size but, if present, would tend to reduce the risk of treatment failure in children with SCT or SCD. The large within-group variability is consistent with previous reports of LUM AUC, with significant overlap in the interquartile ranges.

Pathophysiological changes in SCT/SCD could influence hepatorenal function but this has been identified as significant in only a few of the drugs assessed and the direction of the effect is inconsistent. For example, morphine (Darbari et al., 2011) and cefotaxime (Maksoud et al., 2018), as with the antimalarial amodiaquine (Adjei et al., 2014), have been reported to have a clinically relevant higher clearance in SCD, while for lidocaine there is impaired hepatic clearance via the CYP3A4 pathway (Gremse et al., 1998). The latter is relevant to the present study as CYP3A4 is the main metabolic pathway for LUM. Similar to the present finding that there was no statistically significant effect of SCT/SCD on the LUM AUC, SCD did not influence methadone exposure, a drug which is also largely metabolised through CYP3A4, in a study of children and adults (Horst et al., 2016). These heterogeneous findings for drugs with similar metabolic pathways further emphasizes the importance of investigating the potential effect of SCT/SCD on the disposition on individual drugs including antimalarials.

As acknowledged, the limitations of the present study were restricted plasma and no supportive DBS concentrations for ARM/DHA, and a lack of suitable DBL data for inclusion in the pharmacokinetic modelling. This latter limitation needs to be viewed in the context of the minor contribution of DBL to antimalarial efficacy and the fact that levels are not routinely measured in most pharmacokinetic studies of ARM-LUM as a result. There was strong evidence that *ex vivo* metabolism of LUM in DBS versus plasma samples was uniform and thus that inclusion of both sample types in our modelling was valid. Nevertheless, the data highlight the potential for increased DBL concentrations from ex-vivo degradation of LUM which is present in much higher concentrations *in vivo*. Although there was a non-significant trend in LUM AUC by sickle genotype group, this favoured increased rather than reduced drug exposure in SCT/SCD. Malaria infection itself does not appear to have a significant effect on the disposition of ARM or DHA (Djimde and Lefevre, 2009), but it may reduce LUM exposure compared to that in healthy volunteers (Djimde and Lefevre, 2009; Kloprogge et al., 2018). Although, for ethical reasons, our study design excluded children with malaria, the trend to increasing LUM AUC would likely counteract this latter phenomenon in SCT/SCD.

In conclusion, the present data provide reassurance that the conventional 6 doses of ARM-LUM given over 3 days are appropriate for young children with either SCT or SCD. We found no evidence that this regimen was associated with the risk of sub-therapeutic concentrations of the individual drugs or the active metabolites through impaired absorption or increased elimination, or that there was the potential for toxicity due to impaired metabolism. Although there remain significant issues with DBS antimalarial assays, especially for the artemisinin compounds, there is still significant potential for this sampling modality to facilitate pharmacokinetic studies where conventional venous blood draws are problematic.

Author contributions

SRS collected the data, co-ordinated data analysis and interpretation, and produced the first draft of the manuscript. GMB contributed to data collection, analysis and interpretation, and reviewed/edited the manuscript. BRM contributed to data collection, analysis and interpretation, and reviewed/edited the manuscript. MPS performed the drug assays and reviewed/edited the manuscript. LM contributed to data interpretation, and reviewed/edited the manuscript. KTB LM contributed to data collection and interpretation, and reviewed/edited the manuscript. OMSM contributed to data interpretation, and reviewed/edited the manuscript. BN contributed to data interpretation, and reviewed/edited the manuscript. TMED designed the study, contributed to data analysis and interpretation, and produced the final version of the manuscript. JM contributed to study design, data collection and interpretation, and reviewed/edited the manuscript. SS contributed to study design, performed the PK modelling, and reviewed/edited the manuscript.

Data availability

The data that support the findings of this study are available from the

corresponding author upon reasonable request.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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