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Pharmacokinetic study of rectal artesunate in children with severe malaria in Africa

Caterina Fanello[1,2], Richard M. Hoglund[1,2], Sue J. Lee[1,2], Daddy Kayembe[3], Pauline Ndjowo[3], Charlie Kabedi[3], Benjamin B. Badjanga[3], Phettree Niamyim[2], Joel Tarning[1,2], Charles Woodrow[1,2], Melba Gomes[4], Nick P. Day[1,2], Nicholas J. White[1,2], Marie A. Onyamboko[3]

[1]*Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, United Kingdom*

[2]*Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand*

[3]*Kinshasa School of Public Health, University of Kinshasa, Kinshasa, Democratic Republic of Congo*

[4]*World Health Organization, Geneva, Switzerland*

[#]Corresponding author: Caterina Fanello caterina.fanello@ndm.ox.ac.uk

Running title: Rectal Artesunate in African children

Abstract

When severe malaria is suspected in children, WHO recommends pre-treatment with a single rectal dose of artesunate before referral to an appropriate facility. This was an individually randomized, open-label, 2-arm, cross-over clinical trial in 83 Congolese children with severe *falciparum* malaria, to characterize the pharmacokinetics of rectal artesunate.

At admission, children received a single dose of rectal artesunate (10 mg/kg) followed 12 hours later by intravenous artesunate (2.4 mg/kg) or the reverse order. All children also received standard doses of intravenous quinine. Artesunate and dihydroartemisinin were measured at eleven fixed intervals, following 0- and 12-hour drug administrations. Clinical, laboratory and parasitological parameters were measured. After rectal artesunate, artesunate and dihydroartemisinin showed large inter-individual variability (peak concentrations of dihydroartemisinin ranged from 5.63 to 8,090 nM). The majority of patients however, reached previously suggested *in vivo* IC_{50} (98.7%) and IC_{90} (92.5%) values of combined concentrations of artesunate and dihydroartemisinin between 15 to 30 minutes after drug administration. The median (IQR) time above IC_{50} and IC_{90} was 5.68 hours (2.90-6.08) and 2.74 hours (1.52-3.75), respectively. The absolute rectal bioavailability (IQR) was 25.6% (11.7-54.5) for artesunate and 19.8% (10.3-35.3) for dihydroartemisinin. The initial 12-hour parasite reduction ratio was comparable between rectal and intravenous artesunate: median (IQR) 84.3% (50.0-95.4) *vs*. 69.2% (45.7-93.6), respectively (p=0.49). Despite large inter-individual variability, rectal artesunate can initiate and sustain rapid parasiticidal activity in most children with severe *falciparum* malaria, while they are transferred to a facility where parenteral artesunate is available. (www.clinicalTrials.gov: NCT02492178)

Introduction

Parenteral artesunate is the treatment of choice for severe *falciparum* malaria (1). Intravenous or intramuscular artesunate was associated with a substantial reduction in mortality when compared with the previous first-line treatment, quinine (2, 3).

Unfortunately, many children with severe malaria die before or just after reaching a facility capable of administering parenteral drugs. To address this need, a rectal formulation of artesunate has been developed which has been shown in very large community-based trials to reduce malaria mortality in children unable to tolerate oral medications reliably (4-10). These trials, placebo-controlled, were conducted in Ghana (n=2238, 6-72 months old), Tanzania (n=3802, 6-72 months old) and Bangladesh (n=2010, 6-72 months old; n=4018, older children and adults) (5). In young children in Africa and Asia, rectal artesunate was associated with a reduced risk of death compared to placebo (n=8050, RR=0.74, 95% CI 0.59-0.93). In older children and adults in Bangladesh, rectal artesunate was associated with a more than two-fold increase in the risk of death compared to placebo (n=4018, RR=2.21, 95% CI 1.18-4.15, p=0.01) (7). No satisfactory explanation was found for this paradoxical finding. One concern was the possibility of artesunate toxicity as the absorption of rectal artesunate is erratic (11-13) and the dose given is around four times larger than the parenteral dose. Artesunate is rapidly metabolised, mainly by blood esterase and cytochrome P450 (CYP) 2A6, into its active metabolite dihydroartemisinin (14). Dihydroartemisinin is metabolised via glucuronidation by uridine-diphosphate-glucanosyltransferase (UGT)A1, UGT1A9 and UGT2B7 into inactive metabolites, which are renally eliminated (15, 16). Artesunate and dihydroartemisinin have very short biological half-lives of less than 30 minutes and approximately 1 hour, respectively, after both oral and parenteral administration of artesunate (17). To address concerns about a possible low efficacy and/or toxicity resulting from the erratic absorption of rectal artesunate, that have negatively impacted on its deployment, we conducted a randomised cross-over pharmacokinetic study of rectal artesunate *vs*. intravenous artesunate in children with

severe malaria in the Democratic Republic of the Congo at a time when parenteral quinine was still deployed as part of the first line treatment of malaria.

RESULTS

From the 11th July to the 6th October 2015, 136 patients with severe malaria were screened and 82 enrolled (Figure 1). Ten patients were added to the original sample size (n=72): in 7 cases a protocol deviation in the pharmacokinetic sampling scheme was reported, one patient expelled the study drug twice, one patient's worsening conditions did not allow blood sampling, and one patient died 10 hours after enrolment. The latter was retrospectively evaluated as having not met study inclusion criteria as the child had received a full treatment of artemether–lumefantrine and an unidentified traditional medicine before coming to the hospital, but the information was disclosed to the staff only after enrolment. Available data from all 82 patients who were randomized and allocated to study treatments were included in this intention-to-treat analysis.

Medical history

According to medical history, children were brought to the centre mainly because of fever (82/82, 100.0%; mean fever duration 3.7 days, 95% CI 3.4-4.1), severe prostration (64/82, 78.0%; mean duration 1.6 days, 95% CI 1.4-1.8) and convulsions (7/82, 8.5%). Other symptoms reported were gastrointestinal disorders (vomiting, abdominal pain, nausea), anorexia, asthenia and symptoms of an upper respiratory infection, with no significant differences between arms. Prior to admission, 3 children had received an Artemisinin-based Combination Therapy (ACT), 19 oral quinine, 3 intramuscular artemether and 1 intravenous (IV) quinine. The most common severity signs at screening were prostration (65/82, 79.3%),

73 respiratory distress (64/82, 78.0%), coma (14/82, 17.1%) and severe anaemia (25/82,
74 30.5%), (Table S1). Comorbidities included acute renal failure (n=1), gastritis and suspected
75 gastric ulcer (n=2), upper respiratory infection (n=1), suspected sepsis (n=2) and suspected
76 meningitis (n=3). At admission, the two treatment arms were well matched with no
77 significant differences (Table 1, S2-S4). Twenty-seven children were malnourished (27/79,
78 34.2%), 9 of whom were severely malnourished ('malnourished' was defined as a composite
79 variable of wasted, stunted and underweight). In 8 cases, 2 in the arm that received rectal
80 artesunate first (RAS_f) and 6 in the arm that received IV artesunate first ($IVAS_f$), patients
81 developed complications not present at admission, or not reported by the caregiver. These
82 included black water fever, convulsions, posturing, coma or deterioration of the coma score,
83 severe anaemia and respiratory distress.

84 **Clinical and parasitological response to treatment**

85 **Parasitaemia**

86 Children with symptoms of severe malaria were enrolled if they had a positive malaria Ag
87 Pf/Pan SD BIOLINE Rapid Diagnostic Test at screening. The mean (geometric, 95% CI)
88 peripheral blood parasitaemia at admission was 33,733/μL (15,031-75,702) in RAS_f arm and
89 40,067/μL (19,484-108,920) in $IVAS_f$ arm, p=0.31. The mean (geometric, 95% CI) peripheral
90 blood parasitaemia at first treatment (H0) was 40,111/μL (18,788-85,636) in RAS_f arm and
91 40,658/μL (16,261-101,656) in $IVAS_f$ arm (p=0.29). The median (range) plasma *Pf*HRP2 level
92 was 1,674.1 ng/mL (8.6-21,540.8) in RAS_f arm and 1,442.8 ng/mL (35.8-25,000.0) in $IVAS_f$
93 arm, p=0.33 (Table 1). Retrospectively, 3 patients were negative by microscopy and 2 had
94 only *falciparum* gametocytes; the plasma *Pf*HRP2 in these 5 cases ranged from 8.6 to 522.0
95 ng/mL. Two of these patients had received amodiaquine-artesunate and quinine tablets

prior to admission and reported a history of fever of 5 and 7 days, respectively. Children who received a treatment before arriving at the centre (documented or self-reported) had a lower parasitaemia at admission (n=23, 14,780/μL, 95% CI 4,024-54,285) compared to those who did not (n=53, 62,498/μL, 95% CI 33,601-116,249, p=0.06). The parasite reduction ratio (PRR) from hour 0 to 12 was comparable between arms with a median (IQR) PRR of 84.3% (50.0%-95.4%) in the RAS_f group and 69.2% (45.7%-93.6%) in the $IVAS_f$ group (p=0.49). The estimated median (range) time for parasitaemia to decrease by half was 2.2 hr in RAS_f arm (1.3-7.6) and 2.5 hr in $IVAS_f$ arm (1.2-12.0), with no difference between arms (p=0.64) (Table 2 and Table 3). The limit of detection was 16 parasites/μL and 1 case was excluded for insufficient data points.

Haematology

The mean (SD) haemoglobin (Hb) at H0 was: 7.1 (2.5) g/dL in RAS_f arm and 6.9 (2.3) g/dL in $IVAS_f$ arm. Children with the sickle trait (n=5) had a mean (SD) Hb of 6.5 (3.1) g/dL, while the two children with sickle cell disease had 3.7 and 3.9 g/dL at admission. Fifty-three children received a blood transfusion: 26 (65.0%) in RAS_f arm and 27 (64.3%) in $IVAS_f$ arm (p=0.95). From admission to 12 hours (before the cross-over), the median (IQR) difference in Hb was -2.5 (-4.3, 0.7) in RAS_f arm and -2.2 (-3.9, 0.6) in $IVAS_f$ arm (p=0.75, Table 3). By day 7, the mean Hb (SD) was 9.6 (1.4) g/dL in RAS_f arm (n=38) and 9.3 (1.6) g/dL in $IVAS_f$ arm (n=41, p=0.46 adjusted for baseline Hb) and by day 14, 10.6 (1.0) g/dL in RAS_f arm (n=36) and 10.2 (1.5) g/dL in $IVAS_f$ arm (n=40, p= 0.21 adjusted for baseline Hb).

Follow-up visits and neurological assessment

The median time of hospitalization was 3 days (range 3-14 days). There were no significant differences between arms in the recovery time (median time from admission to sit unsupported (p=0.74), speak (p=0.41), localise painful stimuli (p=0.58), eat/breastfeed (p=0.95). Only one patient (randomised to $IVAS_f$ arm; received 11.8 mg/kg of rectal artesunate at 12 hours) developed neurological sequalae (unable to walk); the patient was admitted with a Glasgow Coma Score of 9/15, 9,546 parasites/μL, Hb 6.7 g/dL and temperature 38.0°C, developed convulsions and posturing after admission and was in hospital for 4 days. The sequelae completely resolved by day 14.

Adverse events

Forty-nine patients (59.8%) reported one or more adverse events of mild or moderate intensity, without differences between arms. Most patients had fluctuations in the electrolytes (n=31) or WBC and platelets counts (n=7) above or below the normal range. One child developed pruritus after IV artesunate administration; this was classified as possibly related. The remaining cases were all classified as unlikely to be related or unrelated to drug administration (Table 4).

Rectal artesunate administration

In RAS_f arm, 5 patients expelled the suppositories within 60 minutes (range, 2-29 minutes), and a new dose was administered. In $IVAS_f$ arm, 5 patients expelled the suppositories within 60 minutes (range, 10-15 minutes) and a new dose was administered. One patient also expelled the second dose. These 10 events were classified as possibly related to rectal artesunate administration. Children in the first weight group (6.0-12.9 kg) received (median,

IQR) 9.1 (8.3-10.0) mg/kg of rectal artesunate, 12.5 (10.7-14.3) mg/kg in the second group (13.0-23.9 kg) and 10.7 (9.8-11.8) mg/kg in the third group (24.0-34.0 kg) (Table S5).

Pharmacokinetic properties of rectal artesunate

Data from 80 patients (two patients with data from only 1 time point were excluded) were used to evaluate the pharmacokinetic properties of artesunate and dihydroartemisinin after intravenous and rectal administration. After rectal administration, both artesunate and dihydroartemisinin reached peak plasma concentrations relatively fast, resulting in a median (IQR) time to reach maximum concentration (T_{MAX}) of 0.5 hr (0.25-0.75) for artesunate and 1.0 hr (0.75-2.00) for dihydroartemisinin. However, individual concentration-time profiles of both artesunate and dihydroartemisinin showed large inter-individual variability in both arms, especially after rectal administration (Figure 2). The absolute peak concentrations of dihydroartemisinin varied between 5.63 nM and 8,090 nM after rectal administration (*i.e.,* 1,000-fold variation). Almost all patients (79/80, 98.7%) reached the putative IC_{50} value of 34.9 nM, and most patients (74/80, 92.5%) reached the IC_{90} value of 314 nM, at a median (IQR) time of 0.25 hr (0.25-0.25) and 0.25 hr (0.25-0.50), respectively (Figure 3). Time above the putative IC_{50} varied between patients, with a median 5.68 hr (2.90-6.08) above the IC_{50} value and 2.74 hr (1.52-3.75) above the IC_{90} value (Figure 3). Two patients had a zero duration above the IC_{90}, since only one observation was above the cut-off. The median (IQR) rectal bioavailability was estimated to be 25.6% (11.7-54.5) for artesunate and 19.8% (10.3-35.3) for dihydroartemisinin, emphasising the large inter-individual pharmacokinetic variability. Similar results were obtained if all patients that expelled the suppositories were excluded from the pharmacokinetic analysis. A detailed description of patients who did not

reach IC_{50} (n=1) or reached IC_{90} later than others (n=2) is presented in the Supplementary Materials.

Compared to intravenous artesunate, rectal artesunate had a lower [median (IQR)] maximum concentration ($C_{max,}$ nM) [442 (213-813) *vs.* 2,560 (1,450-5,200)], later time to reach the maximum concentration (T_{max}, hr) [0.500 (0.250-0.750) *vs.* 0.083 (0.083-0.100)], and lower total drug exposure (AUC_T, area under the concentration-time curve until the last measurable concentration, h x nM/µmole) [2.57 (1.68-4.57) *vs.* 10.20 (5.92-14.00)]. Whereas, the terminal elimination half-life ($t_{1/2}$, hr) was comparable between intravenous and rectal artesunate [0.525 (0.325-0.770) *vs.* 0.571 (0.299-1.08)] (Table 5). The exposure and bioavailability of artesunate and dihydroartemisinin, were comparable between patients who received rectal artesunate at time 0 and those who received it at 12 hours. Similarly, exposure was comparable after IV artesunate regardless the time of administration. Children received a median (IQR) of 10.5 mg/kg (9.1-12.0) of rectal artesunate, corresponding well with the intended target dose (Table 5S). Fourteen children (17.0%) received less than 9.0 mg/kg [median (IQR) 8.3 mg/kg; 8.0-8.3] and 13 children (15.9%) received more than 13.0 mg/kg (14.3 mg/kg; 13.3-15.4). There were no significant differences in the pharmacological parameters (bioavailability, p=0.37 for artesunate and p=0.57 for dihydroartemisinin) or pharmacodynamic parameters (parasite clearance time, p=0.90) between children who received a higher (≥13.0 mg/kg, n=13) or lower dose (<9.0 mg/kg, n=14) compared to the target dose (9.0-12.9, n=55). No significant differences were observed between patients who received a blood transfusion after admission and those who did not, within each treatment arm. After rectal artesunate administration, nourished (n=54), malnourished (n=17) and severely malnourished (n=9) children had

183 significantly different median (IQR) exposure (AUC_T/dose) to artesunate [2.28 (1.12-3.52) h x
184 nM/µmole, 2.32 (1.71-5.19) h x nM/µmole and 4.99 (4.36-6.45) h × nM/µmole, respectively;
185 p=0.04] and dihydroartemisinin [5.87 (3.38-11.1) h × nM/µmole, 7.24 (4.93-14.4) h ×
186 nM/µmole, and 16.5 (7.96-25.8) h × nM/µmole, respectively, p=0.02]. In contrast,
187 bioavailability was comparable between these three groups (p>0.05).

188 **Discussion**

189 This pharmacokinetic study in children with severe *falciparum* malaria showed that
190 artesunate is rapidly absorbed by most patients after rectal administration: 98.7% of
191 children reached IC_{50} within a median (IQR) time of 0.25 hr (0.25-0.50), and 92.5% of them
192 reached IC_{90} within 0.25 hr (0.25-0.50). The median time spent above IC_{50} was more than
193 five hours, indicating that the rectal formulation will start and continue its parasiticidal
194 activity during transportation to a medical facility. Rectal artesunate suppositories were able
195 to reduce parasitaemia rapidly, as shown by the similar rates of parasitaemia reduction after
196 treatment with either rectal or parenteral artesunate. Therefore, any differences in
197 pharmacokinetic parameters between arms and the variability observed, did not translate
198 into a worse pharmacodynamic profile, confirming previous results in studies of patients
199 with severe and moderately severe *falciparum* malaria (6, 11). Artemisinin derivatives clear
200 parasitaemia more rapidly than other drugs, and by acting on ring stages prevent parasites
201 from maturing and sequestering. In contrast, quinine acts only in a limited manner on ring
202 stages (18) and the initial parasitaemia reduction observed, although dependent upon the
203 mean age of development of circulating parasites, is typically slower than with artemisinin
204 derivatives (19). Therefore, we assume that the administration of quinine in our study
205 affected only a negligent proportion of the peripheral parasitaemia and the fast parasite

reduction observed was mainly the result of the absorbed artesunate. These results support giving pre-referral artesunate to all children suspected of having malaria who cannot reliably take oral medications, including children who might have otherwise uncomplicated malaria with repeated vomiting to profoundly ill unconscious children. The quick absorption of artesunate after rectal administration is encouraging, but the formulation also exhibits a high variability in exposure due to the very high variability in absorption. However, as rectal artesunate is not a replacement for intravenous treatment, but instead an early start of the treatment while being transported to the hospital, the total exposure is not the most important pharmacokinetic parameter. Instead, it is important to absorb enough of the drug to reach effective concentrations rapidly. Most children received appropriate doses of rectal artesunate, according to the target dose of 10 mg/kg, and no significant differences in bioavailability or parasitaemia reduction were observed in the groups that received lower or higher doses, allaying any potential concerns related to a reduced efficacy or toxicity. Although the study was not designed or sufficiently powered to detect a difference in the rate of adverse events between treatments, the number was low and comparable between arms. Malnutrition is frequent in malaria endemic areas and it has been associated with an increased risk of reduced antimalarial drug exposure (20). In this study, malnourished and severely malnourished children had a slightly higher drug exposure compared to the other children. The severely malnourished group was small in the present study and a larger study would be needed to investigate this result further. In line with 2010 WHO recommendations (21), rectal artesunate was included in the National Guidelines of DRC in 2012, although its deployment on a large scale has since been delayed (22). The results of this study support country-wide deployment of this intervention in the Democratic Republic of Congo.

Conclusions

Clearly parenteral artesunate is preferable to rectal administration, but this is not an option in many villages and rural health centres in resource limited areas. Despite large inter-individual variability, rectal artesunate can initiate and sustain rapid parasiticidal activity in most children with severe *falciparum* malaria, while they are transferred to a facility where parenteral artesunate is available.

METHODS

Study site

This trial was conducted by the Kinshasa School of Public Health – University of Oxford Medical Research Unit (KIMORU) team, at Kingasani Hospital, Kinshasa, the Democratic Republic of Congo. Malaria transmission in the area is high and perennial.

Trial design

This was an individually randomized, open labelled, 2-arm, cross-over clinical trial in children admitted to hospital with severe malaria (23). A weight group-stratified 1:1 randomization design was used, with three blocks for each arm according to body weight (6.0-12.9 kg, 13.0-23.9 kg and 24.0-34.0 kg), in order to have the same number of patients administered 1, 2 or 3 suppositories. A computer-generated randomisation list was prepared by a study statistician. Treatment allocation was concealed in sequential opaque envelopes prepared by an independent person. The intervention was assigned by the study nurse, after the doctor confirmed eligibility and the caregiver had signed the informed consent. When the envelope was opened and signed, the patient was considered enrolled.

Eligibility criteria for participants

Children were included in the study if they fulfilled the WHO criteria for severe *falciparum* malaria (23), had a weight ≥6.0 kg and ≤34.0 kg, a positive malaria Ag Pf/Pan SD BIOLINE RDT and their parent or carer gave fully informed consent. Children were not included if they had acute diarrhoea (defined as >3 liquid stools in the previous 24 hours), visible anorectal malformations or a disease of the rectum, known hypersensitivity to quinine or artesunate, a documented history of an effective dose of parenteral antimalarial in the preceding 24 hours, a single dose of rectal artesunate in the previous 12 hours, a dose of an ACT in the previous 6 hours, a co-morbidity which could have interfered with the study or put at risk the patient, or participation in another clinical trial or earlier in the same clinical trial.

Interventions

Children were randomised to receive either 1 dose of rectal artesunate (approximating as closely as possible to 10 mg/kg) on admission (time 0) followed 12 hours later by intravenous artesunate (2.4 mg/kg) (RAS_f arm) or the reverse order ($IVAS_f$ arm). Children were observed for 1 hour, and if the suppository was expelled within 60 minutes, a single attempt was made to re-administer a second dose (the second dose was equal to the number of suppositories expelled). As the absorption of rectal artesunate is known to be erratic, all children were given intravenous quinine (20 mg salt/kg loading dose at presentation followed by 10 mg/kg 8 hourly) by rate-controlled infusion for a total of three doses (1). Quinine and artesunate can be administered concomitantly without risk (24). The quinine infusion was started immediately after the study drug. If a blood transfusion was needed at admission, quinine was started after the blood transfusion was terminated,

although the first dose of the study drug was not delayed. After 24 hours, all patients continued antimalarial therapy with parenteral artesunate followed by a full standard course of artemether-lumefantrine as soon they were able to take oral medication. If the child was discharged before the oral treatment was terminated the remaining doses were given to the caregiver for home administration.

Study drugs

Intravenous artesunate (Guilin Pharmaceuticals, China), intravenous quinine (Rotex, Germany) and Coartem® (Novartis) were purchased from Medical Expert Group, Gorinchem, The Netherlands. Rectal artesunate, in suppositories of 100 mg each, was produced by Catalent, Germany Eberbach GmbH, packed by Scanpharm, Copenhagen, Denmark and provided by the World Health Organization for this study.

Outcomes

The primary objective of the study was to assess the pharmacokinetics of rectal artesunate in paediatric patients with severe *P. falciparum* malaria. The secondary objective was characterisation of the clinical and parasitological responses to rectal artesunate compared to intravenous artesunate. A randomised sequence cross-over design was employed to characterise the bioavailability of rectal artesunate and to characterise the individual absorption profiles of both artesunate and dihydroartemisinin. From a therapeutic perspective, rectal artesunate aims to achieve minimum parasiticidal concentrations (MPC) as soon as possible. Both artesunate and dihydroartemisinin exhibit parasiticidal effects and therefore the sum of the molar artesunate and dihydroartemisinin concentrations were evaluated and the time to reach, time above, and the proportion of patients to reach a

putative IC_{50} and IC_{90} (*i.e.,* 34.9 nM and 314 nM, respectively) were considered the primary end-points with conventional pharmacokinetic measures as secondary end-points (e.g., bioavailability). The IC_{50} value was taken from the estimated EC_{50} (concentration in the dihydroartemisinin effect compartment) (25), and the IC_{90} was calculated from the IC_{50}.

Investigations

Malaria at screening was confirmed by Malaria Ag Pf/Pan SD BIOLINE Rapid Diagnostic Test. A malaria blood film was prepared at admission, 0 (pre-dose), 6, and 12 hours, and thereafter every 12 hours until 2 consecutive blood films were negative. Parasites were identified and counted by standard light microscopy. Haemoglobin (Hb) and haematocrit (Hct) were assessed at the same time points as the blood films using HemoCue Hb301+® (Angelholm, Sweden) and Hawksley Haematospin 1400 (Hawksley & Sons, Ltd. UK). Total and differential white blood cell (WBC) counts were measured using QBC Star™ at 0, 24 and 72 hours. Biochemistry tests were performed at 0 and 24 hours by i-STAT using the CHEM8 cartridge for electrolytes and the CG4 cartridge for blood gases. Haemoglobin S trait was detected by electrophoresis using the SEBIA Hydragel Hemoglobin K20 Kit. Quantification of plasma *Pf*HRP2 by ELISA (Celisa, Cellabs, Sydney, Australia) was performed at Mahidol Oxford Tropical Medicine Research Unit (MORU) laboratories, Bangkok, Thailand. Laboratory technicians were blinded to study treatment allocation.

Pharmacokinetic blood sampling

Eleven blood samples were taken at fixed intervals, pre-treatment, 5, 15, 30, 45 minutes, 1, 2, 3, 4, 6 and 8 hours after the administration of the first dose of study drug. The sampling scheme was repeated after 12 hours following administration of the second dose of the

study drug. Blood was sampled through an indwelling cannula in the arm opposite to that used for intravenous drug administration; 1 mL of blood was collected into pre-chilled fluoride oxalate tubes for artesunate and dihydroartemisinin quantification. Samples were centrifuged at 4°C and 2,000*g* for 7 minutes. Plasma samples were stored at −80°C until they were shipped to the MORU Department of Clinical Pharmacology, Bangkok, Thailand, for drug quantification. Artesunate and dihydroartemisinin were quantified using liquid chromatography-tandem mass-spectrometry (26). The coefficient of variation of the assay was less than 7% at each level of quality control samples and the Lower Limit of Quantification (LLOQ) was set to 1.19 ng/mL and 1.96 ng/mL for artesunate and dihydroartemisinin, respectively.

Patient management

Patients were managed according to WHO Guidelines for the management of severe malaria (1). Fever was treated with parenteral paracetamol 20 mg/kg. Hypoglycaemia (blood glucose <3 mmol/l) was treated with an IV bolus of 5 ml/kg of 10% dextrose. A blood transfusion (20 ml/kg) was given to children with haemoglobin concentrations of <5 g/dl. A fluid bolus was given to children with signs of shock. Convulsions were treated with IV diazepam. All children were given ceftriaxone intravenously (75 mg/kg at time 0 and after 12 hours).

Assessment at follow-up visits

Patients were hospitalized for at least 4 days, or longer if they were still unwell, and discharged after at least the first dose of the oral follow-on treatment was administered.

Parents/guardians were asked to bring the child back to the clinic at day 7 (if they were discharged earlier) and 14 for clinical examination, neurological exam and laboratory tests.

Ethics

The study was approved by the Ethical Committee of the Kinshasa School of Public Health, the Ministry of Public Health of DRC and the Oxford University Tropical Research Ethics Committee (OXTREC). The study documents were originally designed in English. The protocol was translated into French, the Patient Information Sheet and Informed Consent into French and Lingala by a certified translator. Safety reporting was performed according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice (1996). ClinicalTrials.gov Identifier: NCT02492178.

Sample size

This was considered a bioequivalence study, comparing a new formulation (rectal) to a reference (intravenous). Previous data in uncomplicated and moderately severe malaria in paediatric patients showed that rectal artesunate is characterised by a large inter and intra-individual variability (11-13). Assuming a within-subject coefficient of variation of 40% (27, 28), CV, a sample size of 72 patients was estimated to be sufficient to assess bioequivalence of the two drugs, with 90% power and 95% confidence, including 10% loss to follow-up. This sample size was calculated using the formula by Julious for cross-over studies (29).

Statistical analysis

Medical histories of patients were described using frequencies (%) for each study arm. Clinical and parasitological responses to treatment were reported using geometric means

(GM) with 95% confidence intervals (CI), median (interquartile range (IQR) or range) or mean (SD), and compared between treatment arms using the Kruskal-Wallis test or Student's t-test. Haemoglobin (Hb) comparisons after baseline were adjusted for baseline values of Hb. Parasite clearance half-life ($PC_{1/2}$), lag time (t-lag), and the time to clear 50, 90, 95 and 99% of parasites was calculated using the Parasite Clearance Estimator developed by WWARN (30), which was modified to allow for a lower threshold of parasitaemia at time 0. The parasite reduction ratio was calculated as the difference at 12 hours from baseline, divided by the baseline parasite count. Pharmacokinetic parameters were compared between treatment arms as well as between children who received higher (≥13.0 mg/kg) or lower doses (<9.0 mg/kg) compared with the target dose (9.0-12.9), between nourished, malnourished and severely malnourished children, and between patients who received blood transfusion or not. The subgroup of children who did not expel their suppositories was also assessed. Statistical analyses were performed using STATA IC 14.0 (STATA Corporation, college station, Texas 77845, USA) and GraphPad Prism Software (San Diego, California 92108 USA).

Pharmacokinetic analysis

Artesunate and dihydroartemisinin concentration-time profiles were analysed on an individual level using a standard non-compartmental approach in Phoenix® 64 (Certara USA, Inc., Princeton, USA). All concentrations at time zero were set to zero. The concentration at the time point when the concentration-time profile went permanently below the LLOQ for the first time was set to half the LLOQ. Artesunate data after IV administration were analysed assuming an infusion using the true injection times for patients, while the rectal administration as well as the dihydroartemisinin data after IV and rectal administration was

handled as extravascular administration. The observed concentration-time profiles were used to derive the maximum concentration (C_{MAX}), the time to maximum concentration (T_{MAX}), and the time to the last measured concentration (T_{LAST}). Total drug exposure (area under the concentration-time curve; AUC_T) were calculated using the observed data, from drug administration to the last time point. The calculations of AUC were based on the trapezoid method, using the linear method before C_{MAX} and the logarithmic method after C_{MAX}. Terminal elimination half-life ($t_{1/2}$) was based on the best fit of the terminal portion of the elimination phase. Absolute rectal bioavailability (F) was estimated based on individual drug exposures after rectal and intravenous administration, according to the equation below:

$$F = \frac{AUC_{\mathrm{T},rectal}}{AUC_{\mathrm{T},IV}} \times \frac{Dose_{IV}}{Dose_{rectal}}$$

Time to reach a putative IC_{50} and IC_{90} value (25), the time spent above these values, and the proportion of patients who reached this value were derived directly from the observed concentration-time profiles. Pharmacokinetic parameters from each mode of administration were compared using a Mann-Whitney U test, to determine the effect of drug administration time (0 or 12 hours) and of blood transfusion, and with Kruskal-Wallis test to compare severely malnourished, malnourished and nourished children.

Study contribution

Study design: NJW, CF, MO, JT, ND, MG. Data collection: MO, CF, DK, CK, PN, BB. Statistical analysis: SJL, CF, RH, JT. Laboratory analysis: CW, BB, PN. Manuscript writing: CF, SJL, RH, NJW.

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Declaration of interests

The are no conflict of interest

Data availability

The data that support the findings are available from the authors upon reasonable request and with permission of the University of Oxford and the Kinshasa School of Public Health.

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530

FIGURES

Figure 1. Flow chart CONSORT

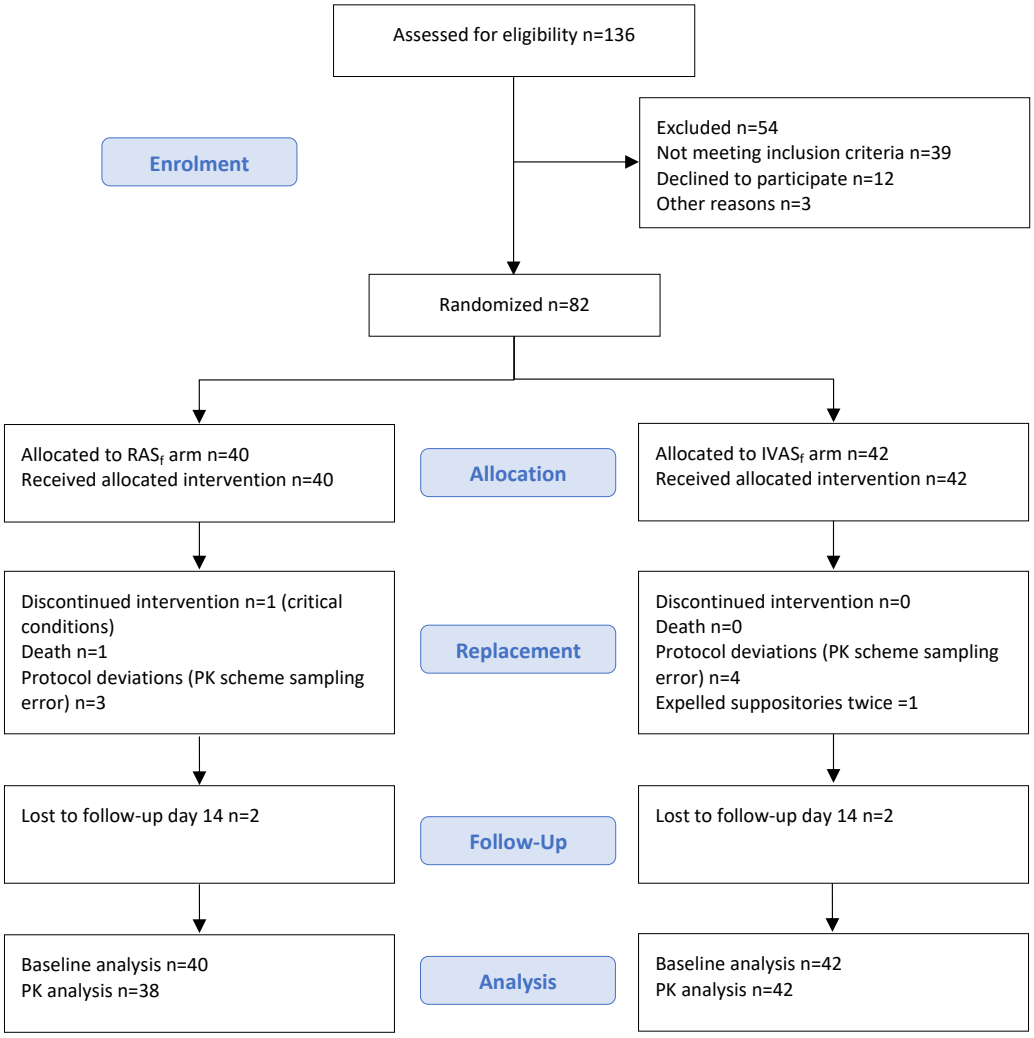


Figure 2. Observed individual artesunate concentration-time profiles. Artesunate and dihydroartemisinin, administrated intravenously or rectally. The dashed horizontal line represents a putative IC_{50} value of 34.9 nM and the dotted horizontal line represents a putative IC_{90} value of 314 nM. The red line represents the patient who did not reach the IC_{50} value after rectal administration.

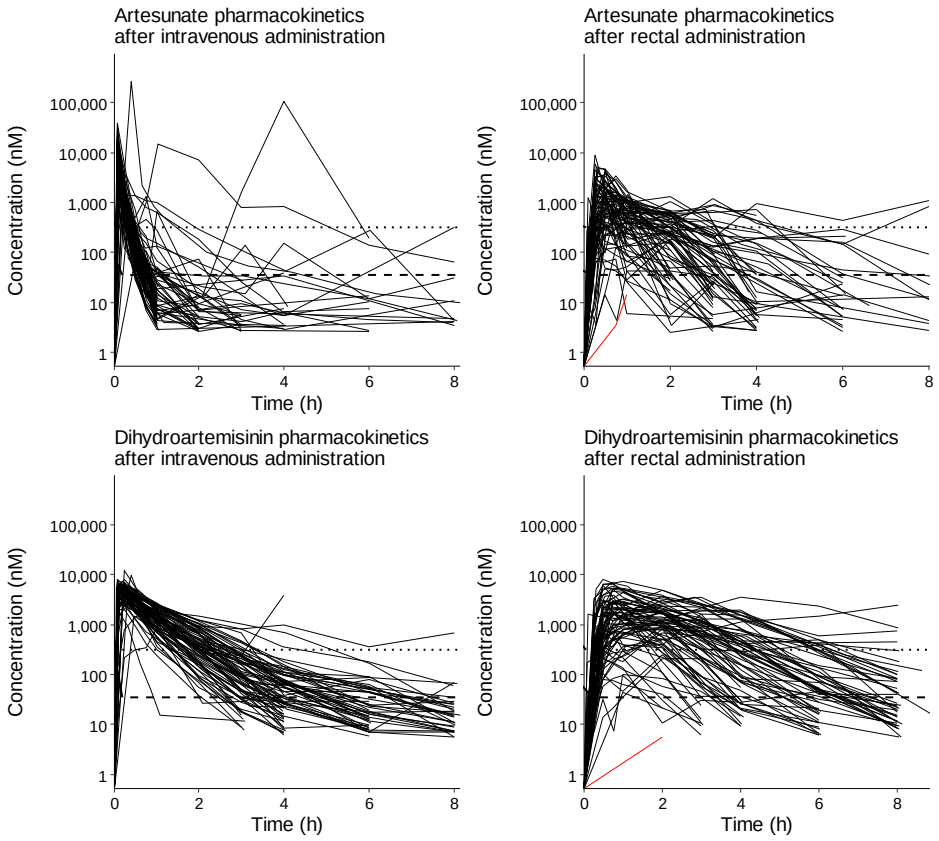
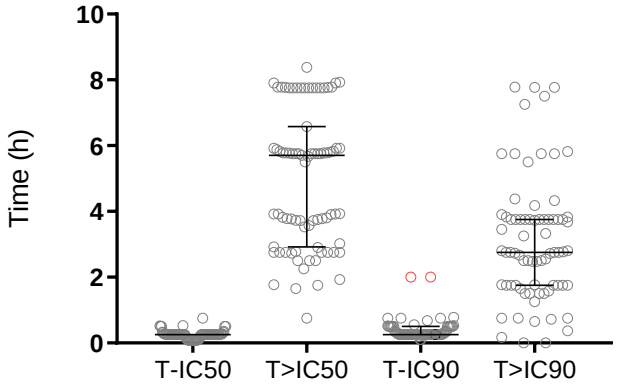


Figure 3. Graphical representation of time to putative IC_{50} (T-IC_{50}), time above IC_{50} (T>IC_{50}), time to putative IC_{90} (T-IC_{90}), and time above IC_{90} (T>IC_{90}) after rectal artesunate administration. Concentrations were measured as the sum of molar artesunate and dihydroartemisinin concentrations. Markers represent individual values and lines represent the median value and its interquartile range. The graphic shows only data for patients who reached the cut-off (IC_{50} or IC_{90}). One patient did not reach IC_{50}. The red dots indicate the 2 cases with a longer time to reach IC_{90}



TABLES

Table 1. Baseline data

Variables	RAS_f	$IVAS_f$
Evaluated [a]	40	42
Median age (IQR), yrs.	4.67 (2.8, 8.1)	4 (2.8, 8.8)
Median weight (IQR), kg.	15.3 (12.0, 25.0)	14.3 (12.0, 24.5)
Median height (IQR), cm.	102 (89.0, 129.0) n=39	98 (88.0, 131.0)
Median MUAC (IQR), cm.	16.0 (14.5, 18.0)	15.2 (14.0, 17.0)
No. male (%)	21 (52.5)	20 (47.6)
No. with enlarged liver (%)	24 (60.0)	28 (66.7)
Median enlarg. (IQR), cm.	2.0 (0, 3.5)	3 (0, 4.0)
No. with enlarged spleen (%)	29 (72.50)	30 (71.43)
Median enlarg. (IQR), cm.	3.0 (0, 4)	3.0 (0,4)
Sickle cell trait (%)	1/39 (2.6)	4/40 (10.0)
Sickle cell disease	0/39	2/40 (5.0)
Mean HCT (SD), %	21.3 (7.6)	20.6 (7.1)
Mean Hb (SD), g/dL	7.1 (2.5)	6.9 (2.3)
Geo. mean parasites/μL (95% CI) Screening	33,733 (15,031- 75,702) n=37	46,067 (19,484 - 108,920) n=39
Median *Pf*HRP2 (range), ng/mL	1,674.1 (8.6 to 21,540.8) n=39	1,442.8 (35.8 to 25,000.0) n=42
Mean BP systolic (SD), mmHg	90.6 (7.8) n=38	92.6 (9.6) n=41
Mean BP diastolic (SD), mmHg	53.7 (7.4) n=38	55 (6.7) n=41
Median heart rate (IQR), bpm	146 (126, 163) n=39	146.5 (123, 154)
Median respiratory rate (IQR), bpm	48 (40, 60)	44 (42, 52)
Mean temperature (SD), °C	38.0 (1.1)	37.9 (1.1)
Blood transfusion (%)	26 (65.0)	27 (64.3)

[a] Unless indicated otherwise

Table 2. Summary of parasite clearance time by study arm

Parameters [a]	RAS_f	$IVAS_f$	p-value
Individual profiles analysed	35	40	
Slope half-life, hrs			0.64
Median	2.2	2.5	
Range	1.3-7.6	1.2-12.0	
Geom. Mean (95% CI)	2.3 (2.0; 2.6)	2.43 (2.1; 2.8)	
t-lag hrs			0.81
Median	0	0	
Range	0-12	0-24	
IQR	0-6	0-6	
Geom. Mean (95% CI)	6.9 (5.9- 8.1) n=5	8.3 (6.2, 11.0) n=15	
Median pc50 (range) hrs	7.1 (0.3, 15.1)	6.8 (0.4, 24.4)	0.28
Median pc90 (range) hrs	11.8 (4.1, 25.7)	13.9 (3.5, 41.8)	0.16
Median pc95 (range) hrs	14.0 (5.7, 33.3)	16.8 (4.8, 53.8)	0.13
Median pc99 (range) hrs	18.8 (9.1, 50.8)	22.1 (7.8, 81.7)	0.14

[a] Unless indicated otherwise

Table 3. Haematology at 0 and 12 hours by arm

Parameters	RAS_f	$IVAS_f$	p-value
Mean (SD) haemoglobin			
At hour 0	7.1 (2.6) n=39	6.88 (2.3) n=42	
At hour 12	9.1 (1.6) n=38	8.7 (2.2) n=42	
Median (IQR) within individual difference (from H0 to H12)	-2.5 (-4.3, 0.7) n=80	-2.2 (-3.9, 0.6) n=80	0.75
Geometric mean (95% CI) parasitaemia			
At hour 0	40,111 (18,788, 85,636) n=36	40,658 (16,261, 101,656) n=40	
At hour 12	5,420 (1,853, 15,851) n=34	8,518 (2,721, 26,667) n=38	
Median (IQR) within individual difference (from H0 to H12)	6.3 (2.0, 18.1) n=72	3.0 (1.8, 12.0) n=72	0.37

Table 4. List of Adverse Events

Adverse Event	RAS_f	$IVAS_f$	Total
Electrolytes changes	15	16	31
WBC/platelets changes	2	5	7
Expelled artesunate suppository [a]	5	5	10
Pruritus/cutaneous rash [a, b]	0	1	1
Urticaria [c]	0	1	1
Viral/bacterial infection suspected	2	5	7
Intestinal parasite infection suspected	1	1	2
Vomit	0	1	1
Epistaxis	0	1	1
Swollen face/Acute Renal Failure	0	1	1
Hypersialosis/Acute Renal Failure	0	1	1
Conjunctivitis (day 14)	1	0	1
Gastroenteritis (day 14)	1	0	1

[a] Classified as possibly related;
[b] Developed after IV AS administration;
[c] Developed 8 minutes after blood transfusion started

Table 5. Pharmacometric parameters of artesunate and dihydroartemisinin after intravenous and rectal administration of artesunate in children with severe malaria

Parameters	Intravenous administration median (IQR)	Rectal administration median (IQR)
Artesunate		
Analysed, n	80	80
T_{MAX} (h)	0.083 (0.083-0.100)	0.500 (0.250-0.750)
C_{MAX} (nM)	6,660 (3,770-13,500)	442 (213-813)
C_{MAX}/D (nM/µmole)	71.0 (40.6-99.1)	2.08 (1.05-4.26)
T_{LAST} (h)	3.00 (2.00-6.00)	6.00 (4.00-8.00)
AUC_T/D (h × nM/µmole)	10.2 (5.92-14.0)	2.57 (1.68-4.57)
$t_{1/2}$ (h)	0.571 (0.299-1.08) [a]	0.525 (0.325-0.770) [b]
F (%)	-	25.6 (11.7-54.5)
Dihydroartemisinin		
T_{MAX} (h)	0.250 (0.083-0.250)	1.00 (0.750-2.00)
C_{MAX} (nM)	5,100 (3,850-5,860)	1,800 (822-3,100)
C_{MAX}/D (n M/µmole)	40.8 (28.6-74.3)	3.34 (1.88-6.44)
T_{LAST} (h)	8.00 (6.00-8.00)	8.00 (7.89-8.00)
AUC_T/D (h × n M/µmole)	37.4 (24.3-61.2)	6.97 (3.94-14.5)
$t_{1/2}$ (h)	0.882 (0.686-1.19) [c]	0.865 (0.681-1.18) [d]
F (%)	-	19.8 (10.3-35.3)

[a] One individual was excluded from the analysis due to a lack of data in the elimination phase.

[b] Six individuals were excluded from the analysis due to a lack of data in the elimination phase.

[c] One individuals were excluded from the analysis due to a lack of data in the elimination phase.

[d] Seven individuals were excluded from the analysis due to a lack of data in the elimination phase.

Abbreviations IQR: inter-quartile range; C_{MAX}: maximum concentration; T_{MAX}: time to reach the maximum concentration; T_{LAST}: time to the last observation; AUC_{LAST}: exposure measured until the last observation; $t_{1/2}$: elimination half-life; F: absolute rectal bioavailability; D: dose