International Journal of Infectious Diseases 139 (2024) 34–40

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Adjunctive rosiglitazone treatment for severe pediatric malaria: A randomized placebo-controlled trial in Mozambican children ☆

Rosauro Varo[1,2,¶,*], Valerie M. Crowley[3,¶], Humberto Mucasse[2], Antonio Sitoe[2], Justina Bramugy[2], Lena Serghides[4,5,6], Andrea M. Weckman[3], Clara Erice[3], Rubao Bila[2], Pio Vitorino[2], Campos Mucasse[2], Marta Valente[1,2], Sara Ajanovic[1,2], Núria Balanza[1], Kathleen Zhong[3], Yiovanna Derpsch[7,8], Melissa Gladstone[9], Alfredo Mayor[1,2,10,11], Quique Bassat[1,2,11,12,13,#,*], Kevin C. Kain[3,4,14,#]

[1] *ISGlobal, Hospital Clínic – Universitat de Barcelona, Barcelona, Spain*
[2] *Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique*
[3] *S. A. Rotman Laboratories, Sandra Rotman Centre for Global Health, University Health Network-Toronto General Hospital, Toronto, Canada*
[4] *Toronto General Research Institute (TGRI), University Health Network, Toronto, Canada*
[5] *Women's College Research Institute, Women's College Hospital, Toronto, Canada*
[6] *Department of Immunology and Institute of Medical Sciences University of Toronto, Toronto, Canada*
[7] *Department of Psychological Sciences, University of Liverpool, Liverpool, United Kingdom*
[8] *School of Psychology, University of East Anglia, Norwich, United Kingdom*
[9] *Department of Women's and Children's Health, Institute of Life Course and Medical Sciences, Faculty of Health and Life Sciences, University of Liverpool, Liverpool, United Kingdom*
[10] *Department of Physiologic Sciences, Faculty of Medicine, Universidade Eduardo Mondlane, Maputo, Mozambique*
[11] *Spanish Consortium for Research in Epidemiology and Public Health (CIBERESP), Madrid, Spain; Department of Medicine, University of Toronto, Toronto, Canada*
[12] *ICREA, Pg. Lluís Companys 23, 08010, Barcelona, Spain*
[13] *Pediatrics Department, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain*
[14] *Tropical Diseases Unit, Division of Infectious Diseases, Department of Medicine, UHN-Toronto General Hospital, Toronto, Canada*

ARTICLE INFO

Article history:
Received 3 August 2023
Revised 22 November 2023
Accepted 22 November 2023

Keywords:
Adjunctive
Treatment
Plasmodium falciparum
Malaria
Severe
Rosiglitazone
Angiopoietin

ABSTRACT

Objectives: We tested the hypothesis that adjunctive rosiglitazone treatment would reduce levels of circulating angiopoietin-2 (Angpt-2) and improve outcomes of Mozambican children with severe malaria.
Methods: A randomized, double-blind, placebo-controlled trial of rosiglitazone vs placebo as adjunctive treatment to artesunate in children with severe malaria was conducted. A 0.045 mg/kg/dose of rosiglitazone or matching placebo were administered, in addition to standard of malaria care, twice a day for 4 days. The primary endpoint was the rate of decline of Angpt-2 over 96 hours. Secondary outcomes included the longitudinal dynamics of angiopoietin-1 (Angpt-1) and the Angpt-2/Angpt-1 ratio over 96 hours, parasite clearance kinetics, clinical outcomes, and safety metrics.
Results: Overall, 180 children were enrolled; 91 were assigned to rosiglitazone and 89 to placebo. Children who received rosiglitazone had a steeper rate of decline of Angpt-2 over the first 96 hours of hospitalization compared to children who received placebo; however, the trend was not significant ($P = 0.28$). A similar non-significant trend was observed for Angpt-1 ($P = 0.65$) and the Angpt-2/Angpt-1 ratio ($P = 0.34$). All other secondary and safety outcomes were similar between groups ($P > 0.05$).
Conclusion: Adjunctive rosiglitazone at this dosage was safe and well tolerated but did not significantly affect the longitudinal kinetics of circulating Angpt-2.

☆ **Trial registration:** ClinicalTrials.gov NCT02694874.

* Corresponding authors: Tel.: +34 93 2275400 (extension 4121) (R. Varo).
E-mail addresses: rosauro.varo@isglobal.org (R. Varo), quique.bassat@isglobal.org (Q. Bassat).

¶ These authors contributed equally to this work and are joint first authors on this manuscript.
# These authors contributed equally to this work and are joint senior authors on this manuscript.

https://doi.org/10.1016/j.ijid.2023.11.031

Introduction

In 2021 malaria caused ~247 million clinical episodes, 2-4 million cases of severe disease and an estimated 619,000 deaths [1]. The highest mortality rates occur in sub-Saharan African children under the age of 5. The majority of the severe sequelae and deaths are due to *Plasmodium falciparum*. The treatment of choice for severe malaria (SM) in both children and adults is parenteral artesunate. However, despite this potent antimalarial, mortality rates remain high (8.5% in children and 15% in adults for SM; 18% and 30% respectively for cerebral malaria [CM]) [2,3]. To improve clinical outcomes, immunomodulatory strategies to enhance survival have been explored. However, to date, none have shown a clear benefit [4]. One strategy to address this barrier includes the use of prognostic markers as surrogate endpoints of efficacy [5], including angiopoietin-2 (Angpt-2), an independent and quantitative marker of malarial disease severity and prognosis [6].

During normal physiological states, the Angpt/Tie axis is involved in maintaining endothelial integrity through the binding of angiopoietin-1 (Angpt-1) to its receptor Tie-2. SM triggers a pro-inflammatory environment that promotes the expression and release of Angpt-2, the antagonist of Angpt-1, which competes for binding to Tie-2 and destabilizes the microvasculature [7]. Preclinical studies in mice have shown a casual and mechanistic link of the Angpt/Tie axis in the pathogenesis of SM [8]. Furthermore, multiple human studies support Angpt-2 as an informative biomarker for malaria disease severity, multi-organ dysfunction, and death. In children, Angpt-2 levels reflect risk of SM and CM [9–12]. Consequently, Angpt-2 may facilitate the choice of participants for inclusion in randomized controlled trials (RCTs) as well as its use as surrogate clinical endpoint and therapeutic target [9,10,13,14]. Peroxisome proliferator-activated receptor-gamma (PPARγ) is a member of the family of nuclear hormone receptors that function as ligand-activated transcription factors via their heterodimerization with retinoic X receptor [15–17]. PPARγ agonists are promising candidates for adjunctive malaria treatment due to their reported anti-inflammatory, anti-oxidant, and neuroprotective properties [18]. The PPARγ agonist rosiglitazone is a thiazolidinedione drug and is approved for the treatment of type 2 diabetes.

Rosiglitazone has shown anti-inflammatory properties and outcome improvements in preclinical *in vivo* models [19–21]. In addition, a RCT in adults demonstrated that rosiglitazone was safe and well tolerated and improved parasite clearance times, decreased levels of pro-inflammatory mediators, and contributed to the maintenance of functionally quiescent endothelia [19]. Rosiglitazone targets multiple pathways implicated in the pathobiology of SM and has an excellent safety profile, supporting its potential for adjuvant therapy.

We previously conducted a trial to evaluate the safety and tolerability of adjunctive rosiglitazone in pediatric uncomplicated malaria as a prerequisite to further investigations in SM. The results of this study further supported rosiglitazone as a safe adjunct therapy for malaria [20]. Here we conducted a phase IIb clinical trial to test the hypothesis that rosiglitazone in addition to standard of care anti-malarial treatment would accelerate the rate of decline in Angpt-2 in children with SM compared to standard of care anti-malarial treatment plus placebo.

Methods

Trial design

This was a prospective, parallel arm, equally randomized, placebo-controlled, double-blind trial of rosiglitazone vs placebo, in 180 Mozambican children with SM. Children were randomized to receive either placebo (n = 89) or rosiglitazone (n = 91) at 0.045 mg/kg/dose twice daily for 4 days in addition to standard of care. All children received the Mozambican standard of care for SM of parenteral artesunate at 2.4 mg/kg/dose on admission, 12 hours (h) and 24 h after, and then once a day for at least 24 h; followed by a full course of oral artemisinin-based combination treatment (Coartem® Dispersible; artemether–lumefantrine 20 mg/120 mg, Novartis) with dosage determined by body weight, twice daily as recommended by national guidelines [21]. An equal randomization list (1:1) was generated using blocks of three, applying the free online randomization software Sealed Envelope™ (https://www.sealedenvelope.com/). Randomization codes were placed inside individual sealed envelopes that were opened only by the nursing staff responsible for the administration of the drug. Participants, caregivers, other investigators, and those assessing clinical outcomes were blinded to treatment allocations. All laboratory tests and statistical analyses were performed blinded to treatment group.

Ethics, consent and permissions

This study was approved by the Mozambican National Bioethics Committee (CNBS) (Ref.230/CNBS/15); the pharmaceutical department of the Mozambican Ministry of Health (Ref. 374/380/DF2016); the Clinical Research Ethics Committee of the Hospital Clínic, Barcelona, Spain (Ref. HCB/2015/0981); and the University Health Network Research Ethics Committee, Toronto, Canada (UHN REB Number 15-9013-AE). All research was conducted according to the principles expressed in the Declaration of Helsinki. The trial was registered with ClinicalTrials.gov on December 09, 2015, (NCT02694874). Phase IIb activities in children with SM only started after the CNBS reviewed safety data from the phase IIa of the study and certified there were no important safety issues, previously analyzed by a Data Safety and Monitoring Board (DSMB).

All participants and their parents/legal guardians were given detailed oral and written information about the trial, and children were recruited only after a written informed consent was signed by their parents/legal guardians. Verbal assent was obtained from children over the age of 8. The DSMB convened after the first 67 patients were enrolled in phase IIb of the study. At this point, an interim analysis was conducted to review trial quality and safety, at which time the DSBM recommended that the trial could proceed without modification.

Setting and participants

The trial was conducted by the *Centro de Investigação em Saúde de Manhiça* (CISM) at the Manhiça District Hospital (MDH), in southern Mozambique. A detailed description of CISM may be found elsewhere [22]. In Mozambique, malaria transmission is perennial, with a seasonal peak from November to April [23]. Parents/caregivers of children presenting to MDH were asked to participate in the trial and were screened for eligibility. Enrollment took place between March 2016 and December 2019. Laboratory parameters were measured at CISM. Biomarker levels were measured at UHN in Toronto, Canada.

Children (aged 1-12 years) were included in the study if they had: a positive rapid diagnostic test (histidine rich protein-II) and/or confirmation of parasitemia of at least >2500 parasites/µl on thick smears, as well as one or more selected features of SM: repeated seizures (two or more generalized seizures in 24 h), prostration, impaired consciousness (Blantyre Coma Score <5 or Glasgow Coma Score <15), respiratory distress (sustained nasal flaring, deep breathing or sub-costal retractions), severe anemia

(hemoglobin ≤5 g/dl), hypoglycaemia (glucose <2.5 mmol/l) or hyperlactataemia (lactate >5 mmol/l); as well as requiring hospitalization and parenteral artesunate for their malaria infection based on admitting clinician assessment. Those presenting with severe malaria anemia alone, were excluded from the study. Potential participants with known underlying illness (neurological or neurodegenerative disorders; cardiac, renal, or hepatic disease; diabetes; epilepsy; cerebral palsy; or children known to be HIV-1 positive); receiving antiretroviral treatment or treatment with a thiazolidine; or unable to remain in research site region for the follow up period were also excluded from the study.

Intervention

Rosiglitazone (Avandia®, GlaxoSmithKline) and an indistinguishable looking placebo manufactured by Hospital Clínic's pharmacology department in Barcelona, Spain, were packaged and labelled to ensure blinding of study staff and hospital personnel. Children received either rosiglitazone (0.045 mg/kg/dose) or placebo twice daily for 4 days [24]. This dose was based on the maximal dose used by the manufacturer in the pediatric evaluation of rosiglitazone in children aged 10-17 years [24]. The study medication was administered at the MDH, within the Clinical Trials Unit, by authorized members of the study team only. The study intervention (rosiglitazone or placebo) was started together with the first dose of artesunate. The interventions were administered orally, and a nasogastric tube was used when children could not swallow. If any patient vomited within 5 minutes of administration, the patient was re-treated. Rosiglitazone and placebo tablets were crushed and administrated as powder mixed in water.

Treatment follow-up and laboratory procedures

Participants had an initial targeted physical examination performed by the study clinician. Anthropometry was conducted for all children using standard World Health Organization (WHO) procedures and anthropometric Z-scores were calculated upon admission using the WHO Anthro Plus Software version 1.0.4 for children 0-19 years old. A blood sample was taken at baseline and prior to the administration of the study intervention, for malaria diagnosis by microscopy, and hematological (hemoglobin, hematocrit, platelets, and white cell full blood count) and biochemical (renal and liver function, glucose, and lactate) evaluations. The 50% and 90% parasite clearance times were calculated using the World Wide Antimalarial Resistance Network (WWARN) parasite clearance estimator [25]. Strict monitoring of glycaemia included finger-prick samples on admission, every 6 h for the first 48 h, and then every 24 h until discharge, and again at the day 7 and day 14 follow-up visits. Lactate was assessed on admission, every 12 h for the first 24 h, and then every 24 h until discharge, and at the day 7 and day 14 follow-up visits. Biochemistry, including aspartate aminotransferase, alanine aminotransferase, urea, creatinine, lactate dehydrogenase, and indirect and direct bilirubin levels, were assessed in venous blood every 24 h from admission until discharge and again on day 7 follow-up. Venous blood extraction for hematology was performed every 24 h from admission until discharge, and again on day 7 and 14 follow-up.

Finally, venous blood extraction for biomarker analysis was collected at admission, and 12, 24, 36, 48, 60, 72, 84, and 96 h after admission, and again at the day 7 and 14 follow-up visits. They were collected in EDTA-coated tubes and plasma was stored at -80°C until analysis. Multianalyte Luminex Human Discovery Assay (R&D Systems, Minneapolis, MN; custom plate) was used to measure Angpt-1 (1:2 dilution) and Angpt-2 (1:2 dilution). Electrocardiographic monitoring was performed using a portable 12 lead electrocardiogram (ECG) machine (Cardioline ECG100+; AB Medica Group SA) at screening (before administration of study interventions), on day 1 (24 h after admission and after the second dose of study intervention), and on day 4 (after the last dose of study intervention). An additional ECG was conducted on day 7 only if abnormalities were recorded on day 4. The study clinicians reviewed all ECG tracings immediately after they were obtained, with attention to the QT segment length and potential prolongations from baseline. All children were kept at the health facility for the 4-day dosing period, and they continued further time under study clinician's criterion. The parents/guardians were asked to return with the child for scheduled visits on day 7 and 14 post-treatment, or earlier if any symptoms occurred. On each visit, a physical examination was performed by the study clinicians, vital signs were recorded, and body temperature measured.

Outcomes

The primary objective of the study was to determine whether supplemental rosiglitazone (0.045mg/kg/dose) twice daily in addition to standard of care anti-malarial treatment accelerated the rate of decline in Angpt-2 from admission levels in children with SM compared to standard of care anti-malarial treatment plus placebo. Secondary objectives of the study included differences in time to recovery (i.e., time to fever resolution, time to sit unsupported, and time to hospital discharge); parasite clearance; intrahospital mortality; blood lactate levels; changes in concentrations of other biomarkers from the angiopoietin family including Angpt-1 and the Angpt-2/Angpt-1 ratio; and safety and tolerability as indicated by blood glucose levels, biochemical and hematological parameters and the occurrence of adverse events.

Statistical analyses

Statistical analyses were performed with SPSS v.24, Graph Pad Prism v.7, and R version 4.0.3 (R Core Team, 2020). Differences between groups were assessed using the Fisher's exact test for categorical demographic values, and by t-test or Mann Whitney U test (two-tailed) for clinical laboratory data based on the distribution of the data. Safety data for hematological and biochemical parameters and vital signs were compared using ANOVA. Time to events were compared with the log-rank test. A P-value <0.05 was considered as statistically significant. The primary outcome was analyzed by intention-to-treat with all available data for all 180 participants. Sample size estimate was based on previous clinical data in SM, in which Angpt-2 levels decreased by 2700 pg/ml/day (95% CI 1800-3600 pg/ml/day) [13]. We assumed that a 50% change in Angpt-2 would represent a clinically significant therapeutic effect. By standard calculations for normally distributed data, 80 patients per group would provide 80% power to detect a difference between the two treatment arms of 1350 pg/ml/day at $P = 0.05$ (two-sided). To account for dropout, loss to follow-up, and/or non-evaluable data of 10% of patients, we planned to recruit 90 patients in each study arm for a total of 180 patients, an efficient sample size supported by a computer simulation to evaluate statistical power [6].

Linear mixed-effects (LME) modeling (using the lme4 package in R [26]) was used to assess the impact of treatment arm on repeated measurements of Angpt-2 and Angpt-1 concentrations, as well as the Angpt-2/Angpt-1 ratio, across 96 h (all blood samples apart from the follow-up visits at day 7 and 14 were used in biomarker analysis). LME supports longitudinal analysis of repeated measures data by accounting for heterogeneity amongst participants in baseline analyte concentrations and within-child correlations between repeated measures (multiple observations measured

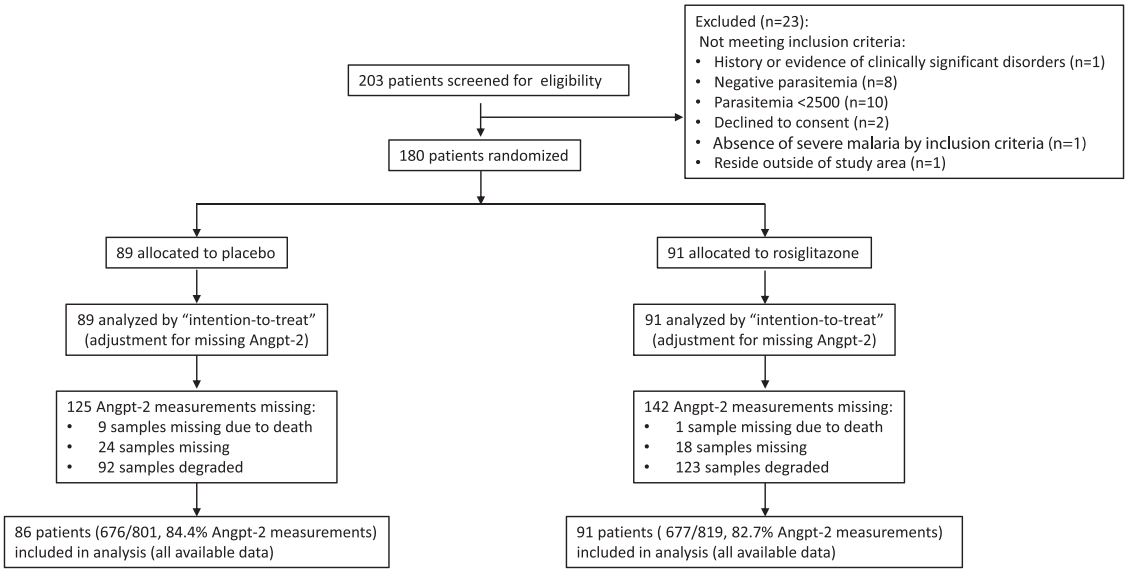


Figure 1. CONSORT diagram. Angpt, angiopoietin.

per child across time). All LME models included time since enrolment, age, sex, and disease severity at enrolment (measured by the Lambaréné Organ Dysfunction Score [LODS]) as fixed effects, and a by-participant random intercept. An interaction term (treatment group * time) was used to assess the effect of treatment group on each analyte by time since enrolment. *P-values* represent the results of a likelihood ratio test for best fit comparing a null model (fixed and random effects only) with a second model that included the interaction term. Analyte data were log-transformed. Residual plots were visually assessed and did not show apparent deviations from normality or homoskedasticity.

Results

Baseline characteristics

Figure 1 outlines the trial's profile. All the enrolled children were negative for HIV-1 and none of them presented with severe acute malnutrition. Apart from repeated seizures prior to admission (higher in the rosiglitazone treatment arm; $P = 0.03$), there were no other differences in the baseline characteristics between the two treatment groups (Table 1). Blood cultures were performed on all but one participant per group. Blood cultures were positive for 3/89 (3.37%) placebo and 4/91 (4.39%) rosiglitazone group. There was one case of *Pantoea agglomerans* in the rosiglitazone group and six cases of probable contamination (two gram-positive bacillus, one gram-negative bacillus, and three coagulate-negative staphylococcus). In-hospital co-treatments did not differ between the two groups (Supplementary Table 1). There was no evidence of bacteriological infection in any of the cerebrospinal fluid samples taking under clinician criteria.

Primary outcome

The median (interquartile range) linear rate of change of Angpt-2 over the first 96 h of hospitalization was −470.4 (−806.4 to −165.6) pg/ml/day in patients receiving rosiglitazone vs −439.2 (−724.8 to −156) pg/ml/day in patients receiving placebo ($P = 0.41$). While there was a trend to a steeper rate of decline in Angpt-2 over 96 h in children treated with rosiglitazone compared to placebo (Figure 2a), the effect of treatment group on Angpt-2 over time was not statistically significant by LME modeling ($P = 0.29$).

Secondary outcomes

The longitudinal dynamics of Angpt-1 ($P = 0.65$) and the Angpt-2/Angpt-1 ratio ($P = 0.35$) did not differ by treatment arm (Figure 2b and c). 50% and 90% parasite clearance, estimated by the WWARN parasite clearance estimator, did not differ between groups (Table 2). All other recovery metrics did not differ between groups (Table 2). Four children died during the study. Mortality rates were low and did not differ between groups, with three (3.4%) and one (1.1%) deaths in the placebo and rosiglitazone groups respectively ($P = 0.37$). The three deaths in the placebo group died at MDH in the first hours after admission. Another child received the standard dosage regimen of rosiglitazone and was transferred, 5 days after admission at MDH, to a higher facility (Maputo Central Hospital). There, the patient received intensive care and the exact cause of death (11 days after admission in this second facility) is unknown but likely due to sequelae of the severe neurological syndrome. A report was analyzed by the local safety monitor and this death was determined to not be related to the study drug.

Rosiglitazone safety

Safety was assessed over the first 96 h of admission as determined by clinical, biochemical, hematological and electrocardiographic observations according to pre-defined local reference ranges. Clinical monitoring of vital signs, including respiratory rate, heart rate, blood pressure, and oxygen saturation rate did not differ between groups (Supplementary Figure 1). Biochemical and hematological parameters did not differ between groups (Supplementary Figure 2).

Adverse events were monitored using pre-defined pediatric toxicity tables modified from the United States National Institute of Allergy and Infectious Diseases for the local population. The number of patients experiencing an adverse event did not differ between treatment groups (Supplementary Table 2).

The number of ECG abnormalities was significantly higher in the placebo vs rosiglitazone group (nine vs two respectively, $P = 0.03$, Supplementary Table 3). None of these ECG abnormali-

Table 1
Baseline characteristics.

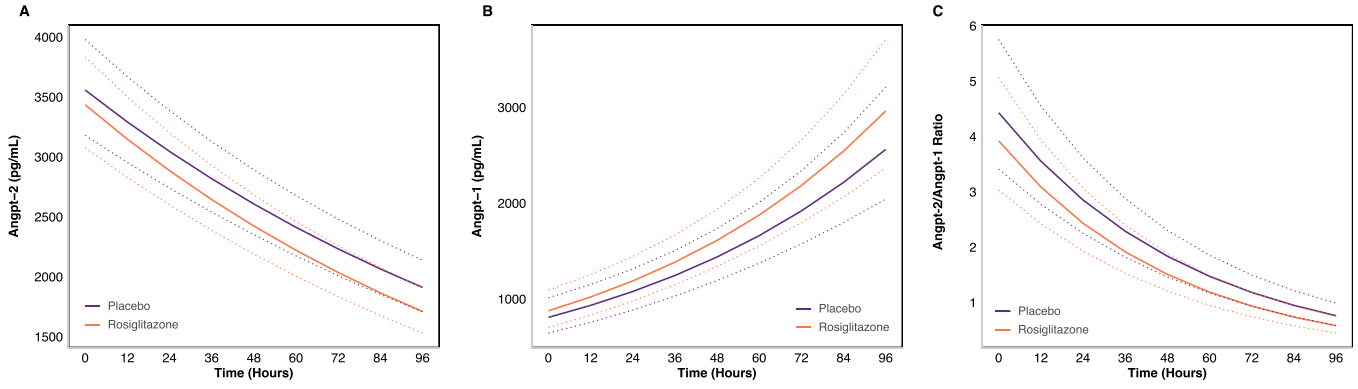
	Placebo (N = 89)	Rosiglitazone (N = 91)
Female sex, number (%)	41 (46.1)	39 (42.9)
Age (years), median (IQR)	3.0 (2.1-5.5)	3.2 (2.2-4.8)
Age <5 years, number (%)	64.0 (71.9)	72 (79.1)
Weight (kg), median (IQR)	13.1 (10.9-18.2)	13.2 (11.1-16.6)
Temperature (°C), median (IRQ)	38.5 (37.4-39.4)	38.4 (37.6-40.9)
Heart rate (beats per minute), mean (SD)	135.80 (23.11)	133.5 (20.8)
Respiratory rate (breaths per minute), median (IQR)	33.0 (27.0-38.0)	32.0 (28.0-36.0)
Blood pressure (mmHg), median (IQR)		
Systolic	90.0 (83.3-97.8)	90.0 (82.0-94.0)
Diastolic	52.0 (47.3-61.8)	51.0 (46.0-56.0)
O_2 saturation (%), median (IQR)	100.0 (99.0-100.0)	100.0 (99.0-100.00
Glucose (mmol/l), median (IQR)	5.6 (4.4-6.8)	5.8 (5.2-7.3)
Lactate (mmol/l), median (IQR)	3.4 (2.3-5.0)	3.2 (2.3-4.5)
Creatinine (μmol/l), median (IQR)	25.2 (19.8-32.8)	27.6 (22.0-33.1)
Urea (blood urea nitrogen) (mg/dl), median (IQR)	12.0 (9.0-16.0)	12.0 (9.0-16.0)
Hematocrit (%), median (IQR)	28.6 (22.4-32.2)	29.5 (24.5-32.6)
White blood cell (n x 10^3/ μl), median (IQR)	9.1 (7.3-12.7)	9.4 (6.6-11.9)
Red blood cell (n x 10^3/ μl), median (IQR)	4.0 (3.0-4.4)	3.8 (3.3-4.4)
Platelets (n x 10^3/ μl), median (IQR)	101.0 (54.0-174.0)	82.5 (54.8-154.3)
Alanine aminotransferase (U/l), median (IQR)	34.5 (25.5-48.0)	33.0 (27.0-52.0)
Aspartate aminotransferase (U/l), median (IQR)	56.0 (38.0-90.3)	66.0 (44.0-66.0)
Parasitemia (parasites per ul), geometric mean (range)	26,484 (3769-159,024)	29,645 (2661-207,063)
Prostration, number (%)	70 (78.1)	66 (72.5)
Respiratory distress, number (%)	21 (23.6)	12 (13.2)
Convulsions (≥2, 24 hours prior to hospitalization), number (%)	37 (41.6)	50 (55.0)
Severe anemia, number (%)	6 (6.7)	3 (3.3)
Hypoglycemia, number (%)	5 (5.6)	3 (3.3)
Hyperlactatemia, number (%)	21 (23.6)	20 (22.0)
Clinical sepsis, number (%)	0 (0.0)	1 (1.1)
Pneumonia, number (%)	3 (3.4)	3 (3.3)
Impaired consciousness, number (%)	24 (27.0)	26 (28.6)
Coma, number (%)	7 (7.9)	11 (12.1)
Lambaréné Organ Dysfunction Score (LODS)		
LODS 0, number (%)	19 (21.4)	25 (27.5)
LODS 1, number (%)	57 (64.0)	54 (59.3)
LODS 2, number (%)	13 (14.6)	10 (11.0)
LODS 3, number (%)	0 (0)	2 (2.2)

IQR: interquartile range.

Table 2
Recovery times and parasite clearance times for study participants.

	Placebo, median (IQR, N)	Rosiglitazone, median (IQR, N)	Hazard ratio (95% confidence interval)	*P-value*
Duration of hospital stay, days	4.1 (4.0-4.1, 86)	4.1 (4.0-4.1, 90)	1.0 (0.7-1.4)	0.68
Time to fever resolution, hours	20 (4-28, 77)	24 (12-32, 86)	1.1 (0.8-1.5)	0.38
Time to first feed, hours	6 (4-20, 27)	8 (4.3-17.8, 32)	0.9 (0.6-1.6)	0.88
Time to localize pain, hours	4 (2-18, 9)	8 (5.5-16.5, 9)	1.1(0.4-2.8)	0.84
Time to first sit, hours	17 (6-20, 35)	18 (12-24, 36)	1.0 (0.7-1.7)	0.83
Time to 50% parasite clearance, hours	11.9 (5.5-16.3, 84)	10.7 (7.2-29.0, 87)	0.9 (0.7-1.2)	0.41
Time to 90% parasite clearance, hours	16.7 (12.1-21.4, 85)	16.6 (12.4-21.3, 89)	1.0 (0.8-1.4)	0.78

IQR: Interquartile range.



Figures 2. a and b. Longitudinal Angpt-2 and Angpt-1 concentrations in placebo and rosiglitazone treatment groups did not significantly differ ($P = 0.29$, and $P = 0.65$) c. The longitudinal ratio of Angpt-2/Angpt-1 did not differ between treatment groups ($P = 0.35$). Best fit curves from linear mixed effects models are shown in solid lines, placebo in purple and rosiglitazone in orange. Dotted lines represent 95% confidence intervals.
Angpt: angiopoietin.

ties were considered clinically significant. No additional intervention was required on these patients and no patient had a corrected QT interval of more than 500 ms at any of the measured time points.

Discussion

Supplemental oral rosiglitazone added to standard antimalarial parenteral therapy was safe and well tolerated in this study of Mozambican children with SM. However, the decline of circulating levels of Angpt-2 during the first 96 h of hospitalization did not significantly differ between children receiving rosiglitazone and those receiving placebo. Additional biomarkers of disease severity and response to treatment (Angpt-1, Angpt-2/Angpt-1 ratio) were not significantly altered by rosiglitazone treatment and no differences in clinical outcomes (e.g., mortality, recovery times) were observed. Therefore, at the dose used, there were no observed stabilizing effect on the endothelium.

In-hospital mortality (2.2%) was lower in the present trial compared to the largest published clinical trial of pediatric SM [3]. At presentation, our cohort had lower incidence of severe anemia and respiratory distress but similar rates of convulsions and altered consciousness. Case fatality ratios for malaria (overall malaria, non-severe and SM) historically reported from the hospital where the study was conducted have been generally lower than those from other African hospitals [27], which may explain these differences. This may be attributable to enhanced health seeking behavior and better case management; as well as the support provided by CISM, including a specific Clinical Trials Unit where this trial occurred. Although COVID-19 pandemic caused population lockdowns and difficulties in research activities in Mozambique, the main impact of the pandemic in our study was due to the problems we experienced to have a correct follow-up of neurological sequelae in our patients between 6 and 12 months after recruitment. However, the pandemic did not impact the results presented here because the recruitment finished in December 2019.

In a previous RCT in adult patients, increased parasite clearance times were observed in those who received rosiglitazone in addition to atovaquone/proguanil [19]. However, parasite clearance times (50 and 90%) did not differ between groups in our study, perhaps due to the faster clearance of early ring stages by artemisinins vs atovaquone-proguanil.

The dosage regimen of rosiglitazone used in this trial was safe and well tolerated but did not result in a significant difference in the decline of circulating Angpt-2 concentrations. The dosage regimen was based on the maximal dose used by the manufacturer in the pediatric evaluation of rosiglitazone in children aged 10-17 with type II diabetes mellitus [24]. However, we have to consider that, in contrast to diabetes, the treatment and course of malaria is acute and the dosage regimen may not have been adequate. We did not perform pharmacokinetic studies of rosiglitazone and other dosage regimens remain potential options for further investigation.

The lack of effect might also be reflecting our limited understanding of the pathobiology underlying malaria infection that ranges from asymptomatic infection to rapid progression and death. WHO criteria for SM are commonly used to recruit patients for RCTs. Nonetheless, these criteria are a broad and overlapping mixture of clinical and laboratory parameters, have variable prognosis, and can present with other co-morbidities, making it challenging to assess and classify children [28]. This may hinder the risk-stratification and recruitment of patients for RCTs and may blur the potential impact of rosiglitazone in different clinical subgroups. We did not risk-stratify participants at enrollment to potentially identify high-risk participants most likely to benefit from rosiglitazone use. For example, those with high initial Angpt-2 levels or high soluble urokinase plasminogen activator receptor (suPAR) levels, both of which are associated with high mortality rates in African children with SM [29,30].

Given the observed trend towards a positive effect of rosiglitazone on Angpt-2 (steeper decline) and considering that Angpt-2 levels decreased at a slower pace than hypothesized [13], an increased sample size would permit more power to confidently confirm or refute a putative effect of adjunctive rosiglitazone.

Conclusion

It is important to consider and account for the multiple processes that culminate in severe and fatal malaria, in order to identify effective adjunctive therapies. Our results do not discount the potential utility of rosiglitazone in a larger RCT as an adjunctive therapy with different doses and routes of administration for pediatric SM. Moreover, the Angpt-Tie2 pathway remains a valid target for adjunctive malaria therapies.

Declaration of competing interest

The authors do not hold a patent for this indication of rosiglitazone. Rosauro Varo had a fellowship from the program Río Hortega of the Instituto de Salud Carlos III (ISCIII) (CD16/00024) while the study was conducted.

Funding

This study was funded in part by the Canadian Institutes of Health Research (CIHR) Foundation Grant FDN-148439 (KCK), a Canada Research Chair in Molecular Parasitology (KCK; and LS) and the Thomas Mather Fund (LS and KCK). Funders had no role in the design, conduct or decision to publish this study. CISM is supported by the Government of Mozambique and the Spanish Agency for International Development (AECID). ISGlobal receives support from the Spanish Ministry of Science and Innovation through the “Centro de Excelencia Severo Ochoa 2019-2023” Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Program. This research is part of ISGlobal’s Program on the Molecular Mechanisms of Malaria, which is partially supported by the Fundación Ramón Areces. The manufacturer of rosiglitazone (Avandia®, GlaxoSmithKline) had no role in this study. NB is supported by an FPU predoctoral fellowship from the Spanish Ministry of Universities (FPU18/04260).

Acknowledgments

We express our sincerest gratitude to the patients and their families for participation in this study. We specially thanks Dr. David Torres for the help in the safety analysis. We also thank the nurses, field assistants, laboratory staff (specially Zumilda Boca, Ana Rosa Manhiça, Crisóstomo Fonseca, Anelsio Cossa and Lazaro Quimice); and other hospital staff who cared for the patients and contributed to study data collection.

Author contributions

KCK, LS and QB conceived of the study and KCK, QB, LS, RV, and VMC and MG contributed to study design. RV, VMC, YD, MG, HM and CM implemented the study. RV, MV, SA, PV, RB, JB and AS acquired the clinical data. CE, NB and CM were involved in data management. AMW, KZ and VMC acquired the biomarker data. KCK, QB, AM, RV, AMW, VMC, SA and LS analyzed and interpreted the data. RV and VMC drafted the manuscript. KCK, QB, RV, VMC, NB and AMW critically revised the manuscript. All authors read and approved the final manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.11.031.

References

[1] World Health Organization *World malaria report 2022*. Geneva: World Health Organization; 2022.

[2] Dondorp A, Nosten F, Stepniewska K, Day N, White N. South East Asian Quinine Artesunate Malaria Trial (SEAQUAMAT) group. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet* 2005;**366**:717–25. doi:10.1016/S0140-6736(05)67176-0.

[3] Dondorp AM, Fanello CI, Hendriksen IC, Gomes E, Seni A, Chhaganlal KD, et al. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet* 2010;**376**:1647–57. doi:10.1016/S0140-6736(10)61924-1.

[4] Varo R, Crowley VM, Sitoe A, Madrid L, Serghides L, Kain KC, et al. Adjunctive therapy for severe malaria: a review and critical appraisal. *Malar J* 2018;**17**:47. doi:10.1186/s12936-018-2195-7.

[5] Jeeyapant A, Kingston HW, Plewes K, Maude RJ, Hanson J, Herdman MT, et al. Defining surrogate endpoints for clinical trials in severe falciparum malaria. *PLoS One* 2017;**12**:e0169307. doi:10.1371/journal.pone.0169307.

[6] Hawkes MT, Conroy AL, Opoka RO, Hermann L, Thorpe KE, McDonald C, et al. Inhaled nitric oxide as adjunctive therapy for severe malaria: a randomized controlled trial. *Malar J* 2015;**14**:421. doi:10.1186/s12936-015-0946-2.

[7] Leligdowicz A, Richard-Greenblatt M, Wright J, Crowley VM, Kain KC. Endothelial activation: the Ang/tie axis in sepsis. *Front Immunol* 2018;**9**:838. doi:10.3389/fimmu.2018.00838.

[8] Higgins SJ, Purcell LA, Silver KL, Tran V, Crowley V, Hawkes M, et al. Dysregulation of angiopoietin-1 plays a mechanistic role in the pathogenesis of cerebral malaria. *Sci Transl Med* 2016;**8**:358ra128. doi:10.1126/scitranslmed.aaf6812.

[9] Erdman LK, Dhabangi A, Musoke C, Conroy AL, Hawkes M, Higgins S, et al. Combinations of host biomarkers predict mortality among Ugandan children with severe malaria: a retrospective case-control study. *PLoS One* 2011;**6**:e17440. doi:10.1371/journal.pone.0017440.

[10] Lovegrove FE, Tangpukdee N, Opoka RO, Lafferty EI, Rajwans N, Hawkes M, et al. Serum angiopoietin-1 and -2 levels discriminate cerebral malaria from uncomplicated malaria and predict clinical outcome in African children. *PLoS One* 2009;**4**:e4912. doi:10.1371/journal.pone.0004912.

[11] Conroy AL, Glover SJ, Hawkes M, Erdman LK, Seydel KB, Taylor TE, et al. Angiopoietin-2 levels are associated with retinopathy and predict mortality in Malawian children with cerebral malaria: a retrospective case-control study*. *Crit Care Med* 2012;**40**:952–9. doi:10.1097/CCM.0b013e3182373157.

[12] Conroy AL, Lafferty EI, Lovegrove FE, Krudsood S, Tangpukdee N, Liles WC, et al. Whole blood angiopoietin-1 and -2 levels discriminate cerebral and severe (non-cerebral) malaria from uncomplicated malaria. *Malar J* 2009;**8**:295. doi:10.1186/1475-2875-8-295.

[13] Yeo TW, Lampah DA, Gitawati R, Tjitra E, Kenangalem E, Piera K, et al. Angiopoietin-2 is associated with decreased endothelial nitric oxide and poor clinical outcome in severe falciparum malaria. *Proc Natl Acad Sci U S A* 2008;**105**:17097–102. doi:10.1073/pnas.0805782105.

[14] Conroy AL, Hawkes M, McDonald CR, Kim H, Higgins SJ, Barker KR, et al. Host Biomarkers are associated with response to therapy and long-term mortality in pediatric severe malaria. *Open Forum Infect Dis* 2016;**3**:ofw134. doi:10.1093/ofid/ofw134.

[15] Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, et al. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature* 2005;**437**:759–63. doi:10.1038/nature03988.

[16] Giannini S, Serio M, Galli A. Pleiotropic effects of thiazolidinediones: taking a look beyond antidiabetic activity. *J Endocrinol Invest* 2004;**27**:982–91. doi:10.1007/BF03347546.

[17] Lehrke M, Lazar MA. The many faces of PPARgamma. *Cell* 2005;**123**:993–9. doi:10.1016/j.cell.2005.11.026.

[18] Serghides L, McDonald CR, Lu Z, Friedel M, Cui C, Ho KT, et al. PPARγ agonists improve survival and neurocognitive outcomes in experimental cerebral malaria and induce neuroprotective pathways in human malaria. *PLoS Pathog* 2014;**10**:e1003980. doi:10.1371/journal.ppat.1003980.

[19] Boggild AK, Krudsood S, Patel SN, Serghides L, Tangpukdee N, Katz K, et al. Use of peroxisome proliferator-activated receptor gamma agonists as adjunctive treatment for Plasmodium falciparum malaria: a randomized, double-blind, placebo-controlled trial. *Clin Infect Dis* 2009;**49**:841–9. doi:10.1086/605431.

[20] Varo R, Crowley VM, Sitoe A, Madrid L, Serghides L, Bila R, et al. Safety and tolerability of adjunctive rosiglitazone treatment for children with uncomplicated malaria. *Malar J* 2017;**16**:215. doi:10.1186/s12936-017-1858-0.

[21] Programa Nacional de Controlo deMalaria (PNCM) *Normas de Tratamento da Malaria em Moçambique*. Ministério de Saúde,Maputo; 2011.

[22] Nhacolo A, Jamisse E, Augusto O, Matsena T, Hunguana A, Mandomando I, et al. Cohort profile update: Manhiça health and demographic surveillance system (HDSS) of the Manhiça Health Research Centre (CISM). *Int J Epidemiol* 2021;**50**:395. doi:10.1093/ije/dyaa218.

[23] Bassat Q, Guinovart C, Sigaúque B, Aide P, Sacarlal J, Nhampossa T, et al. Malaria in rural Mozambique. Part II: children admitted to hospital. *Malar J* 2008;**7**:37. doi:10.1186/1475-2875-7-37.

[24] Zawadzki JK. *Clinical Review Pediatric Study Rosiglitazone (Avandia®)* 2004.

[25] Infectious Diseases Data Observatory (IDDO) (2011): Parasite Clearance Estimator (PCE). [Internet]. Worldwide Antimalarial Resistance Network. 2015. https://www.wwarn.org/parasite-clearance-estimator-pce. Accessed 23 June 2022.

[26] Bates D, Maechler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw* 2015;**67**:1–48.

[27] Guinovart C, Sigaúque B, Bassat Q, Loscertales MP, Nhampossa T, Acácio S, et al. The epidemiology of severe malaria at Manhiça District Hospital, Mozambique: a retrospective analysis of 20 years of malaria admissions surveillance data. *Lancet Glob Health* 2022;**10**:e873–81. doi:10.1016/S2214-109X(22)00125-5.

[28] World Health OrganizationSevere malaria. *Trop Med Int Health* 2014;**19**:7–131. doi:10.1111/tmi.12313_2.

[29] Leligdowicz A, Conroy AL, Hawkes M, Richard-Greenblatt M, Zhong K, Opoka RO, et al. Risk-stratification of febrile African children at risk of sepsis using sTREM-1 as basis for a rapid triage test. *Nat Commun* 2021;**12**:6832. doi:10.1038/s41467-021-27215-6.

[30] Stefanova V, Ngai M, Weckman AM, Wright JK, Zhong K, Richard-Greenblatt M, et al. Soluble Urokinase-type plasminogen activator receptor as a prognostic marker of Ugandan children at risk of severe and fatal malaria. *Clin Infect Dis* 2023;**76**:e1079–86. doi:10.1093/cid/ciac457.