Title:

Acetaminophen as a renoprotective adjunctive treatment in patients with severe and moderately severe falciparum malaria: a randomized, controlled, open-label trial

Authors:

Katherine Plewes \(^{1,2,3}\), Hugh W.F. Kingston \(^{1,4}\), Aniruddha Ghose \(^5\), Thanaporn Wattanakul \(^1\), Md. Mahtab Uddin Hassan \(^5\), Md. Shafiul Haider \(^6\), Prodip K. Dutta \(^5\), Md. Akhterul Islam \(^7\), Shamsul Alam \(^8\), Selim Md. Jahangir \(^9\), A.S.M. Zahed \(^5\), Md. Abdus Sattar \(^5\), M.A. Hassan Chowdhury \(^5\), M. Trent Herdman \(^1\), Stije J. Leopold \(^{1,2}\), Haruhiko Ishioka \(^{1,11}\), Kim A. Piera \(^6\), Prakaykaew Charunwatthana \(^{1,11}\), Kamolrat Silamut \(^1\), Tsin W. Yeo \(^{4,12}\), Sue J. Lee \(^{1,2}\), Mavuto Mukaka \(^{1,2}\), Richard J. Maude \(^{1,2,13}\), Gareth D.H. Turner \(^{1,2}\), Md. Abul Faiz \(^{10}\), Joel Tarning \(^{1,2}\), John A. Oates \(^{14}\), Nicholas M. Anstey \(^4\), Nicholas J. White \(^{1,2}\), Nicholas P.J. Day \(^{1,2}\), Md. Amir Hossain \(^5\), L. Jackson Roberts II \(^{14}\), Arjen M. Dondorp \(^{1,2}\)

Affiliations:

\(^1\) Mahidol Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand

\(^2\) Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK

\(^3\) Department of Medicine, University of British Columbia, Vancouver, Canada

\(^4\) Menzies School of Health Research, Charles Darwin University, Darwin, Australia

\(^5\) Department of Medicine, Chittagong Medical College Hospital, Chittagong, Bangladesh

© The Author(s) 2018. Published by Oxford University Press for the Infectious Diseases Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
6 Department of Nephrology, Chittagong Medical College Hospital, Chittagong, Bangladesh

7 Ramu Upazilla Health Complex, Cox's Bazaar, Bangladesh

8 Department of Anesthesiology, Chittagong Medical College Hospital, Chittagong, Bangladesh

9 Department of Pharmacology, Chittagong Medical College Hospital, Chittagong, Bangladesh

10 Malaria Research Group, and Dev Care Foundation, Dhaka, Bangladesh

11 Department of Clinical Tropical Medicine, Mahidol University, Bangkok, Thailand

12 Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

13 Harvard TH Chan School of Public Health, Harvard University, Boston, USA

14 Department of Internal Medicine, Vanderbilt University School of Medicine, Nashville, USA

**Corresponding author:**

Professor Arjen Dondorp, Mahidol Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand; arjen@tropmedres.ac

**Summary:**

This randomized controlled trial of acetaminophen in severe malaria shows that acetaminophen reduces kidney dysfunction and risk of developing AKI, particularly in patients presenting with high plasma hemoglobin, supporting the hypothesis that acetaminophen inhibits cell-free hemoglobin-mediated renal tubular oxidative damage.
Running title: Acetaminophen renoprotection in malaria
Abstract:

Background. Acute kidney injury independently predicts mortality in falciparum malaria. It is not known whether acetaminophen’s capacity to inhibit plasma hemoglobin-mediated oxidation is renoprotective in severe malaria.

Methods. A phase 2, open-label, randomized controlled trial at two hospitals in Bangladesh was conducted to assess effects on renal function, safety, pharmacokinetic properties and pharmacodynamic effects of acetaminophen. Febrile patients (>12 years) with severe and moderately severe falciparum malaria were randomly assigned to receive acetaminophen (1g 6–hourly for 72 hours) or no acetaminophen, as an adjunct to intravenous artesunate. Primary outcome was the proportional change in creatinine after 72 hours stratified by median plasma hemoglobin.

Results. Between July 2012 and September 2014, 62 patients were randomly assigned to receive acetaminophen (n=31) or no acetaminophen (n=31). Median (interquartile range) reduction in creatinine after 72 hours was 23% (37 to 18%) in patients assigned to acetaminophen, versus 14% (29 to 0%) in patients assigned to no acetaminophen (p=0.043). This difference in reduction was 37% (48 to 22%) versus 14% (30 to -71%) in patients with plasma hemoglobin ≥45,000 ng/mL (p=0.010). The proportion of patients with progressing acute kidney injury was higher among controls (subdistribution hazard ratio, 3.0; 95% CI, 1.1 to 8.5; p=0.034). Pharmacokinetic-pharmacodynamic analyses showed that higher exposure to acetaminophen increased the probability of creatinine improvement. No patient fulfilled Hy’s Law for hepatotoxicity.

Conclusions. In this proof-of-principle study, acetaminophen showed renoprotection without evidence of safety concerns in patients with severe falciparum malaria, particularly in those with prominent intravascular hemolysis.
5 Keywords: Falciparum malaria; acute kidney injury; cell-free hemoglobin; oxidative stress; acetaminophen

Text:
Kidney dysfunction complicating severe falciparum malaria is common and independently predicts mortality in all age groups [1, 2]. Mortality in patients with severe acute kidney injury (AKI) is approximately 75% without and 26% with renal replacement therapy (RRT) [3], but RRT is frequently unavailable in malaria-endemic areas.

Intravascular hemolysis resulting in high levels of plasma cell-free hemoglobin (CFH) contributes to AKI in several conditions, including post-cardiopulmonary bypass [4], paroxysmal nocturnal hemoglobinuria [5], and massive transfusion [6]. After haptoglobin depletion, CFH causes oxidative renal tubular damage through redox cycling between heme-ferric and ferryl states, which results in lipid peroxidation generating \( F_2 \)-isoprostanes (\( F_2 \)-IsoPs) and isofurans (IsoFs) [7, 8]. \( F_2 \)-IsoPs are potent renal vasoconstrictors, acting via thromboxane A\(_2\) receptors [8]. Intravascular hemolysis is an intrinsic component of severe falciparum malaria pathophysiology [9]. Recently it was shown that increased levels of CFH, \( F_2 \)-IsoPs and IsoFs are strongly associated with kidney dysfunction and hemodialysis requirement in adults with severe malaria [10]. Acetaminophen inhibits hemoprotein-mediated lipid peroxidation by reducing heme-ferryl radicals [11]. In an experimental rhabdomyolysis model acetaminophen decreases oxidative stress markers and attenuates AKI [11]. We conducted a randomized controlled trial of acetaminophen versus no acetaminophen in patients with severe and moderately severe malaria to assess acetaminophen as a renoprotective adjunctive therapy. We hypothesized that adjunctive therapy with acetaminophen would improve kidney function, particularly in patients with prominent intravascular hemolysis.
METHODS

Trial Design

The study was a multicenter, randomized, open-label, controlled clinical trial among patients admitted at two hospitals in south-eastern Bangladesh: Ramu Upazilla Health Complex (primary sub-district hospital) and Chittagong Medical College Hospital (CMCH; tertiary referral hospital).

Participants

Eligible patients were >12 years old with microscopy-confirmed *P. falciparum* severe or moderately severe malaria (Supplementary Table 1), and fever (>38°C on admission or fever during preceding 24 hours), provided written informed consent was obtained from the patient or a legally acceptable representative if unconscious. Severe malaria was defined as presence of asexual parasitemia plus ≥1 severity criteria [12]. Moderate disease was defined as need for parenteral therapy without a severity criterion. Exclusion criteria were pregnancy, history of chronic liver disease or alcohol abuse, and contraindications for acetaminophen or nasogastric tube insertion.

Randomization, Masking and Intervention

Randomization to acetaminophen or control was stratified by severity of malaria using a computerized binary sequence number generator in a 1:1 ratio (blocks of 20), generated by a statistician unrelated to the study. Opaque, sealed envelopes containing treatment allocations were opened by a research physician after enrollment. Laboratory staff performing quantification of the primary endpoint and all biochemical tests were masked to the treatment allocation; research physicians and study participants were not. Study codes on samples were
The study data manager was responsible for data entry, cleaning and extraction.

Acetaminophen was given as observed therapy at a 6-hourly dose of 1 g (patient weight ≥50 kg) or 12.5–15.0 mg/kg/dose (<50 kg) for 72 hours, as tablets (500 mg, Bristol Laboratories Ltd., UK) to conscious patients or as syrup (250 mg/5 mL, Rosemont Pharmaceuticals Ltd., UK) via nasogastric tube to comatose patients. This is the standard recommended dosage [12] to achieve therapeutic levels for antipyresis [13] and renoprotection [11, 14]. If patients vomited within one hour, the dose was repeated. In the control arm, patients with fever >40°C were given oral ibuprofen 400 mg (or diclofenac suppository 50 mg), or 500 mg acetaminophen in case of renal impairment, dehydration or dengue infection. Antimalarial treatment was with intravenous artesunate (Guilin Pharmaceuticals, China), followed by artemether/lumefantrine (Coartem, Novartis, Switzerland) once tolerating oral medication. Supportive management was according to WHO guidelines [12]. Patients without shock, anuria or severe dehydration received intravenous normal saline at a rate of 250 mL/h for six hours followed by 100 mL/h until tolerating oral fluids. In case of severe dehydration with oliguria, this was increased to 1000 mL/h for two hours followed by 500 mL/h for two hours if oliguria persisted without signs of fluid overload. Patients in Ramu requiring hemodialysis were transferred to CMCH. Hemodialysis was initiated by attending nephrologists not involved in data collection or analysis.

**Outcomes**

The primary outcome was the relative change in serum creatinine at 72 hours from enrollment stratified by enrollment CFH concentration. Secondary outcome measures included: (i) proportion of patients developing AKI according to ‘Kidney Disease: Improving Global
Outcomes’ (KDIGO) criteria (creatinine increase ≥26.5 µmol/L within 48 hours) [15]; (ii) fever clearance time (time until temperature below 37.5°C (FCT-A) and time until below 37.5°C for 24 hours (FCT-B)); (iii) parasite clearance (time to first of two consecutive negative slides, and parasite half-life) [16]; and (iv) safety (in particular, hepatological parameters at 72 hours).

Population pharmacokinetic (PK) properties and pharmacodynamic (PD) effects on parasitemia, temperature and creatinine were characterized using nonlinear mixed-effects modelling outlined in the Supplementary Material. AKI was staged at enrollment by KDIGO criteria [15], using an estimated baseline creatinine obtained from the Modification of Diet in Renal Disease formula if ≥19 years (GFR 75 mL/min/1.73m²) and the bedside Schwartz formula if <19 years (GFR 100 mL/min/1.73m²) [15].

**Laboratory Methods**

Screening venous blood samples were analyzed for parasitemia and biochemistry (point-of-care iSTAT analyzer (CG4+, Chem8+), Abbott Laboratories, USA). After enrollment, vital signs, urine output, development of complications and parasitemia were assessed 6-hourly until discharge or death. For the first 72 hours, serum creatinine was assessed 12-hourly (Olympus AU400 chemistry analyzer performed in Bangkok), CFH in twice-centrifuged citrated plasma 24-hourly (ELISA; Bethyl Laboratories performed in Darwin) [9] and F₂-isoprostanes and isofurans in lithium heparin plasma 24-hourly (gas chromatography-mass spectrometry at Vanderbilt University) [7, 8]. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed on enrollment and at 72 hours. To determine the PK-PD of acetaminophen on creatinine, parasitemia and fever, plasma EDTA samples for acetaminophen concentration were collected prior to each dose plus dense sampling in the treatment arm after both the first (0 hour) and last (72 hour) dose. Patients in the control arm had samples collected 6-hourly for 72 hours to assess unplanned acetaminophen intake. Detailed procedures are provided in the Supplementary Material.
Statistical Analysis

Inclusion of 62 patients allowed demonstration of a difference in proportional change in serum creatinine at 72 hours of 10% with 5% significance, and 90% power, assuming a mean (SD) admission creatinine value of 150 μmol/L (90 μmol/L). The primary endpoint analysis was modified intention-to-treat principle (ITT) principle including all patients who were randomly assigned. Modified ITT was applied because it was challenging to impute and analyze missing 72-hour creatinine data since most of those with missing data died. Between-group differences were compared with Student’s t-test or Wilcoxon-Mann-Whitney test for continuous and Fisher’s exact test for categorical variables. Primary endpoint comparison adjusted by enrollment CFH was analyzed by linear regression. Temporal data were analyzed using mixed effects models, with the maximum likelihood method of estimation. Stratification was by enrollment median CFH, F_2-isoprostane, and isofuran concentrations. Where interaction terms were significant, the lincom command was used to obtain overall treatment effects. Time-to-event outcome subdistribution hazard ratios (SHRs) were estimated by competing-risks regression to account for death as a competing event for the event of interest (AKI). All patients with a ≥26.5 μmol/L creatinine rise after enrollment were included. Parasite and fever clearance times were assessed using Kaplan-Meier survival analyses. For missing creatinine values in the longitudinal series, totaling 35 of 434 (8%) values, multiple imputation using chained equations with five rounds was used (Supplementary Table 2) [17]. In the mixed effects analyses, it was assumed that in patients on hemodialysis a creatinine rise of 132.6 μmol/L per day was averted, which is the rise proposed for anephric states [18].
Human Research Protections

The study protocol was approved by the Oxford University Tropical Research Ethics Committee and Chittagong Medical College Ethics Committee. Trial sites were monitored by the Clinical Trials Support Group from Mahidol Oxford Research Unit, Bangkok, Thailand, and study outcomes followed by a Data and Safety Monitoring Committee. The study is registered at ClinicalTrials.gov (NCT01641289).

RESULTS

Study Population

Recruitment was from July 10, 2012 until September 11, 2014. Of the 346 patients assessed, 62 were eligible for randomization to receive acetaminophen (n=31) or no acetaminophen (n=31). Trial profile is shown in Figure 1. Baseline characteristics were well matched between treatment arms (Tables 1 and 2). At enrollment, 42% (13/31) and 58% (18/31) had a CFH concentration above the median CFH (45,000 ng/ml) in the acetaminophen and control groups, respectively.

Outcomes

The median (IQR) proportional reduction in serum creatinine at 72 hours compared to baseline was 23% (IQR, 37 to 18%) in patients receiving acetaminophen compared to 14% (IQR, 29 to 0%) in control patients (p=0.043), which was more prominent when adjusted for enrollment CFH (p=0.026; Supplementary Figure 2). In patients with high CFH at enrollment (≥45,000 ng/mL), the median (IQR) reduction in creatinine at 72 hours was 37% (IQR, 48 to 22%) with acetaminophen versus 14% (IQR, 30 to -71%) in control patients (p=0.010). The subgroup of patients with CFH <45,000 ng/mL did not show a difference between treatment groups.
Creatinine concentrations over time are shown in Supplementary Tables 3 and 4. A total of five ibuprofen or diclofenac doses were administered to four patients (1–2 doses/patient) in the control arm, which was not associated with a rise in creatinine (Supplementary Figure 3).

Mixed effects modeling using only the interaction term of treatment arm with time showed there was a significant interaction between treatment and time. In particular, creatinine improved over time in the group receiving acetaminophen but worsened over time in the control group (p<0.001) (Figure 2A; Supplementary Table 5). This difference was more pronounced in patients with high CFH at enrollment (p<0.001) (Figure 2B). Similarly, this beneficial effect of acetaminophen was more prominent in patients with increased F₂-IsoPs and IsoFs on admission (p<0.001; Figure 2D; Supplementary Figure 4B). In the patients with lower CFH and oxidative stress markers at enrollment, there was less difference in rates of creatinine change (Figure 2C and 2E; Supplementary Figure 4C). Mixed effects modelling using the interaction term of treatment group and CFH (as continuous variable), as well as the previously fitted treatment-time interaction term, showed there was a significant interaction between treatment and CFH. Specifically, the effect of acetaminophen on the reduction of creatinine depended on enrollment CFH (interaction p-value=0.016; Supplementary Figure 5 and Table 5).

Among the 17 of 62 patients developing AKI during admission, 12 were oliguric or anuric, and four were non-oliguric (one patient missing this information). Competing-risks regression adjusted by study site showed a higher risk of AKI in patients without acetaminophen administration compared to patients receiving acetaminophen (ITT: SHR 3.0; 95%CI, 1.1 to 8.5; p=0.034; Figure 3). A total of 2/31 (6%) patients in the acetaminophen group and 6/31 (19%) in the control group received hemodialysis during hospitalization (p=0.26; Supplementary Table 6). 15/28 (54%) patients admitted with AKI recovered without hemodialysis; of whom...
5/15 (33%) were in the control group and 10/15 (67%) in the acetaminophen group; OR 4.0 (95%CI, 0.7 to 23.9; p=0.07). Overall case fatality was 4/31 (13%) in the acetaminophen group and 5/31 (16%) in the control group (p=0.72). Among fatal cases, all had severe malaria on admission (median of four severity criteria); 2/9 (22%) had AKI on enrollment and 6/9 (67%) developed AKI after enrollment. Fever and parasite clearance time-to-event analyses found no evidence of a difference between treatment groups by intention-to-treat analysis (Supplementary Table 7, Supplementary Figures 6-8). No infecting parasite strain had a mutation in the PfKelch13 gene (a marker for artemisinin resistance).

**Pharmacokinetics and Pharmacodynamics**

The population pharmacokinetic properties of acetaminophen were best described by a covariate-free one-compartment disposition model with three transit absorption compartments. The predicted median (IQR) \( C_{\text{MAX}} \) reached was 16.1 mg/L (14.0 to 20.9 mg/L) and the estimated median (IQR) terminal elimination half-life was 2.79 hours (2.57 to 2.93 hours). Median (IQR) acetaminophen AUC\(_{0-72h}\) in the treatment arm was 386 mg×h×L\(^{-1}\) (269 to 496 mg×h×L\(^{-1}\)) compared to 7 mg×h×L\(^{-1}\) (0 to 45 mg×h×L\(^{-1}\)) in the control arm. Dosing simulations showed that a 6-hourly dose of 1,000 mg or 1,500 mg resulted in a steady-state mean plasma acetaminophen concentration of 9.21 mg/L and 13.8 mg/L, respectively. A pharmacodynamic mixture model of observed creatinine data including enrollment creatinine and acetaminophen exposure (AUC\(_{0-72h}\)) as covariates best described the data. Higher enrollment creatinine gave a higher probability of a subsequent further deterioration in renal function. The prediction of creatinine change over time was dependent on total acetaminophen exposure where a higher AUC\(_{0-72h}\) increased the probability of an improvement in creatinine over the first 72 hours. For example, an enrollment creatinine of 265 µmol/L confers an 83% probability of further deterioration without receiving acetaminophen, versus probabilities of 0.02%, 1.2%, or 8.1% with acetaminophen exposures (AUC\(_{0-72h}\)) of 500, 300 or 200 mg×h×L\(^{-1}\),
respectively (Table 3). Modelling results showed a positive correlation between acetaminophen exposure and fever clearance time, but no correlation with parasite clearance rate. Detailed pharmacokinetic-pharmacodynamic results are provided in the Supplementary Material.

**Safety**

Median percentage change (IQR) in serum ALT at 72 hours after enrollment was 32% (-9 to 171%) with acetaminophen and -11% (-34 to 57%) in the control group (p=0.030); for serum AST, this was 0% (-27 to 107%) and -18% (-44 to 1%; p=0.06). (Supplementary Table 6). Analysis of the two patients with both an aminotransferase rise >3 times the upper limit of normal (ULN) and a total bilirubin ≥2×ULN revealed that the increase in bilirubin was explained by increased unconjugated bilirubin ≥2×ULN due to intravascular hemolysis, supported by concomitant elevated lactate dehydrogenase, decreased hematocrit, and blood transfusion requirement. Therefore, no patient met criteria for Hy's Law for hepatotoxicity [19].

**DISCUSSION**

This randomized open-label, controlled trial showed that patients with severe and moderately severe malaria receiving acetaminophen had a larger reduction in serum creatinine and a lower risk of developing AKI compared to control patients not receiving acetaminophen. The beneficial effect of acetaminophen on kidney function was distinctly more pronounced in patients with significant intravascular hemolysis and high concentrations of oxidative stress markers (F2-IsoPs and IsoFs). PK-PD modelling showed an acetaminophen exposure-dependent relationship with the improvement in creatinine, which was within the therapeutic dose range. There was a trend toward reduced hemodialysis requirement in the acetaminophen group compared to controls, but the study was not powered to detect an effect towards this endpoint.
The findings support the hypothesis that acetaminophen reduces CFH-mediated oxidative kidney damage, and thus would be most beneficial in patients with significant intravascular hemolysis. The results are consistent with recent studies on a heme-mediated oxidative mechanism of AKI and the renoprotective effect of acetaminophen interfering with this mechanism. We recently showed that elevated plasma CFH, F2-IsoPs and IsoFs are associated with in-hospital creatinine rise, hemodialysis requirement, and mortality in patients with severe malaria [10]. Acetaminophen has been shown to reduce toxic ferryl heme to ferric heme in vitro, and decrease plasma F2-IsoPs and improve kidney function in a rat model of rhabdomyolysis [11]. A recent randomized trial in septic patients with detectable CFH showed reduced oxidative injury, and improved kidney function in patients receiving acetaminophen [14]. While the current study was conducted in patients over age 12, these findings may have important implications for the treatment of severe malaria in African children who carry the major burden of this disease, and in whom the importance of AKI, hemoglobinuria, and increased plasma CFH is increasingly recognized [20-22]. Further, acetaminophen may also be beneficial in severe P. knowlesi malaria, in which high levels of CFH [23] and a high incidence of AKI have been reported [24], as well as in other diseases characterized by a combination of high CFH and incidence of AKI [4-6, 25].

Studies of acetaminophen in uncomplicated malaria lacking PK-PD analyses have questioned its antipyretic efficacy [26, 27] and suggested deceleration in parasite clearance [26]. PK-PD analysis in the current study shows a dose dependent effect of acetaminophen on fever clearance time, whereas acetaminophen concentrations were not associated with parasite clearance rates in these artemisinin-sensitive infections. Studies evaluating acetaminophen in uncomplicated malaria have not reported serious adverse events or hepatotoxicity attributable to acetaminophen [28, 29]. The current study shows that administering the maximum
recommended daily dosage of acetaminophen results in a moderate increase in aminotransferases, but no patient met criteria for Hy's Law for hepatotoxicity [19]. The maximum peak acetaminophen concentration of 31.6 mg/L observed in our study is well below the acute toxicity threshold of 150 mg/L used in guidelines as indication for N-acetylcysteine treatment [30]. Pharmacokinetic dosing simulations showed that 6-hourly dosing of 1,500 mg (6 g/day) achieved therapeutic steady-state acetaminophen concentrations between 10-20 mg/L in this patient population, but this exceeds the maximum recommended daily dosage of acetaminophen in adults (4 g/day). The conventional 6-hourly dose of 1,000 mg used in this trial yielded a simulated average steady state concentration of 9.21 mg/L and median AUC_{0-72h} of 644 mg×h×L^{-1} (Supplementary Figure 12). Comparing this cumulative exposure to that generated from the PK-PD model suggests that this conventional regimen has an important renoprotective effect (Table 3).

The study had some limitations. The sample size was relatively small; a larger study is planned. A total of 35/434 (8%) creatinine values were assigned by multiple imputation because of missing samples. However, no imputation was used for the primary endpoint analysis. Patients who received hemodialysis had artificially lowered creatinine values and according to accepted practice were assigned an anephric rate of creatinine rise in the analysis [18].

This proof-of-principle study provides evidence that acetaminophen improves kidney function and reduces the risk of developing AKI in severe and moderately severe malaria, particularly in patients with elevated plasma CFH, F\textsubscript{2}-isoprostanes and isofurans. Since acetaminophen reduces highly oxidative ferryl heme that can be generated with intravascular hemolysis, the findings support the pathophysiological mechanism that CFH-mediated oxidative stress causes kidney injury in severe malaria, a mechanism also relevant for other disease states characterized by intravascular hemolysis. Acetaminophen is inexpensive and widely used, which would facilitate
rapid implementation for malaria treatment. A larger trial evaluating acetaminophen to reduce renal dysfunction in African children with severe malaria is now warranted.

**Contribution:** KP, RJM, LJR, and AMD designed the study. KP, HWFK, MTH, SL, RJM, and HI performed patient enrollment and data collection. AG, MMUH, MSH, PKD, MAI, SA, SMJ, ASMZ, MAS, MAHC, PC, TWY, MAF, and MAH advised on the protocol and supervised clinical care of the participants. KAP conducted the quantification of the cell-free hemoglobin, and with HWFK interpreted the results. KS assisted in coordinating the laboratory field work. KP, SJL, MM, and AMD conducted the statistical analyses. TW, and JT performed the acetaminophen pharmacokinetic-pharmacodynamic design and analyses. LJR, and JAO provided F$_2$-isoprostane and 16isofurans quantification and interpretation. KP wrote the first draft of the manuscript; AMD, GDHT, and NMA edited the initial drafts; and all authors reviewed the final manuscript.

**Acknowledgments.** We thank the participants consenting to partake in this study, the clinical and laboratory research staff, hospital directors at the study sites and the following contributors: Acetaminophen trial management group: *Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand*, Prof. Arjen Dondorp (chief principal investigator), Dr. Katherine Plewes (principal investigator); *Clinical Trials Support Group*: Dr. Phaik Yeong Cheah, and Zoe Doran; *Data manager*: Thatsanun Ngernseng; *Sample Manager*: Dr. Mehul Dhorda; *Study statisticians*: Dr. Sue J. Lee, Dr. Mavuto Mukaka; *Pharmacokinetic-Pharmacodynamic analyses*: Prof. Joel Tarning and Thanaporn Wattankul; *Bangladesh field site investigators*: *Chittagong*: Prof. Md. Abul Faiz, Prof. Md. Amir Hossain, Dr. Aniruddha Ghose, Dr. Shamsul Alam, Dr. Md. Shaiful Haider, Md. Mahtab Uddin Hassan, Prof. Prodip Dutta, Prof. Selim Md. Jahangir, Dr. A.S.M. Zahed, Prof. Md. Abdus Sattar, Prof. M.A. Hassan Chowdhury, Dr. Katherine Plewes, Dr. Hugh WF Kingston, Dr. M Trent Herdman, Dr. Stije J. Leopold, Dr. Haruhiko Ishioka, Dr. Kamolrat Silamut, Benjamas Intharabut, Ketsanee Srinamon; *Ramu*: Dr. Md. Akhterul Islam, Dr. Katherine Plewes; *Vanderbilt University School of Medicine*: Prof John A. Oates, Prof L. Jackson Roberts; *Medical Monitor*: Prof. Arjen Dondorp; Data Safety and Monitoring Board: Dr. Kasia Stepniewska, Dr. Lorenz Von Seidlein, and Prof. Piet Kager; Study Monitor: Dr. Tom Peto. We would also like to acknowledge William Zachert for performing quantification of F$_2$-isoprostanes and isofurans. Kim A. Piera for conducting quantification of the cell-free
hemoglobin. Dr. Mallika Imwong and Dr. Charlie Woodrow for performing and interpreting the PfKelch13 gene sequencing. Dr. Adeera Levin for input on renal endpoints.

**Disclaimer:** No funding body nor sponsor (University of Oxford) had any role in protocol design, data collection, analysis, interpretation or writing of the manuscript. The corresponding author had full access to all trial data and assumes final responsibility for the decision to submit for publication. There was no payment from any agency or pharmaceutical company for the writing of this manuscript.

**Financial support.** This work was supported by the Wellcome Trust of Great Britain [grant number 089275/Z/09/Z], the Australian National Health and Medical Research Council [grant number 605807, and Fellowships to NMA and TWY], Bill and Melinda Gates Foundation [grant number OPP1134284 to JT], and the National Institutes of Health [grant number GM15431 to LJR and JAO]. KP was supported by the Infectious Diseases Society of America ERF/NFID Young Investigator Merle A. Sande/Pfizer Fellowship in International Infectious Diseases; and the Clinician Investigator Program at the University of British Columbia, Canada. HWFK was supported by an Australian Government University Postgraduate Research Scholarship and Prestigious International Research Tuition Scholarship.

**Conflict of Interest.** All authors declare no competing interests with the exception of JAO and LJR who have a patent for acetaminophen use pending, neither of whom were involved in protocol development or analysis. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.
References:


### Table 1. Demographic and Baseline Clinical Characteristics by Treatment Arm*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Acetaminophen (n=31)</th>
<th>Control (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>19 (61)</td>
<td>21 (68)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28 (20–43)</td>
<td>32 (25–40)</td>
</tr>
<tr>
<td>Fever before enrollment (days)</td>
<td>7 (4–10)</td>
<td>7 (5–8)</td>
</tr>
<tr>
<td>Altered LOC before enrollment (days)</td>
<td>2.5 (1.0–3.0)</td>
<td>1.0 (1.0–2.5)</td>
</tr>
<tr>
<td>Red or black urine before enrollment</td>
<td>0 (0)</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Acetaminophen before enrollment</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Comorbidities</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>3 (10)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>3 (10)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Complications on enrollment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coma&lt;sup&gt;6&lt;/sup&gt;</td>
<td>10 (32)</td>
<td>13 (42)</td>
</tr>
<tr>
<td>Jaundice</td>
<td>3 (10)</td>
<td>5 (16)</td>
</tr>
<tr>
<td>Severe anemia</td>
<td>2 (6)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Medical Condition</td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Hyperlactatemia (lactate &gt;4 mmol/L)</td>
<td>7 (23)</td>
<td>9 (29)</td>
</tr>
<tr>
<td>Hyperparasitemia (&gt;10%)</td>
<td>1 (3)</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Hemoglobinuria</td>
<td>2 (7)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>0 (0)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Severe prostration</td>
<td>18 (58)</td>
<td>19 (61)</td>
</tr>
<tr>
<td>Unable to tolerate oral medications</td>
<td>22 (71)</td>
<td>24 (77)</td>
</tr>
</tbody>
</table>

### Severity

<table>
<thead>
<tr>
<th>Severity Category</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe malaria</td>
<td>24 (77)</td>
<td>25 (81)</td>
</tr>
<tr>
<td>Moderately severe malaria</td>
<td>7 (23)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>Number of severity criteria</td>
<td>3 (1–5)</td>
<td>4 (1–6)</td>
</tr>
</tbody>
</table>

### KDIGO stage on enrollment

<table>
<thead>
<tr>
<th>Stage Description</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>17 (55)</td>
<td>17 (55)</td>
</tr>
<tr>
<td>Stage 1 (≥1.5 × baseline)</td>
<td>7 (23)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>Stage 2 (≥2.0 – 2.9 × baseline)</td>
<td>3 (10)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Stage 3 (≥3 × baseline OR ≥ 353.6 μmol/L)</td>
<td>4 (13)</td>
<td>5 (16)</td>
</tr>
</tbody>
</table>

Data are number (%) or median (IQR) unless otherwise indicated.

Abbreviations: LOC, level of consciousness; KDIGO, Kidney Disease: Improving Global Outcomes.

* There were no significant differences between treatment groups in any of the measured baseline characteristics.
Acetaminophen before enrollment was based on an acetaminophen concentration > 10 mg/L in enrollment pharmacokinetic samples.

Depth of coma was assessed by GCS < 11.

Severe prostration defined as inability to walk or sit up without assistance.
Table 2. Baseline Clinical and Laboratory Investigations by Treatment Arm*  

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Acetaminophen (n=31)</th>
<th>Control (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical examination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>39.0 (37.2–40.0)</td>
<td>38.9 (37.5–39.5)</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>108 (100–116)</td>
<td>110 (95–119)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>61 (50–70)</td>
<td>69 (59–75)</td>
</tr>
<tr>
<td>Glasgow coma score</td>
<td>15 (9–15)</td>
<td>12 (9–15)</td>
</tr>
<tr>
<td>Respiratory rate (breaths/ min)</td>
<td>30 (24–38)</td>
<td>30 (28–40)</td>
</tr>
<tr>
<td>Heart rate (beats/minute)</td>
<td>107 (96–118)</td>
<td>108 (92–130)</td>
</tr>
<tr>
<td><strong>Laboratory investigations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parasite count per μL³</td>
<td>53,426 (24,596–116,048)</td>
<td>17,258 (5,751–51,785)</td>
</tr>
<tr>
<td>Plasma P/HRP2 (mg/mL)</td>
<td>1,356 (146–4,486)</td>
<td>1,308 (154–4,961)</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>134 (130–138)</td>
<td>135 (130–138)</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>3.5 (3.3–4.0)</td>
<td>3.4 (3.0–3.9)</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>103 (99–107)</td>
<td>104 (100–107)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.8 (4.7–8.1)</td>
<td>6.8 (5.5–9.0)</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>23 (13–36)</td>
<td>25 (16–43)</td>
</tr>
<tr>
<td>Measurement</td>
<td>Value 1 (Range)</td>
<td>Value 2 (Range)</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>106 (97–169)</td>
<td>115 (97–168)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.1 (8.0–12.7)</td>
<td>11.6 (10.0–13.4)</td>
</tr>
<tr>
<td>Cell-free hemoglobin (ng/mL)</td>
<td>42,800 (16,800–94,100)</td>
<td>52,500 (23,400–188,800)</td>
</tr>
<tr>
<td>F₂-isoprostanes (pg/mL)</td>
<td>22.0 (14.5–27.7)</td>
<td>23.6 (15.7–38.6)</td>
</tr>
<tr>
<td>Isofurans (pg/mL)</td>
<td>44.7 (25.0–64.2)</td>
<td>44.0 (26.1–84.0)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>1.5 (1.0–2.2)</td>
<td>1.7 (1.2–2.4)</td>
</tr>
<tr>
<td>Indirect bilirubin (mg/dL)</td>
<td>0.7 (0.5–0.9)</td>
<td>0.8 (0.6–1.2)</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>2.89 (1.94–3.70)</td>
<td>2.11 (1.62–4.84)</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>19.2 (17.3–21.8)</td>
<td>18.5 (15.8–19.9)</td>
</tr>
<tr>
<td>Base excess (mmol/L)</td>
<td>-5 (-7 – -3)</td>
<td>-7 (-10 – -3)</td>
</tr>
</tbody>
</table>

Data are median (IQR), unless otherwise indicated by a geometric mean (95% CI).

* There were no significant differences between treatment groups in any of the measured baseline characteristics.

Abbreviations: CI, confidence intervals; PfHRP2, Plasmodium falciparum histidine rich protein 2.
Table 3. Pharmacodynamic Prediction of Creatinine Change Over Time Dependent on Baseline Creatinine and Cumulative Acetaminophen Exposure Over Three Days (AUC\textsubscript{0-72h})

<table>
<thead>
<tr>
<th>Baseline serum creatinine (mg/dL)</th>
<th>Probability of belonging to subpopulation 1 (%) (i.e. increasing serum creatinine over time)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No acetaminophen AUC\textsubscript{0-72h} 100mg×h/ L</td>
</tr>
<tr>
<td>1.25 (110.5μmol/L)</td>
<td>10.0</td>
</tr>
<tr>
<td>1.50 (132.6μmol/L)</td>
<td>16.0</td>
</tr>
<tr>
<td>2.00 (176.8μmol/L)</td>
<td>36.0</td>
</tr>
<tr>
<td>2.50 (221μmol/L)</td>
<td>62.4</td>
</tr>
<tr>
<td>3.00 (265.2μmol/L)</td>
<td>83.0</td>
</tr>
</tbody>
</table>

Pharmacokinetic-pharmacodynamic mixture model of observed creatinine described two subpopulations; where subpopulation 1 had an increasing creatinine over time and subpopulation 2 had a decreasing creatinine over time. All simulations were based on the developed final model, including enrollment creatinine and acetaminophen AUC\textsubscript{0-72h} as predictors of the mixture probability. Full details of pharmacokinetic-pharmacodynamic modeling are shown in Supplementary Material.

Conversion from creatinine mg/dL to μmol/L: multiply by 88.4.
Figure legends:

**Figure 1. Flow chart for the ‘Acetaminophen as a renoprotective adjunctive treatment in patients with severe and moderately severe falciparum malaria’ study.** 30 patients recruited in Chittagong, Bangladesh; 32 patients recruited in Ramu, Bangladesh.

**Figure 2. Effect of acetaminophen on creatinine stratified by intravascular hemolysis.** Creatinine mean percent change from baseline at 12, 24, 36, 60, 48, and 72 hours of: (A) entire cohort, (B-C) patients stratified by level of intravascular hemolysis; B: plasma CFH ≥45,000 ng/mL, C: plasma CFH <45,000 ng/mL (D-E) patients stratified by level of lipid peroxidation: D: plasma F_2-IsoPs ≥22 pg/mL, E: plasma F_2-IsoPs <22 pg/mL. A total of 35 out of 434 (8%) creatinine sampling time points were missing and replaced by imputed values (for details see Supplementary Table 2). Frequencies in rows below figures represent number of patients (n) at each time point. P-value represents overall treatment effect. Abbreviations: Cr, creatinine; CFH, cell-free hemoglobin; F_2-IsoPs, F_2-isoprostanes.

**Figure 3. Kaplan-Meier plot comparing in-hospital AKI development in patients with severe and moderately severe malaria treated with either acetaminophen or no acetaminophen (control).** Patients were classified as developing AKI if they had a creatinine rise of ≥26.5 µmol/L after admission. 17/62 (27%) patients had AKI during admission, 12/17 (38%) in the control group and 5/12 (16%) in the acetaminophen group. Competing risks regression adjusted by study site was used to assess subdistribution hazard ratio (SHR). Patients were censored at the time of creatinine rise meeting KDIGO criteria and death censored as a competing risk preventing the primary event of interest (AKI) from occurring.
Figure 2.
Figure 3.