Safety and Efficacy of Dihydroartemisinin-Piperaquine in Falciparum Malaria: A Prospective Multi-Centre Individual Patient Data Analysis

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Abstract

Background: The fixed dose antimalarial combination of dihydroartemisinin-piperaquine (DP) is a promising new artemisinin-based combination therapy (ACT). We present an individual patient data analysis of efficacy and tolerability in acute uncomplicated falciparum malaria, from seven published randomized clinical trials conducted in Africa and South East Asia using a predefined in-vivo protocol. Comparator drugs were mefloquine-artesunate (MAS3) in Thailand, Myanmar, Laos and Cambodia; artemether-lumefantrine in Uganda; and amodiaquine+sulfadoxine-pyrimethamine and artesunate+amodiaquine in Rwanda.

Methods and Findings: In total 3,547 patients were enrolled: 1,814 patients (32% children under five years) received DP and 1,733 received a comparator antimalarial at 12 different sites and were followed for 28–63 days. There was no significant heterogeneity between trials. DP was well tolerated with 1.7% early vomiting. There were less adverse events with DP in children and adults compared to MAS3 except for diarrhea; ORs (95%CI) 2.74 (2.13 to 3.51) and 3.11 (2.31 to 4.18), respectively. DP treatment resulted in a rapid clearance of fever and parasitaemia. The PCR genotype corrected efficacy at Day 28 of DP assessed by survival analysis was 98.7% (95%CI 97.6–99.8). DP was superior to the comparator drugs in protecting against both P.falciparum recurrence and recrudescence (P = 0.001, weighted by site). There was no difference between DP and MAS3 in treating P. vivax co-infections and in suppressing the first relapse (median interval to P. vivax recurrence: 6 weeks). Children under 5 y were at higher risk of recurrence for both infections. The proportion of patients developing gametocyteemia (P = 0.002, weighted by site) and the subsequent gametocyte carriage rates were higher with DP (11/1000 person gametocyte week, PGW) than MAS3 (6/1000 PGW, P = 0.001, weighted by site).

Conclusions: DP proved a safe, well tolerated, and highly effective treatment of P.falciparum malaria in Asia and Africa, but the effect on gametocyte carriage was inferior to that of MAS3.

Introduction

Over 80 countries worldwide have now implemented WHO recommendations to use artemisinin-based combination therapy (ACT) as first-line treatment of Plasmodium falciparum malaria [1,2]. Dihydroartemisinin-piperaquine (DP) is a fixed dose co-formulated ACT used increasingly in South East Asia, although it is not yet registered by most national drug authorities. Most experience of the use of DP comes from Vietnam, where it is the recommended first-line treatment. The bisquinoline compound piperaquine as a monotherapy was used extensively in China where it replaced chloroquine as the first-line treatment of falciparum and vivax malaria. Between 1976 and 1994 over 300 tons of piperaquine were used in China in antimalarial prophylaxis and treatment. The first combination of DHA and piperaquine (China-Vietnam 8, CV8), also included primaquine and trimethoprim and was first evaluated in Vietnam in 1990 [3]. CV8 was effective, and became part of national treatment policy but, because of primaquine...
toxicity concerns and uncertainty whether trimethoprim contributed to treatment efficacy, these two component drugs were eventually removed. The new two drugs combination became first line treatment in Vietnam in 2007.

Piperaquine has a terminal half-life of several weeks [4]. It is highly active against chloroquine-resistant *Plasmodium falciparum*, and *P. vivax* [5]. Dihydroartemisinin (DHA) is the active metabolite of artesunate and artemether. Recently, several clinical trials have been carried out to study the safety and efficacy of DP for the treatment of *P. falciparum* malaria. The randomized trials included in this individual patient data analysis [6–12] were conducted between October 2003 and June 2006 using a prospectively predefined protocol with a follow-up of at least 28 days and use of PCR parasite genotyping to distinguish new infections from recrudescences.

**Methods**

The trials were conducted in North-western Thailand, Rakhine state, Myanmar, Southern Laos, and Western Cambodia, where mefloquine combined with a three day course of artesunate (MAS3) was the comparator drug, in Uganda where artemether-lumefantrine (AL) was the comparator, and in Rwanda, where artesunate-amodiaquine (AS+AQ) and a non-ACT group amodiaquine+sulfadoxine-pyrimethamine (AQ+SP) were the comparator antimalarial drugs. In Rwanda, following high levels of chloroquine (CQ) resistance, the combination AQ+SP was adopted as the first-line anti-malaria treatment in 2001. However, AQ+SP has always been considered an interim strategy and different artemisinin-based combination treatments (ACT) have been tested in the past few years as possible alternatives.

Patients presenting with acute uncomplicated falciparum malaria were recruited into the treatment studies provided they gave fully informed consent. Eligible patients were 12 to 59 months old patients weighing more than 10 kg in Rwanda, 6 months to 10 years old, weight >5 kg in Uganda, and all patients between 1 and 65 years in Myanmar, Laos, Cambodia, and Thailand. Only uncomplicated cases of *P. falciparum* mono infections were included in Laos, Rwanda, and Uganda, while in Cambodia, Thailand, and Myanmar patients with mixed (*P. falciparum* and *P. vivax*) infections were included. Studies excluded pregnant or breastfeeding women, patients with HIV-AIDS or severe malaria. Malaria on admission and reappearance were determined using power calculations and whether an intention-to-treat (ITT) analysis could be computed. Other markers of quality assessed were whether a sample size was adequate, inadequate, or unclear [17].

**Data pooling**

The databases of randomized controlled trials were sent by the investigators. The following aspects of the quality of trial methodology were evaluated: generation of the allocation sequence, adequacy of concealment of the allocation of treatment, degree of blinding, and completeness of follow-up. Generation of the allocation sequence and allocation concealment was classified as adequate, inadequate, or unclear [17].

Blinding was classified as open, single or double. The proportion of patients lost to follow-up (regardless of failures) was computed and considered acceptable if <10% within 28 days. Other markers of quality assessed were whether a sample size was determined using power calculations and whether an intention-to-treat (ITT) analysis could be computed.

**Ethical Approval**

The clinical trials from each country were approved by appropriate authorities. The Thai studies were approved by the Faculty of Tropical Medicine, Mahidol University Ethical Committee (Bangkok, Thailand). Approval for the Laos study was granted by the Ethical Committee of the Faculty of Medical Sciences, National University of Laos; both of these studies were also approved by the Oxford Tropical Research Ethics Committee (OXTREC), University of Oxford, UK. The protocol for the trial in Myanmar was approved by the Myanmar Department of Health and by the Médecins Sans Frontières (MSF) Ethical Review Board. In Rwanda, the study was reviewed and approved by the Ministry of Health of Rwanda and by the Ethical Committee of the Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium. The Cambodian study received ethical clearance from the Cambodian National Ethical Review Committee (Ministry of Health, Cambodia), and the MSF Ethical Review Board. In Uganda, approval came from the Makerere University Research and Ethics Committee, the Uganda National Council of Science and Technology, and the University of California, San Francisco Committee for Human Research.
Study endpoints and statistical analysis

The analysis was by modified intention-to-treat where patients who did not complete the study were censored on their last day of follow-up, but they were not regarded as a failure as in a “pure” ITT analysis.

The primary endpoint was the treatment efficacy by Day 28. Patients lost to follow-up (or missing a weekly visit) or with a new \( P. falciparum \) infection were censored for the primary outcome at the time they were last seen. All studies followed patients for at least 28 days and the primary endpoint was defined prospectively as the parasitological treatment failure (PCR confirmed: recrudescence, and PCR not corrected: recurrence). Treatment failure was considered as the sum of early and late treatment failures, as defined by the WHO [1] as one of the following: (i) danger signs, death, or severe malaria at Days 1, 2 or 3 with parasitaemia; (ii) parasite density at Day 2>Day 0; (iii) parasitaemia at Day 3>25% than Day 0, and recurrent parasitaemia after Day 4. Patients could be given less than the full dose if they received rescue treatment or withdrew consent from follow-up. Adequate Clinical and Parasitological Response (ACPR) was defined as no parasitaemia until the end of the follow-up without previously meeting any of the criteria for failure.

The efficacy was measured using Kaplan-Meier survival analysis. We applied a statistical correction for cases where PCR genotyping gave an indeterminate result or was unavailable, computing adjusted quotients by determining the probability by site and at any time of a parasite reappearance being either a recrudescence or a new infection [18].

Secondary outcomes were:

i) The risks of recrudescence (reappearance of the same genotype) in the DP groups compared to the comparator groups (stratified by study), using the different lengths of follow-up of each studies.

ii) The risks of recurrence (defined as recrudescence as above and new infections) in the DP groups compared to the comparator groups (stratified by study), using the different length of follow-up of each studies.

iii) The risk of new infection (excluding recrudescences) in DP groups compared to the comparator groups per study and overall result (stratified by study) at Day 28.

The risks of treatment failure (i, ii, iii) were compared in the 2 continents.

iv) The predictors of recrudescence or new infection or recurrence by Day 28 were assessed by Cox regression models. The covariates examined were: sex, age (continuous), food absorption with the drug, anaemia on admission (haematocrit <30%), parasite count on admission (log transformed), elevated temperature on admission (temperature measured by any method at \( \geq 37.5^\circ C \)). As the age groups were different between Africa (under 5 years old) and Asia (children and adults), we conducted this part of analysis separately for the 2 continents.

v) Gametocyte carriage

a. The predictors of gametocyte prevalence on admission were measured using a logistic regression and controlling for site.

b. The time to clearance of gametocytes already present on admission with data censored at the time of gametocyte clearance was calculated based on the results of blood smears.

Within each study, the same sampling schedules were used for all the patients, but between trials parasite counts were performed at different times so any analysis was stratified by site to account for these differences. For patients who cleared gametocytaemia, time of the first negative count (followed by further negative counts) was taken as time of clearance. Differentials in gametocytes clearance were calculated using Kaplan-Meier method using logrank test, stratified by site, and a Cox regression model stratified by site measured the risks of gametocytes carriage between treatment groups.

c. The predictors of gametocyte appearance during the follow-up in patients without gametocytaemia on admission were assessed by a Cox regression model stratified by site. The presence of gametocytaemia after starting treatment was analysed as a binary variable: the Mantel-Haenszel method and the homogeneity test stratified by site were used to estimate a combined odds ratio between treatments. One positive gametocyte count at any time after treatment during the follow-up period was enough to define gametocyte carriage, while a complete set of negative counts during follow-up was required to confirm no carriage.

d. Gametocyte carriage rates were measured in person-gametocyte-weeks, using binary variable calculated within 42 days of follow-up. Person-gametocyte-weeks (PGW expressed per 1000) were defined as the number of weeks in which blood slides were positive for gametocyte divided by the total number of weeks followed up in patients with gametocyte results [19]. Mantel-Haenszel rate ratios (RR) weighted by site were used to measure to the risks between treatment groups.

vi) Haematological changes were measured using the paired t-test. Anaemia was defined as haematocrit <30%, and anaemia recovery during the follow-up by the time for the haematocrit to reach 30% or more.

vii) Adverse events: defined as any sign, symptom, or disease that was not present on admission and was associated with the use of a medicinal product, whether or not it was considered as related to the medicinal product. A serious adverse event was defined as a sign or symptom that was fatal, life threatening or required admission to hospital. Adverse events were standardised and expressed as an incidence density, in person-days at risk within 28 days. The incidence rate ratio test was used to compare the incidence of adverse events. We assumed that young children (<5 years old) were unable to answer questions about dizziness, nausea, headache, confusion, numbness, hearing disturbance, tinnitus or visual disturbance.

viii) The effects of DP on \( P. vivax \) recurrences in the Asian trials with longer follow-up (63 days) was calculated by censoring the data at the time of \( P. vivax \) appearance (binary variable). The predictors of \( P. vivax \) appearance were measured by using Cox regression, and the incidence density of \( P. vivax \) appearance was calculated in person-day.

Heterogeneity was assessed by the Cochran Q test, and \( P^2 \) test. Chi-square, Mann-Whitney, Kruskall-Wallis tests were used as appropriate. Confidence intervals (CI) were measured at 95% by the binomial distribution, or the Wilcoxon procedure, or the Taylor series estimate as appropriate [20]. The statistical programme used was STATA v10 (STATA corp.).

Results

Characteristics of included studies

A total of 3,547 patients were enrolled in six countries from 12 different sites between October 2003 and June 2006. Individually,
the trials enrolled between 75 and 303 patients treated with DP (total = 1,814), 1,475 patients treated with other ACTs, and 258 in the non-ACT (AQ+SP) group (figure 1). The proportion of patients lost to follow-up was <10% in all the trials (4%, 110/3,547 at Day 28).

**Heterogeneity**
In all the trials the methodological quality was high and the randomisation sequence was computer-generated. All trials were open label, and the basis for the sample size studied was provided in all studies. In all studies, the primary treatment outcome was the parasitological treatment failure. All trials reported data on haemoglobin levels during follow-up, and recorded gametocyte carriage at study enrolment and during follow-up. All studies assessed adverse events and 10 different adverse events were documented in all trials. Although there were differences in geographical location, transmission intensity (ranging from low and seasonal in Thailand to very high in the African trial settings), age, treatment and supervision, heterogeneity between trials was not significant (I² test = 26%, P = 0.15, Cochran Q test for heterogeneity), and regarded as low [21].

**Baseline characteristics**
The median age of the recruited patients was 13 years (range 1 to 65) (Table 1). Overall 32% of patients were under five years of age, 28% were 5–14, and 40% were adults. In Asia, age distributions were similar in Cambodia, and in Thailand. The patients were younger in Laos and Myanmar, while in Rwanda and Uganda only children were enrolled. There were no differences detected in admission characteristics between DP groups and comparator treatment groups, except for baseline gametocytaemia in Myanmar [8].

**Clinical recovery**
Overall 54.6% of patients were febrile ($\geq 37.5^\circ C$) on admission. This decreased to 8.8% at 24 h (i.e. median fever clearance <24 hours) and 2.1% at 48 h. No difference was detected between treatment groups. The median time for the spleen to be no longer palpable was 14 days (range 1–42). There was no difference in the time to resolution of splenomegaly between the treatment groups (P = 0.70).

**Parasitological efficacy**
Of the 1,814 DP treated patients, 126 patients (6.9%) were lost to follow-up by Day 28. Of the 221 patients with recurrent infections, 9 cases had indeterminate PCR genotyping results, and 3 results were not available (lost samples). The results of parasitological efficacy per study site are shown in table 2, the number of patients followed-up, recurrent and recrudescent cases, and the daily results by category of follow-up are shown for a hypothetical cohort of 1000 persons with and without the PCR correction (table 3). On Day 1 (24 hours after starting treatment), 31% (95%CI 29–34) of the patients had cleared their parasitaemia, on Day 2, 89% (95%CI 87–90), on Day 3, 98% (95%CI 97–99). All patients had cleared their parasitaemia by Day 7.

**Primary endpoint: parasitological efficacy at Day 28**
In DP groups, the overall observed parasitological efficacy using results from survival analysis at Day 28, corrected by PCR was 98.7% (95%CI 96.8–98.3). In children under 5 years old, the corresponding efficacy was lower: 94.2% (95%CI 91.9–96.5, P = 0.001). For the overall recurrence of *P. falciparum* parasitaemia the corresponding results were 96.1% (95%CI 95.0–97.2) and 90.4% (95%CI 87.8–93.0) in children (P = 0.001).

**Secondary endpoints**
i) **Risk of recrudescence for the full length of follow-up by treatment group.** Based on randomised comparisons by country and using the full length of follow-up of the different trials, DP recipients were at lower risk for a PCR confirmed failure compared to MAS3 in Thailand (P = 0.001), AL in Uganda (P = 0.004), and AQ+SP in Rwanda (P = 0.001)(figure 2, table S1). Overall, using

![Diagram showing the flow of participants through each stage of the randomized trial and the number of patients who completed the follow-up on Day 28](image)

Figure 1. Note: DP; dihydroartemisinin-piperaquine, MAS3; mefloquine-artesunate, AQ+SP; amodiaquine-sulfadoxine-pyrimethamine, AL; artether-lumefantrine, AS+AQ; artesunate-amodiaquine, Pf; *P. falciparum*; loss; loss to follow-up, ACR; adequate clinical response, ACT; artemisinin combination therapy.

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### Table 1. Trials baseline characteristics, patients receiving dihydroartemisinin-piperaquine.

| Country characteristics on admission | Cambodia | Laos | Myanmar | Rwanda | Uganda | Thailand | Total |
|--------------------------------------|----------|------|---------|--------|--------|----------|-------|
| Total number of patients             | 228      | 110  | 327     | 19.2%  | 252    | 14.8%    | 211   |
| Age, median in years (IQR; range)    | 21 (20; 26-65) | 12 (17; 1-50) | 7 (8; 1-42) | 14.8% | 2 (2; 1-9) | 12 (22; 1-65) |
| Age group                            |          |      |         |        |        |          |       |
| 0–4 N, %                             | 7        | 19   | 79      | 24.2%  | 248    | 84.8%    | 200   |
| 5–14 N, %                            | 61       | 49   | 191     | 58.4%  | 121    | 48.0%    | 97    |
| ≥15 N, %                             | 161      | 41   | 57      | 17.4%  | 0      | 0.0%     | 464   |
| Male N, %                            | 160      | 63   | 165     | 50.5%  | 121    | 48.0%    | 446   |
| Haematocrit (%) Mean (SD)            | 35.5 (6.9) | 35.0 (7.0) | 27.8 (6.6) | 31.5 (4.9) | 28.5 (5.66) | 37.3 (6.0) | 33.9 (7.2) |
| Anaemia (<30% hct) N, %              | 40       | 17   | 205     | 62.7%  | 86     | 34.3%    | 87    |
| Geometric mean parasitaemia/μL (range) | 3331 (40–173328) | 18372 (3768–156623) | 8864 (585–99502) | 29425 (32–200000) | 22788 (2080–192800) | 9956 (66–221433) | 11247 (32–221433) |
| Mixed infection N, %                 | n-a      | n-a  | n-a     | 40     | 12.2%  | n-a      | n-a   |
| Gametocyte carriers N, %             | 17       | 0.9% | 137     | 41.9%  | 8      | 3.2%     | 41    |
| Splenomegaly N, %                    | 54       | 19   | 25      | 10.0%  | n-a    | n-a      | 162   |
| Hepatomegaly N, %                    | 24       | 12   | n-a     | 10.9%  | 1      | 0.4%     | n-a   |
| Fever (T.37.5°C) on admission N, %   | 153      | 104  | 148     | 45.4%  | 175    | 69.7%    | 211   |

IQR: interquartile range.

n-a: no observation.

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### Table 2. Dihydroartemisinin-piperaquine efficacy by country and site, Day 28, survival analysis (Kaplan-Meier).

| Country and site | Efficacy (%) Day 28 * |
|------------------|-----------------------|
|                  | PCR uncorrected | PCR corrected |
|                  | Efficacy | Lower 95% confidence interval | Upper 95% confidence interval | Efficacy | Lower 95% confidence interval | Upper 95% confidence interval |
| Uganda, site Apac, Day 28 | 89.0 | 84.8 | 93.2 | 98.0 | 92.9 | 98.8 |
| Rwanda all sites, Day 28 | 88.4 | 83.7 | 91.8 | 95.2 | 91.6 | 97.3 |
| Rwanda site MA | 94.2 | 86.5 | 96.6 | 96.6 | 89.5 | 98.9 |
| Rwanda site KI | 97.3 | 89.4 | 99.3 | 97.3 | 89.4 | 99.3 |
| Rwanda site RU | 75.2 | 64.4 | 89.7 | 89.7 | 82.7 | 95.8 |
| Thailand all sites, Day 28 | 98.2 | 97.2 | 99.2 | 99.5 | 98.0 | 99.7 |
| Thailand site KT | 97.0 | 95.1 | 98.9 | 99.0 | 95.9 | 99.3 |
| Thailand site MT | 100.0 | 98.7 | 100.0 | 100.0 | 98.7 | 100.0 |
| Thailand site TR | 97.8 | 91.1 | 98.5 | 100.0 | 97.8 | 100.0 |
| Cambodia all sites, Day 28 | 98.6 | 94.3 | 99.1 | 99.1 | 95.5 | 99.3 |
| Cambodia site AV | 99.0 | 95.3 | 99.3 | 99.0 | 95.3 | 99.3 |
| Cambodia site KV | 98.1 | 90.6 | 98.6 | 99.1 | 95.4 | 99.3 |
| Myanmar all sites, Day 28 | 98.1 | 97.2 | 99.0 | 99.3 | 95.4 | 99.4 |
| site MN | 98.4 | 93.8 | 100.0 | 100.0 | 97.2 | 100.0 |
| site DB | 97.4 | 93.8 | 98.8 | 99.5 | 98.1 | 99.9 |
| Laos, Day 28 | 100.0 | 96.6 | 100.0 | 100.0 | 96.6 | 100.0 |
| Total, Day 28 | 96.1 | 95.0 | 97.2 | 98.7 | 97.6 | 99.8 |

*Efficacy based on randomised trials was assessed by modified intent to treat analysis for recurrent and recrudescent cases. The Kaplan-Meier results were expressed as percentages.

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multivariate analysis stratified by site and controlling for age, patients receiving DP had a lower risk of PCR confirmed treatment recrudescence than with the comparator treatments (AHR = 0.32, 95% CI 0.21–0.48, P = 0.001). ii) Risk of recurrence for the full length of follow-up, by treatment group. Using multivariate analysis and the full length of follow-up stratified by site and controlling for age and anaemia, the overall risk of recurrence was lower in the DP groups than in the comparator groups (AHR = 0.60, 95% CI 0.51–0.89, P = 0.001)figure 2). DP provided a better protective effect against P. falciparum recurrence than its comparator groups in Rwanda; compared to AQ+SP (P = 0.006) and AS+AQ (P = 0.006); to AL in Uganda (P = 0.005); and MAS3 in Thailand (P = 0.016). There was a longer interval from primary infection to recurrence (suggesting a greater duration of suppressive prophylaxis) compared to MAS3 in Thailand (median: 49 days vs. 37 days, respectively, P = 0.001); in Uganda compared to AL (median 35 vs. 28 days, respectively, P = 0.001); in Rwanda compared to AQ+SP (median 28 vs. 21 days, respectively, P = 0.035), but was not different to AS+AQ (P = 0.23). iii) Risk of new infections (PCR confirmed) by Day 28. At 28 days the risk of new infection was greater in African compared to Asian settings (P = 0.001) reflecting the higher transmission intensity. In these high transmission areas, patients treated with DP were at lower risk for a new infection within 28 days compared to AQ+SP (HR = 0.38, 95% CI 0.19–0.76, P = 0.006); AS+AQ (HR = 0.42, 95% CI 0.21–0.86, P = 0.018) in Rwanda and AL (HR = 0.38, 95% CI 0.22–0.65, P = 0.001) in Uganda. Overall, in the multivariate analysis stratified by site, DP had a greater post treatment prophylactic effect (against new infections) at Day 28 against P. falciparum compared to the other treatments (AHR = 0.47, 95% CI 0.33–0.67, P = 0.001). iv) Predictors of recrudescence, new infection, and recurrence of P. falciparum in DP groups by Day 28. In the DP groups, using multivariate analysis stratified by site at Day 28, age (as continuous variable) was the only predictor of recurrence and recrudescence in Africa or Asia when analyzed separately. In African children, younger patients (per 1 year increase in age) were at higher risks for recurrence (AHR = 0.96, 95% CI 0.93–0.99, P = 0.001, respectively). No significant predictors of new infections were detected in African children, but in Asian patients younger patients were at higher risks for new infections (AHR = 0.96, 95% CI 0.93–0.99, P = 0.001). v) Gametocyte carriage. Admission pre-treatment gametocyteaemia was present in a median (range) of 6.1% (0.9–41.9) of the patients. Using multivariate analysis in DP groups, and controlling by site, younger patients, admission anaemia, and a lower admission parasite count were related to a higher risk of patent gametocyteaemia (table 4). Clearance of gametocyteaemia was slower in DP groups than in the comparators, overall and in individual sites. In Cambodia in the DP treatment arm, 82(95% CI 55–94)% of patients presenting with gametocyteaemia still had gametocyteaemia on Day 3 compared to only 27(7–54)% in the comparator arm. On Day 14 of the follow-up in Thailand 24(10–39)% in DP arm and 11(2–30)% in the other arm, in Uganda 7(2.4–16)% in DP arm compared to 1.8(0.3–13)% in the other arm, in Myanmar 32(23–38)% in DP arm as compared to none in the comparator arm. Overall, using multivariate analysis, the risk of gametocyte carriage by Day 14 was lower in comparators than in DP groups (P = 0.002, stratified by site)figure 3, table S2).
Overall 178 (8.4%) out of 2125 patients presenting without gametocytes developed gametocytaemia after starting treatment. Recurrent parasitaemia as well as anaemia on admission were associated with gametocyte appearance (Table 4). There were no significant overall differences in the risk of developing gametocytaemia during follow-up between DP and all comparator arms (Mantel-Haenszel OR = 1.02 [95%CI 0.73–1.40], P = 0.89; NC: not computable because of no recrudescence cases. *Overall number of failures does not add up because two comparators were used in Rwanda. Note: the forest plot represents the risk of parasite reappearance (PCR corrected; i.e. recrudescence, and not corrected i.e. recrudescence+new infection) of DP versus comparators in comparative studies. Groups size are equivalent except in Thailand where the DP group was twice as large (N = 686). Endpoints were assessed on Day 28 in Rwanda, Day 42 in Laos, Myanmar, and Uganda, and Day 63 in Cambodia, and Thailand. Overall results were stratified by site, and drugs. The size of the boxes is proportional to the number of patients included and thus to the overall effect. The diamond represents the overall hazard ratio and 95% CI.

### vi) Haematological changes.

On admission, 537 out of 1,797 (29.9%) DP recipients with available data were anaemic (Hct<30%). Of these 29.8% (74/537) had severe anaemia (Hct<20%). Using multivariate analysis, anaemia on admission was strongly associated with age and varied by country (Table 1). Children under 15 y were at higher risk for anaemia compared to adults as well as patients from Cambodia, Uganda, and Myanmar compared to Thailand (P = 0.001, for all comparisons). By Day 28, 55% (162/293) of the anaemic patients had recovered from anaemia and 2.8% (24/851) who were not anaemic on admission became anaemic. Overall, in anaemic patients, the median time to recovery (defined as haematocrit ≥30%) ranged from 7 to 42 days. On Day 42, the prevalence of anaemia was 3.4% (34/985). There were no treatment differences in the development of anaemia.

In Cambodia, Laos, Myanmar, Thailand, there was a relative mean paired transient decline of 6.3% (95%CI 5.7–7.3) in haematocrit from admission to Day 7 in DP groups. No difference was detected compared to the MAS3 group. By contrast, in Rwanda, between Day 0 and Day 14, the relative paired mean haematocrit difference was significantly higher in the AS+AQ group (+10%, 95%CI 8–11) compared to the DP group (+6%, 95%CI 4–8); P = 0.021. In Uganda, patients treated with DP (+20%, 95%CI 16–24) had a higher relative paired mean increase in haemoglobin levels on Day 42 compared to AL group (+16%, 95%CI 12–20; P = 0.049).

### vii) Drug vomiting, incidence of adverse events, number of adverse events, and death.

Overall, vomiting on admission and before treatment administration was a risk factor for vomiting the first dose of DP (OR = 6.1, 95%CI 3.2–11.7) and for vomiting DP treatment over the three days (OR = 4.6, 95%CI 2.7–7.7). This was similar in every country. In patients who did not present with vomiting on admission, the overall incidence of early vomiting (defined as vomiting the drug within 1 hour after intake) was low; 1.7% (21/1,231) on Day 0. Over the 3 days of treatment, the overall incidence rate of early vomiting ranged from 2.7% (95%CI 2.4–7.7) in Cambodia to 5.9% (95%CI 5.5–6.3) in Rwanda (Table 5). In DP groups, drug vomiting was more frequent in Rwanda than all other countries (P = 0.001) and was related to age: the 0–4 y age group (OR = 8.2, 95%CI 3.2–21.3), and the 5–14 y age group (OR = 3.9, 95%CI 1.9–8.1) were at higher risk compared to adults. In Rwanda, the incidence of overall vomiting DP was not different to that after AS+AQ (11.5%, P = 0.565), but the risk was much lower than with AQ+SP (19.5%, P = 0.002). No difference in the incidence of early vomiting after drug treatment was observed between DP and MAS3 on Day 0 (3.2%, and 2.4%, respectively, P = 0.400), or overall (3.7%, and 4.2%, respectively, P = 0.671).
We examined the incidence and prevalence of 24 other different adverse events (apart from early vomiting) in 1,267 individuals using available records from DP groups in five countries and 9 different sites. It was not possible to calculate the adverse events duration in Laos, and Uganda. In the remaining DP groups, the five most commonly reported adverse events by Day 28 were 23.3% for headache, 17.0% for dizziness, 13.8% for sleep disturbance, 11.6% for anorexia, 10.5% for nausea (table 6). Hypersensitivity reactions including urticaria were reported in 4 patients in Thailand (0.6%, 4/686, 95%CI 0.2–1.5) [6]. The following adverse events: muscle pain, hearing disturbances, itching, nightmares, visual disturbances, dyspnoea, numbness, skin rash, agitation and confusion, were all reported in less than 5% of cases. The maximum point prevalence rates of the adverse events all occurred on Day 1. Day 1 and Day 2 captured 54% and the first week captured 70% of the reported adverse event incidence.

In the Asian trials, the only adverse event that was significantly more frequent in DP treated patients compared to the MAS3 group was diarrhoea in all age groups (children: 36% 310/852 vs. 17% 108/625, OR = 2.74, 95%CI 2.13–3.51; adults: 46% 248/543 vs. 21% 83/390, OR = 3.11, 95%CI 2.31–4.18, respectively, P = 0.001 for both comparisons, figure 5, table S3). Regarding other gastro-intestinal adverse events, DP was significantly better tolerated than AS+AQ, AS+SP, or MAS3 (P < 0.040 for all comparisons) but was not different compared to AL (only for late vomiting). In adults the risk of nightmare (P = 0.028) and sleep disturbances (P = 0.003) was lower in the DP group than in the MAS3 group. The risk of dermatological events (P < 0.010 for all comparisons), dizziness, palpitation, and muscle pain in all ages was also lower than in the MAS3 group (P < 0.010 for all comparisons). The risks of hearing disturbance (tinnitus, or hearing problems) in adults treated with MAS3 was greater than in the DP group (P = 0.001).

The frequency of patients treated with DP and reporting at least one of the 24 adverse events analyzed was 57.2% (840/1,468, 95%CI 54.7–59.8); among them 38% reported one, and 26% two adverse events (without excluding patients under 5). The total number of adverse events reported per patient was higher in older patients (P = 0.001) and in anaemic patients on admission (P = 0.020 after correcting for age).

The incidence of adverse events was significantly lower in DP recipients compared to MAS3 recipients (on average by 25.9%, 95%CI 22.9 to 29.0%, P = 0.001). More patients treated with MAS3 reported two or more AEs (67%, 454/675, P = 0.034). In Rwanda, the risk of having any adverse event was higher in the AQ+SP group (OR = 2.19, 95%CI 1.33–3.57) and in the AS+AQ group (OR = 1.90, 95%CI 1.15–3.12) compared to the DP group. No differences were detected in Uganda in the AL group compared to the DP group.

A child from Rwanda had a seizure and received a rescue treatment. Overall, 5 deaths occurred in DP groups, all of which were considered to be unrelated to the treatment, except in Thailand where a 43-year-old woman who died from severe toxoplasmosis.

| Table 4. Predictors of patent gametocytaemia, and gametocyte appearance, dihydroartemisinin-piperaquine groups. |
|-----------------------------------------------|-----------------------------------------------|
| Independent variables | Gametocyte on admission | Gametocyte appearance |
| Age continuous | AOR = 0.97, 95%CI 0.95–0.98, p = 0.001 |  |
| Anaemia (ref: no anaemia) | AOR = 1.83, 95%CI 1.43–2.34, p = 0.001 |  |
| Parasite count (continuous) | AOR = 0.69, 95%CI 0.58–0.82, p = 0.001 |  |

Model 1 for recurrence

| Anemia on admission | AHR = 2.71, 95%CI 1.56–4.73, p = 0.001 |
| Recurrence during follow-up | AHR = 2.66, 95%CI 1.32–5.39, p = 0.001 |

Model 2 for recrudescence

| Anemia on admission | AHR = 2.83, 95%CI 2.61–8.66, p = 0.001 |
| Recrudescence during follow-up | AHR = 2.90, 95%CI 0.68–12.45, p = 0.152 |

Note: Age was per 1 year increase in age, and parasite count was per 1 unit increase in the log transformed parasite density. AOR; adjusted odds ratio. AHR; adjusted hazard ratio. CI; confidence interval.

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Figure 3. Note: the forest plot represents the risk of clearing gametocytes comparing DP versus comparator drugs in randomised studies. The endpoint was assessed at Day 14. Overall result was stratified by site. The size of boxes is proportional to the number of patients included and thus to the overall effect. The diamond represents the overall hazard ratio and 95% CI. doi:10.1371/journal.pone.0006358.g003
malaria the day after entering the study, which might have been related to a lack of efficacy of the drug. In Thailand, there were another 2 deaths: a 13-year-old girl who died on Day 7 from probable bacterial sepsis, and a 21 year old male who died on Day 28 from gunshot wounds. In Laos, a two year old child died on Day 37 from cerebral malaria after probable reinfection. In Myanmar, one 11-year-old child died after developing fever on Day 20 and had generalized seizures the next morning (Day 21). The malaria smear was negative. In the comparator groups, one death occurred in MAS3 groups in Thailand involving a malaria-smear negative 13-year-old boy, who was clinically well by the third day of treatment. He was reported to have deteriorated rapidly with worsening abdominal pain and distension, jaundice, and anuria, and he died within a few hours.

viii) Plasmodium vivax and other species appearing during the follow-up period. No difference was detected between DP groups and the comparator (mefloquine-artesunate) in P. vivax recurrence rates (P>0.05 for all comparisons). In Cambodia, Laos, Myanmar, and Thailand, 265 patients receiving DP (28.8/100 person-days within 63 days) had P. vivax parasitaemia detected during the follow-up (figure 6). The median time to the P. vivax parasitaemia was 49 (14–63) days compared with 49 (28–63) days, in MAS3 groups. Patients with P. vivax on admission (mixed infections) were at higher risk of having a second P. vivax episode during the follow-up (HR = 1.70, 95%CI 1.15–2.51, P = 0.008). Children were at increased risk of P. vivax recurrence when compared to adults; 0–4 age group (HR = 2.85, 95%CI 1.81–4.48, P = 0.001) and the 5–14 age group (HR = 1.43, 95%CI 1.05–1.94, P = 0.024). In patients who had mixed infections on admission, the median time to P. vivax recurrence was 42 days (7–63), significantly shorter than in patients presenting with a P. falciparum monoinfection: 49 (16–70) days, (P = 0.029). In Uganda patients treated with DP had a lower risk of recurrent parasitaemia due to P. malariae and P. ovale species compared to patients treated with AL (5.2% versus 0.9%, P = 0.001)[10].

Discussion

Since initial deployment in 1994 of the mefloquine-artesunate (MAS3) combination to treat P. falciparum malaria along the Thai-Myanmar border, there has been increasing use of ACTs throughout the malaria affected world. Dihydroartemisinin-piperaquine (DP) is a relatively new and very promising fixed dose ACT which has been extensively evaluated in the past few years. This analysis of 3,547 patients (1,814 of whom received DP) in randomized comparative clinical trials includes 7 of the 22 published studies (until December 2008), but is broadly represent-

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**Table 5.** Early vomiting (<one hour) after treatment administration, dihydroartemisinin-piperaquine groups.

| Country     | Daily incidence | Total incidence | 95% confidence interval | N  |
|-------------|-----------------|-----------------|-------------------------|----|
|             | Day 0 | Day 1 | Day 2 |  |                  |          |
| Myanmar     | 2.1%  | 1.2%  | 0.6%  | 4.0% | 2.3%–6.8%      | 13/327   |
| Cambodia    | 4.8%  | 1.3%  | 0.0%  | 5.2% | 3.0%–9.1%      | 12/228   |
| Rwanda      | 6.7%  | 2.4%  | 2.8%  | 9.9% | 6.8%–14.4%     | 25/252   |
| Thailand    | 2.6%  | 0.6%  | 0.1%  | 3.2% | 2.1%–4.8%      | 22/686   |
| Total       | 3.5%  | 1.1%  | 0.5%  | 4.8% | 3.7%–5.9%      | 72/1495  |

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Table 6. Adverse event incidence density and prevalence rates, dihydroartemisinin-piperaquine groups.

| Adverse event          | Adverse event cumulative incidence density over 28 days | Incidence within the first week | Adverse event maximum prevalence |
|------------------------|--------------------------------------------------------|---------------------------------|---------------------------------|
|                        | Number of patients without the symptom on admission | % Lower 95%CI Upper 95%CI        | Day % Lower 95%CI Upper 95%CI   |
| Headache               | 124                                                    | 23.3% 15.4% 31.2% 74% Day 1 9.9% 5.8% 17.0% |
| Dizziness              | 457                                                    | 19.3% 15.3% 23.2% 88% Day 1 11.6% 8.5% 14.8% |
| Sleeping problem       | 811                                                    | 18.4% 15.0% 21.7% 83% Day 1 6.3% 4.8% 8.2%  |
| Anorexia               | 521                                                    | 16.7% 13.4% 19.9% 85% Day 1 10.3% 7.7% 13.0% |
| Fatigue                | 628                                                    | 15.0% 12.4% 18.1% 82% Day 1 7.7% 5.9% 10.1% |
| Nausea                 | 628                                                    | 13.6% 10.8% 16.5% 83% Day 1 8.7% 6.4% 11.0% |
| Joint pain             | 451                                                    | 10.0% 7.6% 13.3% 63% Day 1 4.7% 3.1% 7.2%  |
| Abdominal pain         | 1040                                                   | 9.6% 7.8% 11.4% 76% Day 1 4.7% 3.4% 6.0%  |
| Diarrhoea              | 1204                                                   | 9.2% 7.5% 10.9% 84% Day 1 4.8% 3.6% 6.1%  |
| Late vomiting          | 1083                                                   | 7.1% 5.5% 8.7% 86% Day 1 4.7% 3.4% 6.0%  |
| Palpitations           | 753                                                    | 6.7% 5.1% 8.8% 82% Day 1 3.2% 2.2% 4.8%  |
| Hearing disturbance    | 971                                                    | 6.3% 4.5% 8.1% 68% Day 1 2.4% 1.3% 3.5%  |

CI: confidence interval.

*The incidence within the first week is the proportion of cases occurring in the first week divided by the total number of cases within 28 days for each adverse event.

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Asian region are followed by a vivax malaria relapse. In trials with 63 days follow-up the recurrent episode of vivax malaria in patients with mixed infections on admission occurred slightly earlier (6 weeks) than new vivax appearances in patients with a falciparum mono-infection (7 weeks). Thus, the first relapse is suppressed by DP, comparable to the effect of chloroquine on sensitive P. vivax strains [31].

The higher risk of treatment failure in children treated with DP compared with adults is similar to the pattern seen with other antimalarial drugs, and presumably results both from lower immunity and lower blood piperaquine concentrations. The shorter time to P. vivax reappearance in children would also support a pharmacokinetic explanation. The Day 7 piperaquine level is a useful measure of drug exposure. Young children have lower piperaquine levels on Day 7 and higher treatment failure rates than older children and adults [4,25]. In a recently reported population pharmacokinetic study from Thailand, there were therapeutically relevant pharmacokinetic differences between different age groups. Children had a smaller central volume of distribution, a shorter distribution half-life (t1/2, α), and a more rapid fall in initial PQ plasma concentrations compared to the population mean profile [4]. Studies indicated lower plasma piperaquine concentration in children compared to adults in Papua, Indonesia [25] and higher clearance in children in Vietnam [32]. Taken together these data argue for higher weight adjusted doses in children compared with adults. This would also

Figure 5. Note: the forest plot represents the risk of adverse event appearance after the start of dihydroartemisinin-piperaquine treatment in children (<15 y) and adults who did not present this symptom on admission versus comparators in comparative studies. The size of boxes is proportional to the number of patients included. 95% confidence intervals (CI) are calculated for the odds ratio (OR). doi:10.1371/journal.pone.0006358.g005
have the advantage of providing a larger dose of dihydroartemisinin. Further studies in young children to optimize the dose of DP are needed.

The risk of early vomiting of DP was lower than following AQ+SP in Rwanda (P = 0.001). No differences were observed with the other comparator treatments. Early vomiting was more frequent in young children, although it was not a risk factor for treatment failure, presumably because these children were re-dosed successfully after vomiting the drug.

The DP safety profile has been excellent in all published series. The overall safety analysis showed that the risk of the most common adverse events was significantly lower following DP treatment than in the comparator arms in both children (<15 years old) and adults. Adverse events were often related to the disease itself (particularly neurological and gastro-intestinal AEs), although diarrhea was approximately twice as common following DP than with MAS3.

The use of common protocols for data collection to assess antimalarial drug efficacy and tolerability allows combination of these data into larger international databases which can give us more information on the safety and efficacy of these drugs in different patient groups [33]. While methods for assessing antimalarial drug efficacy are well standardized there is little uniformity in safety reporting in antimalarial drug studies, a problem which needs to be addressed.

DP is not yet recognized internationally and its use has been limited by its regulatory status. The formulation used in the trials was donated by Holley and manufactured according to Chinese SFDA Good Manufacturing Practice (GMP) standards. Nevertheless, differences in efficacy might be related to the variability in the composition of the study drug.

This antimalarial combination is currently under evaluation by the WHO pre-qualification process. DP is clearly an important new antimalarial drug. It is well tolerated, highly effective and safe. The higher rates of gametocytaemia compared with other ACTs and lower piperaquine levels early in the terminal elimination phase observed in children suggest that dosage may have to be increased in this important patient group.

Supporting Information

Table S1 (for figure 2): Recurrences (PCR uncorrected) and recrudescences (PCR corrected) comparing the risks in the dihydroartemisinin-piperaquine group versus the comparator arms by drug and country of study. *Overall number of failures does not add up because two comparators were used in Rwanda. Note: the forest plot represents the risk of parasite reappearance (PCR corrected; i.e. recrudescence, and not corrected i.e. recrudescence+novel infection) of DP versus comparators in comparative studies. Groups size are equivalent except in Thailand where the DP group was twice as large (N = 686). Endpoints were assessed on Day 28 in Rwanda, Day 42 in Laos, Myanmar, and Uganda, and Day 63 in Cambodia, and Thailand. Overall results were stratified by site, and drugs. HR: hazard ratio

Found at: doi:10.1371/journal.pone.0006358.s001 (0.07 MB DOC)

Table S2 (for figure 3): Risks of clearing gametocytaemia by Day 14 in patients with gametocytaemia on admission, dihydroartemisinin-piperaquine (DP) group versus comparators arms by drug and country of study. HR: hazard ratio, CI; confidence interval. (NC): not computable because of the day of clearance not available.

Found at: doi:10.1371/journal.pone.0006358.s002 (0.05 MB DOC)

Table S3 (for figure 5): Day 28 adverse event risks for ‘treatment’ DP versus controls [comparators]. Note: The risk of adverse event appearance after the start of dihydroartemisinin-piperaquine treatment in children (<15 y) and adults who did not present this symptom on admission versus comparators in comparative studies. 95% confidence intervals (CI) are calculated for the odds ratio (OR)
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Author Contributions

Conceived and designed the experiments: EAA CK UD FS GD BJ MM PN PS KS NJW FN. Analyzed the data: JZ. Wrote the paper: JZ EAA CK UD FS GD BJ MM PN PS KS NJW FN. Edited the manuscript: JZ.

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