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Research Article

Disposition Kinetics of Plasmodium Falciparum during Treatment with Two Regimens of Chlorpheniramine plus Chloroquine Combination in Acute Uncomplicated Malaria in Children

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Abstract

Background: Chloroquine is receiving a renewed attention in view of the current Corona virus-19 (COVID-19), severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), pandemic. Various options for therapeutic evaluation may be required.

Objective: Evaluation of the kinetics of disposition of Plasmodium falciparum during treatment with chlorpheniramine (CP) plus chloroquine (CQ) in children with acute, uncomplicated malaria and to correlate these with the conventional indices of therapeutic evaluation.

Patients and methods: 106 patients with malaria were randomized to one of two treatment regimens of chlorpheniramine plus chloroquine. Outcome of treatment were assessed using both the conventional fever clearance time (FCT), parasite clearance time (PCT), cure rate and parasite kinetics parameters using the area under the parasite density versus time curve (AUCpd), apparent half time of reduction of parasitaemia (t1/2pd), volume of blood completely cleared of parasite (CLBpd).

Result: The FCT was 1.4 ± 0.7 days and 1.3 ± 0.7 days for the high dose CP plus CQ and the very high dose CP plus CQ groups respectively. The mean PCT was 2.8 ± 0.7 and 2.9 ± 0.7 days, the cure rate was 95.8% and 94.1% respectively.

The parasite kinetics indices area under the parasite density versus time curve (AUCpd), half-lives (t1/2 pd) and volume of blood completely cleared of parasites (CLBpd) were 1.61± 3.28 versus 1.51 ± 2.85 ul h-1 , 3.65 ±1.04 versus 4.00 ± 1.79hr and 0.006 ± 0.005 versus 0.0062 ± 0.0058 ul h-1kg-1 in the high dose CP plus CQ and the very high dose respectively. There was positive and significant correlation between t1/2pd and PC50 r2 = 0.049, p=0.047 and between t1/2 pd and PC90 r2=0.073, p=0.015.

Conclusion: There was a positive and significant correlation between the kinetic and conventional indices suggesting the former may find ready application in therapeutic efficacy monitoring. Surprisingly t1/2 pd correlated more with PC90 than PC50 implying that PC90 may be a better predictor of t1/2pd. This may find application in the evaluation of chloroquine therapy in tCOVID-19. It is novel to employ basic pharmacological principle for clinical application.

INTRODUCTION

Malaria remains the most important parasitic disease afflicting man and the major cause of childhood morbidity and mortality [1]. Chloroquine, 7 chloro 4 (4' diethylamino -1-methylbutylamino) quinolone, is still used for *P. falciparum* despite resistant strains which exist [2], and is currently in focus for the treatment the Corona virus-19 (COVID-19), severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), pandemic [3,4]. Although the World Health Organisation (WHO), has recommended Artemisin-based Combination therapies (ACTs), as the first line anti-malarial drugs [5], the use of ACTs in clinical practice has remained very low due to several limitations including high cost, low public awareness, limited data on safety particularly during pregnancy and insufficient supply relative to demand and lately resistance to artemisinin has been reported [6]. *In vivo* potentiation of chloroquine effect in chloroquine resistant infections has been shown with chlorpheniramine [7,8].

In this study, which is a part of a larger study [9-11], we examined the disposition kinetics of plasmodium falciparum in children with uncomplicated malaria treated with high dose

chlorpheniramine (6-8 mg stat, then 12-18 mg/day X 7days), versus higher dose chlorpheniramine (9-12 mg stat, then 18-24 mg/day X 7days) in combination with high dose chloroquine (30mg/kg body weight). These treatment doses were modified from that used in earlier studies [7,12-14] (Table 1). The basis of the dose modification was a dose ranging/dose escalation of 25% to 50% over the previous doses in order to maximize dose response relationship. Evaluation of therapeutic responses to antimalarial drugs can be and has been determined by conventional indices such as fever clearance time (FCT), various time intervals required to clear specified proportion of patent peripheral parasitaemia which include time to clear 50, 90, 95, or 100% of baseline peripheral parasitaemia (PC50, PC90, PC95, or PCT) [15,16], respectively. Pharmacokinetic principles can be applied to Plasmodium falciparum disposition as a measure of therapeutic responses. It has been proposed that in drug sensitive infections, a constant fraction of peripheral parasitaemia is cleared per unit time [17]. The application of this method in therapeutics has involved using the conventional principles for the disposition of drug to characterize the disposition of parasitaemia during treatment with antimalarial drugs [18]. Indices derived from such applications include area under the parasite density time curve (AUC pd), half-time of reduction of peripheral parasitaemia (t 1/2pd) and the volume of blood completely cleared of peripheral parasitaemia per unit time (CLBpd). These indices have been used to characterize the relative efficacy of antimalarial drugs [18]. The results from such studies give a more accurate characterization of the disposition of parasitaemia than the conventional indices during treatment with antimalarial drugs in children. Earlier studies focused on AUC, t 1/2pd and CLbpd but rarely explored the kinetics of varying times of clearing specified proportions 50, 90, 95% of baseline parasitaemia. There is a need to evaluate the correlation of the disposition kinetics of *P. falciparum* of various time intervals required to clear specified proportion of patent peripheral parasitaemia such as time to clear 50, 90, 95, or 100% of baseline peripheral parasitaemia (PC50, PC90, PC95, or PCT with conventional indices and explore the predictive value for the conventional outcome measures.

This study therefore evaluated the correlation of the kinetics of P. falciparum disposition with the conventional indices of therapeutics during malaria treatment and assessed the predictive value for treatment outcome in children with acute, uncomplicated malaria. It is hoped that this may find an application in the evaluation of the therapy in the current COVID-19 pandemic which therapeutic evaluation is changing [19].

MATERIALS AND METHOD

The study was carried out at the Clinical Pharmacology Department of the University College Hospital Ibadan, Nigeria. The patients were selected from those attending the General out Patient Clinic of the hospital.

The study protocol was approved by the Joint UCH/College of Medicine Ethical Committee. A total of 106 patients aged 6 months to 14 years were enrolled into the study after obtaining a written informed consent from their parents/guardians.

The inclusion criteria were as follows: Children aged 6 months to 14 years, fever or history of fever in the 24-48 hours before presentation; symptoms compatible with acute uncomplicated falciparum malaria; pure Plasmodium falciparum parasitaemia with parasite density >1000 asexual forms/µl of blood and absence of concomitant illness such as bronchopneumonia.

Children who satisfied the inclusion criteria were enrolled into the study. A detailed history was taken with a thorough physical examination. Body weight, height and axillary temperature were recorded. The clinical data were entered into a special Case Record Form. Thick and Thin blood films from finger prick were obtained and Giemsa stained for identification and quantification of parasites. Parasitaemia were quantified in thick film by counting parasites relative to leukocytes. At least 500 leukocytes were counted. The count was then converted to parasite per microlitre of blood using a simple formula.

Number of parasites X Total white cell count/µl = Parasite density

Number of leukocytes

Patients were randomized to receive either of two treatment regimens, using a pre-packed random numbers that were sealed. Each envelope was opened only after a patient had been recruited.

Thick and thin blood films were prepared by collecting the capillary blood from a finger prick after thorough cleaning with methylated spirit. The blood was collected on clean glass slides, a drop for the thin film and two or three separate drops for the thick film. A spreader was used to complete the preparation of the thin film before fixing with methanol, while thick films were air dried, before staining with 20% Giemsa stain. Conventional evaluation of the outcomes of treatment were assessed in terms of clinical and parasitological responses to treatment using the fever clearance time, and time to clear 50 or 90% of baseline parasitaemia, the parasite clearance time and cure rate.

The fever clearance time was defined as time from drug administration until the axillary temperature was below 37.50C and remained so for at least 72 hours. This definition was necessary because of the routine use of paracetamol during the first 36 hours of treatment. The parasite clearance time was defined as the time from drug administration until there was no patent peripheral parasitaemia. Parasite clearance was also assessed using the Parasite clearance50 (PC50), the time for the parasite count to fall by 50% of the enrolment pre-treatment value. Assuming that the baseline parasitaemia was 100%, the 50% was extrapolated using a semi-log graph plot. Similarly the time for the parasite count to fall by 90% of its initial baseline value was assessed -this is the parasite clearance 90 (PC90). The cure rate was defined as the proportion of children who remained free of parasitaemia on day 14 of follow-up. All treatment failures were re-treated with the initial drug regimen on day 14 provided they were not symptomatic before then. They were similarly treated if they became symptomatic between day 7 and day 14. Patients who developed severe symptoms of malaria such as altered level of consciousness, convulsions or oral fluid intolerance were commenced on intramuscular injection of artemether (3.2mg/kg body weight) and referred to the children emergency ward for further management.

Evaluation of the kinetics of disposition of Plasmodium falciparum during treatment: The parasite kinetics parameters were estimated from the parasite densities (parasite concentrations) by non-compartment method using the computer program Turbo ken (Clinical pharmacology Group, University of Southampton, U.K., Courtesy Prof. A.G. Renwick). The areas under the parasite density versus time curve (AUCpd) were obtained by linear trapezoidal rule from time zero to the time of parasite clearance or otherwise. In drug sensitive infection, the final parasite density at time of clearance was assumed to be 1x10-3 asexual forms ul-1 blood; a level below microscopic detection. The apparent terminal elimination rate constant, (z) were obtained by least square regression analysis and the apparent half-time of reduction of parasitaemia (t1/2pd) was obtained from ln/z. The volume of blood completely cleared of parasites (CLBpd) was calculated as parasite density at enrolment/ AUCpd [18].

Statistical Analysis

The data were entered into a specially prepared case record form. They were then entered into the computer and analyzed using the Epi-Info version 6 [20]. The mean and standard deviation of normally distributed, continuous data were compared using student's t-test. Proportions were compared by calculating the chi-square with Yate's correction or by Fisher's exact test. Kruskal-Wallis and Mann-Whitney U tests were used to compare data that were not normally distributed. Relationship between two sets of parameters was done by regression analysis. Numerical values are expressed as means ± standard deviations. Differences were deemed to be significant statistically where P< 0.05

RESULTS

During the study period, a total of 737 patients presented with symptoms suggestive of malaria. Of these, peripheral blood film was positive for malaria parasite in 385, a parasite rate of 52%. Of the 385 children with parasitaemia, 106 patients were studied. Fifty-three were allotted to the high dose chlorpheniramine in combination with chloroquine, and 53 to the very high dose chlorpheniramine in combination with chloroquine group. Seven patients were withdrawn, four due to default of follow up on days 1 or 2, one defaulted on day 5 (parasitaemia cleared in this patients on day 2), and one each other for study protocol violation and development of severe / complicated malaria. Ninety-nine children completed the study and were evaluated. Forty-eight of these children were in the high dose chlorpheniramine plus chloroquine group, while 51 were in the very high dose chlorpheniramine plus chloroquine group. There were 39 males and 60 females. Fifteen males and 33 females were in the high dose chlorpheniramine plus chloroquine group while 24 males and 27 females were in the very high dose chlorpheniramine plus chloroquine group.

The mean age of the children in the high dose group was 6.1 ± 2.9 years (range 0.8-12 years) and in the very high dose 6.1 ± 3.2 years (range 1.5-13.5 years).

The fever clearance time was 1.4 ± 0.7 days (range 1-3 days) and 1.3 ± 0.7 days (range 1-4 days) for the high dose chlorpheniramine plus chloroquine and the very high dose chlorpheniramine plus chloroquine groups, respectively. There was no statistical difference between them. The mean parasite clearance time was 2.8 ± 0.7 days (range 1-5 days) and 2.9 ± 0.7 days (range 2-4 days) for the high dose chlorpheniramine plus chloroquine group and the very high dose chlorpheniramine plus chloroquine group, respectively. The cure rate on day 14 in the high dose chlorpheniramine plus chloroquine group was 95.8% and in the very high dose chlorpheniramine plus chloroquine group, 94.1%. There was no statistical significant difference in the cure rate.

Plasmodium falciparum kinetics during treatment

The t1/2pd was 3.65 ± 1.04 hour in the high dose chlorpheniramine plus chloroquine group and 4.0 ± 1.79 hour in the very high dose chlorpheniramine plus chloroquine group. There was positive and significant correlation between t1/2pd and PC50 in the same patients (regression coefficient r2 =0.049, p= 0.047), Figure 1. Figure 2 shows the relationship between t 1/2pd and PC90 in the same patients. As in the relationship between t1/2pd and PC50, though surprisingly more so, there was a positive correlation between these two sets of parameters (regression coefficient r2 = 0.073, p = 0.015). Figure 3 shows the relationship between the PC50 and the PC90 in the same patient, regression coefficient r2 = 0.802, p < 0.0001 (Table 1).

DISCUSSION

In this study a little more than half (52%), of the patients presenting with symptoms of malaria had microscopically demonstrated parasiteamia on thick blood film. This underscores the need to confirm the diagnosis of malaria to prevent undue exposure to antimalarial drugs in patients who would otherwise have been presumptively diagnosed as having malaria. This will further mitigate the risk of drug resistance which may be a consequence of unnecessary exposure to antimalarial drugs. The measures of therapeutic responses in the children enrolled in the studies were similar in the two treatment groups.

The application of the conventional principle for characterising the disposition of drugs to characterize the disposition of parasitaemia during treatment with antimalarial drugs in children is novel [15]. Its further application for the

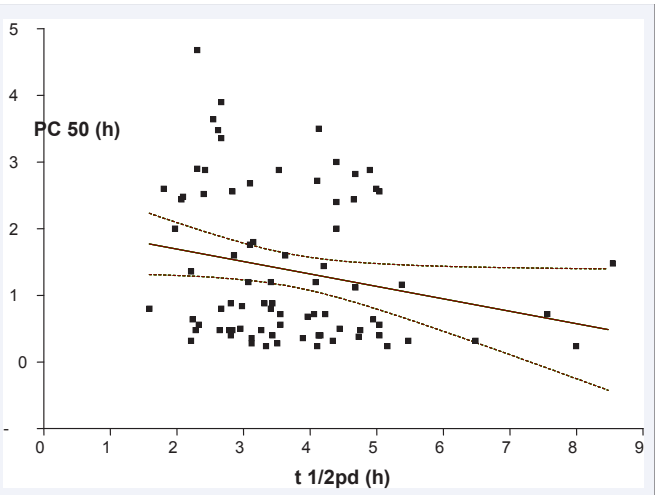

Figure 1 Relationship between T 1/2pd and PC 50.

Table 1: Dose ranging/dose escalation in studies of children with acute uncomplicated falciparum malaria treated with chlorpheniramine plus chloroquine.

Study	Chloroquine dose	Chlorpheniramine dose	Cure rate on day 14 (%)
Sowunmi et al., 1997 [5]	Chloroquine base 25mg/kg over 3 days	≤ 5 yr.: 10 mg in first 24 hr, then 6 mg dly for 6 days; >5yr.: 20mg in first 24 hr, 12mg dly for 6d	85.4
Sowunmi et al., 1998 [10]	Chloroquine base 25 mg/kg over 3 days	≤ 5 yr.: 18 mg in first 24 hr, 12 mg dly for 6 days; >5yr.: 26mg in first 24 hr, 18mg dly for 6d	97.9
Sowunmi et al., 1998 [11]	Chloroquine base 25 mg/kg over 3 days	≤ 5 yr.: 18 mg in first 24 hr, 12 mg dly for 6 days; > 5yr.: 26mg in first 24hr, 18mg dly for 6d	96
Sowunmi et al., 1998 [12]	Chloroquine base 30 mg/kg over 3 days	≤ 5 yr.: 18 mg in first 24 hr, 12 mg dly for 6 days; > 5yr.: 26mg in first 24hr, 18mg dly for 6d	96
Sowunmi et al., 2000 [8]	Chloroquine base 30 mg/kg over 3 days	≤ 5 yr.: 18 mg in first 24 hr, 12 mg dly for 6 days; > 5yr.: 26mg in first 24hr, 18mg dly for 6d And ≤ 5 yr.: 20 mg in first 24 hr, 18 mg dly for 6 days; > 5yr.: 28mg in first 24hr, 24mg dly for 6d	96 94

comparisons of therapeutic efficacy of antimalarial drugs, kinetics of *Plasmodium falciparum* sex ratios and malaria associated anaemia is interesting [18,21,22]. In the present study, those kinetic parameters, namely AUCpd, t1/2pd and CLBpd were similar in the two treatments groups. In addition, there was a positive and significant correlation between the kinetic indices and the conventional indices such as PC50 and PC90 and PCT: t1/2pd ratio. Surprisingly, in this study t1/2 pd correlated more with PC90 than PC50 implying that PC90 may be a better predictor of t1/2pd. The significant correlation suggests that the kinetic indices may find ready applications in therapeutic efficacy monitoring [23]. Ideally, the kinetics of the disposition of parasitaemia during treatment with antimalarial drugs should begin from the time of mosquito-injected sporozoites till the complete clearance of parasitaemia or otherwise. However, in the sick patient kinetic analysis can only begin at presentation and hence during the commencement of drugs and after therapy. In this regards, the kinetic analysis are analogous to those following intravenous bolus injection of drugs. This constraint should encourage the integration of kinetic disposition of both the drugs administered for the treatment of the infection and the kinetic disposition of the parasite during treatment of the infections.

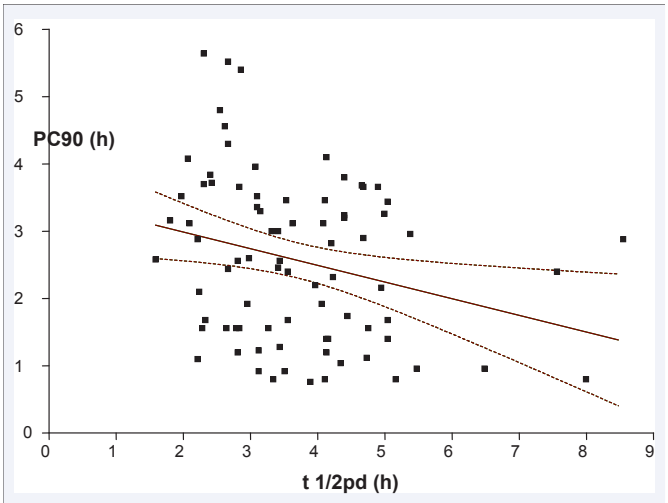

Figure 2 Relationship between t1/2pd and PC90.

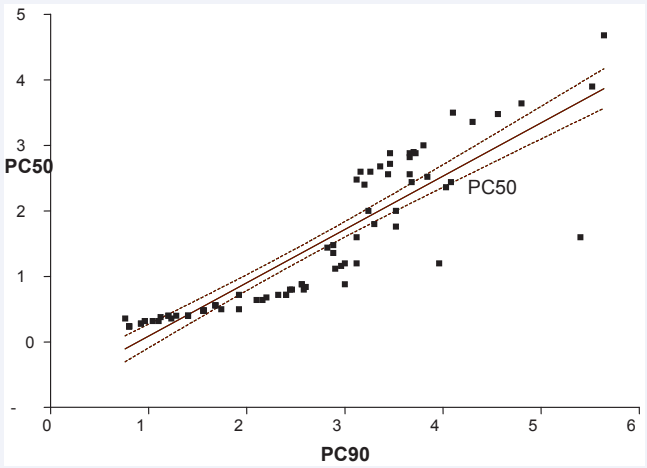

Figure 3 Relationship between PC90 and PC50.

The blood film for parasite density determination and kinetic analysis were taken at 24 h interval a more frequent sample say at 4, 6, 8 or 12 h interval should yield a similar results. In the children studied, ethical considerations prevented more frequent sampling. In addition, treatment was out patient. A model-independent programme used to analyze the kinetic parameters models to describe disposition of parasitaemia may be mono or multi-compartment. Since elimination of circulating parasites from the body is by the spleen, and since parasite sequestration and mobility occur during natural infection, both model find useful application. The use of a model independent programme has the advantage of avoiding the limitations of mono- or multi-compartment models [18].

CONCLUSION AND RECOMMENDATION

Principles of kinetics has not only been applied to Plasmodium falciparum but also to clinical characteristics associated with malaria [18,23]. In view of the t1/2 pd correlating more with PC90 than PC50 it may imply that PC90 may be a better predictor of t1/2pd. It is therefore suggested that future studies should be carried out for the integration of kinetic disposition of both antimalarial drugs and plasmodium falciparum simultaneously.

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