CHLOROQUINE-CHLORPHENIRAMINE INTERACTION IN HUMAN MALARIA

C.A. Okonkwo[1], H.A.B. Coker[2], P. U. Agomo[1], C.O Agomo[1], R. Anyanwu[3], V. N. Asianya[1], S. K. Akindele[1].

[1] Biochemistry Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria.
[2] School of Pharmacy, College of Medicine of the University of Lagos, Nigeria.
[3] Emzor Pharmaceutical Industries, Lagos, Nigeria.

Correspondence: H.A.B. Coker

ABSTRACT

The purpose fo this study was to examine the effect of chloroquine-chlorpheniramine (CQ-CP) combination therapy on the efficacy and disposition of chloroquine (CQ) in acute uncomplicated malaria. A 3-day standard treatment with 25 mg CQ base per kilogram body weight alone or in combination with chlorpheniramine (CP) was orally administered to 17 semi-immune Nigerian children with *Plasmodium falciparum* parasitemia, attending the Massey Street Children's Hospital , Lagos, Nigeria. Parasitemia was determined on thick blood films stained with Giemsa,and treatment failures were established following the WHO classification for CQ resistance. Whole blood CQ concentrations were monitored at pre-determined intervals during the 28 days of follow-up using blood dried on filter-paper. Treatment with CQ-CP combination resulted in a shorter parasite clearance time (2.0+ 0.5 d) and a higher cure rate (87.5%) compared to treatment with CQ alone (3.5+ 0.5 d; 66.7%). CQ pharmacokinetic parameters: maximum drug concentration (C_{max}) and the area under the first-moment drug-concentration-time curve (AUMC) were significantly increased ($p< 0.01$; $p< 0.001$ respectively) by CP administration while the time to achieve the peak was reduced in the presence of CP. We conclude that adminstration of CP increased CQ uptake as judged by an increase in the maximum concentration (C_{max}), and a decrease in the time to attain the concentration (T_{max}), as well as an increase in the area under the curve, which signifies increased systemic availability of CQ in the presence of CP.

INTRODUCTION:

Chloroquine (CQ), an antimalarial drug of the 4-aminoquinoline series, has since the 1940s remained the drug of choice for the treatment of acute attacks of malaria and also for prophylaxis in most parts of Africa (1) It is the first-line drug in the treatment of acute uncomplicated falciparum malaria in Nigeria today. The emergence of CQ-resistant strains of P. falciparum has, however, limited the clinical efficacy of this valuable antimalarial drug. (2)

A number of studies have been focused on development of combination therapy based on various phenomena, including the reversal of resistence by non-antimalarial drugs (3,4) Chlorpheniramine (CP), a histamine H_1-receptor antagonist, has been shown to reverse chloroquine resistance in vitro in isolates from some African countries and (5) has been reported recently to enhance the efficacy of CQ in vivo in acute uncomplicated falciparum malaria. (6,7) CP is a widely used antihistaminic agent (H_1-receptor blocker) and is commonly prescribed with CQ to alleviate CQ-induced pruritus in malarial children and adults in Nigeria. The combination was not indicated for drug-resistant malaria but its use has become common practice among patients in Nigeria. Concomitant administration of two or more drugs to humans often led to drug interactions which may have profound effects on drug distribution and hence concentration in patients. This study examines the resultant effect of CQ-CP combination therapy on the efficacy and disposition of CQ in acute uncomplicated falciparum malaria.

Furthermore, there are no local studies on the analysis of CQ in filter paper-absorbed blood. Thus, we decided to analyze CQ in filter paper-absorbed whole blood during treatment of Nigeria children with malaria infection, using a specific high performance liquid chromatography (HPLC) method.

SUBJECT AND METHODS:

This study took place at the Massey Street Children's

Hospital, Lagos Island, Nigeria; between July and October 1997. The study purpose and procedures were carefully explained to the parent of each child and they gave their consent voluntarily. Patients were enrolled for the study if they fulfilled the selection criteria for the standard World Health Organization in vivo studies. (8). Extended 28 day tests for sensitivity in vivo were then performed according to the WHO procedures. Patients were enrolled into the study based on the following criteria: children aged 5-12 years, history of fever in the 24-48h preceding presentation at the hospital, presence of asexual stages of *P. falciparum* at a parasite density ≥500/uL of blood, no history of antimalarial drug intake in the 2 weeks preceding presentation, and fully informed consent of parent or guardian. Children with any other disease in association with malaria and those with severe illness requiring parenteral therapy were excluded. Also excluded were children with high pyrexia (temp. ≥ 41°C). A child was withdrawn from the study if a concomitant illness developed during the follow-up, if the patient or guardian desired withdrawal, or if there was failure to comply with the study protocol. The enrolled subjects were studied prospectively. This study was approved by the ethical committee of the Nigeria Institute of Medical Research, Yaba, Lagos.

DRUG ADMINISTRATION AND SUBJECT MANAGEMENT.

Chloroquine (CQ) tablets (150 mg base) and Chlorpheniramine (CP)tablets (4mg base) were kindly supplied by Emzor Pharmaceutical Industries (Lagos, Nigeria).

The children were randomly allotted to one of 2 treatments. Each child received a total of approximately 25mg CQ base/kg bodyweight (BW) over 3 days in the following schedule -10 mg base/ kg BW on the first and second days (D 0 and 1) and 5 mg/kg BW on the third day (day 2) according to the WHO treatment schedule. A non-enteric coated phosphate brand was used. For group 2, chlorpheniramine (8mg at presentation, followed by 4 mg every 8 h for 7d, days 0-6) was given concomitantly to each child. this dosing regimen was reported (6,9).

Sample Collection, Determination of Parasitemia And Hematocrit Values

Blood samples for drug analysis were obtained from each subject by finger prick using autolet II-UNILET platforms (0wen Mumford Limited, England) and applied onto filter papers (Whatman No1) in triplicates. The samples were collected on fourteen occasions: immediately before the first treatment dose (0h) and 0.5, 1,1.5, 2, 2.5, 3, and 24 h later, then on day 2,3,7,14,21, and 28 post-treatment. On recruitment and each follow-up, thick and thin blood films were prepared by finger prick and examined for malaria parasites by light - microscopy. All patients had acute *P. falciparum* malaria which were diagnosed by finding the early trophozoites of the parasites in peripheral film. At each follow-up visit, the parents or guardian (and the child) were questioned and the child examined for the presence of adverse drug reactions.

Parasitemia was quantified by counting the number of parasites relative to leukocytes in thick blood films. Giemsa-stained blood films were examined under a x100 oil-immersion objective and10 eye piece of a light microscope. The number of asexual forms of *P. falciparum* correponding to 200 leukocytes were counted and parasite density calculated assuming a WBC density of 6000 cells/mm^3 of blood. The count on the first day, pre-treatment (D o) for each patient was taken as 100% and the count on subsequent days were expressed as percentages of this figure. The parasite species was confirmed by examination of the thin film. The microscopist was blind to the treatments given. Hematocrit values were determined by a microcapillary method.

HANDING OF SAMPLES AND DRUG ANALYSIS

The filter paper blood spottings were dried under room temperature (away from direct sunlight), then sealed tightly in self -sealing plastic envelopes and stored in the 4°C Compartment of a refrigerator until analyzed. Just before drug analysis , the plastic bags were removed removed from the refrigerator to a desiccator, and one spot of each sampling cut off for the assay. Strict routines were followed in order to avoid CQ contamination (10).

Whole blood CQ concentration was determined by the diethylether extraction method (11) with slight modifications. To extract CQ from whole blood (filter paper)

the paper was cut into pieces and processed. Papaverine (20*ul* of 10 ug/ml i.e, 200 ng) was added as internal standard; made alkaline with 100*ul* of 2 M sodium hydroxide solution and then extracted with of diethylether. The tubes were whirlmixed in a vortex-mixer for 1^1/2 minutes, centrifuged (10 minutes, 1000g) to clarify the organic phase, and the ethereal layer transferred into fresh dry tubes to which was added 200 ul of 0.1 N HCL (1.8 sp. gr.). The mixture was whirlmixed for another 1^1/2 min, re-centrifuged (10 min, 1000 g) & the ethereal layer discarded while a 25 ul of the aqueous phase was injected into the HPLC system for chromatographic separation.

Quantitation: For each analysis, a standard curve was generated by adding known, varying amounts of chloroquine base to whole blood and dispensing an aliquot of each serial dilution on filter paper to obtain a concentration range of 0 to 3000 ng/ml. The filter paper blood spottings from spicked samples were dried and treated as the unknown test samples to evaluate the accuracy and precision of the method. Linear calibration curves were obtained in this range with correlation coefficients $\geq$ 0.99. Quatitation was achieved using the peak height ratio of CQ to papaverine.

Pharmacokinetic Analysis

CQ concentration-time profiles were obtained by a non-compartmental analysis of data. The maximum concentration (C_{max}) and the time to reach this concentration (T_{max}) were noted directly from concentration-time data. The area under the first-moment drug concentration-time curve (AUMC) was calculated by the trapezoidal rule for observed values.

Statistic Analysis: Estimates of the pharmacokinetic parameters from the 2 treatments were compared using Student's t-test for unpaired observations, and accepting $p \leq 0.05$ as significant.

RESULTS

P. falciparum was the only species indentified in all the patients except 1 patient (CP8) in whom there was a mixed intection of *P. falciparum* and *P.ovale*. Post-treament, the parasite clearance time (PCT) for the CQ group was recorded as 3.5 $\pm$ 0.5 days while the CQ-CP group had a PCT of 2.0 $\pm$ 0.5 days . The parasitological cure rates recorded were 66.7% for CQ and 87.5% for CQ-CP treatmnet (Tables

TABLE 1a: TREATMENT OUTCOME AND SENSITIVITY PROFILE IN PATIENTS WITH ACUTE UNCOMPLICATED MALARIA TREATED WITH CQ ALONE

PATENT STUDY NO.	TREATMENT GROUP	PARASITE DENSITY, (per ul blood)								RESPONSE CODE
		D0	D1	D2	D3	D7	D14	D21	D28	
CQ1 AM 97	CQ	43,789 (100%)	641 (2.2)	475 (1.1)	33 (0.1)	-	-	-	-	S
CQ3 IQ 97	CQ	10,789 (100%)	1,925 (17.8)	N. D.	-	-	35 589+ (329.9)	297 (2.8)	-	RI
CQ4 SS 97	CQ	9,365 (100%)	N. D.	N. D.	271 (2.9)	204 (2.2)	174 (1.9)	-	-	RII
CQ12 NI 97	CQ	5,886 (100%)	827 (14.1)	570 (9.7)	45 (0.8)	-	-	-	-	S
CQ13 AK 97	CQ	48,176 (100%)	40,170 (83.4)	2,719 (5.6)	-	-	-	-	-	S
CQ14 SO 97	CQ	5,084 (100%)	1,220 (20.7)	420 (0.1)	-	-	-	-	-	S
CQ17 AS 97	CQ	512 (100%)	388 (75.8)	350 (68.4)	98 (19.1)	-	-	-	-	S
CQ21 TO 97	CQ	2,640 (100%)	1,260 (47.7)	610 (23.1)	-	-	1,880 (71.2)	160 (6.4)	-	RI
CQ22 BA 97	CQ	6,854 (100%)	1,680 (24.5)	220 (3.2)	-	-	-	-	-	S

Values in parenthesis are percentage parasite density relative to the pre-treatment value.

TABLE 1b: TREATMENT OUTCOME AND SENSITIVITY PROFILE IN PATIENTS WITH ACUTE UNCOMPLICATED MALARIA TREATED WITH A COMBINATION OF CQ-CP.

PATENT STUDY NO	TREATMENT GROUP	PARASITE DENSITY, (per ul blood)								RESPONSE CODE
		D0	D1	D2	D3	D7	D14	D21	D28	
CP1 OK 97	CQ+CP	2,563 (100%)	340 (13.3)	-	-	-	-	-	-	S
CP2 AA 97	CQ+CP	21,327 (100%)	14,129 (66.3)	N.D.	-	-	8,280+ (85.7)	2,406 (11.3)	-	RI
CP5 OM 97	CQ+CP	640 (100%)	-	-	-	-	-	-	-	S
*CP6 OS 97	CQ+CP	28,976 (100%)	7,540 (26.0)	20 (0.1)	-	-	-	-	-	S
CP7 AA 97	CQ+CP	26,661 (100%)	8,943 (33.5)	34 (0.1)	-	-	-	-	-	S
CP9 IO 97	CQ+CP	1,911 (100%)	69 (3.6)	-	-	-	-	-	-	S
*CP10 DO 97	CQ+CP	4,860 (100%)	180 (3.7)	-	-	-	-	-	-	S
CP16 DD 97	CQ+CP	26,480 (100%)	670 (2.5)	120 (0.5)	-	-	-	160 (6.4)	-	S

*: Cases with history of consistent relapse after previous treatment with CQ.

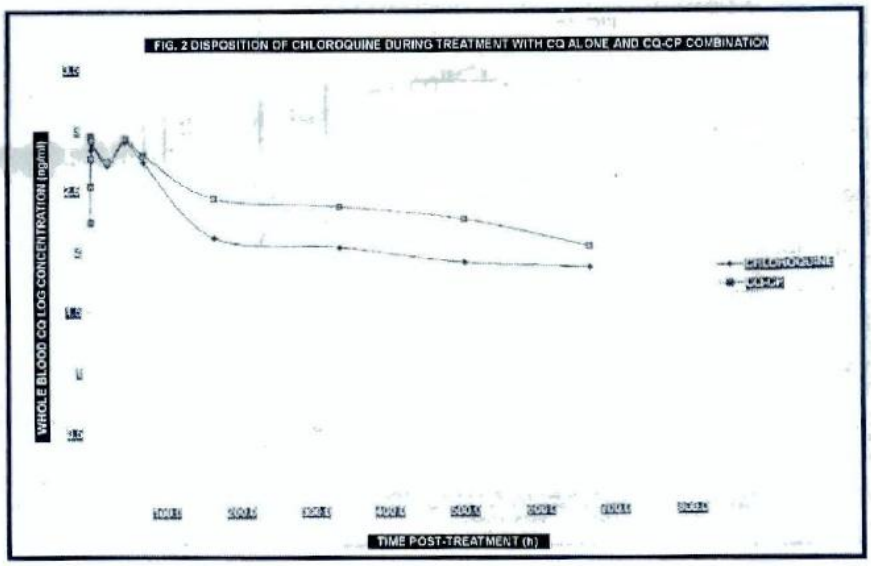
PARASITE CLEARANCE TIME: CQ - 3.5 $\pm$ 0.5 d
CQ - CP - 2.0 $\pm$ 0.5 d

CURE RATE (%): CQ Treatment 66.7%
CQ-CP Treatment..... 87.5%

28 d follow-up period (1) (WHO, 1994).

Patient parasitemia on D 14 after initial clearance on D7 were regarded as recrudescence. CQ-CP combination had a higher parasitologic cure rate (87.5%) than CQ alone (66.7%) and a shorter parasite clearance time with a mean value of (2.0±5d). This shows that CQ-CP combination has a faster clearance rate than CQ alone.

Of particular interest were 2 cases (CQ-CP6 & CQ-CP10) with history of consistent relapse after previous CQ adminstration who were sucessfully treated with the CQ-CP combination, with no recurrence of parasitemia during the 28 d follow-up.
Under the conditions of this study, CP appeared to increase CQ uptake and accumulation as judged by an increase in the maximum concentration, C_{max} a decrease in the time to attain this concentration, T_{max} and an increase in the AUMC. This study further provides more evidence on the enhanced efficacy of CQ-CP combination for the treatment of acute *P. falciparum* malaria infection. This is a preliminary study on the pharmacokinetic disposition of CQ when co-administered with CP in acute uncomplicated falciparum malaria infection in Nigerian children in Lagos and the data presented should provide more basis for the use of CQ-CP combination for the treatment of acute uncomplicated falciparum malaria infection. The filter paper blood spotting method for drug analysis described is a great advance over other techniques which require a large volume of blood. While most children and adults would readily allow a finger prick for kinetic blood sampling, only few people would allow venepuncture.



REFERENCES.

1. WHO (1994). Antimalarial drug policies: Data requirements treatment of uncomplicated malarial and management of malaria in pregnancy. Geneva : World Health Organization, mimeographed document WHO/MAL/94. 1070.

2. Moore DV, Lanier JE (1961). Observation of two *P. falciparum* infections with abnormal response to chloroquine, AM. J, Trop. Med. Hyg., 10, 5.

3. Martin SK, Oduola AMJ, Milhous WK (1987). Reversal of chloroquine resistance in *P. falciparum* by verapamil. Science , 235, 899-901.

4. Peters W, Ekong R, Robinson BL, Warhust DC (1989). Antihistaminic drugs that reverse chloroquine resistence in *P. falciparum* . Lancet , ii, 334-5.

5. Basco LK, Le Bras J (1994). In Vitro reversal of CQ resistance with chlorpheniramine against African isolates of P.falciparum Japanese J. Med. Sci. Bio. 47(1), 59-63.

6. Sowunmi A, Oduola AMJ, Ogundahunsi OAT, Falade CO, Gbotosho GO, Salako LA (1997). Enhanced efficacy of chloroquine chloropheniramine in acute uncomplicated Fulciparum malaria in children.

7. Okonkwo CA, Coker HAB, Agomo PU et al. Effect of chlorpheniramine on the pharmacokinetics of and reponse to chloroquine of Nigerian children with falciparum malarial. Trans. Roy. Soc Trop. Med. Hyg. Accepted for publication12 January 1999, In press.

8. WHO (1973).Chemotherapy of malaria and resistence to antimalarials. Geneva: WHO Technical Report Series, no.529 .

9. Simons FER, Luciuk GH,Simons KJ (1982). Pharmacokinetics and efficacy of chlorpheniramine in children. J. Allergy Clin Immunol. 69, 376-81.

10. Bergqvist Y, Ericsson O, Rais M (1986). Determination of chloroquine in dried blood spots on filter-paper. Importance of sample handling. Ther. Drug Monit.,8,211-3

11. Alvan G, Ekaman L, Lindstrom B (1982). Determination of chloroquine and its desethyl-metabolite in plasma, red cell and urine by liquid chromatography. J. Chromatogr., 229,241-7.

12. Bruce-Chwatt LJ (1981). Drug resistence in malaria. In chemotherapy of malaria, 2nd d., Geneva: WHO p. 102-118.