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3-HMG-Coa Reductase Inhibitor Modulates Parasitological Response in Malaria Patients Treated with Amodiaquine

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ABSTRACT

The occurrence of parasitological resistance to amodiaquine has been reported globally. The 3-HMG CoA reductase inhibitors, otherwise known as statins have been shown to inhibit the growth of malaria parasite.This study was aimed at evaluating the effects of simvastatin in modulating parasitological response to amodiaquine in the chemotherapy of malaria. Subjects with frank malaria (n=60) diagnosed by thick blood film and and confirmed using immunological tests were nominated for the study. Informed written content was obtained and subjects randomized into amodiaquine plus simvastatin (test) and amodiaquine alone (control) groups. The ethical clearance certificate was obtained from the University of Nigeria Teaching Hospital Research Ethics Committee (NHREC/05/01/2008B). The assessment of parasitological response was done in line with WHO criteria and patients followed up on days D0, D3, D7, D14 and D28 post-treatment. The GraphPad Prism 4.0 was employed in the analysis of data which was presented as tables and graphs. Revealed a statistically significant difference in parasitological response ($p<0.05$) between test and control groups. The mean value of low level resistance, RI was given as 1.3±0.14%, mid-level resistance, RII as 2.7±0.15%, high level parasitological resistance, RIII as 2.4±0.17% and the late parasitological failure, LPF as 3.3±0.26% in the test group. This contrasts with the value of RI given as 8.7±0.42%, RII as 12.8±0.49%, RIII as 4.6±0.17% and the LPF given as 6.7±0.21% in the control group. The outcome of this study suggests that the 3-HMG-CoA reductase inhibitor, simvastatin, is implicated in modulating parasitological response to amodiaquine in the chemotherapy of malaria.

Key words: Amodiaquine, Falciparum malaria, HMG-CoA reductase inhibitor, Parasitological failure, Parasitological resistance, Simvastatin.

INTRODUCTION

A review recommended the continued use of amodiaquine in the treatment of uncomplicated malaria, stressing the need to take into consideration local drug resistance patterns[1]. The occurrence of parasitological resistance to amodiaquine has been reported across the globe[2-4]. Cross resistance to antimalarial drugs may derive from single nucleotide polymorphisms (SNPs) in the Pfmdr and Pfcrt genes of *Plasmodium falciparum*. The Pfmdr1 86Y and haplotypes at Pfcrt 72-76 have been lnked to amodiaquine resistance. A study observed rapid but steady percent increase in wild-type parasites with regard to both Pfmdr1 and Pfcrt pointing to a significant change in parasite response[5]. The 3-HMG CoA reductase inhibitors, otherwise known as statins are lipid lowering agents and their clinical benefits could be related to reduction in anti-inflammatory responses. Statins have been shown to regulate inflammatory cell adhesion and endothelial function. Statins prevent lipopolysaccharide (LPS)-induced intracellular adhesion molecule-1 (ICAM-1) expression in endothelial cells via inhibition of Rho activity[6]. Statins have been reported to inhibit growth of *Plasmodium.falciparum in vitro*[7,8]. We hypothesize that a statistically significant difference ($p<0.05$) exists in parasitological response between simvastatin treated subjects in combination with

amodiaquine and subjects treated with amodiaquine alone.

MATERIALS AND METHODS

Subjects

Subjects with acute malaria (n=60) in attendance at eight primary health facilities were selected for this study. Malaria infection was diagnosed using thick blood films and confirmed by immunological test (Paracheck PI®) . Paracheck PI®, a rapid qualitative two site sandwich immunochromatographic dipstick assay, was employed for the determination of *Plasmodium falciparum* specific histidine rich protein-2 (PfHRP-2) in whole blood samples. This was in view of the fact that the classical method of diagnosis by microscopy involving examination of thin and thick blood smears was prone to false negative readings and time consuming.

Study Design

Informed consent was obtained after adequate explanation of the purpose of study, formal written documentation , type of treatment to be administered and clarification of any likely adverse effects or complication that may arise in the course of treatment. Patients enrolled for this study were within the age range 16 to 65 years inclusive, in attendance at eight primary health facilities within Asu Nkanu Local Health Authority in Nkanu East Local Government Area of Enugu State, Nigeria. Routine clinical clerkship and examination including body weight measurement and axillary temperature were carried out to confirm the enrollee's physical condition and ascertain presence of any confounding ailment. Subjects were randomised into test and control groups using a table of random numbers statistically generated. No member of the research team including the principal investigator, microscopist, field supervisor, field assistants, medical officer and nurses involved in the study had any prior knowledge of the patients' medical records nor the treatment group to which any enrollee was assigned. The ethical clearance certification was given by the Institutional Research Ethics Review Committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Nigeria (Ref: NHREC/05/01/2008B) in line with principles guiding human experimentation as enumerated in the Declaration of Helsinki by the World Medical Association General Assembly as last amended (Seoul 2008); while approval for the study was obtained from Enugu State Ministry of Health, Enugu-Nigeria. Amodiaquine (*Camoquin*® from Pfizer West-Africa, Dakar-Nigeria) was given as 15mg/kg at initial presentation D0, then 10mg/kg daily for D1 and D2. Simvastatin (*Simvor*® from Ranbaxy Laboratories, Dewas-India) was given orally in the dosage 0.6mg/kg/d only in the evening for 3 consecutive days. The control group received Amodiaquine only in same dose as test group. Artemether-Lumefantrine (*Coartem*® from Novartis Pharma AG, Basel-Switzerland) was used to salvage subjects who presented with recrudescence or parasitological failure and eventually withdrawn from the study. The Artemether component was given as 3.2mg/kg/d while the Lumefantrine as 19.2 mg/kg/d respectively in two divided doses for 3 days. Baseline monitoring of liver function tests was done before commencement and in the course of therapy. The discontinuation of simvastatin would be indicated following elevation of serum transaminase activity up to three times normal level.

Assessment of Response

The patients were followed up on days D0, D3, D7, D14 and D28. The World Health Organisation (WHO) criteria were applied in the categorization of parasitological response. Parasitological response is classified as low to high level parasitological resistance (RI, RII, RIII) and defined as:

- *High level resistance III (RIII)* is parasitemia on day 3, D3 higher or 25% of parasitemia on D0.
- *Mid-level resistance II (RII)* is parasitemia on day 3, D3 d" 25% of parasitemia on D0; but positive parasitemia between D4 and D7.
- *Low level resistance I (RI)* is a negative blood smear on day 3, D3 and a positive blood smear on any day between D7 and D14.

Statistical Analysis: Graphpad Prism version 4.0 (GraphPad Software, Inc., La Jolla, CA, USA) statistical software was employed and data presented in the form of tables and graph. Test of significance statistically determined using two-

tailed Student *t*-test, at 95% confidence interval, p<0.05 considered significant.

RESULTS

Table 1 depicts the baseline characteristics of subjects in the test and control groups at presentation. A statistically significant difference (p<0.05) in the low, mid and high level parasitological resistance (RI, RII, RIII) between the test and control groups was depicted in Table 2 and Figure 1. A statistically significant difference (p<0.05) in late parasitological failure was also reported between the test and control groups as depicted in Table 2.

DISCUSSION

The current study revealed statistically significant difference (p<0.05) in the cumulative low to high level parasitological resistance (RI + RII + RIII) and late parasitological failure as depicted in Table 2 and Figure 1. Hence, the consideration of late parasitological failure (LPF) alongside sums up to an overall parasitological resistance of 9.7% and 32.8% in the test and control groups respectively. The underlying resistant mechanism to amodiaquine could be related to accumulation within the infected parasite to high levels. This highly specific accumulation causes significant drug depletion from sensitivity assay plates, leading to an under-estimation of drug activity (the innoculum effect). The net effect is to under-estimate the differences in the amodiaquine dose-response of resistant isolates. The accumulation-related resistance to amodiaquine is totally insensitive to the effects of verapamil. The ability of the classic reverser of multi-drug resistance, verapamil to increase chemo-sensitivity to chloroquine in resistant isolates of *Plasmodium falciparum in vitro* has been documented. However, the verapamil insensitive component confers resistance to amodiaquine.

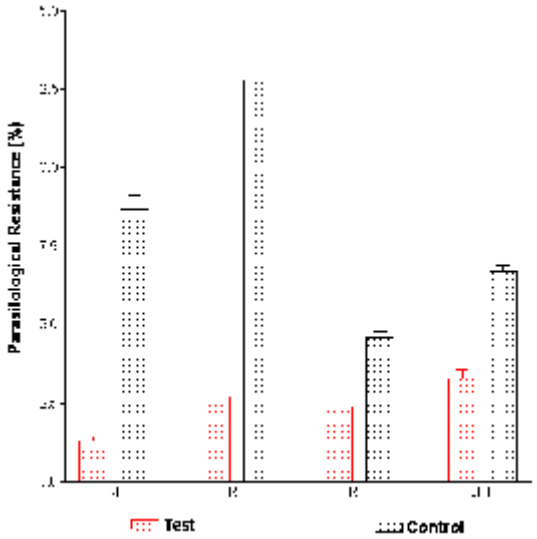
Studies have indicated consensus on the thresholds to define resistance to N-

Table 1. Baseline Characteristics of Test and Control Groups Treated

Characteristics	Test	Control	p-Value
Number of Patients	30	30	-
Male: Female Ratio	2:3	2:3	-
Mean Age (Range: 16-65 years)	38.7±2.6	39.4±3.2	p>0.05
Mean Weight (Range: 43–92 kg)	62.5±4.8	61.8±3.6	p>0.05
Mean Temperature (Range: 37.8–39.2^{o}C)	38.8±1.4	37.9±1.1	p>0.05
Mean Parasite Density (Range: 1260-21500/µL)	9168±932	10723±821	p>0.05
Mean Hemogram (Range: 4.2 – 11.5g/dL)	9.1±1.2	8.8±1.4	p>0.05
Mean WBC Total (Range: 3000 – 11700 x 10^{9}/L)	6720±457	7700±453	p>0.05
Mean Alanine Transaminase (Range: 7.8-31.2U/L)	13.4±3.1	15.7 ±4.1	p>0.05
Mean Aspartate Transaminase (Range: 13.7-28.4U/L)	16.7±5.1	17.3±5.4	p>0.05
Mean Alkaline Phosphatase (Range: 45.2-110.7U/L)	88.7±8.4	92.4±8.1	p>0.05
Mean Total Bilirubin (Range 4.3-13.8µmol/L)	6.4±1.2	7.2±1.2	p>0.05

Table 2: Mean Parasitological Response in the Test and Control Groups

Parasitological Resistance	Test (%)	Control (%)	p-Value
Low Level Resistance (RI)	1.3±0.14	8.7±0.42	p<0.05
Mid Level Resistance (RII)	2.7±0.15	12.8±0.49	p<0.05
High Level Resistance III (RIII)	2.4±0.17	4.6±0.17	p<0.05
Late Parasitological Failure (LPF)	3.3±0.26	6.7±0.21	p<0.05



Above depicts bar chart showing mean low level (RI), mid level (RII), high level (RIII), parasitological resistance and late parasitological failure (LPF) in both test and control subjects treated with amodiaquine. A statistically significant difference ($P<0.05$) is reported between the test and control subjects respectively in all the above parameters assessed fodr parasitological response. The errors bars as shows are indicative of the standard errors of mean (SEM)

Fig. 1:

desethylamodiaquine, the active metabolite of amodiaquine[9-12]. The differences in the reported thresholds to define amodiaquine resistance *in vitro* could be partially explained by: variations in the *in vitro* methodology such as incubation time of the parasite with the drug[13,14], the final hematocrit[15] and the percentage of red blood cell parasitized[16]; the most important probably is the hematocrit, since amodiaquine has the tendency to concentrate inside erythrocytes[17]. The *in vitro* tests are performed with desethylamodiaquine but conclusions refer to amodiaquine[10,12,16]. The use of different commercial presentation of amodiaquine without taking into account their different molecular weights; and finally, the use of parasites adapted to cultures *in vitro* and with incubation time >24h, were likely to show different results from those obtained with fresh isolate. A study emphasized that it is more relevant to monitor *in vitro* resistance to desethylamodiaquine instead of amodiaquine, since desethylamodiaquine exerts the major anti-malarial activity *in vivo*[18]. The same study maintains that understanding the mechanism of resistance to amodiaquine is useful in the design of new drugs, particularly 4-aminoquinoline derivatives.

A receptor Known as SR-BI (class B, type I scavenger receptor) mediates the selective uptake of cholesterol from both high and low density lipoproteins. The SR-BI plays a crucial role in Plasmodium hepatocyte infection[19]. A reduction in SR-BI expression in HUH7 hepatoma cells led to a significant reduction in Plasmodium infection rates and *in vivo* use of SR-BI si-RNAs also significantly reduced liver infection in Plasmodium infected mice[20]. It is postulated that the malaria parasites may have originally selected the SR-BI for an evolutionary reason; since SR-BI plays a direct or indirect role in providing cholesterol for the parasites to build up their cell membranes. The HDL fraction has been implicated as a major substrate for the growth of infective stages of the malaria parasite and used to support growth of *Plasmodium falciparum* with results comparable to those obtained using human serum[21]. Scientific evidence suggest that the parasitophorous vacuole membrane lipids in malaria infected erythrocytes are derived from the host cells[22,23]. An enzyme capable of activating fatty acids which is necessary for incorporation into lipids has been localized to membrane structures found within the cytoplasm of the infected erythrocyte[24]. Hence, the molecular link between malaria infection and cholesterol uptake pathway has been well established. It could be deduced that simvastatin might protect against intrahepatic development of malaria parasites, thereby blocking erythrocyte invasion with its elaboration of toxins associated with increase in morbidity and mortality. Consequently, the outcome of this study suggests that the 3-HMG-CoA reductase inhibitor, simvastatin, is implicated in modulating parasitological response to amodiaquine in the chemotherapy of malaria.

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