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Original Article

3-Hydroxy-3-methyl glutaryl coenzyme A reductase inhibitor modulates parasitological response to artesunate in falciparum malaria

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ABSTRACT

Statin treatment has been reported to inhibit *in vitro* growth of *Plasmodium falciparum*. This study examines the effects of the 3-HMG CoA reductase inhibitor, simvastatin in altering and enhancing parasitological response to artesunate. Patients in attendance at primary health facilities (n=60) suffering from malaria infection were selected for the study and informed consent obtained. Ethical clearance certification was obtained (NHREC/05/01/2008B) and patients categorized into artesunate plus simvastatin (test) and artesunate alone (control) groups. The patients were followed up on days D0, D3, D7, D14 and D28 post-treatment in line with WHO criteria. Graphpad Prism version 4.0 was employed in the analysis of data. Results revealed statistically significant difference in parasitological response between test and control groups ($p<0.05$). The low level resistance, RI given as 0±0.0%, mid-level resistance RII as 0±0.0%, high level parasitological resistance, RIII as 0.5±0.08% and the late parasitological failure (LPF) as 1.4±0.12% in the test group. The consideration of LPF alongside gives an overall parasitological resistance of 1.9±0.13% in the test group. The above contrasts with RI given as 4.6±0.18%, RII as 6.4±0.31%, RIII as 2.7±0.15% and the LPF given as 5.6±0.17% in the control group. Thus, the consideration of LPF alongside gives an overall parasitological resistance of 19.3±0.44% in the control group. In conclusion, the main implication of the foregoing is that the HMG-CoA reductase inhibitor, simvastatin in combination with artesunate exerts modulating influence on parasitological response to antimalarial therapy.

Key words: Artemisinin resistance, artesunate, HMG CoA reductase inhibitor, late parasitological failure, parasitological resistance, simvastatin, uncomplicated malaria.

INTRODUCTION

The emergence of parasitological resistance to artemisinins despite their rapidly acting schizonticidal effects and potency is a major set back in combating the menace of morbidity and mortality associated with malaria infection. The World Health Organisation recommended the use of artemisinins as first line treatment of uncomplicated falciparum malaria in endemic regions[1]. It is well known that there is a more rapid decline in parasitemia associated with artesunate or artemisinin derivatives compared to other antimalarials. The rate of decline in parasitemia is beyond what can be accounted for by inhibition of schizogony alone; suggesting that rapid changes in the circulating ring-stage infected erythrocytes that allow recognition by the host defence system may be induced by artesunate[2]. A study revealed that artesunate acting on young ring form parasites attenuated the reduction in deformability associated with continued parasite growth thereby preventing their development to more rigid mature trophozoites[3]. The decline in the efficacy of artesunate monotherapy and artemisinin-based combination therapy first noticed in the Thai-Cambodian border has been reported[4,5]. Indeed, parasitological resistance to artemisinins poses a significant threat to global efforts geared towards combating malaria. The 3-hydroxy 3-methyl glutaryl coenzyme A (3-HMG CoA) reductase inhibitor, simvastatin has been shown to exhibit important immunomodulatory effects independent of lipid lowering[6,7]. The isoprenoid pathway in the malaria parasite is highly regulated through feedback control at the level of

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two sequential enzymes involved in the synthesis of mevalonate namely: 3-HMG CoA synthase and 3-HMG CoA reductase[8]. Parasite growth inhibition *in vitro* of *Plasmodium falciparum* following statin treatment has been reported[9,10]. This study examines the effects of the 3-HMG CoA reductase inhibitor, simvastatin in altering and enhancing parasitological response to artesunate.

MATERIALS AND METHODS

Patients in attendance at primary health facilities (n=60) suffering from malaria infection were selected for the study. Confirmation of malaria diagnosis done using thick blood films and immunological test (Paracheck PI®) . Paracheck PI® is a rapid qualitative two site sandwich immunochromatographic dipstick assay employed for the determination of *Plasmodium falciparum* specific histidine rich protein-2 (PfHRP-2) in whole blood samples. This was necessary in order to supplement the classical method of diagnosis by microscopy involving examination of thin and thick blood smears which is time consuming and prone to false negative readings. Formal written documentation was employed in obtaining informed consent after adequate explanation of the purpose of study, type of treatment to be administered and clarification of any likely adverse effects or complication that may arise in the course of treatment. Patients attending eight primary health facilities within Asu Nkanu Local Health Authority in Nkanu East Local Government Area of Enugu State, Nigeria within the age range 16 to 65 years inclusive were selected for the study. Routine clinical clerkship and examination including body weight measurement and axillary temperature were done to ascertain the subjects's physical condition and presence of any confounding ailment. Randomization of subjects into test and control groups was done using a table of random numbers statistically generated. The principal investigator, microscopist, field supervisor, field assistants, medical officer, nurses and all other participants in the study did not have any prior knowledge of the patients' medical records nor the treatment group to which each subject was assigned. Ethical clearance certification (Ref: NHREC/05/01/2008B) was obtained from Health Research Ethics Committee, University of Nigeria Teaching Hospital, Ituku-Ozalla, Nigeria; 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 this study was obtained from Enugu State Ministry of Health, Nigeria. Artesunate (*Malmeter*® from Evans Medical) was given as 4mg/kg initial dose orally, then 2mg/kg for the next four days and Simvastatin (Simvor® from Ranbaxy) given orally in the dosage 0.6mg/kg/d only in the evening for 3 consecutive days. Artesunate only was given to the control in same dose as test group. Artemether-Lumefantrine (Coartem® from Novartis Pharma) was employed to salvage patients who presented with treatment failure or recrudescence; and eventually withdrawn from the study. The Artemether component is 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 treatment is inevitable 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 $\leq$ 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.

Graphpad Prism version 4.0 (GraphPad Software, Inc., La Jolla, CA, USA) statistical software was employed for analysis and data presented in tabular and graphical forms. Statistical test of significance between test and control groups ascertained using two-tailed Student *t*-test, $p<0.05$ considered significant at 95% confidence interval.

RESULTS

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

DISCUSSION

The consideration of late parasitological failure alongside values of low to high grade parasitological resistance (RI+RII+RIII), gives an overall parasitological resistance of 1.9±0.13% and 19.3±0.44% respectively in respect of test and control groups. A previous study reported low level resistance, RI of 9.4% but no RII or RIII responses (that is RII= RIII= 0%)[11]. However, they differed significantly from the nil response (RI=0) obtained in respect of test group treated with artesunate and simvastatin. Another study involving artesunate reported RI=65% and nil RII and RIII responses[12]. The sites of action of the artemisinin derivatives are not fully understood, although such information is crucial for the study of the mechanism of resistance of *Plasmodium falciparum* to the anti-malarial drug[13]. The possible roles suggested for artemisinin include: alteration of membrane protein export from the vascular network of *Plasmodium falciparum*[14]; alkylation of one or more essential malaria proteins[15] and inhibition of the protein synthetic machinery of the parasite[16,17]. A *Plasmodium falciparum* cell-free system that contains polyribosomes, transfer RNA, aminoacyl-tRNA synthetases and factors that allow the system to complete the steps of elongation and termination of the protein synthesis mechanism has been developed[18] and using this system, it can be determined that the translation of endogenous mRNA is not the target for artemisinin. Artemisinin has an inhibitory effect on the [^{3}H] hypoxanthine and [^{3}H] isoleucine re-uptake of cultured *Plasmodium falciparum*[19]. Thus, the efficacy of artesunate can be evaluated *in vitro* by the ability to inhibit schizont maturation or to inhibit the uptake of the purine precursor, hypoxanthine[20]. Resistance is related to the parasite's ability to pump artesunate out of the cell. The development of resistance initially commences slowly as treatment regimens are sufficient to eradicate the infecting biomass but sub-therapeutic blood concentrations that occur after drug administration provide selective pressure on any newly acquired infections. There is no selective pressure on treated infections at the early stage as all parasites are removed. The sub-therapeutic level of artesunate can be attributed to auto-induction of metabolic clearance pathway via CYP2C19. This has possible implications in antimalarial chemotherapy as the decreased artesunate concentration during treatment may partly explain recrudescence and the increased risk for resistance development.

Simvastatin can exert inhibitory effects on the hepatocytic development of malaria parasites. This can be attributed to the tendency of simvastatin to concentrate in the liver, thereby blocking the transformation of sporozoites to hepatocytic schizonts[21]. Again, simvastatin which is concentrated in the liver due to its capacity for been highly bound to the plasma proteins inhibits the HMG-CoA reductase enzyme of the malaria parasites; thus, depriving them of the much needed cholesterol for building up their cell membranes. A proportion of the liver-stage parasites go through a dormant period known as hypnozoites, instead of immediately undergoing asexual replication. These hypnozoites will reactivate several weeks to months after the primary infection and are responsible for relapses. However, as a result of the effect of simvastatin on the exo-erythrocytic stage of the parasite, these relapses wll be prevented. The main implication of the foregoing is that the 3-HMG-CoA reductase inhibitor, simvastatin in combination with artesunate exerts modulating influence on parasitological response to antimalarial therapy.

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Ethical Clearance: Obtained from University of Nigeria Teaching Hospital, Health Research Ethics Committee (Ref: NHREC/05/01/2008B)

Table 1: Baseline Characteristics of Test and Control Groups

Characteristics	Test	Control	p-Value
Number of Patients	30	30	-
Male: Female Ratio	2:3	2:3	-
Mean Age (Range: 16-65 years)	40.1±2.2	36.9±2.4	p>0.05
Mean Weight (Range: 43–92 kg)	59.8±3.4	57.5±3.1	p>0.05
Mean Temperature (Range: 37.8–39.2^{o}C)	38.1±1.3	38.9±1.6	p>0.05

Mean Parasite Density (Range: 1260-21500/µL)	9834±747	9530±723	p>0.05
Mean Hemogram (Range: 4.2 – 11.5g/dL)	8.5±1.3	9.0±1.1	p>0.05
Mean WBC Total (Range: 3000 – 11700 x 10^9/L)	7450±40	6880±50	p>0.05
Mean Alanine Transaminase (Range: 7.8-31.2U/L)	15.4±3.6	16.8±3.9	p>0.05
Mean Aspartate Transaminase (Range: 13.7-28.4U/L)	14.9±4.5	18.1±4.3	p>0.05
Mean Alkaline Phosphatase (Range: 45.2-110.7U/L)	73.9±7.2	67.6±7.5	p>0.05
Mean Total Bilirubin (Range 4.3-13.8µmol/L)	7.9±1.5	8.5±1.3	p>0.05

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

Parasitological Resistance	Test (%)	Control (%)	p-Value
Low Level Resistance (RI)	0±0.0	4.6±0.18	P<0.05
Mid Level Resistance(RII)	0±0.0	6.4±0.31	P<0.05
Resistance III (RIII)	0.5±0.08	2.7±0.15	P<0.05
Late Parasitological Failure (LPF)	1.4±0.12	5.6±0.17	P<0.05

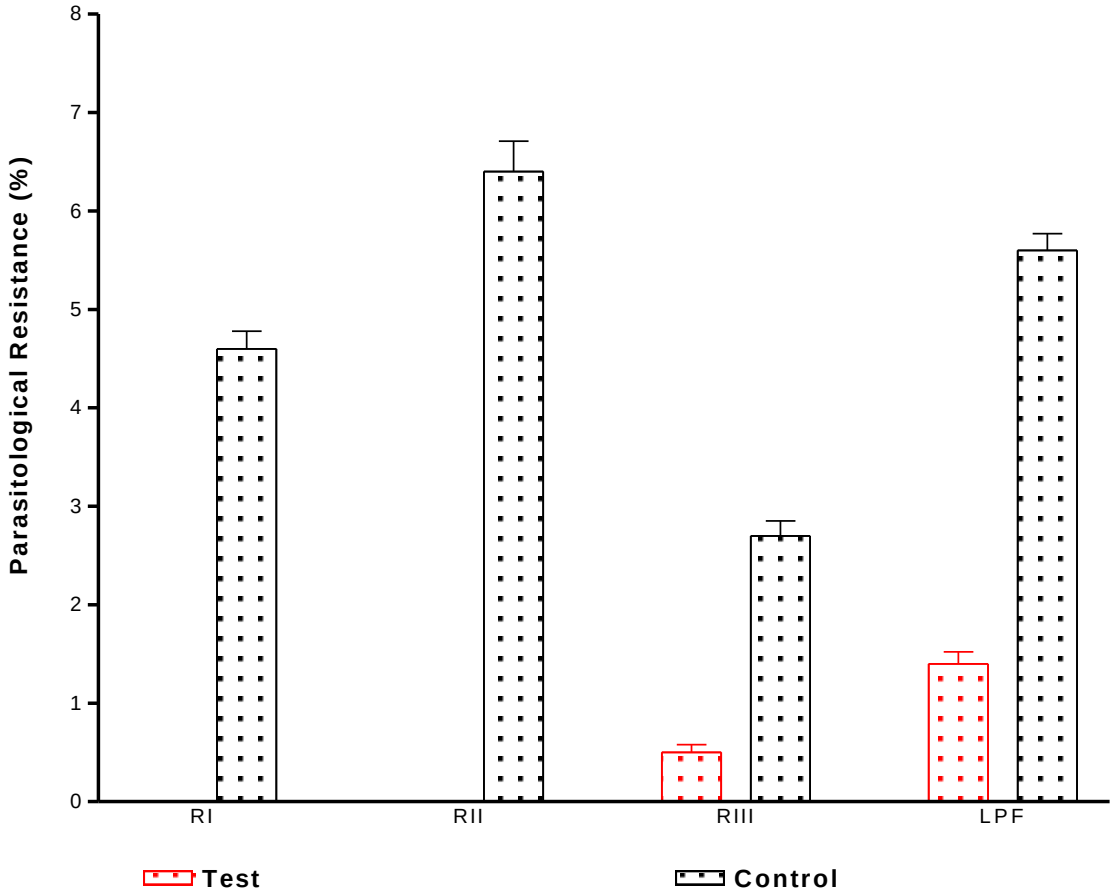

Figure 1: Depicts bar chart showing mean low level (RI), mid-level (RII), high level (RIII) parasitological resistance and late parasitological failure (LPF) in the artesunate plus simvastatin (test) and artesunate alone (control) groups. A statistically significant difference (p<0.05) is reported between test and control groups in all the parameters assessed for parasitological response. The error bars as shown are indicative of the standard error of mean (SEM).

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