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Simvastatin Modulates Parasitological Response to Sulfadoxine-Pyrimethamine in Acute Uncomplicated Malaria

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Mutations in parasite enzymes and sub-optimal dosing associated with poor quality drug administration are considered major causes of parasitological resistance to sulfadoxine-pyrimethamine in the treatment of malaria. This study evaluated the effects of simvastatin in modulating parasitological response to sulfadoxine-pyrimethamine in the treatment of malaria. Malaria patients (n=60) diagnosed by thick blood film and confirmed using immunological tests were selected and informed written consent obtained. Patients were categorized into simvastatin plus sulfadoxine-pyrimethamine (test) and sulfadoxine-pyrimethamine alone (control group). The University of Nigeria Teaching Hospital Research Ethics Committee reviewed the proposal and provided ethical clearance certification (NHREC/05/01/2008B). The WHO criteria was adopted in the assessment of parasitological response and patients followed up on days D0, D3, D7, D14 and D28 post-treatment. The analysis of data was done using GraphPad Prism 4.0 and data presented in tabular and graphical forms. 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 8.5±0.76%, mid-level resistance, RII as 7.7±0.82%, high level parasitological resistance, RIII as 5.2±0.35% and the late parasitological failure, LPF as 3.4±0.29% in the test group. This contrasts with the value of RI given as 17.1±0.61%, RII as 22.6±0.85%, RIII as 15.2±0.76% and the LPF given as 11.4±0.15% in the control group. The implication of present study indicates that the enhanced parasitological response to sulfadoxine-pyrimethamine may be attributed to modulating effects of simvastatin use.

Key words: HMG-CoA reductase inhibitor, Malaria, Parasite resistance, Parasitological response, *Plasmodium falciparum*, Simvastatin.

Parasitological resistance to sulfadoxine-pyrimethamine is associated with mutations in parasite dihydrofolate reductase conferring resistance to pyrimethamine and dihydrofolate synthase for sulfadoxine resistance respectively[1,2]. The prolonged half-life of sulfadoxine-pyrimethamine may exert undue pressure for a considerable time following fall of blood concentration below a critical threshold. Poor quality drug administration associated with suboptimal dosing is a major cause of resistance in disease endemic regions such as Sub-Sahara Africa[3]. The use of sulphur-based antibiotics such as sulphonamides in the management of bacterial infections may complicate management leading to high rate of parasitological failure. Statin treatment causes the exclusion of lipid rafts which are cholesterol and sphingolipid enriched membrane domains that play a role in endovacuolation and macromolecular transport during malaria infection[4]. Simvastatin is known to suppress proliferation of natural killer cell activity *in vitro*[5-7] and function

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as T-cell co-stimulator by binding to leukocyte function antigen 1 (LFA-1) which is thought to have a role in immunity to blood stages of malaria[8,9]. In view of the foregoing, it was hypothesized that no mean difference in parasitological response exists between malaria patients treated with sulfadoxine-pyrimethamine plus simvastatin and those treated with sulfadoxine-pyrimethamine alone. Hence, the present study evaluated the effects of simvastatin in modulating parasitological response to sulfadoxine-pyrimethamine in the treatment of malaria.

MATERIALS AND METHODS

Subjects

The subjects selected for this study were patients in attendance at primary health facilities (n=60) suffering from malaria infection, confirmed using thick blood films and immunological test (Paracheck PI®) . A rapid qualitative two site sandwich immunochromatographic dipstick assay, (Paracheck PI®),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 classical method of diagnosis by microscopy involving examination of thin and thick blood smears was time consuming and prone to false negative readings.

Study Design

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 within the age range 16 to 65 years inclusive attending eight primary health facilities within Asu Nkanu Local Health Authority in Nkanu East Local Government Area of Enugu State, Nigeria were selected for the study. The subject's physical condition and presence of any confounding ailment were ascertained following routine clinical clerkship and examination including body weight measurement and axillary temperature. Subjects were randomised into test and control groups 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. The Health Research Ethics Committee, University of Nigeria Teaching Hospital Ituku-Ozalla, Nigeria provided ethical clearance certification (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 Enugu State Ministry of Health, Nigeria provided approval for this study. Sulfadoxine-pyrimethamine (*Fansidar*® from Swiss Pharma, Lagos-Nigeria) was given as stat dose of 25mg/kg and 1.25mg/kg for the Sulfadoxine and Pyrimethamine components respectively. Each tablet of Sulfadoxine-pyrimethamine contains 500mg Sulfadoxine and 25mg Pyrimethamine. 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 Sulfadoxine-pyrimethamine only in same dose as test group. Patients who presented with treatment failure or recrudescence were salvaged with Artemether-lumefantrine (*Coartem*® from Novartis Pharma AG, Basel-Switzerland); 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. Elevation of serum transaminase activity up to three times normal level will result to discontinuation of simvastatin.

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:

a) High level resistance III (RIII) is parasitemia on day 3, D3 higher or 25% of parasitemia on D0.

b) Mid-level resistance II (RII) is parasitemia on day 3, D3 ≤ 25% of parasitemia on D0; but positive parasitemia between D4 and

D7.

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

RESULTS

The baseline characteristics of subjects in the test and control groups at presentation are as shown in Table 1. Table 2 and Figure 1 revealed statistically significant difference ($p<0.05$) in the low, mid and high level parasitological resistance (RI, RII, RIII) between the test and control groups.There was a statistically significant difference ($p<0.05$) between test and control groups in late parasitological failure (LPF).

Table 1. Baseline characteristics of test and control groups treated with sulfadoxine-pyrimethamine

Characteristics	Test	Control	p-Value
Number of Patients	30	30	-
Male: Female Ratio	2:3	2:3	-
Mean Age (Range: 16-65 years)	41.7±2.5	38.2±2.6	p>0.05
Mean Weight (Range: 43–92 kg)	60.8±3.7	63.4±3.2	p>0.05
Mean Temperature (Range: 37.8–39.2^{o}C)	39.2±1.9	38.0±1.6	p>0.05
Mean Parasite Density (Range: 1260-21500/µL)	9635±791	8791±750	p>0.05
Mean Hemogram (Range: 4.2 – 11.5g/dL)	8.2±1.3	7.9±1.0	p>0.05
Mean WBC Total (Range: 3000 – 11700 x 10^{9}/L)	6955±452	7470±455	p>0.05
Mean Alanine Transaminase (Range: 7.8-31.2U/L)	14.8±3.5	15.6±4.3	p>0.05
Mean Aspartate Transaminase (Range: 13.7-28.4U/L)	15.8±4.6	17.7±4.8	p>0.05
Mean Alkaline Phosphatase (Range: 45.2-110.7U/L)	85.1±7.9	79.6±7.7	p>0.05
Mean Total Bilirubin (Range 4.3-13.8µmol/L)	8.7±1.4	9.1±1.4	p>0.05

Table 2. Mean parasitological response in the test and control groups

Parasitological Resistance	Test (%)	Control (%)	p-Value
Low Level Resistance (RI)	8.5±0.76	17.1±0.61	p<0.05
Mid Level Resistance (RII)	7.7±0.82	22.6±0.85	p<0.05
High Level Resistance III (RIII)	5.2±0.35	15.2±0.76	p<0.05
Late Parasitological Failure (LPF)	3.4±0.29	11.4±0.15	p<0.05

DISCUSSION

The incidence of low to high level parasitological resistance (RI + RII + RIII) and late parasitological failure varies from 21.4% and 4.4% in the test subjects to 54.9% and 11.4% in the control treated with sulfadoxine-pyrimethamine. A Malawian study reported parasitological resistance rates (RII and RIII) which range from 7% to 19%, though 80% of parasitological response is at the RII level[10]. The said study revealed that overall resistance (RII + RIII) rates are slightly higher in the north than in the central and southern regions of Malawi. Higher resistance rates are observed during the rainy than dry season. However, the above differences are not statistically significant. Another study evaluated *in vivo Plasmodium falciparum* response to sulfadoxine-pyrimethamine and reveals that 6% exhibited RII parasitological resistance and 3% late parasitological failure.

A previous study in South-West Nigeria reported low to high level parasitological resistance

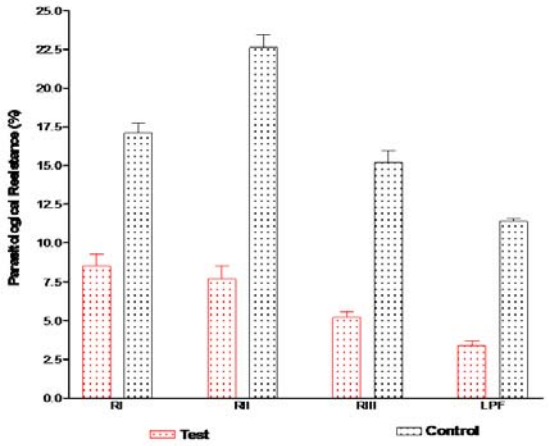

Fig. 1. Depicts bar chart showing mean low level (RI), mid-level (RII), high level (RIII) parasitological resistance and late parasitological failure (LPF) in the sulfadoxine-pyrimethamine plus simvastatin (test) and sulfadoxine-pyrimethamine alone (control) groups. A statistically significant difference ($p<0.05$) 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)

(RI + RII + RIII) in subjects treated with sulfadoxine-pyrimethamine alone, which is readily detectable by the functional viability estimate *ex vivo*[11]. The functional viability in isolates sensitive to sulfadoxine-pyrimethamine becomes significant by 16-20 hours after drug administration and viable parasites are still evident after 36 hours in some isolates. Interestingly, the results of conventional methods employed in the present study correlate with above functional viability estimates *ex vivo*. The finding of viable circulating parasites at 30 to 36 hours after sulfadoxine-pyrimethamine administration is not surprising. This is because the anti-malarial activity of this drug combination and other anti-malarial anti-folates appears to be stage specific and continues to the late stages of the asexual cycle[12]. The implication is that during the critical early phase of drug administration, and also during early infection, sulfadoxine-pyrimethamine may not readily arrest the development of young ring forms to the potentially damaging mature or late stages which sequester in internal organs and are partly responsible for the adverse consequences of falciparum infection[13].

Anti-malarial drug resistance to sulfadoxine-pyrimethamine involves a right shift in the anti-malarial concentration-effect relationship. An infection derived originally from a single sporozoite represents a distribution of concentration-effect relationship which varies with each individual parasite. Naturally, acquired infections without drug pressure, may still contain spontaneous mutants which exhibit significant reductions in susceptibility to anti-malarial drugs. The routine use of sulfonamides in respiratory tract infections may have resulted in the drug pressure effect. Resistance to pyrimethamine component is mediated in wide isolates of *Plasmodium falciparum* by point mutations in the dihydrofalate reductase gene[14]. Spontaneous mutations which confer a marked reduction in pyrimethamine susceptibility occur at relatively high frequency in natural populations.

Thus, the therapeutic use of sulfadoxine-pyrimethamine provides a strong selective pressure to the emergence of resistance. The recrudescent isolates show a marked reduction in susceptibility when tested *in vitro* because the minimal parasiticidal concentration of these compounds for the sensitive parasites cannot inhibit development of the resistant mutants. Again, there would be no therapeutic effect, if patients with such isolates are re-treated with the drug to which the malaria parasites have developed resistance. The emergence of sulfadoxine-pyrimethamine resistant falciparum malaria as confirmed in previous studies is seen in control subjects treated with sulfadoxine-pyrimethamine who present in this study[15].

A receptor identified as scavenger receptor, class B, type I (SR-BI) known to help the malaria parasite sneak inside to fully develop; has been shown to play a critical role in Plasmodium hepatocyte infection[16,17]. However, it is further postulated that SR-BI may have been selected for evolutionary reason, considering the direct or indirect role it plays in providing cholesterol needed by the malaria parasites for biogenesis of their cell membranes. Intriguingly, sporozoites enter the liver through a resident macrophage, bypassing the liver's primary defense mechanism, due to the role of circumsporozoite protein (CSP) in preventing respiratory burst[18]. The ability of sporozoite to traverse through and invade liver cells by membrane disruption allowing for movements in and out of cells[19,20]; has been attributed to thrombospondin-related anonymous protein (TRAP), a micronemal protein that mediates gliding, motility and invasion in the mammalian host[21,22]. It is pertinent to note that simvastatin blocks the transformation of sporozoites to liver schizonts by concentrating in the hepatocytes. The major therapeutic effect attributable to simvastatin in the present study is due to relatively high concentration in the liver. Consequently, the need for further studies to explore and design derivatives of HMG-CoA reductase inhibitors with higher concentrations in plasma can never be over-emphasized.

In conclusion, the implication of present study indicates that the enhanced parasitological response to sulfadoxine-pyrimethamine may be attributed to modulating effects of simvastatin use.

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