World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Statin treatment enhances clinical response to artesunate in acute uncomplicated malaria

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Received: 05-08-2014 / Revised: 21-08-2014 / Accepted: 23-08-2014

ABSTRACT

Several reports have emerged on widespread clinical failures since the introduction of artesunate as first-line treatment for malaria. This study sought to evaluate the synergistic effects of simvastatin plus artesunate combination in antimalarial chemotherapy. 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 and in line with WHO criteria. Graphpad Prism version 4.0 was employed in the analysis of data. Results revealed statistically significant difference ($p<0.05$) in clinical response between test and control groups involving all the parameters assessed. The post-treatment mean geometric parasite density was given as 0±0.0/µL and 139±19.0/µL in the test and control groups. The mean total treatment failure was given as 1.9±0.13% in the test group compared to 19.3±0.44% in the control. A mean parasite clearance time of 1.2±0.9 days in the test group as compared to 2.9±0.29 days in the control was reported. Mean fever clearance time of 12.1±0.8 hours and 38.6±2.8 hours were reported in the test and control groups. The recrudescence rate of 1.1±0.05% given in the test differed from 8.7±0.09% given in the control. Consequently, simvastatin plus artesunate may be considered as novel approach for combinational therapy aimed at enhancing clinical response to artesunate.

Key words: Artesunate, clinical response, fever clearance, parasite clearance, simvastatin, treatment failure, uncomplicated malaria.

INTRODUCTION

Statins are known to inhibit 3-hydroxy 3-methyl glutaryl coenzyme A reductase, that catalyses the rate limiting step in cholesterol biosynthesis. They are generally reputed to be beneficial in reducing incidence of mortality and morbidity associated with cardiovascular disease[1]. However, studies have shown that statins exhibited other effects independent of cholesterol lowering including the potential to inhibit the growth of bacteria and protozoa[2,3]. Simvastatin has been found to have antimalarial effects inhibiting *in vitro* intraerythrocytic development of malaria parasite[4]. World Health Organisation has since recommended the adoption of artesunate as first line treatment of malaria in endemic regions. The effectiveness and contribution of artemisinins in the reduction of malaria burden have been acknowledged globally[5].

The emergence of treatment failure or prolonged parasite clearance time along the Thai- Cambodian border has raised major public health concern due to the risk of possible spread to other regions particularly in Africa where artemisinins are widely recommended and used as first line antimalarial therapy[6,7]. Hence, the need to improve the efficacy of artesunate and enhance its antimalarial effect can never be over-emphasized. This study, therefore, sought to evaluate the synergistic effects of simvastatin plus artesunate combination in antimalarial chemotherapy.

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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 subject'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 therapeutic response as follows:

- Early Treatment Failure (ETF): Development of danger signs of severe malaria on D1-D3 in the presence of parasitemia. Parasitemia on D2 higher than D0 count irrespective of axillary temperature. Parasitemia on D3 with axillary temperature ≥ 37.5ºC.
- Late Treatment Failure (LTF): Development of danger signs of severe malaria after D3 in the presence of parasitemia, without previously meeting any of the criteria of early treatment failure. Presence of parasitemia and axillary temperature ≥ 37.5ºC on any day from D4 to D14, without previously meeting any of the criteria of early treatment failure.
- Late Parasitological Failure (LPF): Presence of parasitemia on D28 and axillary temperature <37.5ºC without previously meeting any of the criteria of early treatment failure or late treatment failure.
- Adequate Clinical and Parasitological Response (ACPR): Absence of parasitemia on D14 irrespective of axillary temperature without previously meeting any of the criteria of early treatment failure or late treatment failure
- Fever Clearance Time (FCT): The time taken from anti-malarial drug administration until axillary temperature falls below 37.4ºC and remains at that value for 72 hours.
- Parasite Clearance Time (PCT): The time taken from anti-malarial drug administration until no patent parasitemia is detected.
- Clinical Clearance Rate (CCR): The proportion of subjects with full resolution of signs and symptoms of malaria on D14.
- Recrudescence Rate (RR): The proportion of subjects in which there is incomplete clearance of parasitemia on D14 and D28 of follow-up.
- Cure Rate: The proportion of patients who remain free of parasitemia on D14 and D28 of follow-up.

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

The baseline characteristics of test and control groups at presentation are as shown in Table 1. The mean geometric parasite densities of test and control groups on follow-up days D0, D3, D7, D14 and D28 are as shown in Figure 1. Mean values of treatment failure in patients treated with artesunate and simvastatin (test) and those treated with artesunate alone (control) are shown in Table 2. The mean values of Parasite Clearance Time (PCT), Fever Clearance Time (FCT), Clinical Clearance Rate (CCR), Recrudescence Rate (RR) and Cure Rate (CR) in both test and control groups are as shown in Table 3.

DISCUSSION

Previous studies reported pre-treatment mean geometric parasite density of 22919/µL[8] and 16943/µL[9] in patients treated with artemisinin alone. The above contrasts from the value of 5437/µL reported in another study for artemisinin[10]. However, there is a statistically significant difference ($p<0.05$) in the post-treatment mean geometric parasite density between the test and control groups as shown in Figure 1. The mean total clearance of parasitemia was achieved on day 7 in the test group as compared to day 28 in control treated with artesunate. A study reported increase in parasitemia after initiation of treatment in 13.2% of subjects; although subsequent counts declined[9]. The underlying mechanism of action is not fully elucidated but appears to involve an interaction with intra-parasitic heme, yielding free radical formation followed by alkylation of parasite proteins and cleavage of the endoperoxide bridge[11]. Artesunate is considered a water soluble analog of artemisinin, a sesquiterpene lactone endoperoxide and rapidly acting blood schizonticide.

It is interesting to note that the early treatment failure in both test and control groups are still below the 10% margin recommended for change in first line anti-malarial therapy. However, it is worthy to note that there is better clinical response in the test relative to control as evidenced by adequate clinical and parasitological response as shown in Table 2. Interestingly, though the total treatment failure of 19.3% in the control differs significantly from the 1.9% in the test subjects, it is still below the 25% margin recommended for centres in the high endemicity of malaria transmission. Previous studies involving artemisinin derivatives reported fever clearance time of 20 hours and 46.1 hours respectively[8,10]. Another study reported a mean fever clearance time of 36.3 hours for all but one of the subjects[9]. A study reported parasite clearance time of 1.73 days in subjects treated with artemisinin derivative alone[8]. A study involving artemisinin derivative reported parasite clearance time of 1.68 days and rapid rate of parasitemia reduction. The mean 50% and 90% reduction times (PC_{50} and PC_{90}) were 0.42 and 0.84 days respectively[9]. It has been generally reported in various studies that artemisinin derivatives can reduce parasitemia by 90% within 24 hours of starting treatment[12-16]. Malaria infection particularly those in high parasite biomass may eventually survive the therapeutic onslaught leading to recrudescence. Recrudescence results because of the failure to eradicate the infecting population before antimalarial drug concentrations have declined below the levels of minimum inhibitory concentration necessary to maintain a parasite multiplication rate of <1. Artemisinin is associated with high rates of recrudescence, particularly when the course of treatment is < 5 days[17]. It remains unclear whether it is the blood concentration of artemisinin or its active plasma metabolite dihydroartemisinin or both; that is the important determinant of successful treatment[18]. The blood levels of an anti-malarial drug should exceed minimum parasiticidal concentration (MPC) values during at least four asexual cycles (>6 days) for maximum cure rates[19]. Mean cure rates reported in the present study are as shown in Table 3. Another study reported cure rate of 90% in subjects treated with dihydro-artemisinin alone[9]. Again, the present study adopted a 5-day treatment course compared to the 7-day course of treatment in a previous study. It has been shown in the treatment of multi-drug-resistant *Plasmodium falciparum* malaria, that therapeutic concentrations must be maintained for at least 2 cycles of schizogony, that is 7 days[20]. However, the 5-day course regimen adopted in the present study is based on empirical observations and still yielded a relatively high cure rate particularly in the test group. It was also observed that the clinical clearance rates reported in the present study for test and control groups respectively correlated closely with the cure rates as shown in Table 3. It has been demonstrated that glycosylphosphatidylinositol (GPI) moieties covalently linked to the surface antigens of malaria parasites are able to induce high levels of tumor necrosis factor α (TNF-α) and interleukin-1β from macrophages and cause pyrexia[21]. The above could be explained by the pleiotropic effects of simvastatin on a variety of host cells when released during merogony or cell death, by substituting for the endogenous GPI-

based second messenger signal transduction pathways of the host[22]. The outcome of present study has conclusively shown that simvastatin has promising prospects in combination with artesunate in enhancing clinical efficacy of antimalarial therapy. However, there is need for further studies using other statins to assess their potential antimalarial effects considering differences in the chemical, pharmacokinetic and pharmacodynamic properties distinct from simvastatin.

ACKNOWLEDGEMENTS

The selfless contribution of the entire staff of 8 primary health facilities in the study site at Asu-Nkanu Local Health Authority and the executive secretary, Mr. M.O. Offu are highly appreciated particularly for their support, understanding and co-operation. My immense gratitude further goes to Mr. E.A. Ahaotu and Mr. B.C. Ezeagwoma, both of whom are chief medical laboratory scientists at University of Nigeria Teaching Hospital for their contribution. I also wish to acknowledge Dr. Nick C. Obitte, Lecturer, Department of Pharmaceutical Technology, University of Nigeria, Nsukka for his contribution and useful advice.

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 Treatment Failure in the Test and Control Groups

Treatment Parameters	Test	Control	P-Value
ETF (%)	0±0.0	7.7±0.15	P<0.05
LTF (%)	1.9±0.12	11.6±0.12	P<0.05
ACPR (%)	98.1±0.72	80.7±0.58	P<0.05

ETF: Early Treatment failure; LTF: Late Treatment failure; ACPR: Adequate Clinical and Parasitological Response

Table 3: Mean Clinical Response in the Test and Control Groups

Clinical Parameters	Test	Control	p-Value
PCT (Days)	1.2±0.09	2.9±0.29	P<0.05
FCT (hours)	12.1±0.8	35.6±2.8	P<0.05
CCR (%)	100±0.0	82.1±1.4	P<0.05
CR (%)	98.1±0.3	80.7±0.7	P<0.05
RR (%)	1.1±0.05	8.7±0.09	P<0.05

PCT: Parasite Clearance Time
FCT: Fever Clearance Time
CCR: Clinical Clearance Time
CR: Cure Rate
RR: Recrudescence Rate

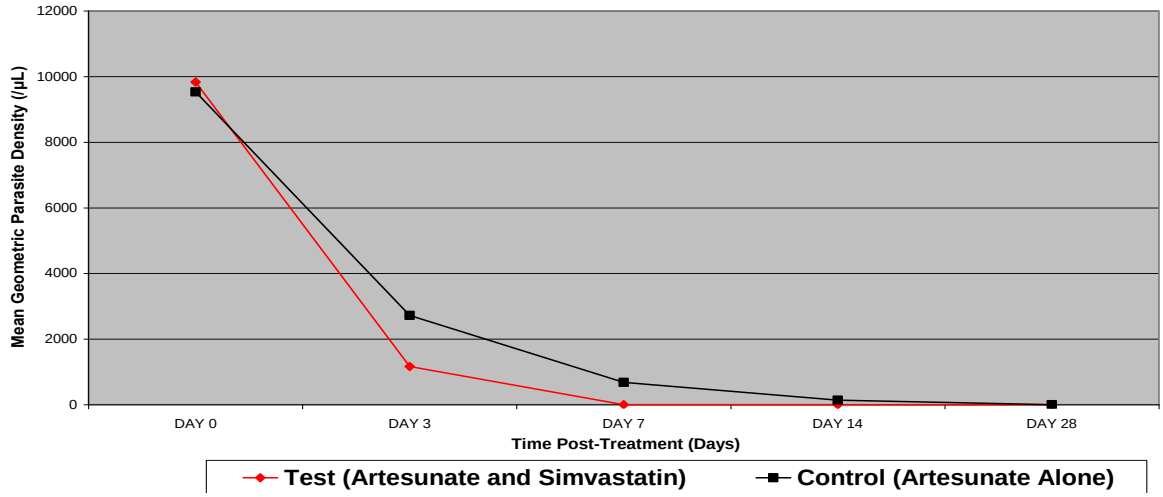

Figure 1: Above depicts linear graphical representation of the progressive decline in the level of parasitemia in both test and control groups treated with artesunate. It reveals mean geometric parasite density of 9834/µL, 1164/µL, 0/µL, 0/µL and 0/µL in respect of test group on days 0, 3, 7, 14 and 28. This is as compared to the mean geometric parasite density of 9530/µL, 2723/µL, 682/µL, 139/µL and 0/µL reported for days 0, 3, 7, 14 and 28 in respect of control group .

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