



Potency of atorvastatin in parasitemia clearance of plasmodium and the effects of the combination of atorvastatin and artemisinin derivatives on parasitemia clearance and organ toxicity

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Abstract

Objective: This study investigated the effects of atorvastatin and artemisinin based combination therapies (ACTs), administered individually and orally co-administered on some hepatotoxic biomarkers and parasitemia counts in *Plasmodium berghei* NK65 strain infected mice.

Methods: Twenty-five male Swiss albino mice were randomly distributed in five groups; Group I: 'parasitized mice administered with atorvastatin'. Group II; 'parasitized mice administered with atorvastatin + artesunate. Group III; 'parasitized mice administered with atorvastatin + artemether. Group IV; 'parasitized negative control group administered normal saline only'. Group V; 'parasitized positive control group administered chloroquine'.

Results: Plasma aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphate (ALP) and procalcitonin (PRO) activities were significantly reduced ($p < 0.05$) in atorvastatin + artesunate, atorvastatin + artemether and chloroquine treated groups when compared with the negative control and atorvastatin treated group. Furthermore, the parasitemia count in *Plasmodium berghei* infected mice was suppressed in mice treated with atorvastatin + artesunate, atorvastatin + artemether and chloroquine when compared to mice treated with atorvastatin alone.

Conclusion: The data from this study indicates that atorvastatin has a moderate potential in the clearance ability of *Plasmodium berghei* NK65 parasites and elicits hepatic and renal injury. It also indicates that the co-administration of artesunate and artemether with atorvastatin in the treatment of malaria could be beneficial with less severe hepatic and renal events.

Keywords: ACTs, Atorvastatin, Aspartate Transaminase, Alanine Transaminase, Alkaline Phosphatase, Procalcitonin, *Plasmodium berghei*

Introduction

Malaria is a deadly and devastating disease caused by *Plasmodium* species. Hundreds of millions of people are infected annually across tropical and subtropical regions, largely Africa, South and Central America, India, Southeast Asia, and Oceania [1]. Human malaria is caused by five species of parasites that belong to genus *Plasmodium*: *P.falciparum*, *P.vivax*, *P.ovale*, *P.malariae* and until recently *P.knowlesi* [2]. *P.falciparum* proves to be the most fulminating [3]. However, *P.berghei* belongs

to a group of *Plasmodium* species that infect rodents and is extensively used in preclinical drug testing [4].

Various drugs are used for malaria prevention and cure and artemisinin-based combination therapies (ACTs) are now recommended worldwide for treatment of infections [5]. The exceptional story of the discovery of artemisinin and demonstration of its antimalarial activity by Chinese scientists represents one of the great discoveries in medicine in the latter half of the 20th century [6]. Chinese scientists isolated the ac-

tive component from the plant *Artemisia annua* also known as sweet or annie woodworm to treat symptoms of malaria [7]. Due to the modest solubility of artemisinin in polar and non-polar solvents, other semi-synthetic derivatives have been reshaped chemically to yield other derivatives; artesunate, artemether, arteether, artelinic acid and dihydroartemisinin [8]. In 1986, derivatives of artemisinin, artesunate, arteether, and artemether were synthesized, showing a better oral bioavailability and a small number of adverse effects. To date, many other derivatives have been synthesized, including SM735, SM905, SM933, SM934, and SM1044. These novel drugs have shown higher activity than their predecessors [9]. Artemisinins are established antimalarial agents with an outstanding safety profile [10]. There is considerable debate regarding the mechanisms of antimalarial action of the artemisinins but several evidence indicates the role of ferrous species in parasite clearance [11].

Atorvastatin (AVA), a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, belongs to a family of lipid-lowering drugs that are presently used for the control of hyperlipidemia and are considered helpful for protection from cardiovascular events. Apart from the cholesterol-lowering activity of statins, the immunomodulatory and pleiotropic effects of statins may remarkably impact infection-related survival [12,13]. Statins inhibit the mevalonate pathway, which involves the synthesis of isoprenoids and the intracellular trafficking of membrane-associated proteins such as G-proteins. GTP-binding proteins have pivotal roles in intracellular inflammatory signaling by acting as molecular switches for different protein kinases. Specific targets, including Ras, Rho and Rac subfamilies, are thought to be important in systemic inflammatory syndromes, such as sepsis, because of their key roles in intracellular signaling [14] There is a need for the discovery of new anti-malarial drugs and combination therapy. A combinatorial approach protects each drug from the development of resistance and minimizes generally the net transmission rate of malaria [15]. Statins were found to interfere severely with the growth of protozoan parasites of the family Trypanosomatidae, such as *Trypanosoma cruzi*, and various *Leishmania* species [16]. However, the effects of statins on the clearance of *plasmodium* parasites remain unclear. Hence, this study was designed to investigate the influence of orally administered statins and other anti-malarial agents on parasite clearance as well as the effect on hepatic and renal biomarkers in mice infected with *P.berghei* NK65 strain.

Materials and methods

Twenty-five Swiss albino mice of both sexes with an average weight of 16g were obtained from the animal house of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria. The animals were observed under 12 hours light/dark cycles in clean and well maintained cages in the biochemistry laboratory and were fed with mice pellet diet (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum* for one

week so as to acclimatize to room temperature of 29°C. This method was done and carried out prior to randomization into various experimental groups of five animals per group based on body weight of the mice and was designated as groups 1 (Atorvastatin), 2 (Atorvastatin + Artesunate), 3 (Atorvastatin + Artemether), 4 (Normal saline negative control) and 5 (Chloroquine positive control). Animals were humanely handled in conformity with the standard operating procedures of the National Research Council [17].

Chemicals

A 0.5 grams salt of chloroquine phosphate, CQ (Emzor Pharma, Nigeria) was dissolved in 10ml of distilled water to final doses of 5mg/kg body weight.

Artemether, artesunate and atorvastatin were also obtained from (Emzor Pharma, Nigeria).

All other chemicals and reagents used were of analytical grade.

Parasite and mice inoculation

NK65 strain of *Plasmodium berghei*, which is sensitive to chloroquine used for this study, was obtained from Dr. O. Aina's Laboratory, Department of Biochemistry and Nutrition, NIMR, Yaba, Lagos, Nigeria. An infected donor mouse with the *P.berghei* strain of the rodent *Plasmodium spp* was used for parasites inoculum preparation. Each mouse was inoculated with 0.1ml of the infected blood containing about 1×10^6 *P.berghei* parasitized red blood cells intraperitoneally.

Experimental design

Animals were assigned randomly into five groups of five animals.

Study design was as follows:

Group I: parasitized mice were orally administered 5mg/kg atorvastatin in 0.9% NaCl.

Group II: parasitized mice were co-administered 5mg/kg atorvastatin in 0.9% NaCl+5mg/kg artesunate in 0.9% NaCl.

Group III: parasitized mice were co-administered 5mg/kg atorvastatin in 0.9% NaCl+5mg/kg artemether in 0.9% NaCl.

Group IV: served as negative control. Parasitized mice were administered with 0.9% NaCl.

Group V: served as positive control. Parasitized mice were administered with 25mg/kg chloroquine in 0.9% NaCl.

Freshly prepared atorvastatin, artesunate, artemether and chloroquine solutions were administered to mice via oral administration for 4 days.

Suppressive treatment

A four-day suppressive test according to Peter's 4-day suppressive treatment was adopted and used for this study. The mice of both sexes weighing 12-20g were divided into groups of five with each group having five animals in a cage. Groups I, II and III were administered atorvastatin, atorvastatin + artesunate, atorvastatin + artemether respectively while group IV served

as a negative control and was given normal saline. The last group V was given chloroquine; the chloroquine was used as a positive control. The animals were administered with the drugs 2 hours after the inoculation on the parasite on day 0 and everyday till day 3 using an oral cannula. On day 4, a thin blood film was made from the tail of each mouse. The smear was prepared by spreading the blood on a clean slide over an area of 1.5 cm X 2.5cm, allowed to dry and fixed with methanol, stained with 3 % Giemsa stain for 45 minutes rinsed, allowed to dry and examined with microscope under the oil immersion objective to determine the parasite density microscopically (Olympus CX, Japan). This is necessary to monitor the level of parasitemia. The suppression of parasitemia in relation to the control was assessed using the recommended formular :

Average (Av) % suppression =

$$\frac{Av \% parasitemia\ in\ negative\ control - Av \% in\ test}{Av \% parasitemia\ in\ negative\ control} * 100$$

Collection of blood

On the fifth day the mice were sacrificed and blood and organ samples were collected for biochemical analysis.

Blood samples were obtained via the orbital sinus using a sterile catheter inserted into the medial canthus of the eye (30 degrees angle to the nose) and immediately transferred into clean and sterile lithium heparin tubes to prevent clotting. The blood samples were immediately centrifuged at 3000 rpm for 10 min to separate plasma from the blood cells. The obtained plasma was used to determine alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and procalcitonin (PRO) activities using Reitman and Frankel spectrophotometric methods in accordance with Radox diagnostic kit assays.

Results

Data in **Table 1** showed that the % parasitemia count in *Plasmodium berghei* infected mice was suppressed when treated with atorvastatin + artesunate, atorvastatin + artemether and chloroquine and when compared to treatment with atorvastatin alone and the negative control.

Data in **Table 2** showed that the average % parasitemia suppression in plasmodium berghei infected mice was increased

when treated with atorvastatin + artesunate, atorvastatin + artemether and chloroquine and when compared to treatment with atorvastatin alone and the negative control.

Treatment groups;

1. Atorvastatin
2. Atorvastatin + Artesunate
3. Atorvastatin + Artemether
4. Negative control
5. Positive control

Data in **Figure 1** showed that the % parasitemia count in *Plasmodium berghei* infected mice was suppressed when treated with atorvastatin + artesunate, atorvastatin + artemether and chloroquine when compared to treatment with atorvastatin alone.

Data in **Figure 2** showed that plasma PRO activities were significantly elevated ($p < 0.05$) in the atorvastatin treated group (37.63 ± 2.54) when compared to the atorvastatin + artesunate (34.93 ± 1.9), atorvastatin + artemether (32.83 ± 2.54) and the positive (25.72 ± 2.67) and negative control (30.65 ± 0.73) groups. Data in **Figure 3** showed that plasma AST activities were significantly elevated ($p < 0.05$) in the atorvastatin treated group (113.7 ± 2.75) and negative control (120.54 ± 2.96) when compared with the atorvastatin + artesunate (36.3 ± 2.85), atorvastatin + artemether (54.93 ± 2.75) and positive control (16.21 ± 3.04) groups. Data in **Figure 4** showed that plasma ALT activities were significantly elevated ($p < 0.05$) in atorvastatin

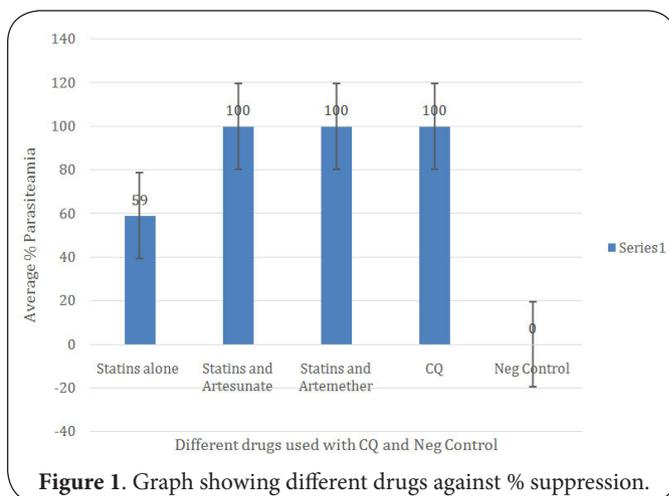
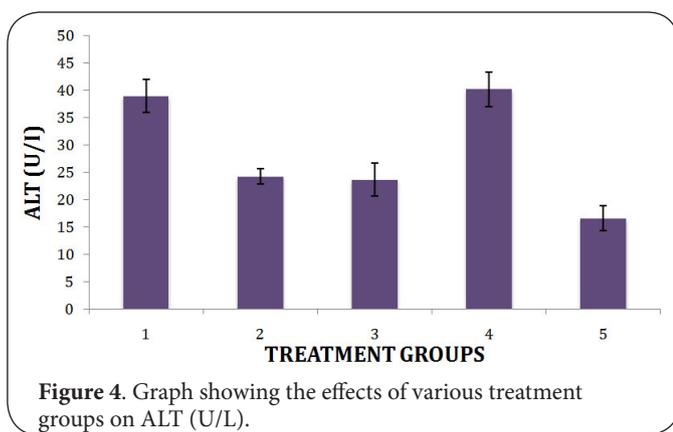
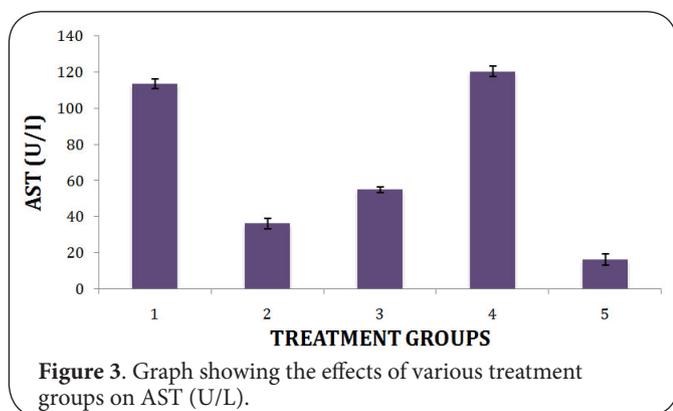
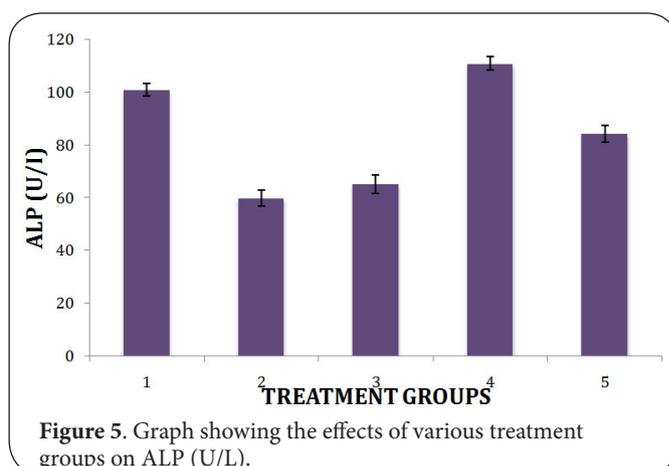
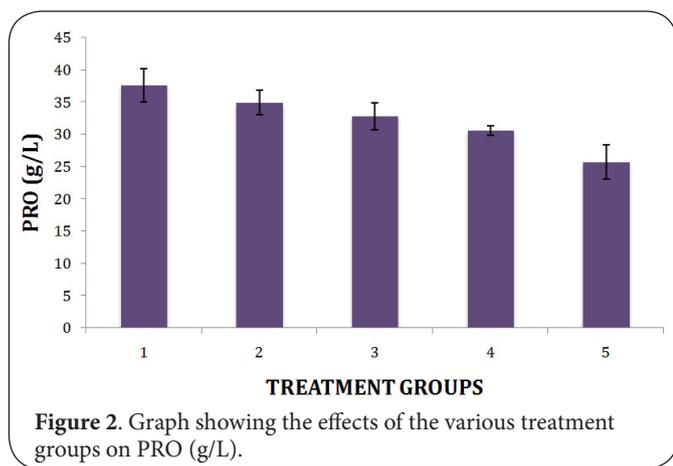


Table 1. Average % Parasitaemia of Drugs.

DRUGS	Atorvastatin	Atorvastatin + Artemether	Atorvastatin + Artesunate	Negative Control	CQ
	2.5 ± 1.64	0	0	5.11 ± 2.13	0

Table 2. Average % Suppression.

DRUGS	Atorvastatin	Atorvastatin + Artemether	Atorvastatin + Artesunate	Negative Control	CQ
	59%	100%	100%	0%	100%



(38.99±3.03) treated group and negative control (40.21±3.15) when compared with atorvastatin + artesunate (24.25±1.42), atorvastatin + artemeter (23.66±3.03) and positive control (16.58±2.27) groups. Data in **Figure 5** showed that plasma ALP activities were significantly elevated ($p < 0.05$) in atorvastatin treated group (100.96±2.24) and negative control (110.89±2.6) when compared with atorvastatin + artesunate (59.82±3.1), atorvastatin + artemeter (65.09±2.24) and positive control (84.22±3.31) groups.

Discussion

The present study demonstrated that the parasitemia count in the treatment group of atorvastatin + artemether, atorvastatin + artesunate were completely suppressed while the treatment group consisting of atorvastatin alone had a moderate suppression of parasitemia count when compared to the negative control. Similarly, atorvastatin coadministered with artemether and artesunate was observed to exhibit high antiplasmodial activity (100%) while atorvastatin exhibited a moderate suppression (59%) against the strains of *plasmodium berghei*. In a previous study, other statins; lovastatin and simvastatin were been shown to have antiplasmodial activity against *plasmodium falciparum* [18]. However, while the mechanism of action of atorvastatin is remarkably contrasting from those of conventional antimalarials, their antiplasmodial clearance ability could be due to the inhibition of HMG-CoA reductase which subsequently represses the formation of ubiquinone and dolichol and an eventual collapse of the parasite mitochondrial membrane potential. This mechanism has been the rationale behind the antimalarial atovaquone where the suppression of ubiquinone binding to cytochrome b kills the infectious agent or parasite [19].

This study also showed that mice infected with *P.berghei* treated with Atorvastatin and the negative control had elevated plasma AST, ALT and ALP activities compared with parasitized mice co-treated with atorvastatin + artesunate and atorvastatin + artemether. Previous studies report that an elevation in plasma ALT and AST activities is an indication that hepatotoxicity abounds [20]. This could be due to the incomplete clearance of parasites in mice that were not treated with antimalarial agents at all. In this study, the elevation of AST was more than ALT when compared to individual groups, presumably because of the contribution of the lysis of red blood cells to this enzyme. It was also indicated in this study that mice infected with *P.berghei* treated with atorvastatin and the negative control had elevated plasma ALP activities as compared to other treatment groups. A previous study had shown that significantly higher ALP activity among malaria

patients compared with healthy patients is evidence of the fact that changes in ALP activity can be used as a potential biomarker in assessing the integrity of the hepatic drainage system during acute falciparum malaria infection. Centri-lobular liver damage is one of the factors involved in hepatic dysfunction in acute falciparum malaria infection, leading to hyperbilirubinemia [21,22] which could be a straight outcome of the compromised drainage capacity of the liver accounting for the ALP elevation observed in this study. Hepatic dysfunction in malaria has been well known for many years and distorted liver functions are commonly seen as a complication of falciparum infections [23,24] and it has been reported that a host of factors are responsible for the hepatic dysfunction in severe malarial infection. This does not appear to be due to direct inflammation of hepatocytes but due to the inability of bilirubin excretion as a result of heavy parasitemia, endotoxemia, ischemia, acidosis or a combination of some or all of these factors [25]. The most common clinical hepatic manifestation of statins is asymptomatic elevation in aminotransferases and this appears to be a class effect of statins [26], this could be an additional contributor to the elevated liver enzyme patterns observed in this study.

The elevation in plasma PRO in the atorvastatin treated group and negative control compared to the groups co-administered with atorvastatin and antimalarial drugs could be due to the incomplete clearance of parasitemia in infected mice. While the pathogenesis of acute kidney injury in malaria is still not clearly understood Some studies have shown that renal injury in falciparum malaria results from acute tubular necrosis and that restricted local blood flow to the kidneys is considered a major contributor for malarial acute renal failure [27]. Blockage of renal microcirculation due to sequestration of infected erythrocytes, immune-mediated glomerular injury and volume depletion are some of the advocated hypotheses [28,29]. Furthermore, heme accumulation in plasma and urine of malaria patients is associated with the development of acute kidney injury (AKI), a clinical hallmark of severe malaria [30]. Lastly, the atorvastatin + artemeter, atorvastatin + artesunate and chloroquine treated groups when juxtaposed with the atorvastatin alone treated group showed a more formidable clearance of parasites perhaps due to the presence of more potent and specific antimalarials than atorvastatin. Studies have shown atorvastatin to be 10 times more active against *P. falciparum* compared to mevastatin, simvastatin, lovastatin, fluvastatin or pravastatin [31], suggesting that atorvastatin is the best candidate among statins for therapeutical prospects in malaria treatment.

Conclusion

This study showed that atorvastatin has a moderate clearance ability of parasitemia in mice infected with *P. berghei* as well as elicits hepatic and renal injury when administered to the infected group. It also shows that the co-administration of artesunate and artemeter with atorvastatin in the treatment of

malaria is beneficial in the clearance of parasites with a lesser degree in severity as per hepatotoxicity and nephrotoxicity.

Competing interest

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	OA	AO
Research concept and design	✓	--
Collection and/or assembly of data	✓	✓
Data analysis and interpretation	✓	✓
Writing the article	✓	--
Critical revision of the article	--	✓
Final approval of article	✓	✓
Statistical analysis	✓	✓

Acknowledgements

We are very grateful to Prof and Mrs. K.S Adeyemi for the financial support to cover the article processing charge for this study. We are also grateful to Dr. O. Aina and Mr. Samuel Akindele of the Nigerian Institute of Medical Research, Yaba, Lagos for all the assistance provided and for providing all the necessary equipment and the use of his laboratory.

Publication history

Editor: Werner Apt Baruch, University of Chile, Holland.

Received: 06-Sept-2020 Final Revised: 08-Nov-2020

Accepted: 10-Nov-2020 Published: 26-Nov-2020

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Citation:

Adeyemi O and Aina OO. **Potency of atorvastatin in parasitemia clearance of plasmodium and the effects of the combination of atorvastatin and artemisinin derivatives on parasitemia clearance and organ toxicity.** *Trends Vector Res Parasitol.* 2020; 7:1.
<http://dx.doi.org/10.7243/2054-9881-7-1>