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A Study of the Effect of Ascorbic Acid on the Antiplasmodial Activity of Artemether in *Plasmodium Berghei* Infected Mice

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ABSTRACT

The main aim of this work was to study the effect of ascorbic acid on the efficacy of artemether against *plasmodium berghei* infected mice. The study was divided into 2 phases and a total of 40 Swiss albino wistars mice of average weight 20g were used in all, 10 for phase 1 and the remaining 30 for phase 2. The phase 1 was mainly to determine the peak parasitaemia in order to estimate the day to commence drug treatment in phase 2. In phase 2 of the study, the mice were divided into 6 treatment groups: artemether alone, artemether with low dose ascorbic acid, artemether with high dose ascorbic acid, low dose ascorbic acid alone, high dose ascorbic acid alone and the control group. The study showed that ascorbic acid, in high dose antagonized the antiplasmodial effect of artemether. Average % suppression in parasitaemia was found to be 51.4 ± 32.21 as against that observed for artemether alone at 60.3 ± 18.70 . The study also showed that high ascorbic acid can slow down the rate of development of malarial parasitaemia in infected mice and these results were found to be statistically significant. In conclusion, co-administration of ascorbic acid may reduce the antimalarial potency of artemether, an artemisinin derivative and high dose of ascorbic acid may also suppress parasite growth.

Keywords: Artemether, ascorbic acid, interaction, *Plasmodium berghei*.

INTRODUCTION

Malaria causes about 250 million cases of fever and approximately 1 million deaths annually, and it remains a devastating global problem representing a medical emergency because it may rapidly progress to complication and death without prompt and appropriate treatment. The vast majority of cases occur in children below 5 years old and pregnant women. Death rate could double in the next 20 years if prevalence stays on its present upward course (WHO, 2005; Trampuz *et al.*, 2003). Several interventions have been instituted for prevention and possibly for eradication of malaria. Some of the control measures are provision of mosquito insecticide impregnated nets and insect repellants, spraying of insecticide inside houses and draining of standing or stagnant water where mosquitoes lay their eggs. Successful treatment outcomes are highly predicated on choice of antimalarial drugs employed.

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Over the past decade, a new group of antimalarial - the artemisinin compounds, especially artesunate, artemether and dihydroartemisinin – have been deployed on an increasingly large scale for treatment of malaria. These compounds produced a very rapid therapeutic response (reduction of the parasite biomass and resolution of symptoms), are active against multidrug resistance *P.falciparum*, are well tolerated by the patients and reduce gametocyte carriage (and thus have the potential to reduce transmission of malaria). If used alone, the artemisinins will cure falciparum malaria in 7 days (Benakis et al., 1997; Sirima et al 2007; Malenga et al, 2005) but studies have shown that recrudescence and drug resistance occur after the treatment. Emergence of multidrug resistant plasmodium falciparum (mdrpf) led to the recommendation by WHO that Artemisinin based Combination therapies (ACTs) be adopted (WHO, 2006). Since then many African countries have accepted the use of ACTS in management and treatment of malaria. However the impact of parasites on the red blood cells cannot be over-emphasized. The stress imposed on humans as a result of parasite effect on red blood cells is still very contentious. Both nutritional and pharmacological treatments have been utilized to exploit the oxidative stress imposed on the host red blood cells (RBC) by the parasite responsible for the causation of malaria (Lavender *et al.*, 1989).

Often, these antimalarials are prescribed alongside other drugs such as ascorbic acid and vitamin supplements. Some of these vitamin preparations contain some medicinal agents that are antioxidants and they act by mopping up free radicals that are often associated with human stress. However, artemisinin derived antimalarials are claimed by their mode of action to generate free radicals (pro-oxidants) which in turn kill the malaria parasites (Krishna *et al.*,2004), an effect directly opposite that of ascorbic acid. Although some drug-related problems develop unexpectedly and the effect may not be predicted while many are related to known pharmacological actions of the drugs and can reasonably be predicted. However, as drug therapy becomes more complex and because many patients are being treated with two or more drugs, the ability to predict the magnitude of a specific action of any given drug diminishes (Hussar, 2000). An interaction is said to occur when the effects of one drug are changed by the presence of another drug, food, drink or an environmental chemical agent (Stockley, 1999). There are limited study on the effect of antioxidant on the mode of action of artemisinin and its derivatives. Available reports posited that coadministration of artemisinin with antioxidants such as vitamins A, E and C may affect the pharmacodynamic antimalarial activity of artemisinin (Oreagba & Ashorobi, 2007; Awodele et al., 2007; Meshnick et al., 1989). This work therefore aimed to study the possible outcome of concurrent therapeutic use of ascorbic acid with artemether in the clearance of *plasmodium berghei* in infected mice.

MATERIAL AND METHODS

This study was conducted at physiology department and Central Research Laboratory of faculty of pharmacy, College of Medicine, University of Lagos, Idi Araba, Lagos and Malaria

Laboratory, Department of Biochemistry, National Institute of Medical Research (NIMR) Yaba, Lagos, Nigeria.

Test Chemicals

Artemether injection 80mg/ml (Hilact®), Ascorbic acid injection, 500mg/5ml (Labeta®), Arachis oil, Giemsa stain, Methanol AR (Sigma-Aldrich), Oil of immersion, Phosphate buffer saline (PBS), 0.9% Normal saline (Dana®) and Water for injection.

Laboratory animals

All the mice employed for this project were obtained from the Animal House, College of Medicine Campus, Idi-Araba, Lagos. Mice collected were of average weight, 20g and they were kept in a well ventilated environment in standard cages. The mice were acclimatized for a period of 2 weeks, maintained on standard pellets (Pfizer Livestock Feeds, Lagos, Nigeria) and water ad libitum.

Parasite

The NK 65 strain of *plasmodium berghei* used in this study was obtained from Dr. O. O. Aina of Malaria Laboratory, Department of Biochemistry, National Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria.

METHOD

A total of 40 Swiss albino Wistars mice of average weight 20g were employed in this study which was carried out in 2 phases.

Phase 1

The essence of which was to determine peak parasitaemia and duration of animal survival served as the preliminary study and 10 mice divided into 2 subgroups of 5 mice (control and test) were used. The 5 mice in the preliminary experimental subgroup were passaged with 0.2mL parasitized blood samples from the donor mouse while the control group had 0.2mL normal saline intraperitoneally.

Blood samples were collected from the tails of the mice 48hrs after initial passaging and used in preparing thick smears on slides, one to a mouse and then fixed with methanol. The smears on the slides were then stained with giemsa stain after which they were viewed under the light microscope using the x100 oil immersion. The essence of this was to confirm the presence or absence of parasite in the passaged mice.

After confirmation of the presence of parasites in the selected mice (via thick blood film microscopy), thin blood film microscopic examinations were commenced 72hrs after initial passaging. The thin blood film microscopic examinations were repeated everyday for 16 days within which the day corresponding to peak parasitaemia was determined.

Phase 2

30 animals were used in the study. They were divided into 6 groups of 5 mice and placed in 6 cages labeled groups A to F. All

animals in the various groups were passaged with 0.2mL parasitized blood samples from the donor mouse. Confirmation of parasitaemia was done by thick film microscopy 48hrs after passaging. Following the confirmation of parasitaemia in mice, blood samples were collected on day 5 after passaging for thin film blood microscopy and drug administration commenced also 5th day post animal passage. Test drugs and arachis oil were administered to the passage experimental mice accordingly, in the following manner:

Group AA mice received artemether (0.25mL, 2.3mgkg⁻¹) only on day 1 and (0.125mL, 1.2mgkg⁻¹) on day 2-5 of drug treatment respectively. Group BB and CC received same doses of artemether concomitantly with low dose ascorbic acid 2.86 mg/kg-1 and high dose 8.56mg/kg respectively for 5 days. Group DD and EE received low dose ascorbic acid (2.86mg/kg) and high dose (8.56mg/kg) alone respectively. Group FF mice served as control and received 0.2ml of arachis oil, being the vehicle for artemether. The drugs and arachis oil were administered intraperitoneally to mimic corresponding parenteral administration of such in humans and drug dosages adjusted in mice (based on average body weight). Blood samples were taken from animal tails for thin film microscopic examination immediately before drug administration on everyday of drug treatment.

DATA ANALYSIS.

All data generated were reported as means \pm S.D, average % parasitaemia suppression was calculated to determine the effect of ascorbic acid (low/high dose) on artemether. The %

parasitaemia reduction within and among groups were compared using analysis of variance (ANOVA). Graph was plotted using Micrococal® Origin software. T-test was used to assess statistically the effect of ascorbic acid on parasitaemia compared with the control. In all cases, the effect was found significant if p value is less or equal to 0.05.

RESULTS

Table 1 represents data obtained from determination of peak parasitaemia on selected mice. Five of the mice died between day 15 and 16 as a result of no chemotherapeutic drug protection/treatment at this stage. Figure 1 shows progressional increase in parasitaemia from day 3 to 8 with a sharp fall and subsequent peak parasitaemia obtained on 11th day.

Table 2 indicates the mean percentage parasitaemia in pre and post drug treatment. Treatment group AA, BB and CC showed therapeutic parasite clearance of artemether and impact of varying concentration of ascorbic acid on the pharmacological activity of artemether. Figure II shows total parasite clearance on the first day of post drug treatment with either artemether alone or in the presence of both low or high doses of ascorbic acid in group AA, BB and CC respectively. In group DD and EE the result did not show any appreciable reduction in parasitaemia throughout the 5th day of drug administration. The degree of percentage parasitaemia suppression in the group taking antimalarial chemotherapy in the presence and absence of an antioxidant, ascorbic acid is represented in table 3. Some level of parasitaemia suppression was observed when high dose of ascorbic was administered alone.

Table. 1: Peak parasitaemia determination conducted for 16 days

Mice Code *Day	% Parasitaemia								
	3	4	5	6	7	8	9		
M1	2.45	7.54	11.36	15.96	18.24	23.13	21.24		
M2	8.53	15.80	14.44	20.03	21.72	27.75	4.36		
M3	3.83	7.66	12.27	15.10	20.03	22.76	10.12		
M4	11.37	15.31	23.54	25.81	31.51	36.90	22.21		
M5	4.78	8.58	11.96	12.89	10.47	14.10	6.71		
Average	6.19 \pm 3.67	10.98 \pm 4.20	14.71 \pm 5.07	17.96 \pm 5.09	20.39 \pm 7.56	24.93 \pm 8.31	12.93 \pm 8.30		
*Post passaging									
Mice Code *Day	% Parasitaemia								
	10	11	12	13	14	15	16		
M1	32.37	44.56	45.51	37.07	44.56	Dead	Dead		
M2	5.02	29.18	24.52	22.83	29.18	36.80	37.19		
M3	17.40	47.85	42.98	47.37	47.85	24.52	25.63		
M4	34.50	52.28	40.21	52.40	12.27	Dead	Dead		
M5	9.38	45.44	47.03	36.60	45.44	29.90	Dead		
Average	19.73 \pm 13.29	43.86 \pm 8.74	40.05 \pm 9.06	39.26 \pm 11.41	35.86 \pm 15.10	30.41 \pm 6.16	31.41 \pm 8.17		

*Post passaging

Table. 2: Average % parasitaemia pre and post-drug treatment in all groups (mean \pm SD).

Groups	*5 th day	*Day 1	*Day 2	*Day 3	*Day 4	*Day 5
AA	2.09 \pm 0.75	0.83 \pm 0.47	NP	NP	NP	NP
BB	1.65 \pm 0.65	0.56 \pm 0.45	NP	NP	NP	NP
CC	2.59 \pm 0.66	1.26 \pm 1.20	NP	NP	NP	NP
DD	3.13 \pm 0.97	3.62 \pm 1.95	6.75 \pm 2.14	7.02 \pm 2.01	7.17 \pm 5.25	7.16 \pm 4.88
EE	1.99 \pm 0.60	2.48 \pm 1.72	3.08 \pm 1.44	4.73 \pm 3.29	6.83 \pm 2.42	4.42 \pm 1.59
FF	2.27 \pm 0.46	5.62 \pm 0.71	8.23 \pm 1.88	7.71 \pm 1.30	6.65 \pm 0.59	8.45 \pm 0.41

NP = No parasitaemia; * = passaging/pre-drug treatment; + = post drug treatment

AA = artemether alone, BB = artemether and low dose ascorbic acid, CC = artemether and high dose ascorbic acid, DD = low dose ascorbic acid alone, EE = high dose ascorbic acid alone, and FF = control (arachis oil).

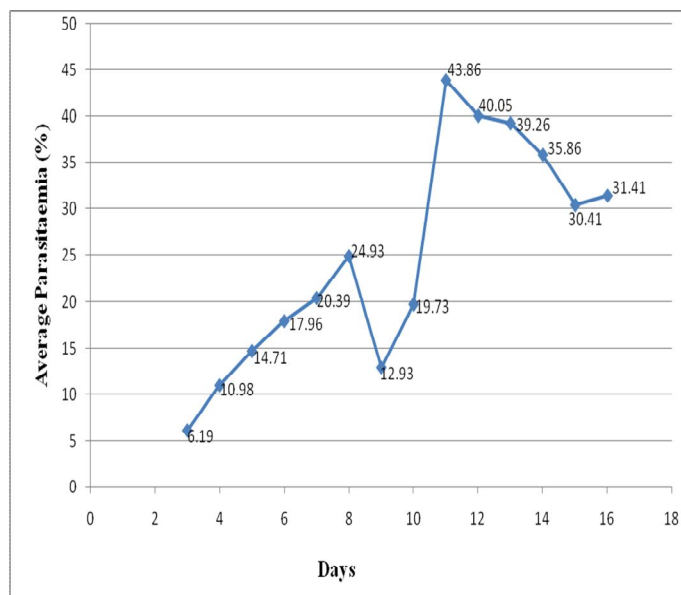


Fig. 1: Profile of parasitaemia (%) in untreated mice (passed with *Plasmodium berghei*) over a period of 16 days.

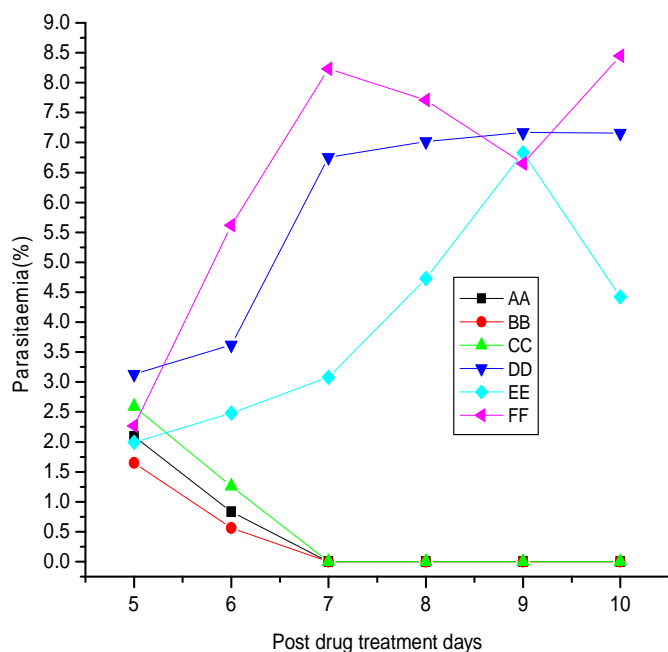


Fig. 2: Effect of artemether alone, artemether in combination with low and high ascorbic acid therapeutic concentration and Low and high dose of ascorbic acid alone on the parasite clearance.

Table. 3: Average % reduction in parasitaemia.

Groups	Average % parasitaemia suppression \pm SD
AA	60.3 \pm 18.70
BB	66.1 \pm 23.60
CC	51.4 \pm 32.21

DISCUSSION

The peak parasitaemia time was established on day 11 which is an indication as to the appropriate time to commence drug treatment in the second phase of the study. Drug administration commenced 5th day post-passaging following confirmation of parasitaemia in the mice. When artemether alone was administered,

there was a significant reduction in parasitaemia values from 2.09 % (pre-drug treatment) to 0.83% (1st day post drug treatment) and no parasitaemia was observed for day 2 – 5 of drug administration. This is in consonance with expected outcome since artemether is an established antimalaria, and its efficacy had been confirmed (Ibrahim et al., 1993; Malenga et al., 2005)

However, when artemether was administered concurrently alongside ascorbic acid the rates of parasite clearance of artemether were observed to be compromised and this effect was noticeable at high dose of ascorbic acid. In all the three groups of artemether/ascorbic acid treatment total parasite clearances were however observed on day 2 to day 5 of drug treatment.

This observed interaction (antagonism) between artemether and high dose of ascorbic acid was further confirmed when average % parasitaemia reduction was evaluated for each of the treatment groups under consideration. The particular interaction observed for the concurrent administration of artemether and high dose ascorbic acid was in line with earlier work carried out by Meshnick *et al.* (1989) in which it was shown that antioxidant vitamins (such as α -tocopherol and ascorbate) interfere with the antimalarial activity of artemisinin derivatives. Likewise, a study carried out by Oreagba and Ashorobi (2007), showed that in vivo, retinol (another antioxidant) impairs the antiplasmodial activity of dihydroartemisinin (an artemisinin derivative) against *P. yoelii*. Another reference point is the result obtained by Awodele *et al.* (2007) in their study of the antagonistic effect of vitamin E on the efficacy of artesunate against *Plasmodium berghei* infection in mice.

Furthermore, evaluation of possible therapeutic activity of ascorbic acid doses in suppression of rate of malarial parasite growth was found to be statistically significant ($p < 0.05$) at both low and high doses when compared with the control. This observed impairment of rapid development of parasitaemia for high dose ascorbic acid is in line with “**mega dose ascorbic acid concept**” which states that ascorbic acid is capable of suppressing parasitaemia at high dose.

CONCLUSION

This study has shown that in vivo ascorbic acid impairs the activity of artemether in the clearance of *P.berghei*. This pharmacodynamic interaction observed in mice therefore forbid co-administration of the two pharmacological agents hence pharmacists and prescribers should advice their patients to complete antimalaria use before commencing with ascorbic acid if need be. Also ascorbic acid when used in high dose has been shown to suppress the rate of progression of parasitaemia in infected mice.

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