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Effect of Chloroquine and Ascorbic Acid Interaction on the Oxidative Stress Status of *Plasmodium berghei* Infested Mice

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Abstract: This study was designed to access the interaction of chloroquine and ascorbic acid in parasite induced oxidative stress, with the aim of ascertaining the relevance of such interaction in the treatment of malaria infection. A total of forty mice comprising of twenty males and twenty females were admitted for this study. Each sex category was divided into four groups of five mice and drugs administered intraperitoneally (ip). The presence of parasitemia in mice induced stress in subjects and elevated significantly ($p < 0.05$) the values of all parameters under consideration except Superoxide dismutase (SOD) activity that decreased ($p < 0.05$) in male and female mice. Chloroquine treatment increased ($p < 0.05$) SOD, Alanine and Aspartate aminotransferases (ALT and AST) respectively in both sexes as against control mice. Combination treatment with chloroquine and ascorbic acid reduced ($p < 0.05$) Malondialdehyde (MDA) concentration in female mice and increased ($p < 0.05$) SOD, AST and ALT compared to control mice. The same treatment increased ($p < 0.05$) the activities of AST and ALT in male mice. Chloroquine used in single or in combination with ascorbic acid in the recommended dosage does not appear toxic to mice. A combination of these drugs shows the potential to reduce significantly parasite induced oxidative stress in female mice.

Key words: Chloroquine, ascorbic acid, malondialdehyde, oxidative stress, *Plasmodium berghei*

INTRODUCTION

Malaria still remains one of the most deadly infectious diseases in African. The World Health Organization (WHO) data approximates 270 million clinical cases of malaria globally resulting in a significant number of deaths each year. The strength of malaria infection is attributed to the development of anaemia that derives from oxidative stress^[1, 2]. Malaria infection place both host and parasite under oxidative stress by increasing the level of Peactive Oxygen Specie (ROS) produced by activated neutrophils in the host and during degradation of haemoglobin in the parasite^[3]. Malaria parasites are highly susceptible to alterations in the redox equilibrium of its system and environments and this is suggested to offer a great potential for the development of novel chemotherapeutic strategy^[4].

Chloroquine the traditional first line treatment for uncomplicated malaria is becoming less effective as a result of the advent and prevalence of chloroquine resistant malaria parasite^[5]. Combination therapy for malaria infection has been advocated to improve the efficacy and delay the development and spread of the drug resistant strains. In line with this advocacy. The use

of safe combination therapies have been reported^[5,6]. The use of this combination therapy has been reported to be severely restricted by cost^[7] especially in African where malaria is endemic. Malaria parasites are susceptible to oxidative stress and nutritional manipulation of host antioxidant nutrients such as riboflavin or ascorbic acid may influence the course of malarial infection under certain condition^[8].

The ability of ascorbic acid to ameliorate oxidative stress induced during normal aerobic respiration without parasitemia in mice has been reported^[9]. It is however not known if ascorbic acid in combination with other cheap and readily available antimalarial drug such as chloroquine will have same effect in parasitized conditions.

This is the backdrop against which this research was designed, with the aim to determine the influence of chloroquine and ascorbic acid combination therapy in mice exposed to malaria parasites.

MATERIALS AND METHODS

Experimental animals: Forty albino mice comprising twenty males and twenty females each aged between

4-8 weeks, bred at the animal house unit of Ambrose Alli University, College of Medicine, were used as subjects for this study. This unit approved the admission of these subjects into experimentation. The animals were observed for seven days for any sign of ill health. Those that showed any sign of weakness were excluded and replaced. Each category of mice was divided into four groups of five mice. Subjects were allowed free access to feed (Grower's mash from Bendel feeds and Flourmills Ltd.) and water. At the end of the experiment, the mice were anaesthetised with chloroform and blood collected by cardiac puncture into sample tubes from where serum used for assay was harvested after clotting and centrifugation.

Parasites: Plasmodium berghei (ANKA strain) sensitive to chloroquine was maintained in the laboratory by syringe passage of parasitized blood into mice. A standard dose of 10^4 parasitized red blood cell (RBC) was inoculated intraperitoneally and parasitemia assessed from the parasitized mice by Giemsa-stained thin blood films^[10].

Test drugs preparations and administration: Chloroquine phosphate 500 mg tablet containing 300 mg chloroquine base (NAFDAC Reg. No. 04 2601) manufactured by Swiss Pharma Nigeria Ltd. was used. Each tablet was dissolved in 100 mL of distilled water and the resulting solution centrifuged to obtain clear chloroquine solution. Three milliliters of ascorbic acid containing 100 mg/5 mL w/v (NAFDAC REG NO. 04-0262) manufactured by Emzor Pharmaceutical industries Ltd. Lagos was made up to 60 mL with sterile distilled water. These preparations brought the active component of each drug to 3 mg mL⁻¹ these were administered intraperitoneally (25 mg kg⁻¹ BW) for three days.

Biochemical assay: Randox laboratories kits (Randox UK) were used to assay for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) activities^[11,12]. Malondialdehyde (MDA) was assayed as described by Gutteridge and Willins^[13]. Superoxide dismutase and catalase activities were assayed using the methods described by Misra and Fridovich^[14] and Cohen *et al.*^[15].

Statistical analysis: Data collected from this study was subjected to Analysis of Variance (ANOVA) using computer software (InStat, Graphpad Software, San Diego, CA). $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Chloroquine treatment increased ($p < 0.05$) the activities of Superoxide Dismutase (SOD), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in male and female mice compared to control (Table 1 and 2). The activity of SOD was observed to be significantly higher in mice group treated with chloroquine compared to mice SOD activity before treatment with chloroquine in male and female mice (Table 1 and 2). Combination treatment with chloroquine and ascorbic acid in mice shows increases ($p < 0.05$) in AST and ALT in both sex categories of mice compared to control. Significant increase in SOD activity after treatment was observed in female mice, in addition to malondialdehyde (MDA) concentration that reduced significantly (Tables 2).

The presence of parasitemia in mice is seen to increase ($p < 0.05$) all parameters considered in this experiment before treatment except SOD activity that reduced ($p < 0.05$) in both mice categories. The reduction

Table 1: Changes in malondialdehyde and enzyme activities in *P. berghei* infested male mice

Parameters	Control	Before treatment	Chloroquine treatment	Chloroquine and Ascorbic acid treatment
Malondialdehyde (nM mL ⁻¹)	2.22±0.22 ^{a,c}	4.68±0.07 ^b	2.34±0.17 ^c	1.96±0.06 ^a
Superoxide dismutase (Units/ mg Protein)	44.58±0.16 ^a	39.75±0.46 ^b	46.92±0.14 ^c	45.31±1.23 ^a
Catalase (Units/min)	1.38±0.13 ^a	2.73±0.14 ^b	1.32±0.16 ^a	1.27±0.08 ^a
Aspartate aminotransferase (Units/L)	39.12±0.71 ^a	44.28±0.94 ^b	42.08±0.34 ^c	42.23±0.22 ^c
Alanine aminotransferase (Units/L)	34.90±0.57 ^a	37.64±0.12 ^b	36.96±0.42 ^c	36.92±0.18 ^c
Gamma glutamyltransferase (Units/L)	43.16±1.03 ^a	46.69±0.17 ^b	43.96±0.11 ^a	43.24±0.39 ^a

Mean±SD of triplicate determinations: Values in the same row with different superscripts are significantly different

Table 2: Changes in malondialdehyde and enzyme activities in *P. berghei* infested female mice

Parameters	Control	Before treatment	Chloroquine treatment	Chloroquine and Ascorbic acid treatment
Malondialdehyde (nM mL ⁻¹)	2.34±0.05 ^a	4.66±0.08 ^b	2.35±0.19 ^a	1.94±0.19 ^c
Superoxide dismutase (Units/ mg Protein)	43.90±0.43 ^a	40.15±0.27 ^b	45.43±0.23 ^c	48.88±0.26 ^c
Catalase (Units/min)	1.40±0.09 ^a	2.70±0.12 ^b	1.50±0.22 ^a	1.26±0.08 ^a
Aspartate aminotransferase (Units/L)	37.97±0.11 ^a	44.40±0.18 ^b	39.18±0.29 ^b	40.33±0.16 ^b
Alanine amino transferase (Units/L)	34.54±0.23 ^a	36.68±0.22 ^b	36.56±0.11 ^c	36.84±0.22 ^d
Gamma glutamyltransferase	43.12±0.33 ^a	46.60±0.42 ^b	43.50±0.29 ^a	43.20±0.30

*Mean±SD of triplicate determinations: Values in the same row with different superscripts are significantly different

in SOD activity in comparison to control mice may suggest that parasitemia in mice may not automatically trigger the synthesis of superoxide dismutase. It may rather point to the fact that the oxidative stress induced as seen in the rise in malondialdehyde concentration before treatment may have necessitated the use of SOD to its threshold. The threshold dose not appear to have been exceeded probably because at this stage of infection, parasitemia in circulation is brief before invading hepatocytes^[16]. The substrate for this enzyme may have emerged as a result of xenobiotics metabolism. The observed increases in mice catalase activities may be as a result of cell mediated immunity involving neutrophils and macrophages that may have provided several highly reactive molecules and ions such as hydrogen peroxide, hydroxyl ions and oxygen ions^[17].

Chloroquine treatment as observed in this study did not restore SOD activity to normal. This is contrary to previous finding of Sarin *et al.*^[18]. These researchers though, did not consider SOD activity in their study. The reason for the observed increase in SOD activity in this study may be as a result of the combined effect of parasitemia and chloroquine metabolism through the biotransformation pathway that may produce Superoxide anion.

The involvement of the liver in malaria infection as shown by increases in the activities of aspartate and ALT and GGT^[19]. Chloroquine treatment on mice has previously failed to show any significant changes in ALT, AST and AP activities but histopathological studies showed cell necrosis and these researchers could not conclude that chloroquine is not toxic to mice^[10]. In this study however, the activity of GGT was used to confirm liver function because, it is reported to be a valuable screening test with a high negative predictive value for liver disease^[20]. The significant increases in the activities of AST and ALT after treatment with chloroquine and a combination of chloroquine and ascorbic acid may not have necessarily arose from liver damage. Because, moderate increases in the activities of these enzymes as observed in this study are found in many other diseases^[21]. Especially in organs as kidney, spleen and lungs that are known to extensively sequester chloroquine^[22]. The fact that gamma glutamyltransferase is with increased in the sera of both males and females exclude the possibility of liver damage.

The increased activity of SOD in groups that received chloroquine and combination treatment in female mice and the male group that received chloroquine only is not followed by the expected concomitant increase in catalase activity compared to control mice. The reason for this observed result may be due to the fact that, it is that the

same site that produces superoxide anion for dismutation to hydrogen peroxide the substrate for catalase and glutathione peroxidase^[23]. The latter enzyme activity was not considered in this study and the possibilities exist that this enzyme may have been involved in the utilization and conversion of hydrogen peroxide. In addition this enzyme have been reported to react with superoxide and hydroxyl radicals^[24].

Combination treatment with chloroquine and ascorbic acid reduced malondialdehyde concentration in female mice. The ability of this combination to reduced lipid peroxidation may be attributed to the reducing power of ascorbic acid. The interaction of ascorbic acid with oxygen radical that may abstract hydrogen atom from membrane lipid are probably converted to hydrogen peroxide and dehydroascorbic acid this suggested reaction pathway does not appear to be feasible in male mice. This may perhaps the due to variation in the physiology of the subjects.

On the basis of this assay we cannot conclude that chloroquine is toxic to mice when administered in the recommended dosage. On the other hand, chloroquine-ascorbic acid combination may have good potential to reduce significantly parasite induce oxidative stress without any form of liver toxicity.

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