



Chloroquine and Vitamin Combination Effects on *P. berghei* Induced Oxidative Stress

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Authors' contributions

This work was carried out between the Authors HOTI and AOO. Author HOTI designed and conducted the study, including the manuscript draft. Author AOO supervised all aspect of the work from study design to manuscript draft revision. Both Authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: The effect of chloroquine, folic and ascorbic acid on malaria parasite induced oxidative stress was the focus of this study. The study relevance derives from the need to understand the specific roles of these individual organic acids used in combination with chloroquine.

Study Design: The design involves five groups of control (non-parasitized-nontreated), parasitized nontreated (PnT), parasitized chloroquine and ascorbic acid treated (Pcq+asT), parasitized chloroquine and folic acid treated (Pcq+faT) and parasitized chloroquine, ascorbic and folic acid treated (Pcq+asT+faT).

Place and Duration of Study: Department of Biochemistry Ambrose Alli University (Faculty of Natural Sciences). This study is part of a research that lasted three years.

Materials and Methods: Treatment regime was for three days after parasitemia in mice was established with Gemsa stain. All biochemical and haematological parameters assayed for in this project were conducted using standard procedures.

Result: Chloroquine and vitamin treatments significantly ($P=.05$) reduced erythrocyte fragility (EF), total bilirubin and increased packed cell volume (PCV) when compared with PnT parameters of mice. Treatments significantly ($P=.05$) increased serum albumin compared with control and had no effect on the serum albumin levels of PnT mice. Treatment with cq+asa and cq+as+fa resulted in significant ($P=.05$) oxidative stress in

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mice compared to control but reduced ($P=.05$) oxidative stress in comparison with PnT mice. Exceptionally, chloroquine and folic acid treatment did not show any significant change in oxidative stress and superoxide dismutase activity in mice when compared with control.

Conclusion: The results suggest chloroquine and folic acid treatment to be more effective than ascorbic acid or other combination treatment employed in this study in the management of malaria induced oxidative stress.

Keywords: Chloroquine; ascorbic acid; folic acid; *P. berghei*.

1. INTRODUCTION

Malaria is a major contributor to morbidity and mortality in Africa due to red blood cell rupturing effect of the parasites that precipitates anaemia, a common complication in both acute and chronic malaria [1]. The emergence of chloroquine resistant malaria parasites is a phenomenon in malaria endemic zones. This phenomenon has been monitored by the World Health Organization (WHO), a body that released protocols for assessing the efficacy of antimalarial drugs. These protocols reflected recognition that parasitologic resistance does not necessarily imply therapeutic failure, and that policy decisions about antimalarial drug use may be best guided by experimental outcomes [2].

In sub-Saharan Africa where malaria, is highly prevalent, poor nutritional status accounts for a large proportion of deaths in children and infants. The protective role of vitamin supplements against morbidity and mortality from infectious diseases particularly malaria suggests that supplementation could also ameliorate the adverse consequences of such conditions among infants [3]. There have been reports suggesting individual vitamin supplements to be crucial in improving malaria treatment outcomes in animal models [4,5,6,7]. It has been shown that ascorbic and folic acid combinations in malaria infection may reduce lipid peroxidation and stimulate cellular pathways that enhance the production of high concentrations of hydrogen peroxide [8].

Arising from this background, there is the need to examine the combine effects of the vitamins that have been used in previous studies [5,7], in combination with antimalarial particularly chloroquine. The outcome of this project may provide empirical information as to the choice of drug intervention that could produce better modulating effect in the management of malarial infection in endemic zones. It is with this rationalization that this research was designed to investigate the effect of chloroquine, folic and ascorbic acid combination effect on malaria parasite induced oxidative stress.

2. MATERIALS AND METHODS

2.1 Animals

Fifty albino male mice of eight old weeks were used in this study. Observation protocols and method used for maintaining ANKA strains of *Plasmodium berghei* in our laboratory had been previously reported [7]. The animals used in this study, were treated and handled in the most humane manner. Five groups of ten mice each respectively categorized as control (non-parasitized-nontreated), parasitized nontreated (PnT), parasitized chloroquine and ascorbic acid treated (Pcq+asa), parasitized chloroquine and folic acid treated (Pcq+fa) and

parasitized chloroquine, ascorbic and folic acid treated (Pcq+asa+fa) were used in this investigation. Feed and water were given freely. Sera used for assay were harvested as previously described [7].

2.2 Drug Preparation and Administration Procedures

Chloroquine phosphate 500mg-tablet containing 300mg-chloroquine base manufactured by Swiss Pharmaceutical Nigeria, was used. Each tablet was dissolved in 100ml of distilled water and the resulting mixture centrifuged to obtain clear chloroquine solution. Ascorbic acid and folic acid were obtained from Emzor Pharmaceutical and Mopson Pharmaceutical respectively (Nigeria). Solutions of ascorbic and folic acids were prepared by diluting 3mL of ascorbic acid mixture (NAFDAC REG NO. 04 -0262) containing 100mg/5mL with sterile distilled water to a final volume of 60mL. Folic acid solution was prepared by diluting 12mL of folic acid mixture (NAFDAC cert. No: 04-4714) containing 2.5 mg/5 ml w/v was diluted with equal volume of sterile distilled water, making the active components in each drug 3 mg/mL. These drugs were administered intraperitoneally (25 mg/kg b.w.) for three days during infection, after establishing the presence of parasites in mice with Giemsa stain.

2.3 Biochemical Assays

Haematological and serum assays for lipid peroxidation [Malondialdehyde (MDA)], superoxide dismutase (SOD) activity, catalase activity, assay of reduced glutathione (GSH) levels, glucose-6-phosphate dehydrogenase activity (G6PD), gamma glutamyltransferase activity (GGT), Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) assay, serum bilirubin (total, conjugated and unconjugated), Total serum albumin and proteins, Globulin concentration and erythrocyte fragility were respectively determined as previously described [8].

2.4 Data Collection and Statistical Analysis

Assay for each parameter was performed in triplicate and the mean and standard deviation computed. The raw data were subjected to one factor analysis of variance (ANOVA) using computer software (InStat, Graphpad Software, SanDiego, CA). $P < 0.05$ was considered significant. LSD was used to determined differences in means at 95% confidence interval.

3. RESULTS AND DISCUSSION

Erythrocyte destruction by malaria parasite was reduced ($P=.05$) by treatment with a combination of chloroquine and vitamins (Table 1). The observed reductions in erythrocyte fragility were pronounced in mice groups administered chloroquine and vitamin combination treatments, with no significant differences among the groups. The packed cell volume (PCV) of the group administered chloroquine and vitamin combination was elevated ($P=.05$). The effect of treatments on total, direct and indirect bilirubin did not follow a clear pattern. In malaria infection both host and parasite are under oxidative stress. Increased levels of reactive oxygen species (ROS) are produced by activated neutrophils in the host and during degradation of hemoglobin in the parasite. Oxidative stress plays a vital role in the development of malarial anemia as seen in the increased levels of bilirubin in PnT mice. In this study, chloroquine and folic acid appears to be better at alleviating the bilirubin effects caused by *P. berghei* compared with other treated groups.

The observed increase in bilirubin level of chloroquine and ascorbic acid treated mice may perhaps be attributed either to the interaction between the malaria parasites that cause increased degradation of haemoglobin or as a result of metabolism arising from the presence of both chloroquine and ascorbic acid within the cellular milieu of the experimental animals. The reductions in total protein and globulin levels in ascorbic acid and folic acid treatments as compared to PnT mice indicate that treatment of chloroquine with these acids could cause drug interaction in the bone marrow that reduced production of immunoproteins in parasitized and treated mice, as ascorbic acid has been implicated in iron absorption in cells and tissues such as the bone marrow [9,10] that requires this mineral for blood cells formation, as confirmed in this study by the recorded increase in packed cell volume (PCV). It does appear from data collected that ascorbic acid and folic acid may employ similar mechanism in reducing serum total protein and globulin in parasitized mice. There are several reported evidence indicating that physiochemical changes in membrane associated with oxidative stress are responsible for the membrane lipids peroxidation products (LPPs) and haemolysis seen in malaria infection [11]. The high erythrocyte fragility and reduced PCV recorded in PnT mice may be attributed to such changes.

Treatments increased ($P=.05$) total protein and albumin levels of mice. These increases both in experimental and PnT groups were higher compared to control group, with no significant effect of treatment on liver enzymes (Table 2).

The decrease in oxidative stress in ascorbic acid supplemented group may be due to the fact that the most potent membrane antioxidant α -tocopherol is probably being used due to this supplementation as ascorbic acid can supply required reducing equivalents to tocopheroxyl radical [12]. Folic acid is capable of sequential double reduction by folate reductase to H_2F and H_4F respectively making the molecule become rich in reducing equivalent as it carries four as against the two possessed by ascorbic acid that has same hydrophilic property as folic acid. This may be the mechanism by which folic acid interacts to cause a reduction in membrane lipid peroxidation as compared to control mice. Unlike ascorbic acid and folic acid used separately, a combination of both does not appear to synergize the effect of the respective acid.

Malaria parasite and treatment impacted significantly on oxidative stress level in mice. The exception is the group treated with chloroquine and folic acid combination which maintained a non significant level of oxidative stress and superoxide dismutase activity in comparison with control group (Table 3).

Treatments of mice generally had the same pattern of reductions ($P=.05$) in catalase activity, glucose-6-phosphate dehydrogenase, and reduced glutathione compared to control, but as against PnT group. The reduced activity in plasma SOD and increased activity of CAT observed in this study is unexpected as the enzymes are known to act in concert. An increase in the activity of SOD is often accompanied by an increase in CAT activity. The result of this work did not follow this pattern.

Table 1. Changes in some haematological and biochemical indices due to chloroquine and vitamin administration

Parameters	Control*	PnT*	Pcq+asa	Pcq+fa	Pcq+asa+fa
Erythrocyte fragility (%)	0.00 ± 0.00a	37.15 ± 0.77b	13.83 ± 0.59c	12.99 ± 0.78c	12.36 ± 0.59c
Packed cell volume (%)	42.71 ± 2.17a	26.83 ± 2.33b	36.69 ± 1.78c	37.23 ± 2.12c	37.41 ± 1.76c
Total bilirubin (mg/dL)	0.16 ± 0.02a	0.76 ± 0.12b	0.26 ± 0.03c	0.23 ± 0.05d	0.21 ± 0.04d
Direct bilirubin (mg/dL)	0.10 ± 0.01a	0.68 ± 0.11b	0.20 ± 0.01c	0.21 ± 0.04c	0.16 ± 0.05a
Indirect bilirubin (mg/dL)	0.06 ± 0.02a	0.08 ± 0.01b	0.06 ± 0.03a	0.02 ± 0.01c	0.05 ± 0.03a

*- Source: Iyawe and Onigbinde, 2010

Mean ± SD triplicate determinations (n=10). Values in same row with different letters are significantly different (P=.05)

Table 2. The effects of chloroquine and vitamin combinations on plasma proteins and liver function enzymes

Parameters	Control*	PnT*	Pcq+asa	Pcq+fa	Pcq+asa+fa
Total protein (g/L)	67.72 ± 4.17a	89.43 ± 6.24b	70.28 ± 2.65a,c	70.02 ± 2.71a,c	71.59 ± 2.03c
Albumin (g/L)	36.63 ± 2.15a	39.14 ± 3.48b	38.52 ± 1.62b	38.62 ± 1.60b	37.78 ± 2.59b
Globulin (g/L)	31.09 ± 2.65a	50.29 ± 6.18b	31.67 ± 3.65a	31.09 ± 4.34a	33.81 ± 3.89a
AST activity (U/L)	32.07 ± 5.41a	36.55 ± 4.93a	34.48 ± 5.14a	32.76 ± 6.55a	33.10 ± 7.31a
ALT activity (U/L)	28.17 ± 4.94a	30.67 ± 2.86a	28.67 ± 3.41a	28.34 ± 5.98a	29.17 ± 5.46a
GGT activity (U/L)	12.22 ± 2.34a	14.27 ± 2.44a	13.06 ± 2.56a	13.61 ± 3.27a	13.98 ± 3.26a

*-Source: Iyawe and Onigbinde, 2010

Mean ± SD triplicate determinations (n=10). Values in same row with different letters are significantly different (P=.05).

Table 3. Chloroquine and vitamin combinations effects on oxidative stress indicators in mice

Parameters	Control*	PnT*	Pcq+asa	Pcq+fa	Pcq+asa+fa
Malondialdehyde (nmole/mL)	3.36 ± 0.71a	6.54 ± 0.45b	4.39 ± 0.37c	3.54 ± 0.20a	4.75 ± 0.33dc
Superoxide dismutase (U/L/min)	148.72 ± 10.81a	58.18 ± 18.78b	127.27 ± 8.22c	141.03 ± 13.51a	116.87 ± 13.69c
Catalase (U/L/min)	199.70 ± 0.14a	166.27 ± 5.92b	196.94 ± 0.17c	197.15 ± 0.20c	195.57 ± 1.23c
Glu-6-P- dehydrogenase (U/L)	29.97 ± 0.78a	33.55 ± 1.68b	27.65 ± 1.55c	27.17 ± 1.77c	27.78 ± 1.99c
Reduced glutathione (ug/mL)	3.83 ± 0.19a	3.48 ± 0.19b	2.37 ± 0.20c	2.35 ± 0.35c	2.32 ± 0.31c

*- Source: Iyawe and Onigbinde, 2010

Mean ± SD triplicate determinations (n=10). Values in same row with different letters are significantly different (P=.05).

4. CONCLUSION

Considering the results and observations made in this study, it is reasonable to draw the conclusion that chloroquine and folic acid treatment could possibly interact to cause an inhibition of parasite induced superoxide anion formation probably through a process of complex formation that may at the same time possibly induced other cellular pathways that generated hydrogen peroxide.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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