CHLOROQUINE AND VITAMIN TREATMENTS ON THE ANTIOXIDANT STATUS OF CRITICAL TISSUES OF MALARIA INFECTED MODELS

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Abstract

Objective: To assess chloroquine and vitamin combination treatment on liver, kidney and heart muscles of malaria parasite challenged mice, in order to evaluate the influence of such treatment regime on the antioxidant status of these organs. **Method:** This study was a 5 by 10 model design, comprising of non-parasitized-nontreated (control), parasitized-non-treated (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). Treatment of challenged animals was for three days after parasitemia was established with Gemsa stain. All biochemical indicators assayed for in this investigation were conducted using standard procedures. **Result:** Collated data shows that parasitemia and treatment regime had no significant ($p<0.05$) effect on heart tissues. Parasitemia in liver tissues caused significant ($p>0.05$) increases in superoxide dismutase (SOD) activity, catalase (CAT) activity, glucose-6-phosphate dehydrogenase (G6PD) activity and reduced glutathione (GSH) in comparison with control and treatment of test groups showed significant ($p>0.05$) reduction in G6PD activity in comparison with control and PnT groups. In kidney tissues there were reductions ($P<0.05$) in GSH molecules in comparison with control and PnT. **Conclusion:** The treatment regime under this condition has the potential to induce high liver G6PD activity and a low GSH concentration in kidney tissues, with its attendant consequences.

Keywords: *P-berghei, ascorbic acid, folic acid, liver, kidney, heart muscle.*

Introduction

Malaria is defined as an acute or chronic disease caused by Plasmodium parasite species. Early diagnosis and prompt treatment are the fundamental components of WHO global strategy for malaria control. The use of antimalarial drugs for chemoprophylaxis, prevention and treatment of uncomplicated malaria had been reviewed at a WHO informal consultation, which also considered diagnosis and the principles of clinical management, resulting to the use of existing and new antimalarial drugs alone, or in combination [1]. The concerns of WHO in the treatment of malaria is largely tied with antimalarial drug resistance that has spread and intensified, leading to decline in the efficacy of the most affordable antimalarial drugs [2, 3],

particularly chloroquine. In spite of reported cases of resistance to malarial parasites, chloroquine, a synthetic derivative of 4-aminoquinoline remains one of the drugs of choice for the control and cure of malaria infection [4]. In addition, there are reports indicating the re-emergence of chloroquine sensitive *P. falciparum* strains in endemic zones [5, 6, 7]. Available data show that chloroquine is concentrated in the liver and in many other tissues following its administration. Furthermore, in toxic doses it is known to cause appreciable cellular damage to liver, kidney and heart muscle [8].

There are considerable reports in literatures of the effects of chloroquine on tissues. Examples of such that include the effect on cat retina [9], liver weight of developing rats [10], on testes [4], on muscle, spleen and brain [11], on the histology of the heart [8], and on mice liver, kidney and heart muscle [12]. It is important to note that these studies focused mainly on chloroquine alone. Since there is a report of chloroquine toxicity on tissues[8], it is in the light of the foregoing necessary to ascertain the influence of some common vitamins in tandem with chloroquine administration in such tissues. From this hindsight, this research was designed to generate empirical data as to how chloroquine and vitamin treatments may impact on the antioxidant status of the liver, kidney and heart muscles of malaria infected mice.

Material and Methods
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, and had been previously reported [13]. 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+asT), parasitized chloroquine and folic acid treated (Pcq+fa) and parasitized chloroquine, ascorbic and folic acid treated (Pcq+asT+faT) were used in this investigation. Feed and water were given freely. Sera used for assay were harvested as previously described [13].

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 (100mg/5mL) with sterile distilled water to a final volume of 60mL. Folic acid solution was prepared by diluting 12mL of folic acid mixture (2.5 mg/5 ml w/v) and 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.

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Table 2: Treatment regime on some antioxidant enzymes and molecules of P. berghe infected liver.

Parameters	Control	PnT	Rcq+asTs	Pcq+faT	Pcq+faT
Malondialdhyde (nmole/ mg)	074 ± 0.14a	0.79 ± 0.12a	0.80 ± 0.11	0.80 ± 012a	0.82 ± 0.10a
Superoxide Dismutase (U/L)	57.32 ± 12.53a	66.67 ± 8.26b	58.17 ± 4.93a	57.57 ± 4.51a	60.25 ± 5.19a
Catalase (U/L)	38.57 ± 4.71a	44.46 ± 3.06b	41.92 ± 2.23b	40.52 ± 2.36b	42.29 ± 2.94b
Glu-6-P-D (U/mg)	0.07 ± 0.01a	0.05 ± 0.01b	0.06 ± 0.01c	0.06 ± 0.01c	0.06 ± 0.00c
Reduced Glutathione (ug/mg)	0.69 ± 0.10a	0.58 ± 0.10b	0.72 ± 0.04ac	0.76 ± 0.05c	0.74 ± 0.05a

Mean ± SD triplicate determination (n=10). Values in same row with different letters are significantly different (P<0.05).

Tissue Extracts Preparation and Biochemical Assays

Kidney, liver and heart tissues of mice were obtained as previously described [12]. Lipid peroxidations, Superoxide dismutase activity, Catalase activity, Assay of glutathione levels, glucose–6–phosphate dehydrogenase activity (G6PD), were respectively determined as earlier reported [14].

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. Least Significant Difference (LSD) was used to determined differences in means at 95% confidence interval.

Tables 3: Treatment regime on some antioxidant enzymes and molecules of P.berghei infected kidney.

Parameters	Control	PnT	Rcq+asTs	Pcq+faT	Pcq+faT
Malondialdhyde (nmole/ mg)	0.66 ± 0.20a	0.71 ± 0.13a	0.67 ± 0.17a	0.68 ± 0.16a	0.72 ± 0.12a
Superoxide Dismutase (U/L)	42.04 ± 8.24a	41.70 ± 7.73a	40.18 ± 4.89a	40.56 ± 4.98a	41.66 ± 4.89a
Catalase (U/L)	24.49 ± 1.30a	24.31 ± 1.06a	24.73 ± 0.80a	24.79 ± 0.97a	24.79 ± 1.01a
Glu-6-P-D (U/mg)	0.04 ± 0.00a	0.04 ± 0.00a	0.04 ± 0.01a	0.04 ± 0.00a	0.04 ± 0.00a
Reduced Glutathione (ug/mg)	0.36 ± 0.02a	0.38 ± 0.02a	0.034 ± 0.03c	0.31 ± 0.02c	0.31 ± 0.02c

Mean ± SD triplicate determinations (n=10). Value in same row with different letters are significantly different (P<0.05).

Results and Discussion

Parasitemia and treatments of infected mice groups did not show significant ($p < 0.05$) effect on heart muscle (Table 1). The vertebrate heart is composed of involuntary cardiac muscle (myocardium) found only within this organ. Its main function is to contract and pump blood out of the heart and then relaxes as the heart refills with returning blood [15]. The contractile actions of muscles are known to be due largely to availability of Ca^{2+} in muscle fibres. The pumping of parasitized blood for distribution through the heart to other parts of mice system may not likely induce any form of oxidative damage to the heart tissues and this perhaps accounted for the pattern of result obtained for heart tissues, particularly when the tissue is not directly involved with xenobiotics metabolism. A similar study using chloroquine alone was reported not to have adverse effect on mice testes [16].

Parasitemia in mice had no significant change in lipid peroxidation in terms of malondialdehyde concentration in liver tissues. It induced significant ($p < 0.05$) increase in SOD activity compared to control group (Table 2). Treatments with chloroquine and vitamins used alone or in combination did not significantly affect SOD activity compared to control, but reduced ($p < 0.05$) liver SOD in comparison with PnT. The same result trends observed for SOD activity were also seen for CAT and G6PD enzymes, with the exception that there were increases ($p < 0.05$) in CAT activity and decrease in G6PD activity respectively in treated groups. Significant increase in GSH was established for cq+faT treated mice compared with other treated groups (Table 2). The kidney antioxidant affected by treatment is GSH that indicated ($p < 0.05$) a reduction in treated groups as compared with PnT and control (Table 3).

compliance of drug dosage as chloroquine is not known to be toxic when administered within normal dosage. A report [17] using chloroquine alone reported a stabilizing effect of the drug on hepatocytes. Moreover, chloroquine was administered in tandem with ascorbic and folic acids separately and jointly. Ascorbic and folic acids are electron donating molecules, which may further protect the liver from oxidative stress, due to parasitemia. The earlier investigations were also corroborated with our findings that ascorbic and folic acids were able to reduce lipid peroxidation in *P. berghei* infectedmice [12, 13].

The increase in SOD activity in PnT group is due to parasitemia. The reason for this rationalisation derives from the fact that the immune system is mobilized in infectious state to generate free radicals against xenobiotics. The reduction of SOD activity in treated mice groups may be due to the possible synergy between chloroquine and the respective vitamins, used in this investigation. The chloroquine effectiveness is not in doubt, as chloroquine sensitive strains were used. This may have impacted greatly on parasites clearance, thereby suppressing the production of T-cells by the immune system. Additionally, the vitamins being electron donors would have contributed to alleviating the effect of parasites on liver cells, by quenching free radicals generated as a result of parasite activity. The synergism between chloroquine and vitamins impacted greatly on the liver cell to reduce SOD activity to near that of control group. However, there is a variation between this observation and an earlier reported investigation in which antimalarial treatment was found to increase liver SOD [18]. The differential in

this case is the involvement of vitamins which was absent in the former. Normally, increases in SOD activity is usually accompanied by increases in CAT activity. There is however, a discordant between liver SOD and CAT activities in treated groups. The implication may be due to the fact that the observed increases in CAT activities of parasitemia and treated groups may not be free radical linked, but rather as a consequence of other metabolic activities that produces hydrogen peroxide the required catalase substrate. The enzyme G6PD generates NADPH for reductive biosynthesis. Its reduction as observed in treated mice is an indication that malaria parasite may disrupt some cellular biosynthetic process that may be reversed with chloroquine and vitamin combination therapies.

Table 1: Treatment regime on some antioxidant enzymes and molecules of P. berghei infected heart.

Parameters	Control	PnT	Pcq+asT	Pcq+faT	Pcq+as+faT
Malondialdehyde(nmole/mg)	$0.53 \pm 0.17a$	$0.58 \pm 0.11a$	$0.56 \pm 0.09a$	$0.56 \pm 0.10a$	$0.56 \pm 0.10a$
Superoxide Dismulase (U/L)	$41.54 \pm 10.23a$	$41.34 \pm 7.63a$	$40.14 \pm 7.05a$	$41.74 \pm 6.77a$	$40.84 \pm 6.07a$
Catalase (U/L)	$24.66 \pm 1.81a$	$24.31 \pm 2.05a$	$23.92 \pm 1.65a$	$23.64 \pm 1.43a$	$23.90 \pm 1.38a$
Glu-6-P-D (U/mg)	$0.04 \pm 0.00a$	$0.03 \pm 0.01a$	$0.04 \pm 0.00a$	$0.04 \pm 0.00a$	$0.04 \pm 0.00a$
Reduced Glutathione (ug/mg)	$0.37 \pm 0.02a$	$0.37 \pm 0.02a$	$0.38 \pm 0.02a$	$0.37 \pm 0.02a$	$0.37 \pm 0.02a$

Authors' contributions

Iyawe HOT : Designed and conducted the research including preparation of manuscript. Onigbinde AO: Supervised the work design, laboratory work and preparation of manuscript.

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