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Full Length Research Paper

The role of ascorbic acid in the treatment of *Plasmodium Berghei* infected mice

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This work aimed at examining the effect of malaria parasites and ascorbic treatments in mice. The relevance of this research derives from the desire to understand the role of ascorbic acid in malaria infection. In this study design, three groups of ten mice each categorized as non-parasitized-non-treated (control), parasitized-non-treated (PnT) and parasitized ascorbic acid treated (P+asT) were used. Results collected and analyzed using adequate statistical software revealed that parasitism in mice had significant ($p < 0.05$) increases in erythrocyte fragility, total and indirect bilirubin, total protein and globulin but decreased ($p < 0.05$) mice packed cell volume (PCV). Plasma malondialdehyde (MDA) significantly ($p < 0.05$) increased while superoxide dismutase (SOD) and catalase (CAT) decreased ($p < 0.05$). Liver SOD and CAT as well as kidney MDA of parasitized non treated mice were observed to increase ($p < 0.05$) following *Plasmodium berghei* infection. Ascorbic acid treatment of parasitized mice was observed to reverse the effects of *P. berghei* in mice. The findings suggest ascorbic acid to be critical in the management of malaria parasite infection.

Key words: *Plasmodium berghei*, ascorbic acid, antioxidants, erythrocyte fragility, oxidative stress.

INTRODUCTION

In the tropics and subtropical regions of the world, the endemic nature of malaria as well as the mortality associated with the infection particularly among children under the ages of five years have been reported (WHO, 2000; Nmorsi et al., 2007).

It is also documented that malaria parasites inside erythrocytes exert oxidative stress within the parasitized red blood cells (Hunt and Stocker, 1990; Potter et al., 2005). The parasites are suggested to generate reactive oxygen species (ROS) from which they are protected (Potter et al., 2005), through one or more of the named pathways: Electron transport chain (Deslauriers et al., 1987), haemoglobin and cytosolic proteins degradation (Atamma and Ginsburg, 1993), or redox reactions of hemin (Har-El et al., 1993). The formation of ROS by malaria parasites if not checked by the host cytoprotective enzymes and antioxidants could led to oxidative damage and there are increasing evidence that injuries contribute to pathophysiology of many diseases (Gora et al., 2006).

The potential toxicity of free radical generated by malaria parasites are counteracted by a large number of cytoprotective enzymes and antioxidants. One of such antioxidant is ascorbic acid (Behrman et al., 2001), a water soluble vitamin that reacts with radicals and effectively protects cell against lipid peroxidation (Frei et al., 1990). For cell protection ascorbic acid has been reported to act as scavenger for superoxide radicals, sulphur-centred radical, singlet oxygen and hydroxyl radical (Gutteridge and Halliwell, 1994). Though the effect of ascorbic acid combination treatment with chloro-quine in malaria infection has been reported (Iyawe et al., 2006), the specific role of ascorbic acid in the management of oxidative stress occasioned by malaria parasites is not clear. Therefore, this study is an attempt to elucidate the possible specific role of ascorbic acid in oxidative stress occasioned by malaria parasites.

MATERIALS AND METHODS

Animals and parasites

Thirty albino male mice of 8 weeks were used in the study and the animals were handled in a very humane manner. Observation protocols and method used for maintaining ANKA strains of *Plasmodium berghei* in our laboratory has been previously described (Iyawe and Onigbinde, 2009).

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Procedures

Three groups of animals respectively categorized as control (None parasitized non–treated), parasitized non–treated (PnT) and parasitized but ascorbic acid-treated (P+asT) were used. The animals were allowed free access to feed on Grower's mash (from Bendel feeds and flourmills Ltd. Ewu Edo State Nigeria) and water. At the end of the experiment, the mice were anaesthetized with chloroform and blood collected by cardiac puncture into sample tubes from where plasma used for assay was harvested.

Drug preparation and administration

Three milliliters of ascorbic acid containing 100 mg/5 ml w/v (NAFDAC REG NO. 04 – 0262) manufactured by Emzor Pharmaceutical Industries Ltd., Lagos Nigeria was made up to 60 ml with sterile distilled water. The preparation brought the active component of each drug to 3 mg/ml. The drug was administered (25 mg/Kg BW) intraperitoneally for three days.

Tissue extracts preparation and assays

Kidney, liver and heart tissues of subjects were obtained as previously described (Iyawe and Onigbinde, 2009). Lipid peroxidation, superoxide dismutase activity, catalase activity, assay of glutathione 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 (Iyawe and Onigbinde, 2009).

STATISTICAL ANALYSIS

Data collected from this study 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.

RESULTS

Parasitized mice had their erythrocyte fragility, total and direct bilirubin increased significantly ($p < 0.05$) but reduced ($p < 0.05$) mice packed cell volume (PCV). Ascorbic acid treatment significantly ($p < 0.05$) reduced erythrocyte fragility, significantly ($p < 0.05$) increased PCV in mice compared to PnT animals (Table 1). *P. berghei* infected mice had their total protein and globulin significantly ($p < 0.05$) increased. Ascorbic acid treatment also reduced ($p < 0.05$) Plasma total protein and globulin levels compared to PnT (Table 2). Parasitized mice plasma MDA increased ($p < 0.05$) while SOD and CAT activities reduced ($p < 0.05$). Treatment of parasitized mice with ascorbic acid reduced ($p < 0.05$) plasma MDA while SOD and CAT enzymes increased significantly ($p < 0.05$) compared to PnT (Table 3). Parasitemia increased liver tissue SOD and CAT, including kidney MDA ($p < 0.05$). Ascorbic acid treatment of parasitized mice liver and kidney reduced kidney MDA compared to PnT and reduced ($p < 0.05$) liver SOD and CAT compared to PnT (Table 4).

DISCUSSION AND CONCLUSION

The reductions in total serum protein and globulin observed in ascorbic acid treated mice as compared to PnT mice group may perhaps indicate the contribution of the organic molecule to erythropoesis. It is most likely that ascorbic acid may perform useful function in bone marrow to enhance blood cells formation, as indicated in this study by the recorded increased packed cell volume (PCV). This may imply that the iron released as a result of parasite degradation of haemoglobin may probably be recycled for blood cells production.

Ascorbic acid and reduced glutathione are important chain breaking antioxidants responsible for scavenging free radicals and suppression of peroxidation in aqueous and lipid region of the cell (Gora et al., 2006). It is reported to exist in blood as oxidised dihydroascorbic acid (DHAA) and reduced ascorbic acid forms (RAA) and its transportation across cell membranes is in the form of DHAA, which is less ionized at physiological pH and therefore, more permeable to membrane (Mann and Newton, 1975). The decrease in oxidative stress in ascorbic acid treated group compared to PnT mice group may be that the most potent membrane antioxidant -tocopherol has probably become more effective as ascorbic acid can supply required reducing equivalents to tocopheroxyl radical (Sies, 1991), a product of -tocopherol reaction with membrane free radical. As a result, the ascorbic acid is oxidized to dehydroascorbic acid (DHAA) which is reconverted to ascorbic acid by plasma reduced glutathione. This reaction may account for the observed reductions in plasma MDA and GSH.

It could be hypothesized that the observed reduction in G6PD activity under parasitized condition may be due to G6PD enzyme inhibition by ascorbic acid. There is evidence that malarial parasites inside the erythrocytes exert oxidative stress within the parasitized red blood cell from which they are protected by a number of parasite encoded enzymes, by ascorbic acid, vitamin E, by glutathione and that parasite antioxidant defence is believed to include export of oxidized glutathione to the erythrocyte cytosol (Postma et al., 1996; Potter et al., 2005). Apart from the parasite induced ROS, phagocytic leucocytes are able to engulf infectious agents within the phagocytic food vacuole leading to the formation of the enzyme NADPH- oxidase involved in the formation of superoxide anion that is involved in the killing of the pathogen (Karupiah et al., 2000). It is therefore not unlikely that in other to control the superoxide anion and its derivatives such as hydrogen peroxide, cellular synthesis of SOD and CAT may have occurred under increased oxidative stress condition to cause an increase in the plasma levels of these enzymes and hence their observed increased activities.

Table 1. Effect of *P. berghei* and ascorbic acid treatments on some biochemical indices in parasitized mice.

Parameters	Control	PnT	P+asT
Erythrocyte fragility (%)	0.00 + 0.00[a]	37.15 + 0.77[b]	25.35 + 3.27[c]
Packed Cell Volume (%)	42.71 + 2.17[a]	26.83 + 2.33[b]	35.17 + 2.65[c]
Total Bilirubin (mg/dL)	0.16 + 0.02[a]	0.76 + 0.12[b]	0.35 + 0.07[c]
Direct Bilirubin (mg/dL)	0.10 + 0.03[a]	0.68 + 0.12[b]	0.28 + 0.04[c]
Indirect Bilirubin (mg/dL)	0.06 + 0.02[a]	0.08 + 0.01[a]	0.06 + 0.03[a]

Mean ± SD triplicate determinations (n = 10). Values in same row with different alphabet are significantly different ($p < 0.05$).

Table 2. Effect of *P. berghei* and ascorbic acid treatments on parasitized mice plasma proteins and some liver function enzymes.

Parameters	Control	PnT	P+asT
Total Protein (g/L)	67.72 ± 4.17[a]	89.43 ± 6.24[b]	70.36 ± 2.70[a]
Albumin (g/L)	36.63 ± 2.15[a]	39.14 ± 3.50[a]	38.62 ± 1.60[a]
Globulin (g/L)	31.09 ± 2.65[a]	50.29 ± 6.18[b]	31.75 ± 3.67 [a]
AST Activity (U/L)	32.07 ± 5.41 [a]	36.55 ± 4.93 [a]	35.61 ± 3.64 [a]
ALT Activity (U/L)	28.17 ± 4.93 [a]	30.67 ± 2.86 [a]	28.74 ± 3.45 [a]
GGT Activity (U/L)	12.22 ± 2.34 [a]	14.27 ± 2.43 [a]	13.07 ± 2.52 [a]

Mean ± SD triplicate determinations (n = 10). Values in same row with different alphabet are significantly different ($p < 0.05$).

Table 3. Effect of *P. berghei* and ascorbic acid treatments on MDA, glutathione and some antioxidant enzymes of parasitized mice.

Parameters	Control	PnT	P+asT
Malondialdehyde (nmole/mL)	3.36 ± 0.71 [a]	6.54 ± 0.44[b]	4.76 ± 0.54[c]
Superoxide Dismutase (U/L)	148.72 ± 10.81 [a]	58.18 ± 18.78[b]	85.21 ± 4.27[c]
Catalase (U/L)	199.70 ± 0.14 [a]	166.27 ± 5.92[b]	186.33 ± 9.16[c]
Glu-6-P Dehydrogenase (U/L)	29.97 ± 0.78 [a]	33.55 ± 1.68 [a]	31.88 ± 1.78 [a]
Reduced Glutathione (ug/mL)	3.83 ± 0.19 [a]	3.48 ± 0.19 [a]	3.49 ± 0.26 [a]

Mean ± SD triplicate determinations (n = 10). Values in same row with different alphabet are significantly different ($p < 0.05$).

The significant reductions in total serum protein and globulin levels observed in parasitized and ascorbic acid treated mice may be linked with the formation of blood cells in the bone marrow as synthesis of blood cells may reduce the stress on the surviving T-cells and its complements in producing and secreting antibody molecules in an attempt to ameliorate the effects of the infecting parasites. Kidney cells appear to be very amenable to ascorbic acid treatment under parasitized condition as treatment is observed to significantly reduce oxidative stress observed in PnT and brought values near that of control group. The reductions in liver GSH level SOD and CAT enzyme activities observed in this study are pointers to the fact that the ascorbic acid may have reacted with other molecule to inhibit these enzymes causing the reduction in their activities, therefore resulting in the utilization of cellular reduced glutathione.

From these observations a conclusion can be drawn, that under parasitized condition in mice, ascorbic acid treatment may affect erythrocytes fragility by possibly in-

Table 4. Effects of parasites and ascorbic acid treatments on MDA, glutathione and antioxidant enzymes of parasitized mice organs.

Parameters	HERAT			LIVER			KIDNEY		
	Control	PnT	P+asT	Control	PnT	P+asT	Control	PnT	P+asT
MDA(nmole/g)	0.54 ± 0.17 [a]	0.59 ± 0.09 [a]	0.58 ± 0.11 [a]	0.74 ± 0.14 [a]	0.79 ± 0.12 [a]	0.67 ± 0.15 [a]	0.66 ± 0.10[a]	0.71 ± 0.12[b]	0.61 ± 0.08 [a]
SOD (U/mg)	41.51 ± 8.22[a]	41.34 ± 7.63[a]	41.34 ± 7.63 [a]	57.32 ± 12.53 [a]	66.67 ±8.26[b]	41.64 ± 7.31[c]	41.51 ± 9.70 [a]	40.91 ± 3.16 [a]	41.34 ± 7.23 [a]
CAT (U/L)	24.89 ± 0.75[a]	24.63 ± 0.50[a]	24.63 ± 0.35 [a]	38.57 ± 4.71 [a]	44.16 ± 3.06[b]	34.32 ± 5.33 [c]	24.66 ±1.80 [a]	23.89 ± 2.47 [a]	24.31± 2.04 [a]
G6PD (U/mg)	0.04 ± 0.11 [a]	0.03 ± 0.08 [a]	0.03 ± 0.10 [a]	0.07 ± 0.01 [a]	0.05 ± 0.01 [b]	0.05 ± 0.01 [c]	0.04 ± 0.10 [a]	0.03 ± 0.08 [a]	0.03 ± 0.04 [a]
GSH (ug/mg)	0.37 ± 0.02 [a]	0.37 ± 0.02 [a]	0.36 ± 0.03 [a]	0.69 ± 0.10 [a]	0.58 ± 0.10 [b]	0.56 ± 0.20 [c]	0.37 ± 0.02[a]	0.37 ± 0.02 [a]	0.37 ± 0.02 [a]

Mean ± SD triplicate determinations (n = 10). Values in same row with different alphabet are significantly different ($p < 0.05$).

ducing erythropoesis in bone marrow and may reduce MDA formation by supplying electrons to membrane tocopherol radicals to reduce lipid peroxidation.

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