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Oxidant versus Antioxidant Activity in Malaria: Role of Nutritional Therapy

[1]O.I. Iribhogbe, [2]E.O. Agbaje, [2]I.A. Oreagba, [3]O.O. Aina and [2]A.D. Ota

Micronutrients are known to have antioxidant activity; however, its role in *plasmodial* infection is still not clearly defined. The present study involves an *in vivo* evaluation of the role of some selected antioxidant micronutrients in the therapeutics of malaria. In this study, rodent malaria model using *Plasmodium berghei* NK-65 strain (chloroquine sensitive) was used. Fourty five mice of either sex weighing 20.05±0.02 g were used for the study. Fourty mice were inoculated intraperitoneally with 1×10^7 million *Plasmodium berghei* infected erythrocyte and were administered with 0.2 mL of distilled water, 0.2 mL of vehicle; Tween 80 (control and vehicle group), chloroquine 25 mg kg^{-1} and artesunate 4 mg kg^{-1} (standard drug group), vitamin A 60 mg kg^{-1}, vitamin E 100 mg kg^{-1}, selenium 1 mg kg^{-1}, zinc 100 mg kg^{-1} (test group F, G, H and I, respectively) 72 h post inoculation. Antioxidant micronutrients demonstrated significant ($p<0.05$) chemosuppressive activity when compared with negative control during the 4 day curative test. Mean parasitemia was significantly reduced ($p<0.05$) in the micronutrient treated groups after the 4 day curative test when compared with negative control. This however, was also significant between micronutrient treated groups ($F = 17.88$; $p = <0.05$). Catalase and glutathione peroxidase activity was significantly ($p<0.05$) higher in the vitamin A, E, selenium and zinc treated groups, respectively when compared to apparently healthy uninfected control. Conclusively, antioxidant micronutrients have antimalarial activity and may be of benefit in malaria therapeutics.

Key words: Antioxidant activity, micronutrients, therapeutic potential, oxidative stress, malaria

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For further information about this article or if you need reprints, please contact:

O.I. Iribhogbe
Department of Pharmacology and Therapeutics,
College of Medicine,
Ambrose Alli University Ekpoma,
Edo State Nigeria

GSM: +2348065794437

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[1]Department of Pharmacology and Therapeutics College of Medicine, Ambrose Alli University Ekpoma, Nigeria
[2]Department of Pharmacology, College of Medicine of the University of Lagos, Nigeria
[3]Malaria Research Unit, Nigerian Institute of Medical Research, Yaba Lagos State, Nigeria

INTRODUCTION

According to report from previous data, membrane percentage polyunsaturated fatty acid and tocopherol was found to be significantly reduced in patients with malaria. This provides support for oxidative stress evidenced by the generation of reactive oxygen species, malondialdehyde and thiobarbituric acid reactive substances (Thurnham *et al*., 1990; Das *et al*., 1993). More recently, Kremser *et al*. (2000) reported an increase in ROS generation in whole blood of African children with malarial anemia compared with mild malaria subjects. Micronutrients are known to have antioxidant action *in vivo*, however there is paucity of data on the relationship between these antioxidant micronutrients and *falciparum* malaria infection in man. Previous studies in Kampala, Uganda have shown that children with acute uncomplicated *falciparum* malaria had a reduced plasma level of antioxidant vitamins at the point of recruitment which was significantly elevated on day 7 of follow-up (Amy *et al*., 2001). In the study, a higher plasma lycopene level on day 0 of recruitment was associated with increased clearance of malaria between day 0 of recruitment and day 3 of follow-up. The aim of the present study is to further elucidate the role of selected antioxidant micronutrients in malaria induced oxidative stress using animal model.

MATERIALS AND METHODS

Chemicals and equipments: Heparinised capillary tubes, Light Microscope (Olympus, Japan), EDTA bottles, Feeding trochars, Syringes (1, 5 mL), Cotton wool, Microscopic slides (Olympus, China), Hand gloves, Giemsa stain (Sigma), 98% Methanol (Sigma) and Tween 80 (sigma).

Drugs: Vitamin A (Clarion Medical Pharmaceuticals, Nigeria), Vitamin E (Clarion Medical Pharmaceuticals, Nigeria), Zinc gluconate (Mason Vitamins Incorporated USA), Selenium-organic (Mason Vitamins Incorporated USA), Chloroquine (Emzor Pharmaceuticals, Nigeria) and Artesunate (Emzor Pharmaceuticals, Nigeria).

Preparation of animals: Fourty five in bred pure Swiss albino mice of either sex weighing between 18-25 g was used for the study. They were obtained from the animal house of the Nigerian Institute of Medical Research, Yaba Lagos State and housed in stainless steel cages with wire screen top. The animals were about 7-8 weeks old and were maintained on commercial feeds (Vital feeds, Jos) and tap water *ad libitum* for the entire duration of the study. The mice were allowed to acclimatize for 1 week in the laboratory environment under a controlled temperature of 20°C and at optimum humidity before being subjected to the experiment (Obernier and Baldwin, 2006). Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from the cages daily.

Preparation of Inoculum of chloroquine sensitive strain of *Plasmodium berghei*: *Plasmodium berghei* NK 65 strain maintained in the laboratory of Nigerian Institute of Medical Research, Yaba by serial blood passage from mouse to mouse was used for the study. Donor mouse with a rising parasitaemia of 20-30% confirmed by thin and thick blood film microscopy was used. Blood (0.2 mL) was collected in a heparinized tube from the auxiliary plexus of veins in the donor mouse using heparinized capillary tubes. The blood was diluted with 5 mL of Phosphate buffer solution (PBS) pH 7.2 so that each 0.2 mL contained approximately 1×10^7 infected red cells (Peter *et al*., 1975; Fidock *et al*., 2004). Each animal received inocula of about 10 million parasites per kilogram body weight which is expected to produce a steadily rising infection in mice.

Drugs/micronutrient administration: A 4-day curative test was performed using the methods of (Peters, 1965; Peters *et al*., 1977: Peter *et al*., 1975; Agbaje and Onabanjo, 1991; Fidock *et al*., 2004). Artesunate (4 mg kg^{-1}) and chloroquine sulphate (25 mg kg^{-1}) was used as a reference drug (Tekalign *et al*., 2010). The antioxidant micronutrients were administered orally as follows; vitamin A (60 mg kg^{-1}), vitamin E (100 mg kg^{-1}), zinc (100 mg kg^{-1}), selenium (1 mg kg^{-1}) using doses based on LD_{50} values as reported by Schrauzer (2000), Oncu *et al*., (2002) and Oreagba and Ashorobi, (2006). Tween-80 (0.2 mL) was used, as vehicle for fat-soluble vitamins A and E agents. Mice were grouped into nine groups of 5 each and drug/micronutrient administration was done daily for 4 days as shown in Table 1.

At the end of the 4 day curative treatment (day 5 post treatment) blood samples were collected via the auxiliary vein into lithium heparin specimen bottles for biochemical analysis. Smears were made on microscopic slides, fixed with methanol and stained with 3% Giemsa at pH 7.2, for parasitaemia. Percentage of chemosuppression was calculated using the formula (Peter and Anatoli, 1998; Fidock *et al*., 2004):

$$\text{Suppression (\%)} = \frac{\text{Parasitemia in negative control-Parasitemia in study group}}{\text{Parasitemia in negative control}} \times 100$$

Table 1: Drug administration (per os) in the animals

Groups	Drugs/micronurients	Dosage
A: Uninfected mice (control group). Not inoculated, no drugs administered	-	-
B: Parasitized mice (negative control group)	Distilled water	0.2 mL
C: Parasitized mice (vehicle control group)	Tween 80	0.2 mL
D: Parasitized mice (positive control) treated	Chloroquine sulphate	25 (mg kg^{-1})
E: Parasitized mice (positive control)	Artesunate	4 (mg kg^{-1})
F: Parasitized mice (test group 1)	Vitamin A	60 (mg kg^{-1})
G: Parasitized mice (test group 2)	Vitamin E	100 (mg kg^{-1})
H; Parasitized mice (test group 3)	Selenium	1 (mg kg^{-1})
I: Parasitized mice (test group 4)	Zinc	100 (mg kg^{-1})

Table 2: Percentage of chemosupression in *P. berghei* Parasitized mice after 4 days of treatment

Group	Dose (mg kg^{-1})	Av % parasitaemia	Suppression (%)
Control (not inoculated/no treatment)	-	-	-
Negative control (distilled H_2 0)	0.2 mL	24.14±2.59	-
Tween-80	0.2 mL	21.82±3.42	9.61
Positive control (chloroquine)	25	0.00±0.00	100.00
Artesunate	4	0.00±0.00	100.00
Vitamin A	60	8.90±2.78	63.13
Vitamin E	100	11.61±2.78	51.90
Selenium	1	5.08±1.85	78.95
Zinc	100	9.89±1.11	59.03
One way ANOVA	F = 17.88		
	p<0.05		

Values are expressed as X±SEM. df = 4, Mean difference is significant at p<0.05

Estimation of lipid peroxidative indices: Lipid peroxidation as evidenced by the formation of malondialdehyde a marker of free radical damage was measured using the method of Niehaus and Samuelsson (1968) and Jiang *et al*. (1992) as adopted by Rukkumani *et al*. (2004).

Determination of superoxide dismutase, catalase and gluthathione peroxidase activity: Superoxide dismutase (SOD) was assayed using the method of Kakkar *et al*. (1984) as adopted by Rukkumani *et al*. (2004). A single unit of enzyme was expressed as 50% inhibition of NBT (Nitroblue tetrazolium) reduction/min/mg protein. Catalase (CAT) was assayed calorimetrically at 620 nm and expressed as µmol of hydrogen peroxide consumed/min/ mg protein as described by Rukkumani *et al*. (2004). Glutathione Peroxidase (GPx) activity was also measured by the method described. Total protein was estimated by using the protein and albumin kit from Qualigens Chemicals, Worli, Mumbai.

Data analysis: Statistical analyses of the data were performed using statistical software package SPSS version 17.0. Student's t test and one way ANOVA were used to compare the mean of laboratory data between groups. The statistical significance level was set at 95% confidence interval and p<0.05 was considered significant.

RESULTS

As shown in Table 2, mean% parasitemia was significantly (p<0.05) reduced in the micronutrient treated groups when compared with control. This was most marked in the selenium treated group (5.08±1.85%) followed closely by the zinc treated group (9.89±1.11%).

As shown in Table 3, catalase activity was significantly (p<0.05) higher when compared to uninfected control in the chloroquine (1.932±0.017 µmol/min/mg protein), artesunate (1.880±0.050 µmol/min/mg protein), vitamin A (2.374±0.170 µmol/min/mg protein), vitamin E (2.745±0.055 µmol/min/mg protein), selenium (2.754±0.056 µmol/min/mg protein) and zinc (2.467±0.106 µmol/min/mg protein) treated groups, respectively. Similarly, glutathione peroxidase activity was significantly (p<0.05) elevated in the vitamin A (5.444±0.100 µmol mL^{-1}), vitamin E (5.420±0.091 µmol mL^{-1}), selenium (6.484±0.189 µmol mL^{-1}) and zinc (5.842±0.037 µmol mL^{-1}) treated groups, respectively when compared to apparently healthy uninfected control.

Increased lipid peroxidation is associated with significant elevation of markers of oxidative stress. As shown in Table 3, malondialdehyde (MDA) levels were significantly higher in the distilled water, tween 80, chloroquine and artesunate treated groups when compared to apparently healthy uninfected control (p<0.05). There was a significant difference in antioxidant activity between groups with the exception of superoxide dismutase (F = 0.81; p>0.05). However, catalase activity was significantly higher (Table 3) in the chloroquine, artesunate, vitamin A, E, selenium and zinc treated groups while glutathione peroxidase activity was significantly

Table 3: Lipid peroxidation and antioxidant markers in *P. berghei* parasitized mice after 4 day curative treatment (n = 5 mice group^{-1})

Group	Dose (mg kg^{-1})	Parasitemia (%)	MDA (mg mL^{-1})	SOD (min mg^{-1}) protein	CAT (µmol/min/mg) protein	GPX (µmol mL^{-1})	Total protein (g L^{-1})
Control (not inoculated)	-	-	2.020±0.020	0.062±0.001	1.085±0.028	4.580±0.045	47.59±0.60
Negative control (Distilled H_2 0)	0.2 mL	24.14±2.59	*6.020±0.296	0.043±0.120	1.396±0.033	4.213±0.056	44.56±0.76
Tween-80	0.2 mL	21.82±3.42	*5.794±0.449	0.054±0.004	1.416±0.144	4.472±0.141	44.00±0.48
Positive control (chloroquine)	25	0.00±0.00	*4.340±0.068†	0.057±0.003	*1.932±0.017†	4.646±0.029	52.64±1.00
Artesunate	4	0.00±0.00	*8.200±0.491†	0.064±0.005	*1.880±0.050†	4.418±0.172	58.00±1.55
Vitamin A	60	8.90±2.78	2.060±0.024†	0.084±0.002	*2.374±0.170†	*5.444±0.100†	46.90±0.62
Vitamin E	100	11.61±2.78	2.080±0.020†	0.075±0.004	*2.745±0.055†	*5.420±0.091†	49.34±0.60
Selenium	1	5.08±1.85	2.080±0.020†	0.092±0.004	*2.754±0.056†	*6.484±0.189†	44.20±0.92
Zinc	100	9.89±1.11	2.040±0.025†	0.078±0.004	*2.467±0.106†	*5.842±0.037†	43.55±0.84
One way ANOVA		F = 19.76 p<0.05	F = 91.65 p<0.05	F = 0.81 p>0.05	F = 47.81 p<0.05	F = 48.69 p<0.05	F = 30.54 p<0.05

Values are expressed as X±SEM. *Mean difference is significant at p<0.05 when compared to control (not inoculated). †Mean difference is significant at p<0.05 when compared to negative control

higher in the vitamin A, E, selenium and zinc treated groups, respectively when compared to apparently healthy uninfected control (p<0.05).

DISCUSSION

The present study showed a marked elevation of MDA in the groups treated with distilled water and tween 80 when compared to uninfected control. This may be due to the high level of parasitemia in these groups. This suggests increased oxidative stress in malaria infection in the groups treated with distilled water and vehicle. The finding is in keeping with a recent study by Reis *et al.* (2010) in which oxidative stress markers where markedly elevated in mice with cerebral malaria.

Similarly, exercise stress and other forms of oxidative stress where found to be linked with marked increase in erythrocyte membrane fragility which was associated with an elevated level of markers indicative of lipid peroxidation of red blood cell membrane (Kelle *et al.*, 1999; Ozturk and Gumuslu, 2004). In the present study, the MDA levels were significantly higher in the artesunate and chloroquine treated groups when compared with the antioxidant micronutrient treated groups. This presupposes that oxidative stress in malaria infection may be further increased by free radicals generated from artesunate and chloroquine metabolism. Earlier studies have also demonstrated a reduction in the level of zinc, copper and endogenous antioxidants such as Glutathione Peroxidase (GPx) and superoxide dismutase (SOD). This was associated with an increase in catalase and oxidized glutathione levels (Kelle *et al.*, 1999; Ozturk and Gumuslu, 2004; Aguilo *et al.*, 2005). A slightly similar pattern was observed in the present study Which showed that no significant difference exist in superoxide dismutase level between the groups treated with standard antimalarials and the group treated with antioxidant micronutrients. However, catalase and glutathione peroxidase activity were significantly elevated in the antioxidant treated groups when compared with the control and antimalarial groups. In the present study, the elevated antioxidant activity observed in the micronutrient treated groups may be responsible for erythrocyte membrane protection. The finding of Kraus *et al.* (1997) indicated that vitamin C and E administration significantly reduced erythrocyte osmotic fragility due to oxidative damage. The presence of selenium as a component of glutathione peroxidase may be responsible for its protective effect on red blood cell membrane. However, GPx mainly distributes in the cytoplasm of erythrocytes, therefore it seems that the protective action of supplemented selenium on the isolated erythrocyte membrane might not be related to the activity of GPx alone.

CONCLUSION

The present study has demonstrated that there is increased peroxidative activity with free radical production during malaria infection which has been implicated in associated pathologies. Hence, antioxidants may play a significant role as adjunctive therapy in the management of clinical malaria.

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