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Effect of an Antimalarial and a Micronutrient Supplementation on Respiration Induced Oxidative Stress

H.O.T. Iyawe and A.O. Onigbinde
Department of Biochemistry, Ambrose Alli University, P.M.B. 14,
Ekpoma, Edo State, Nigeria
E-mail: iyawehanson@yahoo.com

Abstract: This research was designed to access the effect of chloroquine a common antimalarial and ascorbic acid a popular antioxidant, on oxidative stress and liver function in animal models, with the aim of applying the research findings to the treatment of some devastating tropical diseases. A total of forty mice comprising of twenty males and females were divided into four groups per sex category and test drugs were administered intra peritoneally (ip) in mono and combined doses to healthy mice. Chloroquine treatment increased all oxidative stress indices with catalase being significant ($P<0.05$) against control. Significant increases ($P<0.05$) were also indicated in superoxide dismutase (SOD) and in catalase activities in both sexes. Ascorbic acid generally reduced all ($P>0.05$) assayed stress indices but the reduction was significant ($P<0.05$) only in female mice as against control. A combined treatment of chloroquine and ascorbic acid did not show any significant decrease and increase in malondialdehyde (MDA) and SOD, but catalase increased ($P<0.05$). Increases ($P<0.05$) were observed in SOD and catalase activities in both male and female mice. The activities of liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined and confirmed using gamma glutamyl transferase (GGT) activity and increases ($P<0.05$) in the activity of this enzyme were observed in female mice given a combination treatment. This result indicates that ascorbic acid can ameliorate oxidative stress induced during normal aerobic metabolism in mice. Chloroquine and a combination of chloroquine and ascorbic acid treatment can adversely affect GGT in female mice.

Key words: Antimalarial, micronutrient, oxidative stress

Introduction

Oxidative stress arises when the cellular generation of reactive oxygen specie (ROS) overwhelms the antioxidant defense system. This phenomenon has been implicated in most human pathology. Oxidative challenges often arise from sources such as radiation, metabolism of xenobiotics and challenges to the immune system or abnormal immune functions (James, 1994). Though, many cellular organelles produce ROS during normal aerobic metabolism. It has been established that the mitochondria, an organelle that make ATP by coupling of respiration generated proton gradient, with the proton driven phosphorylation of ADP by F_0F_1ATPase, is the major intracellular source, as well as the vulnerable target of ROS (Yau - Huei and Hsin - Chen, 2002). It is also documented that dietary constituents including vitamins have both causative and protective role in oxidative stress status (James, 1994). Nutritional manipulation of host oxidative stress by dietary means have been found to have effect on the growth of parasite and the status of some vitamins such as riboflavin and ascorbic acid also have been found to influence the course of the infection (Levender and Ager, 1993). It has also been reported that oxidative stress experienced by cells during infection with malarial parasite can be restored to near normal after treatment with chloroquine (Sarin *et al*., 1993; Mishra *et al*., 1994). It is necessary to clarify that the mediating role being performed by chloroquine, riboflavin and ascorbic acid as suggested by the report of earlier workers are only with respect to infected cells (Levender and Ager, 1993; Sarin *et al*., 1993; Mishra *et al*., 1994). Whether these drugs will perform the same function in a normal metabolic state where ROS are equally produced is not known. It is on this platform that this investigation anchored its relevance, with the aim to determine the possible effect of ascorbic acid administered mono and in combination with chloroquine to normal mice.

Materials and Methods

Experimental animals: Forty (40) albino mice comprising Fifteen (20) males and females each aged between 4 - 8 weeks, bred at the Ambrose Alli university college of medicine animal house, were used as subjects for this study. The animals were observed for seven (7) days for any sign of ill health. Each category of mice was divided into three groups of five mice per group with a group in each category serving as control. Animals were allowed free access to feed (Grower's mash from Bendel feeds and flour mills Ltd.) and water.

Table 1: Response of mice to chloroquine and ascorbic acid treatment

Parameters	Control	Chloroquine	Ascorbic acid	Chloroquine + Ascorbic acid
Malondialdehyde (nmol/ml)	2.09 ± 0.23	2.24 ± 0.08	1.79 ± 0.15	2.07 ± 0.07
Superoxide dismutase (Units/mg Protein)	44.17 ±0.17	46.33 ±0.35	43.31 ± 0.45	45.61 ±0.19
Catalase (Units/min)	1.39 ± 0.04[a]	3.22 ±0.12[b]	0.90 ± 0.05[a]	3.24 ± 0.21[b,c]
Aspartate aminotransferase (Units/l)	38.46 ± 0.27[a]	39.42 ± 0.34[a]	39.20 ± 0.29[a]	40.78 ± 0.36[b]
Alanine aminotransferase (Units/l)	34.55 ±0.09	34.82 ± 0.13	34.53 ± 0.34	34.75 ± 0.09
AST : ALT	1.11 ± 0.01	1.13 ± 0.01	1.14 ± 0.01	1.17 ± 0.05
Gamma glutamyl transferase (Units/l)	36.74 ± 1.77	38.34 ± 1.56	35.17 ± 1.83	37.47 ± 1.57

Mean + SEM of triplicate determinations: Values in the same row with different superscripts are significantly different (P = 0. 05)

Table 2: Response of male mice to chloroquine and ascorbic acid treatment

Parameters	Control	Chloroquine	Ascorbic acid	Chloroquine + Ascorbic acid
Malondialdehyde(nmol/ml)	2.22 ± 0.13	2.23 ± 0.15	1.72 ± 0.36	1.85 ± 0.36
Superoxide dismutase(Units/mg Protein)	44.58 ± 0.10[a]	47.50 ± 0.22[b]	44.85 ± 0.02[a]	46.23 ± 0.17[c]
Catalase(Units/min)	1.37 ± 0.05[a]	3.21 ± 0.34[b]	0.77 ± 0.34[a]	2.94 ± 0.10[b c]
Aspartate aminotransferase (Units/l)	39.12 ± 0.43[a]	40.14 ± 0.29[a]	40.18 ± 0.05[a]	42.04 ± 0.05[b]
Alanine aminotransferase (Units/l)	34.90 ±0.41	35.19 ± 0.27	34.50 ± 0.10	35.00 ± 0.07
AST : ALT	1.12 ±0.02	1.16 ± 0.02	1.16 ± 0.05	1.18 ± 0.03
Gamma glutamyl transferase (Units/l)	42.76 ± 0.31[a]	43.62 ± 0.44[a]	41.43 ± 0.20[a,b]	42.83 ± 0.08

[a]Mean ± SEM of triplicate determinations: Values in the same row with different superscripts are significantly different (P = 0. 05)

At the end of the experiment, the mice were anaesthetized using chloroform and blood collected from the heart into plain sample tubes from where serum used for assay was harvested after clotting and centrifugation.

Test drugs preparations and administration: Chloroquine phosphate 500mg-tablet containing 300mg-chloroquine base (NAFDAC REG. NO. 04 2601) manufactured by Swiss Pharma Nigeria Ltd. was used. Each tablet was dissolved in 100ml of distilled water. 3 ml of ascorbic acid containing 100mg/5ml w/v (NAFDAC Reg No. 04 – 0262) manufactured by Emzor Pharmaceutical industries Ltd. Lagos was made up to 60ml with distilled water. These preparations brought the active component of each drug to 3mg/ml. These were administered intra peritoneally (25mg/Kg body weight) for three days.

Biochemical assay: Randox laboratories kits (Randox UK) were used for alanine aminotransferase (ALT) activity assay, aspartate aminotransferase (AST) activity determination and the estimation of gamma glutamyl transferase (GGT) activity. Malondialdehyde (MDA) was assayed according to the method described by Gutteridge and Wilkins (1982). Superoxide dismutase and catalase activities were determined by the methods of (Misra and Fridovich, 1972) and (Cohen *et al.*, 1970) respectively.

Statistical analysis: Data collected from this study was subjected to one way analysis of variance (ANOVA) and least significance difference (LSD) was used to determine the significance of difference between the means (Steel and Torrie, 1980). P< 0.05 was considered as statistically significant.

Results and Discussion

Chloroquine treatment increased (P<0.05) catalase (3.22 ± 0.12 Units/min) activities of pooled mice (Table 1). There was no significant change in parameters of mice treated with ascorbic acid. A combined treatment of chloroquine and ascorbic acid increased (P<0.05) catalase (3.24±0.21 Units/min) and aspartate aminotransferase (AST) activities (40.78± 0.36 Unit/L). In males, Superoxide dismutase (SOD) and catalase activities increased (P<0.05) in chloroquine treated groups (47.50±0.22 Units/mg Protein) and (3.21±0.34 Units/min) respectively (Table 2). Ascorbic acid treatment reduced (P<0.05) gamma glutamyl transferase (GGT) activity (43.62 ± 0.44 U/L) in mice and a combination treatment of chloroquine and ascorbic acid increased (P<0.05) the activities of SOD, Catalase and AST in same category of mice. In female mice, ascorbic acid treatment significantly reduced all parameters except alanine aminotransferase (ALT) activity and AST: ALT ratios (Table 3). Chloroquine treatment increased (P<0.05) SOD, Catalase, AST and GGT activities. A combination treatment of this group affected (P<0.05) all parameters under consideration.

The increases (P>0.05) noted in mice treated with chloroquine suggest that this antimalarial probably have the potential to induce stress in normal mice. Increased level of malondialdehyde is an indication of stress

Table 3: Response of female mice to chloroquine and ascorbic acid treatment

Parameters	Control	Chloroquine	Ascorbic acid	Chloroquine + Ascorbic acid
Malondialdehyde (nmol/ml)	2.34 ± 0.05[a]	2.25 ± 0.08[a]	1.85 ± 0.05[b]	2.24 ± 0.07[a]
Superoxide dismutase (Units/mg Protein)	43.90 ± 0.23[a]	45.62 ± 0.08[b]	42.35 ± 0.11[c]	45.24 ± 0.10[b d]
Catalase (Units/min)	1.40 ± 0.09[a]	3.22 ± 0.13[b]	0.98 ±0.10[c]	3.42 ± 0.18[bd]
Aspartate aminotransferase (Units/L)	37.97 ± 0.11[a]	38.98 ± 0.04[b]	38.62 ± 0.07[c]	40.03 ± 0.06[d]
Alanine aminotransferase (Units/L)	34.54 ± 0.23	34.72 ± 0.40	34.55±0.66	34.60± 0.22
AST : ALT	1.10 ± 0.01	1.11± 0.01	1.12±0.03	1.16±0.03
Gamma glutamyl transferase (Units/L)	33.12 ± 0.33[a]	35.17 ± 0.13[b]	31.42 ± 0.27[c]	34.26 ± 0.53

[d]Mean ± SEM of triplicate determinations: Values in the same row with different superscripts are significantly different (P = 0. 05)

produced through the oxidation of membrane lipid polyunsaturated fatty acids (PUFA). These drugs exert its action in the acidic food vacuole of malaria parasite (Rang and Dale, 1991). In the absence of parasitemia chloroquine may be metabolized as xenobiotics through the cytochrome P_{450} pathway generating superoxide anion as byproduct that may gain electron through possible leakage from the electron transport chain to produce superoxide radical that serves as substrate for superoxide dismutase. The activity of this enzyme lead to production of hydrogen peroxide that is required by catalase. These may have explained the observed raise in the activities of these enzymes in male and female mice.

Free radicals are continuously produced during normal aerobic respiration and metabolism of xenobiotics. In this study of chloroquine could have contributed to the observed increase in lipid per oxidation product in serum of the mice. These increases are not enough to induce considerable stress in the category of mice under investigation. This is the result pattern that also sustains for the group that received combination treatment, except for malondialdehyde that is significantly reduced in the female group treated with ascorbic acid. The reason for the observed reduction (P<0.05) in MDA of female mice may be attributed to the reducing nature of ascorbic acid. Ascorbic acid is able to donate electron to alpha tocopherol an effective chain breaker in the propagatory phase of lipid per oxidation in other to regenerate the alpha tocopherol. Alpha tocopherol scavenges lipid radicals much faster than these can react with adjacent fatty acid side chains or with membrane proteins (James, 1994). This property of ascorbic acid may have affected the radicals generated during normal respiration therefore enhancing the function of alpha tocopherol in quenching the generated radicals hence the observed (P<0.05) reduction in lipid per oxidation product in female mice.

The observed reductions in the parameters of ascorbic acid treated groups is pronounced in the female mice. This is expected as SOD and Catalase seem inducible and a reduction in MDA is most likely to affect the activities of these enzymes. Although AST, ALT and GGT did not show any relationship to oxidative stress as observed in this study, the determination of these enzymes activities are necessary because the drugs administered are metabolized in the liver and the functional state of this central organ of metabolism under this condition is noteworthy as this might give insight into possibilities. The slight increases (P<0.05) observed in AST and ALT activities suggest that the metabolism of chloroquine may have elicited damage to either the heart or liver tissue. Serum activities of AST and ALT were employed to access liver status because these enzymes can be used to differentiate between liver and heart disease (Nicholas and Lewis, 1989). In addition, estimation of serum gamma glutamyl transferase level is reported to be a valuable screening test with a high negative predictive value for liver disease (Neha and Rawal, 2000; Nemesanszky, 1996; Kim *et al.*, 1977). The activity of AST increased (P<0.05) in ascorbic acid treated female mice while that of GGT reduced (P<0.05). The increase in AST activity may be due to heart related toxicity. This requires confirmation through assay for creatine kinase that was not determined in this work. Since GGT is a membrane bound enzyme (Chander *et al*., 1994), a decrease in the activity of this enzyme suggests possible membrane protection. It can conveniently be concluded on the basis of the statistical power of these results that ascorbic acid can act in combination with chloroquine to reduce oxidative stress and stabilize membrane in normal mice, while ascorbic acid administered as mono treatment may perform better with potential cardiac toxicity particularly in female mice. However, weather these drugs particularly ascorbic acid will exhibit the same function in a disease state as malarial infection that is endemic in tropical and subtropical regions is being considered.

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