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Chloroquine and Folic Acid Interactions in Respiration Induced Oxidative Stress

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Abstract: This study was designed to access the interaction between chloroquine and folic acid in respiration induced oxidative stress, with the aims of employing these drugs in the treatment of malaria infection in the tropical and subtropical regions where the disease is endemic. A total of forty mice comprising of twenty males and twenty females were used in this investigation. Each sex category was divided into four groups of five mice and designated control, chloroquine, folate and chloroquine-folate group, respectively. All drugs were administered intraperitoneally (ip). Data collected and analyzed from treated mice show that folic acid reduced ($p<0.05$) malondialdehyde concentration in male and female mice. All administered drugs increased ($p<0.05$) the activities of Superoxide Dismutase (SOD) in male and female mice compared to the control groups. Catalase activities increased ($p<0.05$) in females treated with chloroquine and the female group that received combined dose and in chloroquine treated males. Significant differences ($p<0.05$) were recorded in the activities of Aspartate Aminotransferase (AST) and Gamma Glutamyl Transferase (GGT) activities in male and female mice. The finding from this study shows folic acid as having antioxidant property and chloroquine as a suppressant of this antioxidant activity of folic acid in normal mice.

Key words: Antioxidants, malondialdehyde, superoxide dismutase, catalase, gamma glutamyl transferase

INTRODUCTION

Oxidative stress occasioned by the release of Reactive Oxygen Species (ROS) arises as a result of imbalance in the antioxidant/prooxidant mechanism in living systems in favour of the latter. Oxidative challenges often arise from sources as radiation, cellular metabolism and challenges to the immune system or abnormal immune function[1]. The effects of ROS on cells have been reported to be strongly associated with carcinogenesis, mutagenesis, ageing and arteriosclerosis. Endogenous antioxidants such as Superoxide Dismutase (SOD) and catalase constitute primary antioxidants that are expected to protect the biological function of cells against ROS[2].

The mitochondria have been described as the major intracellular source and the most vulnerable target of ROS[3]. Despite this assertion, ROS are known to perform important functions within the cell such as the synthesis of thyroxin and killing of pathogen[4]. However, since modern medicine subscribe to the use of therapeutic agents in complimenting ROS defense functions, then drawing a balance between ROS and therapeutic drugs becomes critical in the treatment or management of pathological conditions. The reason is that while ROS production is minimized not to cause possible damage to cells, the therapeutic agents compliment ROS in clearing pathogens.

Vitamins such as riboflavin and ascorbic acid have been implicated to influence the course of infection[5]. The mediating role played by these vitamins in combination with chloroquine as reported are only with respect to infected cells[5-7].

Because no attention has been directed to folic acid in this regard, this research is therefore, designed as a preliminary study to assess the influence of chloroquine and folic acid on respiration induced oxidative stress in healthy mice. This study derives it importance from the popular use of chloroquine in treatment of malaria infection that is endemic in the tropic and subtropical regions.

MATERIALS AND METHODS

Experimental animals: Forty albino mice comprising twenty males and twenty females each aged between 4-8 weeks, bred at the animal house unit of Ambrose Alli University College of Medicine, were used as subjects for this study. This unit approved the admission of these subjects into experimentation. The animals were observed

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for seven days for any sign of ill health and those that showed any sign of weakness were excluded and replaced. Each category of mice was divided into four groups of five mice. Subjects were allowed free access to feed (Grower's mash from Bendel feeds and flourmills Ltd.) and water. At the end of the experiment, the mice were anaesthetised with chloroform and blood collected by cardiac puncture into sample tubes from where serum used for assay was harvested after clotting and centrifugation.

Test drugs preparations and administration: Chloroquine phosphate 500 mg-tablet containing 300 mg-chloroquine base (NAFDAC Reg. No. 04 2601) manufactured by Swiss Pharma Nigeria Ltd. was used. Each tablet was dissolved in 100 mL of distilled water and the resulting solution centrifuged to obtain clear chloroquine solution. Twelve millimeters folic acid containing 2.5 mg/5 mL w/v (NAFDAC cert. No: 04-4714) manufactured by Mopson Pharmaceutical Ltd. Lagos, was diluted with equal volume of distilled water. These preparations brought the active component of each drug to 3 mg mL^{-1}. Equal concentrations of these were administered intraperitoneally on body weight basis (25 mg/kg-body weight) for three days.

Biochemical assay: Randox laboratory kits (Randox UK) were used to assay for Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Gamma Glutamyltransferase (GGT) activities according to authors instructions[8,9]. Malondialdehyde (MDA) was assayed as described[10]. The assays of superoxide dismutase and catalase activities were performed as previously described[11,12].

Statistical analysis: Data collected from this study was subjected to Analysis of Variance (ANOVA) using computer software (InStat, Graphpad Software, SanDiego, CA). $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Data collected from this study indicates that folic acid reduced significantly ($p<0.05$) Malondialdehyde (MDA) concentrations in male and female mice (Table 1 and 2). A combination treatment with chloroquine and folic acid produced ($p<0.05$) increase in MDA concentration in female mice (Table 2). All drugs used in single and combined dose increased significantly the activities of SOD compared with control mice in males and females (Table 1 and 2). Significant increases ($p<0.05$) were indicated in the activities of catalase in female group that received chloroquine and the combined dose as against the control and folic acid treated groups (Table 2), while the same effect was observed in chloroquine group in male (Table 1). Folic acid reduced ($p<0.05$) the activity of AST in male mice. Differences ($p<0.05$) in the activities of GGT among the treated groups in females were observed in addition to a ($p<0.05$) reduction in GGT activity in folic acid treated male mice.

The observed increases in MDA concentrations in male and female mice may be traced to the metabolism of administered drugs through the cytochrome P_{450} detoxification pathway that produces superoxide anion the substrate for SOD. The recorded ($p<0.05$) increases in SOD activity among all treated groups in both male and female mice are expected to have caused concomitant increases in catalase activities in all treated groups in both sex categories as the activity of SOD provides substrate for catalase. Significant increases ($p<0.05$) in catalase activities were only recorded for chloroquine treated male and female mice and the female group on chloroquine-folic acid combination treatment. In both mice categories, folic acid treatment did not show any significant increase on catalase activities suggesting that folic acid may have an impact that may be repressive or inhibitory on the activity of catalase. However, the effect of folic acid on mice catalase activities is not seen in the activity of the same enzyme in males mice but rather, folic acid significantly increased ($p<0.05$) the activity of the enzyme in female mice. This observation suggests that the repressing or inhibitory effect of folic acid on catalase may either be prevented or controlled in the presence of chloroquine.

Mice group that had their feed intake supplemented with daily folic acid showed significant reduction ($p<0.05$) in serum concentration of MDA and GGT activity in both sex categories. The observed reduction ($p<0.05$) in serum MDA concentration in folic acid treated mice suggests

Table 1: Changes in some serum enzymes and MDA concentration in male mice on folic and chloroquine drugs

Parameters	Control	Chloroquine	Folic acid	Chloroquine + Folic acid
Malondialdehyde (nM mL^{-1})	2.22±0.26^a	2.23±0.30^a	0.49±0.05^b	2.56±0.09^a
Superoxide dismutase (Units/mg Protein)	44.58±0.21^a	47.50±0.44^b	48.65±0.36^c	49.24±0.45^c
Catalase (Units/min)	1.37±0.09^a	3.21±0.68^b	1.38±0.14^a	2.18±0.07^a
Aspartate aminotransferase (Units/L)	39.12±0.87	40.14±0.58^a	38.11±0.37^b	40.51±0.15^a
Alanine aminotransferase (Units/L)	34.90±0.81	35.19±0.54	34.64±1.22	35.46±0.14
AST: ALT	1.12±0.02	1.16±0.02	1.10±0.04	1.14±0.02
Gamma glutamyl-transferase (Units/L)	42.76±0.65^a	43.62±0.89^a	40.22±0.37^b	44.25±0.13^a

Values are Mean±SD of triplicate determinations; n = 5 in each group, Values in the same row with different superscripts are significantly different

Table 2: Changes in some serum enzymes and MDA concentration in female mice on folic and chloroquine drugs.

Parameters	Control	Chloroquine	Folic acid	Chloroquine + Folic acid
Malondialdehyde (nM mL^{-1})	2.34±0.05^a	2.25±0.19^a	0.67±0.04^b	2.74±0.03^c
Superoxide dismutase (Units/mg Protein)	43.90±0.43^a	45.62±0.08^b	46.56±0.79^c	47.26±0.28^c
Catalase (Units/min)	1.40±0.09^a	3.22±0.13^b	1.39±0.06^a	1.62±0.08^c
Aspartate aminotransferase (Units/L)	37.97±0.11^a	38.98±0.04^b	36.59±0.02^c	39.48±0.23^d
Alanine aminotransferase (Units/L)	34.54±0.23	34.72±0.40	34.80±0.89	35.16±0.11
AST: ALT	1.10±0.01^a	1.11±0.01^a	1.06±0.02^b	1.12±0.02^a
Gamma glutamyl transferase (Units/L)	33.12±0.33^a	35.17±0.13^b	29.64±0.59^c	36.26±0.22^d

Values are Mean±SD of triplicate determinations; n = 5 in each group, Values in the same row with different superscripts are significantly different

that folate may probably function as a membrane antioxidant scavenger for free radicals within the lipid membrane. An attempt is made in this presentation strictly on the basis of our result to proffer explanation for the observed seeming antioxidant role of folic acid. In doing so, it is assumed that folate being hydrophillic may have involved part of its constituting component: Pteridine, Para amino benzoic acid or glutamate moiety to possibly interact with either lipid, protein or other lipophilic agent resulting into complex formation that may assist the complex to gain access into the hydrophilic core of the lipid bilayer, carrying along with it, a methenyl group attached to the fifth and tenth nitrogen atom of pteridine and Para Amino Benzoic Acid (PABA) of folate.

Free radical and molecular oxygen are requisite for the onset of lipid peroxidation. When these criteria are met in the presence of folate, abstraction of hydrogen atom from the unstable folate methenyl group may occurs in preference to that of Polyunsaturated Fatty Acid (PUFA) of membrane phospholipid to conserve membrane integrity. The abstraction of hydrogen atom from methenyl carbon at N^5 N^{10} -methenyl-tetrahydrofolate may creates a reactive carbon radical (RCR). The presence of molecular oxygen may produce folate peroxyl radical, that could be reduced with NADH + H^+ releasing hydrogen peroxide from the membrane to the cytosol. Regeneration of N^5, N^{10}-methenyl-tetrahydrofolate is probably made possible by ∝-tocopherol acting in concert with cytosolic ascorbate. Alpha tocopherol is a potent chain breaking antioxidant that functions by transferring phenolic hydrogen to a peroxyl free radical while forming tocopheryl radical in the process. Alpha-tocopherol is regenerated from its derived radical by redox reaction involving cytosolic ascorbate[13]. The pathway described indicates that hydrogen peroxide is formed and released to the cytosol increasing the cytosolic concentration and yet no significant increase in the activity of catalase was observed in folic acid treated mice of both sex categories. This is probably because folic acid in addition to its suggested repressive or inhibitory role on catalase may interact with glutathione peroxidase in a way that would enhance the activity of the cytosolic enzyme to utilize hydrogen peroxide thereby sparing catalase from activity. The status of liver function of the subjects is warranted under this experimental condition, due to the drugs administered. To achieve this, the enzyme AST, ALT and GGT activities were assayed. These enzymes have been found useful in differentiating between liver and heart diseases[14-16]. Also estimation of serum GGT has been reported as a valuable screening test with a high negative predictive value for liver disease[14,17,18]. In a previous study chloroquine treatment failed to reveal any significant changes in biochemical parameters assayed in subjects but histopathological examinations showed cytolysis[19]. Through their subjects were parasitized. These researchers did not determine the activity of GGT in their studies. The observed increase ($p<0.05$) in the activity of GGT in mice confirms[15] damage to liver, as GGT is a liver membrane bound enzyme that is mainly responsible for amino acid transport into hepatocytes. This should indicate that the liver is the most likely target for chloroquine toxicity. Based on the available evidence as obtained in this study, it may be conclude that chloroquine is toxic to hepatocyte while folate could function as a carrier-mediated antioxidant of the lipid bilayer.

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