

Membrane Lipid Peroxidation of HbAA Erythrocyte of Non-Malarious Participants Administered with Quinine

Paul Chidoka Chikezie¹ and Augustine Amadikwa Uwakwe².

¹Department of Biochemistry, Imo State University, Owerri, Imo State, Nigeria. ²Department of Biochemistry, University of Port-Harcourt, Port-Harcourt, Rivers State, Nigeria.

ABSTRACT

The erythrocyte membrane is susceptible to lipid peroxidation due to its high content of polyunsaturated lipids. The present study ascertained levels of lipid peroxidation of non-parasitized erythrocytes of non-malarious male participants administered with quinine. Determination of erythrocyte lipid peroxidation was by a monitor of levels of thiobarbituric acid reactive substances, measured spectrophotometrically by taking the sample absorbance at 432 nm against a reagent blank at 24 °C. Fifteen (15) non-malarious male (59-79 kg) participants of confirmed HbAA genotype between the age brackets of 21-34 yr enrolled for this study. Three (3) participants per group were administered with quinine according to the corresponding doses: 1.5, 3.0, 4.5 and 6.0 mg/kg (control; $n = 3$). At regular time interval of 3 h for 12 h, blood samples were withdrawn for determination of erythrocyte membrane lipid peroxidation. Erythrocyte membrane of participants administered with quinine exhibited relatively high levels of lipid peroxidation in a dose dependent manner. Generally, within 0-6 h, the four administered doses of quinine caused a time dependent elevation of lipid peroxidation. Further increase in time ($t > 6$ h) showed decreasing levels of lipid peroxidation in participants administered with quinine. Generally, the levels of erythrocyte lipid peroxidation in the presence of quinine showed 2 phase profile. The first stage showed increasing levels of lipid peroxidation at $t < 6$ h after drug administration, followed by the second phase which showed decreasing levels of lipid peroxidation. The results showed that administration of quinine to non-malarious individuals elicited flux in erythrocyte lipid peroxidation.

Keywords: Quinine, Lipid peroxidation, Erythrocyte, Antioxidant.

Corresponding author: Paul Chidoka Chikezie; Phone: +2348038935327.

E-mail: p_chikezie@yahoo.com

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INTRODUCTION

Quinine acts primarily as blood schizontocide; it has little or no effect on sporozoites or preerythrocytic forms of malarial parasites. The alkaloid is gametocytocidal for *P. vivax* and *P. malariae* but not for *P. falciparum*.^[1] Because quinine is a weak base, it concentrates in the acidic food vacuoles of *P. falciparum* where the drug inhibits the non enzymatic polymerization of highly reactive, toxic haem molecules into a non toxic polymer pigment called haemozoin. The failure to inactivate haem kills the parasite via oxidative damage to membrane; digestive proteases and possibly other critical biomolecules.^[2,3] This drug action has been proposed to occur in a two-step process whereby the quinoline binds first to haem, and the

resulting haem-drug complex binds to and saturates the haem-polymer chains.^[1] Consequently, the parasites are overwhelmed by rapid accumulation of reactive oxygen species (ROS) and death ensues. Quinine has several other primary modes of action; it directly denatures plasmodial protoplasmic proteins, interferes with the parasite replication process by inhibiting the incorporation of phosphate in the synthesis of DNA and RNA molecules, and diminishes the parasite's ability to fix oxygen, thus interferes with the metabolism of the parasite.^[4] The drug interferes with oxidation and influences the stability of oxyhaemoglobin so that oxyhaemoglobin is relatively unavailable to give up oxygen to peripheral tissues.^[5] This could present clinical cyanosis even in the presence of adequate arterial oxygenation.

The erythrocyte membrane is susceptible to ROS induced lipid peroxidation due to its high content of polyunsaturated lipids.^[6,7] Erythrocyte lipid peroxidation has been extensively used as a basis to investigate the role of oxidative membrane damage in pathological conditions.^[8,9] Extensive lipid peroxidation in biomembranes engenders loss of fluidity, decrease in membrane potential, increase permeability to ions and haemolysis.^[10,11] Furthermore, abnormal susceptibility of erythrocyte membrane to oxidative damage, caused by membrane lipid peroxidation is known to reflect similar abnormalities in organs and tissues.^[12,11] Nevertheless, inhibition of antioxidant enzymes and depletion of low molecular weight antioxidants engender accumulation of ROS which is particularly relevant in chemotherapy of numerous parasitic infections.^[13-15] Although quinine primary target is the *Plasmodia* parasites, the present study ascertained level of lipid peroxidation of non-parasitized erythrocytes when non-malarious individuals were administered with quinine.

MATERIALS AND METHODS

Antimalarial Drug: Quinine (BDH, UK), was purchased from Cimpok Pharmaceuticals, Amakhohia, Owerri, Nigeria.

Selection of Volunteers/Experimental Design: Fifteen (15) non-malarious male (59-79 kg) participants, in a group of five (5), of confirmed HbAA genotype between the age brackets of 21-34 years enrolled for this study. Three (3) participants per group were administered with quinine according to the corresponding doses: 1.5, 3.0, 4.5 and 6.0 mg/kg (control; $n = 3$). At regular time intervals of 3 h for 12 h, blood samples were withdrawn for determination of erythrocyte membrane lipid peroxidation. The Institutional Review Board of the Department of Biochemistry, Imo State University, Owerri, Nigeria, granted approval for this study and all participants involved signed an informed consent form. This study was in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

Collection of Blood Samples/Preparation of Erythrocyte Haemolysate: Five milliliters (5.0 ml) of human venous blood samples of HbAA genotype were collected by venipuncture and stored in Na₂EDTA anticoagulant tubes. The erythrocytes were washed by centrifugation method as described by Tsakiris *et al.*,^[16] Within 2 h of collection of blood samples, portions

of 1.0 ml of the samples were introduced into centrifuge test tubes containing 3.0 ml of buffer solution pH = 7.4:250 mM tris (hydroxyl methyl) amino ethane-HCl (Tris-HCl)/ 140 mM NaCl/ 1.0 mM MgCl₂/ 10 mM glucose). The erythrocytes were separated from plasma by centrifugation at 1200 *g* for 10 min and washed three times by the same centrifugation method with the buffer solution. To remove platelets and leucocytes, the sediment was re-suspended in 3.0 ml of phosphate-buffered saline (PBS) solution, pH 7.4, and passed through a column (3.5 cm in a 30 ml syringe) of cellulose-microcrystalline cellulose (ratio w/w 1:1) as described by Kalra *et al.*,^[17] The eluted fraction was passed twice through a new column of cellulose-microcrystalline cellulose (ratio 1:1 w/w) to obtain erythrocyte suspension sufficiently devoid of leucocytes and platelets. The isolated erythrocytes were lysed by freezing/ thawing as described by Galbraith and Watts,^[18] and Kamber *et al.*,^[19] Hemoglobin-free erythrocyte membrane suspension was prepared using hypotonic lysis procedure as reported by Lam *et al.*,^[20] re-suspended in 1.0 ml of the buffer and stored at -70 °C until analyses.^[21]

Determination of Lipid Peroxidation: Level of erythrocyte membrane lipid peroxidation was measured by the production of thiobarbituric acid reactive substances (TBARS) as described by the modified method of Bernheim *et al.*,^[22] as reported by Ojha *et al.*,^[23] This method used trichloroacetic acid to eliminate interference caused by malondialdehyde precursors. To a reaction mixture of 2.0 ml containing 1.8 ml phosphate buffer (0.1 M; pH = 7.4), 0.2 ml of crude erythrocyte membrane suspension was added. The reaction mixture was incubated at 37 °C in water bath shaker for 1 h. The reaction was terminated by adding 1.0 ml of 10% trichloroacetic acid followed by the addition of 1.0 ml of 0.67% thiobarbituric acid. All the tubes were kept in boiling water bath for 20 min. The tubes were then cooled in ice and centrifuged at 2500 *g* for 10 min. The resulting supernatant containing TBARS was measured spectrophotometrically (SPECTRONIC 20, Labtech – Digital Blood Analyzer®) by taking the sample absorbance at 432 nm against a reagent blank at 24 °C. Estimation of protein standard employed Bradford,^[24] method and lipid peroxidation was reported in nmol TBARS/mg protein.

Statistical Analyses: The results were expressed in terms of arithmetic means (X) ± standard deviation (SD). The statistical significance of the difference between the means was evaluated by Student's-t-test.^[25]

RESULTS AND DISCUSSION

Figure 1 showed that within the experimental period of 0-12 h, values of erythrocyte membrane lipid peroxidation of the control participants revealed relatively low variability. In addition, erythrocyte membrane of participants administered with quinine exhibited relatively high levels of lipid peroxidation in a dose dependent manner. Generally, within 0-6 h, the four administered doses of quinine caused a time dependent elevation of lipid peroxidation. However, further increase in time ($t > 6$ h) showed decreasing levels of lipid peroxidation. Specifically, the highest level of erythrocyte lipid peroxidation occurred at $t = 6$ h in participants dosed with 6.0 mg/kg of quinine. At every given time interval, variations in the levels of lipid peroxidation amongst participants administered with the four doses of quinine showed no significant difference ($p > 0.05$). However, the levels of lipid peroxidation of participants between 6 and 9 h showed significant difference ($p < 0.05$). Contrary, levels of lipid peroxidation between 9 and 12 h showed no significant difference ($p < 0.05$) in this regard. Generally, the levels of erythrocyte lipid peroxidation in the presence of quinine showed 2 phase profile. The first stage showed increasing levels of lipid peroxidation at $t < 6$ h after drug administration to the participants, accompanied by the second phase which showed decreasing levels of lipid peroxidation.

The present study showed that quinine caused flux in erythrocyte lipid peroxidation which indicated oxidative

damage to membrane structural lipid components.^[7,14] Several studies have demonstrated that oxidative stress in various tissues and blood of experimental animals and humans might be prevented by antioxidant interventions.^[26-28] Therefore, the capacity of quinine to alter the levels of erythrocyte lipid peroxidation is as well connected to the capability of quinine to compromise those antioxidant systems that are responsible for erythrocyte membrane structural integrity. Notable endogenous antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase have been well-characterized.^[21,23,29,30] Also, there are low molecular weight antioxidants such as glutathione, ascorbic acid, carotenoids and α -tocopherol that have been reported by various researchers.^[14,23,31-34]

Previous *in vitro* studies by Uwakwe and Ezech,^[35] and Ali and Kadaru,^[36] reported that xenobiotics interfered with erythrocyte membrane integrity and stability. Specifically, the reports of Chikezie *et al.*,^[37] showed that oral administration of quinine and FansidarTM inhibited erythrocyte glutathione-S-transferase (GST) activity of male volunteers. In another *in vitro* study, Chikezie,^[38] revealed that erythrocytes incubated in aqueous quinine solution promoted membrane disruption. All these findings points to the fact that quinine, by virtue of its capacity to inhibit the redox enzyme GST, caused accumulation of ROS that may have triggered membrane disruption as a result of lipid peroxidation of structural lipid molecules that are responsible for erythrocyte structural and functional integrity. The present findings

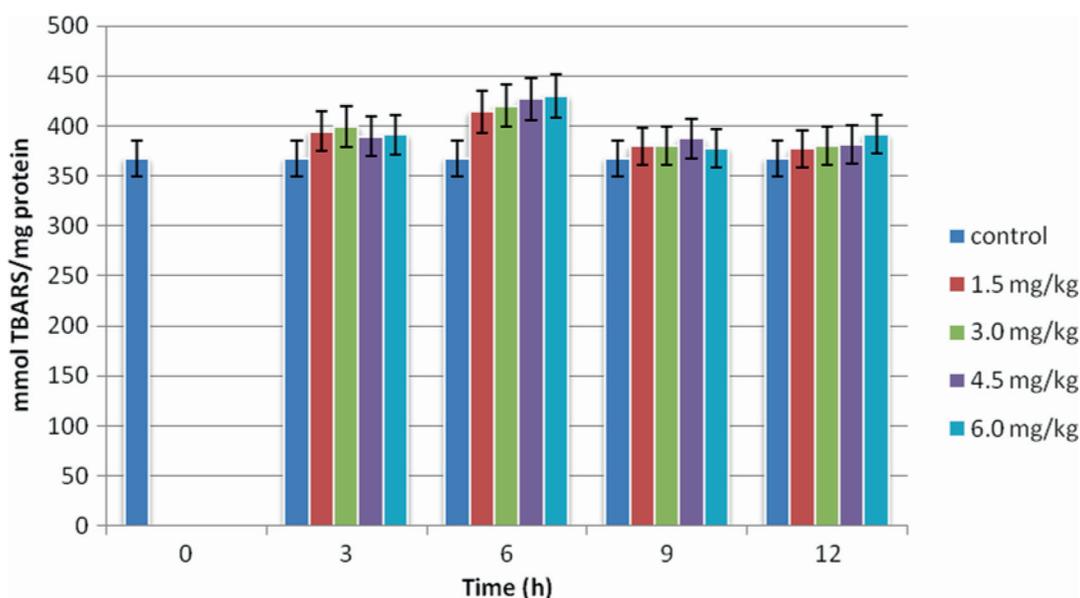


Figure 1. Levels of lipid peroxidation of participants administered with quinine.

is supported by previous reports which stated that oxidative damage of erythrocyte membrane is the primary cause of reduced capacity of the erythrocytes to withstand mechanical and osmotic stress.^[39,40]

The recovery of erythrocyte GST, and probably other redox enzymes, from inhibitory effect of quinine with progression of experimental time,^[37] could be adduced for decreasing levels of erythrocyte lipid peroxidation ($t > 6$ h) reported in this study. This same mode and pattern of inhibition of erythrocyte GST by diverse xenobiotics have been reported elsewhere.^[41-44] The present *in vivo* study confirmed that substances implicated to have compromised/distorted erythrocytes redox equilibrium exacerbates the accumulation of ROS, and therefore, are indirect promoters of membrane lipid peroxidation.

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