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In vitro effects of co-incubation of blood with artemether/lumefantrine & vitamin C on the viscosity & elasticity of blood

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***Background & objectives*: The antimalarial combination drug artemether/lumefantrine has been shown to be effective against malaria parasite through its haemolytic action. This drug is sometimes co-administered with vitamin C in patients with malaria. Vitamin C is associated with antioxidant properties which would be expected to protect against haemolytic effects of this antimalarial drug. This study was designed to investigate *in vitro* effects of co-incubation of artemether/lumefantrine with vitamin C on the viscosity and elasticity of blood.**

***Methods*: Blood was collected from 12 healthy female volunteers with normal haemoglobin genotype (HbAA). A Bioprofiler was used to measure the viscosity and elasticity of untreated blood samples (control) and samples exposed to artemether/lumefantrine (0.06/0.36 mg/ml) alone and with low or high dose vitamin C (equivalent to adult doses of 100 or 500 mg).**

***Results*: Artemether/lumefantrine significantly ($P<0.05$) reduced viscosity of blood from 4.72 ± 0.38 to 3.78 ± 0.17 mPa.s. Addition of vitamin C (500 mg) further reduced blood viscosity to 2.67 ± 0.05 mPa.s. The elasticity of blood was significantly ($P<0.05$) reduced from 0.33 ± 0.04 mPa.s to 0.24 ± 0.03 mPa.s by the antimalarial drug, and further reduced to 0.13 ± 0.02 mPa.s in the presence of vitamin C (500 mg).**

***Interpretation & conclusions*: Co-incubation of blood with vitamin C and antimalarial combination drug potentiates the haemolytic effects of the latter on reducing blood viscosity and elasticity *in vitro*. This may possibly have implications in relation to haemolysis in patients receiving vitamin C supplementation with artemether/lumefantrine during malaria therapy.**

Key words Artemether/lumefantrine - blood - elasticity - haemolysis - viscosity - vitamin C

The combination of artemether and lumefantrine is used as an oral antimalarial agent. Artemether interacts with components of blood to generate free radicals which may damage the malaria parasite and alleviate symptoms of malaria. Lumefantrine eliminates residual parasites, decreases parasite burden, and resolves clinical symptoms of the disease[1]. The antimalarial agents are sometimes co-administered with vitamin C (ascorbic acid) which is predominantly associated with antioxidant properties[2-4], possibly to

oppose oxidative stress and consequent haemolysis and anaemia associated with malaria infection[5]. Vitamin C has been shown to potentiate the effects of the antimalarial agent exifone *in vitro*[6]. The practice of vitamin C supplementation in patients receiving antimalarial therapy may also be supported by the low serum concentration of vitamin C in *Plasmodium* infection[7]. There are however, recommendations against administration of vitamin C to patients with malaria, to avoid possible impairment of therapeutic action of antimalarial drugs[8]. This is supported by reports of the interference of antiplasmodial action of antimalarial agents in the presence of antioxidants[9,10].

The mechanism of action of artemether/lumefantrine involves its haemolytic effects on erythrocytes[11]. This combination has also been shown to reduce blood viscosity, elasticity and erythrocyte aggregation *in vitro*[12]. We undertook this *in vitro* study to investigate the effects of vitamin C on artemether/lumefantrine induced changes in whole blood viscosity and elasticity in healthy human volunteers.

Material & Methods

Participants were 12 healthy female volunteers (students with mean age 20.8±1.5 yr) with normal haemoglobin genotype (HbAA) attending the University of the West Indies, Mona, Jamaica. The study was restricted to female participants as glucose-6-phosphate dehydrogenase (G6PD) deficiency affects haemolysis. The G6PD enzyme deficiency is mainly expressed in males as females are carriers of the gene[13]. Individuals were excluded if they had sickle cell disease (HbSS) or trait (HbAS), or if they had been taking medication for any illness within the last three months. All participants signed a consent form confirming voluntary participation in the study. Ethical approval was obtained from the University of the West Indies / Faculty of Medical Sciences Ethics Committee. The study was conducted in the Department of Basic Medical Sciences at the University of the West Indies, Mona, in April 2013.

Sample preparation: The blood (3 ml) was collected into vacutainer tubes containing K^+ EDTA anticoagulant and kept at 25°C until used for analysis. A Coartem® (artemether 80 mg/lumefantrine 480 mg) tablet (Novartis Pharmaceutical Company, Beijing, China) was dissolved in 80 ml physiological saline (0.9%) solution to prepare stock solution. Soluble vitamin C (Redoxon®, Bayer Inc., UK) tablets were dissolved in 0.9 per cent saline solution to prepare vitamin C solution (1 mg/ml). Blood (0.5 ml) samples from all participants were placed in each of four Eppendorf tubes. Aliquots (30 µl) of Coartem® solution were pipetted into three of those tubes to constitute 0.06/0.36 mg/ml Coartem® in each blood sample, based on therapeutic adult dose of 320/1920 mg artemether/lumefantrine. The fourth tube had no drug added and served as the control sample. Vitamin C solution (10 or 50 ml) was added to each of two tubes containing blood and Coartem®, such that the concentrations of vitamin C were equivalent to adult doses of 100 or 500 mg, respectively. The samples were gently mixed and allowed to equilibrate for five minutes before tests were done.

Viscometry: Blood samples were added to the sample cup of a BioProfiler (Vilastic Scientific Inc., Texas, USA). The haematocrit reading obtained using a Coulter Counter (Coulter Corporation, Florida, USA) was entered into the BioProfiler which measured the viscosity and elasticity of whole blood at a frequency of 2 Hz, shear rate of 62.8 per second. The haemorheologic measurements at those settings approximate microcirculatory flow patterns. Readings were taken at a projected haematocrit of 45 per cent and temperature of 37°C[14]. Tests were performed within one hour of blood collection.

Statistical analysis: Statistical analysis of differences in group means was performed by analysis of variance (ANOVA) and Duncan's multiple range test using SigmaPlot 11.0 software (Systat Software Inc., San Jose, CA, USA).

Results & Discussion

The viscosity of control blood sample was 4.72 ± 0.38 mPa.s. In the presence of antimalarial drug alone, blood viscosity significantly (P<0.05) decreased to 3.78 ± 0.17 mPa.s. Further significant (P<0.05) reduction to 3.20 ± 0.10 mPa.s was observed with 100 mg vitamin C and to 2.67 ± 0.05 mPa.s (P<0.01) with 500 mg vitamin C, respectively. The elasticity of blood in the control sample was 0.33 ± 0.04 mPa.s; whereas in samples exposed to antimalarial drug alone the elasticity was significantly (P<0.05) lower at 0.24 ± 0.03 mPa.s. There were further significant (P<0.05) reduction to 0.21 ± 0.02 mPa.s in samples exposed to antimalarial drug with 100 mg vitamin C and to 0.13 ± 0.02 mPa.s (P<0.01) in samples with 500 mg vitamin C.

The results of the present study in relation to the effects of artemether/lumefantrine on blood viscosity and elasticity were consistent with an earlier study[12]. Free radicals generated due to artemether increase haemolysis, resulting in a decrease of the haematocrit[11]. The haematocrit is the major determinant of whole blood viscosity[15]. It is, therefore, likely that in the present study, artemether/lumefantrine generated the free radicals that caused increased haemolysis resulting in reduced hematocrit and ultimately reduced whole blood viscosity and elasticity.

The effect of vitamin C on viscosity in this *in vitro* study was consistent with the findings in human studies where blood viscosity was significantly inversely associated with acute ingestion and blood concentration of vitamin C[16-18]. Vitamin C was also shown to potentiate the effects of a therapeutic agent on rheological indicators including decreasing blood viscosity and erythrocyte aggregation, and increasing deformability of erythrocytes[19]. Our *in vitro* results represent the situation *in vivo*, and suggest that co-administration of vitamin C with artemether/lumefantrine may increase the risk of haemolysis and possible anaemia in patients with malaria.

In erythrocytes, vitamin C exhibits pro-oxidant or antioxidant activity depending on the presence of glutathione or the source of vitamin C, whether intracellular or extracellular[20,21]. Vitamin C is also reported to induce oxidative stress in *Plasmodium*-infected erythrocytes[22]. The effects observed in the present study may also be related to the doses (100 and 500 mg) of the vitamin used.

In conclusion, the results of the present study showed that the co-incubation of blood with vitamin C and artemether/lumefantrine *in vitro* significantly increased the haemolytic effects of the latter. This observation warrants further scientific validation, especially in relation to the practice of vitamin C supplementation with antimalarial therapy.

***Conflicts of Interest*:** None.

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