Minireview

Low dose vitamin C, vitamin E or L-arginine supplementation and sickle cell disease

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ABSTRACT

Sickle cell disease is a multi-system disease, associated with episodes of acute illness and progressive organ damage. Many mechanisms contribute to the complex pathophysiology of sickle cell disease. The production of excess free radicals causes damage to several organs including the blood, vascular endothelium and liver. The effect of chronic low-dose supplementation with vitamin C (300mg/day for 6 weeks in adults or 100mg/day for 6 weeks in children) or vitamin E (100 IU/day for 6 weeks in adults) or L-Arginine (1g/day for 6 weeks in adults) in ameliorating the pathophysiology and combating the deleterious effects of sickle cell disease in some cell types and organs of the body are examined in this review.

INTRODUCTION

Sickle cell disease (SCD) is a class of hemoglobinopathy, which results from a single mutation in the beta globin chain inducing the substitution of valine for glutamic acid at the sixth amino acid position (Stuart and Nigel, 2004). This mutation leads to the production of abnormal hemoglobin S (HbS). In addition to homozygous sickle cell disease (HbSS), other forms such as hemoglobin SC disease (HbSC) and hemoglobin Sβ thalassemia (HbSβthal) also exist (Hyacinth et al, 2011). It is not clear where the initial mutation occurred but it is generally believed that the initial mutation occurred in West Africa (Kan and Dozy, 1980). However, the gene has spread across the world through migration. The first publication of sickle cell disease was in 1910 by Dr. Herrick in the USA. The ailment was identified by Dr. Earnest E. Irons (an intern) in 1904 who initially examined a patient, Walter Clement Noel, a dental student from Granada (Sergeant, 2001).

The sickle cell disease is the first known human molecular disease (Sergeraft, 2001) and its molecular pathology was established in 1949 by Linus Pauling (Bunn, 1997).

Incidence of SCD in West, Central and East Africa is between 5 - 20%. The disease is less common in Northern and Southern Africa. Gene frequency is higher in low-lying, wet regions with a high prevalence of malaria (Diallo & Tchemia, 2001). In Africa, 120,000 – 200,000 babies are born each year with the sickle cell gene (WHO, 1994) while in Nigeria, it is estimated that 45,000 – 90,000 new babies are born each year with the gene (Shenoy, 2007). In the USA, sickle cell disease is considered the most common hemoglobinopathy and it is estimated that about 100,000 individuals suffer from the disease (Hassel, 2010).

Although there is no cure for SCD, several therapeutic strategies including antioxidant supplementation had been suggested (See Reviews by Hyacinth et al, 2011; Nur et al, 2011; Chirico and Pialoux, 2012; Imaga, 2013). Non-enzymatic antioxidants like vitamin C, Vitamin E and arginine are found in food sources. Food sources of vitamin C include citrus fruits, red and green peppers, cabbage and spinach. Food sources of vitamin E include green leafy vegetables (such as pumpkin and spinach), tomatoes, sweet potatoes, and carrots. Arginine is a naturally occurring basic amino acid which participates in many important biochemical reactions associated with normal physiology. It is found in proteinous foods like meat, poultry, nuts and fish and

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Hypoxia and the generation of free radicals

Hypoxia is the central or primary pathogenic event in SCD that causes intracellular polymerization of HbS. The effect of SCD on every part of the body stems from the fact that under hypoxic circumstances the red blood cells of a sufferer assumes a sickle shape in vivo. This renders the erythrocyte more viscous than normal blood. Increased blood viscosity decreases blood flow in the capillaries, terminal arterioles and veins leading to stasis. A vicious cycle is thus set up. The available oxygen is utilized by surrounding tissue, leucocytes and reticulocytes thus further increasing the level of hypoxia. The resulting hypoxia causes a worsening of the sickling process causing the production of more sickle cells. Sickle red blood cells are more fragile than normal erythrocytes. They easily undergo phagocytosis and are also more easily removed from circulation. The increased destruction of the cells results in chronic anaemia and hemolytic-type jaundice.

The RBC reoxygenation phase is a major source of free radical production in SCD. Repeated cycles of sickling and unsickling result in the production of irreversibly sickle cells. These cells play a role in the pathogenesis of sickle cell crisis (Dean & Schechter, 1978). The irreversibly sickle cells have increased intracellular viscosity due to their elevated hemoglobin concentration (Natta et al, 1980). The increased viscosity and increased membrane rigidity decrease blood flow in the capillaries, terminal arterioles and veins leading to stasis.

During the period of reoxygenation, normal RBCs can generate a significant amount of superoxide due to an electron transfer between the heme iron and oxygen. In the presence of oxygen, heme auto-oxidizes inducing methemoglobin and superoxide formation. Although both hemoglobin A (HbA) and hemoglobin S (HbS) blood have a tendency to auto-oxidize into methemoglobin and superoxide (Aslan et al, 2000; Conran et al, 2009), HbS blood has been shown to auto-oxidize 1.7 times faster than HbA blood (Wood et al, 2008; Hebbel et al, 1998). Unlike HbA, which can counter this reaction to form harmless byproducts, HbS can become overwhelmed by the continual source of superoxide (O$_2^-$) and, via its dismutation, hydrogen peroxide (H$_2$O$_2$) (Aslan et al, 2000). The formation of H$_2$O$_2$, when exposed to methemoglobin, decomposes hemoglobin and releases iron. The iron can then react with remaining H$_2$O$_2$ to further produce hydroxyl radicals (OH), the most reactive and harmful of the reactive species (Aslan et al, 2000). Sickle cells ultimately generate about twofold greater quantities of superoxide, hydrogen peroxide and hydroxyl radicals than HbA (Amer et al, 2006). These free radicals thus constitute oxidative stress burden and cause or are involved in multiple pathophysiologic mechanisms such as accelerated hemolysis (Krajewski et al, 2008), endothelial damage (Kato et al, 2009; Hebbel et al, 2004), reduced NO bioavailability (Sydow and Munzel, 2003; Beckman and Koppenol, 1996), and hypercoagulability (Singer and Ataga, 2008). These contribute to vaso-occlusion and damage to virtually every organ of the body (Nur et al, 2001; Morris et al, 2008). Sickle cell disease is therefore characterized by lifelong continuous oxidative stress which results from an imbalance between oxidants and antioxidants in favor of the oxidants.

Oxidative stress burden and response to antioxidant supplementation

The body's defense mechanisms against reactive oxygen species (ROS) include enzymatic and non-enzymatic systems. The enzymatic systems include catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and nitric oxide (NO) and heme oxygenase-1. The nonenzymatic antioxidants which scavenge free radicals include ascorbic acid, tocopherols, reduced glutathione (GSH), carotenoids, lipoic acid, ubiquinols, selenium, riboflavin, zinc, carotenoids, and uric acid as well as metal-binding proteins. (Chirico and Pialoux, 2012).

Increased production of oxidants and/or decreased availability of antioxidants trigger(s) a cascade of oxidative reactions damaging lipids, proteins, and DNA ultimately leading to premature cell death (Nur et al, 2011). Measurement of malondialdehyde (MDA) levels in blood had been used as an index of free radical generation. On the other hand, measurement of blood levels of antioxidant enzymes or non-enzymatic agents gave an indication of the ability of the subject to withstand the effects oxidative stress (El-Ghamrawy et al 2014; Jaja et al, 2013; Jaja et al, 2016).

Plasma levels of malondialdehyde (MDA), an index of free radical generation, had been shown to be higher in HbSS than in HbAA subjects (Titus et al, 2004; El-Ghamrawy et al 2014; Jaja et al, 2013; Jaja et al, 2016). Gbenebitse et al, (2005) investigated the effects of vitamin E supplementation on plasma vitamin E level, lipid peroxidation status, forearm blood flow and forearm vascular resistance in adult sickle cell anemia
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subjects. Results showed that supplementation increased plasma vitamin E level and forearm blood flow but reduced forearm vascular resistance and lipid peroxidation status. Change in plasma vitamin E level correlated positively with change in forearm blood flow \( (r=0.8; \ p=0.006) \) and negatively with lipid peroxidation \( (r=-0.8; \ p=0.003) \). This showed that a reduction in lipid peroxidation level with vitamin E supplementation accounted for the increase in forearm blood flow in these subjects. Total antioxidant levels are depressed in SCD sufferers (Fasola et al, 2009; Kehinde et al, 2015). Fasola et al, (2009) showed that total antioxidant status (TAS) levels were about 50% lower in the SCA patients compared with NSCA counterparts. Among SCA patients studied, 57.1% of those with TAS levels less than 1.00 mmol/L had bone pain crisis more than 3 times in the preceding year, compared with 16% in those with TAS levels greater than 1.00 mmol/L. Total leucocyte count and platelets were also significantly higher in the SCA patients than controls. (Fasola et al 2009). Plasma vitamin C or E had also been shown to be depressed in HbSS subjects and had been attributed to increased utilization of the vitamins or to increased renal loss (Jaja, et al, 2002; Hyacinth et al, 2011). Plasma l-arginine (also an antioxidant) concentration had also been shown to be lower in HbSS subjects than in their HbAA counterparts (Lopez et al, 2003; Scavella et al, 2010; Jaja et al, 2016). L-Arginine is the substrate for nitric oxide (NO) synthesis and in SCD increased NO consumption by cell-free haemoglobin (Reiter et al, 2003) and reactive oxygen species (Morris et al, 2005) lead to decreased NO bioavailability (Reiter et al, 2003; Gladwin et al, 2003). Low arginine bioavailability had been linked with early mortality in adult sickle cell disease sufferers (Morris et al, 2005).

Micronutrients and antioxidant supplementation.
Trace metals are required as co-factors for efficient functioning of antioxidants which are present as metallo-enzymes or metallo-proteins. Superoxide dismutase requires copper, zinc and manganese as cofactors, while catalase requires iron as a cofactor to catalyse the decomposition of hydrogen peroxide \( (\text{H}_2\text{O}_2) \) to water and oxygen. Glutathione peroxidase requires selenium as a cofactor and catalyses the degradation of \( \text{H}_2\text{O}_2 \) and hydro-peroxides at the expense of reduced glutathione (GSH) (Hyacinth et al, 2010).

Trace metals levels in blood had been reported as similar (Kehinde et al, 2010), lower (Olaniyi et al, 2010) or higher (Akenami et al, 1999) in HbSS subjects in the steady state when compared to their HbAA counterparts. Also, Kehinde et al, (2010) reported that plasma levels of \( \text{Cu}^{+2}, \text{Zn}^{+2} \) or \( \text{Mn}^{+2} \) in HbSS subjects in the acute phase or during pain crises were higher than those of HbAA subjects or HbSS subjects in the steady state. However, Temiye et al, (2011) had shown that in HbSS children in painful crises, serum \( \text{Zn}^{+2} \) level was lower than in HbAA children. They had suggested that painful crisis in SCA may exert greater demand for zinc utilization in children with SCA thereby resulting in lower serum levels. Ogungbemi (2014) had shown that arginine supplementation caused increases in serum levels of \( \text{Zn}^{+2} \) and \( \text{Cu}^{+2} \) in HbAA and HbSS subjects. The effect on serum \( \text{Mn}^{+2} \) was similar in both groups of subjects. Greater increases were seen in the HbSS than in the HbAA group (except for \( \text{Cu}^{+2} \)). It is not clear how arginine supplementation increased the serum levels of the trace metals. However, since the sources of the trace metals are dietary it is likely that arginine may have enhanced intestinal absorption of these trace metals from food. Ogungbemi (2014) also demonstrated significant and positive correlations between change in plasma arginine (\( \Delta[R] \)) and change in each of the trace metals levels and also between \( \Delta[R] \) and change in the various antioxidant enzymes levels suggesting that increased arginine levels resulted in elevated levels of trace metals and antioxidant enzymes. In addition, the study also demonstrated a significant and positive correlation between change in each of the antioxidant enzymes levels and change in each of the trace metals levels. The study also showed that the correlation coefficients \( (r) \) between change in measured antioxidant enzymes and change in trace metal levels were higher in HbSS than in their HbAA counterparts. Taken together, the study showed a relationship between serum trace metals concentrations and plasma antioxidant enzymes levels in the HbSS subjects.

Effect of antioxidant supplementation on haematological parameters.
Sickle cell disease subjects exhibit an elevated WBC, percent irreversibly sickle cells and platelet count and a reduced RBC count, hemoglobin concentration and hematocrit (Natt et al, 1980). Raised WBC count suggests disease severity. They could cause tissue damage and inflammatory reaction in vascular endothelium, increase expression of ligands for adhesion molecules on blood cells and can aggregate with other blood cells to cause vaso-occlusion (Okpala, 2004). On their own part, activated platelets secrete ADP which cause adherence to sickle erythrocytes contributing to platelet plug and formation of microvascular obstruction (Villagra et al 2007). The lower RBC, [Hb] and Hct values seen in the SCA subjects are a direct consequence of the destruction of RBC seen in this condition (Jaja et al, 2000).
In HbSS subjects, oral, low-dose (300mg/day for adults and 100mg/day for children) supplementation with vitamin C (Jaja et al, 2000; 2002b), vitamin E (Jaja et al, 2001) or L-arginine (Kehinde et al, 2015) for 6 weeks reduced mean corpuscular haemoglobin concentration (MCHC), percent of irreversibly sickle cells (%ISC) and also had a membrane stabilizing effect on the red blood cell.

Lower blood pressures in sickle cell disease
Studies in the United States of America, (Johnson & Giorgio, 1981, Saborio & Scheinman 1999) and Nigeria (Jaja et al 2000) have shown that sickle cell disease patients have lower blood pressures than the general population. The mean blood pressures reported by Johnson & Giorgio (1981) were 116/70 mmHg while that reported by Jaja and associates (2000) were 109/65 mmHg. Furthermore, it had been reported in the USA that whereas the incidence of hypertension in the black population is about 29% (Johnson & Giorgio, 1981, Saborio & Scheinman 1999), the incidence of hypertension in SCD patients is between 2 and 6%. Thus, blood pressure values that could be considered as normal or slightly elevated in healthy individuals may have serious cardiovascular and renal consequences in sickle cell disease patients (Sabório & Scheinman 1999). Although several reasons have been given for the lower blood pressures seen in sickle cell anaemia, the exact mechanism is not clear. Some of the reasons given include a renal tubular defect responsible for increased sodium and water excretion (Johnson & Giorgio, 1981) or a defect in the vascular tone (Hatch, 1989). A third reason is a reduced peripheral resistance at the arteriolar level resulting from altered vascular reactivity to circulating vascular hormones like nitric oxide (Nath et al 2000). Using the transgenic sickle mouse model, Kaul et al (2000) had suggested that the microcirculatory flow abnormalities in transgenic sickle mouse could lead to chronic tissue hypoxia that triggers vascular tone modifications secondary to chronic increase in NOS/NO activity. Thus the elevated NOS/NO activity results in systemic hypotension associated with a depressor effect on the vascular tone. Vitamin C supplementation in adult or paediatric HbSS subjects (Jaja et al, 2000, 2002) reduced while vitamin E (Jaja et al, 2001; 2003) or L-arginine (Ogungbemi et al, 2013) supplementation had little or no effect arterial blood pressure.

Abnormal autonomic cardiovascular response in sickle cell disease.
Sufferers of SCD have an abnormal autonomic cardiovascular function (Romero-Veccchione, 1995) by exhibiting a blunted autonomic cardiovascular response to change in posture (Jaja et al., 2008; Ogungbemi et al, 2013) which may result in sudden death (Romero-Mestre et al., 1997). Jaja et al, (2008) showed that change in posture from lying to an upright posture elicited significantly higher tachycardia and myocardial oxygen demand (estimated by the rate pressure product; RPP) in non sickle cell disease (NSCD) than in SCD subjects. The greater increase in RPP seen in the NSCA subjects was attributed to the greater increase in their heart rate response to change in posture. The blunted reflex responses to change in posture seen in the SCA subjects showed that sickle cell disease subjects possess an abnormal autonomic cardiovascular function.

Chronic low-dose vitamin C (300mg/day for 6 wks) or L-Arginine (1g/day for 6 wks) supplementation normalized the blunted hemodynamic changes associated with posture adjustments in HbSS subjects (Jaja et al, 2008; Ogungbemi et al, 2013). The effect of L-arginine was attributed to elevated nitric oxide metabolites (NOx) elicited by arginine supplementation (Ogungbemi et al, 2013).

Haemodynamic responses to antioxidant supplementation.
Various studies have shown the interaction between sickle red cells and vascular endothelium, with researchers demonstrating that almost all major adhesion pathways are involved in this interaction (Hebbel et al, 1980; Barabino et al, 1987). Carotid endothelial damage results in plaque formation, which, when detected sub-clinically, enables early intervention, thereby preventing stenosis, infarction, and stroke (Adams et al, 1997). Olowoye et al, (2011) compared the effects of chronic, oral, low-dose vitamin C supplementation on peak systolic velocity (PSV), end-diastolic velocity (EDV), resistivity index (RI), intima-media thickness (IMT), and cross-sectional diameter (CSD) of the common carotid arteries of HbSS patients with HbAA subjects. Measurements were made using a duplex sonographic scanner (Aloka ProSound SSD-3500; Aloka, Wallingford, Connecticut) with a high-frequency (5–10 MHz), linear-array transducer. Results showed that the pre-supplementation phase, CSD and EDV were significantly higher and RI was significantly lower in the HbSS subjects than in the HbAA subjects. The changes brought about by vitamin C supplementation were more manifest in HbSS than in HbAA subjects. Vitamin C supplementation had a slight effect on all the measured parameters in the HbAA subjects but caused an increase in PSV, EDV and RI in HbSS subjects. Using the same dose and duration, Jaja et al (2002) had earlier shown that vitamin C supplementation or warmth (40°C) decreased arterial blood pressure, forearm vascular resistance but increased forearm blood flow in HbSS subjects. Pretreatment with vitamin
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C enhanced the vasodilator effect of warmth. Vitamin C is known to reduce irreversibly sickle cells (Jaja et al, 2000) which may in turn cause a decrease in blood viscosity and contributing to the increase in blood flow velocity (Olowoyeye et. al, 2011).

Antioxidants and liver function
In sickle cell disease (SCD) decreased liver function by adulthood had been attributed to acute and/or chronic ischaemia in the liver which may result in liver dysfunction (Charlotte et al, 1995). Liver dysfunction results in elevated liver enzymes (Kehinde et al, 2010; Pandey et al, 2012; Jaja et al, 2013; 2016). Liver enzymes, alanine aminotransaminase (ALT), aspartate amino-tranaminase (AST) and alkaline phosphatase (ALP) had been shown to be elevated in HbSS subjects in crises (Kehinde et al, 2010) and in the steady state (Jaja et al, 2013). The elevated levels of these liver enzymes were however higher in subjects in crises than those in the steady state or non sickle cell sufferers (Kehinde et al, 2010). Elevated AST and ALT levels had been linked to damage to hepatic cells and hemolysis (Nsiah et al, 2011; Pandey et al, 2012) while elevated ALP had been related to destruction of the bile duct or bone associated with vaso occlusive crises resulting in delayed growth (Kotila et al, 2005).

In the steady state, vitamin C supplementation caused a reduction in the elevated plasma liver enzymes and malondialdehyde (MDA) levels and an elevation in plasma catalase level (Jaja et al, 2013). The combined effect of elevation of antioxidant enzyme (catalase) activity and reduction in MDA level by vitamin C suggests that reduced oxidative stress burden may account for the reduction in plasma liver enzymes levels (an indication of improved liver function) in these subjects. In another study, Jaja et al. (2016) showed that arginine caused greater percent reductions in ALT and AST in HbSS than in HbAA subjects. It also caused greater percent increases in plasma arginine concentration ([R]), and plasma nitric oxide metabolites concentration ([NOx]) in HbSS than in HbAA subjects. Reductions in [MDA] were similar in both groups. The study thus showed that although l-arginine supplementation improved liver function, oxidative stress, [R] and NOx levels in both groups of subjects the responses in HbSS subjects were more sensitive than in HbAA subjects.

Some other therapeutic strategies
Currently, there is no cure for sickle cell disease. However, hydroxyurea which is the approved therapy for the management of the disease may have certain benefits and drawbacks. It decreases the polymerization rate of Hb S by increasing Hb F concentration (Raghupathy and Billet, 2009). Although it is considered safe in the short term, it may possess myelosuppressive effects on leucocytes and platelets and does not prevent stroke even with elevation of Hb F levels (Atweh and Schechter, 2001).

Nitric oxide (NO) inhalation had also been suggested. In transgenic sickle mice, nitric oxide inhalation reduced red cell density, increased perfusion and decreased lung injury, microvascular vaso-occlusion and mortality (Martinez-Ruiz et al, 2001). However, clinical studies on nitric oxide inhalation in humans had provided divergent results. While inhaled NO significantly reduced pain scores in adult patients (Head et al, 2010) and children with acute vaso occlusive (VOC) pain (Weiner et al, 2003), Gladwin et al. (2011) showed that inhaled NO did not reduce VOC pain severity in SCD. Despite of these controversies, inhaled NO is cumbersome and may have side effects that require close monitoring in an acute care setting, thus limiting its application (Morris et al, 2000). Sildenafil was also used to amplify the effect of endogenous NO. Sildenafil inhibits the breakdown of its downstream signal transduction mediator, cyclic GMP (Machado et al, 2005) thus amplifying the effect of endogenous NO. However, the use of sildenafil has been discontinued because of safety concerns (Machado et al, 2011).

Stem cell transplantation (Piel et al, 2013) and gene therapy (Townes, 2008) are novel methods of treatment that are either in their experimental stages or considered very expensive and sophisticated for the generality of the African population that has a high SCD sub-population.

CONCLUSION
In conclusion, SCD is characterized by increased generation of ROS resulting in oxidative damage of various cell types and playing a significant role in the development of SCD related organ complications. The use of antioxidant supplementation has shown some promise as potential therapeutic agent. By limiting the production of ROS, many of the complications of SCD could be diminished. In endemic regions like sub-Sahara Africa, where the economy and technology are weak, effective therapy must be affordable and available. Antioxidant (vitamin C, E or L-Arginine) therapy is cheap and affordable. Further studies are still required to investigate the effect of antioxidants on glucose metabolism, haemostasis and possible role in the prevention of stroke in SCD.

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