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L-Arginine supplementation enhances antioxidant activity and erythrocyte integrity in sickle cell anaemia subjects

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Summary

The effect of oral, low-dose L-arginine supplementation (1 g/day for 6 weeks) on antioxidant activity, haematological parameters and osmotic fragility of red blood cells was investigated in sickle cell disease sufferers. Twenty eight sickle cell anaemia subjects were recruited for the study. Five millilitres of blood was withdrawn from an ante-cubital vein for the estimation of plasma arginine concentration ([*R*]), total antioxidant enzymes (TAE) activity, malondialdehyde concentration ([MDA]), RBC count, [Hb], PCV, MCHC, MCV, MCH, percent irreversibly sickled cells (%ISC)) and osmotic fragility of red blood cells in the subjects. L-arginine supplementation increased [*R*] ($p < 0.001$), TAE activity ($p < 0.05$) and MCV (<0.05) but reduced plasma [MDA], MCHC, MCH and %ISC ($p < 0.001$, respectively). Δ[*R*] correlated positively with ΔTAE ($r = 0.8$) and negatively with Δ[MDA] ($r = -0.7$) and Δ%ISC ($r = -0.5$). Also ΔTAE activity correlated negatively with Δ[MDA] ($r = -0.7$) and Δ%ISC ($r = -0.6$). Supplementation shifted the osmotic fragiligram to the right and reduced the concentrations of NaCl at which initial and complete lyses of erythrocytes occurred. Study showed that low-dose, oral L-arginine increased antioxidant activity, red blood cell resistance to osmotic lysis but reduced red cell density in SCD.

Keywords: L-Arginine; Antioxidant activity; Erythrocyte integrity; Sickle cell anaemia

Introduction

Sickle cell haemoglobinopathy is common among black populations of the world and arises from a replacement on the beta chain of haemoglobin of gluthamic acid by valine at the 6th position [1]. Sickle cell anaemia is characterized by chronic haemolytic anaemia and periodic, painful vaso-occlusive events [2]. The incidence of sickle cell disease (SCD) in Africa is about 5–40% [3]. It is estimated that in Africa 120,000 to 200,000 babies are born each year with SCD [4] while in Nigeria, estimated annual number of HbSS neonates is about 85,000 [5].

Sufferers of SCD have a high oxidative stress burden. The factors that contribute to the burden include recurrent ischaemia-reperfusion injury [6], elevated cell-free haemoglobin [7] and higher autoxidation of sickle haemoglobin [8]. The consequences of high oxidative stress burden in SCA include increased haemolysis [7], endothelial damage [9], increased degradation or consumption of antioxidant enzymes [10] and reduced nitric oxide (NO) activity [11] resulting in vaso-occlusion and organ damage [12]. The antioxidants include enzymatic antioxidants like superoxide dismuthase, catalase and glutathione peroxidase.

There is no known cure for sickle cell disease. However, hydroxyurea which is being currently used in the management of the disease decreases the polymerization rate of Hb S by increasing Hb F concentration [2,13]. It is considered safe in the short term but may possess myelosuppresive effects on leucocytes and platelets. In addition, it does not prevent stroke even with elevation of Hb F levels [14]. Another therapy that had been suggested is the use of inhaled nitric oxide. In transgenic sickle mice, nitric oxide inhalation reduced red cell density, increased perfusion and decreased lung injury, microvascular vaso-occlusion and mortality [15]. However, clinical studies on nitric oxide inhalation in humans had

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provided divergent results. While inhaled NO significantly reduced pain scores in adult patients [16] and children with acute VOC pain [17], Gladwin et al. [18] showed that inhaled NO did not reduce VOC severity in SCD. Inspite 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 [19]. Sildenafil had also been used to amplify the effect of endogenous NO by inhibiting the breakdown of its downstream signal transduction mediator, cyclic GMP [20]. However, the use of sildenafil was discontinued because of safety concerns [21]. Stem cell transplantation [5] and gene therapy [22] 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.

Arginine therapy has also been suggested. In sickle cell transgenic mice, arginine had been shown to improve microvascular function [23] but decreased inflammatory cytokines and c-reactive protein [24], inhibited red cell Gardos channels and red cell density [25] and protected against oxidative stress [10]. Arginine had been shown in humans with SCD to increase erythrocyte glutathione levels [26], nitric oxide metabolites (NO_x) [19] and caused a significant reduction in total opioid use and pain scores in children that had acute pain crises [27]. However, the doses of L-arginine used in these studies were high and for variable periods.

It had been suggested that low-dose arginine therapy was likely to be sub-therapeutic in SCD since earlier studies had shown that low-dose arginine did not affect NO synthesis [28,29]. Furthermore, it had also been suggested that higher plasma concentrations of arginine are needed to produce beneficial effects in SCD subjects [24] since arginine metabolism had been shown to be different between sickle cell disease subjects in the steady state and those in vaso occlusive crisis or normal non sickle cell disease subjects [19,29].

Since different authors had employed high dose and different periods of administration of arginine we rationalized that low dose, long term administration of arginine may enhance plasma concentrations of arginine which may be beneficial to the disease sufferers. This study therefore sought to investigate in sickle cell anaemia subjects the effect of a low-dose, long term oral supplementation with L-arginine (1 g/day for 6 weeks) on plasma arginine concentration ([*R*]), total anti-oxidant enzymes (TAE) activity, malondialdehyde concentration ([MDA]), osmotic fragility, some haematological parameters, red blood cell indices and percent irreversibly sickled cells (%ISC).

Materials and methods

Twenty eight (28) sickle cell anaemia subjects (14 males and 14 females) were recruited for the study after due ethical approval had been obtained from the Research Grant and Experimentation Committee of the College of Medicine of the University of Lagos. Subjects were out-patients of the Sickle Cell Clinic of the Lagos University Teaching Hospital, Lagos at the time of study. The blood counts and blood film were done for the red cell morphology (which showed sickle cell and some target cells). The sickling test was performed using solubility (hard red band on top and colourless solution, using a freshly prepared buffer mixture and packed red cell from EDTA anticoagulation blood). The haemoglobin electrophoretic pattern was determined for confirmation. [30]. The subjects had not undergone any blood transfusion in the last six months. Persons with the sickle cell trait (HbAS) were excluded from the study.

All subjects gave informed consent before participating in the study.

On entering the laboratory, the age (years), height (metres) and weight (kilograms) of each subject was recorded. Five millilitre of blood was withdrawn from an ante-cubital vein of the subject for the measurement of plasma arginine concentration [*R*], total anti-oxidant enzymes (TAE) activity, malondialdehyde concentration ([MDA]), osmotic fragility, haematological parameters (red blood cell count and haemoglobin concentration), red blood cell indices (packed cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular volume) and irreversibly sickled cells (ISC).

Determination of plasma L-arginine level ([R])

Plasma L-arginine concentration ([*R*]) (μmol/L) was determined using a modification of the Sakaguchi reaction [31]. A standard curve using dilutions of 100% L-arginine was prepared. Briefly, to each test tube containing 1.0 mL of either 0, 4, 8, 12, 16 or 20 mg/L of 100% L-arginine was added 0.2 mL of 0.02% 8-hydroxyquinoline and 0.2 mL of 10% NaOH. The resulting solution was thoroughly mixed and 0.1 mL of 0.4% sodium hypochlorite was added to develop the colour of the solution in each test tube. Further, 0.2 mL of 40% urea was added immediately and mixture kept in a water bath at 0 °C for 40 min before its absorbance was read at 500 nm with a UV-1700 Spectrophotometer (SpectrumLab S23A, Medical Globe, England). A standard curve of absorbance against concentration was then plotted.

In order to determine the concentration of L-arginine in plasma, 1 mL of test plasma was used instead of 100% L-arginine. Absorbance was read on the Spectrophotometer and plasma L-arginine concentration (μmol/L) was calculated from the standard curve. L-arginine concentration [*R*, mg/L] was then converted to μmol/L.

Determination of total anti-oxidant enzymes (TAE) activity

Total anti-oxidant enzymes activity was assessed using the method of Koracevic [32]. Absorbance was read at 532 nm with a UV-1700 Spectrophotometer (SpectrumLab S23A, Medical Globe, England) using deionized water as control.