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Supplementation with L-arginine stabilizes plasma arginine and nitric oxide metabolites, suppresses elevated liver enzymes and peroxidation in sickle cell anaemia

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ABSTRACT

The effect of L-arginine on liver function in SCD has received little or no attention. The effect of a chronic, oral, low-dose supplementation with L-arginine (1 gm/day for 6 weeks) on some liver enzymes, lipid peroxidation and nitric oxide metabolites was studied in 20 normal (non-sickle cell anaemia; NSCA) subjects and 20 sickle cell anaemia (SCA) subjects. Ten milliliters of blood was withdrawn from an ante-cubital vein for the estimation of plasma arginine concentration ([R]), alanine aminotransaminase (ALT), aspartate aminotransaminase (AST) and alkaline phosphatase (ALP), plasma total bilirubin concentration [TB], malondialdehyde concentration [MDA] and nitric oxide metabolites concentration [NOx]. Before supplementation, ALT, AST, ALP ($p < 0.05$ respectively) and TB ($p < 0.001$) were higher in SCA subjects than in NSCA subjects. [R] and [NOx] were higher in NSCA subjects ($p < 0.001$ and $p < 0.05$ respectively). Supplementation caused greater percent increases in [R], and [NO_X] in SCA than in NSCA subjects ($p < 0.001$ in each case). L-Arginine caused greater percent reductions in ALT and AST in SCA subjects but greater percent reduction in ALP in NSCA subjects ($p < 0.001$ in each case). Changes in [MDA] and [TB] in the two groups were similar. Study shows that chronic, oral, low-dose supplementation with L-arginine improved liver function, oxidative stress, plasma arginine concentration and nitric oxide metabolites levels in NSCA and SCA subjects. Responses in SCA subjects to L-arginine were more sensitive than in NSCA subjects.

1. Introduction

Sickle cell disease (SCD) is predominantly seen in Africa and Southeast Asia. It is a multi-system disease, associated with episodes of acute illness and progressive organ damage [1]. There is a high oxidative stress burden in the SCD sufferer. High oxidative stress burden had been attributed to recurrent ischaemia-reperfusion injury [2], elevated cell-free haemoglobin [3] and higher auto-oxidation of sickle haemoglobin [4]. The consequences of high oxidative stress burden in SCD include increased haemolysis [5], endothelial damage [6] reduced NO_X activity [7] decreased levels of antioxidant enzymes [8,9] and elevation of lipid peroxidation levels [10] resulting in vaso-occlusion and organ damage [7].

There is no known cure for SCD. However, the usefulness of arginine in the treatment of sickle cell disease had been suggested by numerous reports based on animal and human studies. In sickle cell transgenic mice [11] or sickle cell disease patients [12] low plasma arginine level was associated with increased oxidative stress. In sickle cell anaemia patients arginine had been shown to increase nitric oxide metabolites (NO_X) bioavailability [12], increased antioxidant activity, red blood cell resistance to osmotic lyses but reduced red blood cell density [13,14]. It had also been demonstrated in sickle cell mice that arginine decreased inflammatory cytokines and C-reactive protein, inhibited red cell Gardos channels, reduced red cell density [15] and improved microvascular function [11]. In sickle cell mouse model, arginine increased erythrocyte glutathione levels [16] and protected against oxidative stress [17]. Clinical studies had also been conducted to test the beneficial effects of arginine on sickle cell disease [18,19]. While Styles et al. [18] had expressed doubts about the usefulness of arginine therapy the study of Morris et al. [19] offered some promise.

The effect of L-arginine on liver function in SCD has received scant attention. However, previous studies had shown that liver lesions leading to liver dysfunction are commonly seen in sickle cell disease sufferers [20,21]. Multiple factors that may contribute

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to the etiology of liver disease include ischaemia, intra-hepatic sinusoidal sickling, transfusion related viral hepatitis, iron overload, and gallstones [21,22]. Hepatomegaly, a frequent finding in sickle cell anaemia had been associated with an increase in expression of liver enzymes. Oparinde et al. [23] had demonstrated a significant increase in serum alanine aminotransaminase, alanine phosphatase and gamma-glutamyl transferase levels in sickle cell anaemia subjects with persistent hepatomegaly over those without hepatomegaly. Kehinde et al. [24] had also shown that during sickle cell anaemia crises aspartate aminotransaminase (AST), alanine aminotransaminase (ALT) and alkaline phosphatise (ALP) were significantly higher than values obtained from sickle cell subjects in the steady state or non sickle cell anaemia subjects. Elevated plasma alkaline phosphatase levels had been attributed to delayed growth or bone destruction due to associated vasoocclusive crises [25] while elevated aspartate aminotransaminase (AST) had been attributed to intravascular haemolysis [26].

The aim of this study therefore was to investigate and compare the effect of low dose, oral, chronic supplementation of L-arginine on some liver enzymes, plasma total bilirubin concentration [TB], malondialdehyde concentration [MDA] and nitric oxide metabolites (NOx) in SCD subjects and non-SCD subjects as controls.

2. Materials and methods

Forty (40) male and female adult subjects were recruited for the study after their medical history had been taken. Twenty (20) participants were subjects without sickle cell anaemia (HbAA). They served as control subjects and were students of some tertiary institutions in Lagos, Nigeria. They were non-smokers and non-alcoholics. Twenty (20) other participants were sickle cell anaemia (HbSS) subjects. 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 [27]. Persons with the sickle cell trait (HbAS) were excluded from the study.

The HbSS subjects were patients attending the Sickle Cell Out-Patients' Clinic of The Lagos University Teaching Hospital, (LUTH), Idi-Araba, Lagos, Nigeria. They were adjudged to be in the steady state since none of them had been admitted to the ward for pain crisis in the preceding six months. They also had no history of blood transfusion in the last twelve months.

Institutional approval was obtained from the Ethics and Experimentation Committee of the College of Medicine of University of Lagos, Lagos, Nigeria and informed consent was obtained from each subject before the commencement of study.

After about 15 min in the laboratory, anthropometric data: age (years), height (meters) and weight (kilograms), were recorded. Ten millilitres (10 mL) of blood was withdrawn from an antecubital vein of each subject for the estimation of haematological parameters, plasma arginine (R) and malondialdehyde (MDA) concentrations, liver enzymes, plasma total bilirubin concentration [TB] and nitric oxide metabolites (NOx). L-Arginine (Mason Vitamins, Inc. Miami Lakes, Florida, USA.) was then administered to each subject orally at a dose of 1 g/day for 6 weeks. After 6 weeks, the parameters were measured again.

2.1. Estimation of some haematological parameters and red blood cell indices

Red blood cell count (RBC count, million/mm^3), haemoglobin concentration [Hb, g/dL], mean corpuscular haemoglobin concentration (MCHC, g/dL), mean corpuscular volume (MCV, fL), mean corpuscular haemoglobin (MCH, pg) and packed cell volume (PCV, %) were estimated using an automated counter (Mindray BC 2800 Haematology Automated System, China).

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

Plasma L-arginine concentration ([R]) (μmol/L) was determined using a modification of the Sakaguchi reaction [28]. 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.

2.3. Estimation of malondialdehyde

Malondialdehyde, (MDA), a secondary product of lipid peroxidation, was determined using the thiobarbituric acid method. Malondialdehyde was estimated with the spectrophotometer at 535 nm based on the principle that lipid peroxides condense with 1-methyl-2-phenyl indole under acidic conditions [29].

2.4. Determination of serum concentration of nitric oxide metabolites ([NOx])

The formation of [NO_x] was measured by determination of its stable end products in serum, i.e. nitrite (NO_2^-) and nitrate (NO_3^-) in mmol/l, as previously described [30]. Briefly, Cayman chemical nitrate/nitrite assay kit was used to measure serum nitric oxide metabolite concentration ([NO_X]). Serum was digested by adding 2.50 mL of 6 N HCl to 2.50 mL of serum and left for 30 min to react. It was then filtered into a 25 mL conical flask and the filtrate was made up to 25 mL with distilled water. The Greiss reagents were then added to the filtrate. The resultant nitrite produced was estimated using a spectrophotometer at 540 nm. Nitric oxide metabolites ([NO_X]) was then determined using nitrate/nitrite ratio [30].

2.5. Determination of plasma total bilirubin concentration ([TB])

Plasma total bilirubin concentration was measured with the method of Sherlock as modified by Sadeek and Abd El-Razek [31]. Sample and blank were read at a wavelength of 578 nm on a spectrophotometer.

2.6. Measurement of plasma concentration of liver enzymes

Colorometric methods utilizing RANDOX® kits were used in the measurement of liver enzymes aspartate aminotransferase, (AST), IU/L; alanine aminotransferase, (ALT), IU/L and alkaline phosphatase, (ALP) IU/L.