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Research Article

Blood Pressure, Haematologic and Biochemical Changes Following L-Arginine Supplementation in Children with Sickle Cell Anaemia already on Hydroxyurea Therapy

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Summary: Hydroxyurea is an approved therapy for management of children suffering from sickle cell disease (SCD). In adult sufferers, co-administration of hydroxyurea and l-arginine had shown some benefits. This study examined the effect of co-administration of hydroxyurea (15-35mg/kg/day) and l-arginine (500 mg/day) for 6 weeks on blood pressure and haematological indices, liver and antioxidant enzymes levels. The levels of these parameters when the subjects were on hydroxyurea alone were taken as control values. Results showed that combined therapy (HU + l-arginine) decreased SBP, DBP, MAP and PP ($p < 0.01$ respectively) but enhanced %HbF, Hb and PCV (p< o.001 in each case). It elevated CAT, SOD, GPX ($p < 0.001$ in each case) but depressed MDA, AST, ALT and ALP ($p < 0.001$ respectively). Study thus showed that combined therapy (HU + l-arginine) provided more useful benefits to children suffering from sickle cell anaemia than hydroxyurea alone.

Keywords*: Hydroxyurea, L-Arginine, children, sickle cell anaemia*

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INTRODUCTION

Sickle cell disease (SCD) results from a single mutation in the beta globin chain inducing the substitution of valine for glutamic acid at the sixth amino acid position. This mutation leads to the production of abnormal haemoglobin S (HbS) (Pauling *et al*, 1949; Ingram, 1956). The sickle cell gene is dispersed around the world and consequently is recognized as a world-wide problem due to its impact on mortality and morbidity. The incidence of this molecular disease in Africa is between 5 – 40%. In Nigeria it is estimated that there are about 88,000 new births annually and about 160,000 new births per annum in Africa. Apart from sub-Saharan Africa where the disease is most frequently found, it is also found in the United States of America, Brazil, Greece Turkey (Diallo and Tchernia, 2002; Piel *et al*, 2013).

Polymerization of the abnormal haemoglobin (HbS) has clinical manifestations. These include chronic haemolytic anaemia resulting in sickle cell anaemia (SCA) and periodic microcirculatory vaso-occlusion which gives rise to high oxidative stress burden, tissue ischemia and pain. Eventually, there is chronic end-organ damage reducing the lifespan of the individual (Hebbel *et al*, 2009).

Hydroxyurea (hydroxycarbamide) and Endari (L-glutamate) are approved therapies for SCD in adults and children (Carden and Little, 2019). Hydroxyurea induces hemoglobin F (HbF) and increases red blood cell volume, thus reducing the chances of polymerization of HbS. In addition, it elevates the level of nitric oxide in plasma thus decreasing mortality and morbidity (McGann and Ware, 2015).

Arginine participates in many biochemical activities of normal physiology. It is a basic amino acid that occurs naturally and found in proteinous foods like meat, poultry, nuts and fish and also in watermelon. L-Arginine supplementation (1g/day for 6wks) in adults normalized the blunted hemodynamic changes associated with posture adjustments in HbSS subjects. The effect of arginine was attributed to increased nitric oxide metabolites (NO_X) (Ogungbemi *et al*, 2013). L-Arginine had also been shown in SCA sufferers to lower elevated liver enzymes and malondialdehyde, but elevating nitric oxide metabolites (NO_X) (Jaja *et al*, 2016).

Some studies had suggested that l-arginine could enhance the therapeutic effects of hydroxyurea when they are co-administered. Morris *et al*. (2003) showed that arginine when given acutely did not increase serum NOx production in SCD patients at steady state, but it did when given together with hydroxyurea. In addition, Eleuterio *et al*, (2019) had shown that co-administering hydroxyurea and l-arginine for four months increased serum NOx levels and relieved pain, thus demonstrating that co-administration of the two drugs may be useful for pain relief and thus improving the quality of life of these patients.

Sickle cell disease eventually affects all systems of the body. However, the effect of a combination of hydroxyurea and l-arginine on several systems of the body has not been investigated in children suffering from sickle cell anaemia. This study investigated the effect of a combination of hydroxyurea and l-arginine on blood pressure, haematologic indices, liver and antioxidant enzymes on children suffering from sickle cell anaemia that were already on hydroxyurea therapy.

MATERIALS AND METHODS

Ethical consideration: Institutional approval was obtained from The Research Grants and Experimentation Ethics Committee of the College of Medicine, University of Lagos, Lagos, Nigeria (CM/COM/8/VOL.XXIII). A parent of each child that participated in the study gave informed consent before the start of the study.

Study population and location: Thirty seven (37) children were recruited for the study. However twenty two (22) children completed the study. Fifteen (15) children were unable to complete the study especially due to school examinations. The children were of both sexes aged between 3 years and 12 years. They were previously confirmed to be suffering from sickle cell anaemia and thus regularly attending the Pediatrics Outpatient Clinic of the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria.

Data collection: Each participating patient was already on hydroxyurea therapy (15 -35 mg/kg/day). Age (yrs) was recorded. Height, (m) and weight (kg) were measured and recorded. Blood pressure was measured before withdrawal of five milliliter of blood from an anti-cubital vein of the patient. Part of the collected blood was stored in anticoagulant bottles for the measurement of haematological indices while the other part was centrifuged, serum collected in plane bottles for the estimation of liver enzymes, antioxidant enzymes and malondialdehyde (lipid peroxidation). Each patient then received 500 mg/day of l-arginine (Mason Vitamins Inc. Miami Lakes, Florida, USA) for six (6) weeks. L-Arginine served as an addition to hydroxyurea therapy. Re-evaluation of the parameters followed after six weeks of combined therapy.

Blood pressure measurement: Auscultatory method was employed in the measurement after the subject had taken a 15-minute rest. Arterial blood pressure was measured on the brachial artery of each subject in the supine position. Disappearance of Korotkoff's sound (fifth phase) marked diastole. Pulse pressure was the difference between systolic blood pressure and diastolic blood pressure. Mean arterial blood pressure was calculated as diastolic blood pressure plus one third of pulse pressure.

Estimation of hematological parameters: Percent foetal haemoglobin (%HbF) and packed cell volume (PCV) were estimated with the microhaematocrit method. Haemoglobin concentration ([Hb] (g/dl) was determined with the colorimetric method (Dacie and Lewis, 1991).

Measurement of liver enzymes: Serum liver enzymes (aspartate amintransferase, AST, IU/L), alanine aminotransferase (ALT, IU/L) and alkaline aminotransferase (ALP, IU/L) were measured with colorimetric method utilizing RANDOX® kits.

Estimation of malondialdehyde: Malondialdehyde was measured with the spectrophotometer using the thiobarbituric acid method (Titus *et al*, 2004).

Determination of blood levels of antioxidant enzymes: Serum CAT and SOD levels were assayed as described by Rukkumani *et al*, (2004) while serum GPx level was measured as described by Ellman (1959).

Data analyses

Results have been expressed as mean ± standard error of mean (χ ± SEM). Paired t' test was used in making statistical comparisons. Significance was accepted when $p< 0.05$.

RESULTS

Children were between the ages of three (3) years and twelve (12) years (Mean ± SEM = 5.60 ± 1.1 years). Their heights ranged from 1.0 m to 1.42 m (1.2 ± 0.05 m). Mean weight slightly increased from 18.6 ± 2.6 kg (range 10.0 kg – 35.0 kg) at the beginning of the study to 19.7 ± 2.2 kg (range 10.0 kg – 38.0 kg) at the end of the study. Mean body mass index (BMI) also increased slightly from 16.4 ± 0.6 kg/m^2 (range 14.0 kg/m^2 to 17.4 kg/m^2) to 17.2 ± 0.4 kg/m^2 (range 13.8 kg/m^2 to 18.0 kg/m^2) within the 6-week study period.

Table 1 compares blood pressure responses to hydroxyurea (HU) therapy alone with the combination (HU + L-Arg) therapy. Combination therapy significantly reduced the blood pressure parameters ($p < 0.001$ in each case) except pulse pressure. Table 1 also shows that combination therapy significantly elevated each of the hematological parameters ($p < 0.001$ in each case).

Table 1:
Comparison of blood pressure and haematological parameters before and after L-Arginine supplementation.

Parameters	HU alone Mean ± SEM (Range)	HU + L-Arg Mean ± SEM (Range)	Δ (mm Hg)	Δ (%)	P level
SBP (mm Hg)	101.1 ± 5.7 (60.0 – 123.0)	89.8 ± 4.5 (58.0 – 120.0)	- 11.2 ± 1.8	- 11.2 ± 1.6	< 0.01
DBP (mm Hg)	65.9 ± 3.5 (40.0 – 98.0)	56.9 ± 2.8 (40.0 - 70.0)	- 8.9 ± 2.6	-13.6 ± 3.2	< 0.01
MAP (mm Hg)	77.6 ± 4.0 (46.6 – 106.3)	67.8 ± 3.2 (50.6 – 87.8)	- 9.8 ± 2.2	-12.6 ± 2.4	< 0.01
PP (mm Hg)	35.2 ± 3.0 (15.0 – 40.0)	32.1 ± 1.9 (15.0 – 40.0)	- 3.0 ± 2.2	-1.0 ± 7.0	NS
HbF (%)	5.3 ± 0.5 (1.6 – 11.9)	7.0 ± 0.5 (3.7 – 13.2)	1.7 ± 0.4	32.1 ± 5.6	< 0.001
Hb (g/dl)	7.9 ± 0.3 (4.8 – 10.9)	9.6 ± 0.2 (6.1 – 12.3)	1.7 ± 0.2	21.6 ± 4.8	< 0.001
PCV (%)	22.4 ± 0.7 (13.7 – 27.3)	27.6 ± 0.7 (17.7 – 32.5)	5.3 ± 0.3	23.5 ± 2.8	< 0.001

KEY
SBP = Systolic blood pressure; DBP = Diastolic blood pressure; PCV = Packed cell volume ; HU = Hydroxyurea
MAP = Mean arterial pressure; L-Arg = L-Arginine; PP = Pulse pressure ; Δ = Change;
HbF = Hemoglobin F; %Δ = Percent change; Hb = Hemoglobin; NS = Not Significant

Table 2:
Effect of combination therapy on malondialdehyde, antioxidant and liver enzymes.

Parameters	HU alone Mean ± SEM (Range)	HU + L-Arg Mean ± SEM (Range)	Δ	%Δ	P Level
MDA (μmol/L)	16.8 ± 1.6 (13.6 – 30.4)	13.2 ± 1.1 (8.6 – 26.4)	- 3.6 ± 0.3	- 22.3 ± 2.1	< 0.001
CAT (μmol/ml)	1.7 ± 0.04 (1.4 – 2.0)	2.2 ± 0.06 (1.9 – 2.6)	0.5 ± 0.5	30.9 ± 2.5	< 0.001
SOD (μmol/ml)	23.1 ± 1.2 (13.7 – 33.8)	26.4 ± 1.2 (18.5 – 35.1)	3.3 ± 0.3	15.4 ± 2.0	< 0.001
GP_X (μmol/ml)	1.8 ± 0.1 (0.3 – 3.0)	3.0 ± 0.1 (2.3 - 10.3)	1.1±0.1	86.8 ± 26.0	< 0.01
AST (IU/L)	10.8 ± 1.0 (3.2 – 15.0)	7.2 ± 0.6 (7.4 – 14.4)	-3.6 ± 0.5	-29.0 ± 2.6	< 0.001
ALT (IU/L)	7.1 ± 0.6 (3.2 – 11.8)	4.2 ± 0.3 (2.6 – 10.5)	-2.9 ± 0.5	-35.1 ± 4.2	< 0.001
ALP (IU/L)	278.2 ± 25.1 (148.0–737.0)	117.4 ± 7.3	-160.8± 27.1	-52.6 ± 4.4	< 0.001

Key:
MDA = Malondildehyde; Δ = Change; CAT = Catalase; %Δ = Percent change; SOD = Superoxide dismuthase ALT = Alanine aminotransferase; GPX = Glutathione peroxidase; ALP = Alkaline phosphatase; AST = Aspartate aminotransferase

Table 2 shows that combination therapy (HU + L-Arg) caused an elevation of antioxidant enzymes levels (p < 0.001 in each case) but lowered MDA (p < 0.001) and each of the liver enzymes levels (p < 0.001 in each case).

DISCUSSION

Subjects were paediatric patients of the Paediatric Out-Patients' Sickle Cell Clinic at the Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria. Subjects were in the steady state. Informed oral consent was obtained from a parent of each child. The children had been on hydroxyurea (HU) therapy.

Although BP was not measured before HU therapy, results of this study showed that HU may not have affected the blood pressure of the subjects. The values reported in this study were higher than values reported for normal Nigerian children (Umar *et al*, 2016). Inspite of its benefits, HU is reported not to prevent stroke in SCA sufferers (Atweh and Schetchter, 2001). Blood pressure values in children (Hussain and Hassan, 2017) and adults (Ajayi *et al*, 2013) suffering from SCA had been reported to be lower than in the general population.

Our results show that the combination therapy (HU + Arginine) significantly reduced the blood pressure of the subjects. Morris *et al* (2013) had demonstrated that oral arginine reduced estimated pulmonary artery systolic pressure in sickle cell patients suffering from pulmonary hypertension. That arginine reduced blood pressure when used as an adjunct to hydroxyurea could be regarded as beneficial to the subjects. It had been suggested that blood pressures which appear normal or slightly elevated in healthy individuals may have serious cardiovascular and renal consequences in sickle cell disease patients (Saborio and Scheinman, 1999).

Arginine supplementation following hydroxyurea therapy caused a significant increase in HbF%, [Hb] and PCV. Elevation of foetal haemoglobin in SCD improved the rheological characteristics of erythrocytes by decreasing intracellular polymerization of HbS (Rodgers *et al*, 1993). Hydroxyurea is known to raise the level of HbF and haemoglobin (Agrawal *et al*, 2014). In an earlier study in adult SCD patients, Little *et al*, (2009) could not demonstrate any increase in HbF% when arginine was used as an adjunct to hydroxyurea. Enhancement of HbF%, [Hb] and PCV as seen in this study would improve oxygen delivery to tissues.

Result of this study showed that combining hydroxyurea therapy with L-arginine supplementation decreased blood levels of measured liver enzymes. A comparable study was not found in literature. There is also no report of the effect of hydroxyurea alone on these liver enzymes in sickle cell anaemia. However, L-arginine (Jaja *et al*, 2016) or Vitamin C (Jaja *et al*, 2013) supplementation had been shown to decrease these liver enzymes in adult SCA subjects. Elevated liver enzymes levels in SCA had been related to hepatic damage and haemolysis (Kotila *et al*, 2005; Nsiah *et al*, 2011).

Combined hydroxyurea and l-arginine elevated antioxidant enzymes levels while decreasing lipid peroxidation (MDA). Thus the combination therapy resulted in reduced oxidative stress burden in the patients. This result is similar to that of Little *et al*, (2009) which showed the elevation of glutathione when hydroxyurea was augmented with arginine. The mechanism of action of l-arginine in SCD sufferers had been shown to be by reducing oxidative stress burden through the elevation of antioxidant enzymes levels and reducing malondialdehyde (Kehinde *et al*, 2015; Jaja *et al*, 2020). L-Arginine also elevates trace metals levels in blood which form an integral part of antioxidant enzymes (Ogungbemi *et al*, 2018). It may also act through the nitric oxide pathway (Ogungbemi *et al*, 2013; Bakshi and Morris, 2016; Eleuterio *et al*, 2019). Hydroxyurea, on its own part, inhibits DNA synthesis by inhibiting the activity of ribonucleotide reductase (RR) – the enzyme that converts ribonucleosides into deoxxyribonucleosides which are building blocks for DNA synthesis (Agrawal *et al*, 2014). It also elevates the level of nitric oxide in blood (McGann and Ware, 2015).

The short coming of this study was that basal values of the parameters were not measured before the commencement of hydroxyurea therapy. A more structured study will overcome that shortcoming. In conclusion, the study suggests that a combination of l-arginine and hydroxyurea in the management of paediatric patients suffering from SCA may be beneficial.

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