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Research Article

Effect of Papaverine on Electrolyte Profile in Sickle Cell Anaemia

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ABSTRACT

In vitro studies were carried out to ascertain the effect of Papaverine, a calcium channel inhibitor, on electrolyte profile in sickle cell anaemia. Blood samples of genotype AA and AS obtained from volunteers were used as control. Hematological and plasma Ca^{2+} status of the samples were ascertained after a preliminary analysis. Results of electrolyte profile revealed that Papaverine significantly ($p<0.05$) restored salinity, conductivity and total dissolved solute in both the test group and controls.

Keywords: Papaverine, Electrolyte, Salinity, Conductivity, total dissolved solutes, erythrocyte, sickle cell anaemia

INTRODUCTION

Sickle cell anaemia results from a point mutation in the genetic code such that glutamic acid is replaced by valine at 6th position of β-globin chain of hemoglobin (Hb). This substitution transforms normal adult hemoglobin (HbA) into sickle hemoglobin (HbS). In a low oxygen tension environment, the replaced valine can bind to a complementary hydrophobic site on beta subunit of another hemoglobin tetramer in a polymerization process that leads to the sickling of the red blood cells (RBCs) (Hundekar *et al.,* 2010). Polymerization of deoxygenated sickle hemoglobin (HbS) tetramers is central to the process of vasoocclusion (Serjeant, 1997). This makes the erythrocyte rigid, distorts its shape, and cause structural damage in the red-cell membrane, all of which alter the rheologic properties of the cell and impairs blood flow through the microvasculature leading to hemolysis and vasoocclussive episodes (Bunn, 1997). In the deoxygenated state, HbS is heavily dehydrated due to loss of electrolyte and its solubility is 10% that of HbA (Stocker *et al.,* 2003; Ortiz *et al.,* 1990). Deoxygenation of sickle RBCs boosts calcium influx by about five folds resulting to net calcium accumulation vesicles (Karaki *et al.,* 1997) and eventual dehydration of red cells, a prominent feature in sickle cell anaemia. The total cellular calcium content of sickle erythrocyte is abnormally high (about 40µmole/liter of cell) when compared with normal red cells (about 16µmole/litre) (Eluwa *et al.,* 1990). Dehydration is also due to loss of cellular K^+ (Kracke *et al.,* 1988). The loss of K^+ is partially offset by an increase in cellular cations probably resulting from cell membrane damage and increased Na^+/Ca^{2+} intrusion. The major mechanisms of K^+ loss and sickle cell dehydration are Ca^{2+} activated K^+ channel (Gardos Channel) in which increased intracellular Ca^{2+} leads to large K^+ loss with accompanying movement of Cl^- and water, higher intracellular haemoglobin concentration and subsequent polymerization of deoxygenated Hb S (Brugnara *et al.,* 1993; Eaton and Hofrichter, 1987). The Gardos channel of sickle cells either alone or in conjunction with K^+ Cl^- cotransport play a major role in cell dehydration (Brugnara *et al.,* 1995). Studies have shown that sickle cells can become dense via loss of K^+ mediated by the K^+ Cl^- co-transport system (Kracke *et al.,* 1988). Increasing cell Mg^{2+} above physiologic levels markedly decresed K-Cl co-transport activity. Thus, any maneuver leading to an increase in cell Mg^{2+} content should inhibit dehydration via K-Cl co-transport (Lal *et al.,* 1996). Deoxygenation and sickling can be induced by Na^+ and K^+ fluxes and Na-K pump. The increase in Na^+ and K^+ permeability and sickling are associated with increased entry of calcium ions (Bookchin *et al.,* 1994; Vestergaard-Bogind *et al.,* 1987). Oxidative damage of erythrocyte membrane contributes to K^+ loss and sickling. Erthrocytes are particularly susceptible to peroxidative damage because they contain hemoglobin, one of the most powerful catalysts for initiation of peroxidative reaction (Chiu *et al*, 1982). However, under ambient oxygen tensions, sickle cells spontaneously generate superoxide radical $O_2^{\bullet -}$, hydrogen peroxide H_2O_2 and hydroxyl radical $OH^{\bullet}$ approximately two times more, when compared to normal RBCs (Hebbel *et al.,* 1982; Moore *et al.,* 1992). The membranes of sickle cells possess higher levels of denatured hemoglobins, hemin, and nonheme iron (Kuross *et al.,* 1988). It is speculated that the nonheme iron component is free iron bound to the membrane, possibly chelated to anionic phospholipids. These iron compounds enhance the oxidative stress in sickle cell membranes, and this has been proposed as the cause for many of the changes that occur in sickle cell anemia, including dehydration, adhesivity (Moore *et al.,* 1992) and electrolyte imbalance. The relationship between sickling and cell transport had been investigated by Tosteson in 1955. Transport

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Table 1: Result of Some Haematological Parameters and Plasma Calcium levels in the different Blood Samples

Parameter	AA	AS	SS	Normal Range
PCV (%)	40	47	39	40 – 52
ESR (mm/hr)	3	4	8	0 - 5
Haemoglobin (g/dl)	14.0	15.8	11.0	13 – 17.5
RBC Count (/mm^3)	$3.58x10^6$	$2.81x10^6$	$3.32x10^6$	
Platelet Count (/mm^3)	$2.5x10^5$	$1.7 x10^5$	$1.5 x10^5$	$1.5x10^5 – 4.0x10^5$
Plasma Calcium (mg/dl)	11.1	8.1	12.9	

Table 2: Results of Salinity (%)

Sample	Salinity (%)		
	Before Incubation	After Incubation	
		Solution B	Papaverine
AA	0 at 31.0^0C	4.3±0.25 at 28.8^0C	0 at 26.2^0C
AS	0 at 28.1^0C	4.8±0.21 at 26.7^0C	0 at 26.8^0C
SS	0 at 31.1^0C	0.1±0.34 at 29.1^0C	0 at 27.2^0C

experiments with tracers of Na^+, K^+ and Cs^+ indicated that sickling induced a reversible and poorly selective increase in the electrodiffusional cation permeability of the erythrocyte membrane (Tanner, 1993) thereby demonstrating the sickling induced permeability pathway referred to as the P. sickle.

Several therapeutic strategies to prevent sickle cell dehydration are aimed at altering ion transport in sickle erythrocytes including clotrimazole and other imidazole antimycotics that are specific inhibitors of Ca^{2+} activated K^+ channel (Anthony *et al.,* 2003; Brugnara *et al.,* 1993). Increased intracellular Mg^{2+} may partially inhibit K^+ Cl-cotransport in sickle cells. P-sickling permeabilizes sickle cells to Mg^{2+} and subsequent increase in Mg^{2+} potentiates Cl^- intrusion to maintain intracellular electroneutrality. Water also moves into the cell causing a decrease in cell Hb concentration and ultimately, ameliorates Hb polymerization (Falke *et al.,* 1984).

Papaverine is a calcium channel blocker that acts in a concentration dependent manner to inhibit voltage-dependent Ca^{2+} inward current in L-type Ca^{2+} channel thereby providing a realistic antisickling strategy (Iguchi *et al.,* 1992). Papaverine also has inhibitory effects on other membrane currents including the voltage-dependent transient outward K^+ current and the Ca^{2+}-activated oscillatory K^+ current or Gardos channel (Iguchi *et al.,* 1992). The aim of the study therefore is to ascertain the effect of Papaverine on the electrolyte profile in sickle cell anaemia.

MATERIALS AND METHOD

Blood samples from three volunteer male donors were used in this study. The haemoglobin genotypes were confirmed to be AA, AS and SS using the electrophoretic tank method with a standard AS blood sample as the control.

1.Determination of Haematological Parameters: Erythrocyte Sedimentation Rate was determined using the method of Westergreen. Micromethod with the aid of a microhaematocrit capillary tube as described by Dacie and Lewis (1994) was used to determine the Packed Cell Volume. Haemoglobin level was determined using the Cyanide Haemoglobin Method. Red blood cell and platelet counts were determined using standard methods. A Randox calcium kit was also used to measure the plasma calcium levels.

2.Membrane Extraction from Blood Samples:To remove the white blood cells, a quantity 3ml of blood sample was filtered through a 10ml syringe containing glass wool initially wetted with distilled water. The filtrate was washed severally through centrifugation at 10,000rpm using a washing buffer. Subsequently, the samples were dialyzed for three (3) hours at 37^0C using 300ml of incubation buffer. Following incubation, the sample was re-centrifuged and the supernatant decanted after which the pellets were washed severally with distilled water until all the haemoglobin was removed leaving the membrane. The membrane was dried and weighed on a filter paper after which it was macerated using a volume of ice cold extraction buffer which is three times the weight of the membrane. Finally, the membrane was refrigerated for further use.

3.Electrolyte Profile Analysis: The salinity, conductivity, and total dissolved solute of the blood samples were determined using a multimeter. Residues of the three blood samples pre-washed in a centrifuge were used in taking measurements. After taking readings for one sample, the electrode was properly washed with deionized water before inserting into the next sample. The readings were taken again after incubation with an incubation buffer Solution B (145mM NaCl, 20mM KCL, 4mM $MgCl_2$, 10mM Glucose, 20mM Tris, 0.5mM Urea and 1.8mM $CaCl_2$) as well as with the calcium inhibitory drug, Papaverine.

4.Statistical Analysis: Values are expressed as mean ± SD, significance of the mean differences between the groups was assessed by two way analysis of variance.

RESULTS

1.0 Results of Haematological Parameters: The PCV, ESR, haemoglobin, RBC and Platelet Counts were found to be 40%, 3mm/hr, 14g/dl, $3.58x10^6$/mm^3 and 2.5 $x10^5$/mm^3 for the AA respectively; 47%, 4mm/hr, 15.8g/dl, $2.81x10^6$/mm^3 and 1.7 $x10^5$/mm^3 for AS respectively; and 39%, 8mm/hr, 11.0g/dl, $3.32x10^6$/mm^3 and 1.5 $x10^5$$mm^3$ for SS respectively. The calcium level

Table 3: Results of Conductivity (µs)

Sample	Conductivity (µs)		
	Before Incubation	After Incubation	
		Solution B	Papaverine
AA	10.4±0.50 at 31.0^{0}C	8.7± 0.65 at 28.9^{0}C	4.0±0.22 at 26.2^{0}C
AS	3.4±0.34 at 28.1^{0}C	11.2±0.22 at 26.6^{0}C	3.9±0.30 at 27.1^{0}C
SS	8.7±0.41 at 31.3^{0}C	232±3.21 at 29.6^{0}C	3.9±0.25 at 27.2^{0}C

Table 4: Results of Total Dissolved Solute (mg/L)

Sample	Total Dissolved Solute (mg/L)		
	Before Incubation	After Incubation	
		Solution B	Papaverine
AA	5.1±0.42 at 31.0^{0}C	4320±2.10 at 28.9^{0}C	2.1±0.67 at 26.2^{0}C
AS	1.0±0.31 at 28.5^{0}C	5880±7.33 at 26.7^{0}C	2.0±0.15 at 27.0^{0}C
SS	17.0±0.01 at 31.1^{0}C	119±1.20 at 29.3^{0}C	2.0±0.05 at 27.1^{0}C

in samples AA, AS and SS were found to be 11.1mg/dl, 8.1mg/dl and 12.9mg/dl respectively.

2.0 Electrolyte Profile: Results of salinity test shows that the salinity before incubation and after incubation with Papaverine were 0% in all the groups. The values were found to increase significantly after incubation with solution B in all groups when compared to values before and after incubation with Papaverine.

Result after incubation with Papaverine showed marked decrease ($p<0.05$) in conductivity values (3.9 – 4.0µs) when compared with values before and after incubation with Solution B. Results of total dissolved solute after incubation with Papaverine decreased significantly ($p<0.05$) in all groups when compared with values before and after incubation with Solution B.

DISCUSSION

Sickling is accompanied by an intraerythrocytic loss of potassium and gain of sodium, thus creating disequilibrium in the ionic strength across the cell membrane (Statius *et al.,* 1971). From the result of haematological studies, the packed cell volume of SS was found to be 39% when compared to AA (40%) and AS (47%). The value in SS sample was below the normal range for male (40-52%) due to anaemia (Dacie and Lewis, 1994). Similarly, haemoglobin level in the SS (11.8g/dl) was below the normal range for male (13.5-17.5g/dl) (Hoffbrand *et al.,* 2001) when compared to the controls AA (15.8g/dl) and AS (14.0g/dl). The erythrocyte sedimentation rate in SS (8mm/hr) increased significantly beyond the normal range (0-5mm/hr) and when compared to AA (3mm/hr) and AS (4mm/hr). This is due to elevated subpopulation of dehydrated dense cells in sickle red blood cell samples (Hebbel, 1991). Plasma Ca^{2+} level of SS sample (12.9mg/dl) are high when compared to the controls AA (11.1mg/dl) and AS (8.1mg/dl). This is due to elevated Ca^{2+} influx as a result of altered membrane permeability under hypoxic condition, a common feature in sickle cell anaemia. Results of electrolyte profile analysis reveal that the salinity before incubation was 0%. However, salinity increased markedly after incubation with Solution B in all test groups due to electrolyte intrusion. The higher values obtained with AA (4.3%) and AS (4.8%) test groups were probably due to NaCl present in the medium (Engelmann and Duhm, 1987) while the lower value (0.1%) in SS test group may be due to the disruption in membrane integrity of erythrocyte that affects the membrane transporters. Salinity was restored in all the test groups after incubation with Papaverine. This drug significantly decreased erythrocyte conductivity in all the test groups when compared to values before and after incubation. The highest decrease was observed in the SS test sample. Similarly, result of total dissolved solute showed that Papaverine had significant decrease in total dissolved solute when compared to values before and after incubation. This again, may be due to a decrease in the level of insoluble salts, especially ($CaCl_2$) after incubation with the drug.

CONCLUSION

From the results, it had been shown that Papaverine decreased the electrolyte profile in sickle cell anaemia in vitro. The drug can be utilized in the management of the disease but in vivo studies should be carried out to determine the possible side effects and toxicity beyond its normal physiological concentration.

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