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Research Article

Ionic fluxes in erythrocyte membranes of sickle cell anaemia subjects at different tonicities

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ABSTRACT

Background: Cells with markedly increased Haemoglobin concentration are a prominent feature of sickle cell disease, as a consequence of the loss of K^+, Cl^- and water from the erythrocyte. This affects the erythrocyte microrheology, resulting in cellular abnormalities. The potentials of reversing this phenomenon is currently being studied as a mechanism of rehydrating the cells. The aim of this study was to investigate ionic fluxes in membrane of erythrocytes at different tonicities with a view to highlighting any selective ionic-fluxing potential of homozygous HBS when compared to normal haemoglobin. **Methods:** 5ml heparinized blood samples were obtained from subjects with different haemoglobin genotypes (AA, AS and SS). Erythrocytes were washed until a clear supernatant was seen and haematocrit was adjusted to 50% for each. Equal volumes were dispensed into 1ml (v/v) of de-ionized water, 40mM high potassium Physiological salt solution (PSS), 80mM K^+ PSS and fresh coconut water respectively. The samples were then centrifuged at 2000 r.p.m. for 10 minutes and electrolyte (Na^+, K^+, and Cl^-) concentrations were determined from the supernatant obtained, using flame photometry. Values are expresse d as means ± SEM and analyzed using 2-way analysis of variance; post-hoc analysis was done using Bonferroni test. **Results:** HbS, at high K^+ PSS, exhibited a significantly higher concentration of potassium ion in the supernatant with relatively lower concentration of Sodium and Chloride ions compared to that of HBA and HBAS ($P<0.05$, respectively). There was no significant difference between ionic fluxes across membrane of erythrocytes with genotype AA and AS. **Conclusion:** Relatively high potassium ion could possibly inhibit Na^+/K^+ -ATPase activities within the membrane of HBS, thereby allowing for significantly higher potassium ion efflux and influx of sodium, Chloride and water to account for the rehydration/ sickling-reversal process.

INTRODUCTION

Concentration gradients of sodium and potassium ions across the erythrocyte membrane are primarily maintained by an active transport of sodium and potassium by the sodium pump (Sakardi and Toseton, 1979). The characteristics of the sodium potassium pump have been extensively studied in sickled erythrocytes. Studies of active pumping mechanism have yielded conflicting results, with Na^+-K^+ -ATPase activity of sickled erythrocyte membrane increased (Luthra and Sears, 1982) and that of intact cells reported to be deficient (Ortiz et al., 1986) or fully intact (Izumo et al., 1987). Deoxygenation of sickle RBCs variably increased passive potassium efflux and sodium influx three-folds to five-folds (Berkowitz and Orringer, 1985). This effect on permeability is probably cation selective and accompanied by stimulation of the Na^+-K^+ -ATPase (Izumo et al., 1987). This leak enhancement cause sickling and cell deformation with areas of membrane affected by spiculation being mostly implicated as leak sites (Mohandas et al., 1986; 2008). The consequence of abnormal cation homeostasis regardless of the specific mechanism involved is directly responsible for several cellular defects observed in HbS. These defects include excessively elevated P_{50}, poor cellular deformability and abnormal

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microrheology, all of which have been implicated in development of vaso-occlusion (Hebbel et al., 1986).

Haemoglobin which is the component of the erythrocyte that transports oxygen is made up of two alpha subunits and two beta subunits. The genes that encode the subunits have alleles, many of which derive from point mutation in the DNA sequence that led to single amino acids substitution in the protein (Cavalli-Sforza and Bodmer, 1971). The effect of this genetic aberration has been variously demonstrated in the sickle cell membrane abnormalities too. Haemoglobin S is much less soluble than the normal haemoglobin and it begins to crystallize when the oxygen concentration falls, as it does in the capillaries of the tissues. This causes the red blood cells, normally biconcave disc-shaped, to assume the shape of a crescent or sickle. With this shape, the red blood cells become less efficient in carrying oxygen (Roberts, 1986).

One of the distinguishing characteristics of sickled erythrocytes is the presence of cell dehydration, which is due to loss of cell potassium, is partially offset by an increase in cell sodium content, probably resulting from cell membrane damage, increased sodium leak and relative sodium pump inhibition (Brugnara et al., 1993). According to Brugnara et al., (1996), there are four major mechanisms responsible for potassium loss and cell dehydration in sickled erythrocytes: A) Ca^{2+}-Activated K^+ Channel (Gardos Pathway); B) K-Cl co-transport; C) Deoxygenation induced Na and K fluxes and Na-K pump; D) Oxidative damage of the cell membrane and K loss. Sickled erythrocytes have an increased haemoglobin S concentration as a consequence of the loss of potassium, chloride and water from the erythrocyte; these dehydrated erythrocytes rapidly sickle when deoxygenated. Blockage of potassium loss from the erythrocyte should therefore prevent the increase in Haemoglobin S concentration and reduce sickling (Brugnara and Tosteson, 1987).

Research findings on ion fluxes via membrane of erythrocytes of different haemoglobin genotypes at different tonicities have not been documented; although it has been established that deoxygenation of sickle erythrocytes increased passive potassium efflux and sodium influx three to five folds (Berkowitz and Orringer, 1985; Glader and Nathan, 1978). Ajayi and Ogbee (2010) demonstrated a significant rehydration of sickled erythrocytes with different high potassium – isotonic solution and the remarkable reversal of the sickled shape. In furtherance to these reports, this study was designed to investigate possible variations in ionic fluxes in membrane of erythrocytes of different haemoglobin genotypes at different tonicities with a view to highlight any selective ionic-fluxing potential of homozygous Haemoglobin S when compared with normal haemoglobin.

METHODS

Subjects: a total of fifty subjects were randomly selected and enrolled for this study. They were apparently healthy individuals with ages 18-30 years. They comprised of 20 each for HbA and AS while the remaining 10 subjects were HBS.

Specimen collection: five milliliters of blood was collected from ante-cubital vein and placed in lithium heparin sample bottles.

Experimental protocol:

Haemoglobin genotypes were confirmed using Helena's Hemoglobin Electrophoresis Procedure after which the erythrocytes were washed Briefly, Samples were centrifuged at 2000 rpm for 5minutes and supernatant was removed, normal saline was then added to wash the samples severally until a clear supernatant was obtained. Haematocrit was adjusted to 50% for each blood sample to approximate the total number of erythrocytes tested in each experiment. 1ml of aliquots was respectively dispensed into 1ml of: De-ionized water; 40mM high potassium PSS; 80mM high potassium PSS and Fresh coconut water – known for its high content of potassium ions but isotonic. The samples were then centrifuged and the electrolytes (Sodium, Potassium and Chloride) concentrations of the supernatants were estimated with flame emission photometry.

Statistical analysis:

All analyses were done with graph pad prism 5.0 statistical software. Values are expressed as means ± SEM and analyzed using 2-way analysis of variance; post-hoc analysis was done using Bonferroni test. $p<0.05$ was regarded as statistical significant.

RESULTS

Figure 1 shows sodium fluxes via membrane of erythrocytes of different haemoglobin genotypes at different tonicities. Concentration of Sodium ion in extracellular medium was significantly ($p<0.05$) lower at different tonicities (except for coconut water) in erythrocytes from HBS genotype compared with HBAA and HBAS, there were no significant differences ($p>0.05$) in the concentrations of sodium when erythrocytes HBAA and HBAS were compared at different tonicities

Figure 2 shows potassium ion fluxes via membrane of erythrocytes of different haemoglobin genotypes at different tonicities. Concentration of Potassium ions in extracellular medium was significantly ($p<0.05$) higher at different tonicity in erythrocytes from HBS genotype compared with HBAA and HBAS, there was no

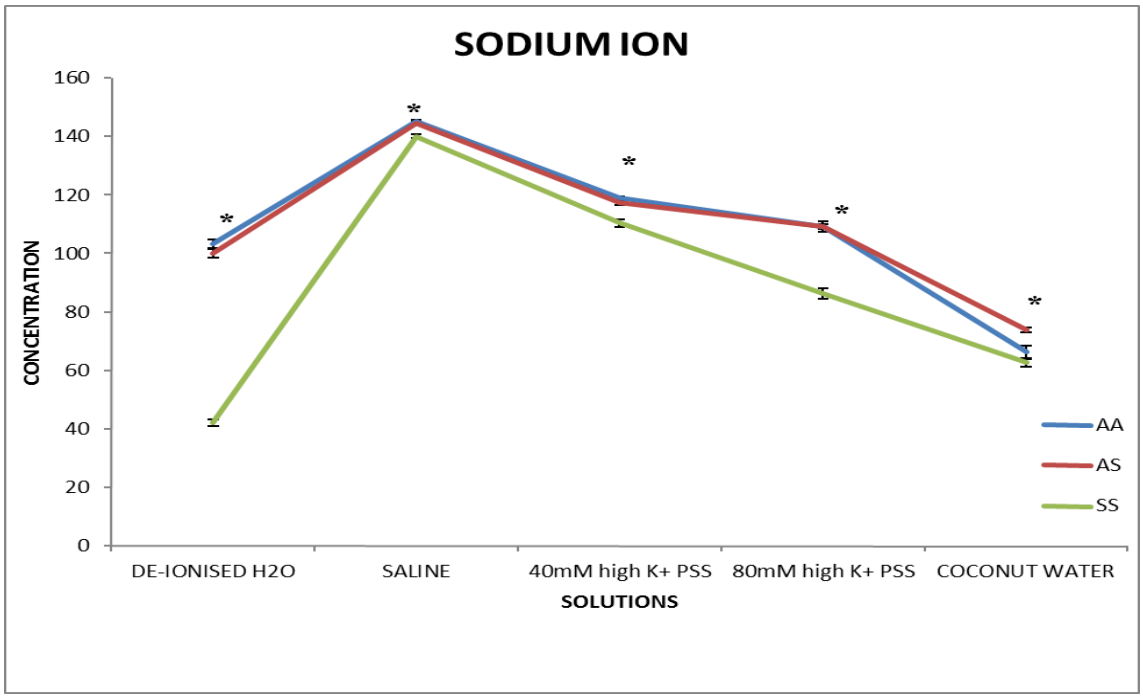

Fig. 1: shows sodium fluxes via membrane of erythrocytes of different haemoglobin genotypes at different tonicities. *P<0.05 compared to SS

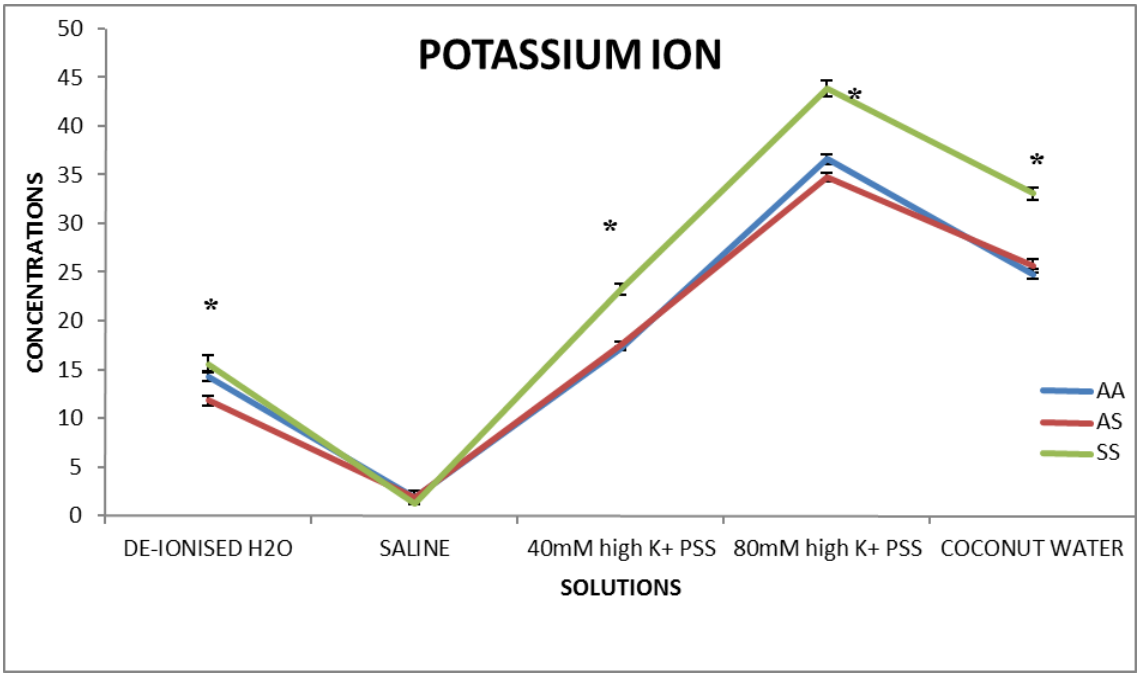

Fig. 2: shows potassium ion fluxes via membrane of erythrocytes of different haemoglobin genotypes at different tonocities *P<0.05 compared to SS

significant difference (p>0.05) in the concentrations of Potassium when erythrocytes AA and AS were compared at different tonicities.

Figure 3 shows chloride ion fluxes via membrane of erythrocytes of different haemoglobin genotypes at different tonicity. Concentration of Chloride ion in extracellular medium was significantly (p<0.05) lower at different tonicity (except for De-ionized water) in erythrocytes from HBS genotype compared with HBAA and HBAS, there was no significant difference (p>0.05) in the concentrations of Chloride ion when erythrocytes AA and AS were compared at different tonicities.

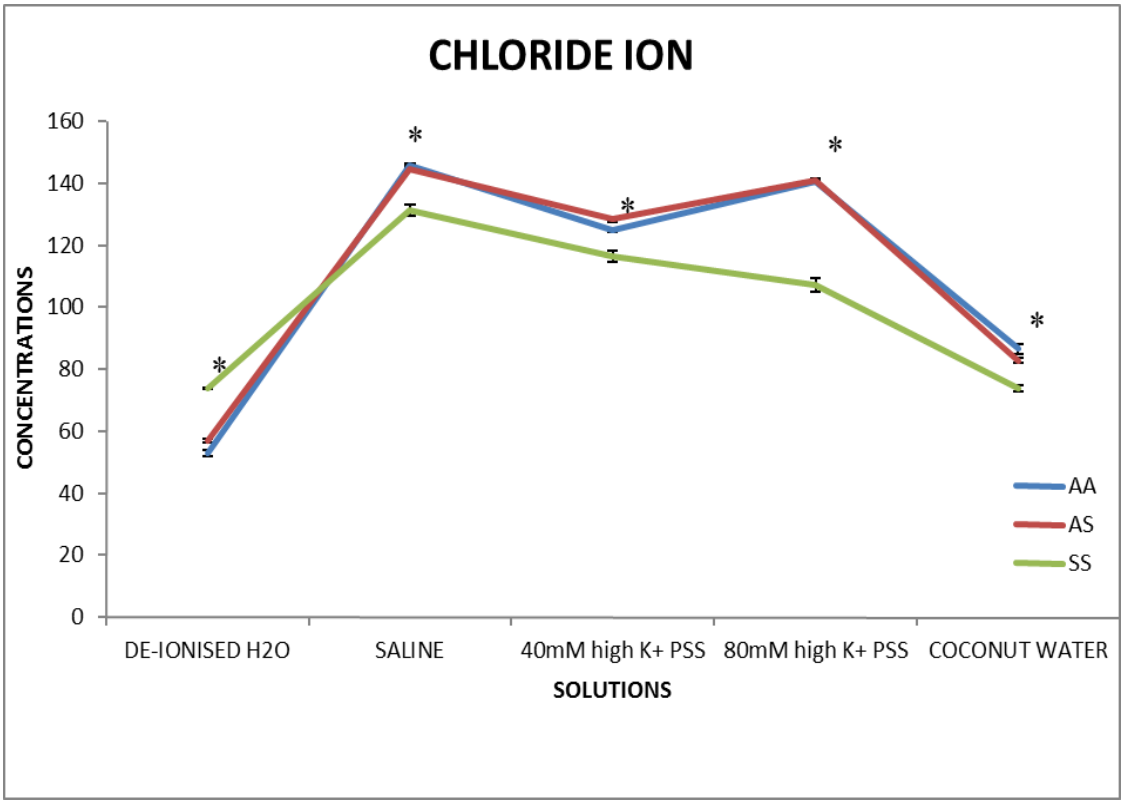

Fig. 3: shows chloride ion fluxes via membrane of erythrocytes of different haemoglobin genotypes at different tonicity *P<0.05 compared to SS

DISCUSSION

The erythrocyte membrane is only slightly permeable to the major cations Na^+ and K^+ and their movement depends greatly on an energy requiring transport mechanism. Within the human erythrocyte, potassium is the predominant cation and sodium is a minor constituent whereas the relationship is reversed in plasma. Research findings on ion fluxes via membrane of erythrocytes of different haemoglobin genotypes have not been documented at different tonicities; although it has been established that deoxygenation of sickle RBCs variably increased passive potassium efflux and sodium influx three-folds to five-folds (Berkowitz and Orringer, 1985; Glader and Nathan, 1978). The increased potassium efflux has been inextricably linked with the increased calcium ion in the HBS plasma via the Gardos channel (Brugnara *et al*., 1996). We have investigated possible variations in ionic fluxes in membrane of erythrocytes with different haemoglobin genotypes at different tonicities. We observed at high potassium PSS, that there was a significantly ($P<0.05$) higher concentration of potassium ion with lower Sodium ion in extracellular medium in HBS compared with that of AA and AS. There were also no significant differences between ionic fluxes via membrane of erythrocytes with genotype AA and AS.

At extracellular high potassium, it seems the sodium pump becomes relatively inefficient in the HBS erythrocyte membrane thereby limiting the rate of potassium influx and thereby allowing for an increase accumulation of sodium, chloride and water within the cell. The rehydration here is via a different mechanism from the previously suggested one by Ajayi and Ogbee, (2010). They investigated via the ca^{++} operated K-Cl pathway and reported a possible inhibition of Gardos channel, the present study utilized blood samples devoid of plasmatic components especially calcium. The increase in potassium ion in the extracellular medium therefore could only be explained through a postulation that without calcium mediated exflux which was reversible by high potassium in the ECF, Sodium influx was more pronounced as an exchange with chloride and water as co-transporters. This will ultimately create rehydration of the cells, but its effects on the mean corpuscular haemoglobin may not be high enough to create a significant reversal of the sickled cell as previously observed. However, there could be other underlying mechanisms for this observation which may be explained by further studies to clearly elucidate the rehydration process with unwasged red cells and the intact plasma of HBS erythrocyte in high potassium media. It is puzzling however to note a possible dependency of the Na^+/K^+ ATPase activity on

calcium ion especially in HBS subjects. Also, HBS demonstrated differential pattern of ionic exchange across their cellular membrane at different tonicities, this indicate an intrinsic genetic alteration (which is dose dependent) of selective permeability to ions in sickle cell anaemia. The close pattern of ionic exchange in HBAS and HBAA confirms this observation.

CONCLUSION

From this study, we conclude that: At high potassium PSS, there was a greater influx of Sodium and Chloride ions via the membrane of erythrocytes with haemoglobin genotype SS compared to that of AA and AS. This indicated a possible rehydration effect of high potassium media by possibly inhibiting Na^+/K^+ - ATPase activities within the membrane, especially for erythrocytes from HBS genotype. It is plausible therefore to indicate a membrane intrinsic aberration due to the homozygous state of HBS in SCA, that enhances selective permeability of cations as an additional build up mechanism towards dehydration and subsequent vaso-occlusion.

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