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Research Article

Increased Resistance to osmotic lysis of sickled erythrocytes induced by *Cocos nucifera* (coconut) water

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ABSTRACT

Background: Various anti-sickling agents (nutrients, drugs, phytochemicals and ions) which by their actions, inhibit the pathophysiological mechanisms leading to sickling or polymerization of sickle red blood cells in the vasculature have been reported. *Cocos nucifera* water: *CNw* (a natural isotonic beverage, with the same level of electrolytic balance as plasma but with a higher concentration of potassium ions in substitution for sodium), also an ultra-filtrate liquid in the endosperm of coconut, has been shown to have tremendous medicinal values. These properties of *CNw* could be employed to block the major pathway in the pathophysiology of dehydration of the cells. In this study, we have examined the effect of *CNw* on osmotic fragility index of sickle red cells. **Materials and Methods**: A total of 30 blood samples comprising of HbSS (10), HbAS (10) and HbAA (10) were obtained from subjects after an informed consent. The samples were divided into two sets with each sample treated in duplicate with graded percentage concentrations of NaCl (0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9). One set was pre-incubated with *CNw* while the other set was exposed directly to access their resistivity to various hypotonic lysis. The absorbance was recorded after 30mins incubation for each set with a standard spectrophotometer at 540nm wavelength. Hemolysis in each tube was recorded and expressed as percentage of the absorbance in distilled water. The average values recorded were plotted against the different NaCl concentrations used. **Results**: Erythrocytes from different haemoglobin genotypes (AA, AS and SS) pre-treated with *CNw* had significantly reduced osmotic lysis when compared with the untreated set ($P<0.05$, respectively) at various hypotonic NaCl concentrations. Various Hb genotypes exhibited a graded increase in osmotic pressure lysis in the order HbSS>>HbAS>>HbAA. **Conclusion:** The incubation of red cells with *CNw* seems to induce a significantly high resistance to osmotic pressure on the red cell membrane thereby reducing the rate of osmotic destruction of the cells during hypotonic stress. This may be a clue towards reversing sickling phenomenon and alleviating the haemolytic crisis in sickle cell patients. We further highlighted that the herozygote HbS state has greater osmotic reistance that nprmal hemogblobin but lesser than that of the homozygous state HbSS.

INTRODUCTION

The homozygous state of SCA causes moderate to severe anemia resulting both from hemolysis and the reduced oxygen affinity of HbS. As a result, the effectiveness of red blood cells is compromised. Instead of being round and soft, the cells become rigid and crescent (sickle) shaped, making it difficult to pass through small blood vessels and capillaries thereby clugging the passage and resulting in characteristic venous and arterial thrombosis, vaso-occlusive and haemolytic crisis coupled with chronic organ damage. HbS is the most common pathological haemoglobin variant worldwide and majority of children born with SCA die before reaching five years of age (Weatherall *et al*., 2006). The prevalence of SCA varies all over the world with Turkey having about 4.6% of sickle cell trait and Western Nigeria with 2.4% in 2006; this figure was expected to have risen by 2015 (Taiwo *et al*.,

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2011). Unfortunately, Nigeria has the largest global population of people with sickle cell disorder, with more than 100,000 Nigerian children being born each year with the disorder (*www.scaf.org.ng/page-the_sickle_cell*). Treatment for sickle cell anemia is usually aimed at avoiding crises, relieving symptoms and preventing complications. Treatments may include medications to reduce pain and prevent dehydration, blood transfusions and supplemental oxygen, as well as blockade of K^+ loss from the erythrocyte to prevent the increase in HBS concentration and reduce erythrocyte sickling and hemolysis. The literature contains reports on various anti-sickling agents (nutrients, drugs, phytochemicals and ions) which by their actions, inhibit the pathophysiological mechanisms leading to sickling or polymerization of sickle red blood cells in the vasculature. Of recent, nanomedical practice has proposed the use of nanorobots to repair damaged erythrocytes (*www.powershow.com/view./Medical_Applications of_Nanotechnology)*, this is considered in view of possible aid to SCA sufferers all over the world. *CNw*, an ultra-filtrate liquid in the endosperm of coconut has been shown to have tremendous medicinal values. It is a natural isotonic beverage, with the same level of electrolytic balance as the plasma but with a higher concentration of potassium ions in substitution for sodium. These properties of the water could be employed to block the major pathway in which sickle cells could easily be dehydrated before sickling processes. This is hoped to provide both immediate and long lasting remedy to the sufferers of this dreaded disease. We have previously demonstrated the reversibility potentials of cocos nucifera water on sickled red cells by a proposed membrane counter-transport of Potassium and Chloride (Ajayi and Ogbee, 2010) and more recently, we demonstrated the relativity in different hemoglobin genotypes of how 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 processes (Ajayi and Arishe, 2015). In furtherance to the on-going studies on *CNw*, to characterize the membrane mechanisms involved in its ability of reversing sickled cells and its other efficacies, the effect of *CNw* on osmotic fragility of red cells with different hemoglobin genotypes was investigated in this study.

METHODS

Subjects:

A total of 30 subjects comprising of HbSS (10), HbAS (10) and HbAA (10) participated in this study after an informed consent. They were apparently healthy subjects within the ages of 15-25 years. The SCA subjects were at stable states during the study.

Blood sampling

Five milliliters (5ml) of blood was collected from each subject into lithium heparin anticoagulants and analyzed within 2hrs of collection

Experimental protocols

The samples were divided into two sets with each sample treated in duplicate with graded percentage concentrations of NaCl (0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9) as described by Dacie and Lewis, (1984). Briefly, one set was pre-incubated with *CNw* while the other set was reacted directly with salt to access their resistivity to various hypotonic stress. Hemolysis in each tube was expressed as a percentage of the absorbance in distilled water. The absorbance was recorded after 30mins incubation for each set with a standard spectrophotometer at 540nm wavelength. The average values recorded were plotted against the different NaCl concentrations used.

Statistical analysis

Data analyses were done with Microcal Origin 8.0. statistical software. Values are expressed as means ± SEM and analyzed using Student t-test. $P<0.05$ was regarded as statistical significant.

RESULTS

Table 1: shows the ionic content of the specie of coconut water used.

Figure I: shows the plot of optical density of red cells from different hemoglobin genotypes at varying concentrations of NaCl at 540nm wavelength. Note that a left-shift in the curve is indicative of greater resistivity to hypotonic stress. Untreated red cells from the different Hemoglobin genotypes exhibited characteristic osmotic fragility curves while the CNw - treated red cells in all the groups showed remarkable significant decreases in osmotic fragility ($P<0.05$, respectively), when compared with the untreated ones. This showed a resistant pattern induced by the CNw on the various erythrocyte membranes.

Table 1: Ionic contents of the *Cocos nucifera*

Ionic content	Concentration (mmol/L)
K^+	63.6 ± 5.2
Na^+	25.2
Cl^-	59.1
Ica^{++}	0.6
TCa^{++}	1.18
pH	5.08
CO_2^-	3.6
Mg^{++}	50.1

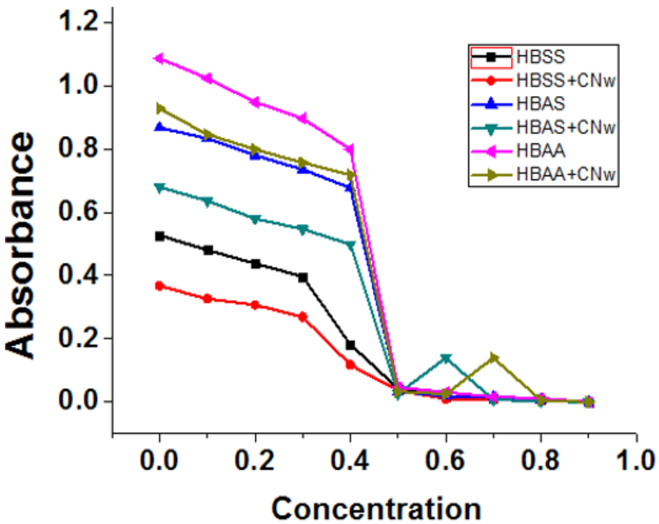

Fig I: Optical densities of hemolysates from different Hb genotypes at various hypotonic concentrations of NaCl. There were significant left shifts of the curve with *CNw* - treated cells compared with the untreated samples ($P<0.05$, respectively). These indicate increases in osmotic burst resistance respectively. CNw – coconut water treated

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DISCUSSION:

The major functions of erythrocyte are relatively simple, consisting of delivering oxygen to the tissues and helping in the disposal of carbon (IV) oxide and protons formed by tissue metabolism, thus it has a much more structure than most human cells, being essentially composed of a membrane surrounding a solution of haemoglobin (Murray, 2003). At low oxygen tension, the mutant hemoglobin, sickle hemoglobin, polymerizes inside the red blood cells into a gel or further into fibers leading to a drastic decrease in the red cell deformability. As a result, micro-vascular occlusion arises which may lead to serious, sometimes fatal crises (Mehanna, 2001). The osmotic fragility test is used to measure erythrocyte resistance to hemolysis while being exposed to varying levels of dilution of a saline solution. Osmotic fragility of erythrocytes reflects the extent of membrane redundancy present when the red blood cell is in equilibrium with an osmotic salt solution (Kretchman *et al*., 1981), it measures the surface/volume ratio of erythrocytes as it exists when they are suspended in an isotonic medium. The osmotic fragility test is common in Hematology, and is often performed to aid with diagnosis of diseases associated with RBC membrane abnormalities. Some diseases linked to increased osmotic fragility include hereditary spherocytosis and hypernatremia, while some linked to decreased fragility include chronic liver disease, iron deficiency anemia, thalassemia, hyponatremia, polycythemia vera, and sickle cell anemia after splenectomy (Clinlab Navigator, 2016). Lipid peroxidation has also been associated with increased osmotic lysis of red cells (Yucel *et al*., 2008; Rohn, *et al*., 1998), Potassium chloride co-transport and calcium activated potassium channel (Gardos Channel) has been postulated to mediate erythrocyte dehydration in sickle cell disease and β-thalaseamia (Brugnara *et al*., 1995) while *Cocos nucifera* has been reported to be a rich source of Potassium and calcium ions. Our previous report of relative higher potassium efflux in SCA erythrocytes than other hemoglobin genotypes accounting for the rehydration/sickling-reversal processes (Ajayi and Arishe, 2015) is further substantiated in the present study.

In this study, we have demonstrated a decreasing osmotic lysis and therefore an induced increase in resistance in erythrocytes from different haemoglobin genotypes (AA, AS and SS) pre-treated with *cocos nucifera* water. The increase in extracellular concentration of potassium coupled with that of calcium than the intracellular concentration may have reversed the osmotic pressure on the red cell membrane in line with Brugnara *et al*., (1995). In the high potassium medium provided by *CNw*, it seems probable that hypotonic stress process is slowed and may indirectly reduce the rate of osmotic destruction of the erythrocyte as seen in this study. It is however not clear the precise mechanism at this present stage of the study, the possibilities are: alterations of the membrane mechanism of water influx, interactions with the hemoglobin molecule to influence polymerization or reducing lipid peroxidation of the red cell membranes. Paradoxically. activation of K-Cl co-transport will lead to increase dehydration and decreased osmotic fragility of red cells (Sears *et al*, 2003). This view is characteristic of HbSS erythrocytes and this could explain in part our findings with *CNw*, appearing to alter the membrane structural characteristics to effect a decreased osmotic fragility. Current studies on lipid peroxidation markers after incubation with *CNw* are on-going in our laboratory to further explain this inference. Also, this study has further demonstrated that heterozygous HbAS state has more resistance to osmotic lysis than the normal hemoglobin under various osmotic stress but less than that of HbSS ($P<0.05$, respectively). Increased Target cell and micro-spherocytes have been associated with decreased fragility (Sears *et al*., 2003) and the characteristic exhibition of Target cells in HbAS may be associated with this finding. The mixture with normal hemoglobin seems to confer some balancing effect as the fragility curve of HbAS has a left shift to that of HbAA and a right shift to that of HbSS. If this observation offers some viability advantage or not could not be substantiated in the present study.

CONCLUSION

The incubation of red cells with *cocos nucifera* water seems to induce a high resistance to osmotic pressure on the red cell membrane thereby reducing the rate of osmotic destruction of the cells during hypotonic stress. This may be a physiological advantage towards erythrocyte viability and reduction in hemolytic episodes especially in SCA subjects with further understanding of its role in reversible sickling phenomenon. The decreased fragility of HbAS erythrocytes compared to the normal HbAA appears novel but with the precise mechanism is yet to be elucidated.

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