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Original Research

GESDAV

Membrane integrity of two human erythrocyte genotypes in the presence of the aqueous solution of sodium metabisulphite and their correlations *in vitro*

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ABSTRACT

Objectives: Membrane integrity is a pre-requisite for cellular functionality. The present study sought to investigate the capacity of normal adult hemoglobin (HbAA) and homozygote hemoglobin S (HbSS) erythrocytes to withstand osmotic stress when incubated over time (0 h $\leq t \leq$ 8 h) in relatively low concentrations of $Na_2S_2O_5$ << 2.0 g/100 mL *in vitro*. **Materials and Methods:** Erythrocyte mean corpuscular fragility (MCF) index and corresponding erythrocyte membrane stability (EMS) were measured using spectrophotometric methods. Furthermore, erythrocyte malondialdehyde (MDA) concentration in the presence of NaS_2O_5 was ascertained by standard methods following pre-mixing of 2.104 mM NaS_2O_5 with erythrocyte hemolysate (ratio: 1:2; *v/v*). **Results:** HbAA erythrocytes MCF indices in the presence of 0.263 mM-2.104 mM $Na_2S_2O_5$ were lower than that of the control HbAA erythrocyte MCF index. The capacity of $Na_2S_2O_5$ to stabilize HbSS erythrocyte membrane was within the range of 2.01-8.44%. Incubation of HbAA and HbSS erythrocytes genotypes in 2.104 mM $Na_2S_2O_5$ for 8 h gave (MDA) = 1.49 ± 0.03 mmol/mL and (MDA) = 4.82 ± 0.03 mmol/mL, respectively. **Conclusion:** The present study suggest that the capacity of HbAA and HbSS erythrocytes treated with Na2S2O5 to withstand osmotic stress was hinged on the free radical scavenging capability of Na2S2O5, whereas declining %EMS with time was an outcome of background reaction of Na2S2O5 with membrane structural components.

KEY WORDS: Erythrocytes, genotypes, malondialdehyde, mean corpuscular fragility, sodium metabisulfite

INTRODUCTION

Sodium metabisulfite ($Na_2S_2O_5$; molecular mass = 190.107 g/mol) is a white or yellowish-white crystalline powder, which can be prepared in the laboratory by evaporating solution of sodium bisulfite ($NaHSO_3$) saturated with sulfur IV oxide (SO_2) [1]. The chemical properties of $Na_2S_2O_5$ make it a multipurpose compound for industrial, domestic, and laboratory applications [2-4]. Notably, in certain physicochemical studies of homozygote hemoglobin S (HbSS) erythrocyte, $Na_2S_2O_5$ by virtue of its reducing potentials is used to establish low oxygen tension, thereby engenders aggregation/polymerization of deoxyhemoglobin S (deoxyHbS) molecules and morphologically distorted (sickle shape) erythrocytes *in vitro* [5-8].

In general, biomembranes are composed of asymmetric trans-bilayer distribution of phospholipids in assemblies with diverse protein and carbohydrate molecules. The erythrocyte membrane is a selective barrier between the interior and exterior compartments of every cell. Thus, erythrocyte membrane is embedded with molecular transporters such as the human ABCG2 protein, which facilitates the transport protoprophyrin IX or heme and normal functions of erythrocytes [9]. The relative high content of polyunsaturated fatty acids and redox active hemoglobin molecules of human erythrocyte membrane, coupled with constant exposure to molecular oxygen renders the erythrocytes membrane structural components vulnerable to peroxidation with concomitant cellular injuries [10]. In addition, the use of $Na_2S_2O_5$ as food additives, cosmetic and general pharmaceutical preservatives can elicit allergy when ingested alongside with food materials or through dermal exposure to industrial products containing $Na_2S_2O_5$ [3]. Some chemical agents compromise cellular integrity through perturbation, modification or peroxidation of biomembrane structural components [11-14]. Several researchers have exploited the readily available erythrocyte membrane, which is a convenient and reliable model, to investigate cellular membrane integrity in the presence of xenobiotics [12,15-17]. On account of the fact that membrane integrity is a pre-requisite for cellular functionality, the present study sought to investigate the capacity of normal adult hemoglobin (HbAA) and HbSS erythrocytes to withstand osmotic stress when incubated over

time in aqueous solution of $Na_2S_2O_5$ *in vitro*. Furthermore, previous reports showed that ($Na_2S_2O_5$) = 2.0 g/100 mL induced the polymerization of deoxyHbS molecules with resultant HbSS erythrocyte membrane deformity and greater susceptibility to lysis *in vitro* [6,8]. However, there are no available reports on the effect of low concentrations of $Na_2S_2O_5$ on human erythrocyte membrane integrity *in vitro*. Thus, by extension, the present study investigated the correlation between two erythrocyte genotypes membrane stability (EMS) incubated in relatively low concentrations of $Na_2S_2O_5$ << 2.0 g/100 mL, which is experimentally unfavorable to induce polymerization of HbS molecules *in vitro*.

MATERIALS AND METHODS

Collection of Blood Samples

A volume of 5 mL of human venous blood samples was collected by venipuncture, from 19 participants who expressed the two erythrocyte genotypes, into sodium EDTA anti-coagulant test tubes. The participants were comprised of clinically confirmed 11 healthy male of HbAA erythrocyte genotype and 8 male of HbSS erythrocyte genotype. The blood samples of HbSS genotype were from patients attending clinics at the Federal Medical Center, Imo State University Teaching Hospital, Orlu, St. John Clinic/Medical Diagnostic Laboratories, Avigram Medical Diagnostic Laboratories, and Qualitech Medical Diagnostic Laboratories. These centers are located in Owerri, Imo State, Nigeria. All blood samples were collected between the months of July and August, 2012. Exclusion criteria for participants included; individuals on tobacco products, anti-malarials or pro-oxidant drugs, vitamin supplements, alcoholics and persons living with human immunodeficiency virus. The participants were further screened for glucose 6-phosphate dehydrogenase deficiency using the MAKO15 Sigma-Aldrich assay kits.

Preparation of Erythrocytes

Erythrocytes were separated from the blood samples and washed by centrifugation methods according to Tsakiris *et al.* [18], as reported by Chikezie and Uwakwe [19]. Within 2 h of collection of blood samples, portions of 3.0 mL of the samples were introduced into centrifuge test tubes containing 3.0 mL of buffer solution pH = 7.4: 250 mM tris (hydroxyl methyl) amino ethane-HCl (Tris-HCl)/140 mM NaCl/1.0 mM $MgCl_2$/10 mM glucose). The erythrocytes were separated from plasma by centrifugation at 1200 × *g* for 10 min and washed 3 times by the same centrifugation method with the buffer solution. The pelleted erythrocytes were finally re-suspended in 6.0 mL of this buffer to obtain approximately 10% hematocrit [7] and used for analyses within 30 min of preparation.

A 2.0 mL portion of the pelleted two erythrocyte genotypes were lysed by freezing/thawing as described by Galbraith and Watts [20], and Kamber *et al.* [21]. Portion of the erythrocyte hemolysate was used for the measurement of malondialdehyde (MDA) concentration.

Measurement of Erythrocyte Osmotic Fragility

A final volume of 5.0 mL of phosphate-buffered saline (PBS) of dilution equivalents; 0.90, 0.72, 0.54, 0.36 and 0.18 g/100 mL were prepared according to Chikezie *et al.* [7], and introduced into corresponding 5 test tubes, whereas 5.0 mL of distilled water was added to the sixth test tube. Portion of 0.5 mL of $Na_2S_2O_5$ (BDH, UK) of varying concentrations (0.263-2.104 mM) was delivered into each corresponding test tubes (1-6). To each test tube, 0.05 mL of the erythrocyte suspension was added and mixed thoroughly by inverting the tubes several times. For the control experiment, $Na_2S_2O_5$ was substituted with equal volume of PBS. For a particular erythrocyte genotype (i.e., HbAA or HbSS), 5 series of control and test analytes were setup as described above and allowed to stand for 8 h, between which at regular intervals of 2 h the assay mixtures were measured for mean corpuscular fragility (MCF) index. Accordingly, at the end of each incubation time intervals of 2 h, the erythrocyte suspensions were further allowed to stand for 30 min at room temperature (24 ± 5°C) after which the contents were centrifuged at 1200 rpm for 15 min. The relative quantity of hemoglobin released into the supernatant was measured with a spectrophotometer (Digital Blood Analyzer®; SPECTRONIC 20, Labtech) at wavelength maximum λ_{max} = 540 nm. PBS osmotically equivalent to 0.9 g/100 mL NaCI and distilled water served as blank and 100% lysis point, respectively. The cumulative erythrocyte osmotic fragility curve, the plot of percentage of erythrocyte lysis versus concentrations of PBS solution, was used to obtain the MCF values. The corresponding concentration of PBS solution that caused 50% lysis of erythrocytes defined the MCF index [11]. Reference MCF indices of HbAA and HbSS erythrocytes were measured by methods previously described by Chikezie and Uwakwe [19].

Evaluation of EMS

The relative capacities of varying concentrations of $Na_2S_2O_5$ to stabilize or disrupt erythrocyte membrane was evaluated as a percentage of the quotient of the difference between MCF values of the test and control sample to the control sample [19].

Calculation

Percentage score of EMS is given thus:

$$\%EMS = \frac{(MCF_{control} - MCF_{test})100}{MCF_{control}} \qquad (1)$$

Measurement of MDA

MDA levels of HbAA and HbSS erythrocytes were measured at the commencement (t = 0 h) and end (t = 8 h) of the experimental time according to the methods of Tjahjani *et al.* [22], with minor modifications as described by Chikezie [7]. A mixture of 20% trichloroacetic acid and 0.67% thiobarbituric acid in a ratio of 2:1 was introduced into a test tube. Next, 0.2 mL of erythrocyte hemolysate was added to the mixture and boiled for 10 min in a water bath and cooled to 24 ± 5°C. The mixture was centrifuged at

3000 × *g* for 10 min, followed by measurement of the absorbance of the supernatant with a spectrophotometer at λ_{max} = 532 nm. Erythrocyte MDA concentration in the presence of NaS_2O_5 was ascertained by pre-mixing 2.104 mM NaS_2O_5 with erythrocyte hemolysate (ratio: 1:2; *v*/*v*) and allowed to stand at 24 ± 5°C for 8 h before analysis. The values of absorbance of the samples were converted to MDA concentrations using the MDA standard curve [23].

Ethics

The Institutional Review Board of the Department of Biochemistry, Imo State University, Owerri, Nigeria, granted approval for this study and all participants involved signed an informed consent form. This study was in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

Statistical Analysis

The results were expressed as arithmetic mean (ẍ) ± standard deviation. The correlation coefficients were determined with Excel Software (Microsoft Excel 2010.lnk) and data were analyzed by Student's *t*-test as described by Pearson and Hartley [24]. Values of $P < 0.05$ were considered statistically significant.

RESULTS

The MCF index of HbSS erythrocyte was significantly ($P < 0.05$) higher than that of HbAA erythrocyte [Table 1]; i.e., $MCF_{HbSS} > MCF_{HbAA}$ erythrocyte. By implication, HbSS erythrocyte exhibited relatively lower tendency to withstand osmotic stress.

Figure 1 shows that within the experimental time of 0 h ≤ *t* ≤ 8 h, MCF indices of the incubated control samples varied within a narrow range of 0.3675 ± 0.02 g/100 mL-0.3728 ± 0.02 g/mL, with values not significantly ($P > 0.05$) higher than the control HbAA erythrocyte MCF index (0.3670 ± 0.03 g/100 mL) [Table 1]. Notably, %EMS of HbAA in the presence of $Na_2S_2O_5$ was significantly ($P < 0.05$) higher than that of the control samples. Furthermore, an overview of Figure 1 showed that HbAA erythrocytes MCF indices in the presence of the four experimental concentrations of $Na_2S_2O_5$, within 0 h ≤ *t* ≤ 8 h, were lower than that of the control HbAA erythrocyte MCF index. Incubation of HbAA erythrocytes for 0 h ≤ *t* ≤ 8 h in 0.263 mM $Na_2S_2O_5$ gave MCF indices within the range of 0.2669 ± 0.04 g/100 mL to 0.3259 ± 0.03 g/100 mL. Specifically, incubation of HbAA erythrocytes in 0.263 mM $Na_2S_2O_5$ for 6 h gave MCF index of 0.2669 ± 0.04 g/100 mL, which represented EMS = 10.01%. Further increase in incubation time, i.e., at *t* = 8 h, MCF index recorded 0.3026 ± 0.03 mg/100 mL, corresponding to EMS = 6.44%. The MCF index at *t* = 2 h represented the lowest EMS level of 4.11%; MCF = 0.3259 ± 0.03 mg/100 mL.

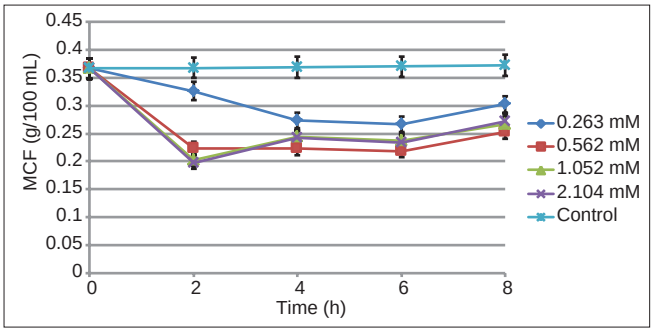

Figure 1: Mean corpuscular fragility indices of human erythrocyte adult hemoglobin genotype treated with varying concentrations of sodium metabisulfite

Table 1: MCF indices of two human erythrocyte genotypes

Erythrocyte genotypes	HbAA	HbSS
MCF g/100 mL (ẍ±SD)	0.3670±0.03[b]	0.5341±0.09[a]

Values are mean±SD of six (*n*=6) determinations. Means with the different letters are significantly different at *P*>0.05. MCF: Mean corpuscular fragility, HbAA: Adult haemoglobin, HbSS: Homozygote haemoglobin S, SD: Standard deviation, [a,b]: ???

A general overview of Figure 1 showed that HbAA erythrocyte incubated in 0.562 mM $Na_2S_2O_5$ exhibited the lowest MCF indices compared with the other three experimental concentrations of $Na_2S_2O_5$, except at *t* = 2 h. On the average, HbAA EMS was 13.75% with peak value of 14.92% at *t* = 6 h. Similarly, HbAA erythrocytes incubated in 1.052 mM $Na_2S_2O_5$ exhibited relatively higher MCF indices than those incubated in 0.562 mM $Na_2S_2O_5$, except at *t* = 2 h. peak level of stability was at *t* = 2 h; EMS = 16.51%; MCF = 0.2019 ± 0.06 g/100 mL. In addition, at the end of the incubation time, *t* = 8 h, loss in HbAA EMS level was 6.49%.

An overview of Figure 1 showed that the highest HbAA EMS level occurred at *t* = 2 h in the presence of 2.104 mM $Na_2S_2O_5$; EMS = 17.01%. Paradoxically, the same experimental concentration of 2.104 mM $Na_2S_2O_5$ caused 7.57% decay in EMS at *t* = 8 h. From another perspective, within the experimental period of 0 h ≤ *t* ≤ 4 h, HbAA EMS increased in the approximate proportionate order with increasing concentrations of $Na_2S_2O_5$ (0.263-2.104 mM). Incubation of HbAA erythrocytes in the four separate experimental concentrations of $Na_2S_2O_5$ for 8 h engendered diminished HbAA %EMS compared with each corresponding peak values of %EMS at the given time intervals and $Na_2S_2O_5$ concentrations.

The MCF index of the control sample was greater in numerical value than that of HbSS erythrocytes incubated in the four experimental concentrations of $Na_2S_2O_5$ (0.263-2.104 mM) [Figure 2]. Furthermore, incubation of HbSS erythrocytes control sample caused a progressive elevation of the erythrocyte MCF index with increasing experimental time. The loss in HbSS %EMS was calculated to be between 2.93% and 4.95%. The capacity of the four experimental concentrations of $Na_2S_2O_5$ to stabilize HbSS erythrocyte membrane within the experimental period of 0 h ≤ *t* ≤ 8 h was in the range of 2.01-8.44%. Again, within the experimental time, HbSS erythrocytes incubated in 0.263 mM $Na_2S_2O_5$ gave peak level of 4.93% stability at *t* = 6 h. Also, peak level of HbSS erythrocyte stability incubated in 0.562 mM $Na_2S_2O_5$ was 8.44% at *t* = 6 h, followed by resultant loss in stability by 4.27% after 2 h of incubation ($P < 0.05$).

The lowest level of HbSS erythrocyte stability occurred following the incubation of the erythrocyte in 1.052 mM $Na_2S_2O_5$ at t = 8 h, which represented 2.71% loss in HbSS EMS compared with %EMS of HbSS at t = 2 h. Likewise, peak level of HbSS EMS was 6.15% at t = 2 h in the presence of 2.104 mM $Na_2S_2O_5$, but gave HbSS EMS = 2.13% at t = 8 h ($P < 0.05$). Overall, Figure 2 showed that there was a declining capacity of the four experimental concentrations of $Na_2S_2O_5$ to stabilize HbSS erythrocyte membrane, exemplified by the increased HbSS erythrocyte MCF index (i.e., at t = 8 h) at each corresponding four concentrations of $Na_2S_2O_5$ when compared with the peak values of HbSS %EMS.

From comparative analyses, incubation of the two erythrocyte genotypes (HbAA and HbSS) for 8 h in the absence of $Na_2S_2O_5$ caused declining levels of %EMS (destabilizing effect) that gave a strong positive correlation (r = 0.960027). A quick inspection of Table 2 showed that %EMS of the two erythrocyte genotypes exhibited positive correlations between the range: r = 0.378316 – 0.960027 in the presence of relatively low concentrations of ($Na_2S_2O_5$) < 0.562 mM, whereas %EMS of HbAA erythrocytes incubated in relatively high concentrations of ($Na_2S_2O_5$) > 0.562 mM exhibited a fairly positive correlations with %EMS of HbSS erythrocytes incubated in relatively high concentrations of ($Na_2S_2O_5$) > 0.562 mM. In addition, %EMS of HbAA and HbSS genotypes incubation in comparatively low concentrations of ($Na_2S_2O_5$) < 0.562 mM gave strong negative correlations with those incubated in high concentrations of ($Na_2S_2O_5$) > 0.562 mM. Finally, incubation of HbAA and HbSS erythrocytes in 2.104 mM $Na_2S_2O_5$ exhibited strong negative correlations with their corresponding control samples.

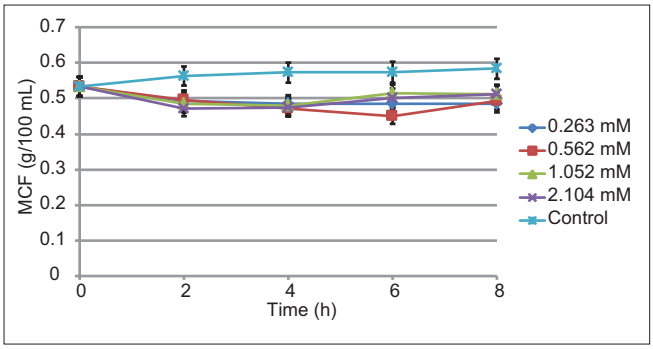

Figure 2: Mean corpuscular fragility indices of human erythrocyte homozygote hemoglobin S genotype treated with varying concentrations of sodium metabisulfite

At t = 0 h, MDA concentration of the control samples of HbSS erythrocyte was 2.8 folds greater than that of HbAA erythrocyte. Figure 3 showed that at the end of the incubation time, t = 8 h, the control samples exhibited marginal increases ($P > 0.05$) in MDA concentrations in HbAA erythrocyte = 5.19% and HbSS erythrocyte = 12.27%. Conversely, incubation of the two erythrocyte genotypes in 2.104 mM $Na_2S_2O_5$ for 8 h gave (MDA) = 1.49 ± 0.03 mmol/mL and [MDA] = 4.82 ± 0.03 mmol/mL for HbAA and HbSS erythrocytes respectively. These values represented HbAA = 35.50% and HbSS = 26.07% reductions in final MDA concentrations.

DISCUSSION

Cellular disintegration is most often prompted by mechanical perturbation or/and chemical modifications of biomembrane structural components [12,16]. The present findings showed that HbSS erythrocytes exhibited lower tendency to withstand osmotic stress than the corresponding HbAA erythrocytes under equivalent control experimental conditions [Table 1]. Previous reports had hinged the variability in osmotic stabilities of different human erythrocyte genotypes on their peculiar physicochemical attributes [17,25]. Studies have shown that massive generation and accumulation of reactive oxygen species (ROS) promoted peroxidation of membrane structural components, of which the lipid molecules were mostly affected [26,27]. Particularly, the HbSS erythrocyte genotype has been reported to generate twice as much superoxide (O_2^-), hydroperoxide (HO_2^-) and hydroxyl (OH^-) radicals than the normal adult erythrocyte [17,28,29], with consequential accelerated level of membrane lipid peroxidation and damage [30]. In general, oxidative damage to biomembrane leads to alteration and distortion in cell rigidity and shape. Furthermore, the failure of HbSS erythrocyte antioxidant enzyme systems to arrest oxidative stress and counter membrane injuries occasioned by overwhelming levels of reactive oxygen and nitrogen species (RONS) in sickle cell anaemias have been previously noted elsewhere [30,31]. The present *in vitro* study showed that HbSS EMS was substantially low compared with that of HbAA erythrocytes ($\%EMS_{HbSS} < \%EMS_{HbAA}$; $P < 0.05$). Likewise, *In vivo* studies have revealed that the erythrocyte membranes of sickle cell anaemias are osmotically and mechanically more fragile than those from normal subjects [32,33].

Both *in vitro* and *in vivo* studies have revealed that erythrocytes treated with RONS antagonist exhibited

Table 2: Correlation coefficient (r) between HbAA and HbSS erythrocytes membrane stabilities at varying concentrations of sodium metabisulphite

HbSS	HbAA				
	($Na_2S_2O_5$)=0.000 mM	($Na_2S_2O_5$)=0.263 mM	($Na_2S_2O_5$)=0.562 mM	($Na_2S_2O_5$)=1.052 mM	($Na_2S_2O_5$)=2.104 mM
($Na_2S_2O_5$)=0.000 mM	0.960027	0.378318	−0.72207	−0.98577	−0.98882
($Na_2S_2O_5$)=0.263 mM	0.478261	0.876278	−0.12904	−0.82701	−0.78809
($Na_2S_2O_5$)=0.562 mM	0.071843	0.900246	0.573061	−0.15875	−0.0965
($Na_2S_2O_5$)=1.052 mM	−0.79978	−0.25762	0.396507	0.526474	0.520909
($Na_2S_2O_5$)=2.104 mM	−0.94637	−0.23052	0.627272	0.739528	0.746087

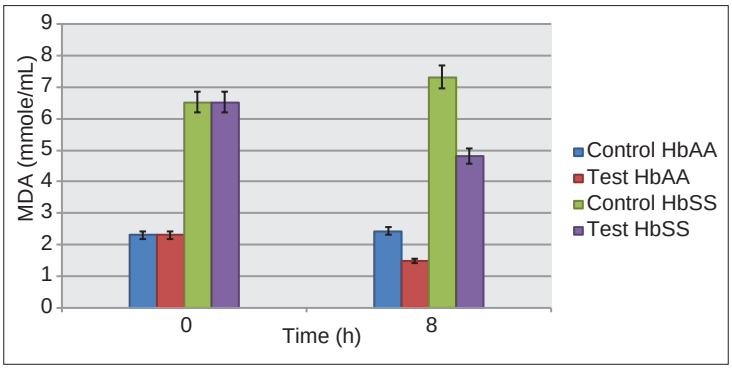

Figure 3: Malondialdehyde concentrations of adult hemoglobin and homozygote hemoglobin S erythrocytes incubated in ($Na_2S_2O_5$) = 2.104 mM and control samples

enhanced membrane stability as a result of reduced membrane lipid peroxidation [10,34,35]. The membrane stabilizing effect by the four experimental concentrations of $Na_2S_2O_5$ (0.263-2.104 mM) on the two human erythrocyte genotypes were in connection to the capacity of $Na_2S_2O_5$ to reduce molecular O_2 and related ROS [36]. The strong negative correlations between control samples of HbAA and HbSS erythrocyte and that incubated in 2.104 mM $Na_2S_2O_5$ and vise visa was an indication of the stabilizing effect of $Na_2S_2O_5$ [Table 2]. However, progressive declining %EMS of HbAA and HbSS erythrocytes in the presence of $Na_2S_2O_5$ with experimental time was probably because of reversibility in the reductive potentials of $Na_2S_2O_5$ with the progression of experimental time, which elicited decreased capacity of $Na_2S_2O_5$ to protect the erythrocyte membrane against ROS destabilization. Furthermore, chemical modification of membrane structural components by background reaction of $Na_2S_2O_5$ might have progressively promoted disarrangement and distortions of three-dimensional structures the erythrocyte membrane. The proposed implication of $Na_2S_2O_5$ in facilitating chemical modification of erythrocyte membrane structural components and membrane stability outcomes showed molecular evidence that were analogous to the observations by Watala and Winocour [37], in which they noted that non-enzymatic erythrocyte membrane protein and plasma lipoprotein glycation engendered reduced erythrocyte membrane fluidity and increased lipoprotein peroxidation in diabetic patients. In a related study, Ramana *et al.* [11], posited that glycosylation of hemoglobin and erythrocyte membrane proteins promoted erythrocytes osmotic fragility of gestational diabetics.

Experimental methods have demonstrated the capability of relatively high concentrations of $Na_2S_2O_5$ > 2.0 g/100 mL to elicit mechanical distortion of HbSS erythrocyte membrane, incidental to $Na_2S_2O_5$ induced polymerization of deoxyHbS molecules [7,8]. Mechanical distortion and perturbation of HbSS erythrocyte membrane assemblies, engendered by deoxyHbS polymerization, was not profoundly evident as exemplified by the experimental %EMS parameters, which was to the effect that HbSS erythrocytes were incubated in relatively low experimental concentrations of $Na_2S_2O_5$ << 2.0 g/100 mL. In a similar characteristic manner, according to Ibrahim *et al.* [38], human erythrocytes incubated in relatively high concentrations of the hydrophilic vitamins (niacin, pyridoxine, thiamine and ascorbic acid; > 2.0 mM) paradoxically promoted oxidative hemolysis. In the same manner, *in vivo* studies of babies receiving metabisulfite at concentrations outside the range for parenteral nutrition, as reported by Lavoie *et al.* [36], showed that the reduction of HO_2^- by $Na_2S_2O_5$ could ironically engender the production of toxic oxidant radical.

The reduced levels of MDA, a product of lipid peroxidation, in the two erythrocyte genotypes incubated in $Na_2S_2O_5$ [Figure 3] appeared to suggest the protective dynamics of the four experimental concentrations of $Na_2S_2O_5$ (0.263-2.104 mM) against membrane peroxidation. In addition, increased MDA concentration of the control sample of HbSS erythrocytes as against decreased level of MDA of the test samples of HbSS erythrocytes confirmed the capability of $Na_2S_2O_5$ to protect the erythrocytes against oxidative stress *in vitro*. Previous studies had also used MDA levels as basis for ascertaining the capacity of antioxidant to eliminate ROS and ameliorate cellular oxidative stress [25,36,39,40].

The present study proposes that the capacity of HbAA and HbSS erythrocytes to withstand osmotic stress over time (0 h ≤ t ≤ 8 h) in the presence of the four experimental concentrations ($Na_2S_2O_5$) = (0.263-2.104 mM) were hinged on the following molecular interplay:

i. Free radical scavenging capability of the relatively low experimental concentrations of $Na_2S_2O_5$, which promoted EMS.
ii. Reversibility in the reductive potentials of $Na_2S_2O_5$ over time.
iii. The declining %EMS in spite of corresponding decreasing erythrocyte MDA levels with progression of experimental time was, for the most part, the outcome of background reaction of $Na_2S_2O_5$ with membrane structural components, which elicited erythrocyte membrane modification and perturbation.

REFERENCES

1. Housecroft CE, Sharpe AG. The group 16 elements. Inorganic Chemistry. 3rd ed. New Jersey: Pearson; 2008. p. 520.
2. Milne GW. Gardner's Commercially Important Chemicals: Synonyms,

Trade Names, and Properties. New York: Wiley-Interscience; 2005. p. 568.
3. García-Gavín J, Parente J, Goossens A. Allergic contact dermatitis caused by sodium metabisulfite: A challenging allergen: A case series and literature review. Contact Dermatitis 2012;67:260-9.
4. Available from: http://www.ultramarineblueindia.com/sodium-metabisulfite.html. [Last retrieved on 2014 Jul 20].
5. Uwakwe AA, Nwaoguikpe RN. *In vitro* antisickling effects of *Xylopia aethiopica and Monodora myristica*. J Med Plants Res 2008;26:119-24.
6. Oyewole OI, Malomoand SO, Adebayo JO. Comparative studies on anti-sickling properties of thiocyanate, tellurite and hydroxyurea. Pak J Med Sci 2008;24:18-22.
7. Chikezie PC, Akuwudike AR, Chikezie CM. Membrane stability and methaemoglobin content of human erythrocytes incubated in aqueous leaf extract of *Nicotiana tabacum* product. Free Rad Antioxid 2012;2:56-61.
8. Pauline N, Cabral BN, Anatole PC, Jocelyne AM, Bruno M, Jeanne NY. The *in vitro* antisickling and antioxidant effects of aqueous extracts *Zanthoxyllum heitzii* on sickle cell disorder. BMC Complement Altern Med 2013;13:162.
9. Leimanis ML, Georges E. ABCG2 membrane transporter in mature human erythrocytes is exclusively homodimer. Biochem Biophys Res Commun 2007;354:345-50.
10. Mikstacka R, Rimando AM, Ignatowicz E. Antioxidant effect of trans-resveratrol, pterostilbene, quercetin and their combinations in human erythrocytes *in vitro*. Plant Foods Hum Nutr 2010;65:57-63.
11. Ramana Devi CH, Hema Prasad M, Padmaja Reddy T, Reddy PP. Glycosylation of hemoglobin and erythrocyte membrane proteins mediated changes in osmotic fragility of erythrocytes. Indian J Med Sci 1997;51:5-9.
12. Bazzoni G, Rasia M. Effects of an amphipathic drug on the rheological properties of the cell membrane. Blood Cells Mol Dis 1998;24:552-9.
13. Cruz Silva MM, Madeira VM, Almeida LM, Custódio JB. Hydroxytamoxifen interaction with human erythrocyte membrane and induction of permeabilization and subsequent hemolysis. Toxicol *in vitro* 2001;15:615-22.
14. Azeez OI, Oyagbemi AA, Iji OT. Haematology and erythrocyte osmotic fragility indices in domestic chicken following exposure to a polyvalent iodophorous disinfectant. Jordan J Biol Sci 2012;5:99-103.
15. Xia J, Browning JD, O'Dell BL. Decreased plasma membrane thiol concentration is associated with increased osmotic fragility of erythrocytes in zinc-deficient rats. J Nutr 1999;129:814-9.
16. Richards RS, Wang L, Jelinek H. Erythrocyte oxidative damage in chronic fatigue syndrome. Arch Med Res 2007;38:94-8.
17. Tamer L, Gurbuz P, Guzide Y, Birol G, Fikri B. Erythrocyte membrane Na+-K+/Mg++ and Ca++/Mg++ adenosine 5' triphosphate in patients with sickle cell anaemia. Turk J Haematol 2000;17:23-6.
18. Tsakiris S, Giannoulia-Karantana A, Simintzi I, Schulpis KH. The effect of aspartame metabolites on human erythrocyte membrane acetylcholinesterase activity. Pharmacol Res 2006;53:1-5.
19. Chikezie PC, Uwakwe AA. Membrane stability of sickle erythrocytes incubated in extracts of three medicinal plants: *Anacardium occidentale*, *Psidium guajava*, and *Terminalia catappa*. Pharmacogn Mag 2011;7:121-5.
20. Galbraith DA, Watts DC. Changes in some cytoplasmic enzymes from red cells fractionated into age groups by centrifugation in Ficoll/Triosil gradients. Comparison of normal humans and patients with Duchenne muscular dystrophy. Biochem J 1980;191:63-70.
21. Kamber E, Poyiagi A, Deliconstantinos G. Modifications in the activities of membrane-bound enzymes during *in vivo* ageing of human and rabbit erythrocytes. Comp Biochem Physiol B 1984;77:95-9.
22. Tjahjani S, Puji BS, Syafruddin D, Agoes R, Hanggono T, Immaculata M. Oxidative stress in *Plasmodium falciparum* culture incubated with artemisinin. Proc ASEAN Congr Trop Med Parasitol 2008;3:47-50.
23. Schmuck G, Roehrdanz E, Haynes RK, Kahl R. Neurotoxic mode of action of artemisinin. Antimicrob Agents Chemother 2002;46:821-7.
24. Pearson ES, Hartley HQ. Biometric Tables for Statistians. 3rd ed. London: Cambridge University Press; 1966.
25. Hundekar PS, Karnik AC, Valvi R, Ghone RA, Bhagat SS. An approach to ameliorate the effect of oxidative stress in sickle cell anaemia. J Clin Diagn Res 2011;5:1339-42.
26. Bast A, Haenen GR, Doelman CJ. Oxidants and antioxidants: State of the art. Am J Med 1991;91:2S-13.
27. Boon JM, Smith BD. Facilitated phosphatidylcholine flip-flop across erythrocyte membranes using low molecular weight synthetic translocases. J Am Chem Soc 2001;123:6221-6.
28. Hebbel RP, Eaton JW, Balasingam M, Steinberg MH. Spontaneous oxygen radical generation by sickle erythrocytes. J Clin Invest 1982;70:1253-9.
29. Essien EU. Increased susceptibility of erythrocyte membrane lipids to peroxidation in sickle cell disease. Cent Afr J Med 1994;40:217-20.
30. Titus J, Chari S, Gupta M, Parekh N. Pro-oxidant and anti-oxidant status in patients of sickle cell anaemia. Indian J Clin Biochem 2004;19:168-72.
31. Anosike EO, Uwakwe AA, Monanu MO, Ekeke GI. Studies on human erythrocyte glutathione-S-transferase from HbAA, HbAS and HbSS subjects. Biomed Biochim Acta 1991;50:1051-6.
32. Ohnishi ST, Ohnishi T. *In vitro* effects of aged garlic extract and other nutritional supplements on sickle erythrocytes. J Nutr 2001;131:1085S-92S.
33. Oboh G. Nutritive value and haemolytic properties (*in vitro*) of the leaves of *Vernonia amygdalina* on human erythrocyte. Nutr Health 2006;18:151-60.
34. Tsuchiya M, Asada A, Kasahara E, Sato EF, Shindo M, Inoue M. Antioxidant protection of propofol and its recycling in erythrocyte membranes. Am J Respir Crit Care Med 2002;165:54-60.
35. Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO, Sipeolu FO. Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions. Int J Plant Physiol Biochem 2010;2:46-51.
36. Lavoie JC, Lachance C, Chessex P. Antiperoxide activity of sodium metabisulfite. A double-edged sword. Biochem Pharmacol 1994;47:871-6.
37. Watala C, Winocour PD. The relationship of chemical modification of membrane proteins and plasma lipoproteins to reduced membrane fluidity of erythrocytes from diabetic subjects. Eur J Clin Chem Clin Biochem 1992;30:513-9.
38. Ibrahim IH, Sallam SM, Omar H, Rizk M. Oxidative hemolysis of erythrocytes induced by various vitamins. Int J Biomed Sci 2006;2:295-8.
39. Elmas O, Aslan M, Cağlar S, Derin N, Agar A, Alicigüzel Y, *et al*. The prooxidant effect of sodium metabisulfite in rat liver and kidney. Regul Toxicol Pharmacol 2005;42:77-82.
40. Olaifa F, Ayo JO, Ambali SF, Rekwot PI. Effect of packing on changes in erythrocyte osmotic fragility and malondialdehyde concentration in donkeys administered with ascorbic acid. Onderstepoort J Vet Res 2012;79:E1-5.

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