ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 10, Issue, 06(E), pp. 33047-33050, June, 2019

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

WATER INTOXICATION: RED CELLS MORE RESILIENT TO CHANGES IN OSMOLALITY THAN BRAIN CELLS

Hannah Tom-Johnson[1], Olusegun Ariwodola[2] and William A. Anong[1, 2],*

[1]Department of Clinical Laboratory Science, School of Health Sciences, Winston Salem State University, Winston Salem, North Carolina, USA
[2]The Biomedical Research Center, Winston Salem State University, Winston Salem, North Carolina, USA

DOI: http://dx.doi.org/10.24327/ijrsr.2019.1006.3594

ARTICLE INFO

Article History:
Received 12th March, 2019
Received in revised form 23rd April, 2019
Accepted 7th May, 2019
Published online 28th June, 2019

Key Words:
Erythrocyte; Hemoglobin; Osmolality; Sodium chloride; hemolysis

ABSTRACT

Background: Postmortem findings of edema to the brain and lungs following water intoxication have been documented withserum sodium concentration of 108-mEq/L (216-mOsm/Kg), far lower than the physiological osmolality of 135 -145-mEq/L (275 – 295-mOsm/Kg). In this study, we investigatedthe effect and extent of low sodium, resulting from drinking too much water, on the erythrocyte membrane architecture.

Methods: Appropriately, collected whole blood wascentrifuge to separate plasma from red cells. The packed cellswere washed three times and then re-suspended to ~25% hematocrit in isotonicsolution. 50-µl of the 25% suspension was incubated insolution of various tonicity ranging from 290 to 65-mOsm/kg sodium chloride (NaCl). Following incubation, the supernatant and pellets were analyzed for hemoglobin content, respectively by spectrometry and western blotting techniques.

Results: Red cells hemolyzed in solution when sodium salt concentration dropped to less than 95-mEq/L (190-mOsm/Kg). Below 190-mOsm/Kg, membrane rupture was rapid -displaying an S-shaped "cooperativity" pattern similar to that of oxygen-hemoglobin binding curve. Complete (100%) red cell hemolysis occurred at ≤80-mEq/L (160-mOsm/Kg).Hemoglobin content was approximately 50% lower in cellsexposed to hypotonic compared to isotonic or hypertonic solutions.

Conclusion: The human red blood cellsshow more resilience to changes in osmolality compared to reported data from other cells such as the brain and hung. Erythrocytes ruptured in-vitro when osmolality fell below 190-mOsm/kg/kg, whereasedema to the brain and lungs from water intoxication led to death and with reported postmortem serum osmolality of 216-mOsm/kg/kg. Red cell remained intact at 216-mOsm/kg because of itsflexible membrane and cytoskeletal network of proteins

INTRODUCTION

Metabolic and physiological processes are largely dependent on or affected by electrolyte concentrations. Abnormal concentrations may be either the cause or the consequence of many disorders.With water, accounting for 40% to 75% of human body weight (1),serum electrolytes contributes to the maintenanceof the osmotic pressure and the distribution of fluid in and around the body, tissueand cell compartments. The water content of an individualdeclines with age and obesity,and generally less for women compared to men due to the higher proportion of body fat (1). Water intoxication resulting from drinking or rarely from intravenous administration can significantly alter the electrolyte balance with detrimental effects. Although rehydration of the tissues and cells are necessary,appropriate IV fluid administration depends on accurately assessing the water deficit and slowly correcting that deficit in elderly patients suffering from dehydration (2).Over correction can lead to central pontine myelinolysis; characterized by damage to the myelin sheath of nerve cells, brain impairment and death (3-5).

Electrolyte disorder, specifically sodium, largely determines serum osmolality. Water intoxication causes edema to the brain, lung tissues damage and death (3, 4). Individuals diagnosed with water intoxication, generally have their

Corresponding author:* **William A. Anong
Department of Clinical Laboratory Science, School of Health Sciences, Winston Salem State University, Winston Salem, North Carolina, USA

potassium, urea, and glucose within the serum reference ranges, despite the low sodium (3, 4). Potassium is twenty-three times higher inside the cell than outside, while sodium is much higher outside than inside the cell. The Na+ K+-ATPase pump maintains the gradient of a higher concentration of sodium extracellularly and a higher level of potassium intracellularlyby pumping 3Na+ out of the cell and 2K+ into the cell, for every adenosine triphosphate (ATP) molecule consumed (6). The sodium-potassium pump maintains the sodium and potassium imbalance against the concentration gradients outside and inside the cell. Water intoxication significantly alter/decreases the concentration of sodium in the plasma, forcing the movement of water into the cell. The resulting effect are brain swelling, lung edema and death when plasma osmolality drops to 216-mOsm/kg. However, the effect and to what extent such decrease in osmolality poses on red cell membrane architecture/hemolysis has not been documented.

This study investigates the optimal osmolality (salt concentration) for red cell damage and hemolysiswhen red cells are exposed to solution of various salt (sodium chloride) concentrations. We found that red cells are more resilient to changesin serum osmolality. The elasticity, "spring-like" and on/off membrane-cytoskeletal protein interactions contributes to the resilience of red cells to changes in serum osmolality. The threshold for red cell hemolysis offers practical clinical and valuable insights on fluid intake or intravenous administration to correct electrolytes disorders in hospitalized patients, especially babies under one year old and the elderly who are more likely to suffer from dehydration (7).

EXPERIMENTAL METHODS

Whole blood drawnfrom a healthy adult volunteer intoan EDTA tube and washed 3 times with 0.85% (290-mOsm/kg) saline solution. The packed red cells was diluted four folds with saline to make~25% hematocrit suspension. 50-µl of the 25% suspension was added to 2 ml salt solution of various tonicity ranging from 290 to 65-mOsm/kgSodium chloride (NaCl). The homogenous mixture incubated for 30 min at room temperature on a rocking platform. The cells were centrifugefor4 min at 3000 rpm to separate the supernatant from the pellet. The hemoglobin content in the supernatant was determined from the absorbance at 540-nm. The pellets was analyzedfor hemoglobin content bysodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (SDS-Page) and Western blotting techniques.

SDS-Page and Western blot analysis

The packed cells were re-suspended in saline and protein concentration determined by BCA assay. After mixing with loading buffer, the sample was incubated at 93 ^{0}C for 10 minutes. A final protein concentration of 20-µg was separated onto a 10% SDS-Page gels (BIORAD) and stained by a solution of Coomassie brilliant blue R-250 or the proteins contained in the gels were transferred onto nitrocellulose membrane. After blocking for 2 hours at room temperature with 5% nonfat dry milk in TBST, the membranes were washed three times in TBST each for ten minutes, then probed overnight at 4°C with either 1:5000 dilution of sheep anti-human hemoglobin antibody (BIORAD) or 1: 1000 dilution rabbit anti-GPA antibody (BIORAD) in TBST. After 3 washes of ten minutes each in TBST, the blots were incubated at room temperature for 1 hour with of rabbit anti-sheep HRP (1:10000 dilution)or goat anti-rabbit HRP(1:2000 dilution). The blots werewashed 3 times for ten minutes in TBST and 0.5% nonfat dry milk and finally developed using the Clarity Western ECL Substrate chemiluminescence detection kit (BIO-RAD).

Following the development, hemoglobin and GPA bands volume were determined using the BIO-RAD Chemidot Software. The proportion of hemoglobin was determined to compare the amount of hemoglobin retained in pellets from red cells exposed to hypotonic, isotonic and hypertonic solutions.

RESULTS

We observed significant hemolysis in erythrocytes exposed to hypotonic solution. The degree of hemolysis was a function of the salt concentration of the solution.

Red cell hemolysis as a function of Osmolality

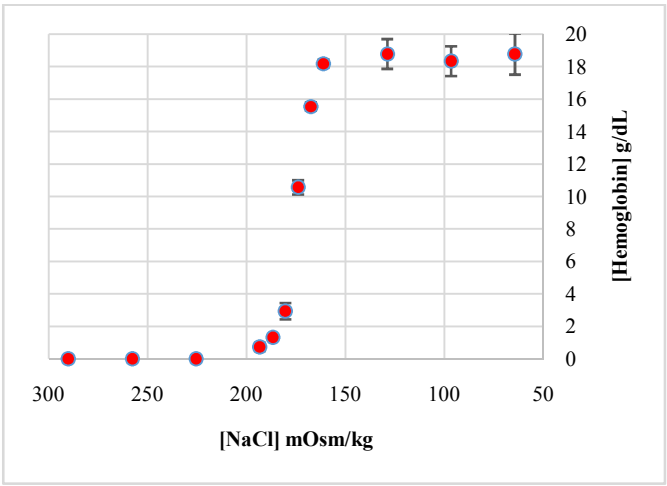

Figure 1 Erythrocytes incubated in NaCl solution of various concentrations and hemoglobin content determined from the absorbance at 540 nm. Mean and SD from triplicate measurements. Age and size distribution of the red cells accounts the S-shaped "cooperativity pattern" of the curve.

Red cells ruptured when exposed to osmolality of190-mOsm/kg, far lower than the 290-mOsm/kg (isotonic) or the 216-mOsm/kg associated brain edema and death (3-5). To determine how quickly the cells hemolysed, a concentration dependent study of reds cells incubated in solution of various NaCl concentration was performed (figure 1). The hemoglobin content, following hemolysis, in the supernatant was measured at an absorbance of 540 nm. The graph represents mean of triplicate measurement of the hemoglobin content in the supernatant following incubation of red cells in decreasing salt concentration as described under Methods. Red cell hemolysis was not evident until the solution osmolality decreased to less than 190-mOsm/kg. Below 190-mOsm/kg solution concentration, membrane rupture was rapid and exponential (between 170 and 160-mOsm/kg), reaching a maximum after 160-mOsm/kg. In figure 2below,we demonstrate the disappearance of the hemoglobin band from red cell pellets as a function of solution osmolality on the SDS-page stained with Coomassie Brilliant Blue R250 Staining Solution.

SDS-Page of Red Cell Pellets

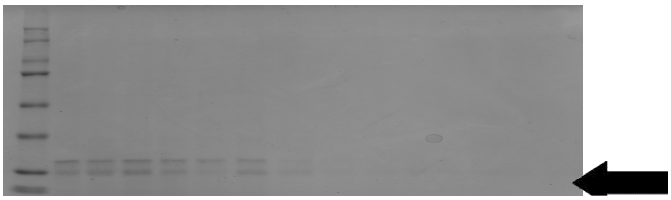

Figure 2: Coomassie Blue Stain of SDS-Page gel showing the disappearance of α, β subunits of hemoglobin (Hb) bands (arrow) in pellets from cells exposed, as described under Methods, to decreasing concentration (-mOsm/kg) of NaCl solution. Each well loaded with 20-µg protein. MMW= Molecular Weight Marker

To determine the hemoglobin retained in the packed cells following incubation in hypotonic (150-mOsm/kg), isotonic (290-mOsm/kg) and hypertonic (600-mOsm/kg) solution, the cell pellet,as already described, were probedwith antibodies for hemoglobinand glycophorin A (GPA) as seen in Figure 3(A-C). Unlike hemoglobin (cytoskeletal proteins) in red cell, GPA is a transmembrane protein with 800,000 copies per cell. GPA prevents cell-cell interaction (red cell aggregation) because of the net negative charge on the cell surface (8). The proportion of hemoglobin content in red cells exposed to hypotonic (150-mOsm/kg) solution was 50 ± 8% lower compared to isotonic or hypertonic solution. This represents a fifty percent decreased in the hemoglobin content resulting from hemolysis in the cells exposed to hypotonic solution. Because hemolysis was not evident in cells exposed to isotonic or hypertonic solution, the hemoglobin content retained was virtually the same.

Red Cell Pellets from Hypotonic, Isotonic & Hypertonic Solution

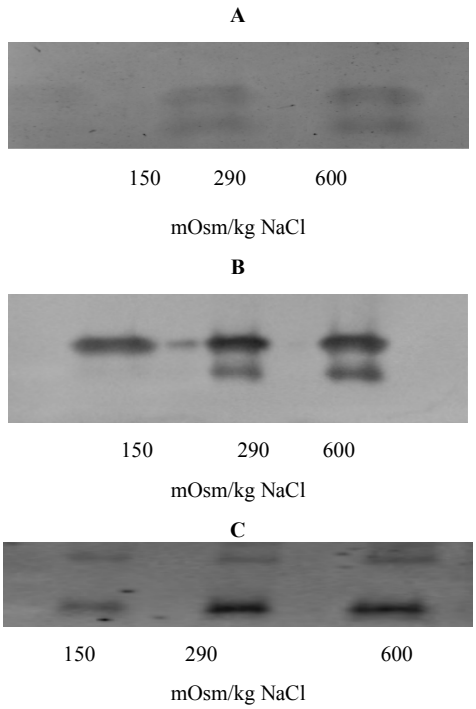

Figure 3 Coomassie Stain (A) and probes of hemoglobin (B) and GPA (C) in pellets derived from red cells exposed to hypotonic (150), isotonic (290) and hypertonic (600) NaCl solution. Each well (B-C) was loaded with 20-µg protein. The bands volume were quantified using the BIORAD Chemidot software. Approximately 50 ± 8 % of hemoglobin (B) is retained in the pellets derived from red cells exposed to the hypotonic solution

DISCUSSION

We have defined the minimum osmolality for red cell hemolysis to be 190-mOsm/kg. This is significantly lower than the 216-mOsm/kg linked to water intoxication and death from brain and pulmonary edema (3, 4). Above 190-mOsm/kg, red cells are stable in solution with no evidence of hemolysis. Approximately, 50 % and 100% of red cells were hemolyzed,respectively at 170 and 160-mOsm/kg (fig. 1). The degree of red cell rupture, follow anS-shaped pattern, similar to the oxygen-hemoglobin cooperativity curve described in most physiology or biochemistry textbooks.

Cooperative binding of ligands and receptors with multiple binding sites such as the oxygen-hemoglobin interaction, occurs when an oxygen atom binds to one of hemoglobin's four binding sites. The initial binding triggers and increases the binding of subsequent oxygen to the three remaining available binding sites. The sigmoidal shape reflects the increased affinity of hemoglobin for subsequent oxygen triggered by initial interaction of an oxygen atom to one of the four sites on the hemoglobin molecule.

However, the S-shaped pattern seen in figure 1 is not associated with cooperative binding betweenligands and receptors. The pattern is explained by the following: (i) variation in the sizes of the red cells. In a red cell population, the cells are of different sizes with an average diameter of 6-8-µm. Compared to cells of with large diameter, smaller cells are more likely to reach their membrane elastic limit first as they take in water, swell and rupture on exposure to hypotonic solution. Cells to the left of the curve are more likely to be smaller in diameter. Second, (ii) age distribution of the cells. The average lifespan of a red cell is 120 days. Older cells are more fragile and likely to rupture first thanyounger cells of the same size[9]. Third, (iii) different proportion and variants of hemoglobin. Hemoglobin variants such as hemoglobin C (HbC), D (HbD), E (HbE), S (HbS) and other blood disorders affect red cell hemolysis (10). In sickle cell disease, the abnormal red cell results from a mutation in the β-globin gene in which valine substitutefor glutamate at position 6 of the β-chain of hemoglobin.Defective β-globin gene also leads to various types (silent, mild and severe) of β-thalassaemia (10). These mutations result in red blood cells that are abnormal (sickled) in shape, decreased osmotic fragility and decreasedlife span (2-4 weeks). In one study, red cells with normal hemoglobin (HbAA) was found to be most susceptible to saline hypotonicity, followed by HbAC and HbAS while HbSC and HbSS were highly resistant to lysis when exposed to varying degrees of hypotonicity(11). The susceptibility of red cells with defective membrane associated proteincompared to normal red blood cells, under any solution osmolality is currently under investigation.

The red cells traverses blood vessels of much smaller diameter than its size.The ability to do so is a function of the flexible interaction between and within membrane bound and cytoskeletal protein networks. These proteins, especially Spectrinis highly flexible and assumes a variety of conformations and critical for normal membrane pliancy, for maintaining the normal shape and overall morphology of the red cell (12, 13).Spectrin is capable of stretching threefold (14)and the on/off interaction gives the red cell the ability to

deform and reform as moves through narrower blood vessels. It also allows the erythrocytes the ability to expand when place in a hypotonic environment. This explains why at 216-mOsm/kg, the red cells are still intact with no sign of hemolysis, whereas reported brain edema, lung damage and death from water intoxication. Because of its membrane elasticity, red cells are more resilient to changes in plasma osmolality than brain cells and lungs tissue.

Water intake is a function of body weight, gender and age and serum osmolality. Generally, the water volume in obese individuals with more fat content is small. For an average healthy 70 kg adult with approximately 42 liters of total body water, 2-3 liters is the recommended intake of water over a 24-hour period. Exceeding this amount, especially for those whose serum osmolality hovers around the lower end of normal (270-300-mOsm/kg) and kidney related problems risks exposing the cells to hypotonicity. In a hypotonic environment, an influx of water occurs (15): the cells swell, disrupting the integrity of the membranes, and if significantly low (<190-mOsm/kg) for the red cells, hemoglobin escapes and dissolves in the surrounding medium. Dilutional hyponatremiathat decreases serum osmolality to 216-mOsm/kg leads to death from brain and lung damage without destroying the red cells.The osmolality of a NaC1 solution (170-mOsm/kg) in which 50% of red cells lyse is a value of practical clinical importance for predicting erythrocyte fragility (9).

CONCLUSION

The present study was an attempt to clarify the impact of osmolality on red cell hemolysis. Postmortem examination showed brain, lung edema and death from water intoxication in which serum osmolality was 216-mOsm/kg. We demonstrated that 190-mOsm/kg is the threshold for red cell hemolysis human red blood cells exposed to differing solution osmolality. Death from water intoxication is less impactful to the red cells compared to the brain and lungs. Although red cells remain intact and resilient at 216-mOsm/kg, such dilutional hyponatremia kills because of edema and damage to the brain and lungs.The osmolality of a NaC1 solution (170-mOsm/kg) in which 50% of red cells lyse is clinically important for predicting erythrocyte fragility.

Supported by WSSU PDC Grant #211260

Reference

1. Chumlea WC, Guo SS, Zeller CM, Reo NV &Siervogel RM (1999). Total body water data for white adults 18 to 64 years of age: The Fels Longitudinal Study, Kidney International, Volume 56, Issue 1, 1999, Pages 244-252, ISSN 0085-2538, https://doi.org/10.1046/j.1523-1755.1999.00532.x.
2. Lavizzo-Mourey R.J. (1987). Dehydration in the elderly: a short review. *Journal of the National Medical Association*, 79(10), 1033–1038.
3. Yamashiro M, Hasegawa H, Matsuda A, Kinoshita M, Matsumura O, Isoda K. &Mitarai, T. (2013). A case of water intoxication with prolonged hyponatremia caused by excessive water drinking and secondary SIADH. Case reports in nephrology and urology, 3(2), 147-52. doi:10.1159/000357667
4. Kawashima W, Hatake K, Kudo R, Nakanishi M, Tamaki S, *et al*. (2015) Two Autopsy Cases of Water Intoxication. J Forensic Res 6: 271. doi: 10.4172/2157-7145.1000271
5. Farrell DJ & Bower L. (2003). Fatal water intoxication. *Journal of clinical pathology,* 56(10), 803-
6. Pirahanchi Y, Aeddula NR. (2019). Physiology, Sodium Potassium Pump (Na+ K+ Pump) [Updated 2019 Jan 21]. In: Stat Pearls [Internet]. Treasure Island (FL): Stat Pearls Publishing; 2019 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537088/
7. Malpas PJ. (2017). Dying Is Much More Difficult Than You'd Think: A Death By Dehydration. The Permanente journal, 21, 16–148. doi:10.7812/TPP/16-148
8. Auffray I, Marfatia S, de Jong K, Lee G, Huang C, Paszty C, Tanner MJ, Mohandas N, &Chasis JA. (2001). Glycophorin A dimerization and band 3 interaction during erythroid membrane biogenesis: in vivo studies in human glycophorinA transgenic mice. Blood, 97(9), 2872-2878. Accessed May 21, 2019. https://doi.org/10.1182/blood.V97.9.2872.
9. SALDANHA C, NUNES M &MARTINS E SILVA J (1991). Hemolysis Studies on Erythrocytes of Different Shapes: a Laboratory Class Experiment. Biochemical education 19(1) 1991 p34-35
10. Galanello r. (2013) screening and diagnosis for haemoglobin disorders. In: Angastiniotis M, Eleftheriou A, Galanello R*et al*., authors; Old J, editor. Prevention of Thalassaemias and Other Haemoglobin Disorders: Volume 1: Principles [Internet]. 2nd edition. Nicosia, Cyprus: *Thalassaemia International Federation*; 2013. Chapter 4. Available from: https://www.ncbi.nlm.nih.gov/books/NBK190467
11. Ilesanmi AO, Banjoko V, Olayanju AO, Akele RY, Akinleye WA, Okamgba O. &Adesina OO (2016). Susceptibility of Hemoglobin Variants to Descending Grades of Hypotonic Saline is Inversely Related to Degree of Clinical Morbidity. Advances in Life Science and Technology www.iiste.org ISSN 2224-7181 (Paper) ISSN 2225-062X (Online) Vol.42, 2016
12. Gallagher PG (2012) Hemolytic anemias. Goldman's Cecil Medicine (Twenty Fourth Edition), 2012
13. Blanc L & Wolfe LC (2016). General Considerations of Hemolytic Diseases, Red Cell Membrane, and Enzyme Defects. Lanzkowsky's Manual of Pediatric Hematology and Oncology (Sixth Edition), 2016
14. Brown JW, Bullitt E, Sriswasdi S, Harper S, Speicher DW& McKnight CJ (2015). The Physiological Molecular Shape of Spectrin: A Compact Supercoil Resembling a Chinese Finger Trap. PLoS computational biology, 11(6), e1004302. doi:10.1371/journal.pcbi.1004302
15. Goodhead, K. & MacMillan, FM. (2017). Measuring osmosis and hemolysis of red blood cells.Advances in Physiology Education 2017 41:2, 298-305