African Journal of Biomedical Research, Vol. 7 (2004); 9 – 12
ISSN 1119 – 5096 © Ibadan Biomedical Communications Group

Full Length Research Article

LONG TERM STORAGE STABILIZES HUMAN ERYTHROCYTE MEMBRANE IN NIGERIAN BLACK MALES

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Osmotic fragility (OF) test was conducted in Nigerian human black male erythrocytes stored for Oh, 12h, 24h and 48h. Storage of these human erythrocytes for up to 24h failed to alter significantly their membrane characteristics. A leftward shift in osmotic fragiligrams was noted suggestive of storage-time (age) dependent erythrocyte stability. Mean corpuscular fragility (MCF) diminished with and was negatively correlated (r = - 0.972052) with storage time. A 48h-storage-time MCF exhibited the greatest variance compared to Oh, 12h and 24h (P< 0.00 1). These results support OF-test data as viable indices of membrane stability of human erythrocytes stored for upto 24h. Beyond this time under-estimation of OF may ensue due to storage-time (ex-vivo ageing) dependent human erythrocyte membrane stabilization.

Keywords: erythrocyte, human, storage, stability, black, Nigerian, males

INTRODUCTION

The Cell membrane generally constitutes an initial line of protection for the cell and hence for the entire organism for that matter. This basic tennet underlines the various interests and hi-tech methods for evaluating the intrinsic membrane characteristics: its stability, fluidity, deformability and other viscoelastic properties'. Extended to the human red blood cells (RBC) specific alteration of any of these factors are frequently implicated in many physiological and pathophysiological processes, including various erythrocyte based hereditary disorders Estimation of the osmotic fragility (OF) of RBCs for example exploits the specific structural changes which the RBC membrane undergoes when the cell is subjected to osmotic stress Properly employed, tests such as cell osmotic fragility test (OF test) may serve a potent yet an inexpensive evaluative test in etiology of hemolytic disorders and of use both in blood banking and improved clinical transfusion efficiency. A seeming draw back of OF tests has to do with the large sample size usually generated during such test, especially when the test is applied to a large test subjects and the evaluation is done over a wide NaCl gradient. As a consequence, the problem of storage of samples before analysis is ever present. Obvious questions frequently arise as to the validity of OF-test results of large sample size since analysis of such a large sample size may take quite a long time (up to days) by this technique in a modest, low -cost research laboratory.

Recently, the effect of storage and other variables on the OF of RBCs have been determined in various mammalian species These studies established time-windows for meaningful OF analysis in these mammalian species. However, it was not clear whether longer delay in analysis would further yield as well unreliable data. Indeed, whether a similar time window or ceiling obtains in human RBC samples stored over a period of time is yet to be fully delineated. This present study was undertaken to assess the influence of storage on the OF of human erythrocytes stored for varying periods of time: Oh, 12h, 24h, and 48h.

MATERIALS AND METHODS

MATERIALS

Blood Samples: Five (5ml) milliliters of fasted blood samples were freshly collected from ten (10) apparently healthy consenting undergraduate medical students of age 20-35 years in accordance with the prevailing local University's human research ethics. The samples were obtained by clean venipunctures carried out by clinically qualified personnel into 10 ml heparinized vacutainers. All blood samples were placed on ice. The blood samples were subsequently divided into 4 equal aliquots. Each aliquot was stored for designated period of time: Oh, 12h, 24h and 48h before OF assay. Effect of temperature was kept minimal by maintaining all sample aliquots at 10°C until assayed Each aliquot was used once without a re-storage.

OF-Test: Osmotic fragility test was performed on neat, unwashed RBCs essentially as described by Oyewale The assays were conducted in a total final volume of 5m1 using 1% phosphate buffered saline (1% PBS) solution, pH 7.7 at 10°C as summarized in Table 1

Statistical Analysis: All values are reported as mean ± standard error of the mean (SEM). The effect of storage was evaluated by comparing the mean ± SEM of hemolysis of RBCs stored for 12h, 24h and 48h with the mean (hemolysis) ± SEM of freshly collected blood samples stored for Oh'° using analysis of variance (ANOVA). A p< 0.05 was considered significant.

Table 1:
Summary of Osmotic Fragility Assay Protocol

Tube No.	Blood vol. (ml)	1% PBS vol.(ml	Distilled water vol. (ml	Final Total Assay+	Final [NaCl] %
1	0.02	0.00	4.98	5.00	0.00
2	0.02	0.50	4.48	5.00	0.10
3.	0.02	1.00	3.98	5.00	0.20
4	0.02	1.50	3.48	5.00	0.30
5	0.02	2.00	2.98	5.00	0.40
6.	0.02	2.25	2.73	5.00	0.45
7.	0.02	2.50	2.48	5.00	0.50
8	0.02	3.00	1.98	5.00	0.60
9.	0.02	3.50	1.48	5.00	0.70
10.	0.02	4.00	0.98	5.00	0.80

+Assay was conducted in a total final volume of 5ml. PBS, phosphate buffered saline

RESULTS

The hemolysis profile of human RBCs stored for varying periods of time are summarized in Table 2. For freshly collected blood samples not previously stored (Oh), evidence of hemolysis (minimal) was noted at NaCl concentration gradient of 0.6 — 0.7%; and hemolysis was essentially complete (maximum) at NaC1 concentration of 0.3%.

In blood samples stored for 12h, hemolysis commenced at NaC1 concentration gradient 0.6-0.7%, a salt gradient not significantly different from that of blood samples stored for Oh. Hemolysis in these 1 2h-stored blood samples was complete at more or less the same NaC1 concentration gradient as that of freshly collected samples, 0.2-0.3% (Table 2). Thus, a 12h storage time did not significantly alter the RBC membrane characteristics.

In the blood samples stored for 24h, minimum hemolysis occurred at NaCl concentration of 0.6%. This salt concentration was not significantly different from those of samples stored for 0h and 12h. Maximum hemolysis was however, noticed at NaCl concentration of 0.1% (Table 2). This salt concentration was significantly lower than those of samples stored for 0h and 12h (P<O.05). Hemolysis in 48h-stored sample commenced at a salt concentration of 0.5%, and was maximal at NaCl concentration gradient of 0.1-0.0%. At 48h storage period, hemolysis of the erythrocytes was significantly (P<O.0O1) lesser than in the blood samples stored for 0h, 12h or 24h at each hemolyzing NaCl concentration evaluated in this study (Table 2).

Figure 1 depicts the osmotic fragiligrams of the human erythrocytes stored for varying periods of time. Evident from fig. 1, the osmotic fragility curves exhibited a leftward shift suggestive of a storage time (age) dependent stabilization of the membrane of the RBCs stored over a period of time. The mean corpuscular fragility (MCF) (Table 3) were extrapolated from the osmotic fragility curves (fig.1). MCF represents the concentration of NaC1 in which 50% hemolysis occurred". As depicted in Table 3, the older these samples got (longer storage up to 48h), the less susceptible to lysis in hypotonic (lower NaCl gradient) medium, the erythrocytes became. A decrease in MCF occurred with an increase in storage time and exhibited a negative correlation coefficient, r = -0.972052 (fig. 2).

Table 2
Hemolysis Profile of human Erythrocytes stored for varying Time Periods

Storage Time (h)	[NaCl] (%)									
	0.0	0.1	0.2	0.3	0.4	0.45	0.5	0.6	0.7	0.8
0	100	98±10	97±10	92±9	75±7	48±5	25±3	11±2	6±2	3±0.5
12	100	97±8	95±9	85±8	55±6	34±3	21±2	5±1	4±l^{ns}	2±0.5^{ns}
24	100	93±6	87±7*	70±6*	35±4*	22±2*	14±1*	4±0.5*	4±l^{ns}	3±0.5^{ns}
48	100	84±8**	69±7**	40±5**	23±2**	13±2**	5±1**	3±0.5**	2±0.5**	2±0.5

*+, mean ± SEM; ns, not significant versus Oh; *p<0.05 versus 0h; **p<0•001 versus 0h, 12h and 24h;*

Table 3
Summary of mean corpuscular fragility of human RBC stored for varying Periods of Time

Storage Time (h)	MCF x 10^{-2}
0	$45 \pm 3^{+}$
12	42 ± 2^{ns}
24	36 ± 3^{ns}
48	$26 \pm 2^{*}$

*Mean corpuscular fragility (MCF) is [NaCl] for 50% hemolysis and was extrapolated from the plot of % Hemolysis versus [NaCl] depicted in fig. 1; +, mean ± SEM; ns, not significant versus 0h; *p<0.05 versus 0h, 12h and 24h*

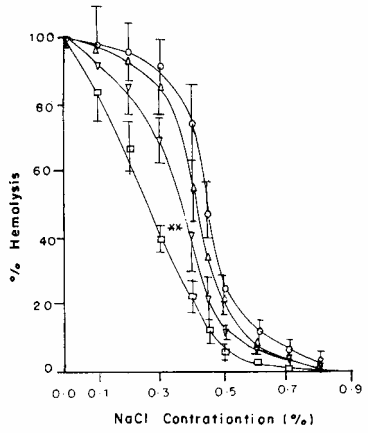

Figure 1
Osmotic fragility of human erythrocytes stored for varying periods of time: Oh (O); 12h (Δ); 24h (∇); and 48h (□). Each point represents mean ± SEM (vertical bars); ** p<O.OO1 versus Oh, 12h and 24h.

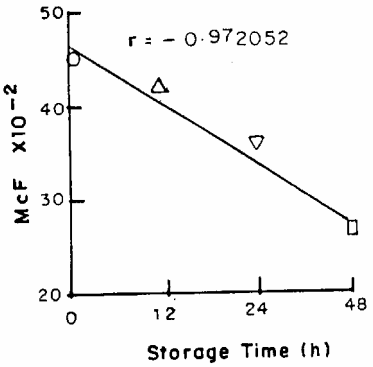

Figure 2
The correlation between mean corpuscular fragility (MCF) and storage time (h) of human erythrocytes stored for varying periods of time: 0h (O); 12h (Δ); 24h (∇) and 48h (□); r = - 0.972052. MCF is [NaCl] for 50% hemolysis and was extrapolated from the plot of % Hemolysis versus [NaCl] depicted in fig 1.

Changes in membrane stability indexed as changes in MCF (fig. 3) were accomplished by normalizing the MCF data to the data on samples stored for Oh. Obvious from fig. 3, the greatest change was observed in the samples stored for 48h, when compared to Oh, 12h or 24h samples (P<O.OO1). The changes in the 24h-stored samples, especially the NaC1 concentration for maximum hemolysis differed significantly from those of Oh and 1 2h-stored samples (P<O.05). The salt gradient for minimum hemolysis in these samples were however not significantly different (fig. 3).

DISCUSSION

OF-test has the potential of serving as a potent and relatively cheap evaluative test of membrane stability in both experimental investigations or in clinical evaluation s of membrane based RBC pathology.

The test exploits characteristic physiologic structural changes which the RBC membrane undergoes when the cell is subjected to osmotic stress for example Problems frequently limit the utility and acceptability of the OF-test results and data. A host of factors (such as changes in pH, temperature, blood gas level, age, humoral environment and so on) influence OF and the values of OF-tests so obtained" Consequently, various investigators tend to establish their own in-house normal OF-test values. Often, these test values may or may not obtain in other laboratories. Another limitation to the viability and utility of OF-test results has to do with promptness in the analysis of large blood sample size immediately upon collection. This study therefore investigated some aspects of this latter problem.

Specifically, the effects of storage of human erythrocytes for varying periods of time (Oh, 1 2h, 24h and 48h) on the stability of erythrocyte membrane osmotic fragility (OF) were evaluated in apparently healthy Nigerian black male undergraduate Medical Students. The results of this study support the thesis of a storage-time (age) dependent stabilization of the membrane of erythrocytes stored over a period of time. Evidence from literature shows a wide species specific variations in mammalian OF data Whereas mammals such as goats and pigs with more fragile RBCs exhibit increases in fragility, RBCs osmotic fragility decreases with storage time in less fragile, more stable RBCs of cattle, mouse, rabbit and rat Human RBC appears to exhibit a varied response to OF-test, depending on the ex-vivo age of the samples.

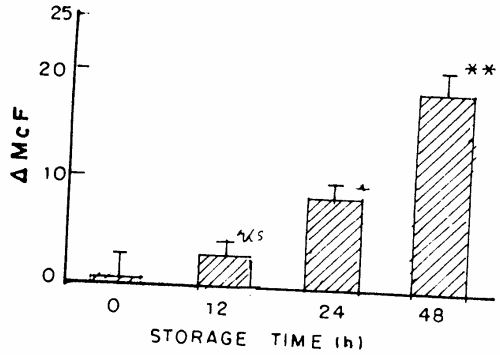

Figure 3
Change in mean corpuscular fragility (MCF) as index of membrane stability of human erythrocyte stored for varying periods of time: Oh, 12h, 24h and 48h. Vertical bars represent standard error of the mean (SEM); ns, not significant versus Oh; *p<0•05 versus Oh, 12h; versus Oh, 12h and 24h. MCF is [for 50% hemolysis and was extrapolated from the plot of % Hemolysis versus [depicted in fig 1. AMCF was obtained by normalizing the MCF data to the data on samples stored for Oh.

In the present study, storage of human blood samples for up to 24h did not significantly alter the RBC membrane characteristics. This result is similar to the observations in the sheep erythrocytes data where a 24h storage had no effect on OF-test. In contrast, beyond a 24h storage period, decreases in OF were noted in human RBC similar to the OF data obtained in rats, rabbits and cattle Thus, OF of human RBCs during storage appear to progress through a period of relative membrane quiescent up to 24h of storage as in the sheep RBCs, where no effect of storage was observed following a 24h storage.

After this period, the human RBCs become less fragile as storage time increases and OF decreases in these samples. The osmotic flagiligrams in these human samples become shifted to the left suggestive of storage time dependent membrane stability. Finally, changes in membrane stability seems most pronounced at 48h storage where on the average lesser RBCs hemolyzed per unit rise in storage time (r = -0.972052)

In conclusion therefore, the results of the present study suggest that OF-test values are viable indices of membrane stability of human erythrocytes stored for up to 24h. Data obtained beyond this period may show some underestimation due to membrane stabilization over a long term storage or ex-vivo ageing of the erythrocytes.

Acknowledgement
This work was supported by a Grant-in-Aid of Research from NIGCAN Packers Co. Ltd. I express my gratitude to all the sophomore and senior medical students of LAUTECH College of Health Sciences who donated their blood free.

REFERENCES

Assal, A. N. & Poulsen J. S. D (1978). Acid-base status of equine blood during storage. Nord. Vet. Med.; 30: 3 54-360

Assal, A. N. Arnbjerb, J., & Poulsen, J. S. D (1978);. Acid-base status of canine blood during storage. Nord. Vet. Med. 30: 345-353.

Coldman, M. F., Gent, M., & Good, W (1969). The Osmotic fragility of mammalian erythrocytes in hypotonic solutions of sodium chloride. Comp. Biochem. Physiol. 31: 329-331.

Godfrey, K (1985). Statistics in practice. Comparing the means of several groups N. Eng. J. Med. 313: 1450-1456.

Mohandas, N., Clark, M. R., Jacobs, M. S., & Shohet, S. B (1980). Analysis of factors regulating erythrocyte deformability. J. Clin. Invest. 1980; 66: 563-573.

Murphy, J. R (1969). The influence of pH and temperature on some physical properties of normal erythrocytes and erythrocytes from patients with hereditary spherocytosis. J. Lab, Clin. Med. 1967; 69: 758-775.

Orcutt, R. H., Thurmond, T. S. & Ferslew, K. E. (1995): Mathematical modeling of the osmotic fragility of rabbit red blood cells. J. Pharmacol. Toxicol. Meth. 1995; 34(3): 169-174.

Oyewale, J. 0. (1993): Effect of storage of blood on the osmotic fragility of mammalian erythrocytes. J. Vet. Med. A40: 258-264.

Parpart, A. K. (1947); The osmotic resistance (fragility) of human red cells. J. Clin. Invest. 26: 636-638.

Poulsen, J. S. , & Surynek, D. (1977): Acid-base status of cattle blood. Sampling and storing. Nord. Vet. Med. 1977; 29: 27 1-277.

Van Der Wal, P. G. Hulshof, H. G., & Essen, 0. V. (1981): Changes in the acid-base parameters of venons porcine blood caused by the period of storage and the method of sampling. Vet. Quart. 1981; 3: 200-220.

Received: December, 2002
Accepted in final form: December 2003