THE OSMOTIC FRAGILITY OF ERYTHROCYTES OF THE ASIAN ELEPHANT *(Elephas maximus)*

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ABSTRACT

Elephant erythrocytes are biconcave in shape and are the largest among mammals. The in-vitro osmotic fragility of erythrocytes of clinically healthy elephants was determined to study the behaviour of these cells in circulation. The NaCl concentration giving minimum percent hemolysis was 0.47 ± 0.03% and the maximum percent hemolysis was observed at 0.25 ± 0.08% NaCl solution. Fifty percent nemolysis occurred at 0.40 ± 0.03% NaCl concentration. These findings suggest that elephant erythrocytes have a high resistance to osmotic lysis, which may be related to the increase surface / volume ratio of the large biconcave erythrocytes and this high osmotic resistance may be of functional importance to the elephant in that a single consumption of a large volume of water following severe dehydration or thirst could prevent the occurrence of erythrocyte lysis in circulation.

INTRODUCTION

The lifespan of an erythrocyte varies among species. Destruction of red cells or hemolysis may occur extravascularly through phagocytosis by the monocyte-phagocytic system, or intravascularly where the erythrocyte lyses within the circulation as a consequence of changes in the membrane permeability and / or cellular fragmentation [4, 5, 17]. Resistance of erythrocytes to hemolysis could be increased or decreased in disease depending upon the rigidity or the deformability of the cell [2,5,8,9,10,18]. The maintenance of the biconcave shape is one of the factors which controls the deformability of a normal erythrocyte[5]. Since the elephant erythrocyte is a biconcave disc and the largest among mammals (80-160 fl (10^{-15}L))[5,13] the objective of this study was to determine the osmotic fragility of erythrocytes of clinically healthy elephants *in vitro* in an attempt to suggest similar behaviour of these cells in circulation.

MATERIALS AND METHODS

Blood samples

Thirteen (4 females, 4 males, and 5 male tuskers) clinically healthy domesticated Asian elephants *(Elephas maximus)*, were used in this study. Their ages varied from 9 to 67 years. A blood sample from each elephant was collected from the ear vein into vacutainers containing the anticoagulant EDTA. The following blood measures were analyzed using these standard techniques: packed cell volume (PCV) using the microhematocrit method; the erythrocyte (RBC) count using the hemocytometer; hemoglobin concentration (Hb) using the Cyanmethemoglobin method[4,5]. The mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) were calculated using the values obtained for PCV, RBC and Hb[4,5]. The morphology of erythrocytes was normal and the above erythrocyte parameters of these elephants were in the normal range for the species (MCV, 118 ± 18 fl (10^{-15}L); MCH, 36 ± 7 pg (10^{-12}G); MCHC, 30% ± 5)[13,14].

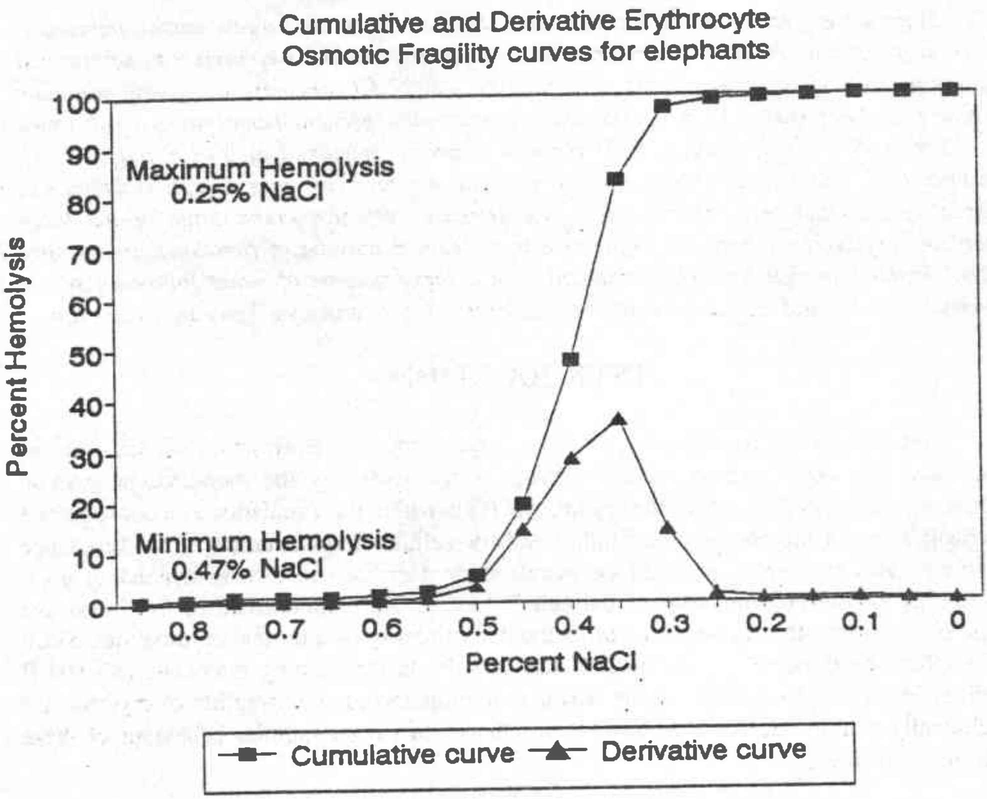

Fig. 1. The cumulative and derivative Erythrocyte osmotic fragility curves for elephants.

Erythrocyte osmotic fragility test

Erythrocyte osmotic fragility was determined according to the method of Parpart *et al*[11]. Briefly, 5 ml each of NaCl concentrations ranging from 0.85% and 0% were prepared using a 1% buffered (pH 7.59) NaCl stock solution and distilled water. Twenty μl of blood was added to each tube of NaCl, the contents were mixed and incubated at room temperature (approximately 27°C) for 30 minutes. The tubes were centrifuged at 2,000 rpm for 10 minutes and the optical density of the supernatants was determined at 540 nm. Percent hemolysis was calculated by using the percent hemolysis at 0% NaCl (100% distilled water) as 100%.

The cumulative osmotic fragility curve was obtained by plotting the percent hemolysis against the concentration of NaCl. The derivative osmotic fragility curve was obtained by plotting the additional amount of hemolysis which occurred in each successive solution of decreasing NaCl concentration, against the NaCl concentration. The derivative curve was used to observe the populations of erythrocytes present in each sample.

RESULTS

Figure 1 shows the cumulative and derivative erythrocyte osmotic fragility curves obtained from the mean values for the 13 clinically healthy elephants. A single peak derivative curve indicated the presence of one osmotically similar population of erythrocytes in all samples tested. Minimum hemolysis occurred at 0.47 ± 0.03% NaCl and maximum hemolysis occurred at 0.25% ± 0.08% NaCl. Fifty percent hemolysis occurred at 0.40 ± 0.03% NaCl concentration.

DISCUSSION

The rigidity or deformability of red cells is important in determining the survival of erythrocytes in circulation[16]. The deformability of normal erythrocytes depends on the maintenance of the biconcave shape, the normal internal or hemoglobin fluidity and the intrinsic membrane deformability or viscoelastic properties[5]. In most cells, the cytoskeleton is involved in the maintenance of cell shape and cell movement. Non-nucleated erythrocytes rely solely on the membrane skeleton to confer shape, reversible deformability and membrane structural intergrity[2,9]. Spectrin, the principle protein of the membrane skeleton, has been found to be defective in certain hemolytic diseases[2,9]. The osmotic fragility test is a simplified method of estimating the surface / volume ratio of erythrocytes and is a precise measure of how nearly spherical a cell is at the time of exposure to hypotonic medium[1,17]. An excess uptake of water renders the cell membrane porous and permits leakage of intracellular contents[5,17]. A positive correlation has been shown between the shortened erythrocyte survival and erythrocyte osmotic abnormalities and mechanical fragilities[3].

The NaCl concentrations giving minimum and maximum percent hemolysis of the elephant erythrocytes in the present study (0.47% and 0.25%, respectively) were in between the values reported for the camel (0.3% & 0.21, respectively) and dog (0.5% & 0.29%, respectively)[5]. Out of the animals tested in previous studies the thin, wafer-like elliptical red cell of the camel (7.7 x 4.4 μm) has a high resistance since it can increase in volume by 200% without undergoing lysis[5,6,12]. Erythrocytes of the dog (7 μm diameter) showed the second highest resistance to osmotic lysis. In contrast, the small biconcave discoid erythrocyte of the goat (3.2 μm diameter) and the horse (5.8 μm diameter) have a low resistance to osmotic lysis swelling only 25% and 42%, respectively, before undergoing lysis[5,12]. The minimum and maximum percent hemolysis of goat erythrocytes were 0.66% and 0.44%, and those of horse erythrocytes were 0.54% and 0.34%, respectively[5]. These results suggest that the surface / volume ratio of the erythrocyte determines the ability of the cell to survive in the circulation. The findings of the present study show that the large biconcave erythrocytes of the elephants, which are the largest among mammals[5,13], have a high resistance to osmotic lysis, although, not to the same extent as the elliptical erythrocytes of the camel. This resistance may be related to the large surface/volume ratio of the erythrocytes of elephants.

In the event of overhydration, transient hypervolemia can occur in the peripheral circulation of healthy animals resulting in hemodilution, a decline in plasma sodium concentration and osmolality[7]. When the camel gets access to water following long term water deprivation, it drinks a large quantity of water in one attempt. The elongated shape of the camel erythrocyte seems to be of functional importance to the camel in that a single consumption of large quantities of water following severe dehydration or thirst does not induce hemolysis[12]. The free ranging elephants usually get access to water only once a day, or even less when water resources are limited especially in the dry season, and they consume about 80-120 L of water in one attempt[15]. Therefore, the high osmotic resistance of the large biconcave erythrocytes of the elephant may also be of functional importance to the elephant, as in the camel. Since the resistance of erythrocytes to hemolysis is known to change in disease[2,5,8,9,10,18] similar information on the elephant erythrocytes could be useful in future to study the changes in hematologic diseases and in anemia in elephants.

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