EFFECT OF VARIOUS SOLUTE CONCENTRATIONS ON THE MEMBRANE PERMEABILITY OF RBCS IN HUMAN BLOOD

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Abstract: The development in the field of medicine has been immense in the past few decades. As the rate of diseases in humans as well as accidents is increasing, there is a constant need of the blood for the transfusion. The number of blood donors is limited, so the blood needs to be stored. A great care is needed to preserve the blood components specially RBCs. The integrity and physiology of the RBCs must be maintained to tackle the demand of blood in hospitals. A great care has to be taken to choose the possible donors with healthy blood. An increased level of the normal constituents may be harmful for the integrity of the RBCs. In the present study, the effect of abnormal levels of glucose, acetone, urea as well as saline on the membrane permeability of the RBCs was considered. These components i.e. 0.2 M glucose, 0.4 M glucose, 4% acetone, 5% acetone, 0.5% urea, 2% urea and hypotonic saline solutions, were shown to promote membrane permeability in RBCs, once RBCs were placed in them. However, the RBC membrane permeability was also found to be influenced by the temperature at which the RBCs were kept. Some solutions like 0.2% saline at 20°C, 5% saline at 20°C and 4% acetone at 20°C were found to have delayed the swelling and rupture of RBCs as compared with RBCs at 4°C and 37°C. However, 0.5% urea was seen to fasten the rupture of the RBCs at 4°C and 20°C as compared to that at 37°C where it was significantly delayed.

Key words: Hemolysis, Membrane permeability, RBCs,

INTRODUCTION

The various components of the blood have some role or the other in the physiology of the humans. Whereas WBCs and platelets play a major role in providing immunity to the body, the plasma maintains the necessary physiological conditions for the survival of the cells and also contains various ions, solutes and protein components. Many proteins are involved in the clotting of the blood, if any damage occurs to the blood vessels. Of all the components, RBCs appear to have many important roles including their role as the carriers of gases like oxygen. The RBCs are unique and highly specialized cells in human body. They also contain many markers on their cell surface that play an important role in the immune system activation, for example, presence or absence of antigens A and B divides the blood of humans into four groups i.e. A, B, AB and O and this knowledge is essential for the transfusion of blood between individuals.

The membrane of the RBCs is maintained by its membrane lipids and proteins. The changes in the osmotic and physiological conditions around and inside the RBCs may lead to morphological and functional abnormalities which can cause pathological consequences [8]. Further, the blood donated by the donors needs to be stored for longer durations to be available for transfusion medicine
and care must be taken to maintain optimal physiological conditions for maintaining the integrity and functionality of cells [7].

However, in many diseased conditions, the concentration of one or more solutes may increase or decrease, which may change the concentration gradient inside and outside the RBCs. Under such conditions RBCs may be exposed to ‘physiological stress’ and this may interfere with their normal role of gaseous exchange at cellular level. The membrane permeability of the RBCs may change and may have influence on the entire cascade of the functions in vivo. Several pathologies have been associated with the altered membrane permeability of RBCs like congenital hemolytic anemia [2]. However, human body has several organic osmolytes that have important regulatory role on human RBC membrane ATPases that protect the RBCs against hypoosmotic stress [4].

The membrane permeability in RBCs is very crucial for maintaining the physiology of the cell, which is important for the RBCs to assay various roles. Hence, the present experiment was designed to look into the membrane permeability changes of the RBCs when the RBCs are exposed to various abnormal concentrations of the normal constituents of the blood in vitro.

MATERIALS AND METHODS

The heparinized blood samples were collected from forearms of 24 voluntary young donors in sterile tubes. The blood was collected as per the ethical guidelines. The blood samples were divided into three groups of 8 samples each as follows:

Group A: Samples were kept at room temperature in sterilized heparinized tubes (~20°C).
Group B: Samples were kept at 4°C in sterilized heparinized tubes.
Group C: Samples were kept at 37°C in sterilized heparinized tubes.

The RBC morphology was examined by adding a drop of blood onto a solution on the slide. The RBCs then were immediately observed using the Nikon Eclipse E100 microscope at 600 X and the time taken for hemolysis/swelling/shrinking of the RBCs was noted down. The various stages of osmotic RBCs were photographed using Samsung SDC-313B. Similar procedure was repeated, for all the solutions and the blood, at three different temperatures. All the chemicals were procured from HIMEDIA laboratories, Mumbai.

Statistical analysis: The data was subjected to one sample ‘t’ test using IBM SPSS Statistics Data Editor with 95% confidence limit and is expressed as Mean ± SE.

RESULTS

The RBC membrane showed different reactions when exposed to solutions of different concentrations. Even the temperature at which the RBCs were placed had influence on the permeability of the RBC membrane. When the RBCs were placed in 0.2% saline, the membrane ruptured at all three temperatures studied, but it took longer time to rupture at 20°C. At 0.4% saline, there was only swelling of RBCs at 20°C, while they ruptured at 4°C and 37°C. The 5% saline is hypertonic to human RBCs, hence the RBCs showed shrinking at all three temperatures while shrinking was also followed by breaking at 4°C (Table 1).

When the RBCs were placed in a 0.2 M glucose solution, they showed rupture at all three temperatures. Similarly, at 0.4 M glucose concentration, the RBCs ruptured at 4°C and 37°C but at 20°C showed swelling and then rupture. When the RBCs were placed at 4% acetone, the effects were different at all three temperatures. At 4°C, the RBC membrane showed breaking, whereas at 20°C it was swelling of RBCs and then rupture. At 37°C, the RBCs ruptured quickly. The results were almost similar, with those of 4% acetone, for 5% acetone at all the three temperatures. RBCs started breaking down when placed in 0.5% urea at 4°C, while at 20°C and 37°C, they ruptured. At 4°C, the RBC membrane showed breaking whereas at 20°C and 37°C, the RBC membrane immediately burst.

Although in almost all cases the time factor was almost comparable for each solute at all three temperatures, with there not being any drastic difference in the time taken. However, few solutions showed a very conspicuous difference in the trend in the diffusion across the RBC membrane with respect to time taken. For example, in 0.2% saline,
Fig. 1: RBCs in 0.2% saline: The arrow points to the swollen, engorged RBC. Fig. 2: RBCs in 0.4% saline: The arrow points to comparatively less swollen RBC. Fig. 3: RBCs in 5% saline: The arrows point to the shrunk RBCs. Fig. 4: RBCs in 5% acetone: The arrow points to the swollen RBC. Fig. 5: RBCs in 0.4 M Glucose: Extensive plasma membrane breaks in the RBCs. Fig. 6: RBCs in 2% urea: The plasma membrane of the RBCs can be seen breaking.
The effect of various solute concentrations on the RBC membrane permeability at three different temperatures (i.e. 4°C, 20°C and 37°C).

<table>
<thead>
<tr>
<th>Solute</th>
<th>Effect of solutes on the RBCs at 4°C</th>
<th>Effect of solutes on the RBCs at 20°C</th>
<th>Effect of solutes on the RBCs at 37°C</th>
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<tbody>
<tr>
<td>0.2% Saline</td>
<td>Rupture</td>
<td>Swelling &amp; bursting</td>
<td>Rupture</td>
</tr>
<tr>
<td>0.4% Saline</td>
<td>Rupture</td>
<td>Swelling</td>
<td>Rupture</td>
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<tr>
<td>5% Saline</td>
<td>Shrink (crenate) and break</td>
<td>Shrink (crenation)</td>
<td>Shrink (crenation)</td>
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<tr>
<td>0.2 M Glucose</td>
<td>Rupture</td>
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<tr>
<td>0.4 M Glucose</td>
<td>Rupture</td>
<td>Swelling and rupture</td>
<td>Rupture</td>
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<tr>
<td>4% Acetone</td>
<td>Breaking</td>
<td>Swelling and rupture</td>
<td>Rupture</td>
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<tr>
<td>5% Acetone</td>
<td>Breaking</td>
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<td>Rupture</td>
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<tr>
<td>0.5% Urea</td>
<td>Breaking</td>
<td>Rupture</td>
<td>Swelling and rupture</td>
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<tr>
<td>2% Urea</td>
<td>Breaking</td>
<td>Bursting</td>
<td>Bursting</td>
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The RBCs took around 118 ± 1.336 seconds to swell and burst at 20°C as compared to 73.5 ± 4.072 seconds at 4°C and 38.875 ± 2.939 at 37°C. Similarly, RBCs showed delayed shrinking at 20°C in 5% saline, taking 38.875 ± 3.445 seconds as compared to 21 ± 1.751 seconds and 19.667 ± 2.304 seconds at 4°C and 37°C respectively (Table 2).

At 20°C the RBCs ruptured in 4% acetone taking longer time i.e. 65.625 ± 6.279 seconds as compared to RBCs at 4°C and 37°C. However, RBCs placed in 0.5% urea took a whopping 113 ± 4.115 seconds to swell and rupture at 37°C when compared to those at 4°C and 20°C. The significant morphological changes in the RBCs were captured by the camera and are reproduced in microphotographs (Figs. 1-6).

The amount of time that is taken by the RBCs to swell, burst or crenate is directly proportional to the rate of osmosis across the cell membrane. Further, the rate of osmosis is related to the degree of permeability of cell membrane in RBCs. As seen during the experiment the time taken by the RBCs to swell or burst varies from person to person, the membrane permeability is different in different individuals. The molecules can cross the cell membrane of the RBCs either by lipid bilayer, carrier-mediated transport or by ion channels. Small non-polar molecules and small uncharged polar molecules like water, alcohol, urea and amides can dissolve in cell membrane and cross it. The charged molecules, including inorganic ions (Na⁺, K⁺, Ca²⁺), use the aqueous channels to cross the plasma membrane. The larger non-charged polar molecules (ex. sugars) and large charged molecules (ex. acids) use carrier-mediated transport [10].

In the present study all the solutions i.e. 4% acetone, 5% acetone, 0.5% urea, 2% urea, 0.2 M glucose, 0.4 M glucose, 0.2% saline and 0.4% saline, induced swelling and rupture of the RBC membrane except 5% saline, which is hypertonic to the RBCs. Interestingly 4% acetone and 5% acetone acted as hypotonic solutions and allowed the osmosis to happen in the RBCs leading to the rupture of the RBC membranes. This indicates that while in case of 5% saline, water gushed out of the RBC rendering them to shrink but with 4% and 5% acetone, the water from the surrounding seems to enter the RBCs leading to their swelling and rupture. The acetone molecules being larger might not have entered the RBCs as they need a carrier molecule for the same. Hence, an increase in the acetone levels in the plasma might have led to hemolysis. The levels of ketone bodies including acetone have been found to be insignificant in the blood as well as urine.
When plasma levels increase beyond 0.1 to 0.2 mM, their excretion increases and measurable amounts of ketone bodies appear in the urine [6], which may pose a danger of hemolysis in vivo.

Similarly, with other solutes like urea and glucose, the RBCs may undergo swelling and further rupture, if their levels rise in the blood. The normal range of urea in the blood or serum is 10-40 mg/dl or 1.8 to 7.1 mmol/liter [1]. The higher than the normal values of urea in blood might pose a threat of RBC rupture by allowing more osmosis to occur as is seen in the present study. Above that, when the RBCs were placed at temperatures lower than 37°C, the rupture of RBC membranes was found to be faster. The normal glucose concentration is maintained at 80-120 mg/dl in the blood [5]. Both, 0.2 M glucose and 0.4 M glucose, accelerated the swelling of RBCs and then their rupture. This effect was, however, found to be independent of the temperature as the time taken by the RBCs to rupture was almost similar at all three temperatures studied.

The storage of blood might be a problem if the blood levels of these solutes are high. Of the three temperatures studied for the osmosis in RBCs, it has been found that the rupture of RBC membrane was delayed in most of the cases when the RBCs were kept at 20°C as compared with those at 4°C and 37°C. This suggests that the rate of osmosis is dependent on the temperature and so is the rupture of RBC membrane as higher temperatures usually lead to hemolysis [3].

The rate of diffusion through plasma membrane through lipid bilayer is determined by the size and steric configuration or shape of the molecule. The RBCs have the property to deform to pass through the narrow capillaries. The decrease in deformability plays a role in hemolysis and can lead to many medical conditions like hereditary spherocytosis, elliptocytosis. Several medical conditions such as uremia, sickle cell anemia, diabetes etc can influence the deformability of the RBCs [9].

The temperature greatly affects the deformability of the membrane and subsequently its stability. The threshold concentration of the solutions at which the RBCs may be damaged can be altered by changing the temperature of the solution in which the RBCs are placed [9]. Thus, the concentrations of the glucose, acetone and urea, not to forget the saline concentrations, need to be taken care of along with the temperature regulation, for the long term storage of the RBCs without disturbing its integrity and physiological status.

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