Effect of osmotic pressure to bioimpedance indexes of erythrocyte suspensions

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Abstract. In the paper we studied effects of osmotic modification of red blood cells on bioimpedance parameters of erythrocyte suspension. The Cole parameters: the extracellular (Re) and intracellular (Ri) fluid resistance, the Alpha parameter, the characteristic frequency (Fchar) and the cell membranes capacitance (Cm) of concentrated erythrocyte suspensions were measured by bioimpedance analyser in the frequency range 5 – 500 kHz. Erythrocytes were incubated in hypo-, hyper- and isoosmotic solutions to achieve changes in cell volume. It was found that Re and Alpha increased in the suspensions with low osmolarity and decreased in the hypertonic suspensions. Ri, Fchar and Cm were higher in the hyperosmotic and were lower in the hypoosmotic suspensions. Correlations of all BIS parameters with MCV were obtained, but multiple regression analysis showed that only Alpha parameter was independently related to MCV (β=0.77, p=0.01). Thus Alpha parameter may be related the mean corpuscular volume of cells.

1. Introduction
The electrical properties of the erythrocyte suspensions with standard hematocrit are dependent to three factors: the volume of single red blood cells, their number in the measured sample of blood, as well as the concentration of ions in the suspending solution [1]. Osmotic pressure affects both the electrical resistance of the extracellular water, and the size and shape of cells [2]. It is known that the impedance parameter Alpha may depend on those parameters of red blood cells [1, 3]. However, the relationship between the osmotic modification of erythrocyte suspensions and bioimpedance parameters of the suspensions is not fully understood. In this report, we tested the proposition that the osmotic effects of suspension medium on red cell volume will be relationships to the bioimpedance parameter Alpha.

2. Materials and methods
2.1. Preparation of hypotonic, isotonic, and hypertonic erythrocyte suspensions
The blood samples with volume 9 ml (n=8) were divided into three aliquots by 3 ml. First aliquot was mixed with 6 ml of the hypotonic 0.7% NaCl solution (osmolarity – 240 mosmol/l), second – with 6 ml of the isotonic 0.85% NaCl solution (osmolarity – 290 mosmol/l) and third – with 6 ml of the hypertonic 1.1% NaCl solution (osmolarity – 375 mosmol/l). Then the erythrocytes were washed in these solutions by triple centrifugation at 3000 rpm for 10 minutes. Then washed erythrocytes were incubated for 1 hour in solutions with different osmolarity. After incubation concentrated erythrocyte suspensions with 92% hematocrit were prepared.
2.2. Estimation of mean corpuscular volume, hematocrit and red blood cells concentration in suspensions with standardized hematocrit

Hematocrit (Ht) was derived by centrifugation of the suspensions in the microcapillaries at 5000 rpm for 15 minutes. Red blood cells concentration (RBC) was measured by the photocolorimetric method. Mean corpuscular volume (MCV) was calculated by the formula: MCV = Ht/RBC.

2.3. Bioimpedance measurements of the erythrocyte suspensions

Prepared concentrated erythrocyte suspensions with standardized hematocrit (Ht=92%) were analyzed with the BIS method. The electric measurements were performed at room temperature on the BIA analyzer ABC-01 “Medass” (Russia) at the frequency range 5 – 500 kHz (31 frequencies). The erythrocyte suspensions of 1 ml were drawn into conductivity cell. The cell is a plastic tube with two pairs of potential and current electrodes. The electrodes were connected to the BIA analyzer. Data from the analyzer were processed on the PC with special software. The method of electrical measuring is described in our previous work in details [4]. The impedance locus was built by bioimpedance analyzer software. The example of the hodograph is shown in the figure 2.

Figure 2. The example of the hodograph. Each point of the graph shows the ratio of reactance to resistance at the appropriate frequency marked with the number.

2.4. Statistics

Results are expressed as mean + SD. Paired Student’s t-tests were used for comparison of BIS parameters in suspensions with different osmolarity. Pearson’s correlation (r) was utilized to study the relations between the bioimpedance spectroscopy parameters and MCV. Multiple regression analyze was used to establish independent correlations between the BIS parameters and MCV. The multiple standardized regression coefficient (β) was calculated.

3. Results

The values of hematocrit, red blood cells concentration and mean corpuscular volume are presented in the table 1.

Table 1. The values of hematocrit, red blood cells concentration and mean corpuscular volume in solutions with different osmolarity

| Osmolarity, mosmol/l | 240   | 290   | 375   |
|----------------------|-------|-------|-------|
| Ht, %                | 92.4±2.5 | 93.6±2.5 | 91.9±2.0 |
| MCV, fl              | 106.3±7.6*** | 94.7±7.1 | 85.8±6*** |
| RBC, 10^{12}/l       | 13.1±0.8*** | 14.9±1   | 16.1±1.2*** |

(*** – p <0.001 compared to 290 mosmol/l suspension)

Ht of the erythrocyte suspensions was equal. MCV was higher in the hypotonic and lower – in the hypertonic solutions. Vice versa, RBC decreased in the hypoosmotic and increased in the hyperosmotic solutions.

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The BIS parameters of the erythrocyte suspensions in solutions with different osmolarity are shown in the table 2.

| Osmolarity, mosmol/l | 240      | 290      | 375      |
|---------------------|----------|----------|----------|
| Re, Ohm             | 660.0±102.5** | 562.9±72.6 | 450.7±28.9** |
| Ri, Ohm             | 64.3±4.6*   | 68.7±1.5  | 73.0±6.1*  |
| Fchar, kHz          | 219.5±31.1* | 245±31.3  | 291.4±19.3** |
| Alpha               | 0.318±0.002* | 0.316±0.002 | 0.314±0.002* |
| Cm, pF              | 47.4±5.6**  | 53.4±4.6  | 59.7±3.4**  |

(*, ** – p<0.05, <0.01 respectively compared to 290 mosmol/l suspension)

We found that Re and Alpha increased in the suspensions with low osmolarity and decreased in the hypertonic suspensions. On the contrary, Ri, Fchar and Cm were higher in the hyperosmotic and were lower in the hypoosmotic suspensions.

The results of correlation analysis of RBC and MCV with the BIS parameters are presented in the table 3.

Table 3. Correlations of RBC and MCV with the BIS parameters

|                       | Re       | Ri       | Fchar    | Alpha    | Cm       |
|-----------------------|----------|----------|----------|----------|----------|
| RBC                   | -0.55**  | 0.50*    | 0.52**   | -0.56**  | 0.59**   |
| MCV                   | 0.62**   | -0.46*   | -0.60**  | 0.65**   | -0.68**  |

(*, ** – p<0.05, <0.01 respectively)

As it’s shown, RBC was positively related to Ri, Fchar and Cm and was negatively related to Re and Alpha. We found that the correlations of MCV with Re and Alpha were positive, and with Ri, Fchar and Cm were negative.

The results of multiple regression analysis of MCV with the BIS parameters are presented in the table 4. One can see that only correlation of the Alpha parameter with MCV was statistically independent.

Table 4. The results of multiple regression analysis of MCV with the BIS parameters

|                       | Re, Ohm  | Ri, Ohm  | Fchar, kHz | Alpha    | Cm, pF   |
|-----------------------|----------|----------|------------|----------|----------|
| β                     | 0.70     | -0.35    | 0.60       | 0.77     | 0.12     |
| p                     | 0.56     | 0.07     | 0.40       | 0.01     | 0.86     |

4. Discussion

The main results of our study showed that all BIS parameters differed in suspensions with different osmolarity. The BIS parameters were related to MCV and RBC values, but only correlation of Alpha parameter with MCV was statistically independent.

The changes of RBC and MCV in hypertonic and hypotonic suspensions were determined by the movement of water on the osmotic gradient. As soon as in the solutions with low osmolarity water entered the erythrocytes they swelled, their volume increased and their shape became more spherical. Since total cell volume of the suspensions was equal, RBC in the hypotonic solutions decreased. On the contrary, in the hypertonic solutions water leaved the erythrocytes, so the cells shrunk and their volume decreased, and RBC of the suspensions increased[2].

Obviously in our experiment condition, corpuscular volume of erythrocytes, red blood cells concentration and ions concentration in suspending solution influence the electric properties of measured suspensions.

It’s known that Re reflects extracellular and Ri – intracellular fluid volume [5]. Since hematocrit of the erythrocyte suspensions was equal, total extracellular and intracellular fluid volume didn’t change in the suspensions with different osmolarity. Evidently, the Re and Ri changes associated with difference in the ions concentration in the hypotonic and hypertonic solutions. Electrical conductivity was higher and Re value was lower in the hyperosmotic solution with high NaCl concentration, in the
hypoosmotic solution with low ion concentration – vice versa. However changes in the osmotic concentration of the suspending media cause flow of water into or out of the erythrocyte until osmotic equilibrium is restored [2]. In the solutions with high osmolarity water leaves red blood cells and hemoglobin concentration in the erythrocytes increases. Since hemoglobin reduces ion mobility in the erythrocytes, high hemoglobin concentration causes a decrease of conductivity and an increase of Ri [6]. In low osmolarity suspensions hemoglobin concentration is lower, and Ri decreases. Thus in our experiment conditions the Ri changes are related to the hemoglobin concentration changes in red blood cells and doesn’t depend on MCV and RBC.

Fchar value is inversely proportional to resistance and capacitance of measured object [1]. In biological objects capacitance is determined by cell membranes quantity or, in other words, by a number of cells. Since in the hypertonic solutions RBC is higher, it may be expected that Fchar increases. On the contrary, in the hypotonic solutions RBC is lower, so Fchar should decrease. However we found that Fchar increased in the hyperosmotic and decreased in the hypoosmotic suspensions (table 2). Obviously, resistance influenced the Fchar value in this case. The resistance, in turn, depends on the ions concentration. Hence, we suggest that Fchar was determined by the ions concentration, like Re. In the hypotonic suspensions the resistance increased so that Fchar decreased, in the hypertonic solutions – vice versa. This is confirmed by close correlation of Fchar with Re in our study (r=-0.94, p<0.001) and in the article [7].

It was found that Cm increased in hypertonic and decreased in hypotonic suspensions (table 2). Obviously, since in the hypertonic solutions RBC was higher and total cell membranes area increased, Cm value grew. In the hypotonic solutions – vice versa, RBC and total cell membranes area decreased, so Cm value diminished. In the papers [4, 8] a correlation between Cm and Ht was also obtained. The authors also believe that a gain of the cell membranes number in the blood samples with high hematocrit causes a Cm increase. Thus it is possible that Cm depended on RBC and didn’t reflect MCV.

The Alpha parameter and osmotic effects. All BIS parameters were related to MCV (table 3) but the multiple regression analysis showed that only correlation of MCV with Alpha was statistically independent (table 4). This indicates that Alpha parameter may be determined by erythrocyte corpuscular volume. Besides, Grimnes S. et al [1] postulated that the Alpha depends on the cell size. Obviously cell volume can also influence Alpha. However, it’s known that the Alpha parameter is also related to the cell size and shape heterogeneities [3]. Alterations of the heterogeneity of erythrocytes in our experiments were induced by changes in solutions osmolarity. In the hypotonic solutions erythrocytes shape became more spherical, suspensions heterogeneity decreased, so Alpha should be increased. In the hypertonic solutions the erythrocytes shrunk, cell heterogeneity in suspensions increased, and Alpha should be decreased. Thus Alpha parameter may be related both to the cell heterogeneity and mean corpuscular volume.

5. Conclusions
Thus, the results showed that all investigated bioimpedance parameters changed in according to the osmotic modifications of erythrocyte suspensions. Changes in Re, Ri, Fchar were caused concentrations of NaCl ions, Cm led to the red blood cell concentrations, however, the Alpha parameter is related to mean corpuscular volume in concentrated erythrocyte suspensions.

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