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Concerning the importance of changes in hemorheological parameters caused by acid-base and blood gas alterations in experimental surgical models

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Abstract. Acid-base equilibrium and pH of blood have important clinical consequences in numerous diseases and patho-physiological conditions. The micro-rheological parameters of blood, such as red blood cell deformability and red blood cell aggregation are influenced by several metabolic factors, and provide information regarding inflammatory, septic and tissue or organ ischemia-reperfusion processes.

Despite the anticipated logical relation of the blood acid-base condition, blood gas parameters and pH to red blood cell deformability and aggregation, controversial data can be found in the literature. Furthermore, related to ischemia-reperfusion hemorheological studies little is known about this issue.

In this paper we aimed to thought-provokingly overview some aspect of acid-base changes, blood pH and hemorheological parameters, discussing certain results from ischemia-reperfusion experimental surgical models (local versus systemic changes), laboratory technical and experimental design protocols related to *in vitro* and *in vivo* studies.

Keywords: Acid-base changes, blood pH, micro-rheological parameters, red blood cell aggregation, red blood cell deformability.

1. Introduction

It is known that blood micro-rheological parameters, such as red blood cell deformability and red blood cell aggregation play an important role in determination of blood viscosity and resistance at various regions of the circulation in healthy and pathological conditions [4, 18, 24, 28, 32, 56].

Red blood cell deformability is determined by morphological properties of the cell (shape, volume, surface-to-volume ratio, cytoskeletal structure), own viscosity of the cell membrane, inner viscosity (hemoglobin content). *Red blood cell aggregation* –reversible coupling of erythrocytes at low shear

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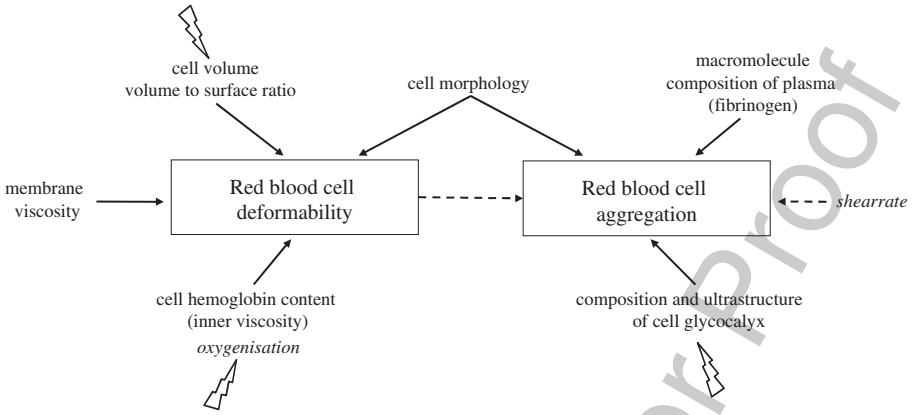

Fig. 1. Factors determining red blood cell deformability and red blood cell aggregation. Dashed lines reflect indirect relations between parameters. Thunder-like arrows show potential targets to be altered by changes in acid-base condition or blood gas partial pressure (pO_2, pCO_2).

rate– is influenced by cellular (shape, deformability, membrane glycocalyx structure) and plasmatic factors (fibrinogen concentration and composition, micro-environmental conditions) [4, 5, 9, 15, 16, 31, 52] (Fig. 1).

In surgical research *ischemia-reperfusion (I/R)* is still an important topic because of its highly important clinical relevance and numerous unsolved questions concerning the time factor, local versus systemic changes, as well as prevention and therapeutic possibilities during and after surgery. Most of the experimental models are related to I/R injuries of solid organs and tissues, transplantation models or technical refinements of vessel anastomoses.

During I/R oxygen-derived free radical reactions, activated complements and leukocytes and related inflammatory processes, as well as local physical and metabolic changes may occur in blood, such as accumulation in lactate, decrease of pH, changes in oxygenation (oxygenation-deoxygenation state) and fluid equilibrium, and micro-environmental alteration of blood osmolarity [20, 26, 33]. Red blood cell deformability and red blood cell aggregation are known to be affected by most of these alterations [e.g. 20, 25, 36–38]. However, the magnitude and reversibility of the micro-rheological changes are still unclear. Few number and controversial studies can be found in the literature in which red blood cell deformability and red blood cell aggregation have been investigated in parallel with *acid-base, blood gas parameters and blood pH*. The lack of this kind of studies might be related to technical difficulties or poor experimental design, but on the other hand it also seems that this question has still not yet been clarified.

In this paper our aim was to briefly and thought-provokingly overview some aspects and concerns on the alterations of acid-base and hemorheological parameters, discussing some results from ischemia-reperfusion experimental surgical models, laboratory technical, measuremental issues and experimental design.

2. Data on acid-base changes and alterations in hemorheological variables

2.1. Physiological conditions

Although several *in vitro* studies demonstrated the effect of pH [11, 15, 23, 27, 55], pO_2 [11, 43, 51] and lactate concentration [17, 30, 41] on red blood cell properties, the *in vivo* studies (using *ex vivo*

samples) often show controversial data. The most complex literature data on acid-base, lactate and pH changes is being related to hemorheology have been originated from studies in exercise physiology and pathophysiology [10].

Many authors concluded that increased lactate level has impairing effect on red blood cell deformability and aggregation [8, 9, 12–14, 46, 50]. However physical exercise itself shows wide individual range and differs among well-trained and not trained volunteers [13, 45]. In well-trained athletes red blood cell aggregation could not be modified by exercise (cyclo-ergometer) or *in vitro* incubation with sodium-lactate solution (2, 4 and 10 mM in HEPES; osmolarity = 210 mOsmol/kg; pH = 7.4) [13]. Probably due to the altered rate of lactate influx into red blood cells [45], in untrained subjects erythrocyte rigidity coefficient (Tk) –an indirect, estimating parameter for red blood cell deformability– increased significantly when incubated in sodium-lactate of 4 mM compared to 2 mM. In well-trained athletes Tk slightly decreased [13]. Also Smith and co-workers reported exercise-induced increase of lactate level, red blood cell osmotic fragility and mean cell volume. These impairments could be simulated *in vitro* by putting 15 mM l-lactic acid (dissociating into lactate anion and H^+) into the samples [46].

Senturk and co-workers reported that contrary to trained subjects in sedentary volunteers exercise induced significant impairment in red blood cell deformability. Interestingly, the exercise-induced rise in oxidative stress markers (TBARS, carbonyl derivative) were almost identical in those groups as well as the lactate level (sedentary: 9.4 ± 0.7 mmol/l; trained: 10.2 ± 1.2 mmol/l) [44].

Animal experiments have also demonstrated that strenuous exercise significantly influences micro-rheological parameters. Yalcin and co-workers described that 60-minute swimming exercise of trained (gradually built up protocol of 60-minute swimming 5 days per week for 6 weeks) rats did not cause enhancement of red blood cell aggregation nor definitive impairment of red blood cell aggregation compared to untrained animals [2, 54].

In normal metabolism the l-lactate is produced from pyruvate by the lactate dehydrogenase (LDH) enzyme. The main pathways of removal of lactate are oxidation to pyruvate or conversion to glucose (Cori-cycle). Lactate anion enters into the red blood cells and independently from the pH (H^+) may cause impairment in erythrocyte rheological properties [12, 30, 41]. About 90% of the total lactate uptake is linked to the red blood cell monocarboxylate transporter (lactate/H^+ co-transporter) [14, 17].

However exercise-induced changes in red blood cell micro-rheological properties are controversial: red blood cell aggregation has been reported exercise induced increase or decrease, or even without change as well [14, 50]. From the exercise physiology studies we could learn many data about the effects of lactate concentration, blood pH, acid-base parameters on red blood cell deformability and aggregation tested in systemic blood samples. Changes in lactate level, pH are also important in ischemia-reperfusion condition, and so the I/R-related hemorheological research; and not only in systemic, but in local circulatory aspects, too.

2.2. Ischemia-reperfusion studies

The complex pathophysiological processes during ischemia and reperfusion have been widely investigated [e.g. 20, 33]. Several components of the ischemia-reperfusion is responsible for impaired red blood cell deformability or enhanced red blood cell aggregation. These include direct effects oxygen-derived free radical reactions and activated neutrophils, endothelial interactions, nitric-oxide-related processes, mechanical trauma as well as micro-environmental changes (e.g. pH, osmolarity) [2, 20, 25, 26, 33, 36, 38].

After ischemia the systemic consequences are partly originated from the washout of locally accumulated metabolites: production of lactate and H^+ and consequent change of acid-base balance toward acidosis,

increased potassium concentration [33]. These changes may cause further local tissue and endothelial damage, contributing to the characteristic microcirculatory disturbances, such as the "no-reflow" phenomenon [6, 39]. Systemic activation of complement system and polymorphonuclear leukocytes and the release of intracellular myoglobin (in case of skeletal muscle ischemia) may lead to life-threatening organ damage (renal-, pulmonary-, cardiac failure, MOF) [33].

Numerous ischemia-reperfusion models have been studied by us. In these models one of the most important questions was the local versus systemic relation of hemorheological changes related to I/R injury of intestines [7], liver [19], hind limb [36, 38, 48] and muscle flap [49]. According to the available instrumental possibilities, and depending on the available-requested blood sample volume, we could test acid-base balance parameters, blood pH, metabolites. In a rat model of 2-hour hind limb ischemia arterio-venous values of pH, pO_2, pCO_2 and hematocrit have been monitored in the first hour of reperfusion, showing significant decrease in venous blood pH compared to arterial and control values [48]. A canine hind limb ischemia-reperfusion model demonstrated serious hemorheological impairment of the excluded blood in the ischemic extremity [38]. Also cerebral hypoperfusion model in a porcine study clearly showed that lactate accumulation in the superior sagittal sinus causes significant impairment of red blood cell filterability [37].

During ischemia, while the blood is in stasis in the excluded tissue region, organ or extremity, local metabolic and physical changes may directly influence the blood rheological parameters. Lactate accumulation in blood and tissues may influence blood rheological properties: decreasing red blood cell deformability and increasing blood viscosity [41, 50].

Morphologically red blood cells may show definitive variety of cell shapes depending on the micro-environmental conditions. It is known as stomatocyte-discocyte-echinocyte sequence [5]. Anionic amphipaths, alkalic pH, ATP depletion induce echinocytes, which forms are initially reversible, but may turn into sphero-echinocyte irreversibly. Cationic amphipaths, acidic pH induce concave stomatocytes, which may irreversibly become sphero-stomatocyte [5, 29]. It is known that both echinocyte and stomatocyte forms have impaired deformability and disturbed aggregation. For aggregation the biconcave shape is ideal, echinocytes do not aggregate well [35, 40].

Therefore, determining acid-base parameters and blood pH has importance to evaluate red cell properties. However, several questions raise. What is the range of red blood cell deformability and aggregation changes and where is the border of reversibility-irreversibility in this sequence? How could it be determined technically?

3. Experimental and technical considerations

Results obtained from *ex vivo* samples and the original *in vivo* conditions can be definitely altered to each other. In many hemorheological methods blood samples are processed to produce cell suspension in various buffers and/or macromolecule solutions [3, 21], taking out of the original sampling tube into other tubes, having contact with laboratory air, changing in temperature, eliciting trauma of the samples (e.g. centrifugation, suspension preparations), the problem of sample storage (*in vitro* aging) [3, 35].

In experimental surgical models when investigating hemorheological parameters it is important to consider the inter-species differences [34, 53] and the potential discrepancy between the quantity of available and required blood sample volume, in addition to many questions of experimental design.

Concerning the local versus systemic hemorheological changes and their investigation, the blood sampling site may vary according to the experimental protocol. Therefore, data on arterio-venous differences

are important [22, 47]. Sample handling and sample oxygenation level may also influence the results, when the aim is to analyze hemorheological variables together with blood pH and gas parameters (pO_2, pCO_2) [3, 51].

4. Effects of oxygenation-deoxygenation and pH on rheological parameters of erythrocytes

To understand the local versus systemic changes of red blood cell deformability and aggregation, the cell oxygenation, blood pH and lactate concentration have importance.

Concerning oxygenation-deoxygenation, pH dependent red blood cell morphology and aggregation behavior, Cicha and co-workers reported very interesting data, suggesting that the magnitude of the changes was dependent on the method of deoxygenation (i.e. 95% N_2 – 5% CO_2 or N_2 alone) [11]. Oxygenated red blood cell (95% O_2 – 5% CO_2) showed significantly higher aggregation (increased rouleaux formation rate) than the cells incubated with the N_2/CO_2 gas mixture. If the cells were incubated with air (pO_2 = 155–160 mmHg) the aggregation increased, but not as much as with deoxygenation by incubation in N_2. The rouleaux formation rate positively correlated with increasing pH. The lowest data were found by O_2/N_2 and N_2/CO_2 incubation (mean pH = 7.54 and 7.5, respectively), the highest in case of air and N_2 incubation (man pH = 8.23 and 8.41, respectively). Similar correlation was found with mean corpuscular hemoglobin concentration; with increasing pH the MCHC increased with flattened cell forms (increased surface-to-volume ratio), showing enhanced red blood cell aggregation [11].

The physiological blood pH has an accurately and well regulated range of 7.35–7.45 with well known respiratory and metabolic compensatory mechanism. Local pH in tissues, at the microcirculatory level is influenced by several factors (e.g. Donnan membrane equilibrium intracellular buffering, ion exchange) and may vary in wider range [11, 42]. However, the pH range used in the study by Cicha and co-workers is much more over the physiologically tolerable range.

Uyuklu and co-workers used a closer pH range for investigation the effect of oxygenation or deoxygenation on red blood cell aggregation and deformability [51]. Oxygenated samples (mean pO_2 = 142 mmHg; pH = 7.8) had significantly lower aggregation index and better red blood cell deformability versus deoxygenated samples (mean pO_2 = 28.6 mmHg; pH = 7.74). The native, control blood samples (mean pO_2 = 42.4 mmHg; pH = 7.4) showed aggregation and deformability values between the two incubated samples' data [51]. These data are very useful for standardization of laboratory measurement techniques, sampling and handling conditions [3].

5. Concluding thoughts

It is seen from the literature that the magnitude of changes in red blood cell deformability and aggregation and the range or border of reversibility-irreversibility are still controversial, being technically very difficult to test objectively. Therefore the accurate design of experiment, standardization of methods (sampling site and method, sample preparation and handling, measurement conditions and if necessary instrumental adaptations) all have great importance to have enough valuable and objective data to clarify better the relation of acid-base and blood pH changes to blood rheological parameters.

We believe that one of the most important factors is the blood sampling-handling to obtain valuable data in *ex vivo* samples [3, 35]. In ischemia-reperfusion models the comparison of local versus systemic changes has important clinical relevance. However, when evaluating results the relation of *in vivo* hemorheology with the *ex vivo* samples means always a limit of extrapolation [1].

Both in small and large animal ischemia-reperfusion models –depending on the available/requested blood sample volume– blood gas, pH, lactate level determinations are necessary. According to the experimental design local and systemic blood samples are also needed to be tested. Hopefully, in the future there will be minimally invasive devices/sensors that are capable to test *in vivo* local and systemic hemorheological parameters in their complexity. Till that these parameters can be tested separately and only on *ex vivo* samples.

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