Are there arterio-venous differences of blood micro-rheological variables in laboratory rats?

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Abstract

In animal experiments blood samples are often taken from various parts of the circulation. Although several variables including blood gas parameters are known to alter comparing arterial to venous system, arterio-venous (A-V) differences of blood micro-rheological variables (erythrocyte deformability and aggregation) tested by ektacytometry and aggregometry are not completely known in laboratory rats. In 12 outbred rats we investigated red blood cell deformability (RheoScan-D200 slit-flow ektacytometer), red blood cell aggregation (Myrenne MA-1 erythrocyte aggregometer), hematological variables (Sysmex F-800 microcell counter), blood pH and blood gases (ABL555 Radiometer Copenhagen) in blood samples taken parallel from the abdominal aorta and from the caudal caval vein. Blood pH did not differ, blood gas partial tensions showed physiological A-V differences, as it was expected. White blood cell count, red blood cell count and hematocrit were significantly higher in samples from the caval vein. Erythrocyte aggregation values (at 3 1/s shear rate) were significantly higher in samples taken from the abdominal aorta. Erythrocyte deformability (elongation index) did not show obvious A-V differences. Arterio-venous hemorheological differences -mostly of erythrocyte aggregation- can be found in rats, thus, the standardization of the studies and planning appropriate control measurements are necessary for safe evaluation of the obtained results.

Keywords: hemorheology, arterio-venous difference, red blood cell deformability, red blood cell aggregation, rat

1. Introduction

Physiology of normal circulation has been widely studied in the past century and fundamental data are known on hemodynamics at various levels of the circulation (Whitmore, 1968; Schmid-Schönbein, 1988; Cokelet and Meiselman, 2007). Also growing number of studies have been conducted revealing in vivo hemorheology, which is an important concern when evaluating findings (Whittaker and Winton, 1933; Fan et al., 1980; Lipowsky, 2005; Baskurt and Meiselman, 2007; Baskurt, 2008). However, studying in vivo alterations of hemorheological variables (blood- and plasma viscosity, hematocrit, red blood cell aggregation and deformability, etc.) is difficult, since the majority of the measurements are done on ex vivo blood samples.

Several methodological studies reported that oxygenation status of blood samples may influence the results, thus its standardization is important (Rusch et al., 1981; Hardeman et al., 1994 and 2001; Baskurt et al., 2009a, Uyuklu et al., 2009). Although arterio-venous (A-V) differences in blood gas partial tensions are also well known in physiology, A-V differences of hemorheological parameters are still controversial.

In animal experiments arterial and venous blood samples are often used in studies of ischemia-reperfusion and circulatory disturbances, in which hemorheological factors are informative (Bishop et al., 2001; Kayar et al., 2001; Mchedlishvili et al., 2004; Nemeth et al., 2006; Szokoly et al., 2006; Furka et al., 2008; Brath et al., 2009). The extrapolation of the results is influenced by inter-species differences, too, which are particularly known in hemorheology and still show controversies (Nemeth et al., 2009a-d; Windberger and Baskurt, 2007). Furthermore little is known about A-V data of animal red blood cell aggregation and deformability tested by currently available modern devices.

Our aim was to evaluate the supposed A-V differences of selected hemorheological variables in laboratory rats, providing information for further experimental studies.
2. Methods

2.1. Experimental animals and blood sampling

The experiments were approved by the University of Debrecen Committee of Animal Research (Permit No.: 37/2007. and 6/2008. UD CAR).

Twelve female CD rats (bodyweight: 328.91 ± 53.68 g) were anesthetized using sodium-thiopental (60 mg/kg, i.p., Thiopental®, Biocheime GmbH, Austria). A midline laparotomy was performed and the infrarenal part of the abdominal aorta and the caudal caval vein were gently explored. The abdominal aorta and the caudal caval vein were punctured separately for blood sampling (1.5 ml per vessels) using a 26 G needle and a connecting syringe that contained sodium-EDTA as anticoagulant (1.5 mg/ml).

The body temperature was 37.51 ± 1.05°C measured by a rectal probe.

2.2. Laboratory tests

2.2.1. Blood pH and gas analysis

In closed system, the blood samples were immediately filled into a blood gas analyzing automate (ABL555 Radiometer Copenhagen, Denmark), that determines blood pH, $pCO_2$ and $pO_2$ values [mmHg]. Values were corrected for body temperature.

2.2.2. Red blood cell deformability

Just after blood gas analysis the red blood cell deformability measurements were performed using a RheoScan-D200 slit-flow ektacytometer (RheoMeditech, Seoul, Korea) (Shin et al., 2005). The sample preparation was made by taking 5 µl of arterial or venous blood sample into 0.5 ml of a viscous isotonic solution (in normal PBS) of 360 kDa polyvinylpyrrolidone (viscosity – 30.51 mPas, osmolarity – 327 mOsm/kg; pH – 7.37). The blood was taken directly from the sampling syringe, trying to avoid direct contact with air.

2.2.3. Red blood cell aggregation

Red blood cell aggregation was determined using a Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) (Schmid-Schönbein et al., 1990). The method is based upon the measurement of light transmittance through the blood sample (Baskurt et al., 2009a). The determined aggregation index parameters are the followings: “M” indicating the extent of aggregation at stasis following an abrupt cessation of high shear to disperse pre-existing aggregates, and measured at 5 or 10 seconds; “M1” indicating the extent of aggregation at a low shear rate of 3 s⁻¹, again following an abrupt cessation of high shear, and measured at 5 or 10 seconds (Schmid-Schönbein et al., 1990). Both M and M1 increase with enhanced aggregation. The device lets the sample to contact with air.

2.2.4. Hematological parameters

White blood cell count (WBC count [x10³/µl]), red blood cell count (RBC count [x10⁶/µl]), platelet count (Plt count [x10³/µl]), hemoglobin concentration (Hgb [g/dl]), mean corpuscular volume (MCV [fl]), mean corpuscular hemoglobin (MCH [pg]), mean corpuscular hemoglobin concentration (MCHC [g/dl]) and mean platelet volume (MPV [fl]) were determined by a microcell counter (Sysmex F-800 microcell counter, TOA Medical Electronics Corp., Ltd., Japan). During the measurement the blood sample (70 µl) is diluted by the automate and has contact with air.

2.3. Statistical analyses

For comparison between arterial and venous blood samples Student t-test or Mann-Whitney rank sum test were made according to the normality of data distribution. A p value of <0.05 was considered as statistically significant.

3. Results

3.1. Blood gas and pH

As it was expected in a physiological point of view blood $pO_2$ values were significantly (p<0.001) higher in aorta, and $pCO_2$ in caudal caval vein blood samples: the $pO_2$ values in aorta were 110.24 ± 4.01 mmHg, and 54.37 ± 4.07 mmHg in caudal caval vein; the $pCO_2$ values in aorta were 42.69 ± 2.78 mmHg, and 59.9 ± 1.8 mmHg in caudal caval vein. The pH values did not show important arterio-venous differences (aorta: 7.22 ± 0.01, caudal caval vein: 7.24 ± 0.02; p=0.561).

3.2. Red blood cell deformability

Fig. 1 shows elongation index (EI) – shear stress (SS) curves. The arterial and venous sample did not differ; at most of the shear stress values the EI values were overlapping. Concerning comparative data of EI-SS curves, there were slight alteration in EI at 3, 5 and 20 Pa in venous samples EI values were slightly higher. At 10 and 20 Pa the difference was significant using paired t-test.
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EI\textsubscript{max} was minimally higher and SS\textsubscript{1/2} values were smaller in venous samples, however, there were no statistical differences between (Table 1).

3.3. Red blood cell aggregation

At stasis (shear rate: 0 s\textsuperscript{-1}) many measurements showed zero aggregation index values. At low shear rate (3 s\textsuperscript{-1}) the tests were stable, therefore, for the comparison M1 values were used. Both at 5 and 10 seconds the M1 values were higher in arterial samples, reaching significant arterio-venous difference at 5 seconds (2.03±0.14 vs 1.46±0.11, p=0.003; means±S.E.). At 10 seconds the arterio-venous difference was close to significant level (4.42±0.36 vs 3.54±0.4, p=0.068) (Fig. 2).

3.4. Hematological parameters

White blood cell count, red blood cell count and hematocrit values were significantly higher in venous blood sample. Platelet count was slightly increased in venous blood. Hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin content and its concentration as well as mean platelet volume did not show important arterio-venous differences (Table 2).

4. Discussion

Red blood cell deformability and aggregation are known to be affected by micro-environmental factors, such as osmolarity and pH (Weed et al., 1969; Kuzman et al., 2000), and influenced by the oxygenation status of hemoglobin molecules. Changes in oxygenation status is known to affect several red blood cell functions and properties (Cicha et al., 2003; Barvitenko et al., 2005; Uyuklu et al.,...
2009) and nitric oxide scavenging (Azarov et al., 2005), which may play a role in modulation of red blood cell deformability (Bor-Kucukatay et al., 2003). Uyuklu et al. found that oxygenated human red blood cells show significantly lower aggregation index values and better elongation index values compared to de-oxygenated cells. However, they used 100% nitrogen for de-oxygenation resulting in almost five-fold lower pO2 than in oxygenated samples by air equilibration, but in the same time, resulting in lower pCO2 and higher pH values than physiological (Uyuklu et al., 2009). They concluded that different degrees of hemoglobin oxygenation during the measurement of red blood cell deformability and aggregation may cause significant variations, and therefore the standardization of sample oxygenation may improve the precision of the measurements.

We found marked arterio-venous differences in several hematological parameters (white blood cell count, red blood cell count and hematocrit) and in red blood cell aggregation index values in rats, testing blood samples taken from the abdominal aorta and from the caudal caval vein. Red blood cell deformability did not show obvious arterio-venous difference, however, at selected shear stresses (10 and 20 Pa) arterial and venous samples differed significantly.

In previous experimental surgical studies in our laboratory we have investigated local versus systemic alterations of hematological and hemorheological parameters, where marked arterio-venous differences were found in red blood cell deformability and aggregation (Nemeth et al., 2006; Szokoly et al., 2006; Furka et al., 2008; Brath et al., 2009). In control or base values of these studies A-V differences in various manners were also observable. In a porcine model red blood cell deformability measured by bulk filtration method showed moderate, but non-significant differences comparing femoral arterial to femoral venous samples, where venous samples showed slightly elongated cell transit time (Nemeth et al., 2006). In a beagle canine study red blood cell aggregation index values were found to be higher in normal arterial samples (femoral artery) compared to venous blood (external jugular vein), where rather hematocrit and white blood cell count were elevated (Furka et al., 2008). In rats slight, but non-significant A-V differences of hematocrit and white blood cell count were observable in samples taken from the femoral artery and vein (Szokoly et al., 2006).

However, Kayar et al. did not find in their hindlimb ischemia-reperfusion experiment on rats important A-V differences in red blood cell deformability (by LORCA ektacytometer) and red blood cell aggregation (by their custom-built photometric aggregometer) in control values of the experiment using blood samples taken from the carotid artery and from the femoral vein (Kayar et al., 2001). These results suggest that the A-V hemorheological differences are complex, may have controversy and depend on the laboratory techniques, sampling site and measurement conditions, too.

It is important to note that arterial or venous hematocrit does not represent the blood composition at all levels of the circulation. Differences can be observed in large vessels throughout the circulatory system (Mchedlishvili and Varazashvili, 1986; Mchedlishvili et al., 2003) and it is also known that microvascular hematocrit is lower than in large vessels and show wide variety (Schmid-Schönbein, 1988; Bishop et al., 2001; Pries and Secomb, 2003; Lipowsky, 2003; Pospel and Johnson, 2005). Both plasma skimming and Fähraeus effect form the difference between hematocrit at various parts of the circulation (Gaefgen 1980; Baskurt and Meiselman, 2007). Therefore the in vivo hemorheological profile cannot be expressed by investigating samples taken only from large arteries or veins (Yilmaz and Gundogdu, 2008).

The limitation of our current study is the lack of using a controlled oxygenation or de-oxygenation system. Furthermore, we could not avoid contact with air in case of hematological and aggregation tests. However, the findings suggest that not only hemoglobin oxygenation status may contribute to arterio-venous differences. Red blood cell aggregation tests were performed at 5 or 10 seconds mode by the light transmittance aggregometer used (Schmid-Schönbein et al., 1990). Using this method the red blood cell aggregation kinetics cannot be tested over 10 seconds, however, further changes are expected during the entire process of the aggregation.

The main conclusion of this simple comparative study is that results of red blood cell deformability tested by slit-flow ektacytometry and erythrocyte aggregation measured by light transmittance aggregometer are not uniform when comparing normal samples obtained from the abdominal aorta or the caudal caval vein in rats. The findings support the recommendation that the standardization of the studies and planning appropriate control measurements are necessary for safe evaluation of the obtained results. However, to reveal all the in vivo aspects, further studies and supposedly new methods and devices are needed in the future.

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