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Comparative osmotic gradient ektacytometry data on inter-species differences of experimental animals

Abstract. It is known that red blood cell deformability may show colorful inter-species differences, influenced by inner viscosity, cell membrane viscosity, morphology and surface-volume ratio of the erythrocytes. It is also well-known that the cell volume is changing depending on the micro-environmental osmolarity. These changes can be well observable using osmotic gradient ektacytometry (osmoscan). Interestingly, there is a lack of base and comparative osmoscan data regarding the experimental/laboratory animal species. In this study mouse, rat, canine and porcine blood samples were analyzed using a LoRRca MaxSis Osmoscan ektacytometer. The highest elongation index values were found in mouse, typically above shear stress of 1 Pa. Some lower values than these were shown in rat and more lower in canine, while the lowest values were detected in porcine, typically above 3–5 Pa. The optimal osmolarity point value, so the measurable maximal EI osmolarity was in a wide range among the species. While the lowest values were detected in canine, the highest ones in porcine and mouse, the rat values were in between. Further analysis and wider comparison of the osmotic gradient ektacytometry may contribute to the better understanding of the erythrocyte micro-rheological properties, their induced changes and inter-species differences.

Keywords: Red blood cell deformability, osmotic gradient ektacytometry, osmoscan, experimental animals, inter-species differences

1. Introduction

Till date growing number of evidences emphasized the importance of deformability of the erythrocytes in various clinical conditions and pathophysiological processes [e.g. 6, 20–22]. Red blood cell deformability is determined by several factors, such as cell morphology, surface-volume ratio, inner viscosity as well as the own viscosity of the cell membrane [11, 16, 19]. For investigating the changes of erythrocyte deformability in various pathophysiological processes, experimental models may provide useful information. However, the comparative hemorheological investigations provide wider and wider knowledge also about the differences of the deformability parameters among the species [e.g. 5, 25]. Using the modern hemorheological measurement methods and devices, by now it has been a possibility to discover more precisely the differences as well as to clarify further the contradictions or similarities [3, 5].

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Regarding the red blood cell deformability the cell morphology and the cell volume are significant determinants and these may be, what at least partly originates the differences among the species [5, 14, 25]. It is also well-known that erythrocyte volume is changing depending on the osmotic conditions [2, 7, 8, 24]. However, only a few data is known about what differences can be measured between species by osmotic gradient ektacytometry [8]. In the PubMed database (data on 20 June, 2012), when searching for terms 'osmotic gradient ektacytometry' altogether 20, and for 'osmotic gradient ektacytometry animal' only 3 matches could be found. If adding the search field with 'osmoscan' word, 3–4 more articles could be appeared.

Although in several pathophysiological processes, in which the alterations of the micro-environment surrounding the erythrocytes alters [2], the changes of the osmoscan parameters can be predicted. In reference to this some promising data have already been revealed, even in regional relations of the vasculature [15].

The demand presents itself in animal research to collect control, base osmotic gradient ektacytometry data from different species what we have already started in our hemorheological laboratory.

We hypothesized, that osmoscan parameters may show differences among experimental animal species. In this present descriptive study, mouse, rat, beagle canine and porcine blood samples have been tested and their base data analyzed.

2. Materials and methods

2.1. Experimental animals and blood sampling

The experiments were approved and registered by the University of Debrecen Committee of Animal Research (registration Nr.: 19/2011. UD CAR), in accordance with the Hungarian Animal Protection Act (Law XVIII/1998).

Because of the hemorheological gender differences [17], only female animals were used in this study. Twelve adult C57BL/b strain inbred mice (bodyweight: 22.09 ± 1.3 g), 12 Sprague-Dawley outbred rats (bodyweight: 285.25 ± 12.43 g), 7 inbred beagle dogs (bodyweight: 10.05 ± 0.87 kg) and 12 outbred juvenile pigs (Seghers OptiMus X Bigwhite F2; bodyweight: 19.2 ± 2.1 kg) were involved into the study.

In rats and mice the blood samplings were carried out under general anesthesia (using sodium-thiopenthal, 60 mg/kg, i.p.), by puncturing the right ventricle with 26 G needle connected to a syringe (anticoagulant: sodium-EDTA, 1.5 mg/ml). In beagle dogs the cephalic vein, in pigs the lateral ear vein were used for letting blood samples directly into Vacutainer tubes (K_3-EDTA, 7.5%, 0.04 ml, BD Vacutainer®, Belliver Industrial Estate, U.K).

The samples were immediately taken into the laboratory to complete the measurements within the possibly shortest time (total *in vitro* time was <60 min).

2.2. Laboratory measurements

2.2.1. Red blood cell deformability

A LoRRca MaxSis Osmoscan device (Mechatronics BV, The Netherlands) was used to determine red blood cell elongation index in the function of shear stress. The device is composed of the well-known LORCA ektacytometer as red blood cell deformability measuring system [13]. The included osmoscan technique is also known and has been used previously in other devices [e.g., 8, 9, 23].

During the regular red blood cell deformability measurements blood sample of 5 μl was taken into 1 ml of isotonic polyvinyl-pyrrolidone solution (360 kDa PVP in normal phosphate buffered saline; viscosity = 27 mPa.s, osmolarity = 290–300 mOsm/kg; pH ∼ 7.3) and gently mixed. The suspension was injected into the bob-cup system of the device, and elongation index (EI) values were determined in a shear stress (SS) range of 0.3–30 Pa, based upon the laser diffraction pattern changes. The EI is equal to (L − W)/(L + W), where L is the length and W is the width of the diffractogram [3]. EI increases with red blood cell deformability. The measurements were carried out at constant temperature of 37°C. For the comparison of individual EI-SS curves Lineweaver-Burk analyses were performed, calculating the maximal elongation index (EI_{max}) and the shear stress values at half EI_{max} ($SS_{1/2}$ [Pa]), according to the following formula: $1/EI = SS_{1/2}/EI_{max} \times 1/SS + 1/EI_{max}$ [4].

By the 'osmoscan function' of the device the osmotic gradient ektacytometry measurements were also completed, using 250 μl blood in 5 ml PVP solution. The device generates a constant shear stress of 30 Pa, while continuously aspirating the sample into the measurement site with changing the osmolarity of the medium using gradual mixtures of PVP solutions of 0 and 500 mOsmol/kg, and so the EI was continuously registered [3, 8]. The measured and calculated parameters by the device (all at shear stress of 30 Pa) were the followings: minimal elongation index values measured at low osmolar environment (minimal EI), maximal elongation index values (maximal EI), half of the maximal elongation index values at high osmolar environment (EI_{hyper}), osmolarity at minimal EI, osmolarity at maximal EI ('optimal' osmolarity), osmolarity at EI_{hyper} and the area under the individual EI-osmolarity curves (AUC).

2.2.2. Hematological parameters

A Sysmex F-800 semi-automated microcell counter (TOA Medical Electronics Co., Japan) was used to determine the general hematological parameters, from which red blood cell count (RBC [$\times 10^6$/ μ l]), hemoglobin concentration (Hgb [g/dl]), hematocrit (Hct [%]), mean corpuscular volume (MCV [fl]), mean corpuscular hemoglobin content (MCH [pg]), mean corpuscular hemoglobin concentration (MCHC [g/dl]) and red cell distribution width (RDW-CV% [%]) were analyzed in this study.

2.3. Statistical analyses

Data are expressed as means and standard deviations (S.D). For comparison *t*-test or Mann-Whitney rank sum test were used, according to the data distribution. A p value of <0.05 was considered as statistically significant.

3. Results

3.1. Red blood cell deformability (EI-SS curves)

The cumulated elongation index (EI) data in the function of shear stress (SS [Pa]) of mice, rats, dogs and pigs are shown on Fig. 1. Except for porcine blood samples, the shape of the EI-SS curves were similar, however, the differences between species could be well observable.

Calculated parameters from the individual EI-SS curves also showed obvious differences. EI_{max} values were 0.587 ± 0.03 in mice, 0.559 ± 0.02 in rats, 0.567 ± 0.02 in beagle dogs and 0.518 ± 0.03 in pigs. We found significant differences between mice and rats ($p < 0.001$), mice and dogs ($p = 0.037$), mice and pigs ($p < 0.001$), rats and pigs ($p < 0.001$), as well as dogs and pigs ($p < 0.001$).

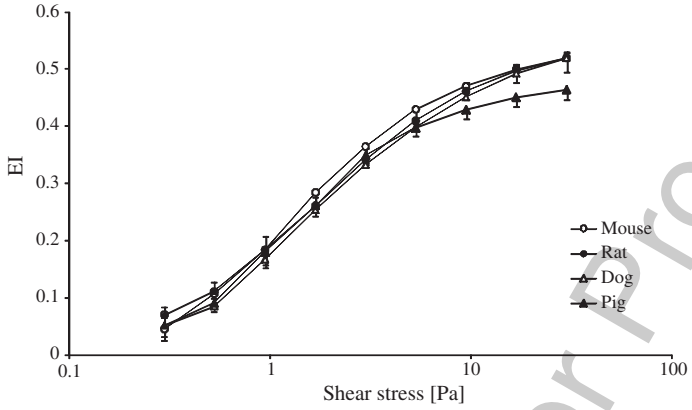

Fig. 1. Elongation index (EI) values (means ± S.D.) in the function of shear stress (SS [Pa]) in blood samples of mice ($n = 12$), rats ($n = 12$), beagle dogs ($n = 7$) and pigs ($n = 12$). Description of significant differences among species in Section 3.1.

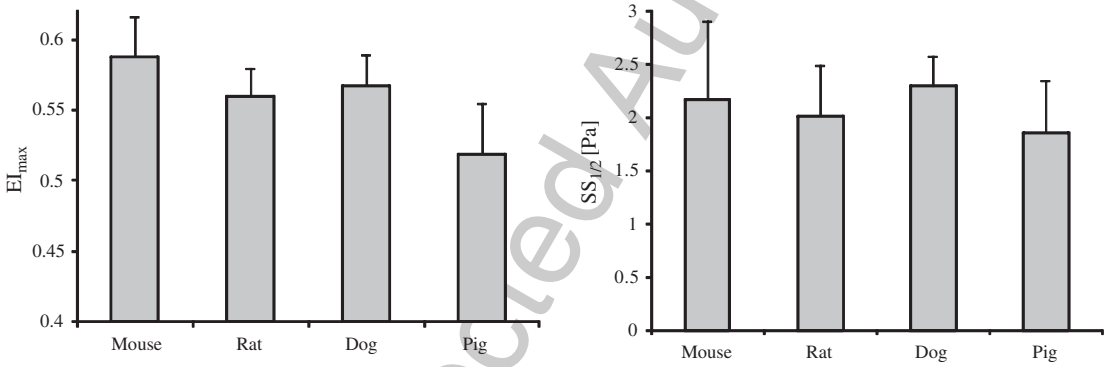

Fig. 2. Calculated maximal elongation index (EI_{max}) and shear stress values at EI_{max} ($SS_{1/2}$ [Pa]) (means ± S.D.) of mice ($n = 12$), rats ($n = 12$), beagle dogs ($n = 7$) and pigs ($n = 12$). Description of significant differences among species in Section 3.1.

$SS_{1/2}$ values [Pa] differed in smaller range, except for pigs, because their EI-SS curve shape was characteristically different compared to the other investigated species. $SS_{1/2}$ values were found to be 2.17 ± 0.74 Pa in mice, 2.02 ± 0.47 Pa in rats, 2.3 ± 0.28 Pa in beagle dogs and 1.86 ± 0.49 Pa in pigs. We found very weak significant differences between mice and dogs ($p = 0.049$), rats and dogs ($p = 0.044$), and stronger between dogs and pigs ($p = 0.003$) (Fig. 2).

3.2. Osmotic gradient ektacytometry (osmoscan curves)

Comparative data of the osmoscan measurements (elongation index in the function of osmolarity at constant 30 Pa shear stress) are summarized in Table 1.

Minimal EI values may reflect an interesting point: at the minimal osmolarity values red blood cells were still intact, but being swelled in hypoosmolar environment (lowest point of osmotic resistance). Testing minimal EI values, significant differences were found only between rats and dogs ($p = 0.028$),

Table 1
Parameters of osmotic gradient ektacytometry (osmoscan) measurements at shear stress of 30 Pa

Variable	Mouse	Rat	Dog	Pig
Minimal EI	0.090 ± 0.019	0.102 ± 0.008	0.093 ± 0.007	0.085 ± 0.006
Maximal EI	0.519 ± 0.016	0.509 ± 0.014	0.513 ± 0.007	0.481 ± 0.007
EI_{hyper}	0.260 ± 0.008	0.254 ± 0.007	0.257 ± 0.003	0.240 ± 0.003
Osmolarity at minimal EI [mOsm/kg]	167.5 ± 1.7	161.2 ± 6.1	153.7 ± 8.3	179.6 ± 8.6
Osmolarity at maximal EI [mOsm/kg]	349.6 ± 44.8	311.2 ± 9.1	288.6 ± 19.4	348.2 ± 15.5
Osmolarity at EI_{hyper} [mOsm/kg]	484.8 ± 8.1	422.4 ± 19.3	449.4 ± 14.9	454.1 ± 11.9
AUC (EI-osmolarity curve)	132.2 ± 14.7	118.5 ± 9.7	128.6 ± 4.1	110.6 ± 7.1

Means ± S.D.; Description of significant differences among species in Section 3.2; Maximal EI = maximal elongation index (EI) values measured; Minimal EI = minimal EI values measured at low (hypo-) osmolar environment. EI_{hyper} = half of maximal EI values in high (hyper-) osmolar environment. AUC = area under curve.

rats and pigs ($p < 0.001$), dogs and pigs ($p = 0.002$). However, the osmolarity at minimal EI values were significantly different between all species: mice and rats ($p = 0.042$), mice and dogs ($p = 0.005$), mice and pigs ($p = 0.005$), rats and dogs ($p = 0.025$), rats and pigs ($p < 0.001$), as well as dogs and pigs ($p < 0.001$).

The maximal EI values at shear stress of 30 Pa reflected the differences in the previously demonstrated EI_{max} of EI-SS curves (see in 3.1). Significant differences were found between mice and pigs ($p < 0.001$), rats and pigs ($p < 0.001$) as well as dogs and pigs ($p < 0.001$). The osmolarity values at maximal EI (*quasi* 'optimal' osmolarity) differed more obviously. In rats this osmolarity point was significantly lower compared to mice ($p = 0.007$), gradually lower in dogs ($p = 0.004$ vs. mice, $p = 0.008$ vs. rats). In pigs the optimal osmolarity values were close to mice ($p < 0.001$ vs. both rats and dogs).

The half point of maximal EI in the hyperosmolar region is used as a third anchorage of the EI-osmolarity curves, calling EI_{hyper} and its osmolarity point. EI_{hyper} values were relatively close to each other in these animal species. However, in pigs the values were relatively lower ($p < 0.001$ vs. all). In contrast, the osmolarity values at EI_{hyper} were the highest in mice, being significant versus rats ($p = 0.002$), dogs ($p < 0.001$) and pigs ($p < 0.001$). Rat values were significantly lower versus dogs ($p = 0.002$) and pigs ($p < 0.001$), too.

3.3. Hematological data

Red blood cell related hematological variables are summarized in Table 2. Interestingly, in pigs we found the lowest hemoglobin concentration and hematocrit values.

Mean corpuscular volume (MCV [fl]) was the lowest in mice ($p = 0.025$ vs. rats; $p < 0.001$ vs. dogs and pigs). Rats' MCV was lower that of dogs ($p < 0.001$) or pigs ($p = 0.002$), however, porcine MVC was significantly lower than the canine ($p < 0.001$). Although MCV showed obvious inter-species differences, it did not show strong correlation with EI_{max} (linear regression R^2 values of mice = 0.033, rats = 0.005, dogs = 0.0344 and pigs = 0.0187) or $SS_{1/2}$ (linear regression R^2 values of mice = 0.0326, rats = 0.0831, dogs = 0.2445 and pigs = 0.0192) values. Concerning the osmoscan values, we did not found important correlation between the variables. Interestingly, the highest MCH and MCHC values were found in rats and dogs together with the lowest osmolarity data at minimal EI, maximal EI and at EI_{hyper} values (Tables 1 and 2).

Table 2
Red blood cell related quantitative and qualitative parameters

Variable	Mouse	Rat	Dog	Pig
RBC count [$\times 10^6/\mu$ l]	6.84 ± 0.48	6.88 ± 0.48	6.97 ± 0.37	5.79 ± 0.5
Hct [%]	41.27 ± 2.13	40.05 ± 3.18	48.65 ± 2.91	$35.19 \pm 2{,}07$
Hgb [g/dl]	10.11 ± 1.61	11.56 ± 0.71	14.4 ± 0.85	8.95 ± 0.52
MCV [fl]	55.57 ± 1.58	58.26 ± 3.45	69.77 ± 3.21	60.88 ± 2.82
RDW-CV% [%]	16.34 ± 1.58	14 ± 0.51	13.92 ± 0.65	17.07 ± 0.79
MCH [pg]	14.57 ± 0.48	16.83 ± 0.65	20.65 ± 1.18	15.5 ± 0.94
MCHC [g/dl]	25.96 ± 0.87	28.93 ± 1.39	29.65 ± 1.97	25.45 ± 0.72

means $\pm$ S.D.; Description of significant differences among species in section 3.3.

4. Discussion and conclusion

In hemorheological animal research the exploration of differences among the species, their properly standardized investigation and the evaluation of results are essentially important to provide comparable data and proper extrapolation [5, 17, 25]. By the osmotic gradient ektacytometry, changes can be detected not only in irreversible red blood cell morphological changes (e.g. hereditary morphological disorders, sickle cell disease) [1, 8, 10, 12, 18], but in inflammatory, ischemic-reperfusionic processes, when the blood composition, its pH value and osmolarity in regional vascular territories (e.g. excluded region during clamping or obturation of vessels) all change [2, 15]. Of course in these latter cases, not only the red blood cells but their surrounding micro-environment is changing and may alter to that point where the results are detectably influenced [2, 7, 24]. Thus, in animal experiments, in which the osmotic gradient ektacytometry is applied, control measurements, base values and comparative hemorheological data are important. Using the new LoRRca MaxSis Osmoscan device, the collection of these base data could have been started, and as the aim of this present study, being analyzed as a descriptive comparison.

In reference to red blood cell deformability, congruous differences were found among the investigated animal species to our previous studies and literature data [17, 25]. The highest EI values were found in mouse, typically above shear stress of 1 Pa. Some lower values were shown in rat and more lower in canine blood, while the lowest EI data were detected in the porcine blood, typically above shear stress of 3–5 Pa. In porcine the morphology of EI-SS curves were obviously different compared to the other species' ones (see Fig. 1). These differences could also be seen in the calculated parameters of the curves. Possible correlation was searched between red blood cell volume (MCV) and deformability or osmoscan data, but no definite correlation was found. This is strengthened by other literature data, that there is no uniform explanation and coherent theory for understanding the backgrounds of the hemorheological differences among the species [5, 25].

The osmoscan curves also showed differences among the investigated species. The optimal osmolarity point value, so the measurable maximal EI osmolarity was in a wide range among the species, too. While the lowest values were detected in canine, the highest ones in porcine and mouse, the rat values were between these poles. The comparison of the lowest osmolarity point values might be of interest, since in this zone the cells are swelling then in a lower zone after a certain osmolarity value the erythrocytes burst. Regarding the differences, the lowest values were detected again in canine and the highest ones in porcine blood, while the values of the two rodent species were between them, but in a well-defined region by species. The investigation of the hyperosmolarity zone ended around 500 mOsmol/kg, since that was the

osmolarity of the applied PVP solution. Of course the biologically tolerable and definable hyperosmotic range is wide (e.g. values even above 1000 mOsmol/kg can appear in vessels running directly by the tubules and Henle loops of the kidney). Therefore the device uses the osmolarity values belonging to the half-maximal EI for comparison. Even here differences can be seen among the species, depending mainly on the slope of the descending part of the EI-osmolarity curve.

The changes in the cell volume provoked by the changes of the osmotic environment depend on the cellular morphological factors [16, 19] and also on the properties of the protein network taking place in the membrane and running under it [11, 16, 24]. Katyukhin et al. have investigated the rheological properties of erythrocytes in mouse, rat, hamster, guinea pig, rabbit, canine and human, and they found the differences being correlated to the activity of cation transport-ATPases, to which osmoscan data can be brought into correlation [14].

Unfortunately, little amount of information can be found in the literature about osmotic gradient ektacytometrial results and their background or explanation. Further more, detailed analysis and wider comparison can raise new questions, and we believe that it can contribute to the better understanding of the micro-rheological properties of red blood cells, their induced changes as well as inter-species differences.

Acknowledgments

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The authors comply with the Ethical Guidelines for Publication in *Clinical Hemorheology and Microcirculation* as published on the IOS Press website and in Volume 44, 2010, pp. 1-2 of this journal.

References

[1] S.K. Ballas and E.D. Smith, Red blood cell changes during the evolution of the sickle cell painful crisis, *Blood* **79** (1992), 2154–2163.

[2] O.K. Baskurt, Mechanisms of blood rheology alterations, in: *Handbook of Hemorheology and Hemodynamics*, O.K. Baskurt, M.R. Hardeman, M.W. Rampling and H.J. Meiselman, eds, IOS Press, Amsterdam, The Netherlands, 2007, 170–190.

[3] O.K. Baskurt, M. Boynard, G.C. Cokelet, P. Connes, B.M. Cooke, S. Forconi, M.R. Hardeman, F. Jung, F. Liao, H.J. Meiselman, G. Nash, N. Nemeth, B. Neu, B. Sandhagen, S. Shin, G. Thurston and J.L. Wautier, International Expert Panel for Standardization of Hemorheological Methods, New guidelines for hemorheological laboratory techniques, *Clin Hemorheol Microcirc* **42** (2009), 75–97.

[4] O.K. Baskurt, M.R. Hardeman, M. Uyuklu, P. Ulker, M. Cengiz, N. Nemeth, S. Shin, T. Alexy and H.J. Meiselman, Parameterization of red blood cell elongation index –shear stress curves obtained by ektacytometry, *Scand J Clin Lab Invest* **69** (2009), 777–788.

[5] O.K. Baskurt and H.J. Meiselman, Lessons from comparative hemorheology studies, *Clin Hemorheol Microcirc* **45** (2010), 101–108.

[6] O.K. Baskurt, P. Ulker and H.J. Meiselman, Nitric oxide, erythrocytes and exercise, *Clin Hemorheol Microcirc* **49** (2011), 175–181.

[7] J.F. Brun, Hormones, metabolism and body composition as major determinants of blood rheology: Potential pathophysiological meaning, *Clin Hemorheol Microcirc* **26** (2002), 63–79.

[8] M.R. Clark, N. Mohandas and S.B. Shohet, Osmotic gradient ektacytometry: Comprehensive characterization of red cell volume and surface maintenance, *Blood* **61** (1983), 899–910.
[9] K. de Jong, S.K. Larkin, S. Eber, P.F.H. Franck, B. Roelofsen and F.A. Kuypers, Hereditary spherocytosis and elliptocytosis erythrocytes show a normal transbilayer phospholipid distribution, *Blood* **94** (1999), 319–325.
[10] J. Delaunay, The hereditary stomatocytoses: Genetic disorders of the red cell membrane permeability to monovalent kations, *Semin Hematol* **41** (2004), 165–172.
[11] S. De Oliveira and C. Saldanha, An overview about erythrocyte membrane, *Clin Hemorheol Microcirc* **44** (2010), 63–74.
[12] J.W. Deuel, H.U. Lutz, B. Misselwitz and J.S. Goede, Asymptomatic elevation of the hyperchromic red blood cell subpopulation is associated with decreased red cell deformability, *Ann Hematol* (2012) DOI: 10.1007/s00277-012-1467-5 [Epub ahead of print].
[13] M.R. Hardeman, P.T. Goedhart and S. Shin, Methods in hemorheology, in: *Handbook of Hemorheology and Hemodynamics*, O.K. Baskurt, M.R. Hardeman, M.W. Rampling and H.J. Meiselman, eds, IOS Press, Amsterdam, The Netherlands, 2007, 242–266.
[14] L.N. Kathyukhin, A.M. Kazennov, M.N. Maslova and Yu.A. Matskevich, Rheologic properties of mammalian erythrocytes: Relationships to transport ATPases, *Comp Biochem Physiol B Biochem Mol Biol* **120** (1998), 493–498.
[15] Z. Klarik, F. Kiss, I. Miko and N. Nemeth, Aorto-porto-caval micro-rheological differences of red blood cells in laboratory rats: Further deformability and ektacytometrial osmoscan data, *Clin Hemorheol Microcirc*. (2012) DOI: 10.3233/CH-2012-1539 [Epub ahead of print]
[16] H.J. Meiselman, Morphological determinants of red blood cell deformability, *Scand J Clin Lab Invest* **41** (Suppl. 156) (1981), 27–34.
[17] N. Nemeth, F. Kiss, I. Furka and I. Miko, Gender differences of blood rheological parameters in laboratory animals, *Clin Hemorheol Microcirc* **45** (2010), 263–272.
[18] B. Pautard, C. Feo, D. Dhermy, H. Wajcman, V. Baudin-Chich and J. Delobel, Occurrence of hereditary spherocytosis and beta thalassaemia in the same family: Globin chain synthesis and visco diffractometric studies, *Br J Haematol* **70** (1988), 239–245.
[19] W.H. Reinhart, Peculiar red cell shapes: Fahraeus Lecture 2011, *Clin Hemorheol Microcirc* **49,** (2011), 11–27.
[20] B. Sandhagen and L. Lind, Whole blood viscosity and erythrocyte deformability are related to endothelium-dependent vasodilation and coronary risk in the elderly. The prospective investigation of the vasculature in Uppsala seniors (PIVUS) study, *Clin Hemorheol Microcirc* **50** (2012), 301–311.
[21] I.A. Tikhomirova, A.O. Oslyakova and S.G. Mikhailova, Microcirculation and blood rheology in patients with cerebrovascular disorders, *Clin Hemorheol Microcirc* **49** (2011), 295–305.
[22] A. Vaya, A. Hernandez-Mijares, E. Bonet, R. Sendra, E. Sola, R. Perez, D. Corella and B. Laiz, Association between hemorheological alterations and metabolic syndrome, *Clin Hemorheol Microcirc* **49** (2011), 493–503.
[23] R.E. Waugh, M. Narla, C.W. Jackson, T.J. Mueller, T. Suzuki and G.L. Dale, Rheologic properties of senescent erythrocytes: Loss of surface area and volume with red blood cell age, *Blood* **79** (1992), 1351–1358.
[24] R.I. Weed, P.L. La Celle and E.W. Merrill, Metabolic dependence of red blood cell deformability, *J Clin Invest* **48** (1969), 795–809.
[25] U. Windberger and O.K. Baskurt, Comparative hemorheology, in: *Handbook of Hemorheology and Hemodynamics*, O.K. Baskurt, M.R. Hardeman, M.W. Rampling and H.J. Meiselman, eds, IOS Press, Amsterdam, The Netherlands, 2007, 267–285.

Comparative osmotic gradient ektacytometry data on inter-species differences of experimental animals

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Abstract:

It is known that red blood cell deformability may show colorful inter-species differences, influenced by inner viscosity, cell membrane viscosity, morphology and surface-volume ratio of the erythrocytes. It is also well-known that the cell volume is changing depending on the micro-environmental osmolarity. These changes can be well observable using osmotic gradient ektacytometry (osmoscan). Interestingly, there is a lack of base and comparative osmoscan data regarding the experimental/laboratory animal species. In this study mouse, rat, canine and porcine blood samples were analyzed using a LoRRca MaxSis Osmoscan ektacytometer. The highest elongation index values were found in mouse, typically above shear stress of 1 Pa. Some lower values than these were shown in rat and more lower in canine, while the lowest values were detected in porcine, typically above 3-5 Pa. The optimal osmolarity point value, so the measurable maximal EI osmolarity was in a wide range among the species. While the lowest values were detected in canine, the highest ones in porcine and mouse, the rat values were in between. Further analysis and wider comparison of the osmotic gradient ektacytometry may contribute to the better understanding of the erythrocyte micro-rheological properties, their induced changes and inter-species differences.

Keywords: red blood cell deformability, osmotic gradient ektacytometry, osmoscan, experimental animals, inter-species differences

1. Introduction

Till date growing number of evidences emphasized the importance of deformability of the erythrocytes in various clinical conditions and pathophysiological processes [e.g. 6, 20, 21, 22]. Red blood cell deformability is determined by several factors, such as cell morphology, surface-volume ratio, inner viscosity as well as the own viscosity of the cell membrane [11, 16, 19]. For investigating the changes of erythrocyte deformability in various pathophysiological processes, experimental models may provide useful information. However, the comparative hemorheological investigations provide wider and wider knowledge also about the differences of the deformability parameters among the species [e.g. 5, 25]. Using the modern hemorheological measurement methods and devices, by now it has been a possibility to discover more precisely the differences as well as to clarify further the contradictions or similarities [3, 5].

Regarding the red blood cell deformability the cell morphology and the cell volume are significant determinants and these may be, what at least partly originates the differences among the species [5, 14, 25]. It is also well-known that erythrocyte volume is changing depending on the osmotic conditions [2, 7, 8, 24]. However, only a few data is known about what differences can be measured between species by osmotic gradient ektacytometry [8]. In the PubMed database (data on 20 June, 2012), when searching for terms ‘osmotic gradient ektacytometry’ altogether 20, and for ‘osmotic gradient ektacytometry animal’ only 3 matches could be found. If adding the search field with ‘osmoscan’ word, 3-4 more articles could be appeared.

Although in several pathophysiological processes, in which the alterations of the micro-environment surrounding the erythrocytes alters [2], the changes of the osmoscan parameters can be predicted. In reference to this some promising data have already been revealed, even in regional relations of the vasculature [15].

The demand presents itself in animal research to collect control, base osmotic gradient ektacytometry data from different species what we have already started in our hemorheological laboratory.

We hypothesized, that osmoscan parameters may show differences among experimental animal species. In this present descriptive study, mouse, rat, beagle canine and porcine blood samples have been tested and their base data analyzed.

2. Materials and Methods

2.1. Experimental animals and blood sampling

The experiments were approved and registered by the University of Debrecen Committee of Animal Research (registration Nr.: 19/2011. UD CAR), in accordance with the Hungarian Animal Protection Act (Law XVIII/1998).

Because of the hemorheological gender differences [17], only female animals were used in this study. Twelve adult C57BL/b strain inbred mice (bodyweight: 22.09 ± 1.3 g), 12 Sprague-Dawley outbred rats (bodyweight: 285.25 ± 12.43 g), 7 inbred beagle dogs (bodyweight: 10.05 ± 0.87 kg) and 12 outbred juvenile pigs (Seghers OptiMus X Bigwhite F2; bodyweight: 19.2 ± 2.1 kg) were involved into the study.

In rats and mice the blood samplings were carried out under general anesthesia (using sodium-thiopenthal, 60 mg/kg, i.p.), by puncturing the right ventricle with 26 G needle connected to a syringe (anticoagulant: sodium-EDTA, 1.5 mg/ml). In beagle dogs the cephalic vein, in pigs the lateral ear vein were used for letting blood samples directly into Vacutainer tubes (K_3-EDTA, 7.5%, 0.04 ml, BD Vacutainer®, Belliver Industrial Estate, U.K.).

The samples were immediately taken into the laboratory to complete the measurements within the possibly shortest time (total *in vitro* time was < 60 min).

2.2. Laboratory measurements

2.2.1. Red blood cell deformability

A LoRRca MaxSis Osmoscan device (Mechatronics BV, The Netherlands) was used to determine red blood cell elongation index in the function of shear stress. The device is composed of the well-known LORCA ektacytometer as red blood cell deformability measuring system [13]. The included osmoscan technique is also known and has been used previously in other devices [e.g., 8, 9, 23].

During the regular red blood cell deformability measurements blood sample of 5 µl was taken into 1 ml of isotonic polyvinyl-pyrrolidone solution (360 kDa PVP in normal phosphate buffered saline; viscosity = 27 mPa.s, osmolarity = 290-300 mOsm/kg ; pH ~ 7.3) and gently mixed. The suspension was injected into the bob-cup system of the device, and elongation index (EI) values were determined in a shear stress (SS) range of 0.3 - 30 Pa, based upon the laser diffraction pattern changes. The EI is equal to (L-W)/(L+W), where L is the length and W is the width of the diffractogram [3]. EI increases with red blood cell deformability. The measurements were carried out at constant temperature of 37 °C. For the comparison of individual EI-SS curves Lineweaver-Burk analyses were performed, calculating the maximal elongation index (EI_{max}) and the shear stress values at half EI_{max} ($SS_{1/2}$ [Pa]), according to the following formula: $1/EI = SS_{1/2}/ EI_{max} \times 1/SS + 1/EI_{max}$ [4].

By the 'osmoscan function' of the device the osmotic gradient ektacytometry measurements were also completed, using 250 µl blood in 5 ml PVP solution. The device generates a constant shear stress of 30 Pa, while continuously aspirating the sample into the measurement site with changing the osmolarity of the medium using gradual mixtures of PVP solutions of 0 and 500 mOsmol/kg, and so the EI was continuously registered [3, 8]. The measured and calculated parameters by the device (all at shear stress of 30 Pa) were the followings: minimal elongation index values measured at low osmolar environment (minimal

EI), maximal elongation index values (maximal EI), half of the maximal elongation index values at high osmolar environment (EI_{hyper}), osmolarity at minimal EI, osmolarity at maximal EI ('optimal' osmolarity), osmolarity at EI_{hyper} and the area under the individual EI-osmolarity curves (AUC).

2.2.2. Hematological parameters

A Sysmex F-800 semi-automated microcell counter (TOA Medical Electronics Co., Japan) was used to determine the general hematological parameters, from which red blood cell count (RBC [$x10^6$/µl]), hemoglobin concentration (Hgb [g/dl]), hematocrit (Hct [%]), mean corpuscular volume (MCV [fl]), mean corpuscular hemoglobin content (MCH [pg]), mean corpuscular hemoglobin concentration (MCHC [g/dl]) and red cell distribution width (RDW-CV% [%]) were analyzed in this study.

2.3. Statistical analyses

Data are expressed as means and standard deviations (S.D.). For comparison t-test or Mann-Whitney rank sum test were used, according to the data distribution. A p value of <0.05 was considered as statistically significant.

3. Results

3.1. Red blood cell deformability (EI-SS curves)

The cumulated elongation index (EI) data in the function of shear stress (SS [Pa]) of mice, rats, dogs and pigs are shown on Figure 1. Except for porcine blood samples, the shape of the EI-SS curves were similar, however, the differences between species could be well observable.

Calculated parameters from the individual EI-SS curves also showed obvious differences. EI_{max} values were 0.587 ± 0.03 in mice, 0.559 ± 0.02 in rats, 0.567 ± 0.02 in beagle dogs and 0.518 ± 0.03 in pigs. We found significant differences between mice and rats ($p<0.001$), mice and dogs ($p=0.037$), mice and pigs ($p<0.001$), rats and pigs ($p<0.001$), as well as dogs and pigs ($p<0.001$).

$SS_{1/2}$ values [Pa] differed in smaller range, except for pigs, because their EI-SS curve shape was characteristically different compared to the other investigated species. $SS_{1/2}$ values were found to be 2.17 ± 0.74 Pa in mice, 2.02 ± 0.47 Pa in rats, 2.3 ± 0.28 Pa in beagle dogs and 1.86 ± 0.49 Pa in pigs. We found very weak significant differences between mice and dogs ($p=0.049$), rats and dogs ($p=0.044$), and stronger between dogs and pigs ($p=0.003$) (Figure 2).

3.2. Osmotic gradient ektacytometry (osmoscan curves)

Comparative data of the osmoscan measurements (elongation index in the function of osmolarity at constant 30 Pa shear stress) are summarized in Table 1.

Minimal EI values may reflect an interesting point: at the minimal osmolarity values red blood cells were still intact, but being swelled in hypoosmolar environment (lowest point of osmotic resistance). Testing minimal EI values, significant differences were found only between rats and dogs ($p=0.028$), rats and pigs ($p<0.001$), dogs and pigs ($p=0.002$). However, the osmolarity at minimal EI values were significantly different between all species: mice and rats ($p=0.042$), mice and dogs ($p=0.005$), mice and pigs ($p=0.005$), rats and dogs ($p=0.025$), rats and pigs ($p<0.001$), as well as dogs and pigs ($p<0.001$).

The maximal EI values at shear stress of 30 Pa reflected the differences in the previously demonstrated EI_{max} of EI-SS curves (see in 3.1.). Significant differences were found between mice and pigs ($p<0.001$), rats and pigs ($p<0.001$) as well as dogs and pigs

($p<0.001$). The osmolarity values at maximal EI (*quasi* 'optimal' osmolarity) differed more obviously. In rats this osmolarity point was significantly lower compared to mice ($p=0.007$), gradually lower in dogs ($p=0.004$ vs. mice, $p=0.008$ vs. rats). In pigs the optimal osmolarity values were close to mice ($p<0.001$ vs. both rats and dogs).

The half point of maximal EI in the hyperosmolar region is used as a third anchorage of the EI-osmolarity curves, calling EI_{hyper} and its osmolarity point. EI_{hyper} values were relatively close to each other in these animal species. However, in pigs the values were relatively lower ($p<0.001$ vs. all). In contrast, the osmolarity values at EI_{hyper} were the highest in mice, being significant versus rats ($p=0.002$), dogs ($p<0.001$) and pigs ($p<0.001$). Rat values were significantly lower versus dogs ($p=0.002$) and pigs ($p<0.001$), too.

3.3. Hematological data

Red blood cell related hematological variables are summarized in Table 2. Interestingly, in pigs we found the lowest hemoglobin concentration and hematocrit values.

Mean corpuscular volume (MCV [fl]) was the lowest in mice ($p=0.025$ vs. rats; $p<0.001$ vs. dogs and pigs). Rats' MCV was lower that of dogs ($p<0.001$) or pigs ($p=0.002$), however, porcine MVC was significantly lower than the canine ($p<0.001$). Although MCV showed obvious inter-species differences, it did not show strong correlation with EI_{max} (linear regression R^2 values of mice = 0.033, rats = 0.005, dogs = 0.0344 and pigs = 0.0187) or $SS_{1/2}$ (linear regression R^2 values of mice = 0.0326, rats = 0.0831, dogs = 0.2445 and pigs = 0.0192) values. Concerning the osmoscan values, we did not found important correlation between the variables. Interestingly, the highest MCH and MCHC values were found in rats and dogs together with the lowest osmolarity data at minimal EI, maximal EI and at EI_{hyper} values (Table 1 and 2).

4. Discussion and Conclusion

In hemorheological animal research the exploration of differences among the species, their properly standardized investigation and the evaluation of results are essentially important to provide comparable data and proper extrapolation [5, 17, 25]. By the osmotic gradient ektacytometry, changes can be detected not only in irreversible red blood cell morphological changes (e.g. hereditary morphological disorders, sickle cell disease) [1, 8, 10, 12, 18], but in inflammatory, ischemic-reperfusionic processes, when the blood composition, its pH value and osmolarity in regional vascular territories (e.g. excluded region during clamping or obturation of vessels) all change [2, 15]. Of course in these latter cases, not only the red blood cells but their surrounding micro-environment is changing and may alter to that point where the results are detectably influenced [2, 7, 24]. Thus, in animal experiments, in which the osmotic gradient ektacytometry is applied, control measurements, base values and comparative hemorheological data are important. Using the new LoRRca MaxSis Osmoscan device, the collection of these base data could have been started, and as the aim of this present study, being analyzed as a descriptive comparison.

In reference to red blood cell deformability, congruous differences were found among the investigated animal species to our previous studies and literature data [17, 25]. The highest EI values were found in mouse, typically above shear stress of 1 Pa. Some lower values were shown in rat and more lower in canine blood, while the lowest EI data were detected in the porcine blood, typically above shear stress of 3-5 Pa. In porcine the morphology of EI-SS curves were obviously different compared to the other species' ones (see Figure 1). These differences could also be seen in the calculated parameters of the curves. Possible correlation was searched between red blood cell volume (MCV) and deformability or osmoscan data, but no definite correlation was found. This is strengthened by other literature

data, that there is no uniform explanation and coherent theory for understanding the backgrounds of the hemorheological differences among the species [5, 25].

The osmoscan curves also showed differences among the investigated species. The optimal osmolarity point value, so the measurable maximal EI osmolarity was in a wide range among the species, too. While the lowest values were detected in canine, the highest ones in porcine and mouse, the rat values were between these poles. The comparison of the lowest osmolarity point values might be of interest, since in this zone the cells are swelling then in a lower zone after a certain osmolarity value the erythrocytes burst. Regarding the differences, the lowest values were detected again in canine and the highest ones in porcine blood, while the values of the two rodent species were between them, but in a well-defined region by species. The investigation of the hyperosmolarity zone ended around 500 mOsmol/kg, since that was the osmolarity of the applied PVP solution. Of course the biologically tolerable and definable hyperosmotic range is wide (e.g. values even above 1000 mOsmol/kg can appear in vessels running directly by the tubules and Henle loops of the kidney). Therefore the device uses the osmolarity values belonging to the half-maximal EI for comparison. Even here differences can be seen among the species, depending mainly on the slope of the descending part of the EI-osmolarity curve.

The changes in the cell volume provoked by the changes of the osmotic environment depend on the cellular morphological factors [16, 19] and also on the properties of the protein network taking place in the membrane and running under it [11, 16, 24]. Katyukhin et al. have investigated the rheological properties of erythrocytes in mouse, rat, hamster, guinea pig, rabbit, canine and human, and they found the differences being correlated to the activity of cation transport-ATPases, to which osmoscan data can be brought into correlation [14].

Unfortunately, little amount of information can be found in the literature about osmotic gradient ektacytometrial results and their background or explanation. Further, more

detailed analysis and wider comparison can raise new questions, and we believe that it can contribute to the better understanding of the micro-rheological properties of red blood cells, their induced changes as well as inter-species differences.

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6. References

[1] S.K. Ballas and E.D. Smith, Red blood cell changes during the evolution of the sickle cell painful crisis, *Blood* **79** (1992), 2154-2163.

[2] O.K. Baskurt, Mechanisms of blood rheology alterations, in: *Handbook of Hemorheology and Hemodynamics,* O.K. Baskurt, M.R. Hardeman, M.W. Rampling and H.J. Meiselman, eds., IOS Press, Amsterdam, The Netherlands, 2007, pp. 170-190.

[3] O.K. Baskurt, M. Boynard, G.C. Cokelet, P. Connes, B.M. Cooke, S. Forconi, M.R. Hardeman, F. Jung, F. Liao, H.J. Meiselman, G. Nash, N. Nemeth, B. Neu, B. Sandhagen, S. Shin, G. Thurston and J.L. Wautier; International Expert Panel for Standardization of Hemorheological Methods, New guidelines for hemorheological laboratory techniques, *Clin. Hemorheol. Microcirc.* **42** (2009), 75-97.

[4] O.K. Baskurt, M.R. Hardeman, M. Uyuklu, P. Ulker, M. Cengiz, N. Nemeth, S. Shin, T. Alexy and H.J. Meiselman, Parameterization of red blood cell elongation index – shear stress curves obtained by ektacytometry, *Scand. J. Clin. Lab. Invest.* **69** (2009), 777-788.

[5] O.K. Baskurt and H.J. Meiselman, Lessons from comparative hemorheology studies, *Clin. Hemorheol. Microcirc.* **45** (2010), 101-108.

[6] O.K. Baskurt, P. Ulker and H.J. Meiselman, Nitric oxide, erythrocytes and exercise, *Clin. Hemorheol. Microcirc*. **49**, (2011), 175-181.

[7] J.F. Brun, Hormones, metabolism and body composition as major determinants of blood rheology: potential pathophysiological meaning, *Clin. Hemorheol. Microcirc.* **26** (2002), 63-79.

[8] M.R. Clark, N. Mohandas and S.B. Shohet, Osmotic gradient ektacytometry: comprehensive characterization of red cell volume and surface maintenance, *Blood* **61** (1983), 899-910.

[9] K. de Jong, S.K. Larkin, S. Eber, P.F.H. Franck, B. Roelofsen and F.A. Kuypers, Hereditary spherocytosis and elliptocytosis erythrocytes show a normal transbilayer phospholipid distribution, *Blood* **94**, (1999), 319-325.

[10] J. Delaunay, The hereditary stomatocytoses: genetic disorders of the red cell membrane permeability to monovalent kations, *Semin. Hematol.* **41** (2004), 165-172.

[11] S. De Oliveira and C. Saldanha, An overview about erythrocyte membrane, *Clin. Hemorheol. Microcirc.* **44** (2010), 63-74.

[12] J.W. Deuel, H.U. Lutz, B. Misselwitz and J.S. Goede, Asymptomatic elevation of the hyperchromic red blood cell subpopulation is associated with decreased red cell deformability, *Ann. Hematol.* (2012) DOI: 10.1007/s00277-012-1467-5 [Epub ahead of print]

[13] M.R. Hardeman, P.T. Goedhart and S. Shin, Methods in hemorheology, in: *Handbook of Hemorheology and Hemodynamics,* O.K. Baskurt, M.R. Hardeman, M.W. Rampling and H.J. Meiselman, eds., IOS Press, Amsterdam, The Netherlands, 2007, pp. 242-266.

[14] L.N. Kathyukhin, A.M. Kazennov, M.N. Maslova and Yu.A. Matskevich, Rheologic properties of mammalian erythrocytes: relationships to transport ATPases, *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **120** (1998), 493-498.

[15] Z. Klarik, F. Kiss, I. Miko and N. Nemeth, Aorto-porto-caval micro-rheological differences of red blood cells in laboratory rats: Further deformability and ektacytometrial osmoscan data, *Clin. Hemorheol. Microcirc.* (2012) DOI: 10.3233/CH-2012-1539 [Epub ahead of print]

[16] H.J. Meiselman, Morphological determinants of red blood cell deformability, *Scand. J. Clin. Lab. Invest.* **41** Suppl. 156 (1981), 27-34.

[17] N. Nemeth, F. Kiss, I. Furka and I. Miko, Gender differences of blood rheological parameters in laboratory animals, *Clin. Hemorheol. Microcirc.* **45** (2010), 263-272.

[18] B. Pautard, C. Feo, D. Dhermy, H. Wajcman, V. Baudin-Chich and J. Delobel, Occurrence of hereditary spherocytosis and beta thalassaemia in the same family: globin chain synthesis and visco diffractometric studies, *Br. J. Haematol.* **70** (1988), 239-245.

[19] W.H. Reinhart, Peculiar red cell shapes: Fahraeus Lecture 2011, *Clin. Hemorheol. Microcirc.* **49**, (2011),11-27.

[20] B. Sandhagen and L. Lind, Whole blood viscosity and erythrocyte deformability are related to endothelium-dependent vasodilation and coronary risk in the elderly. The prospective investigation of the vasculature in Uppsala seniors (PIVUS) study, *Clin. Hemorheol. Microcirc.* **50**, (2012), 301-311.

[21] I.A. Tikhomirova, A.O. Oslyakova and S.G. Mikhailova, Microcirculation and blood rheology in patients with cerebrovascular disorders, *Clin. Hemorheol. Microcirc.* **49**, (2011), 295-305.

[22] A. Vaya, A. Hernandez-Mijares, E. Bonet, R. Sendra, E. Sola, R. Perez, D. Corella and B. Laiz, Association between hemorheological alterations and metabolic syndrome, *Clin. Hemorheol. Microcirc.* **49** (2011), 493-503.

[23] R.E. Waugh, M. Narla, C.W. Jackson, T.J. Mueller, T. Suzuki and G.L. Dale, Rheologic properties of senescent erythrocytes: loss of surface area and volume with red blood cell age, *Blood* **79**, (1992), 1351-1358.

[24] R.I. Weed, P.L. La Celle, E.W. Merrill, Metabolic dependence of red blood cell deformability, *J. Clin. Invest.* **48** (1969), 795-809.

[25] U. Windberger and O.K. Baskurt, Comparative hemorheology, in: *Handbook of Hemorheology and Hemodynamics,* O.K. Baskurt, M.R. Hardeman, M.W. Rampling and H.J. Meiselman, eds., IOS Press, Amsterdam, The Netherlands, 2007, pp. 267-285.

7. Tables

Table 1. Parameters of osmotic gradient ektacytometry (osmoscan) measurements at shear stress of 30 Pa.

Variable	Mouse	Rat	Dog	Pig
Minimal EI	0.090 ± 0.019	0.102 ± 0.008	0.093 ± 0.007	0.085 ± 0.006
Maximal EI	0.519 ± 0.016	0.509 ± 0.014	0.513 ± 0.007	0.481 ± 0.007
EI_{hyper}	0.260 ± 0.008	0.254 ± 0.007	0.257 ± 0.003	0.240 ± 0.003
Osmolarity at minimal EI [mOsm/kg]	167.5 ± 1.7	161.2 ± 6.1	153.7 ± 8.3	179.6 ± 8.6
Osmolarity at maximal EI [mOsm/kg]	349.6 ± 44.8	311.2 ± 9.1	288.6 ± 19.4	348.2 ± 15.5
Osmolarity at EI_{hyper} [mOsm/kg]	484.8 ± 8.1	422.4 ± 19.3	449.4 ± 14.9	454.1 ± 11.9
AUC (EI-osmolarity curve)	132.2 ± 14.7	118.5 ± 9.7	128.6 ± 4.1	110.6 ± 7.1

means ± S.D.; Description of significant differences among species in section 3.2.

Maximal EI = maximal elongation index (EI) values measured

Minimal EI = minimal EI values measured at low (hypo-) osmolar environment

EI_{hyper} = half of maximal EI values in high (hyper-) osmolar environment

AUC = area under curve

Table 2. Red blood cell related quantitative and qualitative parameters.

Variable	Mouse	Rat	Dog	Pig
RBC count [$x10^6/\mu l$]	6.84 ± 0.48	6.88 ± 0.48	6.97 ± 0.37	5.79 ± 0.5
Hct [%]	41.27 ± 2.13	40.05 ± 3.18	48.65 ± 2.91	35.19 ± 2,07
Hgb [g/dl]	10.11 ± 1.61	11.56 ± 0.71	14.4 ± 0.85	8.95 ± 0.52
MCV [fl]	55.57 ± 1.58	58.26 ± 3.45	69.77 ± 3.21	60.88 ± 2.82
RDW-CV% [%]	16.34 ± 1.58	14 ± 0.51	13.92 ± 0.65	17.07 ± 0.79
MCH [pg]	14.57 ± 0.48	16.83 ± 0.65	20.65 ± 1.18	15.5 ± 0.94
MCHC [g/dl]	25.96 ± 0.87	28.93 ± 1.39	29.65 ± 1.97	25.45 ± 0.72

means ± S.D.; Description of significant differences among species in section 3.3.

8. Figure legends

Figure 1.

Elongation index (EI) values (means ± S.D.) in the function of shear stress (SS [Pa]) in blood samples of mice (n=12), rats (n=12), beagle dogs (n=7) and pigs (n=12).

Description of significant differences among species in section 3.1.

Figure 2.

Calculated maximal elongation index (EI_{max}) and shear stress values at EI_{max} ($SS_{1/2}$ [Pa]) (means ± S.D.) of mice (n=12), rats (n=12), beagle dogs (n=7) and pigs (n=12).

Description of significant differences among species in section 3.1.

9. Figures

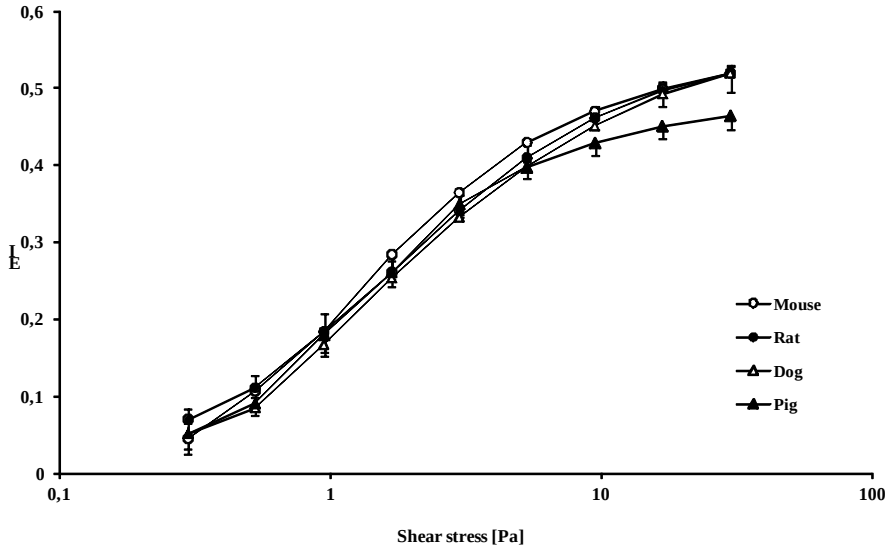

Figure 1.

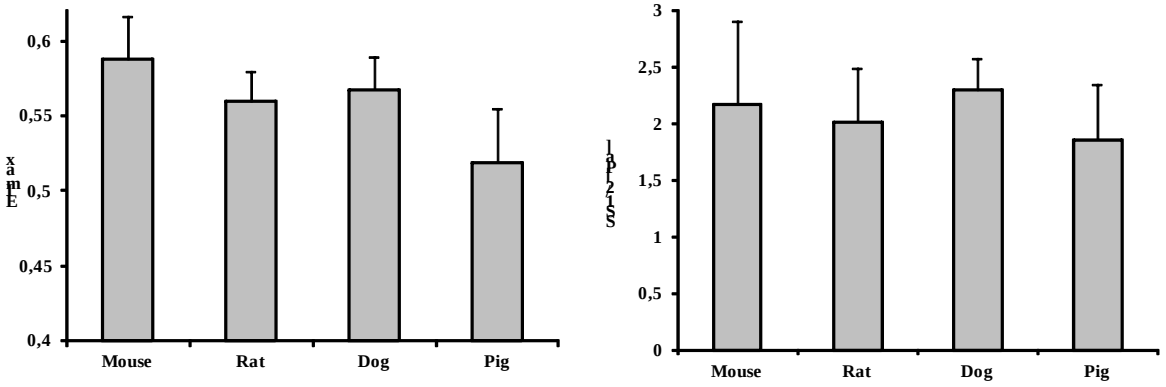

Figure 2.