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Red blood cell deformability and aggregation behaviour in different animal species

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Abstract. Comparative animal studies showed the wide variation of whole blood and plasma viscosity, and erythrocyte aggregation among mammalian species. Whole blood viscosity and red blood cell aggregation is influenced by red cell fluidity. To evaluate differences in erythrocyte deformability in mammals, three species were investigated, whose erythrocytes have a different aggregation property: horse, as a species with high, dog with medium, and sheep with almost unmeasurable aggregation tendency. Erythrocyte deformability was tested ektacytometrically (Elongation Index [EI], LORCA, Mechatronics, Hoorn, Netherlands) at shear stresses from 0.30 to 53.06 Pa. Equine erythrocytes showed EI-values from 0.047 at low shear stress to 0.541 at high shear stress. The EI from dog's erythrocytes ranged from 0.035 to 0.595. Sheep's erythrocytes had an EI of 0.005 at low and 0.400 at high shear stress. Although it might be presumed from the aggregation property that horse had the highest EI among the three species, the EI of canine erythrocytes exceeded the value in horses by 10% at high shear stress. Further, equine erythrocytes started to deform at higher shear stresses (1.69 Pa) than did canine and ovine cells, whose EI increased continuously with increasing shear stress. At moderate shear stress (1–5 Pa) deformability was even higher in the sheep than in the horse. However, at shear stresses higher than 5.34 Pa, equine red cell elongation clearly exceeded the values of sheep. We conclude that erythrocyte elongation is different between the animal species, not clearly linked with the aggregation property, and that the degree of deformability at various shear stresses is species-specific.

Keywords: Erythrocyte, deformability, fluidity, whole blood viscosity, aggregation, rheology

1. Introduction

Comparative animal studies showed the wide variation of whole blood and plasma viscosity, and erythrocyte aggregation among different mammalian species [1,9,20]. The qualitative rheologic properties of erythrocytes include aggregation and flexibility. Aggregation and flexibility are linked, and it is presumed that cells with enhanced aggregation tendency presumably show good flexibility [17].

The following study provides a description of erythrocyte deformability and aggregation in three animal species. A total of 120 animals were used in this study. These species were horse, dog, and sheep, which are known to be animals with "hyper aggregating" erythrocytes, normal aggregating erythrocytes and erythrocytes with almost immeasurable aggregation. The objective of this study was to investigate if red cell fluidity can be predicted by its aggregation property. Blood from three species of 40 animals

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each was analysed employing the same protocol for all animals. It was thus possible to standardize the shear history for each sample and, at the same time, keep procedures at a minimum. Experienced investigators carried out measurements. Incomplete data sets were eliminated. Aside from our reference values for standard hemorheological parameters, we show the differences of erythrocyte flexibility in relation to their aggregation tendency.

2. Material and methods

Whole blood was taken by puncture of the cephalic vein from 40 healthy dogs and by puncture of the jugular vein from 40 healthy horses and sheep. The samples were put into an insulated box and measurements started, due to transportation, with a maximum delay of 2 hours. The blood was rotated at room temperature and analysed hematologically and rheologically. All measurements were finished within four hours following blood sampling.

2.1. Hematology and blood chemistry

A routine hematologic and blood chemistry profile was obtained from each animal. The measurements of red blood cell (RBC) count (RBC count in cells $\times$ 10^{12}/l), mean corpuscular volume (MCV in fl), mean corpuscular haemoglobin concentration (MCHC in %), white blood cell count (WBC count in cells $\times$ 10^{9}/l), and platelet count (PLT in cells $\times$ 10^{6}/l), were done by Cell-Dyn 3500 (Abbot, Abbot Park, Illinois, USA).

Hematocrit (HCT in %) was measured by centrifugation (Hettich, Tuttlingen, Germany).

The routine blood chemistry profile including plasma cholesterol (CHOL in mg/dl), triglyceride (TRI in mg/dl), glucose (GLU in mg/dl), creatinine (CREA in mg/dl), total protein (TP in g/dl), blood uremic nitrogen (BUN in mg/dl), alanine amino transferase (ALT in U/l) and aspartat amino transferase (AST in U/l), was performed by Hitachi 904 (Hitachi, Tokyo, Japan).

Fibrinogen was measured according to the method of Clauss.

2.2. Measurement of RBC deformability

RBC deformability was measured at various fluid shear stresses by laser diffraction analysis, using an ektacytometer (LORCA, Mechatronics, Hoorn, Netherlands). A thin layer of an EDTA blood sample was sheared in a Couette system composed of a glass cup and a precisely fitting bob with a gap of 0.3 mm between the two cylinders. A laser beam traverses the diluted blood suspension and is diffracted by RBC present in that area.

Resting cells produce a circular diffraction pattern, while cells, which are submitted to shear stress, elongate, resulting in an ellipsoid pattern [2]. The diffraction pattern is analysed by the computer. Elongation indices (EI) are calculated for shear stresses between 0.3–53.06 Pascal (Pa) [11,15]. Measurements were carried out at 37°C, 25 μl of anticoagulated EDTA blood was diluted in a phosphate buffered saline (pH 7.4) – polyvinylpyrrolidone (PVP, M = 360,000 Sigma-Aldrich, Steinheim, Germany) medium at a viscosity of 25.7 mPa·s at 37°C.

2.3. Measurement of plasma viscosity

Plasma viscosity (PV) was measured by OCR-D (Anton Paar, Graz, Austria) at 25°C; 1 ml of plasma was filled into a plastic cup, which was mounted at the bottom of a glass capillary (diameter: 0.9864 mm length: 100 mm). The plastic cup was compressed at 2 Hz exerting shear rates inside the capillary. The viscosity at the shear rate of 10 s^{-1} was determined for plasma viscosity.

2.4. Protocol of measure

After withdrawal of blood, the hematologic measurements were done within two hours. Meanwhile the rheologic data were determined. The blood was then centrifuged and plasma viscosity was measured.

2.5. Statistic

For each of the 10 viscosity measurements we performed pair-wise comparisons between the 3 species with a bootstrap multiple testing procedure to correct for the large number of comparisons (SAS Proc Multtest). The according multiplicity adjusted p-values are reported. The sex distribution and proportion of caponised/sterilized animals between species was compared with a chi-squared test. To assess the influence of the factors age, sex and caponised/sterilized on the viscosity within the species we performed for each species an analysis of covariance for the viscosity averaged over the measurements at 0.3, 0.53, 0.95, 1.69, 3, 5.34, 9.48, 26.9, 40.08, 53.06. The two-sided significance level was set to 0.05.

3. Results

Table 1 shows hematologic and blood chemistry data.
Table 2 shows mean values of the EI and the PV of the three animal species.
Figure 1 shows the EI of horse, dog, and sheep.

Table 1
Hematology, blood chemistry, and fibrinogen of horse, dog, and sheep. Data are expressed as mean ± standard deviation

	Horse	Dog	Sheep
HCT (%)	38.09 ± 3.78	46.22 ± 4.65	38 ± 5.18
MCV (fl)	41.98 ± 1.801	63.25 ± 4.41	32.38 ± 2.42
MCHC (%)	39.08 ± 0.48	37.11 ± 5.76	35.23 ± 0.77
MCH (pg)	16.40 ± 0.77	24.03 ± 0.86	11.45 ± 0.81
TP (g/dl)	6.93 ± 0.33	6.72 ± 0.58	7.04 ± 0.52
GLU (mg/dl)	77.09 ± 10.72	53.50 ± 16.4	77.11 ± 13.87
BUN (mg/dl)	17.26 ± 3.35	16.75 ± 5.78	15.03 ± 4.57
CREA (mg/dl)	1.40 ± 0.17	0.96 ± 0.23	1.16 ± 0.40
AST (U/l)	169.61 ± 35.13	13.24 ± 3.79	68.38 ± 54.37
ALT (U/l)	10.60 ± 3.99	30.41 ± 13.91	10.68 ± 5.58
CHOL (mg/dl)	79.50 ± 10.90	245.89 ± 64.32	72.05 ± 20.43
TRI (mg/dl)	26.55 ± 8.94	76.24 ± 53.88	20.80 ± 8.23
Fibrinogen (mg/dl)	169.24 ± 34.28	253.84 ± 60.75	268.49 ± 90.99

Table 2

Plasma viscosity and elongation indices of horse, dog, and sheep. Data are expressed as mean ± standard deviation

	Horse	Dog	Sheep
PV (mPa·s)	1.580 ± 0.050	1.600 ± 0.150	1.610 ± 0.160
EI 0.3 Pa	0.047 ± 0.042	0.035 ± 0.029	0.005 ± 0.015
EI 0.53 Pa	0.054 ± 0.024	0.044 ± 0.018	0.045 ± 0.016
EI 0.95 Pa	0.061 ± 0.020	0.103 ± 0.027	0.095 ± 0.016
EI 1.69 Pa	0.091 ± 0.042	0.191 ± 0.037	0.155 ± 0.021
EI 3 Pa	0.170 ± 0.060	0.290 ± 0.044	0.233 ± 0.035
EI 5.34 Pa	0.290 ± 0.053	0.381 ± 0.046	0.293 ± 0.043
EI 9.48 Pa	0.400 ± 0.032	0.457 ± 0.043	0.339 ± 0.047
EI 16.9 Pa	0.466 ± 0.018	0.518 ± 0.037	0.372 ± 0.051
EI 30.08 Pa	0.511 ± 0.016	0.565 ± 0.030	0.393 ± 0.055
EI 53.06 Pa	0.541 ± 0.019	0.595 ± 0.026	0.400 ± 0.056

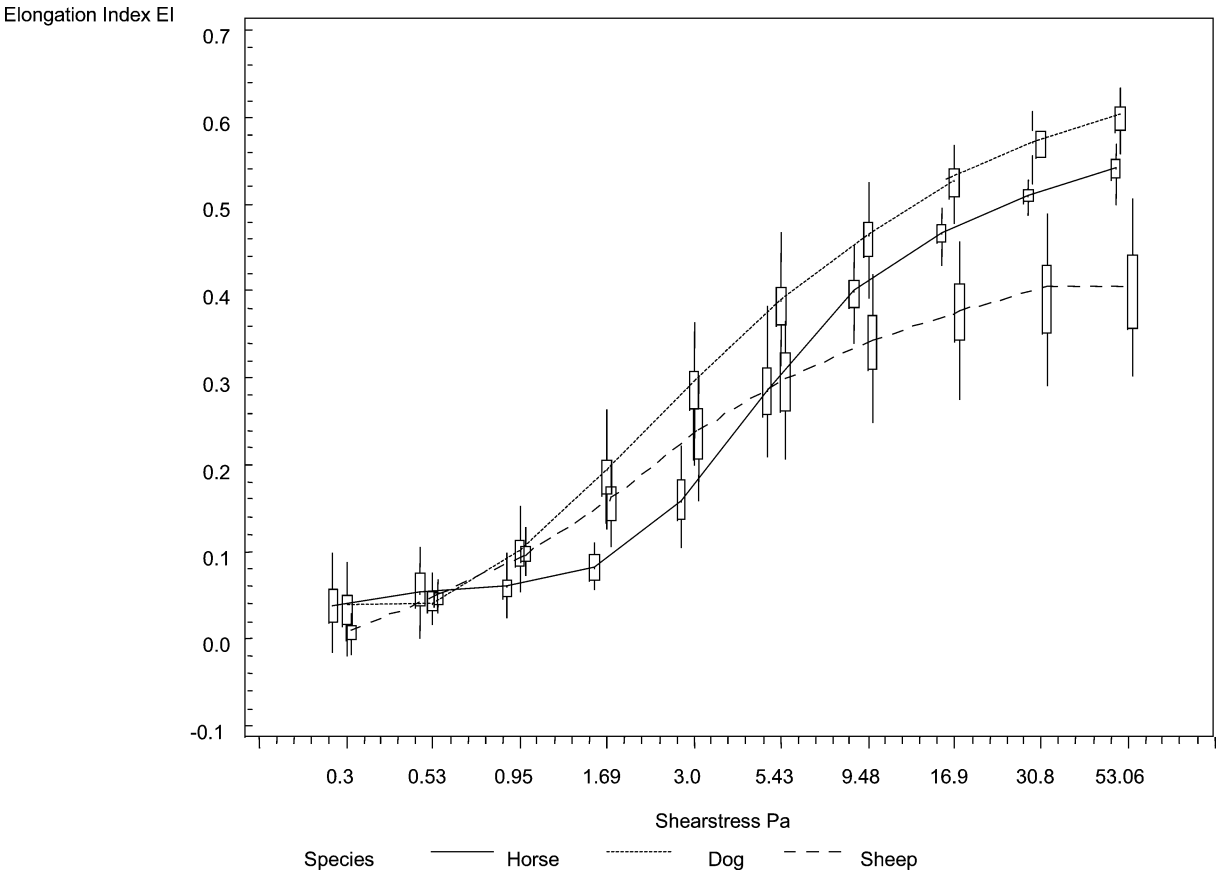

Fig. 1. Boxplots of the elongation index at a different shearstress for horses, dogs, and sheep. The curves connect the median values of the elongation indices. The vertical lines extend from the boxes to the minimal and maximal EI values. To avoid overlapping the boxes for horse and sheep were shifted horizontally.

3.1. Hematology and blood chemistry

All hematologic data were within physiological limits. MCV was greatest in dogs followed by horses and sheep. MCHC was greatest in horses followed by dogs and sheep. MCH was greatest in dogs followed by horses and sheep.

All subjects showed a physiological blood chemistry profile. Plasma fibrinogen could be measured in only 30 of 40 dogs due to methodological reasons. Four dogs showed plasma fibrinogen levels above physiological limits, although showing no clinical signs [22]. Plasma cholesterol and triglyceride concentration was highest in dog followed by horse and sheep according to the species-specific lipid intake.

3.2. Hemorheology

All Elongation Indices at shear stresses greater than 5.34 Pa were different between the three species ($p < 0.001$). At lower shear stresses the EI were different ($p < 0.001$) except for horse versus dog at 0.3 and 0.53 Pa, horse versus sheep at 0.53 Pa, and dog versus sheep at 0.53, 0.95, and 5.34 Pa.

Under low shear stress (0.3 Pa) the horse had the highest elongation index (EI: 0.046 ± 0.04) followed by dog (EI: 0.041 ± 0.028) and sheep (EI: 0.005 ± 0.015).

Under moderate shear stress (3 Pa) the highest value was measured in dog (EI: 0.290 ± 0.044) followed by sheep (EI: 0.233 ± 0.035) and horse (EI: 0.170 ± 0.060).

Under high shear condition (53.06 Pa) the dog had the highest elongation index (EI: 0.595 ± 0.026) followed by horse (EI: 0.541 ± 0.019) and sheep (EI: 0.400 ± 0.056).

Equine erythrocytes started to deform at higher shear stresses (1.69 Pa) than did canine and ovine cells. At moderate shear stresses (1.69–5.34 Pa) deformability was higher in the sheep than in the horse. At shear stresses above 5.34 Pa, equine red cell flexibility exceeded the values of sheep. In sheep and dog, the EI increased continuously with increasing shear stress whereas in horse the line up of the EI showed a rather sigmoid course (see Fig. 1).

3.3. Plasma viscosity

Plasma viscosity was highest in sheep followed by dog and horse, according to reference values obtained in the same laboratory [20].

4. Discussion

In the present study, erythrocyte fluidity was tested by means of red cell elongation, which was measured ectacytometrically. Three animal species were elected according to their aggregation property. The pronounced aggregation of equine erythrocytes just as the almost undetectable aggregation of ovine erythrocytes and the moderate aggregation tendency of canine erythrocytes was demonstrated recently [11,20]. Focusing on the fibrinogen concentration, the sheep's low aggregation tendency is surprising because ovines have the highest plasma fibrinogen among the species tested. According to the assumption that erythrocyte aggregation is linked to flexibility [3,7,18] the study was undertaken to investigate if red cell flexibility correlates with its aggregation property.

At high shear stresses (above 9.48 Pa), erythrocyte flexibility was higher in horse and dog, than in sheep. Canine erythrocytes showed a greater red cell elongation than equine erythrocytes, a result which

was surprising from the pronounced aggregation property, known from the horse. However, equine red cell flexibility clearly exceeded the values of sheep.

At low shear stresses, equine erythrocytes remained stable whereas the EI of canine and ovine erythrocytes increased continuously with increasing shear stress. Surprisingly, at shear stresses between 1 and 5 Pa deformability was even higher in the sheep than in the horse.

These results can be demonstrated graphically in the course of the elongation indices over the range of shear stresses (see Fig. 1). Similar to human data obtained in the same laboratory (unpublished data), the dog showed an exponential increase of values with increasing shear stresses. The sheep showed a matchable exponential increase, however, the elongation indices were low, as mentioned before. In contrast, horse showed a sigmoid course of these values, which seems to be species-specific, since it was neither observed in man, nor in dog or sheep.

The ability of the red blood cells to deform is an important determinant of capillary passage of erythrocytes and oxygen supply to the tissues. In the narrow capillaries, whose diameter is smaller than that of the red blood cells, the erythrocytes must deform in order to pass through [8,14].

Erythrocyte flexibility, like aggregation, is linked to the ability of shape regulation and passive reversible shape change. The shape of the cell is a matter of cytoskeleton and cell membrane. The cytoskeleton can be explained by the protein framework and its spectrin head sliding on the short actin fibres [4,21], and its regulation is dependent on various factors such as intracellular calcium and magnesium ion, adenosine triphosphate [3,23], extracellular and intracellular pH and water content [12,13] and various crenators and cup formers [5]. The lipid bilayer of the cell membrane depends on energy for extension and shrinkage and is naturally coupled with the deformation of the erythrocyte's protein cage [23]. The tank treat motion of the erythrocyte membrane enables the cell to stabilise the axis and to produce a transverse shift from the wall [10,16].

The ability of the erythrocyte to deform is a consequence of: (a) a large surface to volume ratio, which is inherent to the biconcave shape of the erythrocyte, (b) the viscoelastic properties of the protein framework and the lipid bilayer and (c) the inner viscosity of the erythrocyte's hemoglobin solution. In case of dehydration, as in hypertonically shrunken cells, reduction in deformability occurs due to a higher mean cell hemoglobin concentration (MCHC) and thus a higher internal viscosity, in spite of an even more favourable surface area to volume ratio [6,14].

Ovine red blood cells are very small compared to the erythrocytes of horse and dog [19], which results in a disadvantageous ratio between surface and volume. Accordingly, the EI were lower than those from horses and dogs. Recently, differences in the phospholipid composition of the erythrocyte membrane were observed showing an increase of saturated acids in contrast to horse (Windberger, unpublished data), which might influence erythrocyte flexibility further. Unfortunately, the protein framework of the mammals' membrane is yet nearly unexplored to allow assumptions about its influence on erythrocyte flexibility.

The canine red blood cells were larger than the cells from horse and sheep, which led to a more beneficial surface and volume ratio compared to the other two animals. Additionally, the MCHC was lower in dog than in horse. Both facts might result in the high EI measured in this study.

In contrast the inner viscosity of the erythrocyte as assumed by the MCHC was highest in the horse. Additionally equine erythrocytes were medium sized. If it is assumed that dense erythrocytes have a lower flexibility [3], the increased density of the erythrocytes in the horse together with unfavourable geometric factors compared to the dog might be indicative for the observed lower elongation index in this species.

Focusing on the sheep, as the animal with almost unmeasurable erythrocyte aggregation, it can be concluded that ovine cells are small and unflexible and for that reason aggregation is limited. We found out that the dog, whose erythrocytes have medium aggregation property, showed greater erythrocyte flexibility than the horse. Although this is the animal with "hyper aggregating" erythrocytes.This observation can be presumed by geometric differences and differences in the inner viscosity of the red cell.

From this point of view the huge aggregation property of equine erythrocytes seems further unclear.

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