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Interspecies diversity of erythrocyte mechanical stability at various combinations in magnitude and duration of shear stress, and osmolality

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Abstract. We hypothesized that the results of red blood cell mechanical stability test show interspecies differences. The comparative investigations were performed on blood samples obtained from rats, beagle dogs, pigs and healthy volunteers. Mechanical stress was applied in nine combinations: 30, 60 or 100 Pa shear stress for 100, 200 or 300 seconds. Generally, rat erythrocytes showed the highest capability of resistance. With the applied combinations of mechanical stress pig erythrocytes were the most sensitive. On human erythrocytes 60 Pa for 200 s was the minimum combination to result significant deformability deterioration. By increasing the magnitude and duration of the applied mechanical stress we experienced escalating deformability impairment in all species. 100 Pa shear stress for 300 seconds on human erythrocytes showed the largest deformability impairment. The mechanical stability test results were also dependent on osmolality. At hypoosmolar range (200 mOsmol/kg) the mechanical stress improved EI data mostly in rat and porcine blood. At higher osmolality (500 mOsmol/kg), the test did not show detectable difference, while in 250–300 mOsmol/kg range the differences were well observable. In summary, erythrocytes' capability of resistance against mechanical stress shows interspecies differences depending on the magnitude and duration of the applied stress, and on the osmolality.

Keywords: Red blood cell deformability, mechanical stability, membrane stability, comparative hemorheology, osmotic gradient ektacytometry

1. Introduction

Due to physiological and pathophysiological changes, including extraphysiological effects (e.g., extracorporeal circulation, intravascular devices and implants), that affect red blood cell deformability determining parameters (cytoskeleton and morphological properties, surface-volume ratio, inner viscosity, cell membrane viscosity) the erythrocytes' capability of resistance against shear stress may alter [2, 5, 16, 17, 22]. In this aspect the red blood cell mechanical (membrane) stability test may provide useful information [3]. The mechanical stress, depending on its magnitude and duration, may cause trauma to the erythrocytes (and to other blood cells as well), resulting in decreasing deformability and enhanced aggregation if the stress is 'sub-lethal', and fragmentation/hemolysis, if being larger [5, 17, 18].

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When using mechanical stability test, various shear stress magnitude-duration combinations can be applied. However, the effect is depending on the cells' mechanical properties. Increasing amount of data is available in the literature on interspecies diversity of blood composition, including hematological and hemorheological parameters as well [15, 27, 31]. However, there is a lack of data on mechanical stability.

We hypothesized, that just like other micro-rheological parameters, the mechanical stability may also show interspecies differences. We aimed to conduct a comparative, descriptive study using rat, canine, porcine and human blood samples investigating the possible diversity of erythrocyte mechanical stability at various combinations in magnitude and duration of shear stress, and osmolality.

2. Materials and methods

2.1. Experimental animal and human blood samples

The animal experiment parts were approved and registered by the University of Debrecen Committee of Animal Research (permission Nr.: 19/2011/UD CAR), in accordance with national and EU regulations (the Hungarian Animal Protection Act (Law XVIII/1998) and the Edict 63/2010). Human blood samples were obtained from volunteers under Clinical Ethical Committee approval (permission Nr.: DE OEC RKEB/IKEB 3625-2012).

2.2. Study design

2.2.1. Mechanical stability tests at various combinations of shear stress magnitude and duration

In the morning hours blood samples were taken from 6 healthy male Sprague-Dawley outbred rats (age: 4 months, bodyweight: 522 ± 42.9 g) via lateral tail vein puncture (anesthesia: 60 mg/kg, i.p. thiopenthal); 8 healthy male inbred beagle dogs (age: 9–11 months, bodyweight: 13.75 ± 0.78 kg) via cephalic vein puncture; 11 healthy female Hungahib pigs (age: 10–12 weeks, bodyweight: 19.05 ± 2.89 kg) via medial saphenous vein puncture (anesthesia: 15 mg/kg, i.m. ketamine, 1 mg/kg, i.m. xylazine); and 7 female volunteers via median cubital vein puncture (age: 31–48 years). Blood samplings were carried out using 21 G BD Eclipse™ blood collection needle into 3 ml BD Vacutainer® tube containing 1.8 mg/ml K_3-EDTA as anticoagulant (Becton, Dickinson and Company, USA). Laboratory measurements were completed within 2 hours [14, 26].

Each blood sample was subjected to mechanical stability test (see below) using nine combinations of shear stress magnitude and durations as the followings: 30, 60, or 100 Pa for 100 s, 200 s or 300 s.

2.2.2. Effects of osmolality on mechanical stability results

Five aliquots of blood samples per each abovementioned species were investigated further. On those samples the mechanical stability test at 100 Pa for 300 sec were carried out using 200, 250, 300, and 500 mOsmol/kg PVP solutions.

2.3. Laboratory investigations

Hematological parameters were tested by a Sysmex F-800 semi-automated microcell counter (TOA Medical Electronics Co., Ltd., Japan). Red blood cell count (RBC [T/l]), hemoglobin concentration

(Hgb [g/dl]), hematocrit (Hct [%]), mean corpuscular volume (MCV [fl]), mean corpuscular hemoglobin (MCH [pg]) and mean corpuscular hemoglobin concentration (MCHC [g/dl]) are presented in this paper.

Red blood cell deformability was determined by LoRRca MaxSis Osmoscan rotational ektacytometer (Mechatronics BV, The Netherlands), in which the cells' elongation index (EI) was tested in the function of shear stress (SS [Pa]) [14]. Measurements were carried out at 37°C. Polyvinylpyrrolidone (PVP) – phosphate buffered saline (PBS) solution was used as high-viscosity suspending media (PVP: 360 kDa, Sigma-Aldrich Co. USA; PVP-PBS solution viscosity = 30.83 mPas, osmolality = 298 mOsmol/kg, pH = 7.2). For the comparison of the EI-SS curves the following parameters were used: EI values at 3 Pa, maximal elongation index (EI_{max}) and the shear stress belonging to the half EI_{max} ($SS_{1/2}$, [Pa]) calculated by the device's software according to the Lineweaver-Burk equation, and their ratio (EI_{max} / $SS_{1/2}$) was also used [4].

After regular ektacytometry measurements, the cell membrane stability tests were carried out. The method consists of two regular deformability tests, before and after a shearing period with controlled magnitude and exposure time of the shearing force [3]. Every sample was tested with nine combinations of shearing force and duration: 30, 60 or 100 Pa shear stress for 100, 200 or 300 seconds. Measurements were carried out under the same conditions described for the regular deformability test. For evaluating the effect of the various mechanical stress combinations, the EI-SS curves obtained before and after the shearing were compared with the parameters described above, together with their ratio (after versus before values).

In the study part (described in sub-chapter 2.2.2.) the shearing protocol at 100 Pa for 300 sec was applied as mechanical stress. Here the measurements were carried out in PVP-PBS solutions at various osmolality: 200, 250, 300, and 500 mOsmol/kg (pH 7.2, viscosity = 29–31 mPas).

2.4. Statistical analysis

Data are presented as means ± standard deviation (S.D.). For comparing EI values before versus after the mechanical stress paired *t*-test or Wilcoxon signed-rank test was used, depending on data distribution and equality of variances. For comparing the effect of various mechanical stress combinations on deformability impairment one way ANOVA with Bonferroni's *post hoc* test or one way ANOVA on ranks with Dunn's test were used. For inter-species comparison two sample *t*-test/Mann-Whitney rank sum test was applied depending on the normality of data distribution. A $p < 0.05$ value was considered statistically significant.

3. Results

3.1. Red blood cell describing hematological parameters

Erythrocyte-related quantitative and qualitative hematological parameters are shown in Table 1. Red blood cell count was the highest in the rat, then in an order of dog, pig and human. Mean corpuscular volume was the highest in the human, then in an order of dog, pig and rat. Besides dog mean corpuscular hemoglobin concentration, all parameters were significantly different from the human values (for rat hemoglobin concentration: $p = 0.032$, for the other parameters: $p < 0.001$).

Table 1
Selected quantitative and qualitative hematological parameters and red blood cell deformability describing parameters of rat ($n = 6$), dog ($n = 8$), pig ($n = 11$) and human ($n = 7$)

	rat	dog	pig	human
RBC [T/l]	$7.29 \pm 0.87^*$	$6.5 \pm 0.45^*$	$5.92 \pm 0.60^*$	4.64 ± 0.37
Hgb [g/dl]	$13.03 \pm 0.98^*$	$13.44 \pm 0.98^*$	$9.45 \pm 0.75^*$	11.59 ± 0.65
Hct [%]	$46.68 \pm 4.11^*$	$50.28 \pm 3.98^*$	$38.44 \pm 4.03^*$	42.76 ± 4.14
MCV [fl]	$59.75 \pm 5.14^*$	$77.48 \pm 5.00^*$	$65.31 \pm 7.32^*$	92.26 ± 8.19
MCH [pg]	$18.01 \pm 1.46^*$	$20.71 \pm 1.06^*$	$16.05 \pm 1.04^*$	25.04 ± 1.35
MCHC [g/dl]	$30.39 \pm 4.36^*$	26.83 ± 2.07	$24.72 \pm 1.84^*$	27.26 ± 2.15
EI at 3 Pa	$0.312 \pm 0.02^*$	$0.267 \pm 0.013^*$	$0.306 \pm 0.018^*$	0.248 ± 0.011
EI_{max}	$0.579 \pm 0.017^*$	$0.551 \pm 0.025^*$	$0.505 \pm 0.025^*$	0.528 ± 0.029
$SS_{1/2}$ [Pa]	$2.28 \pm 0.38^*$	4.33 ± 0.8	$1.99 \pm 0.33^*$	4.2 ± 0.74
EI_{max} / $SS_{1/2}$ [Pa^{-1}]	$0.24 \pm 0.04^*$	0.13 ± 0.03	$0.26 \pm 0.05^*$	0.13 ± 0.03

means±S.D., $^*p < 0.05$ vs. human.

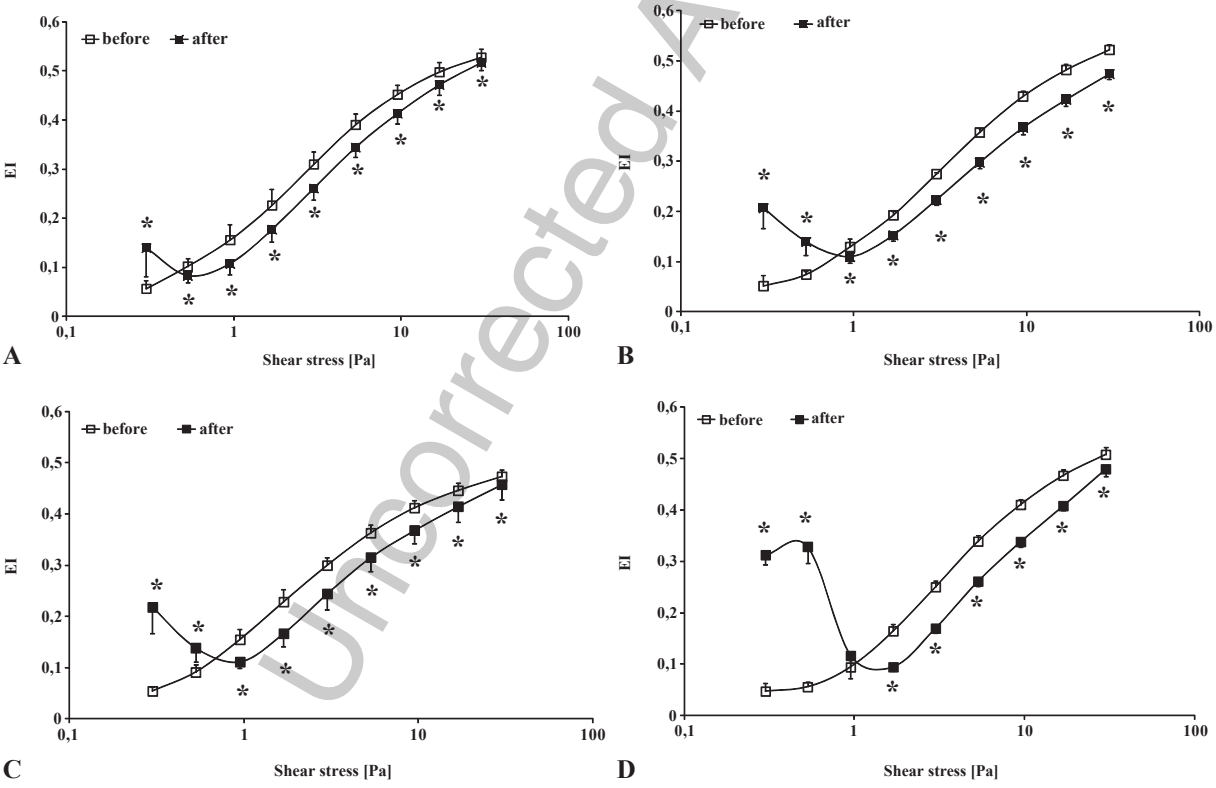

Fig. 1. Red blood cell elongation index (EI) – shear stress [Pa] curves of rat, canine, porcine and human blood samples before and after 100 Pa shear stress for 300 seconds. means ± S.D., $^*p < 0.05$ vs. before.

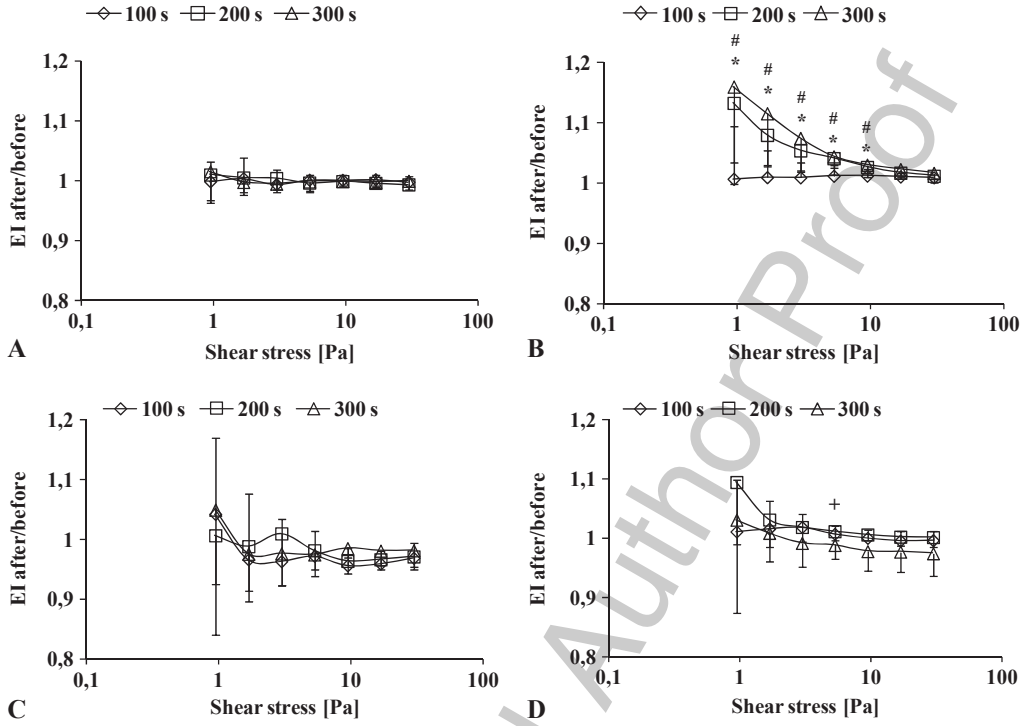

Fig. 2. Ratio of the elongation index (EI) values measured after and before the 30 Pa mechanical stress in rat (A), dog (B), pig (C) and human (D). Data under 0.95 Pa are not plotted. means ± S.D., $^{*}p < 0.05$:100 s vs. 300 s; $^{\#}p < 0.05$:100 s vs. 200 s; $^{+}p < 0.05$:200 s vs. 300 s.

3.2. Red blood cell deformability

Red blood cell deformability describing elongation index (EI) – shear stress (SS) curves showed inter-species differences both in the shape of the curves and in the EI values. Generally, the highest EI values were measured in rat blood and the lowest in the human. The shape of the canine EI – SS curves was the most similar to the human ones but with higher values. Rat and porcine EI values ran parallel to each other at lower shear stress levels, and then above 3 Pa the slope of the porcine curves became flatter. The calculated parameters (Table 1) reflected well the inter-species differences of the EI – SS curves. All values, except for canine $SS_{1/2}$ and EI_{max} / $SS_{1/2}$ values, were differed highly significantly from the human values ($p < 0.001$).

3.3. Red blood cell membrane stability at various combinations of shear stress magnitude and duration

The most expressed differences were observed when the mechanical stress with 100 Pa was used for 300 seconds (Fig. 1). In Fig. 1 the elongation index – shear stress curves determined before and after the mechanical stress can be observed. Using smaller shear stress and by shorter duration, the differences were diminished, but not in the same manner in the investigated species.

Due to the 30 Pa mechanical stress rat erythrocytes did not show significant EI impairment, as the ratio of EI values measured after and before the mechanical test – as a representative parameter for the magnitude of EI impairment – was close to 1 and its value did not change as exposure time increased (Fig. 2A, Table 2). Canine erythrocytes showed improved deformability –higher EI values– after the mechanical stress of 30 Pa. At 100-second duration significantly higher EI values were measured generally at the 10–30 Pa shear stress zone, while in case of 200 and 300-second duration at all measured shear stress levels. As it is shown on Fig. 2, the magnitude of the improvement significantly depended on the exposure time (under 10 Pa) (Fig. 2B, Table 3). In case of the pig the lowest combination of the mechanical stress (30 Pa for 100 seconds) already caused significant lowering in the EI values measured generally at the 10–30 Pa shear stress zone, but its magnitude –EI after/before– was independent on the exposure time (Fig. 2C, Table 4). Human erythrocytes expressed slight, but not obvious deformability improvement that could also be seen at 100 and 200-second duration time (Fig. 2D, Table 5).

The application of the mechanical stress at 60 Pa caused significant impairment in the EI values measured at all shear stress values, except for dog, in which blood samples this shear stress did cause important changes in EI values (Fig. 3A-D, Table 2–5). The magnitude of the EI impairment was basically independent from the length of the exposure time. However, slight difference was seen in the human, and also in the rat at lower shear stress levels.

As shown on Fig. 1 (A-D), the highest mechanical stress (100 Pa for 300 seconds). The shape of the EI – SS curves were highly irregular under 0.95 Pa, mostly in the human and the least in the rat (Fig. 4A-D, Table 2–5). Due to the 100 Pa shear stress applied for 300 seconds human erythrocytes showed the largest deformability impairment, the elongation index dropped by 17.6–42.4% between the 0.95–10 Pa range and by 5.5–12.6% between 10–30 Pa (except at 0.95 Pa shear stress, $p < 0.001$ at all tested shear stress values points: 1.69, 3, 5.33, 9.49, 16.87 and 30 Pa). The same values in rat were 8.6–21.5% and 2–5.2% ($p < 0.001$ at all tested shear stress values), in dog 14.5–20.8% and 9.2–12.3% ($p < 0.001$ at all shear stresses, except for 0.95 Pa: $p = 0.007$), and in pig 10.6–26.8% and 3.3–7% ($p < 0.001$ at most of the shear stress levels, except for 16.87 and 30 Pa were $p = 0.002$ and $p = 0.024$ values existed).

Figure 5 summarizes the erythrocytes' capacity of resistance against increased in the four investigated species by the ratio of the EI_{max} / $SS_{1/2}$ values determined from EI-SS curves after and before the mechanical stress. The values did not change obviously with the exposure time when we used the shearing protocol at 30 Pa. In canine blood even a slight improvement was seen. Using 60 Pa shearing, the values moderately decreased in rat, increased in dog and decreased in porcine and human blood. In all the four species EI_{max} / $SS_{1/2}$ values decreased with the increase of exposure time when tested at 100 Pa stress level (Fig. 5).

3.4. Osmolality-dependent alterations of red blood cell membrane stability data

Osmolality changes both in hypo- or hyperosmolar direction strongly influenced the mechanical stability test results (100 Pa for 300 s) (Figs. 6–9). Interestingly, the tests carried out on using hypoosmolar PVP-PBS solution (200 mOsmol/kg) showed improvement of the EI values after the mechanical shearing in all the four investigated species, most expressedly in rat and porcine blood (Figs. 6 and 8A). Tests at 250 mOsmol/kg reflected the deterioration described above in the main results, expect for porcine blood, in which still an improvement was seen (Fig. 8B). At 500 mOsmol/kg no obvious changes were detected, because of the irregular curves caused by the presence of shrunken cells. (Figs. 6–9D).

Table 2
Rat red blood cell deformability parameters measured before and after the various combinations of mechanical stress (30, 60 or 100 Pa for 100, 200 or 300 seconds)

		30 Pa			60 Pa			100 Pa		
		100 s	*200 s*	*300 s*	*100 s*	*200 s*	*300 s*	*100 s*	*200 s*	*300 s*
EI at 3 Pa	before (B)	0.309 ± 0.02	0.311 ± 0.017	0.314 ± 0.019	0.310 ± 0.018	0.314 ± 0.021	0.317 ± 0.02	0.314 ± 0.019	0.31 ± 0.029	0.31 ± 0.025
	after (A)	0.307 ± 0.018	0.312 ± 0.014	0.312 ± 0.02	$0.300 \pm 0.02^{*}$	$0.300 \pm 0.016^{*}$	$0.298 \pm 0.017^{*}$	$0.282 \pm 0.013^{*}$	$0.269 \pm 0.022^{*}$	$0.261 \pm 0.023^{*}$
	A/B ratio	*0.994 ± 0.023*	*1.004 ± 0.019*	*0.994 ± 0.014*	*0.967 ± 0.014*$^{\#}$	*0.956 ± 0.022*$^{\#}$	*0.94 ± 0.028*$^{\#}$	*0.897 ± 0.024*$^{\#}$	*0.871 ± 0.028*$^{\#}$	*0.842 ± 0.033*$^{\#}$
EI_{max}	before	0.573 ± 0.027	0.59 ± 0.016	0.583 ± 0.017	0.585 ± 0.018	0.578 ± 0.012	0.578 ± 0.013	0.575 ± 0.015	0.581 ± 0.017	0.572 ± 0.016
	after	0.573 ± 0.015	$0.579 \pm 0.018^{*}$	0.582 ± 0.022	0.574 ± 0.025	0.586 ± 0.009	0.576 ± 0.017	$0.553 \pm 0.011^{*}$	$0.541 \pm 0.013^{*}$	$0.53 \pm 0.016^{*}$
	A/B ratio	*1.001 ± 0.033*	*0.981 ± 0.011*$^{\#}$	*0.998 ± 0.014*	*0.98 ± 0.022*	*1.013 ± 0.03*$^{\#}$	*0.998 ± 0.021*$^{\#}$	*0.962 ± 0.018*	*0.932 ± 0.044*$^{\#}$	*0.928 ± 0.027*
$SS_{1/2}$ [Pa]	before	2.48 ± 0.39	2.53 ± 0.32	2.53 ± 0.34	2.55 ± 0.29	2.51 ± 0.37	2.38 ± 0.34	2.38 ± 0.45	2.47 ± 0.56	2.49 ± 0.56
	after	2.53 ± 0.26	2.66 ± 0.25	2.67 ± 0.27	$2.84 \pm 0.44^{*}$	$2.89 \pm 0.32^{*}$	$3.09 \pm 0.28^{*}$	$3.59 \pm 0.28^{*}$	$4.12 \pm 0.48^{*}$	$4.44 \pm 0.4^{*}$
	A/B ratio	*1.032 ± 0.081*	*1.058 ± 0.066*$^{\#}$	*1.056 ± 0.059*	*1.113 ± 0.104*$^{\#}$	*1.16 ± 0.125*$^{\#}$	*1.313 ± 0.193*	*1.537 ± 0.203*$^{\#}$	*1.722 ± 0.304*$^{\#}$	*1.834 ± 0.308*$^{\#}$

$n = 6$, means ± S.D., $^{*}p < 0.05$ vs. before, $^{\#}p < 0.05$ vs. human (data in Table 5).

Table 3
Canine red blood cell deformability parameters measured before and after the various combinations of mechanical stress (30, 60 or 100 Pa for 100, 200 or 300 seconds)

		30 Pa			60 Pa			100 Pa		
		100 s	*200 s*	*300 s*	*100 s*	*200 s*	*300 s*	*100 s*	*200 s*	*300 s*
EI at 3 Pa	before (B)	0.258 ± 0.013	0.265 ± 0.004	0.265 ± 0.008	0.26 ± 0.02	0.262 ± 0.022	0.272 ± 0.003	0.274 ± 0.009	0.275 ± 0.005	0.275 ± 0.003
	after (A)	0.26 ± 0.012	0.28 ± 0.009*	0.285 ± 0.017*	0.256 ± 0.022	0.258 ± 0.028	0.272 ± 0.01	0.231 ± 0.013*	0.224 ± 0.011*	0.223 ± 0.01*
	A/B ratio	*1.009 ± 0.026*	*1.053 ± 0.035#*	*1.074 ± 0.053#*	*0.982 ± 0.029#*	*0.983 ± 0.041#*	*1 ± 0.033#*	*0.844 ± 0.035#*	*0.816 ± 0.033#*	*0.812 ± 0.036#*
EI_{max}	before	0.564 ± 0.028	0.573 ± 0.018	0.565 ± 0.023	0.556 ± 0.027	0.537 ± 0.023	0.537 ± 0.019	0.543 ± 0.018	0.535 ± 0.021	0.546 ± 0.025
	after	0.562 ± 0.027	0.579 ± 0.009	0.576 ± 0.022	0.549 ± 0.021	0.535 ± 0.026	0.549 ± 0.022	0.499 ± 0.017*	0.49 ± 0.17*	0.477 ± 0.007*
	A/B ratio	*0.995 ± 0.024*	*1.013 ± 0.047*	*1.02 ± 0.027*	*0.989 ± 0.056*	*0.998 ± 0.048#*	*1.024 ± 0.05#*	*0.919 ± 0.037*	*0.917 ± 0.049*	*0.876 ± 0.048*
$SS_{1/2}$ [Pa]	before	4.08 ± 0.58	3.82 ± 0.71	4.1 ± 0.83	4.42 ± 0.98	4.74 ± 0.79	4.63 ± 0.75	4.42 ± 0.65	4.55 ± 0.81	4.21 ± 0.92
	after	4 ± 0.49	3.41 ± 0.33	3.8 ± 0.54	4.54 ± 0.8	4.95 ± 1.12	4.38 ± 0.68	5.96 ± 0.55*	6.71 ± 0.58*	6.26 ± 0.6*
	A/B ratio	*0.985 ± 0.091*	*0.91 ± 0.136*	*0.939 ± 0.095*	*1.053 ± 0.21#*	*1.067 ± 0.263#*	*0.968 ± 0.223#*	*1.379 ± 0.271#*	*1.522 ± 0.337#*	*1.567 ± 0.44#*

$n = 8$, means ± S.D., $^{*}p < 0.05$ vs. before, $^{\#}p < 0.05$ vs. human (data in Table 5).

Table 4
Porcine red blood cell deformability parameters measured before and after the various combinations of mechanical stress (30, 60 or 100 Pa for 100, 200 or 300 seconds)

		30 Pa			60 Pa			100 Pa		
		100 s	*200 s*	*300 s*	*100 s*	*200 s*	*300 s*	*100 s*	*200 s*	*300 s*
EI at 3 Pa	before (B)	0.313 ± 0.018	0.301 ± 0.018	0.304 ± 0.024	0.317 ± 0.016	0.303 ± 0.017	0.304 ± 0.021	0.306 ± 0.016	0.308 ± 0.018	0.3 ± 0.016
	after (A)	0.301 ± 0.02	0.303 ± 0.017	0.297 ± 0.025	0.3 ± 0.023*	0.283 ± 0.025*	0.287 ± 0.02*	0.274 ± 0.022*	0.255 ± 0.017*	0.244 ± 0.031*
	A/B ratio	*0.963 ± 0.072*	*1.009 ± 0.087*	*0.976 ± 0.054*	*0.947 ± 0.076*	*0.935 ± 0.087*	*0.946 ± 0.044#*	*0.899 ± 0.072#*	*0.83 ± 0.057#*	*0.815 ± 0.092#*
EI_{max}	before	0.498 ± 0.018	0.518 ± 0.027	0.507 ± 0.023	0.496 ± 0.03	0.503 ± 0.014	0.501 ± 0.018	0.509 ± 0.027	0.506 ± 0.029	0.508 ± 0.032
	after	0.485 ± 0.031	0.492 ± 0.03	0.496 ± 0.021	0.489 ± 0.03	0.478 ± 0.017*	0.468 ± 0.025*	0.474 ± 0.016*	0.47 ± 0.02*	0.449 ± 0.032*
	A/B ratio	*0.975 ± 0.053*	*0.951 ± 0.075#*	*0.981 ± 0.073*	*0.987 ± 0.064*	*0.952 ± 0.044*	*0.935 ± 0.045*	*0.932 ± 0.034*	*0.931 ± 0.04*	*0.885 ± 0.068*
$SS_{1/2}$ [Pa]	before	1.97 ± 0.31	1.94 ± 0.42	1.9 ± 0.36	1.92 ± 0.3	2.15 ± 0.3	2.05 ± 0.35	2.11 ± 0.21	2 ± 0.39	1.85 ± 0.31
	after	2.12 ± 0.31	2.02 ± 0.32	2.12 ± 0.37	2.6 ± 0.41*	3.15 ± 0.54*	3.23 ± 0.31*	4.22 ± 0.49*	5.18 ± 0.49*	5.14 ± 0.89*
	A/B ratio	*1.096 ± 0.243*	*1.071 ± 0.189*	*1.151 ± 0.031#*	*1.367 ± 0.215*	*1.483 ± 0.309*	*1.624 ± 0.35*	*2.018 ± 0.306*	*2.681 ± 0.626*	*2.812 ± 0.443*

$n = 11$, means ± S.D., $^{*}p < 0.05$ vs. before, $^{\#}p < 0.05$ vs. human (data in Table 5).

Table 5
Human red blood cell deformability parameters measured before and after the various combinations of mechanical stress (30, 60 or 100 Pa for 100, 200 or 300 seconds)

		30 Pa			60 Pa			100 Pa		
		100 s	*200 s*	*300 s*	*100 s*	*200 s*	*300 s*	*100 s*	*200 s*	*300 s*
EI at 3 Pa	before (B)	0.249 ± 0.012	0.248 ± 0.013	0.25 ± 0.015	0.252 ± 0.011	0.248 ± 0.009	0.247 ± 0.01	0.245 ± 0.014	0.246 ± 0.012	0.25 ± 0.012
	after (A)	0.253 ± 0.011	0.253 ± 0.016	0.248 ± 0.021	0.233 ± 0.013*	0.224 ± 0.016*	0.218 ± 0.017*	0.193 ± 0.009*	0.174 ± 0.01*	0.17 ± 0.009*
	A/B ratio	*1.018 ± 0.022*	*1.018 ± 0.024*	*0.991 ± 0.04*	*0.924 ± 0.029*	*0.902 ± 0.037*	*0.88 ± 0.042*	*0.786 ± 0.035*	*0.709 ± 0.033*	*0.679 ± 0.022*
EI_{max}	before	0.54 ± 0.032	0.528 ± 0.024	0.524 ± 0.011	0.534 ± 0.027	0.533 ± 0.028	0.525 ± 0.031	0.523 ± 0.026	0.519 ± 0.052	0.522 ± 0.026
	after	0.546 ± 0.026	0.547 ± 0.032	0.53 ± 0.041	0.511 ± 0.016*	0.495 ± 0.02*	0.471 ± 0.04*	0.467 ± 0.036*	0.469 ± 0.031*	0.478 ± 0.012*
	A/B ratio	*1.011 ± 0.022*	*1.037 ± 0.024*	*1.013 ± 0.04*	*0.959 ± 0.029*	*0.929 ± 0.037*	*0.899 ± 0.042*	*0.908 ± 0.035*	*0.908 ± 0.033*	*0.917 ± 0.022*
$SS_{1/2}$ [Pa]	before	4.09 ± 0.73	4.23 ± 0.65	4.38 ± 0.86	4.19 ± 0.62	4.1 ± 0.57	4.27 ± 1	4.2 ± 0.78	4.12 ± 0.93	4.23 ± 0.82
	after	3.71 ± 0.68	3.82 ± 0.84	3.65 ± 0.56	5.53 ± 0.64*	6.01 ± 1.02*	5.9 ± 0.98*	7.82 ± 0.62*	9.44 ± 0.71*	10.13 ± 0.78*
	A/B ratio	*0.915 ± 0.113*	*0.906 ± 0.146*	*0.865 ± 0.214*	*1.333 ± 0.126*	*1.47 ± 0.195*	*1.419 ± 0.251*	*1.953 ± 0.351*	*2.379 ± 0.493*	*2.456 ± 0.392*

$n = 7$, means ± S.D., $^*p < 0.05$ vs. before.

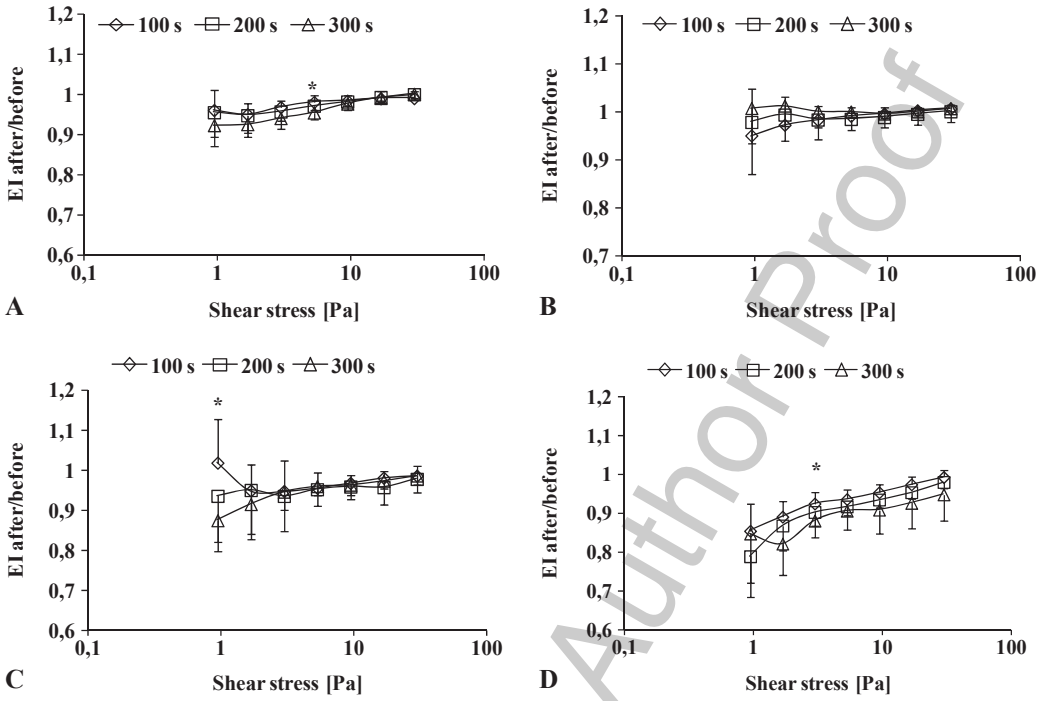

Fig. 3. Ratio of the elongation index (EI) values measured after and before the 60 Pa mechanical stress in rat (A), dog (B), pig (C) and human (D). Data under 0.95 Pa are not plotted. means ± S.D., $^{*}p < 0.05$:100 s vs. 300 s.

Comparing the ratio of EI_{max} / $SS_{1/2}$ values of EI-SS curves determined after and before the mechanical stress, inter-species differences could be observed (Fig. 10). The highest ratio values were observed at low osmolality, while 250 and 300 mOsmol/kg data showed similar results, the hyperosmolar condition triggered a decrease of the values, expect for pig, where it rather increased. In rat blood the difference between the values tested at 250 and 500 mOsmol/kg was the smallest, and it hardly changed. While in canine blood the 200–300 mOsmol/kg range was relatively stable, and at higher osmolality the values dropped. Human data changed in the smallest range, but with obvious direction: decreasing values as osmolality increased (Fig. 10).

4. Discussion

Mechanical stability of red blood cells is essential for their survivor in the circulation. Under physiological circumstances the shear stress on erythrocytes are generally under 5 Pa and usually not exceeding 10 Pa [13, 17, 19]. However, pathophysiological processes or non-physiological circulatory conditions can cause the increase of shear stress, which can lead to membrane injury of erythrocytes [3, 7]. Extent of the mechanical injury –that causes sub-lethal and later hemolytic trauma to the red blood cells– depends on the magnitude and exposure time of the shear stress [7, 17, 18], as well as on the mechanical stability of the cells [7, 28, 29]. Hereditary membrane disorders and enzymopathies of the erythrocytes or any pathophysiological processes that causes injury to the red blood cells result impaired membrane

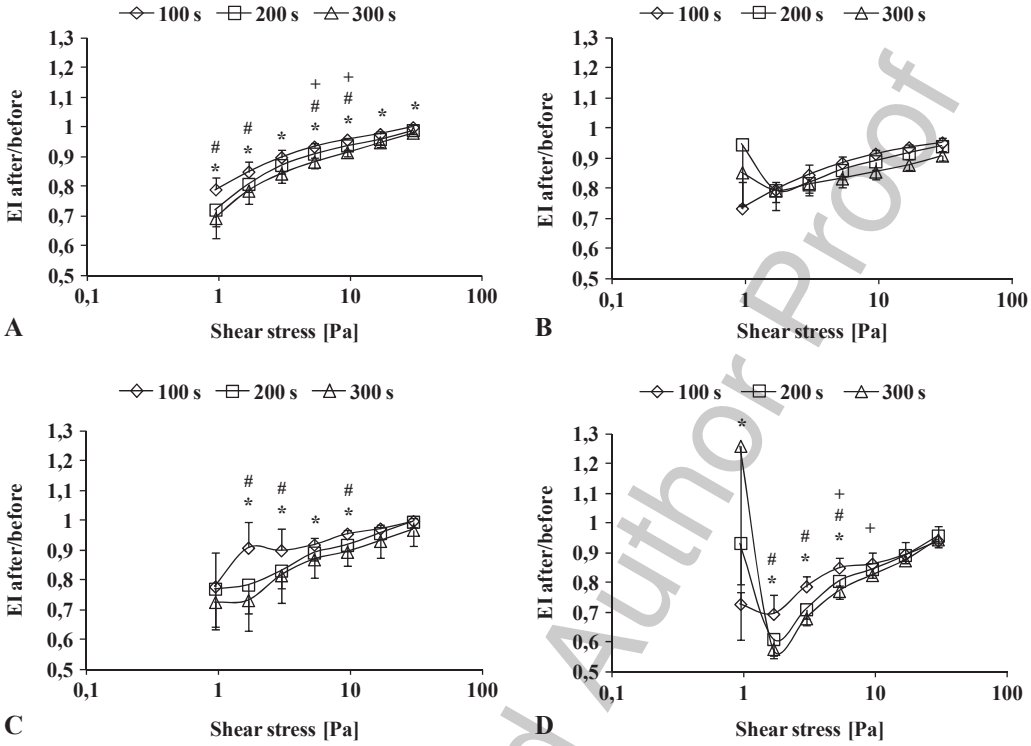

Fig. 4. Ratio of the elongation index (EI) values measured after and before the 100 Pa mechanical stress in rat (A), dog (B), pig (C) and human (D). Data under 0.95 Pa are not plotted. means ± S.D., $^{*}p < 0.05$:100 s vs. 300 s; $^{\#}p < 0.05$:100 s vs. 200 s; $^{+}p < 0.05$:200 s vs. 300 s.

stability and lower capacity of resistance against increased shear stress [8, 11, 12, 24, 25]. The end point of the mechanical injury is the lysis of the cells due to membrane rupture. The mechanical trauma that does not cause hemolysis yet but results in deterioration of cells' micro-rheological parameters, such as deformability and aggregation, is called sub-lethal trauma [17]. It was firstly mentioned by Brinsfield et al in 1962, and they experienced a decrease in red blood cell count after extracorporeal circulation lasting 10–48 hours in experimental animals [6]. It causes impaired red blood cell deformability and increased aggregation, which have a negative effect on microcirculation and tissue perfusion. Through several cascade-like mechanisms and by effect on leukocyte- and platelet functions supra-physiological shear stress causes alteration in the hemodynamic parameters that will lead to further increase in the shear stress, and the process turns into a vicious circle [17, 18]. For investigating various pathophysiological processes that may alter shear forces in the circulations, and for developing-testing intravascular devices (e.g., stents, grafts, vascular prostheses, artificial valves and hearts, devices for extracorporeal circulation, special intravascular circulation-supporting devices, etc) *in vivo* studies are necessary [2, 3, 16, 17, 22].

We hypothesized that just like other physiological and hemorheological parameters this red blood cell property, the mechanical (membrane) stability, as capacity of resistance against increased shear stress, may also show interspecies differences. We investigated rat, dog, pig and human red blood cells using nine mechanical shear stress variations by combinations of magnitude (30, 60 and 100 Pa) and duration

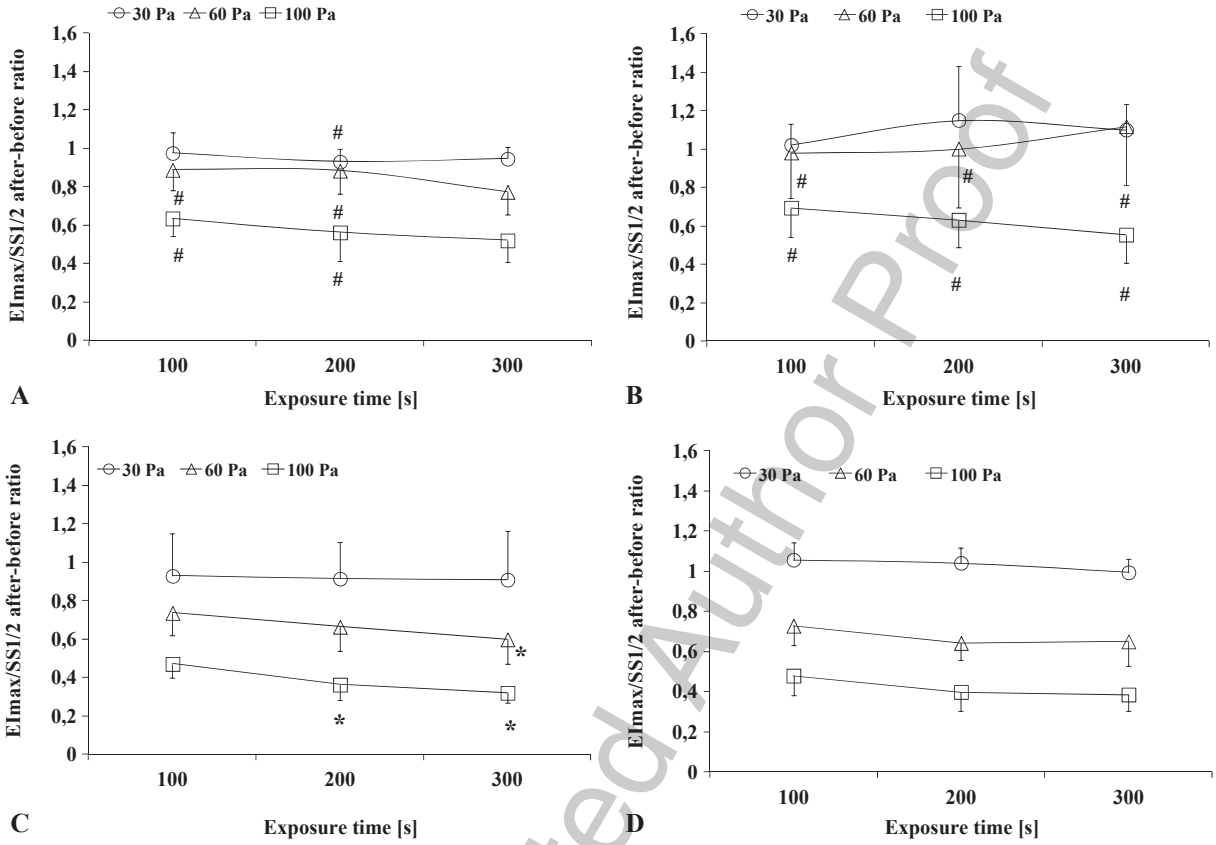

Fig. 5. Ratio of the EI_{max} / $SS_{1/2}$ values measured after and before the various mechanical stress combinations in rat (A), dog (B), pig (C) and human (D).means ± S.D., $^{*}p < 0.05$ vs. 100 s at same Pa, $^{\#}p < 0.05$ vs. human relative change by equal stress.

(100, 200 and 300 s). Furthermore, we analyzed the effect of osmolality on the mechanical stability test results.

Our main findings were the followings: (1) Red blood cell describing hematological parameters and red blood cell deformability describing elongation index – shear stress curve's parameters showed obvious interspecies differences, which enforce the literature data. (2) With the applied combinations of mechanical stress pig erythrocytes were the most sensitive (30 Pa for 100 s caused significant deformability worsening). Generally, rat erythrocytes showed the highest capability of resistance. On human erythrocytes 60 Pa for 200 s was the minimum combination to result significant deformability deterioration. (3) As the magnitude and the duration of the mechanical stress was increased the shape of the elongation index – shear stress curves became more and more prominently irregular under 0.95 Pa, and its magnitude was different among the species. (4) Due to the 100 Pa shear stress applied for 300 seconds human erythrocytes showed the largest deformability impairment among the investigated species. (5) Out of the applied combinations for mechanical stress, in case of the 30 Pa canine erythrocytes showed improvement in elongation index values, of which magnitude was larger if duration of exposure was longer. The improvement of elongation index values were the largest when it was measured under 5 Pa, and it continuously decreased as elongation index was determined at higher shear stress levels. (6) Osmolality

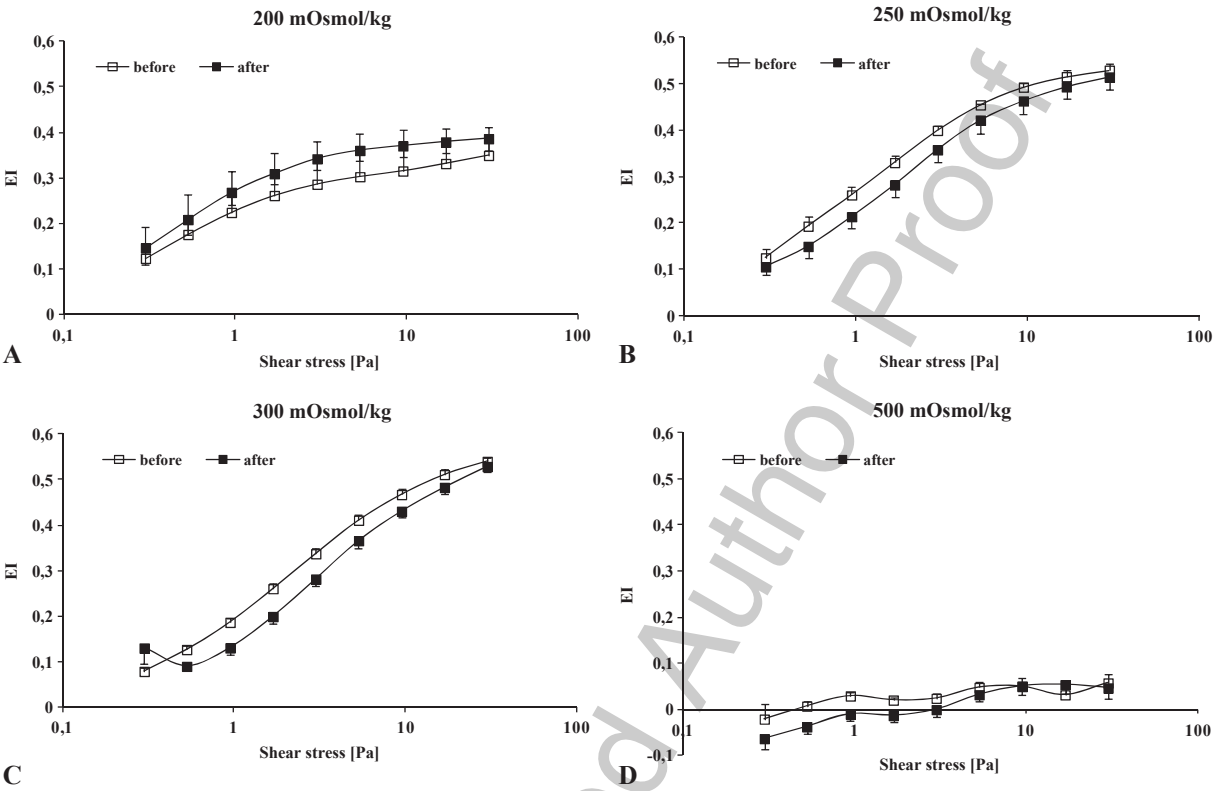

Fig. 6. Red blood cell elongation index (EI) – shear stress [Pa] curves of rat blood samples before and after a mechanical stress of 100 Pa for 300 seconds, tested in PVP-PBS solutions with osmolality of 200 mOsmol/kg (A), 250 mOsmol/kg (B), 300 mOsmol/kg (C) or 200 mOsmol/kg (D). means ± S.D., $^{*}p < 0.05$ vs. before.

changes both in hypo- or hyperosmolar direction strongly influenced the mechanical stability test results in all species. In hypoosmolar range EI values rather improved after the mechanical shearing in all the four investigated species, mostly in rat and porcine blood. This phenomenon was not observable at 250, 300 or 500 mOsmol/kg.

It is known that and already widely investigated that like other physiological parameters, hemorheological ones also show interspecies differences [27, 31]. Red blood cell deformability is determined by several cellular factors [9, 10, 21, 24], and one of the most important in the maintenance of mechanical stability is the integrity of spectrin-based membrane skeleton [5, 10, 24]. Interspecies differences in the mechanical stability of red blood cells can be partly explained by the quantitative and qualitative difference in the spectrin-network and the levels of protein phosphorylation, especially for the protein 4.1 R that modulates spectrin and actin affinity and membrane stability of erythrocytes [10, 20, 28, 30]. However, it should be taken under consideration that this mechanical stability measurement cannot be performed *in vivo*, and although all measurements were completed within 2 hours under the same protocol, different species red blood cells are sensitive to *in vitro* conditions at a different manner [14, 15, 26, 31]. Changes in the erythrocytes' metabolic state may also cause membrane stiffening due to reduced skeletal junction phosphorylation [28].

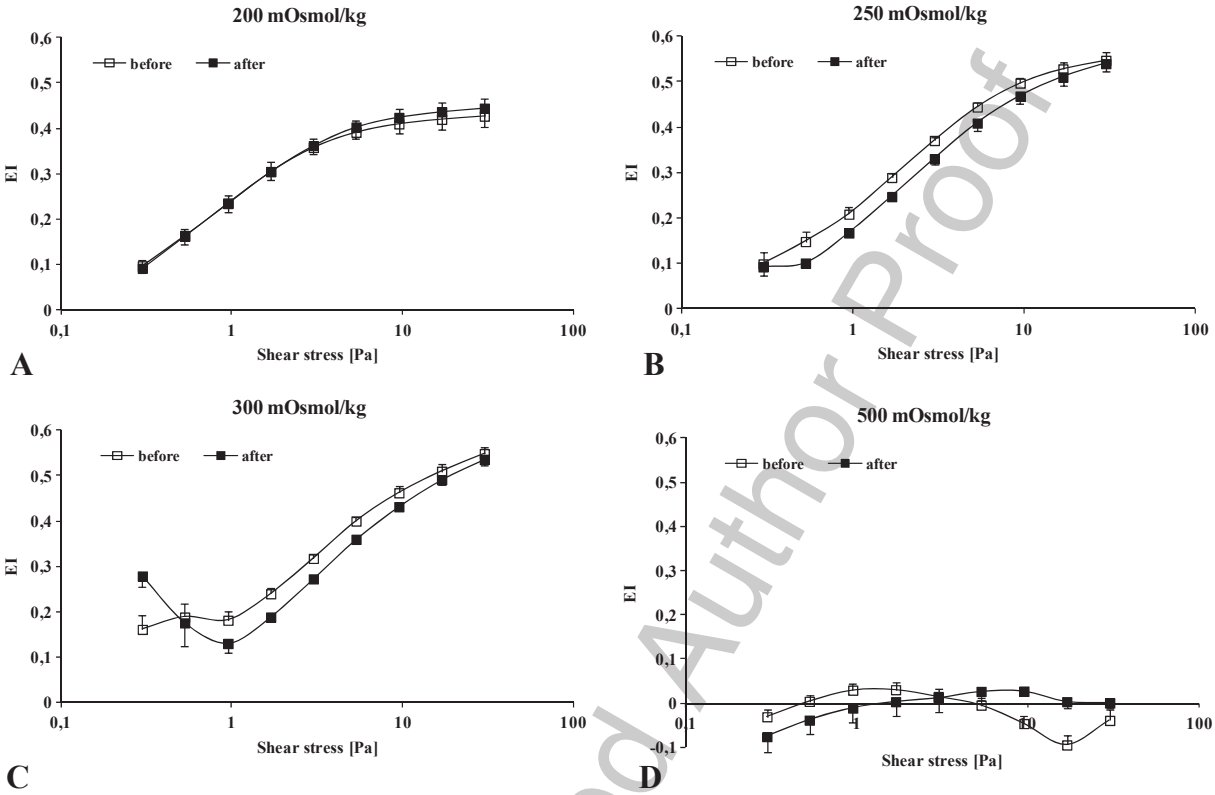

Fig. 7. Red blood cell elongation index (EI) – shear stress [Pa] curves of canine blood samples before and after a mechanical stress of 100 Pa for 300 seconds, tested in PVP-PBS solutions with osmolality of 200 mOsmol/kg (A), 250 mOsmol/kg (B), 300 mOsmol/kg (C) or 200 mOsmol/kg (D). means ± S.D., $^{*}p < 0.05$ vs. before.

Similarly to our findings on dog erythrocyte deformability improvement, recently it was reported by Meram et al. that a very brief (few-second) duration of 5–20 Pa shear stress may even improve deformability of human red blood cells up to by 8% [23]. Simmonds et al. also observed improved deformability on human red blood cells under physiological shear stress and they found that the sub-hemolytic threshold for human erythrocytes was 30–40 Pa with 300 s exposure time [29]. However, Arwatz and Smits, investigating two whole blood samples using a custom-made Taylor-Couette apparatus, found only 1-2% hemolysis when 50 Pa shear stress for 50 seconds was applied, 5% at 50 Pa for 300 seconds, and 10–12% at 200 Pa for 300 seconds [1]. In our experiment elongation index – shear stress curves became more and more prominently irregular under 0.95 Pa shear stress as the magnitude and exposure time of the applied mechanical stress increased. It was probably due to increasing amount of erythrocyte fragmentation and hemolysis [3, 17].

We have not found explanation in the literature for the strange observation on osmolality-dependency of the mechanical stability results together with their inters-species differences of this study. In hypotonic environment the cells are swelling, their shape become more spherical and their surface-to-volume ratios are changing accordingly resulting in decreased deformability and increased stretching-straining of the membrane. If a shear stress is applied on this condition it might cause altered shear stress distribution on the cells compared to a discocyte formation. It is hypothesized that due to the elastic characteristics

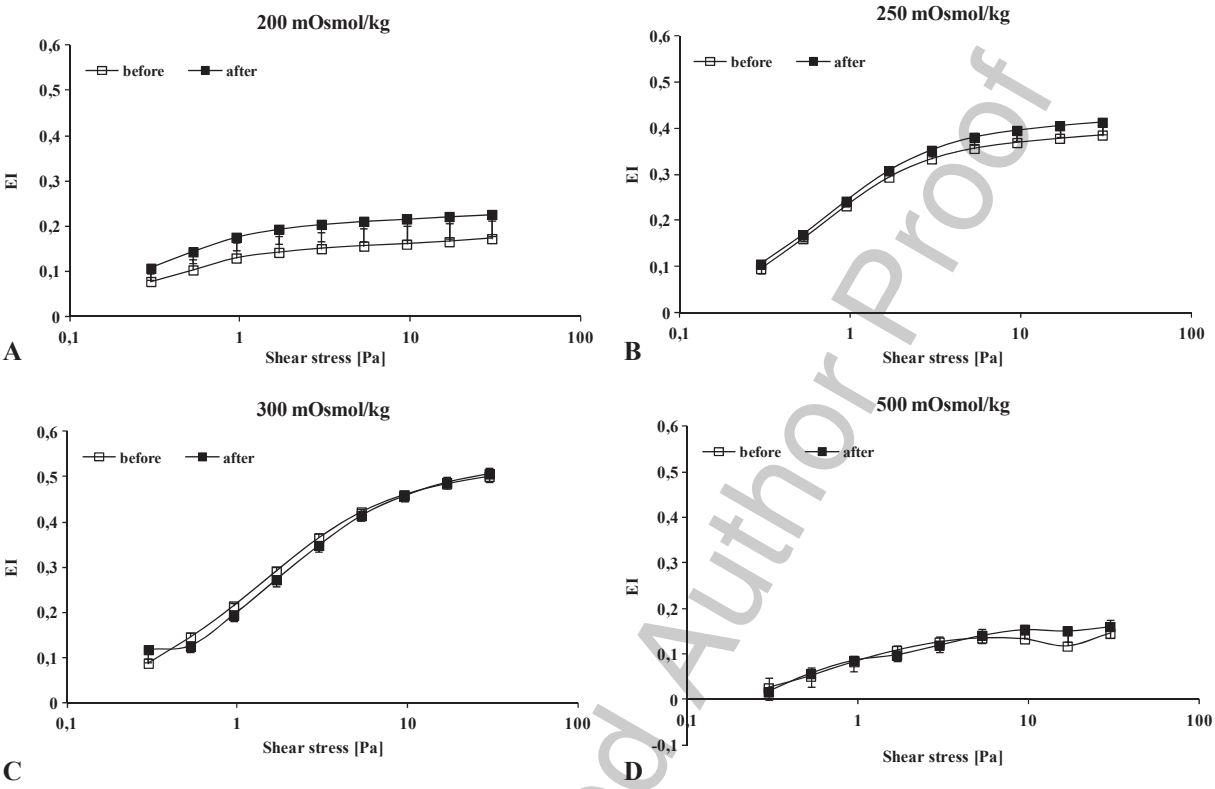

Fig. 8. Red blood cell elongation index (EI) – shear stress [Pa] curves of porcine blood samples before and after a mechanical stress of 100 Pa for 300 seconds, tested in PVP-PBS solutions with osmolality of 200 mOsmol/kg (A), 250 mOsmol/kg (B), 300 mOsmol/kg (C) or 200 mOsmol/kg (D). means ± S.D., $^{*}p < 0.05$ vs. before.

of the cells (membrane), the stretching effect of mechanical shearing might be more expressed under this condition (mechanical shearing at low osmolality). Rat and pig erythrocytes, having smaller MCV (Table 1), showed more expressed 'improvement' during mechanical stability test at 200 mOsmol/kg compared to dog or human. Previously we also observed significant difference in of the elongation index – osmolality (osmoscan) curves were shifted to right compared to rat, dog or human [27]. It seems that the interspecies diversity of hemorheological factors become much more complicated as we investigate with further and further techniques.

5. Conclusion

In summary, erythrocytes' capability of resistance against mechanical stress shows interspecies differences depending on the magnitude and duration of the applied stress, and on the osmolality. The differences can be significant, and the behavior of red blood cells against shear stress is not uniform among species. It have to be taken into consideration when the red blood cell mechanical (membrane) stability test is applied in research and/or in testing vascular grafts, prostheses and artificial devices that can be implanted into the circulation or blood can be perfused through it extracorporeally.

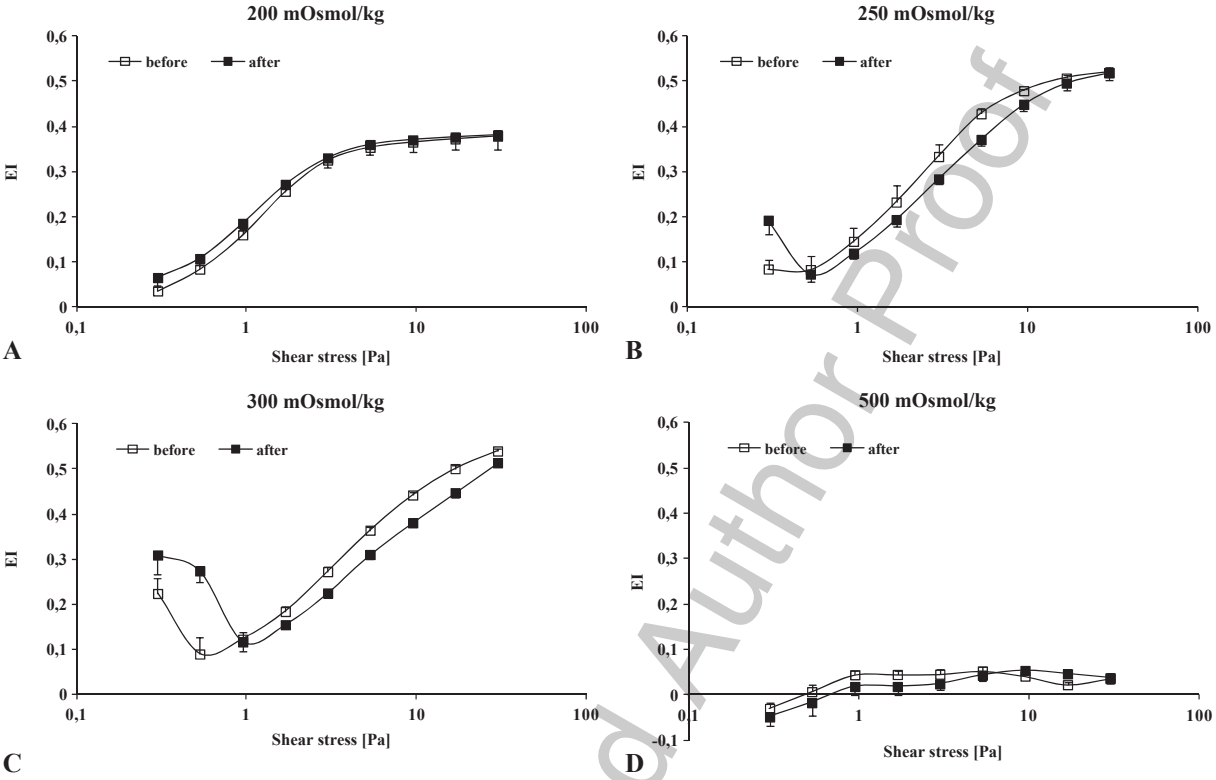

Fig. 9. Red blood cell elongation index (EI) – shear stress [Pa] curves of human blood samples before and after a mechanical stress of 100 Pa for 300 seconds, tested in PVP-PBS solutions with osmolality of 200 mOsmol/kg (A), 250 mOsmol/kg (B), 300 mOsmol/kg (C) or 200 mOsmol/kg (D). means ± S.D., $^{*}p < 0.05$ vs. before.

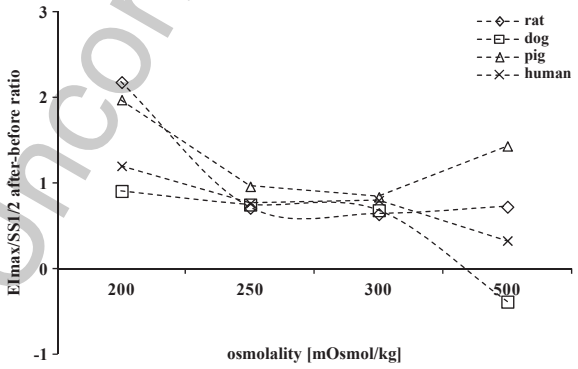

Fig. 10. Mean values of the ratios of $EI_{max}/SS_{1/2}$ values [Pa^{-1}] determined after versus before the mechanical shearing (100 Pa, 300 s) in the function of osmolality [mOsmol/kg] in rat, dog, pig and human.

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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.

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