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Assessment of the hemorheological profile of koala and echidna

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Abstract

Koala, a marsupial, and echidna, a monotreme, are mammals native to Australia. Blood viscosity ($62.5\text{--}1250\text{ s}^{-1}$), red blood cell (RBC) deformability, RBC aggregation, aggregability and surface charge, and hematological parameters were measured in blood samples from six koalas and six echidnas and compared to adult human blood. Koala had the largest RBC mean cell volume ($107.7 \pm 2.6\text{ fl}$) compared to echidna ($81.3 \pm 2.6\text{ fl}$) and humans ($88.4 \pm 1.2\text{ fl}$). Echidna blood exhibited the highest viscosity over the entire range of shear rates. Echidna RBC were significantly less deformable than koala RBC but more deformable than human RBC. Echidna RBC had significantly lower aggregability (i.e., aggregation in standardized dextran medium) than koala or human RBC, while aggregation in autologous plasma was similar for the three species. Erythrocyte surface charge as indexed by RBC electrophoretic mobility was similar for human and echidna cells but was 40 % lower for koala RBC. Data obtained during this preliminary study indicate that koala and echidna have distinct hemorheological characteristics; investigation of these properties may reveal patterns relevant to specific behavioral and physiological features of these animals.

Keywords

Blood viscosity; Erythrocyte deformability; Erythrocyte aggregation; Marsupials; Monotremes

Introduction

Mammals share many common anatomic and physiological properties, including their circulatory system, and except for obvious species differences, the anatomy of all mammalian circulations follows the same design and function. Moreover, the physical rules governing blood flow and hence tissue perfusion are the same regardless of the diverse

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biology and different vessel diameters and lengths ranging from several microns to centimeters. Irrespective of these dimensional variations, blood flow in this complex vascular system is a function of blood pressure, blood vessel geometry and the viscosity of blood (Secomb and Pries, 2007). However, the application of standard hydrodynamic concepts to pressure–flow relations in animals is often made difficult due to: (i) non-constant blood vessel geometry that can change due to the compliance and elastic properties of the vessel wall or the activity of vascular smooth muscle; (ii) non-constant blood viscosity that can change over a wide range depending on flow conditions and shear forces. Consideration of shear forces is essential since blood exhibits non-Newtonian flow behavior in which viscosity decreases with increasing shear forces.

The flow behavior of blood is affected by the properties of both plasma and the cellular elements, especially red blood cells (RBC) (Cokelet and Meiselman, 2007). Two aspects of RBC are of particular importance: (i) RBC deformability, which denotes the degree of reversible shape change of RBC due to the influence of external mechanical forces such as flow forces; (ii) RBC aggregation, which denotes the reversible formation of linear and three-dimensional cellular structures of cells at stasis or low-flow conditions (Meiselman, 1993). Both RBC deformability and aggregation are strongly influenced by the structure, morphology and physiology of the RBC (Baskurt and Meiselman, 2003; Rampling et al., 2004; Cooke and Lim, 2007; Meiselman, 2009). Mammalian blood shares many features that affect its flow behavior. Circulating mature RBC are non-nucleated (Gascoyne and Hawkey, 1992), and this characteristic makes them highly deformable compared to nucleated RBC of other vertebrates. Most mammalian RBC have a biconcave discoid shape (Gascoyne and Hawkey, 1992), which also aids deformation and the formation of cell–cell aggregates. Plasma composition is generally similar among mammals, yet differences can exist in the levels of macromolecules that are an important determinant of plasma viscosity and RBC aggregation. Some hematological indices such as RBC size and mean cell volume vary over a very wide range, while other indices (e.g., hemoglobin concentration, volume fraction of RBC) are relatively constant (Gascoyne and Hawkey, 1992). To date, the existence of a pattern for these indices that matches prominent features of different species is not obvious (e.g., RBC size does not correlate with body size; cf. Dittmer, 1961; Gascoyne and Hawkey, 1992).

Differences of RBC characteristics and of plasma composition result in significant variations of RBC deformability and the flow properties of blood among species. Similarly, blood viscosity of mammals has a very wide range when measured at low shear rates, although at high shear rates it exhibits a much narrower range (Johnn et al., 1992). Since low-shear blood viscosity is markedly affected by red blood cell aggregation, this finding indicates a high variability of RBC aggregation among mammalian species (Ohta et al., 1992; Popel et al., 1994; Baskurt et al., 1997; Plasenzotti et al., 2004). Additionally, RBC deformability differs among species (Smith et al., 1979; Waugh, 1992; Baskurt, 1996; Plasenzotti et al., 2004); an extended description of hemorheological properties of different mammals can be found elsewhere (Windberger and Baskurt, 2007).

The rheological diversity among mammals has stimulated comparative physiologists to search for matching patterns in hemorheological and physiological characteristics (Popel et al., 1994; Elsner et al., 2004; Castellini et al., 2006). One such proposed relationship is a higher RBC aggregation in athletic species compared to sedentary species, with the athletic versus sedentary classification based on maximal oxygen consumption (Popel et al., 1994). This relationship has been shown to be valid by several groups, although there is a paucity of information to satisfactorily explain the possible mechanisms relating RBC aggregation to athletic performance. Another interesting approach has been the search for hemorheological patterns of marine mammals (Meiselman et al., 1992; Popel et al., 1994; Elsner et al., 2004;

Castellini et al., 2006), yet these studies were unable to describe a well-defined pattern: for example, two closely related marine mammals living near the North or South Pole have markedly different features of RBC aggregation. These findings suggest that the diversity of hemorheological parameters may reflect different adaptations under different environmental conditions.

One of the distinct branches of mammals is the marsupial infraclass, which is accepted to have evolved concurrently, although under different environmental conditions, with placental mammals toward the end of the Mesozoic Era (Gardner et al., 2005). Marsupials dominated in Australia and New Guinea while placental mammals were more successful throughout the rest of the world. The primary difference between marsupials and placental mammals relates to their reproductive biology: marsupials have a very short pregnancy (8–43 days) and give birth to “embryo-like” young weighing less than 1 % of their mother’s body weight (Renfree, 1993). Monotremes represent another order of non-placental mammals, with platypus and echidna the only two remaining species. The short-beaked echidna (*Tachyglossus aculeatus*) and platypus (*Ornithorhynchus anatinus*) are native to Australia and the long-beaked echidna (*Zaglossus bruijnii*) is only found in New Guinea. Monotremes are unique as they are the only egg-laying mammals, and although they lactate to feed their young, they do not have developed teats but rather secrete milk through pores on the skin over the mammary gland.

Marsupials and monotremes have been the subject of limited hematological (Parsons et al., 1971), immunological (Jurd, 1994) and energy metabolism studies (Parkinson et al., 1995). Parsons and co-workers report that several hematological parameters of echidnas are within the normal mammalian range, although hemoglobin properties differed from those for placental mammals (Parsons et al., 1971). Based upon the findings of Parsons et al., it may be plausible that if hemorheological differences can be detected among marsupials and monotremes, some of these parameters could potentially reflect responses to special environments. Correlating these parameters with specific biological features and life styles may yield a greater understanding of possible adaptations of their cardiovascular systems. Hence the purpose of this pilot study was to assess and compare hemorheological and hematological properties of marsupial (koala) and monotreme (echidna) blood with values for human blood.

Materials and methods

Blood sampling and preparation

Blood samples from six male koalas (*Phascolarctos cinereus*) were collected from the cephalic vein under manual restraint with the aid of a tourniquet. Blood samples from four male and two female short-beaked echidnas (*Tachyglossus aculeatus*) were collected from their beak sinus; the echidnas were anesthetized with 2–5 % isoflurane in 2 l/min oxygen via a mask over the beak. Samples from both species were collected by swabbing the skin with 70 % alcohol and using a 25 g needle and a 5 ml syringe to collect the blood; the sample was immediately transferred to a tube containing ethylenediaminetetraacetic acid (EDTA, 1.5 mg/ml) as anticoagulant. Human venous blood samples were obtained from six healthy male volunteers using EDTA-containing vacuum tubes. All cell preparations and measurements were completed within four hours after sampling.

Complete hematological analysis was performed using an electronic hematology analyzer (Sysmex XT 2000i-Vet; Roche Diagnostics AG, Switzerland). Fibrinogen levels were determined by enzymatic analysis of the conversion of fibrinogen to fibrin using a clinical chemistry analyzer (AU400; Olympus, Tokyo, Japan). A 1 ml aliquot of each sample was used for the measurement of blood viscosity at native hematocrit, and a 1.5 ml aliquot of

each sample was adjusted to a hematocrit of 0.4 l/l either by removing or adding autologous plasma. The remaining portion of the sample was centrifuged at 1400 g for 5 min, the plasma removed and saved, and the RBC washed in isotonic phosphate-buffered saline (PBS, pH = 7.4, 290 mosmol/kg H₂O). The washed RBC were re-suspended in the following media at 0.40 l/l hematocrit: (i) 3 % (wt/vol) dextran 70 (MW = 70 kDa) in PBS; (ii) 0.5 % (wt/vol) dextran 500 (MW = 500 kDa); (iii) 1 % (wt/vol) dextran 500 in PBS. Both dextrans were obtained from Sigma Chemical Co. (St Louis, MO, USA).

For scanning electron microscopy (SEM) studies, 20 µl of each native blood sample was added to 1 ml of 0.5 % glutaraldehyde in PBS for fixation. The fixed samples were then dehydrated through an alcohol series, immersed in hexamethyldisilazane and air dried on a silicon wafer mounted on a standard SEM stub. Micrographs were obtained using a field emission SEM (JEOL JSM-6300F; JEOL Ltd., Tokyo, Japan).

Viscosity measurements

The apparent viscosities of RBC suspensions in plasma at native or at 0.4 l/l hematocrit were measured at shear rates of 62.5–1250 s⁻¹ with a rotational cone-plate viscometer (model 1/2 RVT-200; Brookfield Engineering Laboratories, Stoughton, MA, USA). Plasma viscosities were measured at 625 s⁻¹ using the same instrument. All viscosity studies were carried out at 37 °C.

Assessment of red blood cell deformability

RBC deformability was assessed using a laser diffraction ektacytometer with a thin microchannel as the shearing geometry (RheoScan-D; RheoMeditech, Seoul, Korea). Blood diluted to ~0.01 l/l hematocrit in a polyvinylpyrrolidone (PVP) solution is sheared in the microchannel under continuously decreasing pressure differentials; a laser beam is directed through the sheared sample and the diffraction pattern produced by the deformed cells is captured by a CCD camera and analyzed by a computer (Shin et al., 2005). Based upon the captured elliptical diffraction patterns, an elongation index (EI) is calculated as:

$$EI = (L - W) / (L + W),$$

where *L* and *W* are the length and width of the diffraction pattern. EI values were determined at sixteen shear stresses between 0.5 and 20 Pa. All measurements were performed at room temperature (22 ± 1 °C).

The shear stress required for half-maximal deformation (SS_{1/2}) and the maximum EI at infinite shear stress (EI_{max}) were calculated using EI–shear stress data for each sample by a Lineweaver-Burke model as described elsewhere (Baskurt and Meiselman, 2004). Briefly, shear stress–EI data were fitted using a non-linear curve fitting algorithm available in a commercial statistical package (Prism 4.0, GraphPad Software Inc., La Jolla, CA, USA), and SS_{1/2} and EI_{max} were determined. Obviously, impairment in RBC deformability leads to increased SS_{1/2} values (i.e., greater stress required to achieve one half of EI_{max}).

Assessment of red blood cell aggregation

RBC aggregation in various 0.4 l/l hematocrit suspensions was quantified at room temperature (22 ± 1 °C) using a photometric rheoscope (Myrenne aggregometer; Myrenne GmbH, Roetgen, Germany) interfaced to a digital computer. This technique is based on the increase of light transmission (LT) through an RBC suspension that occurs when individual cells aggregate; gaps between the aggregates in the suspending medium allow more light to pass through the suspension. This instrument provides two indexes of RBC aggregation as

described previously (Kiesewetter et al., 1982): (i) “M”, the extent of aggregation at stasis; (ii) “M1”, the extent of aggregation at a low shear rate of 3 s^{-1} . M thus reflects aggregation in the absence of shear, and M1 reflects aggregation when fluid movement tends to promote cell–cell interaction; both M and M1 increase with increasing RBC aggregation (Bauersachs et al., 1989). In order to provide additional information relevant to RBC aggregation, LT data from the Myrenne aggregometer were recorded via computer for the first 120 s during RBC aggregation at stasis and several indices of aggregation determined (Hardeman et al., 2001):

- a. Amplitude (AMP): the difference between minimum LT at the start of RBC aggregation and LT at 120 s, and hence the extent of RBC aggregation.
- b. Half-time ($T_{1/2}$): the time required for LT to reach one half of AMP.
- c. Aggregation index (AI): an index to LT integrated over the 120 s period that also reflects the extent of aggregation.
- d. Aggregation time constants (T_{FAST} and T_{SLOW}): time constants for the fast and slow components of RBC aggregation by fitting LT–time data to a double-exponential equation ($\text{LT}(t) = a + b e^{-t/T_{\text{FAST}}} + c e^{-t/T_{\text{SLOW}}}$).

Note that while reflecting somewhat different aspects of RBC aggregation, both AMP and AI increase with aggregation tendency, with $T_{1/2}$, T_{FAST} and T_{SLOW} reflecting the kinetics of the aggregation process.

RBC electrophoretic mobility

The electrophoretic mobility (EPM) of cells previously fixed with 0.5 % glutaraldehyde was measured at 25 °C using an automated system (Zetasizer, model ZS90; Malvern Instruments Ltd., Malvern, UK). To improve sensitivity, the cells were suspended in PBS of 0.075 M ionic strength, prepared by mixing equal parts of PBS and 0.3 M sorbitol dissolved in water. The EPM of each sample was measured four times and the average value assigned to the sample.

Statistics

Values are presented in mean $\pm$ standard error (SE). One way ANOVA followed by Neumann-Keuls post-test was used for statistical comparisons. A criterion of significance was set at an alpha level of $p < 0.05$.

Results

Subject characteristics

Age and biometric variables of the subjects in each group are presented in Table 1. All koalas and echidnas were confirmed to be in a healthy condition at the time of blood sampling.

Hematological parameters

Hematological parameters for koala, echidna and human subjects are shown in Table 2. Hematocrit, total hemoglobin and platelet count were not significantly different between groups. The mean cell volume (MCV) and mean corpuscular hemoglobin (MCH) of koala RBC were significantly higher than those of human and echidna RBC, with the mean corpuscular hemoglobin concentration (MCHC) of koala RBC lower than that of human RBC and higher than that of echidna; the white blood cell (WBC) count was highest for echidna. Representative scanning electron microscope (SEM) photos of koala, echidna and human RBC are shown in figure 1. Similar shapes and sizes were found for each species and

were characterized by a biconcave discocytic morphology and a diameter of approximately 8 μm . Note that although echidna cells do appear smaller (Fig. 1B), the differences in cell volume noted in Table 2 are not easily detected in these images.

Blood and plasma viscosities

Blood viscosity versus shear rate results are presented in Figure 2 for samples with native hematocrit and after adjustment of hematocrit to 0.4 l/l. Koala, echidna and human blood all had non-Newtonian flow behavior with viscosity decreasing as shear rate is increased. Echidna blood exhibited the highest viscosity for both native and adjusted hematocrit samples, although blood viscosity in echidna was only significantly greater than in koala for the hematocrit-adjusted samples. Interestingly, koala blood had viscosity values closer to those of human blood for the samples with native hematocrit (Fig. 2A), while after hematocrit adjustment the viscosity of human blood was significantly higher than that of koala blood and approached that of echidna (Fig. 2B).

Figure 3 presents plasma viscosity results for koala, echidna and human. Echidna plasma had higher viscosity compared to koala and human, with the difference between echidna and human being statistically significant.

Red blood cell deformability

Koala RBC deformability was significantly higher compared to both echidna and human RBC. This difference is evident from Figure 4 which shows significantly greater EI values at shear stresses between 0.5 and 2.5 Pa and is also evident from Figure 5A where the $SS_{1/2}$ for koala is significantly smaller than that for echidna and human RBC. EI_{max} was found to be lower for koala and echidna RBC versus human RBC (Fig. 5B).

Red blood cell aggregation

RBC aggregation, as determined by the Myrenne M and M1 indexes, was similar in all three species for cells suspended in plasma (Fig. 6). This similarity was confirmed by analyzing the LT data recorded for 120 s: neither AI, $T_{1/2}$ nor T_{SLOW} were significantly different between koala, echidna and human red cell suspensions (Table 3). However, the fast-phase time constant for RBC aggregation (T_{FAST}) was significantly higher (i.e., slower rate of aggregation) for echidna RBC in plasma compared to human samples (Table 3).

Aggregation results for cells re-suspended in the dextran-PBS solutions revealed interesting differences between the three species (Fig. 7): (i) human RBC aggregation was slightly higher in 3 % dextran 70 and significantly higher in 0.5 % and 1 % dextran 500 compared to aggregation in plasma; (ii) koala RBC also exhibited enhanced aggregation in the dextran solutions compared to aggregation in plasma; (iii) echidna RBC exhibited a marked *decrease* of aggregation in 3 % dextran 70 compared to their aggregation in plasma and to the aggregation of koala and human RBC in the same dextran medium. Aggregation of echidna RBC in 0.5 % dextran 500 was approximately the same as their aggregation in plasma and was only significantly higher in 1 % dextran 500.

Parameters reflecting the time course of aggregation also revealed significant differences between echidna, koala and human RBC suspended in 3 % dextran 70 (Table 4): (i) $T_{1/2}$ for echidna RBC was about five times longer than for human and almost 7-fold longer than for koala RBC; (ii) both the fast and slow time constants for echidna were significantly longer than those in human and koala samples, thus indicating the exceptionally slow aggregation kinetics of echidna RBC in this polymer solution.

Electrophoretic mobility of red blood cells

Koala RBC had the lowest electrophoretic mobility (i.e., lowest negative surface charge density) that was about 50 % smaller than the echidna and human values; human RBC exhibited the highest values, and echidna values were not significantly different (Fig. 8). The differences between koala, echidna/human RBC were statistically significant ($p < 0.01$).

Discussion

Mammalian evolution has resulted in very diverse biological features among species, thus indicating successful adaptations to different environments and life styles. Differences between placental mammals, marsupials and monotremes reflect one of the most striking and fundamental areas of diversification. A detailed comparison of morphological and physiological features specific to these mammalian subclasses is not within the scope of this paper. However, it should be noted that placental mammals, marsupials, and monotremes are accepted to have diverged from the class Mammalia ~200 million years ago (Grutzner et al., 2003). Marsupials and placental mammals belong to the two infraclasses (Eutheria and Metatheria) of the subclass Theria, while monotremes belong to another, small subclass, the Prototheria. Thus, the common features of these three branches of the Mammalia should represent the earliest adaptations in this diversification process.

Being a monotreme, echidnas exhibit plesiomorphic (i.e., primitive compared to other mammalian species) characteristics, having a mixture of reptilian and mammalian features (Nicol, 2003). Echidnas have a low metabolic rate and maintain a low body temperature (~32 °C) (Nicol and Andersen, 2003). This low rate is related to specific adaptations that are consistent with their living habits, including control of respiration and the gaseous exchange of oxygen and carbon dioxide in blood (Lutz et al., 1989; Nicol and Andersen, 2003). Echidna has a lower cardiac output and higher peripheral resistance compared to similarly sized mammals (Parer and Metcalfe, 1967b).

The koala is a member of the marsupial infraclass. Studies on other marsupials indicate that their circulatory and respiratory physiology systems are, in general, closer to those of placental mammals than those of the echidna (Snyder et al., 1999). Agar et al. (2000) have reviewed the hematological and biochemical parameters of 30 marsupial species and indicate a diversity similar to the wide range observed in placental mammals (Gascoyne and Hawkey, 1992).

The results of this pilot study indicate that the properties of blood which are determinates of circulatory function are basically similar among the species, although differences in detail do exist. Our findings (Table 2) are consistent with the well-known hematological differences between koala, echidna and human blood (Parer and Metcalfe, 1967a; Parsons et al., 1971; Clark, 2004), with echidna having the lowest values for MCV, MCH and MCHC. Basic hemorheological features of the three species can be summarized as follows:

1. Koala and echidna blood exhibit non-Newtonian flow behavior with viscosity values within the normal range for human blood (Cokelet and Meiselman, 2007) both at native hematocrit and after adjustment of hematocrit to 0.4 l/l. However, hematocrit-adjusted koala blood viscosity was significantly lower compared to that of human and echidna blood at all shear rates used in this study (Fig. 2). It should be noted that due to the limitations of the viscometer used, shear rates in this study (i.e., 62.5–1250 s⁻¹) are considered to be “moderate to high” and thus do not reflect the strong effects of RBC aggregation at much lower rates of shear (e.g., 0.1–1 s⁻¹) (Cokelet and Meiselman, 2007).

2. RBC deformability for koala and echidna is also within the normal range expected for mammalian RBC (Windberger and Baskurt, 2007). However, koala RBC were more deformable and had the lowest shear stress required for one half maximum deformation ($SS_{1/2}$, see Figs. 4 and 5), while human RBC were the most rigid.
3. RBC aggregation indexes measured in autologous plasma for the three species were not different except for a much slower rate of initial aggregation for echidna RBC (Table 3). Conversely, RBC aggregation in dextran/PBS media did exhibit species-specific behavior (Fig. 7).

Although echidna maintains a lower body temperature ($\sim 32^\circ\text{C}$) (Nicol and Andersen, 2003) compared to the other two mammalian species included in this study, our viscosity measurements could only be conducted at 37°C . Measurements at temperatures closer to echidna's body temperature should yield higher blood and plasma viscosity values, thereby further increasing the differences from the other two species.

The significant difference in the hematocrit-adjusted viscosity of koala blood (Fig. 2B) can be explained by the greater deformability of koala RBC (Fig. 5). RBC deformability is determined by cell geometry (i.e., surface area/volume ratio optimized by the biconcave-discoid shape), cytoplasmic viscosity that is a function of intracellular hemoglobin concentration (i.e., MCHC), and the viscoelastic properties of membrane (Baskurt, 1996; Cooke and Lim, 2007). SEM images suggest that the biconcave discoid shape is a common feature of RBC from the three species, although echidna RBC appear to be more cup-shaped (Fig. 1). The mean hemoglobin concentration of koala RBC was significantly lower than that of human RBC, but higher than that of echidna RBC. This lower hemoglobin concentration, and therefore lower cytoplasmic viscosity, of koala RBC may explain the difference of deformability in comparison to human RBC. However, given both the lower deformability and the lower MCHC for echidna RBC compared to koala cells, factors other than cytoplasmic viscosity seem to be responsible for the lower deformability of echidna RBC. Differences in membrane structure and mechanical behavior may account for such distinct RBC mechanical behavior, yet unfortunately this information does not appear to exist for marsupials and monotremes.

While RBC aggregation in plasma did not differ among the species (Fig. 6), there were striking differences for cells in 3 % dextran 70 solutions (Fig. 7): the M and M1 indexes as well as the kinetic parameters for echidna RBC were significantly lower than those for koala and human RBC, with the latter two being very close to each other. These findings strongly suggest marked differences in echidna RBC surface properties compared to those of koala and human RBC. RBC surface charge density is an important determinant of aggregation since it opposes cell-cell adhesion by polymers or proteins (Rampling et al., 2004). RBC electrophoretic mobility (EPM) results (Fig. 8) indicate a significantly higher charge density for echidna than koala, providing a rationale for the differences between these two species when measured in 3 % dextran 70 (Fig. 7). Since echidna and koala aggregations in plasma do not differ, this charge density effect in plasma is apparently countered by echidna's higher level of the pro-aggregant protein fibrinogen (Cokelet and Meiselman, 2007). Interestingly, human aggregation data are not consistent with this explanation: human RBC have the highest EPM that is more than 50 % greater than that of koala, have similar aggregation in both plasma and dextran media, and have significantly higher aggregation compared to echidna red cells in spite of their higher surface charge density. These aggregation results clearly indicate that RBC "aggregability" (i.e., aggregation tendency in the same medium) differs among species and that the differences may be attributed to surface charge, glycocalyx thickness and polymer or protein levels near the RBC membrane surface (Meiselman, 2009).

It should be noted that the echidna group included both sexes (4 males and 2 females) while the two other species groups were composed of male subjects only. Inspection of the data for male and female echidnas indicated that, on average, there was a tendency for RBC-related differences: higher hematocrit, hemoglobin, RBC counts, MCV and whole blood viscosity for male echidnas (data not shown). Such differences may account for the larger range of data in the echidna group, but they do not affect the basic hemorheological patterns seen in this pilot study. However, further studies evaluating sex-related differences may reveal interesting findings.

In conclusion, the basic hemorheological properties of the three species included in this comparative study seem to be surprisingly similar despite the distance between them in terms of evolutionary history (Grutzner et al., 2003). The differences in hemorheological properties are within the range that is seen among species taxonomically closer to each other (e.g., among placental mammals), underlining that these features of blood should be among the basic evolutionary properties of the mammalian class.

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(A)

(B)

(C)

Fig. 1. Scanning electron micrographs of (A) koala, (B) echidna, and (C) human red blood cells. Original magnification of 3500 for each panel; horizontal white bar equal to 10 μ m.

Fig. 2. Blood viscosity at (A) native hematocrit and (B) with hematocrit adjusted to 0.4 l/l for koala, echidna and human blood. N = 6 for each species. Data are mean $\pm$ standard error. Difference of human and echidna from koala: ** $p < 0.01$.

Fig. 3. Plasma viscosity for koala, echidna and human samples. N = 6 for each species. Data are mean $\pm$ standard error. Difference from echidna: [†] p < 0.05.

Fig. 4.

Red blood cell elongation indexes measured at shear stresses between 0.5 and 20 Pa for koala, echidna and human RBC. N = 6 for each species. For clarity, elongation indexes measured at only seven representative shear stresses are presented. Data are mean $\pm$ standard error. Difference of human from echidna: $\dagger$ $p < 0.05$; $\dagger\dagger$ $p < 0.01$. Difference of human from koala: $**$ $p < 0.01$; $***$ $p < 0.001$. Difference of echidna from koala: $\dagger\dagger$ $p < 0.01$; $\dagger\dagger\dagger$ $p < 0.001$.

Fig. 5. (A) Shear stress for half-maximal deformation ($SS_{1/2}$) and (B) maximum EI at infinite shear stress (EI_{max}) for koala, echidna and human red blood cells. $N = 6$ for each species. Data are mean $\pm$ standard error. Difference from koala: ** $p < 0.01$; *** $p < 0.001$. Difference from echidna: †† $p < 0.01$.

Fig. 6. Red blood cell aggregation indexes (M and M1) for koala, echidna and human measured in plasma (hematocrit = 0.4 l/l). N = 6 for each species. Data are mean $\pm$ standard error.

Fig. 7. Red blood cell aggregation indexes (M and M1) for (A) koala, (B) echidna and (C) human RBC suspended in plasma and three dextran solutions (hematocrit = 0.4 l/l). N = 6 for each species. Data are mean $\pm$ standard error. Difference from aggregation in plasma: * p < 0.05; ** p < 0.01; *** p < 0.001.

Fig. 8.

Electrophoretic mobility of koala, echidna and human red blood cells. N = 6 for each species. Data are mean $\pm$ standard error. Difference from koala: *** $p < 0.001$. Difference from echidna: †† $p < 0.01$.

Table 1

Age, sex and weight of the subjects in each group.

	Koala	Echidna	Human
Age (y)	8.2 ± 0.4	5.5 ± 0.4	32.2 ± 4.6
Sex (M/F)	6/0	4/2	6/0
Weight (kg)	8.2 ± 0.2	4.6 ± 0.3	80.3 ± 7.4

y = years; M = male; F = female

Table 2

Hematology and fibrinogen data for koala, echidna and human blood.

	Koala	Echidna	Human
Hematocrit (l/l)	0.428 ± 0.018	0.480 ± 0.039	0.422 ± 0.008
Hemoglobin (g/l)	139.7 ± 5.3	142.5 ± 11.7	147.8 ± 3.1
RBC count (10 ¹² /l)	4.0 ± 0.2	5.9 ± 0.4 ^{***}	4.8 ± 0.1 [†]
MCV (fl)	107.7 ± 2.6	81.3 ± 2.6 ^{***}	88.4 ± 1.2 ^{***}
MCH (pg)	35.2 ± 0.9	24.0 ± 0.4 ^{***}	30.9 ± 0.6 ^{**, †††}
MCHC (g/l)	326.5 ± 3.5	296.8 ± 6.8 ^{**}	349.8 ± 3.2 ^{**, †††}
WBC (10 ⁹ /l)	7.3 ± 0.3	14.1 ± 2.9 [*]	6.5 ± 0.6 [†]
Lymphocyte (%)	45.7 ± 4.5	51.7 ± 7.0	40.2 ± 4.4
Monocyte (%)	3.3 ± 1.1	1.5 ± 0.7	7.2 ± 0.9 ^{*, ††}
Granulocyte (%)	50.8 ± 3.8	47.0 ± 7.0	52.6 ± 4.5 ^{**, †}
Platelet (10 ⁹ /l)	214.0 ± 27.5	151.8 ± 17.8	224.3 ± 17.8
Fibrinogen (g/l)	3.4 ± 1.1	4.6 ± 1.2	3.26 ± 0.12

N = 6 for each species. Data are mean ± SE. Difference from koala:

*
p < 0.05;**
p < 0.01;***
p < 0.001.

Difference from echidna:

†
p < 0.05;††
p < 0.01;†††
p < 0.001.

Table 3

Kinetic parameters of red blood cell aggregation for koala, echidna and human RBC in plasma.

	Koala	Echidna	Human
Aggregation index; AI	76.0 ± 1.7	74.0 ± 1.7	75.7 ± 1.6
Aggregation half time; T _{1/2} (s)	13.4 ± 1.2	17.0 ± 2.5	12.8 ± 1.1
T _{FAST} (s)	7.1 ± 0.7	10.6 ± 2.1	4.2 ± 0.5 [†]
T _{SLOW} (s)	63.4 ± 6.2	66.5 ± 8.5	59.2 ± 7.4

Hematocrit adjusted to 0.4 l/l for all samples. N = 6 for each species. Data are mean ± standard error. Difference from echidna:

[†]
p < 0.05.

Table 4

Kinetic parameters of red blood cell aggregation for koala, echidna and human RBC suspended in 3 % dextran 70.

	Koala	Echidna	Human
Aggregation index; AI	85.5 ± 0.7	56.5 ± 2.8 ^{***}	81.6 ± 2.9
Aggregation half time; T _{1/2} (s)	6.5 ± 0.8	43.8 ± 4.0 ^{***}	8.9 ± 1.3
T _{FAST} (s)	2.2 ± 0.4	49.7 ± 18.6 ^{**}	2.5 ± 0.5
T _{SLOW} (s)	35.1 ± 1.2	95.9 ± 11.0 ^{***}	35.1 ± 1.3

Hematocrit adjusted to 0.4 l/l for all samples. N = 6 for each species. Data are mean ± standard error. Difference from koala and human:

^{**}
p < 0.01;

^{***}
p < 0.001.