Zoology 113 (2010) 110–117

Contents lists available at ScienceDirect

Zoology

journal homepage: www.elsevier.de/zool

Assessment of the hemorheological profile of koala and echidna

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ARTICLE INFO

Article history:
Received 31 May 2009
Received in revised form
13 July 2009
Accepted 22 July 2009

Keywords:
Blood viscosity
Erythrocyte deformability
Erythrocyte aggregation
Marsupials
Monotremes

ABSTRACT

Koala, a marsupial, and echidna, a monotreme, are mammals native to Australia. Blood viscosity (62.5–1250 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 ± 2.6 fl) compared to echidna (81.3 ± 2.6 fl) and humans (88.4 ± 1.2 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.

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 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

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0944-2006/$ - see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.zool.2009.07.003

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 bruijni*) 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 et al. (1971) report that several hematological parameters of echidnas are within the normal mammalian range, although hemoglobin properties differed from those for placental mammals. 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 4 h 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 H_2O). 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