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Effect of Osmotic Stress on Human Red Cell Rheology: Cell Deformability, Aggregability and Blood Viscosity

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Abstract

The present study investigated the effects of the osmotic environment on the rheological properties of erythrocytes and their suspensions. In an iso-osmotic medium, erythrocytes forming a biconcave discocyte under resting conditions, exhibited high deformability. In a low-osmotic medium, the deformability of erythrocytes, which swelled and exhibited a spherical shape, significantly decreased at a high shear stress and the high-shear viscosity of the cell suspension was slightly higher than that of normal blood. Hyper-osmotic stress, however, which caused to form echinocytes, decreased cell deformability but exhibited smaller viscosity in low shear rates than iso-osmotic blood viscosity. These results showed a close relation with the aggregability measurements, in that hypertonic blood showed lower aggregability than the hypotonic and isotonic RBC suspensions. These findings indicate that the physicochemical environment has a strong influence on the rheological properties of the erythrocyte and its suspensions.

Keywords: erythrocyte, osmotic stress, deformability, viscosity, shear rate.

Introduction

Rheological characteristics of blood including blood viscosity, cell deformability and aggregability play a key role in blood circulation. These rheological properties are not independent of each other. For example, human blood viscosity may increase markedly by either decreasing the deformability[1] or increasing the aggregability of the erythrocytes.[2] In fact, erythrocyte deformability is known to be responsible for passing through capillaries whose diameter is smaller than their size and which have surprisingly low viscosity at high shear rates.[3] In addition, erythrocyte aggregation is a reversible dynamic process, which may affect the flow resistance of erythrocytes through a capillary network. Erythrocyte aggregation is also affected by erythrocyte deformability. Furthermore, blood viscosity is strongly affected by the above consequences.

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A number of studies have investigated the effects of the deformability of erythrocytes on hemorheological characteristics. In fact, erythrocyte deformation in shear flow is a consequence of continuous viscous deformation, in that the erythrocytes are often described as fluid droplets with tank-treading.[4] The ability to deform under external stress, deformability, is the combined result of several mechanical and geometrical properties of the erythrocytes. The major determinants of the overall deformability of the erythrocytes are the fluidity of the cytoplasm, a favorable surface area-to-volume ratio of the cells, and elasticity and viscosity of the membrane. Dintenfass[3] first demonstrated the surprisingly low viscosity of blood, even at hematocrits of 0.95 and above. He proposed that erythrocytes were not rigid particles, but fluid droplets with low internal viscosity. The importance of erythrocyte deformation was revealed by comparing the high-shear viscosity of normal human blood with

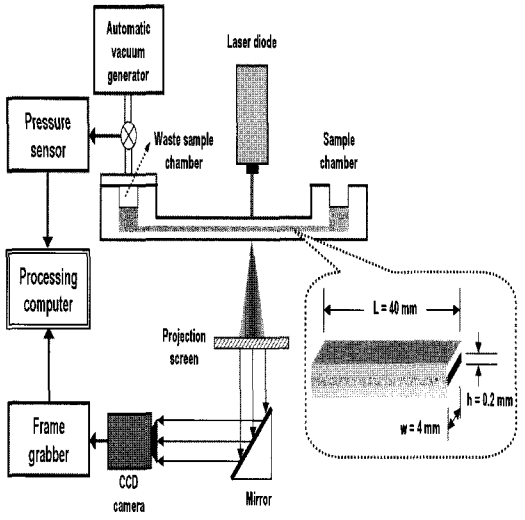

Figure. 1. Schematic diagram of a laser-diffraction slit rheometer (LDSR)

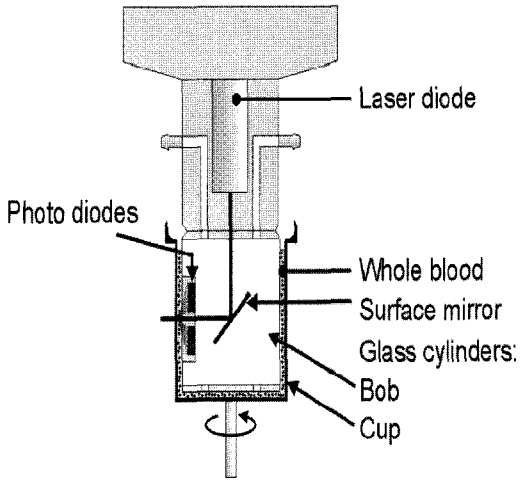

Figure. 2 Schematic diagram of a Laser-assisted Optical Rotational Cell Analyzer (LORCA)[16]

other suspensions and emulsions.[5] In addition, the effect of erythrocyte deformability on high-shear viscosity was demonstrated by poorly deformable sickle cells, or erythrocytes hardened by chemical treatment.[1] It is worthy to note that such highly hardened cells do not exhibit a shear-dependence of viscosity, since they cannot aggregate due to the loss of deformability.

In addition, erythrocytes can easily transform into other shapes when the osmotic stress varies.[6] The osmotic stress-induced shape changes in erythrocytes cause the fluidity of blood, such as erythrocyte deformability, to deteriorate. The osmotic stress-induced rheological disturbances, occasionally, have been found in circulatory disorders such as in stroke and peripheral diseases, whose causes may differ. As described above, the osmotic stress-induced deformability loss may disturb the rheological characteristics of blood. In fact, erythrocyte aggregation requires the deformation of an erythrocyte membrane.[7, 8] Moderately hardened cells, however, exhibit the shear-dependence of blood viscosity due to shear-induced deformation.[9, 10] The correlation between osmotic stress and rheological characteristics, however, has not been fully understood since there have not been many experiments conducted based on the rheological point of view.

Therefore, the present study was designed to provide further insight into the effect of osmolality on the rheological behavior of human RBCs and whole blood. In particular, the in-vitro effects of osmolality on RBC deformation and aggregation and whole blood viscosity were evaluated. Our results indicate the marked influence of osmolality on hemorheological characteristics and thus, provide comprehensive understanding that osmolality has an important regulatory effect on this cellular rheological property.

Materials and Methods

Sample preparation

Blood was obtained from five normal, healthy volunteers who were not on any medications and who provided informed consent (age range 25-40 years and male/female participants). The samples of venous blood were drawn from the antecubital vein and collected in EDTA-containing Vacutainers (BD, Franklin Lakes, NJ). Erythrocytes were separated from whole blood by centrifugation at 1,200 *g* for 10 *min* and washed with a 1 *mM* phosphate buffered saline (PBS; pH = 7.4; osmolality 300 $mOsm/kgH_2O$). Then, the erythrocytes were resuspended in three different osmotic solutions: (i) iso-osmotic medium, (ii) hyper-osmotic medium (osmolality 430 $mOsm/kgH_2O$), and (iii) hypo-osmotic medium (osmolality 160 $mOsm/kgH_2O$). All RBC preparations and measurements were carried out within 4 hours after blood collection.

Apparatus and operation procedures

Deformability measurement

RBC deformability was measured with a laser- diffraction

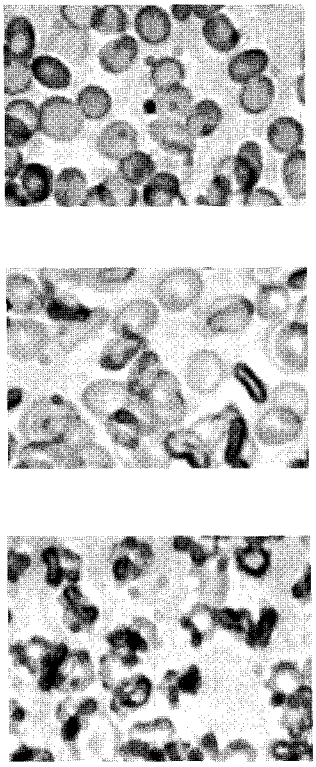

Figure. 3 Photomicrographs of the effects of osmotic stress on erythrocyte morphology

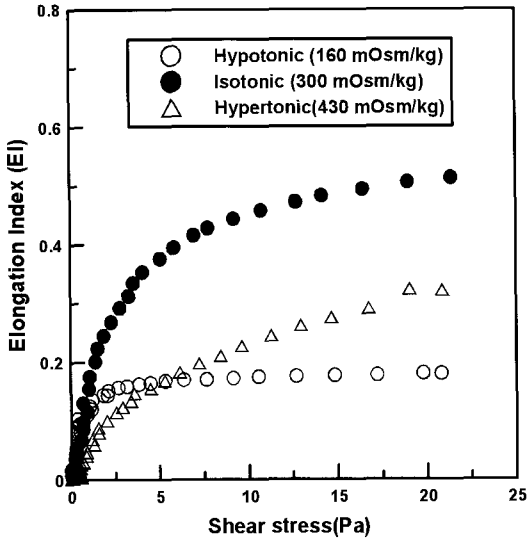

Figure. 4 Comparison of EI values for erythrocytes in hypo-, iso, and hyper-osmotic stress media.

slit-rheometer (Rheoscan-D), which was developed in our previous studies.[11,12] The basic apparatus of the Rheoscan-D, which contains a laser, the operating principle of the Rheoscan-D can be found elsewhere.[13, 14] The blood sample is sheared in the slit channel with a gap of 0.2 mm, a width of 3.8 mm and a length of 42.3 mm. The slit, which is a CCD video camera, a screen, and pressure driven disposable-slit rheometry, is shown in Fig. 1. Details of integrated with the two chambers, is designed to be disposable. The slit is made of transparent polystyrene using micro-injection molding. The length and gap of the slit were chosen to ensure that the friction loss in the slit was the dominant loss in the system.[11] The diode laser (635 nm, 1.5mW) and a CCD camera (SONY-ES30), combined with a frame grabber, were used to obtain laser-diffraction patterns.

Typical tests are conducted as follows: When the preset vacuum chamber is pushed into the connecting needle, the vacuum pressure rapidly propagates to the waste sample chamber tube, which drives the test fluid to flow through the slit by the differential pressure between the two chambers. As the waste sample chamber is being filled with the incoming fluid, the differential pressure is gradually released. When the differential pressure reaches an equilibrium, the test fluid stops flowing. While the test blood is flowing through the slit, a laser beam emitting from the laser diode traverses the diluted erythrocyte suspension and the laser beam is diffracted by the erythrocytes in the volume. The diffraction pattern projected onto the screen is captured by a CCD-video camera, which is linked to a frame grabber integrated with a computer. While the differential pressure is decreasing, the erythrocytes change gradually from a prolate ellipsoid shape toward a biconcave morphology. The diffraction pattern is analyzed by an ellipse-fitting-program and the elongation indexes (*EI*) are calculated for shear stress levels between 0 ~ 20 *Pa*. The length and gap of the slit were chosen to ensure that the friction loss in the slit was the dominant loss in the system. A detailed description of the stress-shear rate relation can be found in a previous study. [14]

For calibration purposes, the present results were compared with those of a commercial laser-diffractometer, LORCA (Laser Assisted Rotational Cell Analyzer, R&R Mechatronics, Hoorn, Netherlands). In order to check the reproducibility of the present slit diffractometer, ten measurements

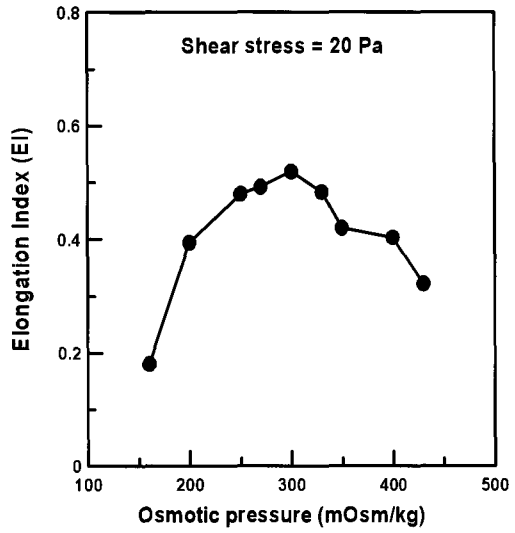

Figure. 5 Elongation Index versus osmotic stress at a high shear stress (20 Pa)

carried out with the same, normal blood samples and the degree of variation was found to be lower than 1.7%.

Aggregation measurements

RBC aggregation was measured using the Couette flow system LORCA (Laser Assisted Rotational Cell Analyzer, R&R Mechatronics, Hoorn, Netherlands). The basic instruments, as well as the methodological aspects, have been described in detail elsewhere.[15, 16] A brief description is as follows: The system for the measurement of RBC aggregation consists of two concentric-cylinders with a gap of about 0.3 mm that can hold about 2 ml of test fluid. The outer cylinder can rotate at various speeds with the aid of a stepping motor. The light source, a laser diode (670 nm, 4mW), is integrated in the fixed inner cylinder, as is a photo-diode and a temperature control unit.

Typical tests were conducted as follows: RBCs in the PVP suspension are shared at 500 s^{-1} for 5 s of dis-aggregation and laser light back-scattering from the sample is recorded for 120 s after a sudden stop. The obtained light intensity-time curve is analyzed by a micro-computer and several parameters of the RBC aggregation are calculated.

Viscosity and osmolality measurement

In measuring the viscosity of various erythrocyte suspensions, a commercial rheometer (Physica model UDS-200, Parr Physica, Inc.) was used. For the purposes of calibration, first, the present study measured the viscosity of distilled water and standard oil (Brookfield Inc, USA), which had a standard viscosity of 4.9 cP and 9.7 cP at 25°C, respectively.

The osmolality of solutions was determined by a freezing-point depression osmometer (Automatic semi-micro osmometer, Model A0300, Knauer, Berlin, Germany). The instrument was calibrated with osmometry standards of 0 and 400 *mOsm/kg* H_2O (B.Braun Melsungen AG Pharma, Berlin, Germany).

Results and Discussion

Figure 3 shows three photographs of the RBCs suspended in hypotonic, iso-tonic and hypertotic media, respectively. The RBCs suspended in the hypotonic medium were transformed from biconcave discocytes to typical sphrocytes due to the osmotic pressure difference between the medium and inner-cell membrane. On the other hand, the RBCs incubated in a hypertonic medium lost water, which led to a decrease of the cell volume and cell sphericity. The transformation of the RBC shapes may affect their deformability, aggregability and viscosity Figure 4 shows a comparison between the mean values of *EI* (elongation index) as a measure of deformability of erythrocytes suspended in three different media. For the hypo- and hyper-osmotic media, the mean *EI* values decreased significantly. It is worthy to note that the erythrocytes in the hypo-osmotic medium show the same or slightly higher values of *EI* in a range of low shear stresses (0 ~ 5 Pa). Beyond 5 Pa, however, it seems that there is no further elongation for the hypo-osmotic medium erythrocytes which have swelled significantly. For the hyper-osmotic medium erythrocytes, the EI values increase slowly and become greater than those for the hypo-osmotic case at a high shear stress ($\tau > 5$ Pa).

Figure 5 depicts the characteristic behavior of deformability over osmotic pressure at a shear stress ($\tau =$ 20 Pa). The EI curve shows an upward convex along the osmotic pressure and the maximum is found at the iso-osmotic pressure (osmolality 300 *mOsm/kg*H_2O). This fact implies that the erythrocytes tend to have the largest deformability within the iso-osmotic environment and lose it when the osmotic stress deviates from the in-vivo condition.

In the present experiments, the osmolalities were altered in order to induce changes in cell volume with a constant surface area and in cellular viscosity. The

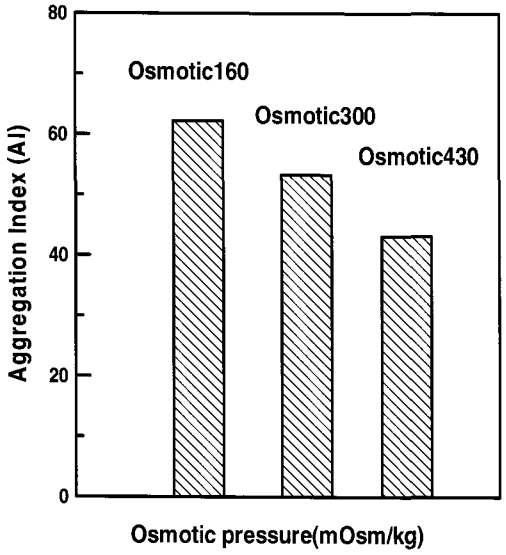

Figure. 6 Comparison of AI values for erythrocytes in hypo-, iso, and hyper-osmotic stress media.

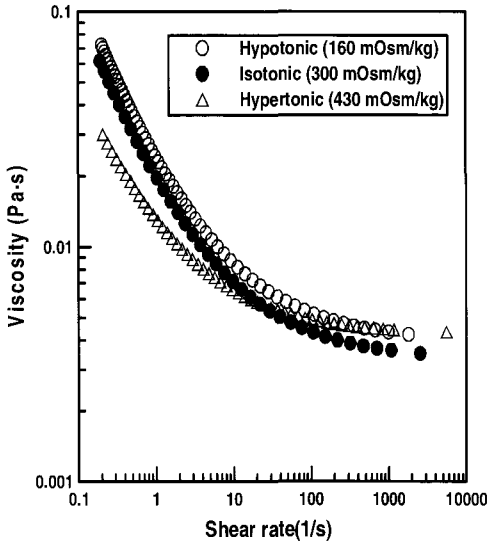

Figure. 7 Blood viscosity versus shear rate for various erythrocyte suspensions

decreased elongation index of the RBCs in a hypotonic medium is caused by erythrocyte swelling and increased sphericity. The decreased elongation index of the RBCs in a hypertonic medium is caused by an increase in cellular viscosity, which includes an increase of the membrane viscosity and an increase in the viscosity of the hemoglobin solutions.[17]

Figure 6 shows the aggregation indexes (*AI*) for the RBC suspensions in three different osmotic media. As the osmotic stress increases, the *AI* decreases. In other words, the erythrocytes in the hypo-osmotic medium tend to aggregate more than others. When changes of osmolality occurred at a constant hematocrit level, the RBC aggregation increased with a decrease in osmolality, as shown in Fig. 6. This is in agreement with Reinhart et al. (1990) who described an increase in the ESR of RBCs within osmolality-decreased plasma. With a decrease in osmolality, the RBC intracellular hemoglobin becomes diluted, which leads to a decrease of cellular viscosity and an increase of cellular aggregability.[18, 19]

After measuring the deformability and aggregability of erythrocytes, the corresponding suspension viscosity was measured by a rotational viscometer (Physica model UDS-200). Figure 7 shows the viscosity over the shear rate ($\dot{\gamma}$ = 10^{-1} ~ 10^{3} s^{-1}) for RBC suspensions in three different osmotic media. This figure demonstrates the smallest in Fig. 6, shows a lower viscosity than the iso- and hyper- osmotic erythrocyte suspensions. At a high shear rate, however, the iso-osmotic erythrocyte suspension, whose deformability was the largest among others in Fig. 5, shows the lowest viscosity.

These results illustrated in Figs 5-7 imply that osmotic stress affects not only the erythrocyte shape, but also rheological characteristics including deformability, aggregability and blood viscosity. Moreover, erythrocyte suspension viscosity is found to be strongly dependent upon the deformability and aggregability of erythrocytes. Deformability and aggregability, however, affect suspension viscosity within a range of shear rates. In fact, it has been known that deformability affects mainly the high shear viscosity of blood and aggregability targets low shear viscosity. This, however, may not be true for a high hematocrit such as a physiological condition (H = 0.45), since there are various possibilities for erythrocyte aggregation and deformation, depending on their shape. Recently, Baskurt and Meiselman [20] also confirmed that erythrocyte aggregation is affected by cellular properties including erythrocyte deformability. Unfortunately, it is not clear whether the morphological change of erythrocytes directly causes rheological characteristics within the present study.

Conclusion

The present study attempted to make a correlation between osmotic stress and the rheological characteristics of erythrocytes including deformability, aggregability and suspension viscosity. It was

determined that osmotic stress causes changes in the rheological characteristics of erythrocytes as well as morphological changes. High-shear blood viscosity was delineated with cell deformation, whereas low-shear blood viscosity was interpreted with aggregation.

Acknowledgments

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