ASIATRIB 2002International Conference
JEJU ISLAND, KOREA

The Effect of the Aging of Red Blood Cells on Rheological Properties and Hemolysis

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It is well known that red blood cells (RBCs) are suffered from chronic stresses in systemic circulation. The objective of this study is to clarify the effect of the aging of RBCs on rheological properties and hemolysis. Initially, RBCs age fractionation was performed by using a high-speed centrifugation (15[min] at 1500[G]), then young and aged RBCs were suspended in plasma to adjust the hematocrit level of 40[%]. After this pretreatment, the viscosity was measured by using a capillary type and a cone-plate type viscometers, respectively, and the hemolysis test was carried out by a seesaw type shaker. Results from these experiments showed that the viscosity of the aged RBCs measured by the capillary viscometer was increased by 10[%] as compared with that of the young RBCs. Under the condition of all shear zones, the viscosity of the aged RBCs was increased in case of using the cone-plate type viscometer. And the hemolytic level was increased twice as the aging. The data obtained in this study indicated that the ability of aggregation of RBCs was increased and the deformability of RBCs' membrane got lower with the aging. Furthermore, it was exhibited that the fragility of RBCs' membrane was increased with the aging.

Keywords : Red blood cell, Aging, Rheological properties, Hemolysis

1. INTRODUCTION

It is well known that red blood cells (RBCs) are suffered from chronic stresses in systemic circulation. The RBCs have a limit life-span which is about 120 days in human blood. Young RBCs are continuously coming into circulation and old RBCs are absorbed by a spleen. The objective of this study is to clarify the effect of the aging of RBCs on rheological properties and hemolysis.

2. METHODS

2.1 Adjustment before an experiment

To clarify the effect of the aging of RBCs, the RBCs were separated on the basis of their density between the young and the old RBCs by the Murphy method[1]. Blood sample was centrifugated at 1500g for 15[min]. Then, the young and the aged RBCs were suspended in plasma to adjust the hematocrit level of 40[%].

2.2 The measuring methods of rheological properties

Firstly, the viscosity of the blood was measured by using an Ubbellohde type viscometer which is a kind of the capillary viscometer. In this experiment, temperature was changed from 293[K] to 313[K].

Secondly, the viscosity of the blood was measured by a cone-plate type viscometer which is a kind of the rotational viscometer. The angle between cone and plate is 1.74×10^{-2} [rad], the radius of a cone-plate is 0.037[m]. In this experiment, the shear rate was gradually changed from 10[1/s] to 1000[1/s].

2.3 The measuring methods of hemolysis level

The hemolysis level of the blood was measured by using a seesaw type shaker. The blood was put into a PET tube with 1/8 inch stainless balls, and it was placed on a seesaw type shaker. In this experiment, Ht level, testing time, frequency, and number of 1/8 inch stainless ball were fixed, while the temperature of blood was changed from 298[K] to 318[K]. The seesaw type shaker was placed into the thermostat bath. Samples were exposed to roking movement at 40[min]. The frequency of the roking movement was 0.6[Hz].

In this study, the hemolysis level was evaluated by MFI[%] (Mechanical fragility index)[2] as follows.

$$MFI = \frac{Hb_{final} - Hb_{base}}{Hb_{bl} - Hb_{base}} \times 100 \qquad (1)$$

where Hb_{final}[mg/dl] is plasma free hemoglobin in blood sample exposed to rocking, Hb_{base}[mg/dl] is plasma free hemoglobin in blood sample which was not rocking, and Hb_{bl}[mg/dl] is hemoglobin concentration in the whole blood. The plasma free hemoglobin in blood sample was measured by the cyanmethemoglobin method.

3. RESULTS AND DISCUSSION

3.1 The rheological properties

Figure 1 shows the effect of the aging of RBCs on the viscosity measured by the capillary viscometer. This shows that the viscosity of the aged RBCs was increased by 10[%] as compared with that of the young RBCs. The data indicated that the deformability of RBCs' membrane got lower with the aging.

Figure 2 shows the effect of the aging of RBCs on the viscosity measured by the cone-plate type viscometer. An experiment result shows that the viscosity of the aged RBCs is higher than that of the young RBCs under the condition of all shear zones.

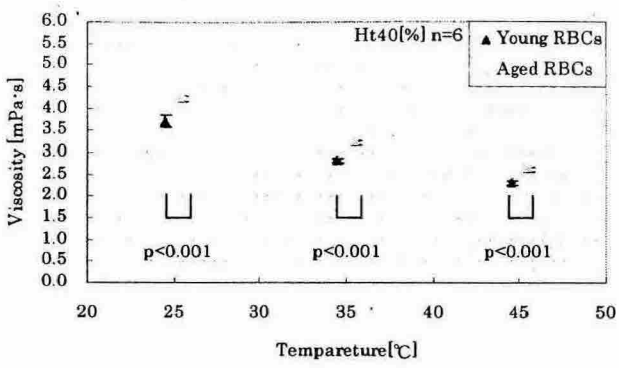

Fig.1 Effect of the aging of RBCs on the viscosity measured by the capillary viscometer

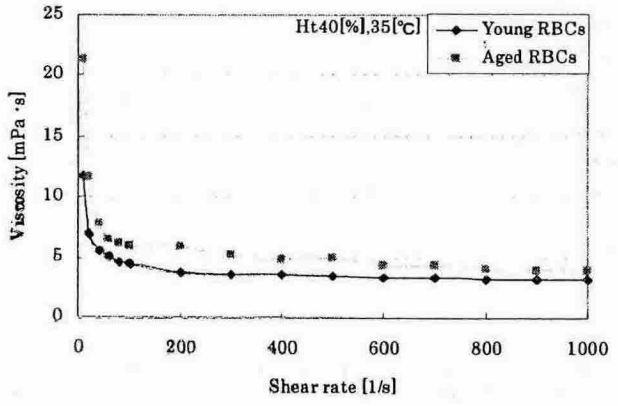

Fig.2 Effect of the aging of RBCs on the viscosity measured by the cone-plate type viscometer

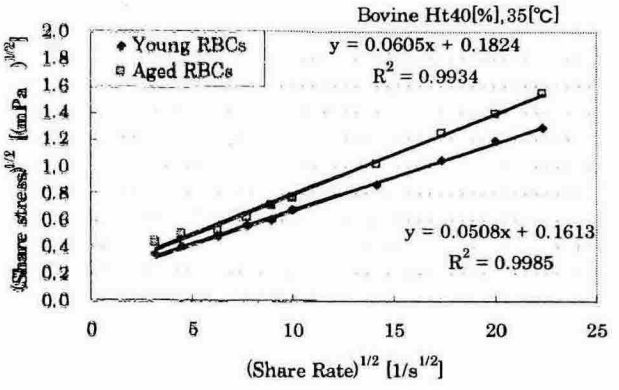

Fig .3 Casson's plot

Table1 Casson's yield stress and Casson's viscosity

Denomination of RBCs	Casson's yield stress mPa	Casson's viscosity mPa·s
Young RBCs	26.01	2.58
Aged RBCs	33.27	3.66

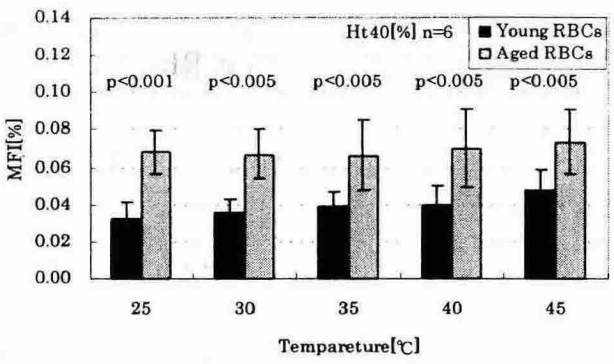

Fig.4 Effect of the aging of RBCs on the hemolysis

It is well known that the flow curve of blood can be described approximately by the Casson's equation as follows.

$$\tau^{1/2} = \tau_c^{1/2} + \eta_c^{1/2}\dot{\gamma}^{1/2} \qquad (2)$$

where τ_c is the Casson's yield stress and η_c is the Cassons viscosity. Figure 3 shows the Casson's plot of the young RBCs and the aged RBCs. This shows that the both experimental data points fall quite accurately on straight line.

Table 1 shows the Casson's yield stress and the Casson's viscosity. Since the Casson's yield stress of the aged RBCs is larger than that of the young RBCs, the ability of aggregation is increases with the aging.

3.2 The hemolysis level

Figure 4 shows that the effect of the aging of RBCs on the hemolysis level by the roking movement on the seesaw type shaker. Results from these experiments showed that the hemolysis level of the aged RBCs was increased as compared with that of the young RBCs. It is possible that the fragility of RBCs' membrane got higher and hemolysis level was affected by the aging of RBCs. Moreover, since the hemolytic level increased with the rise of temperature, it is thought that the RBCs' membrane turned more fragile with temperature.

4. CONCLUSION

Results from these experiments showed that the viscosity of the aged RBCs measured by the capillary viscometer was increased by 10[%] as compared with that of the young RBCs. Under the condition of all shear zones, the viscosity of the aged RBCs was increased in case of using the cone-plate type viscometer. The hemolysis level was increased twice as the aging. The data obtained in this study indicated that the ability of aggregation of RBCs was increased and the deformability of RBCs' membrane got lower with the aging. Furthermore, it was exhibited that the fragility of RBCs' membrane was increased with the aging.

5. REFERENCES

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