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BRIEF COMMUNICATION

THE INFLUENCE OF SURFACE CHARGE OF RBC MEMBRANE UPON RBCs ORIENTATION IN A SHEAR FLOW FIELD OF LOW VISCOSITY

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INTRODUCTION

It is well known that the RBCs in a shear flow field can orientate themselves almost parallel to the flow direction . It is the tank—treading motion(1) of the membrane around the cell content that enables the RBC to take on a stable orientation. The transition threshold of RBC from a flipping motion to a definite orientation depends both on the viscosity ratio η_i/η_o and RBC elongation (2) (where η_i is the internal viscosity and η_o is the viscosity of suspending medium). But the flipping and tank—treading motion of RBCs is not the only possible one. Goldsmith et al(3) found that when the shear rate in plasma—Ringer was increased, the normal RBCs took more time to align with the flow among each orbit than that predicted by theory for hardened RBCs. In the meantime, an increasing number of cells drifted into an orbit "C=0" in which the axis of symmetry is aligned with vorticity axis, and the cells exhibit spin without angular rotation. Moreover, the experiment showed that the value of transition threshold for shear force is about $1N/m^2$.

Wen et al proposed an Ektacytometry with PBS suspending medium instead of dextran one(4), in fact, RBC hemorheology was studied through the orientation and small deformation of RBCs in an orbit "C=0". According to RBC dynamic behaviour in a shear flow field of low viscosity, we showed(5) that there is an obvious difference between a shear flow field of low viscosity and one of high viscosity. As a suspending medium for Ektacytometry, PBS of low viscosity would be better than PVP buffer of

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high viscosity, especially, in low shear stress area. RBC deformation index DI measured with Ektacytometry during relax process of RBCs in a shear flow field of low viscosity consists of small deformation index $(DI)_d$ and orientation index $(DI)_{or}$, namely, $DI=(DI)_d+(DI)_{or}$. The characteristic time (20—30ms) of deformation is 100—1000 times smaller than that (>20 s) of the orientation. Based on this fact, we can get curves $(DI)_{or}-\dot{\gamma}$ (shear rate) and $(DI)_d-\dot{\gamma}$ from curves DI—t by means of microcomputer treatment and special mathematical treatment. Therefore, more rheology information can be obtained. This is the principle of the new Ektacytometry. The mechanical structure and signal detecting system is the same for both new and old Ektacytometry. The main difference between them lies in the control system to produce different patterns of shear flow field. With this new Ektacytometry, we studied rheology behaviour of RBCs and found that RBC deformability and shape exerted obvious influence upon $(DI)_{or}$. This prompted us to investigate the changes (biochemical or biophysical) of RBC membrane through orientation index $(DI)_{or}$ of RBCs in a shear flow field of low viscosity.

We have studied the influence of macromolecule absorption on RBC surface upon RBC orientation in a shear flow field of low viscosity(7). In this paper, using biochemical *methods (removing surface charge of RBC in different degree by means of* neuraminidase of different doses), we studied the relation between RBC orientation and *RBC surface charge and found that the less the surface charge of RBCs*, the smaller the orientation index of RBCs. The small deformability index in a shear flow field of low viscosity, however does not change evidently. This fact reminds us that orientation *index of RBCs in a shear flow field of low viscosity is more sensitive* than the deformability index $(DI)_d$ *of RBCs in a shear flow field of low viscosity and DI of RBCs* in a shear flow field of high viscosity in reflecting the characteristics of microstructure variance of RBC membrane.

MATERIALS AND METHODS

1. Heparinized rabbit blood of 80ml was taken from the carotid (altogether 8 rabbits were involved in the tests) and centrifuged (3000×g) for 15 min and washed three times with PBS (the same centrifugation condition). The washed RBCs of 1ml were put into four test tubes with 5ml graduation respectively and prepared four blood samples as follows.

(1) control sample. (2) blood sample treated with 10 mu neuraminidase. (3) blood sample treated with 30 mu neuraminidase and (4) blood sample treated with 50 mu neuraminidase.

The four blood samples were incubated at 37℃ under constant shaking for 60 min, washed two times with PBS and after removal of PBS, the above packing RBCs were left to be used.

2. The RBCs of above four blood samples were suspended in PBS (adding albumin 2g/L and 0.02% ATP) and adjusted cell concentration to 2.1×10^7/ml. Each blood sample was divided into 5×6 ml parts.

3. RBCs of above four blood samples were suspended in PVP buffer (osmotic pressure, 290 mosm/kg, pH 7.4 and viscosity, 25 cp) and cell concentration was adjusted to 2.1×10^7/ml). Each blood sample was divided into 5×6ml parts.

4. Four blood samples in 2 were measured with the new Ektacytometry and curves

$(DI)_{or}-\dot{\gamma}$ and $(DI)_d-\dot{\gamma}$ were obtained.

5. Four blood samples in 3 were measured with traditional Ektacytometry and curves $DI-\dot{\gamma}$ were obtained.

6. Electrophoretic mobilities of RBCs of the above four blood samples were measured with an electrophoresis apparatus.

All blood samples was measured five times then average value was taken. Tests for all samples were finished within 2 hours after collection.

RESULTS AND DISCUSSION

1. The data measured with Ektacytometer and electrophoretic apparatus are given in table 1. and table 2. , respectively. The curves $(DI)_{or}-\dot{\gamma}$ and $(DI)_d-\dot{\gamma}$ measured with new Ektacytometry and the curves $DI-\dot{\gamma}$ measured with traditional Ektacytometry are shown in Fig. 1 and Fig. 2, respectively. From table 2. we can see that electrophoretic mobilities of RBC decrease noticeably because of a reduction of surface charge of RBCs after being treated with neuraminidase.
2. The negative surface charge of RBCs results primarily from the presence of ionogenic carboxyl groups of sialic acids on the RBC surface(8). Neuraminidase, which cleaves acids from sialoprotein, markedly reduces the electrophoretic mobility of RBCs. Since RBCs were subjected to different doses of neuraminidase , the microstructure of RBC membrane is injured to different degrees so that the orientation index and the deformation index for RBCs decrease in different degrees. The greater the dose of neuraminidase, the more serious the injury to RBC microstructure, therefore, the smaller the orientation index $(DI)_{or}$ of RBCs in a shear flow field of low viscosity.

Table 1. The measured rheology indexes with two Ektacytometric methods

suspending medium	PBS ($\dot{\gamma}=150\ s^{-1}$)		PVP ($\dot{\gamma}=800\ s^{-1}$)
maximum indexes(%)	$(DI)_{or}$	$(DI)_d$	DI
control	30.4±0.6	9.6±0.4	56.0±0.6
10 mu	28.9±0.4	9.8±0.2	55.5±0.7
30 mu	26.7±0.3	10.2±0.5	51.5±0.5
50 mu	24.1±0.5	10.0±0.5	50.0±0.9

T−test results: $(DI)_{or}$: $P<0.05$ for each neuraminidase dose group with control group.
$(DI)_d$: $P>0.05$ for each neuraminidase dose group with control group.
DI : $P<0.05$ for each neuraminidase dose group except for 10mu group with control group.

Table 2. The electrophoritic mobilities (μm/s/v/cm) of RBC treated with neuraminidase of different doses

control	0.65±0.01
10 mu	0.60±0.02
30 mu	0.55±0.02
50 mu	0.51±0.03

T−test results: $P<0.05$ for each neuraminidase dose group with control group.

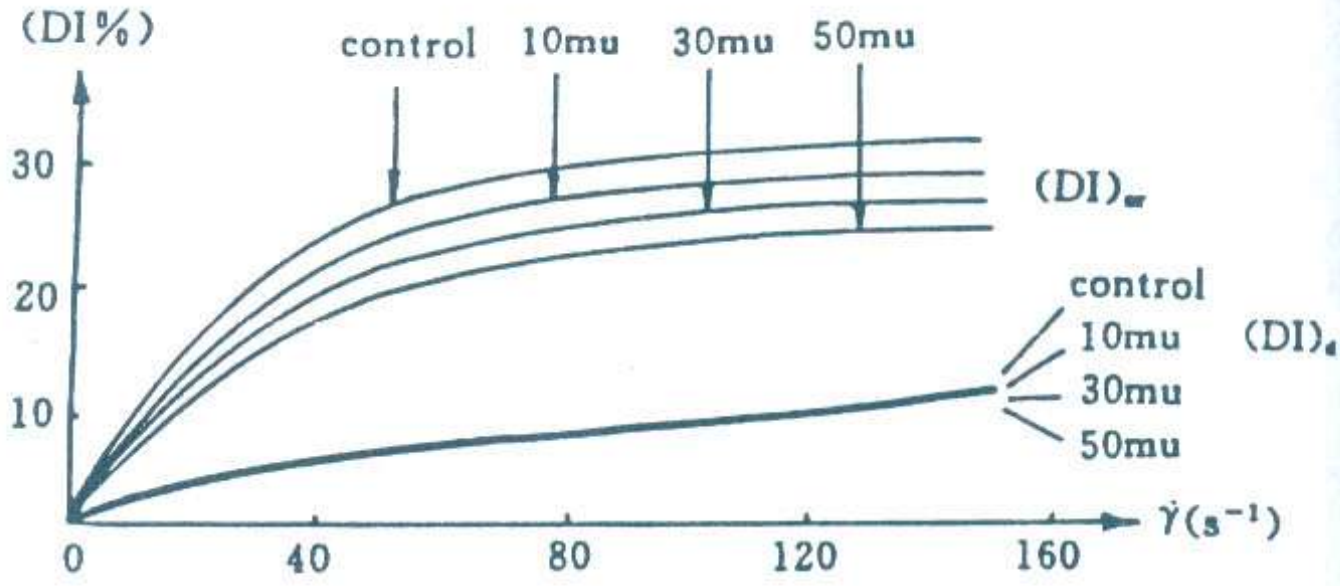

FIG. 1
The measured curves $(DI)_{or}-\dot{\gamma}$ and $(DI)_d-\dot{\gamma}$ in PBS suspending medium

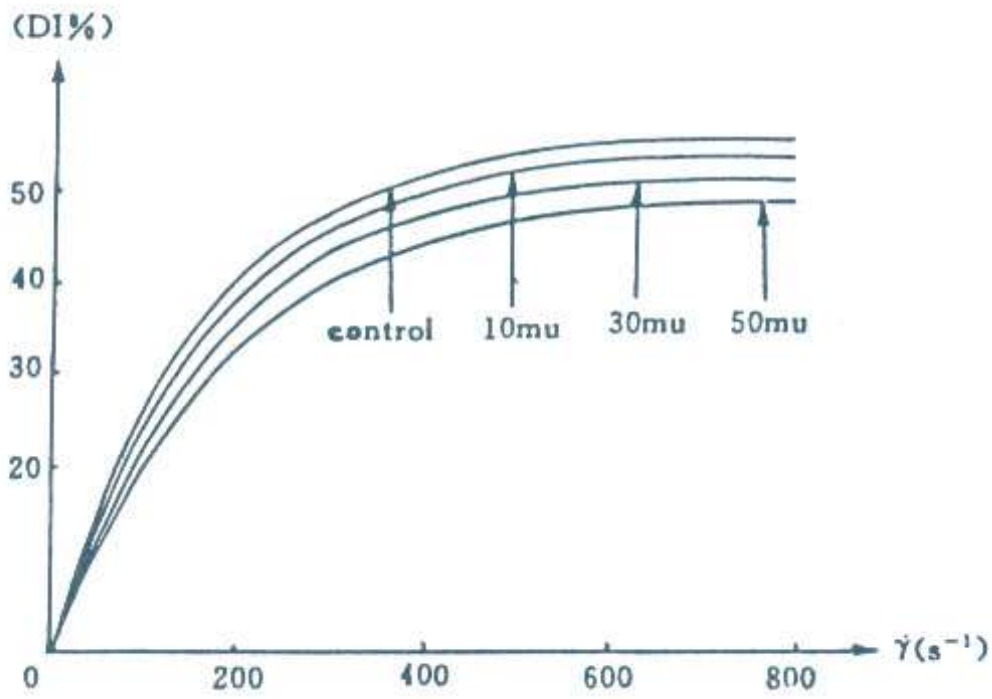

FIG. 2
The measured curves DI—$\dot{\gamma}$ in PVP suspending medium with traditional Ektacytometry

3. The small deformation index $(DI)_d$ reflects primarily isochoric deformation of RBCs subjected to small shear stress in a shear flow field of low viscosity. The experiment showed that $(DI)_d$ has no manifest relation with surface charge of RBCs. This fact reminds us that the damage to the structure of RBCs ($P>0.05$) treated with neuraminidase is so small that the difference between the $(DI)_d$ of RBCs treated with neuraminidase of different doses can not be detected, but $(DI)_{or}$ can reflect the different damage to the structure of RBCs treated with neuraminidase of different doses. In fact, only the non—isochoric deformation index DI of RBCs subjected to great stress in a shear flow field of high viscosity can reflect the variance of the microstructure of RBC membrane.
4. From table 1. and table 2. we can see that, compared with the control group, the DI of RBCs treated with neuraminidase of 50 mu decreases about 11% and the $(DI)_{or}$ of RBCs treated with neuraminidase of 50 mu decreases about 21%. This fact

shows that the new Ektacytometry is more sensitive than traditional Ektacytometry in reflecting change of microstructure of RBC membrane.

5. The orientation index $(DI)_{or}$ describes the orientation at "C=0" orbit for RBCs in a shear flow field of low viscosity. The variance of $(DI)_{or}$ represents the change of the number of RBCs at "C=0" orbit because of the variance of microstructure of RBC membrane.

As Goldsmith's work (3) and Wen's work (4) showed that the number of RBCs at "C=0" orbit in a shear flow field of low viscosity is related to RBC deformability, $(DI)_{or}$ can also reflect RBC deformability. Moreover, $(DI)_{or}$ is more sensitive than DI for estimating the microstructure change of RBC membrane. Therefore, the new Ektacytometry is a useful tool for studying the microstructure of RBC membrane.

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