CLINICAL HEMORHEOLOGY, Vol. 11, pp. 507-510, 1991
0271-5198/91 $3.00 + .00 Printed in the USA.

MINI-REPORT

THE INFLUENCE OF DIFFERENT SUSPENDING MEDIA ON EKTACYTOMETRIC OSMOSCAN CURVE

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(Received 16.1.1991; accepted in revised form 20.4.1991 by Editor F. Liao)

INTRODUCTION

It is well known that erythrocytes (RBC) life-span shortens obviously when their deformability decreases. This change of RBC deformability might be attributed to the decrease of S/V or the increase of intracellular viscosity[1]. Using Ektacytometer and changing osmostic pressure of suspending medium continuously, we could measure RBC deformability at a wide range of osmotic pressure and obtain the Ektacytometric osmoscan curve. The Ektacytometric osmoscan curve for a normal subject is distinct from that for a patient, demonstrated by clinical study [2].

M. R. Clark et al have studied the Ektacytometric osmoscan curve for suspending medium of high viscosity[3]. Considering that DI (deformability index) of RBC is related to the viscosity of suspending medium and PBS buffer can be used as Ektacytometric suspending medium to measure RBC deformability [4], [5], we studied the influence of suspending media PVP and PBS on Ektacytometric osmoscan curve experimentally.

MATERIALS AND METHODS

5 ml fresh heparinized blood was taken from rabbit vein. The RBC were washed three times with PBS (0.12M NaCl, 0.002M Na_2HPO_4, 0.005M KH_2PO_4, PH=7.4, Osmotic pressure 295 mOsm/kg) buffer containing 0.25% human albumin. Taking the PBS buffer or 15% PVP (polyvinylpyrrolidone, mol wt 40000) buffer as Ektacytometric suspending media (each 50 ml), the concentration of RBC suspension was adjusted to 2×10^7/ml.

Another two blood samples for suspending media PBS and PVP (osmostic pressure 800 mOsm/kg and 488 mOsm/kg respectively) were used to study RBC deformability versus time. The whole experiment was completed within 5 hours at 27±1°C.

The experiment consisted of the following approaches:

1. Using Ektacytometer, Osmoscan curve of RBC suspension in PVP was measured over 100-500 mOsm/kg at $\dot{\gamma}$ (shear rate) $=800S^{-1}$.

KEY WORDS: Osmostic presure, deformability, Ektacytometric osmoscan curve.

2. Osmoscan curve for RBC suspension in PBS was measured over 140-800 mOsm/kg range at $\dot{\gamma}$ = 800S^{-1}.

3. DI-$\dot{\gamma}$ curves for RBC suspension in PVP (448 mOsm/kg) and PBS (715 mOsm/kg) at $\dot{\gamma}$= 800s^{-1} were measured every 20 minutes for 5 hours.

4. Using inversion microscope, the photographs of RBC in PBS and in PVP at hypertonic osmolality were taken, at 10 minutes and at 1 hour respectively.

5. Interchanging of suspending media PBS and PVP: RBC was centrifuged from PBS suspension. Then, Putting them into hypertonic suspending medium PVP (480 mOsm/kg), DI-$\dot{\gamma}$ curve was measured with Ektacytometer. Taking RBC from PVP suspension, it was washed three times in PBS, putting them into hypertonic suspending medium PBS (480 mOsm/kg), DI-$\dot{\gamma}$ curve was measured with Ektacytometer.

RESULTS AND DISCUSSION

Ektacytometric osmoscan curves for RBC suspension of PBS and PVP were shown in Fig. 1. The osmoscan curve for RBC suspension of PVP is consistent with that obtained by M. R. Clark et al[3]. However, there is obvious difference between osmoscan curve for RBC suspension of PVP and osmoscan curve for RBC suspension of PBS. Over hypotonic range, the increase of RBC volume leads to a steep reduction of DI. It is different from osmoscan curve for PVP, there is no $(DI)_{min}$ on osmoscan curve for PBS because of hemolysis of great majority of RBC in PBS (Fig. 1). The observation demonstrated that over hypertonic range, initially, intracellular viscosity increased and RBC deformability decreased bacause of osmosis of the water out from the interior of RBC. After about 2 hours, the water osmosed back into RBC from suspension through cell membrane again (the cause is not clear) but the water osmosing back into RBC from PBS was more than that from PVP at the same hypertonic pressure. The loss of intracellular liquid of RBC in PBS was less, the increase of intracellular viscosity for PBS was not larger than that for PVP so that RBC deformability in PBS was better than that for PVP, namely, a more gradual reduction in DI for PBS as shown in Fig. 1.

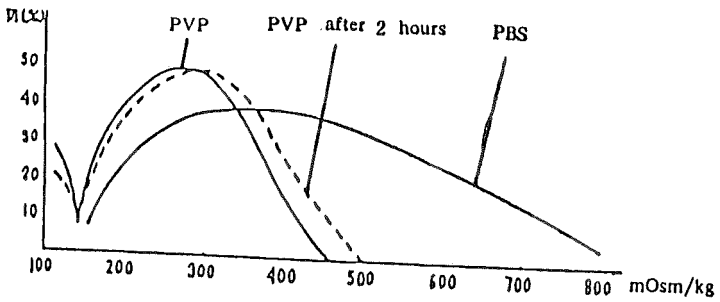

Fig. 1. Ektacytometric osmoscan curves for RBC suspension of PBS and PVP

In addition, we found at the first time that RBC deformability over hypertonic range for both PBS and PVP at constant $\dot{\gamma}$ increased with time (Fig. 2.). Does the difference between osmoscan curves for PBS and PVP reflect the difference of RBC deformability or does this difference result from the different suspending media? As shown in Fig. 3, the experiment of interchanging suspending media shows that RBC becomes more deformable due to the difference of two suspending media. It may be

resulted from the difference of osmosis water for cell membrane in different suspending media. By means of microscope, we found that the size of RBC in hypertonic suspending medium PBS was bigger than that in the same hypertonic suspending medium PVP. The reason might be that the colloid osmotic pressure in suspending medium PVP prevented partially the osmosis of water entering RBC through cell membrane from suspending medium PVP. Nevertheless, a part of water could be osmosed back into RBC through cell membrane from PBS.

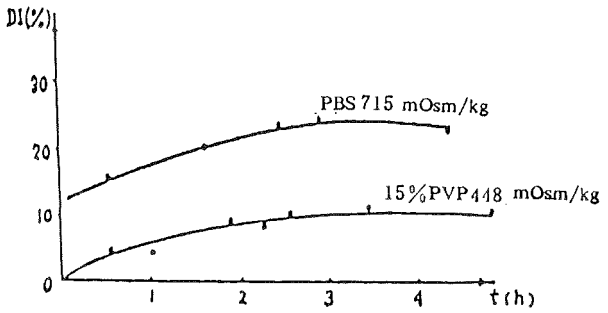

Fig. 2. DI-t curves ($\dot{\gamma}=800s^{-1}$) of hypertonic range for suspending media PBS and PVP

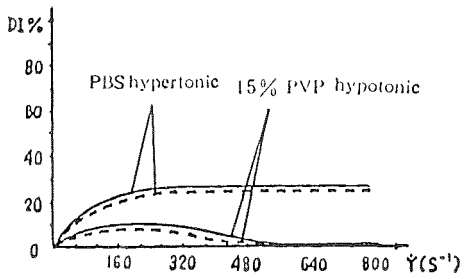

Fig. 3. the experiment of interchanging suspending media

Acknowledgement: This research was supported by the National Natural Science Foundation of China.

REFERENCES

1. HEATH B. P. Deformability of isolated red blood cell membranes. Biochimica et Biophysica Acta., 691 : 211-219. 1982

2. ROSS DW, WATKINS RW. The clinical utility of red blood cell deformability measurements with the Ektacytometer. J Clin Lab Automation, 2 : 348, 1982

3. CLARK M. R. MOHANDAS, N., SOHET, S. B., Osmotic gradient Ektacytometry: Comprehensive characterization of red cell volume and surface maintenance. Blood, 61 (5) : 899, 1983

4. WEN Zong-yao, TAO Zu-lai, CAO shi. The influence of suspending medium viscosity on Ektacytometric measurement of RBC deformability. Proceedings of far eastern conference on medical and biological engineering 194, 1990

5. CHEN sheng, TAO Zu-lai, WEN Zong-yao, CAO shi, Deformation and Orientation: How to get more information from Ektacytometry, Proceedings of far eastern conference on medical and biological engineering214, 1990