Effect of *Qinglongyi* Polysaccharides on Complex Mobility of Erythrocytes in S_{180} Mice

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Abstract: **Objective** To study the effect of *Qinglongyi* polysaccharides (QP) in the exocarp of *Juglans mandshurica* on the complex mobility of erythrocytes in S_{180} mice. **Methods** Erythrocytes were collected and prepared into suspensions, and the complex mobility of cells was measured using high performance capillary electrophoresis (HPCE). Optimized experimental conditions were as follows: 50 cm × 75 μm capillary, buffer for electrophoresis; phosphate solution containing hydroxypropylmethyl cellulose (0.1 mol/L, pH 7.4), injection pressure 3.448 kPa, injection time 10 s, separation voltage 20 kV, and column temperature 25 ℃. **Results** The migration time of erythrocytes in S_{180} mice was longer than that in normal mice, which was 18.09 min for the model group and 12.11 min for the control group, and the complex mobility of erythrocytes in S_{180} mice was lower than that in normal mice, which was 0.92×10^{-4} $cm^2/(V \cdot s)$ for the model group and 1.38×10^{-4} $cm^2/(V \cdot s)$ for the control group. It was also found that S_{180} mice treated by QP could shorten the migration time and increase the complex mobility of erythrocytes. **Conclusion** QP could improve the complex mobility of erythrocytes in S_{180} mice, and HPCE could be used as a powerful tool for determining the physiological state and functions of erythrocytes.

Key words: complex mobility; erythrocyte; polysaccharide; *Qinglongyi*; S_{180} mice
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Introduction

The electrophoretic mobility is one of the parameters describing the surface charges on cells (Sheremet' and Sheremet', 2003). Many diseases, such as tumor, rheumatism, and certain types of inflamemation, could lead to changes in the electromigration speed of erythrocytes, which is used for the clinical diagnosis (Kuo and Chen, 2007; Purlo *et al*, 2005). When using HPCE in the diagnosis, it only needs to compare the differences of peak time between the sick and the normal people. By now, there are still few study reports on differences of erythrocyte mobility between cancer and healthy organisms (Ji *et al*, 2007).

Qinglongyi is the exocarp of immature fruit of *Juglans mandshurica* Maxim. (Juglandaceae, walnut in English). It is frequently used in folk medicine in China with broad pharmacological effects. In recent years, *Qinglongyi* has been found to be widely used in clinic, mainly in antitumor therapeutics. Polysaccharide is one of the active components in *Qinglongyi* and is composed of galactosyl, glucosyl, arabinosyl, rhamnosyl, and fructosyl residues (Ji, Chen, and Ji, 2006). Many researches showed *Qinglongyi* polysaccharide (QP) had antitumor effect and could enhance the immunological functions of erythrocytes in S_{180} mice as well as improve physiological and biochemical functions (Ji *et al*, 2007; Ji, Xiao, and Ji, 2008).

In this study, S_{180} mice were selected as the object and HPCE was used to measure the effect of QP on the complex mobility of erythrocytes in S_{180} mice. According to the changes in migration time and complex mobility of erythrocytes in normal organism and tumor-bearing organisms, HPCE will be a powerful method for clinical diagnosis of disease in future.

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Materials and methods

Animals and tumor cells

Kunming mice, weighing (20.0 ± 2.0) g, half male and female, were provided by the Section of Laboratory Animals, Harbin Medical University (China); S_{180} sarcoma was purchased from Heilongjiang Cancer Hospital (China).

Reagents

Qinglongyi polysaccharide from *Juglans mandshurica* Maxim. with the purity of 76.08% was provided by Center of Research and Development on Life Sciences and Environmental Sciences, Harbin University of Commerce (China). Saline was purchased from Harbin Sixth Pharmaceutical Factory, and HCl, NaOH, dipotassium phosphate, monopotassium phosphate, and DMSO were all purchased from Tianjin Chemical Reagent Co., Ltd. (China). Hydroxypropylmethyl cellulose (HPMC) was purchased from Shanghai Bangcheng Chemical Co., Ltd. (China).

Apparatuses

P/ACE System MDQ capillary electrophoresis and capillary (50 cm × 75 μm) were from Beckman (Duarte, USA). Centrifuge was from Beijing Medical Centrifuge Factory (China).

Establishing mice tumor model

Abdominal dropsy of S_{180} mice was drawn in aseptic condition and diluted with saline. Each mouse was inoculated at armpit with 0.2 mL dropsy.

Grouping and drug administration

Mice were randomly divided into five groups with 10 mice in each group. The five groups consisted of control, model (treated with saline), and three experimental groups treated with QP at doses of 25, 50, and 100 mg/(kg·d), respectively. Drugs were given 24 h after inoculation, and each mouse was administered with the dose of 0.2 mL once a day for continuous 7 d. Blood was drawn from the retrobulbar venous sinus of each mouse after the drug administration and heparin was added as an anticoagulant.

Preparation of cell suspension

Fresh blood was drawn and mixed with 3-fold volume of PBS (0.1 mol/L, pH 7.4). After centrifugation at 1500 r/min for 5 min, the sample was rinsed for three times. A total of 100 μL of erythrocyte residue was drawn and diluted with 3 mL PBS (0.1 mol/L, pH 7.4) containing 0.2% HMPC. Using blood counting plate, the concentration of cell was determined to be 1×10^7 cell/mL.

Erythrocytes complex mobility analysis

Voltage: 10, 15, 20, and 25 kV were tested for optimization. The results showed that at the condition of 20 kV, the electric current was steady and the peak was sharp without inference. The strength of electric field was of 500 V/cm.

Concentration and pH of PBS: Phosphate buffer with concentration of 0.05, 0.1, and 0.2 mol/L (all concentration with pH 7.0, 7.2, 7.4, and 7.6) was tested for optimization. The results showed at the condition of 0.1 mol/L and pH 7.4, there was basically no hemolysis, and good peak forms were obtained.

Other conditions: Capillaries with ID of 75 μm and an effective length of 40 cm. Samples were injected in the positive direction, with a pressure of 3.448 kPa, injection time of 10 s, a separation temperature of 25 ℃, and a detection wavelength of 210 nm. Cleansing liquid: 0.1 mol/L HCl and 0.1 mol/L NaOH, deionized water.

Measurement: Electroosmosis was carried out with DMSO as a marker, hydrodynamic injection at 3.448 kPa, injection time of 10 s, and detection wavelength of 210 nm.

Data processing

The data were processed using SPSS15.0. All results for various groups were expressed as $\bar{X} \pm s$. Variance test was used to compare the samples.

Results

At first, we optimized the experimental conditions and the samples were separated effectively with little heat and few interference peaks. The conditions were that: 50 cm × 75 μm capillary and 0.1 mol/L pH 7.4 phosphate solution buffer containing hydroxylpropylmethyl cellulose, with injection pressure 3.448 kPa, injection time 10 s, separation voltage 20 kV, and column temperature 25 ℃. Secondly, it was found there were obvious differences in migration time and complex mobility of erythrocytes between tumor-bearing and normal organisms. The migration time of erythrocytes in tumor-bearing organisms was longer than that in normal organisms, which was 18.09 min for the model group and 12.11 min for the control group, and the complex mobility of erythrocytes in tumor-bearing organisms was lower than that in normal

organisms, which was 0.92 × 10^{-4} $cm^2/(V·s)$ for the model group and 1.38 × 10^{-4} $cm^2/(V·s)$ for the control group. It was also found that the migration time and complex mobility of erythrocytes with the tendency to normality in tumor-bearing organisms treated by QP could be improved (Table 1 and Fig. 1).

Table 1 Electrophoretic complex mobility of erythrocytes (n = 10, $\bar{X} \pm s$)

Groups	Dose / ($mg·kg^{-1}$)	Migration time / min	Complex mobility / ($cm^2·V^{-1}·s^{-1}$)
control	—	12.11 ± 0.52**	1.38 × 10^{-4} ± 0.06 × 10^{-4}**
model	—	18.09 ± 0.69	0.92 × 10^{-4} ± 0.04 × 10^{-4}
QP	25	14.68 ± 0.63**	1.14 × 10^{-4} ± 0.05 × 10^{-4}**
	50	14.03 ± 0.58**	1.19 × 10^{-4} ± 0.03 × 10^{-4}**
	100	13.06 ± 0.41**	1.28 × 10^{-4} ± 0.06 × 10^{-4}**

**$P < 0.01$ *vs* control group

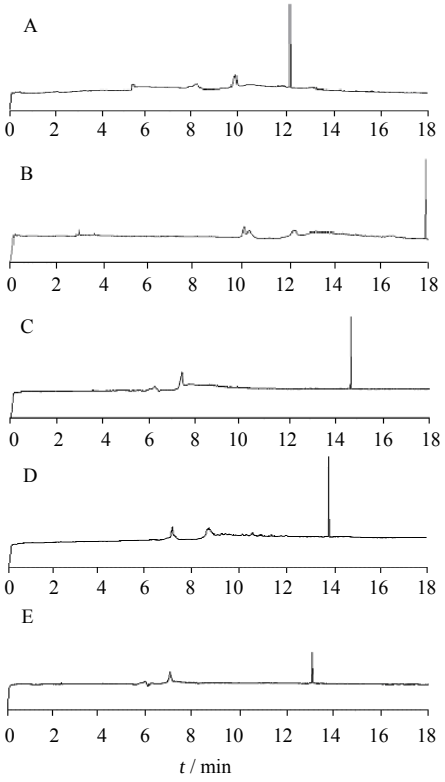

Fig. 1 HPCE of erythrocytes in control (A), model (B), 25 (C), 50 (D), and 100 $mg·kg^{-1}$ (E) QP groups

Discussion

Electrophoretic mobility is the average electrophoresis velocity of ions in a field-intensity unit. The electrophoretic mobility of cells is one of the parameters describing the surface charges on cells. Cells usually carry negative electric charges on their surface and could be treated as simple globular charged particles. When the property of the medium is constant, the electrophoretic mobility of cells mainly depends on their effective electrical charges, size, and shape. It is determined by the density of the electric charges on cell surface (Matyushichev, Shamratova, and Akhunova, 2004). The changes in density and nature of the electric charges on cell surface are mainly caused by the changes in the cell membrane structure, resulting in the changes in the content of neuraminidase, AEP activity, and the function of sodium pumps. These changes lead to a decrease in the potential across the cell membrane, which eventually leads to the change in electrophoretic mobility (Matyushichev and Shamratova, 2005; 2006).

Electroosmosis, or electro-osmotic flow (EOF), is the phenomenon that ionized liquid locating in the electric double layer (EDL) drags the electrically neutral liquid under applied external electric field. Due to the existence of EOF, we detect the complex mobility of erythrocytes in actual experiments, and the complex mobility is the vector sum of electrophoretic mobility and electroosmotic mobility (Krylov and Deryugina, 2005). The results showed that the complex mobility of erythrocytes in normal mice was higher than that in tumor-bearing mice. But the complex mobility of erythrocytes in tumor-bearing mice could be increased markedly after QP treatment and gradually trend to normal, which was markedly compared with tumor-bearing mice.

The electroosmotic mobility in the experiment was 2.10 × 10^{-4} $cm^2/(V·s)$, according to the equation: $\mu_H = \mu_{em} + \mu_{os}$. We got the electrophoretic mobility of erythrocytes in mice: The electrophoretic mobility in tumor-bearing mice was −11.8 × 10^{-5} $cm^2·/(V·s)$ and in normal mice was −7.2 × 10^{-5} $cm^2/(V·s)$. Because of the negative charges on erythrocytes, the direction of electrophoretic mobility was reverse, and the result showed the electrophoretic mobility of erythrocytes in tumor-bearing mice was higher than that in normal mice, which was in accordance with the report that tumors could increase the speed of electromigration of

erythrocytes (Chen, 2006). After QP treatment, the electrophoretic mobility of erythrocytes was decreased [-9.6×10^{-5}, -9.1×10^{-5}, and -8.2×10^{-5} $cm^2/(V \cdot s)$], which indicated that charging density on erythrocytes was changed and the physiological function of erythrocytes was improved.

Charging density on cell surface plays an important role in cell differentiation, recognition, adhesion, phagocytosis, and transformation. Charging density is one of the important physical parameters in the study of cell structures and functions, and it is used to speculate cell structure and study the interactions between cells and biomacromolecule or other cells (Matyushichev and Shamratova, 2004; Haugg *et al*, 2009). The major charged group on the surface of erythrocyte is sialic acid. Our early experiment showed that sialic acid on erythrocytes in tumor-bearing mice is less than that in normal mice, which means the charge quantity is decreasing, and this trend is in accordance with the complex mobility.

There are many influencing factors in HPCE analysis, such as buffer solution kinds, concentration, pH value, additives, voltage, temperature, and capillary property. As to the buffer solution, mostly the pKa value should be matched with pH value, and the increasing buffer solution concentration could improve the sample separation, but EOF decreased and the migration time was prolonged. Buffer solution with high concentration may also cause much joule heat. The pH value of buffer solution could affect EOF and sample complex mobility. High voltage could improve separation efficiency and shorten migration time, while it could even cause much more heat. As to the temperature, it could affect the viscosity of buffer solution and change EOF. The additives used in buffer solution could inhibit sample adhesion to capillary layer, lessen heat, and make peak better.

In this experiment, we optimized the analysis conditions, finally 0.1 mol/L PBS (pH 7.4) with 0.2 g/L HPMC was selected as electrophoretic buffer, which overcame the problem of absorban hesiveness of erythrocytes and minimized the effect of the heat, and the samples were separated effectively.

To summarize, this experiment proves that there are obvious differences in erythrocyte complex mobility between tumor-bearing and normal organisms, and QP could change the complex mobility in tumor-bearing organisms with the tendency to the condition in normal organisms, since it could change the density of surface charges on erythrocytes. And HPCE could be used as an auxiliary assistant tool for determining the physiological state and functions of erythrocytes.

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