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Study on Hemorheological Properties of Erythrocytes in Asymptomatic Hyperuricemia Rat Model

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ABSTRACT

Background: *Hyperuricemia causes gout syndrome, which can manifest as hemorheological damage. However, hemorheological properties have not been well characterized in asymptomatic conditions.* ***Aims:*** *The present study was aimed to investigate the hemorheological changes in rats with asymptomatic hyperuricemia.* ***Methods and Materials:*** *The rat model was established by intraperitoneal injection of 250 mg/(kg·d) oxonate acid for 8 weeks. In one group of rats, 70 mg/(kg·d) allopurinol was also administrated for the latter 4 weeks. Control rats were administered CMC-Na solution and water.* ***Results:*** *Hyperuricemic rats had higher blood viscosity than control rats did. Moreover, their erythrocytes had reduced deformation index, orientation index, surface charges, and osmotic fragility. Treatment with allopurinol decreased the blood uric acid amounts and improved the hemorheological parameters.* ***Conclusions:*** *Our study suggests that there is deterioration in the hemorheology of hyperuricemic rats and that allopurinol can play a protective role on the deterioration. Therefore, hemorheology can be used as a diagnostic tool in asymptomatic hyperuricemia.*

Keywords: Hyperuricemia, Hemorheology, Blood viscosity, Erythrocyte

INTRODUCTION

Uric acid is the end product of purine catabolism in humans. Several factors such as high-protein food, high cell turnover, and renal failure can lead to high levels of uric acid [1,2]. Hyperuricemia is defined as increased levels of uric acid in the blood (≥416 μmol/L for men and ≥ 357 μmol/L for women); hyperuricemia without symptoms of gout is called asymptomatic hyperuricemia [3]. Given that patients do not experience any discomfort, asymptomatic hyperuricemia is thought to be harmless and lowering the levels of uric acid is not listed in the clinical guideline for treatment [4]. However, recent studies suggest that hyperuricemia may have direct relationships with hypertension and metabolic syndrome, and that it increases the risk for subclinical coronary atherosclerosis [5,6]. Asymptomatic hyperuricemia without comorbidities has been suggested to predict cardiometabolic diseases [7].

Hemorheology parameters are critical for blood circulation and microcirculation. Hyperuricemia is associated with increased blood viscosity [8] and increased number and activation of platelets [9,10], leading to thrombosis and inflammation [11]. However, the effects of hyperuricemia on the properties of erythrocytes themselves are not clear, and no systematic study has explored the effects of asymptomatic hyperuricemia on hemorheology. In this study, we aimed to investigate the hemorheological changes caused by asymptomatic hyperuricemia, so as to provide references for clinical treatment.

METHODS

Reagents

Potassium oxonate was purchased from Shanghai Jinsui Bio-Technology Co. Ltd (Shanghai, China). Allopurinol was purchased from Hefei Jiulian Pharmaceutical Co. Ltd (Hefei, China). Polyvinyl pyrrolidone -K30 (PVP) was provided by Shanghai Chemical Instrument Co. (Shanghai, China). The measurement kit for serum uric acid concentration was obtained from Beijing BHKT Clinical Reagent Co. Ltd (Beijing, China). Other agents were provided by Beijing BD Bio-Tech Co. Ltd (Beijing, China).

Animals and treatment

Male Sprague Dawley (SD) rats weighing 200 ± 10 g (provided by Peking University Animal Breeding Unit) were housed under standard laboratory conditions (25 ± 2°C, a 12-hour light/dark cycle). All rats were allowed to get acclimatized for 1 week before the experiments. The animal protocol of this work was approved by the Ethical Committee of Peking University, Health Science Center. The rats were randomly divided into 3 experimental groups with 8 rats in each group. The hyperuricemic group (OA) was intraperitoneally (i.p.) injected with 250 mg/(kg·d) oxonate for 8 weeks; the oxonate was dissolved in 0.5% CMC-Na solution. The hyperuricemic plus allopurinol group (OA+AP) was additionally administered allopurinol (70 mg/kg·d) intragastrically during the latter 4 weeks. The control group was given CMC-Na solution and water for the same period. All rats were weighed twice a week to adjust the doses of oxonate and allopurinol. Both drugs were prepared and mixed well before use to eliminate experimental error. Before the administration of oxonate, blood samples (200 μL) were drawn from the inner canthus of the rats. During the 2nd, 4th, and 6th weeks of the experiment, blood samples were drawn from the inner canthus of the rats 2 hours after the injection of oxonate. At the end of the experimental period of 8 weeks, all rats were anesthetized by intraperitoneal injection of 100 mg/kg ketamine and 5 mg/kg xylazine 2 hours after the final injection of oxonate. Blood was taken from the right ventricle of the rats.

Measurement of serum uric acid

Blood samples were coagulated naturally for 1 hour and centrifuged at 1000 × g for 10 minutes. The serum was collected and stored at −20°C until further analysis. The serum uric acid concentration was measured by the colorimetric uricase method using a commercial kit [12-14].

Measurement of blood viscosity

The blood samples were anticoagulated by 2% heparin and used for the measurement of whole blood viscosities in an automatic cone-plate viscometer (LBY-N6C, Precil, China). The viscosities at high (150 s^{-1}), medium (60 s^{-1}), and low (10 s^{-1}) shear rates were recorded. The plasma was separated from the anticoagulated blood by centrifuging at 1000 × g for 10 min. The plasma viscosities were measured in a capillary viscometer (LBY-N6A, Precil, China).

Measurement of erythrocyte deformation index (DI), small deformation index $(DI)_d$, and orientation index $(DI)_{or}$

Forty microliters of blood were suspended in 1000 μL of 15% (w/v) PVP buffer (pH 7.4, 295 mOsm/kg, and viscosity 15 mPa·s) to measure DI at shear rates ranging from 50 s^{-1} to 1000 s^{-1}, using an LBY-BX2 Ektacytometer (Precil, China). For the $(DI)_d$ and $(DI)_{or}$ of erythrocytes, 20 μL of blood was suspended in 1000 μL of 3% (w/v) PVP buffer (pH 7.4, 295 mOsm/kg, and viscosity 1.2 mPa·s). $(DI)_d$ and $(DI)_{or}$ were measured at the shear rate 100 s^{-1}.

Measurement of erythrocyte osmotic fragility

Aliquots of 30 μL blood samples were resuspended in 3000 μL of PBS, with osmotic pressures ranging from 0 to 295 mOsm/kg. The erythrocyte suspensions were centrifuged at 1000×g for 6 min after 1 hour of equilibration at 25°C. The transmission rate (Tr) of the supernatant was determined at 540 nm, using a UNICO UV-2000 spectrophotometer (Shanghai, China). Hemolysis rates (Hrs) were calculated using the equation:

$$Hr=[Tr(295) - Tr] /[Tr(295) - Tr(0)] \times 100\%$$ [15].

Measurement of erythrocyte electrophoresis rate

Erythrocytes were adjusted to 2 × 10^6/mL with 0.9% NaCl solution. A cell electrophoresis apparatus (LIANG-100, Shanghai Medical University, Shanghai, China) was used to measure the electrophoresis rate.

Data analysis

Data are expressed as the mean ± standard error (SE). Comparisons of means from multiple populations were made by one factor analysis of variance (ANOVA), using the SPSS 13.0 statistical software. P<0.05 was accepted as statistically significant.

RESULTS

Validation of the hyperuricemia rat model

To prove the presence of hyperuricemia in our rat model, we monitored the serum uric acid level every two weeks (Figure 1). We found that the uric acid level in OA was significantly higher than that in the control from the 2nd week. The level reached the highest in the fourth week, and then declined slightly, but remained significantly high until the end of the experiments. As expected, the uric acid level in OA+AP decreased significantly after four weeks. The serum uric acid level in the control decreased slightly after i.p. injection of CMC-Na, but there was no statistical difference.

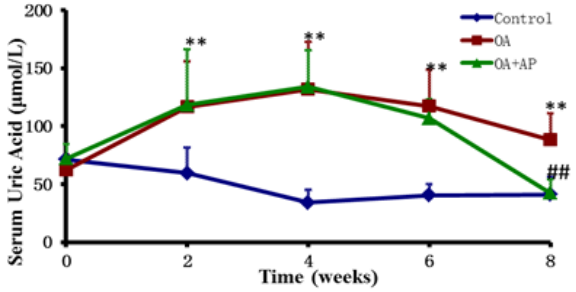

Figure 1 Effects of OA and/or AP on serum uric acid concentration. Data are expressed as the mean ± SE. **P<0.01 vs control, ##P<0.01 vs OA

Increase of plasma and whole blood viscosities in hyperuricemia rats

The plasma and whole blood viscosities of OA were significantly higher than those of the control. The intervention with allopurinol induced the plasma and whole blood viscosities to decrease significantly, compared with those of OA alone (Figure 2).

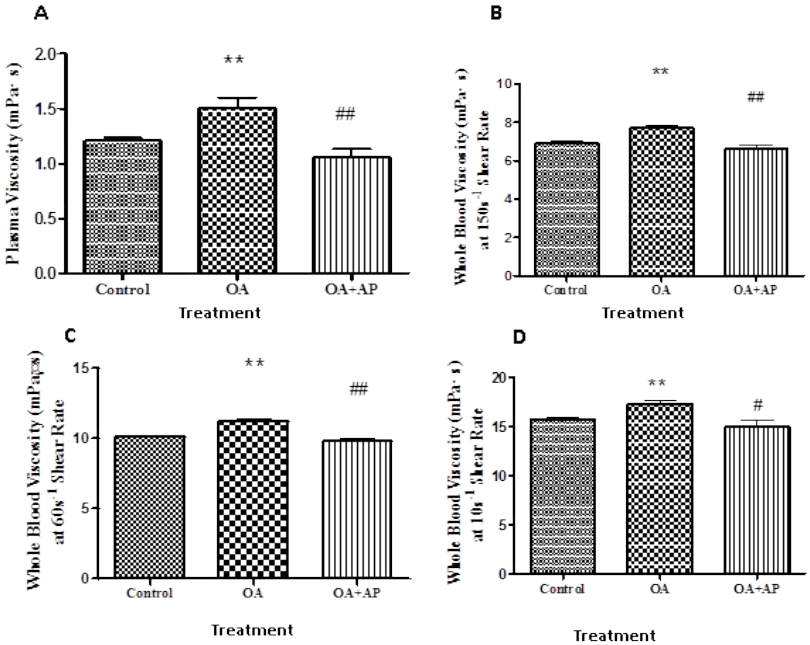

Figure 2 Effects of OA and/or AP on plasma and whole blood viscosity. (A) Plasma viscosity in different groups. Whole blood viscosity at high (B), medium (C), and low (D) shear rates. Data are expressed as the mean ± SE. **P<0.01 vs control, # P<0.05, ##P<0.01 vs OA

Decrease of (DI), $(DI)_d$, and $(DI)_{or}$ of erythrocytes in hyperuricemia rats

We found that DI at each shear rate decreased, but did not change significantly in OA (Figure 3A). The (DI)d of OA and OA+AP was lower than that of the control, with OA+AP showing statistical significance (Figure 3B). There was a remarkably reduction in $(DI)_{or}$ of both OA and OA+AP, as with that of the control (Figure 3C).

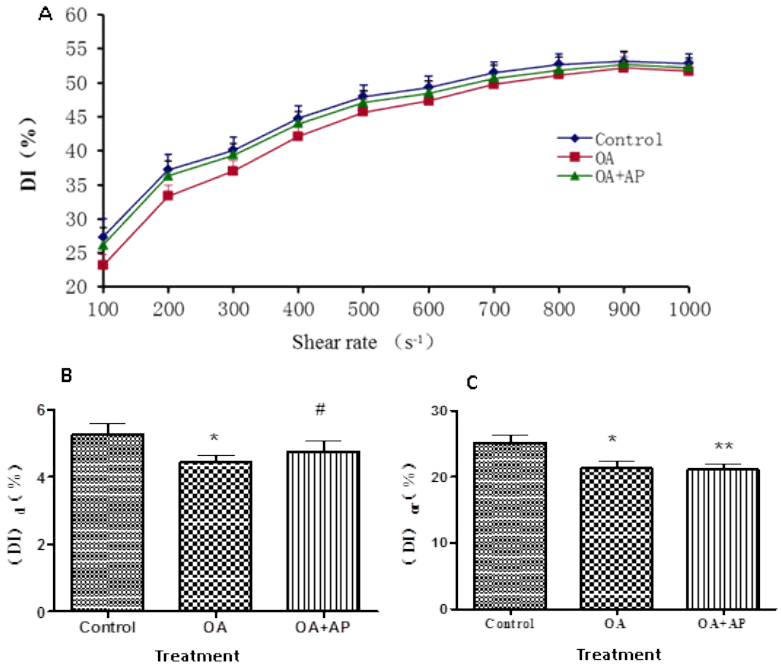

Figure 3 Effects of OA and/or AP on the deformability of erythrocytes. (A) Deformation index (DI), (B) small deformation index $(DI)_d$, and (C) orientation index $(DI)_{or}$. Data are expressed as the mean ± SE. *P<0.05, **P<0.01 vs control; #P<0.05 vs OA

Increase of the haemolysis rate of erythrocytes in hyperuricemia rats

In our experiment, the haemolysis rate was 0% in the isotonic solution and the cells maintained a good integrity. With the decrease of osmolalities in the solutions, the haemolysis rate gradually increased. After a "plateau" period, the hemolysis rate finally reached 100%; all the cells were broken in the solution of 0 mOsm/kg (Figure 4A). Haemolysis rate at 145 mOsm/kg is shown in Figure 4B. The haemolysis rate of OA was significantly higher than that of the control, but that of OA+AP was significantly decreased.

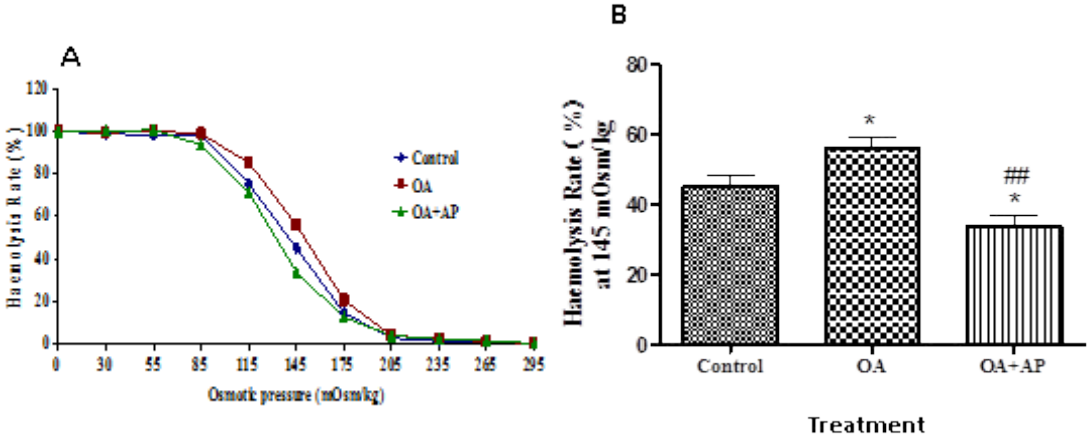

Figure 4 Effects of OA and/or AP on the haemolysis rate of erythrocytes. (A) Change in haemolysis rate with different osmotic pressure. (B) Haemolysis rate at 145 mOsm/kg. Data are expressed as the mean ± SE. *P<0.05 vs control, ## P<0.01 vs OA

Low electrophoresis rate in hyperuricemia rats

The electrophoresis rate in OA was significantly lower than that in the control, whereas that in OA+AP was significantly higher than that in both OA and the control (Figure 5).

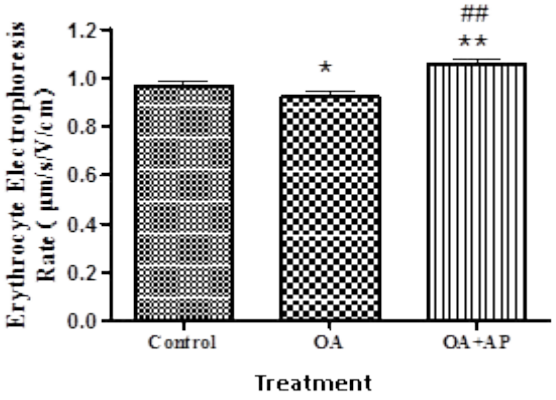

Figure 5 Effects of OA and/or AP on electrophoresis rate. Data are expressed as mean ± SE.
*** P <0.05, **P<0.01 vs control, ## P<0.01 vs OA**

DISCUSSION

In this study, we investigated the effects of hyperuricemia on blood circulation in a rat model. The uric acid levels in the model were twice as much as those in the control. This model has shown the absence of urate crystals in the joints and kidneys, and therefore mimics asymptomatic hyperuricemia [16]. Blood uric acid levels can be lowered when rats are treated with either a xanthine oxidase inhibitor (allopurinol) or a uricosuric agent (benziodarone). Here, treatment with 70 mg/(kg·d) allopurinol was introduced from the 5th week, unlike many other studies where the drug was administered from the beginning [12-14,17,18]. We found a significant reduction in uric acid after treatment with allopurinol.

Hemorheological parameters, especially blood viscosity, had been widely used to predict cardiovascular and cerebrovascular diseases in clinics [19]. In this study, we found that high levels of uric acid were usually accompanied with higher viscosity of blood. Blood viscosity was determined by plasma viscosity and red blood cell characteristics [15]. Indeed, the plasma viscosity increased in hyperuricemia rats. We also observed significant changes in the hemorheological properties of erythrocytes, such as the electrophoresis rate, $(DI)_{or}$, $(DI)_{d}$, and haemolysis rate.

The electrophoresis rate of erythrocytes decreased in hyperuricemia rats, suggesting that the surface charge decreased. Therefore, the repulsive force among cells would decrease and the red blood cells would aggregate. Such aggregates slow the blood flow and increase its viscosity, therefore, reducing the microcirculation perfusion and increasing the incidence of thrombotic diseases.

$(DI)_{or}$ is a rheological index reflecting changes in the membrane microstructure and morphology at the macromolecular level, whereas $(DI)_{d}$ reflects the erythrocyte membrane lipid fluidity [20]. Our results suggest that hyperuricemia reduced the ability of erythrocytes to orient along the flow field, making it disadvantageous in microcirculation perfusion. The morphological change in erythrocytes may be caused by the high levels of uric acid. The haemolysis rate indicates the osmotic fragility of erythrocytes. The fragility is closely related to the state of the cell membrane skeleton. The increase in the haemolysis rate caused by hyperuricemia showed that the osmotic fragility was increased. In fact, the resistance to lower osmotic pressure was changed, suggesting that hyperuricemia might affect the red cell membrane skeleton, and that hyperuricemia could further affect the characteristics of red blood cells and regulate blood viscosity.

CONCLUSION

In summary, our study suggests that asymptomatic hyperuricemia induces high blood viscosity, decreasing the erythrocytes abilities of deformation, orientation, chargeability, and osmotic fragility. Allopurinol treatment decreased the uric acid amount in the blood and, hence, the blood viscosity, and seemed to partially protect the hemorheological

deterioration. Thus, an assessment of hemorheological properties would improve both the early diagnosis and health intervention in patients with asymptomatic hyperuricemia, leading to the reduction of gout and other diseases caused by high uric acid levels. While hyperuricemia is associated with numerous disease states in clinic, our conclusion built on the rat model is difficult to adopt in complicated human disease patients. Establishing a hyperuricemia model system in rodents to study hemorheological properties as well as using a patient treatment model where allopurinol is used to reverse the established disease state is commended.

DECLARATIONS

Acknowledgement

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Competing Interests

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

Authors' Contributions

Kuihua Li and Weijuan Yao performed the experiments and prepared the manuscript contents. Kuihua Li, Xifu Wang, and Xiaobo Tong established the rat model and performed the hemorheology measurements. Atushi Suzuki and Nobuo Suzuki played a part in evaluating the experimental results and preparing the manuscript.

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