Springer

Journal of Mechanical Science and Technology 23 (2009) 868~873

Journal of
Mechanical
Science and
Technology

www.springerlink.com/content/1738-494x
DOI 10.1007/s12206-009-0211-0

Effect of dextran on rheological properties of rat blood †

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(Manuscript Received October 24, 2008; Revised January 12, 2009; Accepted February 5, 2009)

Abstract

There has been little information available on how the use of dextran in vivo influences plasma viscosity and yield stress of blood. Hence, the main purpose of this study is to provide such information when level of red blood cell aggregation in rats was raised to levels seen in normal human blood with administration of Dextran 500. Our results show that plasma viscosity ($P < 0.003$) and yield stress ($P < 0.01$) of rat blood increased significantly after dextran infusion. Whole blood viscosity also significantly increased with the dextran treatment over the entire range of shear rates we measured. The viscosity changes at high shear rates after dextran infusion would be due mainly to elevation of plasma viscosity, whereas the changes at low shear rates would be attributed to red blood cell aggregation induced by the dextran treatment.

Keywords: Dextran; Red blood cell aggregation; Plasma viscosity; Yield stress

1. Introduction

Red blood cell aggregation is present in athletic species and humans, but is absent in sedentary species [1]. Aggregate formation is induced by macromolecular proteins such as fibrinogens and globulins found in the plasma of these athletic animals. Similar aggregation behavior can be observed by the addition of high molecular weight dextran such as dextran 500 or 70 into a suspending medium of red blood cells [2, 3]. Effects of the dextran-induced aggregation on blood flow resistance have been studied extensively, but in vivo and in vitro results are not in agreement. Although red blood cell aggregates are present in both in vivo and in vitro studies under low flow conditions, the distribution pattern of these aggregates in the vessel greatly influences the overall vascular resistance. The favored axial migration of aggregates and subsequent formation of a cell-poor layer near the wall of capillary tubes (30.2 to 132.2 μm ID) in vitro is attributed as a primary cause for the decrease in flow resistance [4]. On the contrary, the frequent branching of the microcirculatory network and constant infusion of red blood cells and aggregates from tributaries in vivo [5, 6] resulted in an absence or reduction of cell-free layer and thus increased vascular resistance [7, 8].

Dextran 500 has been known to be effective in alteration of level of red blood cell aggregation and often used for in vivo and in vitro rheological studies [4, 5, 7, 8]. However, it should be noted that addition of Dextran 500 would change plasma viscosity and yield stress of blood as well. Previous studies [9, 10] have reported that a significant increase in plasma viscosity caused by addition of dextran might play a primary role in elevating whole blood viscosity. Considering red blood cells in plasma as particles in a medium, red blood cell aggregation induced by dextran causes an increase in effective particle size [11, 12], which may result in elevation of yield stress of blood.

Although many in vivo rheological studies have been performed with use of dextran, there has been

† This paper was recommended for publication in revised form by Associate Editor Haecheon Choi
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little quantitative information available on the change in plasma viscosity or yield stress of blood due to the dextran addition. Hence, the main purpose of the present study is to provide such information. In this study, the level of red blood cell aggregation in rats was raised to that seen in normal human blood with administration of Dextran 500. Blood samples from rats before and after dextran infusion were withdrawn and rheological properties of the rat blood including whole blood viscosity, plasma viscosity, and yield stress were investigated with a conventional viscometer and a newly developed scanning capillary rheometer [13, 14].

2. Materials and methods

2.1 Human blood preparation

Human blood was obtained from two healthy male donors aged 23 and 24. Venous blood samples of about 3 ml were withdrawn into vacutainer tubes containing an anticoagulant (Na-heparin 100 IU/ml). All the human blood samples were stored at approximately 5 ºC and examined at room temperature of 25 ºC within 5 hours after the blood collection.

2.2 Animal preparation

Six male rats weighing 327.4 ± 41.5 g were used in the present study. Animal handling and care were provided in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals (NIH, National Research Council, 1996). Rats were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital sodium (Abbott). The animal was placed on a heating pad to maintain a body temperature of 37^{o}C during the preparation. A tracheal tube (ID = 1.57 mm, OD = 2.08 mm) was inserted to aid ventilation. The carotid artery was catheterized for blood withdrawals and the jugular vein was catheterized for administration of Dextran 500. All catheters were filled with a solution of heparinized saline (30 IU/ml) to prevent clotting.

2.3 Hematocrit and level of red blood cell aggregation determination

Blood samples of approximately 35 μl were used for hematocrit measurement. Hematocrit (volume fraction of red blood cells in whole blood) was determined after centrifugation at 13000 rpm for 5 min with a microhematocrit centrifuge (Sigma 1-14, Goettingen, Germany). The degree of red blood cell aggregation was determined from duplicate measurements on a 20 μl blood sample with a photometric rheoscope (Myrenne Aggregometer MA1, Roentgen, Germany). The aggregation index (M) based on the 10-s setting was used for the present study.

2.4 Alteration of red blood cell aggregation

To raise the degree of rat red blood cell aggregation to levels of normal human blood, Dextran 500 (200 mg/kg body) was dissolved in saline (6%) and infused in 50mg/kg increments over the course of 2-3 min. This represents a plasma dextran concentration of approximately 0.6%. Blood samples of approximately 0.1 ml were withdrawn from the carotid artery catheter for measurement of the degree of red blood cell aggregation during both the control period and after infusion of Dextran 500. In addition, there was no discernable adverse reaction (e.g., visible swelling of the limbs) to the dextran infusion in any of the rats used for this study. All the physiological and rheological measurements were taken 15 min after dextran infusion.

2.5 Viscosity and Yield Stress Measurement

Rat blood samples of approximately 3 ml were withdrawn from the carotid artery catheter for measurements of blood viscosity and yield stress before and after infusion of Dextran 500. A scanning capillary-tube-viscometer and the data analysis techniques described previously [13, 14] were employed to determine the viscosity and yield stress of blood at room temperature of 25ºC. The scanning capillary-tube-viscometer was capable of completing each measurement within 2-3 minutes without addition of any anticoagulants. Plasma was obtained by centrifugation at 2500 g for 15 min with a centrifuge (Sigma 2-6, Goettingen, Germany), and a cone-and-plate viscometer (Brookfield DV-II+, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) was used for the plasma viscosity measurement.

2.6 Statistical Analysis

An unpaired t-test was used to determine differences in aggregation index (M), plasma viscosity and yield stress of rat blood before and after dextran infusion. Data are reported as means ± SD and $P < 0.05$

was considered statistically significant.

3. Results and discussions

Hematocrit and red blood cell aggregation index (M) of the human blood samples was 45.4 ± 0.10% and 13.3 ± 1.9, respectively. In case of rat blood, hematocrit was 41.2 ± 1.5% and dextran infusion increased the M value from 1.5 ± 1.4 to 12.2 ± 2.2 with a significant difference ($P < 0.004$). There was no significant difference between the M values of human blood and dextran-treated rat blood, indicating that dextran plasma concentration of approximately 0.6% in rats could simulate the aggregation levels seen in normal human blood.

Fig. 1 shows two typical viscosity results of rat blood on a log-log scale with and without dextran treatment. As expected, rat blood viscosity was highly shear-dependent and elevated with reducing shear rate. The difference between the two blood viscosities appeared small at high shear rates and became more pronounced as shear rate decreased. Fig. 2 shows mean values of yield stress and plasma viscosity before and after dextran infusion, respectively. Yield stress of blood significantly increased from 5.8 ± 0.8 to 23.2 ± 3.2 mPa after dextran treatment ($P < 0.01$). Similarly, there was a significant difference ($P < 0.003$) in plasma viscosity before (1.29 ± 0.03 mPa·s) and after dextran infusion (1.53 ± 0.08 mPa·s).

The results shown in Fig. 1 indicate that dextran addition significantly influences whole blood viscosity over the entire range of shear rate. In particular, pronounced effects of the dextran-induced aggregation on the blood viscosity can be seen at low shear rates. Figure 3 shows the changes of whole blood viscosity at different shear rates before and after dextran infusion. There was a significant increase (> 110%, $P < 0.02$) in whole blood viscosity at the shear rate of 2 s^{-1} after dextran infusion, while at a shear rate of 300 s^{-1} the viscosity increased by about 26% ($P < 0.03$) after the dextran treatment. This phenomenon would be mainly attributed to the fact that the tendency of red blood cell aggregation increases as shear rate decreases. In general, the number and size of red blood cell aggregates increase with reduction of shear rate, which would enhance heterogeneity in blood flow and lead to additional energy loss, resulting in elevation of blood viscosity.

In the present study, quantitative information on effects of red blood cell aggregation on yield stress of blood was obtained when the aggregation level was raised to normal human levels. The result showed that the yield stress increased by about 300% from 5.8 to

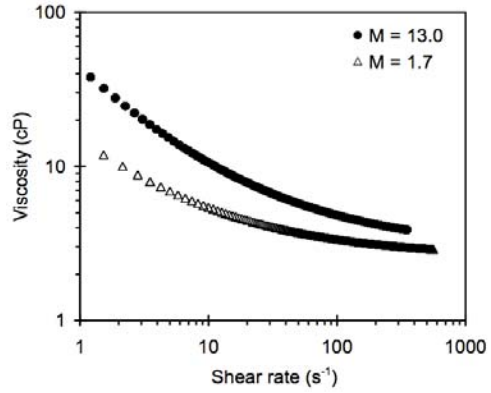

Fig. 1. Two typical viscosity results of rat blood with dextran treatment (●) and without dextran treatment (Δ) at 25 ºC. M values indicate the degree of red blood cell aggregation. Hematocrit of rat blood was 41.2 ± 1.5%. 1 cP = 1 mPa·s.

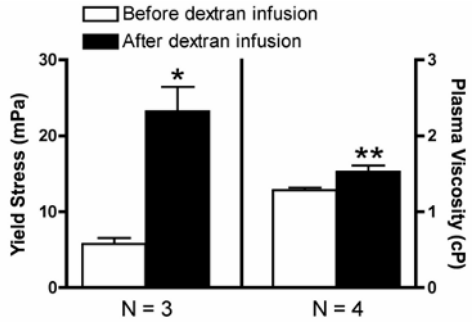

Fig. 2. Mean values of yield stress of rat blood and plasma viscosity before (open bars) and after dextran infusion (solid bars). Hematocrit of rat blood was 41.2 ± 1.5%. Values are mean ± SD. 1 cP = 1 mPa·s. * $P < 0.01$. ** $P < 0.003$.

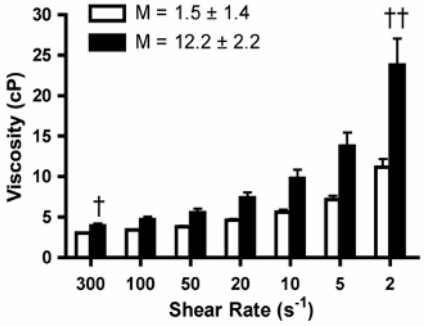

Fig. 3. Mean values of whole blood viscosity at different shear rates before (open bars) and after dextran infusion (solid bars). Values are mean ± SD. 1 cP = 1 mPa·s. † $P < 0.03$ for shear rates ≥ 5 s^{-1}. †† $P < 0.02$ for 2 s^{-1}.

23.2 mPa after the dextran addition. Based on this result, it appears that the yield stress of blood is highly correlated with the level of red blood cell aggregation. The yield stress value of rat blood at normal human aggregation levels obtained in this study was in the yield stress range for human blood reported in previous studies. Charm and Kurland [15] found a yield stress of 9.5-40 mPa for human blood with hematocrits from 18% to 56%. Yield stress was reported to increase with hematocrit, being of the order of 1 mPa at 30% hematocrit to 30 mPa at 95% [16]. Lee and coworkers [17] showed that yield stress of blood was elevated with increasing extent of aggregation as also found in this study. High yield stress with increasing level of red blood cell aggregation may lead to plug formation in arterioles, resulting in an increase in energy spent at the capillary entrance for disaggregation of red blood cells [18]. As a consequence, the dextran-induced aggregation and yield stress can cause the cessation of red blood cell flow in some capillaries [18]. House and Johnson [19] noted the stop-start nature of venular flow and its physiological influence. To restart the blood flow in venules, the venular pressure should be high enough to overcome the yield stress of blood. If the yield stress is high, the duration of the stasis may become longer, resulting in tissue damage.

High molecular weight polymers such as Dextran 500, which is often used to manipulate the level of red blood cell aggregation in vivo, may significantly increase plasma viscosity when added into blood. There was a significant increase (~20%) in plasma viscosity after raising the aggregation level up to the levels seen in normal human blood as shown in Fig. 2. As indicated in Fig. 3, whole blood viscosity was significantly affected by Dextran 500. The dextran effect on whole blood viscosity seemed much larger at low shear rates compared to that at high shear rates. As discussed above, the main reason for the whole blood viscosity increase at low shear rates might be red blood cell aggregation induced by dextran infusion. The change in the blood viscosity at high shear rates might be attributed to the plasma viscosity change after dextran infusion. Whole blood viscosity significantly increased after dextran infusion by about 37% ($P < 0.03$) and 26% ($P < 0.03$) at shear rates of 100 and 300 s^{-1}, respectively. At such high shear rates, the aggregation of red blood cells no longer plays an important role since the high shear flow leads to break-up of aggregates [20]. Thus, the higher blood viscosity after the dextran treatment at such high shear rates would be due mainly to the elevation in plasma viscosity. It should be noted that the increase in plasma viscosity due to dextran addition is independent of shear rate [10]. Therefore, if we assume that this increase is a constant for the entire range of shear rates studied, any further change in whole blood viscosity in addition to the increase in plasma viscosity may be attributed to the contribution by red blood cell aggregate formation and is a useful indicator of the relative degrees of aggregation present at the different shear rates.

The physiological importance of the viscosity and yield stress of blood is highlighted by the fact that they are the major determinants of endothelial shear stress in the microcirculation. Wall shear stress is determined by the product of the velocity gradient (shear rate) adjacent to the vessel wall and blood viscosity. Red blood cell aggregation influences distribution of red blood cells in microcirculatory vessels [21]. The yield stress, which is highly correlated with the level of red blood cell aggregation, alters the bluntness of the velocity profile [22]. Wall shear stress is one of the principal stimuli for the release of a vasodilator, nitric oxide (NO), which plays a critical role in regulating vascular smooth muscle tone and maintaining vascular resistance [23-26]. Therefore, the quantitative information obtained in this study on the changes in viscosity and yield stress of blood due to red blood cell aggregation would be useful to better understand vascular resistance in the microcirculation.

Acknowledgment

The authors thank Peng Kai Ong for valuable discussions on this study. This work was supported by NUS Grant R-397-000-048-133 and performed with funding from the Korea Institute of Industrial Technology (KITECH).

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