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Effect of Dextran 500 on Radial Migration of Erythrocytes in Postcapillary Venules at Low Flow Rates

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Abstract: Recently, we reported that collision efficiency (fraction of total collisions that result in the formation of aggregates) between red blood cells was an important factor in the formation of aggregates in postcapillary venules. In the present study, we focus on how high molecular weight dextran influences the overall radial migration trend of red blood cells in the postcapillary venule along a longitudinal distance of 50 μm from the bifurcation which would in turn affect collision behavior of these cells. A radial migration index, which defines the extent of radial migration of individual cells relative to the vessel center, was found to have a larger magnitude after infusion of dextran (1.9 ± 2.73) compared to that before dextran infusion (1.48 ± 3.89). This implied that dextran-induced aggregation might provide an external force to actively move cells towards the centerline of the vessel, which could contribute to the greater number of red blood cells participating in collision (16% increase) and aggregate formation. Further analysis of the collision behavior of individual red blood cells revealed that collision frequencies of individual cells decreased from a wide range (1 to 14) to a narrow range (1 to 5) after dextran treatment, indicating the alteration of collision behavior of red blood cells by the presence of aggregates along the flow stream.

Keyword: Radial Migration, Erythrocytes

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1 Introduction

Erythrocyte aggregation is a prominent feature of humans and other athletic species but is absent in sedentary animals [25]. In the venous microcirculation, the occurrence of red blood cell aggregation under low flow conditions influences venous vascular resistance [11,29]. These studies on venous resistance had shown that in vivo hemodynamics is under the influence of erythrocyte aggregation and significantly affects normal physiological functions.

Physical interactions in the form of collisions between red blood cells are necessary for the formation of red blood cell aggregates. In our previous study [19], collision efficiency between red blood cells was found to correlate well with the number of aggregates formed in different regions of the postcapillary venule. With reduction in blood flow, more numerous and larger aggregates are seen in the flow stream [10].

During blood flow in microcirculatory vessels, the presence of the vessel wall and the shear rate gradient will lead to an asymmetry of forces acting on the red blood cells. Forces that tend to displace the cells and aggregates towards the tube center are offset by dispersion forces such as shear-induced particle diffusion that arises from the cell-cell and cell-wall interactions during shear flow [6]. However, the force balance between these forces may be influenced by the aggregability of the red blood cells. In addition, the net effect of these forces also depends greatly on the distance over which this process can take place and the characteristic time required for radial migration [1,2,24].

Macromolecules such as dextrans of high molecular weight have been extensively used in previous studies to induce or raise the aggregabil-

ity of red blood cells to desired test conditions [3]. Two important models have been proposed for the red blood cell aggregation, which are the bridging model [12,16,26] and depletion model [4,22]. The bridging hypothesis postulates that macromolecules may be adsorbed onto the surface of more than one cell, leading to a bridging effect between cells while the latter postulates that lowering the macromolecule concentration in the vicinity of red blood cells leads to an osmotic gradient which draws fluid away from the intercellular gap and enhances the tendency for adjacent cells to come together. It is clear that with both mechanisms, macromolecules have a common role of reducing the effective distance between red blood cells, thus possibly increasing the likelihood of collisions between the cells due to their close proximity and favoring the formation of aggregates.

In our previous study [19], we compared the spatial distributions of cell collisions along different regions of the postcapillary venule. It was found that the collision frequency in different regions of the venule is independent of red blood cell aggregation since collision frequencies were similar both in the presence or absence of aggregation. In the initial region (0-15 μm from bifurcation) of the venule, the flow streamlines did not meet except at the vessel center where they overlapped. Therefore considerable separation may exist between cells in this region and collision frequency was found to be the lowest although radial displacement was the largest. Such low collision frequency was observed in this region both before and after dextran infusion, which suggested that the same physical forces were present under both conditions at the entrance region of the venule. At a distance of 15-20 μm downstream from bifurcation, a sharp rise in collision frequency of red blood cells was observed for both normal and dextran-treated blood. This could be attributed to the fully developed flow in this region where streamlines started to converge and move the adjacent red blood cells closer to one another, which greatly increased their probability of colliding. Therefore it is possible that attractive forces induced by dextran are only obvious after flow becomes fully developed in the middle and distant region of the venule.

The aim of this study is to better understand how high molecular weight dextran (Dextran 500) alters the radial displacement of red blood cells under low flow conditions and affects the collision behavior of these cells as they move along the postcapillary venule. The collision behavior of red blood cells was analyzed in terms of fraction of cells participating in collision as well as the collision frequency of individual cells. We hypothesize that in the presence of aggregation induced by dextran, more red blood cells migrate radially towards the tube center, which would increase their probability of contact with each other and result in more cells colliding. However subsequent collision behavior of formed aggregates with remaining cells may be different as compared to that of single cells which are predominantly present in the absence of aggregation.

2 Materials and methods

2.1 Experimental setup and animal preparation

We utilized video recordings obtained from our previous studies [17,19] of red blood cell aggregation in postcapillary venules of rat spinotrapezius muscles for the analyses in this study. A detailed description of the experimental setup and animal preparation can be obtained in those reports and will not be repeated here. Animal handling and care were provided in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 1996). The setup included an intravital microscope transilluminated with a 100-W mercury lamp and $\times$40 water immersion objective. This system provided a full-frame field of 200 $\times$ 200-μm^2 view with a spatial resolution of $\sim$0.4 μm. A high-speed camera was used to record the movements of the red blood cells at frame rates of up to 2,250 s^{-1}.

2.2 Experimental procedures

Data shown in this study were obtained at reduced arterial pressure before and after infusion of Dex-

tran 500 (200 mg/kg). The dosage used was sufficient to induce red blood cell aggregability at the level seen in normal humans. The degree of aggregability was determined with a Myrenne Aggregometer using a 0.035-ml blood sample based on the 10-s setting. To obtain clear and distinct images of both individual cells and aggregates in the postcapillary venules, we reduced systemic hematocrit by exchanging the same amount of blood with autologous plasma (1-2 ml) from a donor animal. To reduce the arterial pressure to $\sim$50 mmHg, an average of 1.0 $\pm$ 0.5 ml of blood was withdrawn from the carotid artery into a heparinized syringe and reinfused back at the end of the 1-min hypotensive period.

2.3 Determination of erythrocyte and vessel wall location

Adobe Premier 5.1 was used to convert the video files into image files (either TIFF or BMP format). The x-y coordinate data for the center points of each red blood cell was obtained from these image files using SigmaScan Pro 5. The center position of the cell in x-y coordinates was manually determined in two independent trials and then averaged. Error associated with marking the center of red blood cell images was estimated to be $\pm$ 0.5 μm [17]. The location of vessel wall was used to determine the inner diameter (ID) of individual venules. To minimize human measurement error associated with the measurement of vessel wall, five separate measurements were used.

2.4 Index of radial migration

Red blood cells movements from frame to frame were used to determine radial migration. The procedure for the measurement is described in our previous studies [17,19]. We defined an index of radial migration to describe the radial migration of individual red blood cells along the length of the vessel, 0 to 50 μm from the bifurcation. A positive value of migration index indicates red blood cell movement towards the centerline of venule while a negative value represents cell movement away from the centerline. To determine the radial migration index of a red blood cell, the following equation was used.

$$\text{Index of radial migration} = \frac{\text{radial distance travelled}}{\text{total distance travelled}} \times 100 \quad (1)$$

2.5 Collision and collision frequency

In the present study, collisions between two individual red blood cells and between a single cell and an aggregate were all included. A total of 147 red blood cells in five venules before dextran infusion and 138 cells in five venules after dextran infusion were analyzed. The total number of collisions for each red blood cell was tabulated to obtain the collision frequency of each cell.

2.6 Statistical analysis and data presentation

A paired t-test is used to determine differences in experimental and physiological parameters between normal and dextran-treated animals. Data are reported as means $\pm$ SD and $P < 0.05$ was considered statistically significant. We tested for statistical difference between distributions with the two-sample Kolmogorov-Smirnov (K-S) test. The movements of red blood cells before and after dextran treatment are presented as a function of radial migration index while number of collisions for each red blood cell were presented as a function of collision frequency.

3 Results

3.1 Systemic values

Control arterial pressure was 113 $\pm$ 6.1 and 109 $\pm$ 6.5 mmHg before and after dextran infusion respectively. After blood withdrawal, arterial pressure dropped to 53 $\pm$ 10.4 and 51 $\pm$ 11.7 mmHg in normal and dextran-treated rats respectively; these two values was not significantly different from each other. We reduced systemic hematocrit (43 $\pm$ 2.9%) by hemodilution with plasma. The reduced hematocrit before dextran infusion (28 $\pm$ 2.6%) and after dextran infusion (29 $\pm$ 2.1%) was not significantly different from each other. Before dextran infusion, this index was 0.0 and after dextran infusion the index rose to 15.6 $\pm$ 4.2, which is similar to the aggregability level reported for blood of healthy humans [5,17].

3.2 Hemodynamics in capillaries and postcapillary venules

The inner diameter (ID) of postcapillary venules used in this study was 11.5 $\pm$ 1.5 μm. The tube hematocrit in the venules before and after dextran infusion was 9.2 $\pm$ 1.4% and 9.6 $\pm$ 2.9% respectively which were not significantly different. Two supply capillaries of slightly different diameter (7.9 $\pm$ 0.9 and 7.1 $\pm$ 1.0 μm ID) were connected to the postcapillary venules. Figure 1 shows a schematic diagram of a postcapillary venule (13.7-μm ID) with two feeding capillaries. Red blood cell flow pathways shown in Fig. 1 were obtained by connecting the instantaneous locations of individual cells. No significant difference was found in the mean cellular velocity in the postcapillary venule before (213.4 $\pm$ 58.7 μm/s) and after (182.9 $\pm$ 35.2 μm/s) dextran infusion. The pseudoshear rate (ratio of mean cellular velocity to vessel diameter) was 20.9 $\pm$ 6.5 and 17.3 $\pm$ 6.7 s^{-1} before and after dextran infusion respectively with no significant difference.

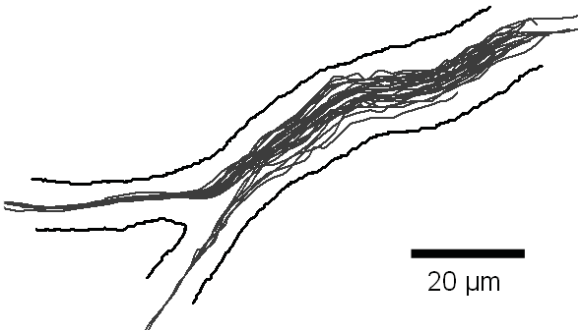

Figure 1: Diagram of a postcapillary venule (13.7-μm ID) with two supply capillaries. The solid lines in the venule show the pathways followed by the centers of red blood cells in this venule over a time period of $\sim$0.5 s.

3.3 Radial migration

As shown in Fig. 2, cells of the same radial migration index were grouped together and the data was presented as the percentage of total cells versus index of radial migration for both conditions. The index of radial migration was 1.48 $\pm$ 3.89 and 1.90 $\pm$ 2.73 before and after dextran infusion. There was no significant statistical difference between the two distributions. However, it should be of note that the larger magnitude of mean radial migration index after dextran infusion indicates more radial migration of cells towards the centerline of vessel while the smaller standard deviation of the radial migration implies more restricted movement in the radial direction. The distribution before dextran infusion consisted of more negative indexes for radial migration than after dextran infusion, indicating a slightly lower degree of random movement of red blood cells after dextran infusion.

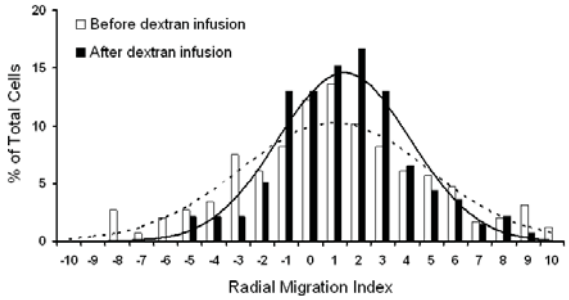

Figure 2: Cell distributions of radial migration index before and after dextran infusion. Dotted and solid lines represent the normal distribution of the radial migration index before and after dextran infusion, respectively.

3.4 Collision frequency

Cells exhibiting the same number of collisions were grouped together and presented before and after dextran infusion. Statistical comparison by K-S test indicated a significant effect ($P < 0.05$) of dextran on the distribution of collision frequency of individual red blood cells. The majority (76.8%) of the cells had collision frequencies ranging between 1 and 5 after dextran infusion whereas there were widespread collision frequencies between 1 and 14 for cells before dextran infusion. Another change after dextran infusion was the decrease (from 35% to 18%) in fraction of cells having zero collision frequency, which indicates that more cells participated in col-

lision with dextran treatment. Figure 3B compares the collision frequency distribution of aggregating cells and non-aggregating cells which accounts for 46% and 54% of the total red blood cells after dextran infusion, respectively. The collision frequency distribution of aggregating cells was similar to that of non-aggregating cells.

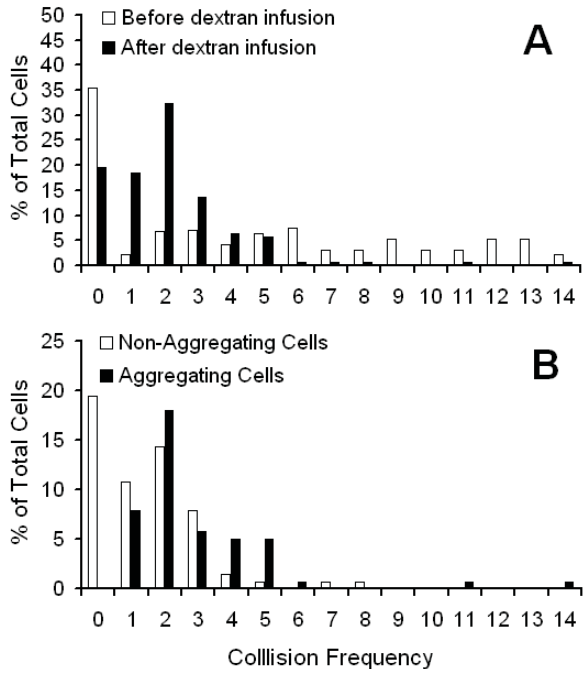

Figure 3: Collision frequency of individual red blood cells. **A**: Comparison before and after dextran infusion. **B**: Comparison between non-aggregating and aggregating cells after dextran infusion.

4 Discussion

Previous studies [19] reported that the rate of collisions between cells might play a key role in the rate of aggregate formation, which might be influenced by radial migration of red blood cells. In this study, slightly greater radial migration was observed in cells after dextran infusion. Although comparing spatial distribution of total collisions in our previous study [19] appeared to be unaffected by dextran infusion, this study shows that collision behavior of individual cells as they move in the flow stream was altered by dextran infusion. We found in this study that more cells participated in collisions after dextran infusion as shown in Fig. 3A. Interestingly, majority of these colliding cells exhibited a much lower collision frequency in contrast to cells before dextran infusion. We deduce that Dextran 500 induces more cells to collide and form aggregates possibly by increasing radial migration of cells towards the tube center but subsequently the presence of aggregates in the flow stream could reduce the collision probability between cells.

As shown in Fig. 2, red blood cells tend to migrate more towards the centerline after dextran infusion than before. During flow, there would be forces acting on the surface of red blood cells, and these forces might influence the migration pattern of the cells. Net effect of these forces in vivo, which in turn affects the radial displacement of cells relative to the tube center, depends partly on the distance over which this process can take place. In in-vitro studies [27] with human blood cells in small glass tubes, a low flow rate allowed aggregates to form and radial migration of these aggregates formed a red blood cell core at the tube center. In contrast to these findings, radial migration of aggregates in vivo was seldom observed in the microvascular network partly due to the highly irregular and branched nature of this system in which the segment length-diameter ratio of 3.5 to 1 [7,23] was much lower than 100:1 to 1000:1 ratios used for glass tubes experiments [13]. In the present study, the segment length to diameter ratio was 4:1 which was similar to that observed in vivo previously and hence pronounced radial cell migration was not expected.

As found previously in studies [7,9] conducted in vivo under normal blood flow conditions, the pseudoshear rate of $\sim$100 s^{-1} in the venous system was not favorable for radial migration of red blood cells. Lowering pseudoshear rate to 5 s^{-1} increased the radial migration of cells. In this study, the pseudoshear rate was reduced to 20.9 $\pm$ 6.5 and 17.3 $\pm$ 6.7 s^{-1} before and after dextran infusions, respectively. Although this value was higher than that achieved in the earlier studies [7,9], this pseudoshear rate still caused a certain degree of radial migration as shown by the posi-

tive values of radial migration index after dextran infusion in Fig. 2.

Red blood cell aggregation enhances the radial migration of red blood cells towards the centerline of the vessel through the formation of larger aggregates which tend to migrate towards the vessel center more rapidly [24]. Previous studies [1,2] have also shown the importance of a characteristic time factor for the radial migration. An earlier study with 25-100 μm ID glass tubes showed that pronounced radial migration of red blood cells required more than 10 seconds [2]. The time required is expected to be dependent on the tube or vessel dimensions. We deduce that the transit time ($\sim$0.3 s) from the bifurcation to the maximum length of vessel (50 μm) may be insufficient for the pronounced radial migration of red blood cells to take place even under the effect of aggregation induced by dextran. However, this effect of radial migration would exist to a small extent as shown in Fig. 2. In small venules (11.5 $\pm$ 1.5 μm ID) used in this study where the red blood cells were confined to a small flow volume, slight radial movement of red blood cells along the direction of flow would be important as it could increase the probability of adjacent cells to come into contact and directly affect the formation of aggregate.

High molecular weight polymers such as Dextran 70 or 500 have been known not only to change the rheological behavior of blood by inducing aggregation of red blood cells but also to increases plasma viscosity [15]. As plasma is the medium for the transport of red blood cells in the microcirculatory blood vessels, a change in plasma viscosity could alter the movement of red blood cells in the medium. An increased plasma viscosity would offer an additional resistance to the movement of red blood cells in the plasma either along or between streamlines, which would reduce radial migration towards the vessel center. However, our experimental findings revealed otherwise. This might imply that the aggregation force induced by dextran overcame the effect of increased plasma viscosity, resulting in the greater radial migration of red blood cells towards the vessel center seen after dextran infusion.

Our previous study [19] showed that in dextran-treated blood, radial migration towards the vessel centerline takes place in the region mainly within 30 μm from the bifurcation. Hence radial movement in the region was likely to contribute to the higher index (1.9 $\pm$ 2.73) of radial migration after dextran infusion in this study. The lower radial migration index (1.48 $\pm$ 3.89) seen in blood before dextran infusion might reflect greater degree of radial dispersion of cells in the absence of aggregation. This finding was supported by a previous study [8] where the fluctuations in radial position of red blood cells were found to be significantly greater in normal blood than dextran-treated blood under reduced flow conditions.

Figure 3 shows that collision frequency for individual cells decreased greatly from a wide range of 1-14 before dextran infusion to a narrow range of 1-5 to after dextran infusion, possibly due to aggregate formation. Schmid-Schonbein et al. [28] suggested that the glycocalyx of red blood cell provided an adhesive film that allowed the unspecific adsorption of the macromolecules such as dextran resulting in the bridging effect. As red blood cells join to form aggregates, there is a net reduction of the adhesive surface area to volume ratio exposed for this adsorption effect. This effect increases as the size of the aggregate increases. This reduction in the effective surface area would lead to fewer collisions in subsequent regions of the vessel, as shown in the much lower range of collision frequencies (1-5) of colliding red blood cells after dextran treatment (Fig. 3A). Without dextran infusion, it is possible for cells to participate in another collision soon after the previous one since the effective surface area of cells remains unchanged. This probably explains the higher collision frequencies before dextran infusion (Fig. 3A). In the present study, there were slightly more non-aggregating cells (56%) than aggregating cells (44%) after dextran infusion. As presented in Fig. 3B, the aggregating cells showed a higher range (1-5) of the collision frequency compared to that (0-3) for non-aggregating cells. As these aggregating cells, which have higher intrinsic aggregability, i.e. older cells [21], form new or larger ag-

gregates, the non-aggregating cells would have a lower possibility of collision.

In a study on erythrocyte aggregate size with flow rate in skeletal venules, it was found that aggregates formed in dextran-treated blood were 2 to 3 times wider than the diameter of a single red blood cell with a pseudoshear rate of less than 40 s^{-1} [10]. Goldsmith and Karino [14] had observed that the magnitude of particle movements decreased when the particle diameter increased and Bishop and coworkers [8] suggested that this increase in particle size from red blood cell aggregation might be responsible for the decrease in the shear-induced dispersion of cells. As random particle movements could increase the probability of cells from adjacent flow streams to come into contact, a decrease in magnitude of such movements due to an increase in particle size from aggregate formation could reduce the number of collisions between particles.

The decrease in effective particle numbers accompanied by the transient increase in size of the aggregates along the direction of the flow stream could affect the number of collisions between the aggregating cells themselves as well as with the non-aggregating cells. To understand the effect of particle numbers on collision frequency, we compare the collision of single red blood cells between themselves and their aggregates. Based on observations made in our previous studies [17,19] and for simplicity, in Fig. 4, we consider only the red blood cells traveling with no overlap relative to the flow direction for the cases of 4 and 5 total red blood cells. When 4 cells are present in the absence of aggregation, a maximum number (M) of 2 possible collisions can occur at one time (Fig. 4A). On the other hand, when these cells are present in the form of single cells and aggregates, only 1 collision is possible at one time (Fig. 4B). When the number of red blood cells increases to five, similarly there would be a loss in the potential collisions that can take place as many possible configurations with aggregation would favor a single collision at any one time (Fig. 4D) compared to 2 collisions that can occur in the absence of aggregation (Fig. 4C). A maximum loss in potential collision ratio at any one time (maximum possible collisions without aggregation to minimum possible collisions with aggregation) is defined to quantify the effect of aggregates presence on collision numbers. This ratio was found to increase with the total number of red blood cells. Previously it was found that 3 or more red blood cells formed 50% of the total aggregates seen in the postcapillary venules [17], which emphasized the significance of this ratio.

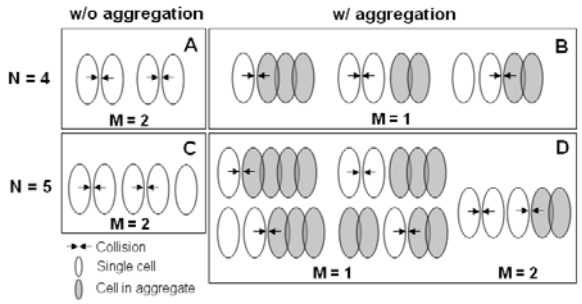

Figure 4: Maximum possible number (M) of collisions at one time with and without aggregation for total number (N) of red blood cells. **A** and **B**: N = 4. **C** and **D**: N = 5. **A** and **C**: Without aggregation. **B & D**: With aggregation. A decrease in M is possible when aggregation occurs as after Dextran 500 infusion. An increase in particle size will lead to a loss in the potential collisions as compared to the condition in which aggregates do not form.

The radial migration of the red blood cells in the vessel, which is influenced by the presence of Dextran 500 as shown in Fig 2, plays a significant role in the formation of the cell-free layer near the vessel wall. A previous study [18] in our laboratory found that the mean thickness of the cell-free layer was approximately 1 μm in arterioles with 11.5-μm ID both before and after dextran infusion. However, this study was done with high pseudoshear rates at normal arterial pressure. In the present study, radial migration index of red blood cells in the postcapillary venules (11.5 $\pm$ 1.5 μm ID) had mean values of 1.48 and 1.90 before and after dextran infusion, respectively. The radial displacement of red blood cells towards the vessel centerline calculated from the mean index

of the radial migration was approximately 0.7 and 1 μm before and after dextran infusion, respectively. Therefore, under low flow situations, the cell-free layer thickness might be significantly affected by red blood cell aggregation as seen in the cell migration index in this study. Since the cell-free layer thickness should be closely related to the radial migration of the red blood cells in the vessel, the index of radial migration could be a useful indicator of the rate of cell-free layer formation.

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