Role of red blood cell aggregation in tissue perfusion: New findings

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Abstract

Red blood cell (RBC) aggregation is a process in which single cells form face-to-face linear aggregates then combine to form three-dimensional structures. This process is reversible: increased shear forces disperse aggregates while they reform at low shear or stasis and hence blood is a non-Newtonian fluid whose viscosity increases with decreasing shear. The in vivo effects of enhanced aggregation are yet to be fully defined, but include: 1] increased flow resistance in larger (i.e., > 1 mm) venous blood vessels; 2] abnormal RBC flux in microcirculation resulting in decreased tissue hematocrit; 3] more-pronounced cell-poor zone at vessel wall and hence decreased near-wall blood viscosity; 4] lower wall shear stress and thus impaired endothelial cell function. It is important to note that the influence of RBC aggregation on endothelial function should depend on the mechanism by which aggregation is increased: enhanced aggregation as the result of changes of RBC properties without altering plasma characteristics or as a result of modified plasma composition and hence higher plasma viscosity without altering RBC characteristics. The effects of enhanced RBC aggregation on wall shear stress may thus be partially or totally offset by increased plasma viscosity, and it is possible that discrepancies between studies investigating relations between RBC aggregation and vascular pathophysiology may be due to the manner by which higher red cell aggregation is achieved.

Key words: red blood cell aggregation, flow resistance, tissue hematocrit, vessel wall, endothelial cell function

Résumé

Le phénomène d’agrégation érythrocytaire : de nouvelles découvertes

Le phénomène d’agrégation érythrocytaire est un processus au cours duquel les globules rouges s’empilent les uns sur les autres comme des assiettes pour former des rouleaux linéaires. Ensuite ces rouleaux s’associent pour former une structure en trois dimensions. Ce phénomène est réversible : l’augmentation des forces de cisaillement disperse les agrégats érythrocytaires alors que ces derniers se reforment dans des zones à cisaillement faible ou de stases. Ainsi, le sang est un fluide non
newtonien dont la viscosité augmente lorsque le cisaillement diminue. Les effets in vivo d’une agrégation érythrocytaire accrue ne sont pas encore complètement connus, mais il est d’ores et déjà établi qu’une hyper-agréagtion conduit à : 1] l’augmentation des résistances vasculaires dans les compartiments vasculaires veineux larges (> 1 mm) ; 2] un flux érythrocytaire anormal dans la microcirculation provoquant une diminution de l’hématocrite tissulaire ; 3] une zone vasculaire au niveau de la paroi endothéliale appauvrie en éléments figurés conduisant ainsi à une réduction de la viscosité sanguine au niveau de la paroi vasculaire ; 4] une contrainte de cisaillement exercée sur l’endothélium plus faible participant ainsi à l’altération de la fonction endothéliale. Il est important de souligner que l’influence de l’agréagation érythrocytaire sur la fonction endothéliale est fonction du mécanisme par lequel l’agréagtion est augmentée. Une agrégation érythrocytaire accrue peut être soit la conséquence de changements au niveau des propriétés cellulaires des globules rouges sans altération des caractéristiques plasmatiques, soit le fruit d’une modification de la composition plasmatique, et ainsi de la viscosité plasmatique, sans modification des propriétés cellulaires des globules rouges. Les effets d’une agrégation érythrocytaire accrue sur la contrainte de cisaillement à la paroi vasculaire peuvent ainsi être partiellement ou totalement controbalé par une augmentation de la viscosité plasmatique, et il est probable que les divergences d’une étude à l’autre à propos des relations entre les phénomènes d’agréagtion érythrocytaires et la pathophysiologie vasculaire soient liées à la manière dont une agrégation érythrocytaire accrue est obtenue.

Mots clés : agrégation érythrocytaire, résistance vasculaire, hématocrite tissulaire, paroi endothéliale, fonction endothéliale

**RBC aggregation is the major determinant of blood viscosity at low shear stress**

Blood is a complex, two-phase fluid consisting of various cell types suspended in a salt and protein solution termed plasma. The rheological (i.e., flow) behavior of blood is also complex, with its resistance to flow dependent on flow conditions. Blood viscosity is a function of shear rate or applied shear stress during flow and hence is a non-Newtonian fluid [1]. In vitro measurements of blood viscosity over a range of shear rates clearly demonstrate this shear dependence: normal blood has a viscosity on the order of $10^2$ mPa.s at shear rates of $10^2$ s$^{-1}$ and $10^0$ mPa.s at shear rates of $10^2$ s$^{-1}$ [2]. This flow behavior is entirely due to the presence of cellular elements, most importantly red blood cells (RBC) which constitute 99.9% of the formed elements and have a volume fraction of 0.4 to 0.5. The flow behavior of plasma is Newtonian and is not affected by shear.

RBC deformation and alignment with flow streamlines accounts for the marked decrease of blood viscosity at high shear rates [2]. As shear rate is decreased, RBC return to their biconcave-discoid shape and tend to form multi-cell aggregates at lower shear forces or at stasis [3]. These aggregates are comprised of linear, face-to-face RBC-RBC structures termed rouleaux, with rouleaux associating to form three-dimensional structures. RBC aggregation is physiological phenomena which strongly depends on both plasma composition (e.g., fibrinogen concentration) and cellular properties [4]. Shear forces are important determinants of the extent of aggregation since aggregates can be dispersed by such forces yet reform when the forces are lower or absent [4]. Plasma composition and cellular factors also determine the time course of aggregation during the transition between two shearing conditions (e.g., from high to low shear rate or to stasis) [5].

Given the above brief discussion of factors affecting RBC aggregation, it should be anticipated that RBC aggregates are expected to be formed in low flow regions of the in vivo circulatory system (e.g., post-capillary region including venules and larger venous blood vessels). Kim et al.
demonstrated the formation of RBC aggregates in post-capillary venules using high-speed videomicroscopy [6] and suggested that the rate of aggregate formation in this region is determined by RBC collision frequency [7]. These findings strongly support earlier reports indicating the importance of physiological levels of RBC aggregation in maintaining normal venous flow resistance and hence microcirculatory flow dynamics [8].

The influence of RBC aggregation on microcirculatory blood flow has been studied using intravital microscopy techniques, with results suggesting increased flow resistance with enhanced aggregation induced by infusion of high molecular dextran solutions [9-12]. However, in experiments using whole-organ preparations rather than a particular vascular bed, the effects of RBC aggregation were found to be determined by the degree of aggregation, thus suggesting that enhanced aggregation may tend to decrease, have no effect or increase flow resistance [13-15]. The reasons for such conflicting findings in different studies have been previously discussed [16, 17] and include factors related to methodological differences and also to various in vivo mechanisms sensitive to the degree of RBC aggregation.

In vivo hemodynamic mechanisms affected by RBC aggregation

The influence of RBC aggregation on blood flow resistance in larger venous blood vessels (i.e., diameter >1 mm) can be explained by the increased effective particle size with aggregation [3]. This mechanism of enhanced flow resistance may only be effective in flow regions with shear forces low enough to allow sufficient RBC aggregation. If the mean values of shear forces in the arterial blood vessels are considered, it might be assumed that such high shear forces indicate that RBC aggregates are unlikely to exist in a wide range of arterial vessels. However, as presented below, there is experimental evidence suggesting a significant influence of altered RBC aggregation on arterial hemodynamic mechanisms.

It has been observed that tissue hematocrit values are affected by modified levels of aggregation. Using a double-isotope labeling technique, myocardial tissue hematocrit has been demonstrated to be reduced after experimentally enhanced RBC aggregation [18, 19]. Lower tissue hematocrit values compared to large vessel hematocrit reflect a hematocrit reduction as blood approaches the microcirculation [20]. Hemodynamic mechanisms underlying this hematocrit reduction are mainly related to the migration of RBC into the central zone of vessels, a phenomena known as axial migration [21]. Axial migration of RBC results in the formation of a cell-poor zone adjacent to the vessel wall and a relatively higher concentration of RBC (i.e., higher hematocrit) in the central zone. One important consequence of this cell-poor zone is more effective plasma skimming at side branches originating from blood vessels, especially for diameters below 1000 μm, since side branches tend to receive blood from near the wall of the larger vessel. If this cell-poor zone is considered together with the higher flow velocity in the central zone, it can be seen that the average RBC velocity in a given vessel segment would be higher than the average velocity of plasma. This difference in velocity results in the Fahraeus Effect in which the hematocrit of blood flowing in a vessel is less than the hematocrit of blood leaving that segment [21].

Enhanced RBC aggregation tendency may theoretically be expected to promote axial migration and related hemodynamic mechanisms, and these effects of aggregation have been demonstrated experimentally. An excellent example is the study by Cokelet and Goldsmith in which they show decreased flow resistance in vertically oriented glass tubes during flow of RBC suspensions with enhanced degrees of aggregation [22]. These findings can be interpreted as the result of more effective axial migration and cell-poor zone formation, thereby reducing the hematocrit and viscosity in the marginal zone which results in lower frictional resistance. Further, it strongly suggests that other mechanisms closely related to axial migration may also be influenced by RBC aggregation.

The above discussion may have several important outcomes related to hemodynamic-vascular mechanisms, especially in blood vessels near and within the microcirculation.

1) The observed effect of enhanced RBC aggregation on tissue hematocrit strongly suggests that RBC aggregates should exist on the arterial side of the circulatory system, at least locally in the central flow zone of the blood vessels where RBC tend to accumulate and where shear forces are low enough to allow aggregation;

2) If RBC aggregates exist in the arterial side of the microcirculation, even locally in the central flow zone, these aggregates must be dispersed into individual RBC in order to transit micro vessels, and this disaggregation has an energy cost. This disaggregation energy should contribute to flow resistance;

3) The observed decrease of hematocrit in the marginal cell-poor zone due to enhanced RBC aggregation, and thus the lower viscosity in this region, should also be considered in terms local wall shear stress which is an important determinant of endothelial function.
Role of RBC aggregation in the regulation of vascular tone

The diameter of blood vessels can vary widely, especially for vessels near and in the microcirculation. This variation is an important component for the local regulation of blood flow at the tissue level, and is also a major determinant of flow resistance in a given circulatory region. Vascular smooth muscle tone as a determinant of diameter changes is affected by a variety of mechanisms including metabolic autoregulation and autonomic control. Vascular smooth muscle tone is also modulated by endothelium-related factors, nitric oxide (NO) being an important example of such mediators.

NO is synthesized by endothelium in response to various stimuli, including local shear stress acting on the vessel wall; NO reduces the tone of underlying vascular smooth muscle thereby dilating the blood vessel. In addition to the other factors (e.g., pO₂, acetylcholine), wall shear stress (WSS) is an important factor for up-regulation of the NO synthesizing mechanisms in endothelial cells [23]. WSS is, in turn, determined by the velocity gradient near vessel wall and the viscosity of the flowing blood in that region, with the latter being a function of plasma viscosity and local hematocrit in the marginal flow zone [24]. Therefore, it can be hypothesized that WSS can be affected by the extent RBC aggregation via altering the local hematocrit in the marginal flow zone. Endothelial function may thus be expected to be altered by modified RBC aggregation with effects not necessarily limited to the control of vasomotor tone.

Direct clinical or experimental evidence regarding the relations between RBC aggregation and endothelial function have only recently been presented. Enhanced aggregation is associated with a variety of circulatory disorders whose origins might be considered as related to endothelial function (e.g., hypertension [25]). However, such relationships cannot be interpreted in terms of the mechanisms of these alteration or in terms of their consequences: enhanced RBC aggregation in hypertension could be the cause or the result of an ongoing pathophysiological process [26].

Using an experimental model of chronically enhanced RBC aggregation, it has been shown that NO related mechanisms are down-regulated in resistance arteries [27]. The model was based on a novel method of increasing RBC aggregability (i.e., intrinsic tendency of RBC to aggregate) by surface coating RBC with co-polymers of specific molecular size. These hyperaggregating RBC were suspended in normal rat plasma and exchange transfused into rats. The results of this experiment approach are summarized in table 1: 1) RBC aggregation was significantly enhanced during the 4-day period following transfusion; 2) Mean arterial pressure of hyperaggregating rats was not different from the control animals on the 1st day and was gradually increased during the 4-day period; 3) The animals were sacrificed on the 4th day and nitric oxide related vascular mechanisms were investigated in arterial segments of ~300 μm diameter isolated from skeletal muscles. Both acetylcholine-induced and flow-mediated dilation responses were attenuated in the vessels of hyperaggregating rats compared to control animals. Specifically, there was a ~70% increase of the half-maximal efficient concentration (EC50) value for acetylcholine for blood vessels from hyperaggregating animals. Flow-mediated dilation of the same blood vessels was characterized by lower percentage of maximal dilation and increased flow rate for maximal response. Both changes indicate significant down-regulation of NO-synthesis mechanisms in these vessels. 4) eNOS expression in the tissue samples obtained from the same skeletal muscles was found to be significantly decreased in rats with enhanced aggregation. This study provides direct evidence for increased blood pressure consequent to enhanced RBC aggregation and the role of down-regulated NO-synthesis mechanisms in this effect; the changes were suggested to be mediated via modified WSS.

Table 1. Effect of RBC hyperaggregation on arterial blood pressure and NO-related vascular control mechanisms.*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hyper-aggregating</th>
<th>Change</th>
</tr>
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<tbody>
<tr>
<td>Microscopic aggregation index (4th day)</td>
<td>1.14 ± 0.03</td>
<td>2.68 ± 0.09</td>
<td>↑ 135%</td>
</tr>
<tr>
<td>1st day Mean arterial pressure (mmHg)</td>
<td>82.3 ± 2.1</td>
<td>85.7 ± 4.2</td>
<td>↔</td>
</tr>
<tr>
<td>4th day Mean arterial pressure (mmHg)</td>
<td>83.2 ± 1.8</td>
<td>117.4 ± 5.7</td>
<td>↑ 41%</td>
</tr>
<tr>
<td>Acetylcholine induced dilation (EC50, M)</td>
<td>2.07 x 10⁻⁷</td>
<td>3.48 x 10⁻⁶</td>
<td>↑ 168%</td>
</tr>
<tr>
<td>FMD (maximum dilation, %)</td>
<td>48.2 ± 7.4</td>
<td>22.3 ± 9.6</td>
<td>↓ 53%</td>
</tr>
<tr>
<td>FMD (flow rate at maximum dilation, μl/min)</td>
<td>17 μl</td>
<td>26 μl</td>
<td>↑ 53%</td>
</tr>
<tr>
<td>eNOS expression (% G6PD expression)</td>
<td>65.2 ± 5.6</td>
<td>18.1 ± 5.5</td>
<td>↓ 72%</td>
</tr>
</tbody>
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*Results are summarized from [27]. EC50: Half maximal effective concentration; FMD: Flow mediated dilation.
The relationship between RBC aggregation and endothelial function has been further investigated using an in vitro model to provide better control of experimental conditions [28]. These experiments utilized 1 mm ID glass tubes coated with human umbilical vein endothelial cells (HUVEC) on their inner surface, and were perfused for 30 min with RBC suspensions with normal and enhanced aggregation at a flow rate to generate a nominal WSS of 15 dyn/cm². It was observed that the degree of activation of HUVEC NO-synthesizing mechanisms, in terms of serine-1177-phosphorylated eNOS expression [29], depended on the aggregation properties of RBC suspensions used for perfusion, being significantly diminished with hyperaggregating RBC suspensions (figure 1). Additionally, it was observed that the influence of enhanced aggregation could be counter-balanced if the suspending phase viscosity of the RBC suspension was also increased (figure 1). This is obvious with comparisons between the experiments using polymer coating to enhance RBC aggregability and hence aggregation in native plasma (HA-I group) and uncoated cells in plasma containing dextran 500 kDa, a polymer that induces strong aggregation, at a concentration of 0.5 % (HA-II group). Due to the presence of dextran 500 the suspending phase viscosity was ~40 % higher in the HA-II group compared to control or to HA-I; both HA-I and HA-II suspensions exhibited a similar degree of enhanced RBC aggregation.

The in vitro and in vivo experimental findings confirm that RBC aggregation can significantly influence endothelial function, thereby lending support to a mechanism involving changes of WSS. This effect might be viewed as a result of more effective axial migration of RBC and phase separation, leading to lower hematocrit and viscosity in the marginal flow zone. However, in the case of more effective phase separation due to elevated polymer or protein levels, plasma viscosity becomes an important factor significantly influencing WSS. Using a hemodiluted hamster model, Tsai et al. demonstrated that plasma viscosity is an important determinant of perivascular NO concentration [30]; they also reported increased eNOS expression in experiments with elevated plasma viscosity. Other studies by the same group also confirmed the important role of plasma viscosity in the regulation of microcirculatory blood flow [31, 32]. Their reports suggest that the influence of plasma viscosity is especially prominent in case of reduced hematocrit (i.e., hemodilution). Note that the modified distribution of RBC in a vascular segment due to hyperaggregation (i.e., enhanced axial migration) is essentially “local hemodilution” near the vessel wall.

**Clinical considerations**

It follows from the above discussion that RBC aggregation should be considered as an important determinant of circulatory function, not only because it has a strong influence on low-shear blood viscosity but also because it is an important feature affecting endothelial function. RBC aggregation should thus not only be considered as a factor influencing vascular control mechanisms that regulate the distribution of blood flow to various organs and tissues, but should also be considered, in a more general sense, as a phenomenon that interferes with endothelial function and vascular health.

Vascular endothelium is now considered as being central to a number of important vascular functions, including the regulation of hemostasis, inflammatory response, angiogenesis and vasomotor control, in addition to its classically-known permeability barrier function [33, 34]. These endothelial functions are regulated by a variety of biomolecules as well as mechanical forces affecting endothelial cells [34]. It is well established that physiological levels of WSS are required to maintain normal endothelial function and quiescent status of endothelial cells [35, 24]. Exposure of endothelial cells to reduced or geometrically altered shear forces results in the disturbance of this quiescent status which may lead to the development of vascular pathophysiological processes (e.g., atherosclerosis) [35]. NO generation by endothelial cells is a requirement for the continuation of this quiescent status, and therefore NO should be viewed as an important element of vasomotor control and as an essential factor for maintaining vascular health.

![Figure 1. eNOS expression of HUVEC on the inner surface of a glass tube exposed to 15 dyn/cm² nominal wall shear stress for 30 min via perfusion with normally-aggregating (i.e., control, normal RBC in native plasma) and hyperaggregating human RBC suspensions. Hyperaggregation was achieved by either suspending surface-modified RBC in native plasma (HA-I) or suspending unmodified RBC in plasma containing 0.5 % dextran 500 kDa (HA-II). The level of RBC aggregation was the same in both HA groups whereas plasma viscosity was ~70 % higher in the HA-II group (Redrawn from [28]).](image-url)
If the proposition that decreased WSS due to enhanced RBC aggregation is deemed worthy, it seems possible to extend this concept to include endothelial function in a broader sense and hence the overall status of vascular health. That is, enhanced RBC aggregation may have an important influence on endothelial function by reducing WSS and NO synthesis accordingly, thereby interfering with the quiescence status of endothelium. However, it should be noted that the influence of RBC aggregation on endothelial function should depend on the mechanism by which aggregation is altered : enhanced aggregation as the result of changes of RBC properties without altering plasma characteristics or as a result of modified plasma composition and hence plasma viscosity without altering RBC characteristics. RBC cellular properties (i.e., RBC aggregability) can be modified in clinical conditions such as diabetes mellitus [36], sepsis [37], ischemia-reperfusion injury [38]; plasma composition can be modified due to increased concentrations of certain plasma proteins (e.g., fibrinogen) as seen frequently in case of acute or chronic inflammatory reactions, including infectious diseases [39] and rheumatoid disorders [40]. And, in some clinical situations, both plasma-mediated and cell-mediated mechanisms may co-exist. With both mechanisms operational, the effect of enhanced RBC aggregation on WSS may be partially or totally offset by increased plasma viscosity. It seems clear that the impairment of endothelial function due to enhanced RBC aggregation should be more pronounced if the enhanced aggregation is the result of increased RBC aggregability since, in this case, plasma viscosity is unaffected. It is therefore possible that the discrepancies between clinical studies investigating the relations between enhanced RBC aggregation and vascular pathophysiology may be due to the manner by which higher red cell aggregation is achieved. 

References


