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Hemodynamic effects of red blood cell aggregation

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The influence of red blood cell (RBC) aggregation on blood flow *in vivo* has been under debate since early 1900's, yet a full understanding has still has not been reached. Enhanced RBC aggregation is well known to increase blood viscosity measured in rotational viscometers. However, it has been demonstrated that RBC aggregation may decrease flow resistance in cylindrical tubes, due to the formation of a cell-poor zone near the tube wall which results from the enhanced central accumulation of RBC. There is also extensive discussion regarding the effects of RBC aggregation on *in vivo* blood flow resistance. Several groups have reported increased microcirculatory flow resistance with enhanced RBC aggregation in experiments that utilized intravital microscopy. Alternatively, whole organ studies revealed that flow resistance may be significantly decreased if RBC aggregation is enhanced. Recently, new techniques have been developed to achieve well-controlled, graded alterations in RBC aggregation without influencing suspending phase properties. Studies using this technique revealed that the effects of RBC aggregation are determined by the degree of aggregation changes, and that this relationship can be explained by different hemodynamic mechanisms.

Keywords: Hemodynamic mechanism, Red blood cell aggregation

Red blood cell (RBC) aggregation occurs when biconcave discoid cells "stick" face-to-face to form the so-called "rouleaux" at sufficiently-low shear forces[1]. This type of cell association is strongly dependent on the macromolecular composition of the suspending medium; both molecular size-shape and concentration of this macromolecular content affect the degree of aggregation[1]. RBC aggregation is also dependent on the local shear forces, as the aggregates are reversible and easily broken into smaller aggregates or individual cells under increasing shear forces.

An important consequence of this shear-dependent nature of RBC aggregation is the non-Newtonian behavior of blood (i.e., decreasing blood viscosity with increasing shear rate). This behavior is mainly related to the reduced energy loss as the particle size decreases as a result of disaggregation under increasing shear forces. Blood viscosity increases as RBC aggregates grow, holding more cells into three-dimensional rouleaux, under low-shear conditions[2]. Accordingly, RBC aggregation is accepted as one of the main determinants of low-shear blood viscosity[2]. Despite these well-known rheologic observations, the *in vivo* hemodynamic effects of RBC aggregation remain uncertain; various *in vivo* hemodynamic-hemorheological mechanisms have been proposed, with at least some supported by convincing experimental data.

Observations on the role of RBC aggregation in tube flow

Robin Fåhraeus was the first to publish interesting observations on the behavior of RBC suspensions with different aggregating properties when flowing in glass capillaries[3]. He pointed out that increased aggregation of RBC in tube flow resulted in more effective axial migration of RBC and thickening of a marginal, cell-poor layer[3]. Furthermore, he suggested that a RBC suspension would flow through a tube with minimal energy dissipation if the cells were accumulated in the axial region of the tube[3]. These observations were confirmed by various groups; flow of RBC suspensions with increased aggregation in capillary-size tubes is characterized by decreased relative apparent viscosity and hydrodynamic resistance[4-8]. The effect of RBC aggregation was more pronounced at low flow rates, but could not be observed at sufficiently-high flow rates at which RBC aggregates were dispersed into individual RBC[5].

The critical flow rate for the onset of decreasing hydrodynamic resistance depends on the size of the tube, being greater in smaller diameter tubes[5]. The reduction in apparent viscosity is time dependent at a given flow condition, reaching a maximum when the phase-separation is completed [6]. Careful studies also revealed that the effect of RBC aggregation on flow resistance and relative apparent viscosity of RBC suspensions depends on the orientation of the flow system. In straight horizontal tubes at low flow rates, enhanced sedimentation of RBC due to aggregation results in increased apparent viscosity due to sedimentation and elevation of tube hematocrit[9,8]. Alternatively, in straight vertical tubes at low flow rates RBC, aggregation leads to the formation of a cell-poor layer at the tube wall, an irregular core of RBC at the tube center, decreased flow resistance and lower apparent viscosity[7,5].

Cylindrical glass tubes have been used as highly simplified models of the vascular system to study the pressure-flow relationship of blood. It should be noted that these observations in glass tubes are not directly applicable to *in vivo* flow conditions in as much as the geometry of the microvasculature is very complex[10]. Frequent branching may limit the unbranched length of microvessels and the residence time of blood may not be long enough for the phase-separation necessary for decreased flow resistance[7]. The orientation of the individual microvessels may also play a significant role in the development of cell-poor marginal zone and related hydrodynamic effects[5]. Vascular control mechanisms may further complicate this relationship.

Role of RBC aggregation in *in vivo* blood flow

It is known from the 1933 work of Whittaker and Winton that the viscosity of blood *in vivo* can be different from that measured *in vitro*[11]. Using the isolated dog hind limb, they observed that the apparent viscosity of blood *in vivo* was lower than that measured *in vitro*[11]. Lower *in vivo* viscosity has also been observed in dilated cat muscle by Djojosugito *et al*[12]. Conversely, Benis *et al.*[13], using an isolated dog hind paw, indicated agreement between *in vivo* and *in vitro* viscometry if both viscous and inertial pressure losses are considered. Thus, the Fahraeus-Lindqvist effect under *in vivo* flow conditions[11], inertial energy loses or altered vessel geometry[14] and phase-separation related to RBC aggregation may play roles in the differences between *in vivo* and *in vitro* measurements of blood viscosity.

The effects of RBC aggregation on *in vivo* blood flow resistance have been investigated at various levels of the circulatory system and a variety of approaches to pressure-flow relationships and methods to alter the degree of aggregation have been utilized.

Effect of RBC aggregation on microcirculatory blood flow—In studies employing intravital microscopy it has been observed that intensified RBC aggregation increased microvascular flow resistance[15-17]. In most of these studies RBC aggregation was increased by infusing high-molecular weight dextran solutions. Durussel *et al.*[15] used rat mesentery and cremaster preparations to measure microcirculatory flow resistance following infusion of dextran 500 (500 kDa). They used an anti-aggregating agent (troxeuritine) to inhibit aggregation after dextran infusions in an attempt to distinguish between the effects of hyperaggregation and plasma hyperviscosity, both of which resulted from dextran infusion. They concluded that dextran-induced aggregation markedly increased microvascular flow resistance independent from suspending phase hyperviscosity[15]. Soutani *et al.* reported that microvascular flow resistance increased with increasing dextran 70 concentration, with the increase being correlated with the velocity of RBC aggregation. Cell-poor layer thickness was also increased with enhanced RBC aggregation[18], a finding that would be expected to lead to decreased hydrodynamic resistance based on the *in vitro* observations mentioned above[5]. The effect of RBC aggregation in arteriolar and/or capillary microcirculation should thus reflect both the influence of RBC aggregation on bulk viscosity of blood in larger blood vessels and the increased energy cost of disaggregation at the entrance of microcirculation (e.g., bifurcations)[19].

Functional capillary density, which reflects the number of capillaries with RBC flow, has also been investigated as a function of RBC aggregation. Kim *et al.*[20] reported that the number of RBC perfused capillaries was only slightly affected by enhanced RBC aggregation under normal arterial pressures. However, at reduced arterial pressures the effect of RBC aggregation on functional capillary density was very significant. They explained their observations by the effects enhanced plasma skimming and thus an increased number of capillaries that do not contain

RBC[20]. Alternatively, when RBC aggregation was enhanced, Mchedlisvili *et al.*[16] reported capillaries filled with non-flowing RBC. Therefore both studies indicate decreased functional capillary density, with the results explained by two separate mechanisms.

Effect of RBC aggregation on venous flow resistance—Several studies have focused on the role of RBC aggregation in venous side of the circulatory system. Blood flow in the venous circulation is characterized by lower shear rates compared to the arterial side and the microcirculation, and it has been demonstrated that RBC aggregates are formed in venous circulation[19,21]. Therefore this part of the circulation is the most likely site for observing the effects of RBC aggregation.

Using dextran 250 (250 kDa) to increase normal RBC aggregation, Cabel *et al.*[22] studied the contribution of red cell aggregation to venous vascular resistance in a cat muscle preparation. They observed an inverse relation between venous conductance and blood flow for normal (aggregating) blood, and that this effect was reduced or absent for *either* non-aggregating suspensions (RBC in dextran 40-Ringer) or for highly aggregating suspensions (dextran 250). They thus concluded that normal RBC aggregation significantly contributes to venous vascular resistance in resting muscle and plays an important role in vascular system homeostasis[22]. It has also been demonstrated that RBC aggregation affects velocity profiles in venous blood flow, especially under reduced flow rates[23]. This blunting is expected to increase the energy loss and consequently flow resistance.

Direct application of *in vitro* viscometric findings related to RBC aggregation to the *in vivo* rheological behavior of blood would predict the observations mentioned above. However, as demonstrated in glass capillaries *in vitro*[5] and in microvascular vessels *in vivo*[24], RBC aggregation is expected to lead to enhanced axial migration of RBC and the formation of cell-poor layers near the vessel wall. The resulting decrement in hydrodynamic resistance due to reduced frictional resistance at the vessel wall counteracts the effect of enhanced low-shear blood viscosity, and the net result of enhanced RBC aggregation is determined by the balance between these two effects [22,20]. Therefore, various degrees of RBC aggregation may have different effects on flow resistance.

There are other factors that may further complicate the overall relationship between RBC aggregation and *in vivo* flow resistance. It has been demonstrated that a critical time in a given vessel segment is required for the formation of an effective cell-poor layer[25]. Obviously, in order to have an impact of cell-poor layer formation on venous hemodynamics, transit times through a given vessel segment should be longer than this critical time. Transit times in turn depend on the unbranched length of the segment and flow rate[25]. Frequent branching in venules usually prevents the formation of an effective cell-poor layer near vessel wall. This is especially important in venous vessels, since with each branching a new stream of RBC is introduced in the peripheral zone of the venous vessel, abolishing the phase separation [22]. It has been demonstrated that shear rates should be lower than 5 sec^{-1} for the formation of an effective cell-poor marginal layer in cat skeletal muscle and this low level cannot be achieved with physiological perfusion pressures[25].

Obviously, time constants for RBC aggregation need to be considered when evaluating the role of aggregation in axial accumulation. However, Kim *et al.*[21] demonstrated that aggregation time constants may be at least an order of magnitude smaller *in vivo* than those measured *in vitro*[21]. This difference is most probably due to the smaller scale of the system in which aggregation takes place and should be considered in evaluating the relation between time constants of RBC aggregation and transit times in vascular segments[21].

Effect of RBC aggregation on whole organ perfusion—In whole organ preparations, enhanced RBC aggregation has been reported to either decrease, increase or have no effect on flow resistance. Charansonney *et al.*[26] used a perfused isolated rat heart preparation to study the effects of modified RBC aggregation[26], with 1% and 2% dextran 70 (70 kDa) used to increase RBC aggregation. At 1% concentration dextran 70 causes relatively mild aggregation with much greater aggregation at 2%. Compared to control, non-aggregating RBC suspensions (e.g., RBC in buffer), they observed that flow resistance was reduced with 1% dextran and elevated with 2% dextran[26]. Their results suggested that relatively low levels of RBC aggregation may reduce flow resistance, whereas greatly enhanced aggregation increases resistance[26]. However, other reports in which whole organ blood flow was used to calculate flow resistance indicated that increased RBC

aggregation caused by high molecular weight dextrans either increased blood flow resistance in the liver[27] or had no effect on uteroplacental blood flow[28].

In studies of *in vivo* flow resistance, RBC aggregation is usually modified by introducing either large proteins (e.g., fibrinogen) or high molecular weight polymers (e.g., dextran 70) into the blood. However, it is well known that addition of such macromolecules to plasma results in elevated suspending medium viscosity in addition to enhanced RBC aggregation[29]. Increased suspending phase viscosity may have significant effects on various factors affecting hydrodynamic resistance[30]. Recently, a new technique has been developed to modify RBC aggregation without modifying suspending medium[31]. This technique is based on the attachment of specific polymers onto the membrane surface of RBC: these polymers, termed poloxmers, have self-association (micellization) properties and can induce aggregation in the absence of dissolved macromolecules. RBC aggregation can be enhanced or inhibited by selecting the molecular size of the polymer and graded alteration can be achieved by modifying the concentration of the polymer during covalent attachment process[31]. This method can be used for *in vivo* studies[32-36]. The influence of *in vivo* RBC aggregation can be studied by this technique without interference from altered suspending phase properties or related mechanisms (i.e., vascular control mechanisms).

This polymer coating method has been used in recent studies to investigate the effects of graded alterations of RBC aggregation on flow resistance in isolated guinea pig hind limb preparations[34]. RBC aggregation was experimentally enhanced at five different levels by modifying the poloxmer concentration between 0.0125 to 0.5 mg/mL, yielding 63-200% increments in the degree RBC aggregation as judged by erythrocyte sedimentation rate[34]. The alterations in flow resistance during perfusion with RBC suspensions of various degrees of aggregation were not monotononic, with both the degree and the direction of the effect (i.e., decrement or increment) dependent on the degree of RBC aggregation (Fig. 1). There was a significant enhancement in flow resistance at a moderate level of RBC aggregation, followed by a return to control level with further increased aggregation. The highest degree of RBC aggregation was again characterized by enhanced flow resistance [34]. This experiment underlined the concept of different modes of action of RBC aggregation on various *in vivo* hemodynamic mechanisms[20,22,25].

Another important finding of this hind limb study was the effectiveness of vascular control mechanisms to compensate for the influence of RBC aggregation on *in vivo* hemodynamics[34]. By using the same polymer coating technique to modify RBC aggregation it has been demonstrated that RBC aggregation increased flow resistance in isolated guinea pig heart preparations only at perfusion pressures below 60 mmHg[35]. This study also confirmed the importance of vascular control mechanisms in the relationship between RBC aggregation and flow resistance[35].

It should be noted that the results of whole organ perfusion studies and microcirculatory studies may often conflict with each other. Vicaut[37] mentioned that this controversy could be explained by the opposite effects of RBC aggregation on blood flow resistance at two different circulatory levels. The apparent viscosity reduction discussed above may be counteracted by the higher energy cost at the entrance of capillaries for disaggregation of RBC in aggregating blood samples. Obviously, this higher energy cost should be reflected by microcirculation studies, while the whole organ studies should reflect a balance between the two opposing effects[37]. Another explanation for this contradiction may be the orientation of blood vessels under investigation. Microcirculation studies employing intravital microscopy technique are usually carried out on tissues spread on a microscope stage and hence blood flow orientation is usually horizontal. RBC aggregation has been demonstrated to increase flow resistance in tubes with such an orientation relative to

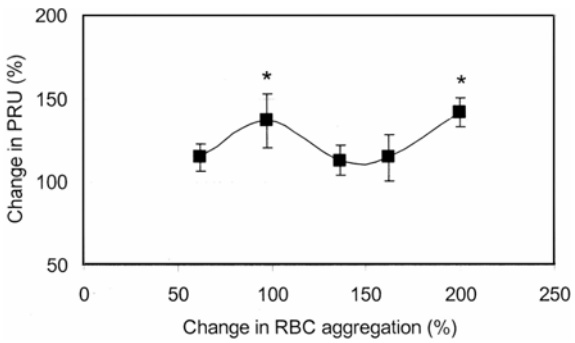

Fig. 1—Relative blood flow resistance in isolated guinea pig hindlimbs as a function of RBC aggregation as percentage of normal blood. *: significant difference from flow resistance during perfusion with normal blood (P<0.05) (Ref. 34)

the gravitational force, while flow resistance was found to be decreased by enhanced aggregation in vertically oriented tubes[5]. In whole organ perfusion studies the orientation of blood vessels may be closer to the somewhat random physiological orientation.

Effect of RBC aggregation on tissue hematocrit—The mean hematocrit value for blood in the vasculature of all sizes in a given tissue is known as tissue hematocrit. This value is always smaller than the venous (or arterial) hematocrit value because there is a significant difference between the hematocrit values of blood in larger blood vessels and microcirculation[38,39]. The two main mechanisms that result in lower microcirculatory hematocrit values are plasma skimming and the Fahraeus effect[40,41]. Both of these mechanisms depend on the non-uniform distribution of RBC in the cross-section of smaller blood vessels as a result of axial accumulation of RBC, which is in turn promoted by enhanced RBC aggregation[5]. Based on these considerations, RBC aggregation should be expected to be one of the determinants of tissue hematocrit values. It has been previously demonstrated that alterations in RBC aggregation induced by fibrinogen infusions affected tissue hematocrit values in rat myocardium[42]. Recently, these experiments were repeated using the newly developed poloxmer coating method to alter RBC aggregation, and it was confirmed that, in the absence of suspending medium alterations, enhanced RBC aggregation alters myocardial tissue hematocrit values[36].

Effect of aggregation on the distribution of other cells—At low flow rates, enhanced RBC aggregation promotes the margination of other blood elements, such as white blood cells (WBC) and platelets[43]. This effect can be regarded as an inverse-Fahraeus effect with in respect to WBC and platelets[44]. Goldsmith *et al.*[43] also demonstrated that the exclusion effect of RBC aggregation affects platelet functions. Pearson and Lipowsky[45] studied WBC rolling velocity and sticking to endothelium in rat mesentery and found that WBC margination was greatly enhanced by RBC aggregation induced by dextran 500. Margination of leukocytes may, in turn, affect flow resistance, especially in blood vessels near the microcirculation[25].

RBC aggregation and vascular control mechanisms—Vascular control mechanisms play an important role in the hemodynamic consequences of hemorheological alterations[46]. A significant hemorheological alteration may be easily compensated by counteracting vasomotion, attenuating or totally preventing the expected change in hemodynamic resistance[46]. From a different point of view, it should also be expected that hemorheological properties of blood may affect vascular control mechanisms. In fact, experimental data indicate that hemorheological alterations may have a direct influence on vascular regulatory mechanisms, especially those related to nitric oxide (NO) generation[30]. Shear stress affecting the endothelial cells is an important factor in controlling NO release from these cells[47,48]. If the wall shear stress is elevated in a vascular bed this would result in increased NO release and vascular relaxation, decreasing vascular hindrance. Wall shear stress is the product of fluid viscosity at the vessel wall and local fluid velocity[49]. It has been recently shown that elevated plasma viscosity may have beneficial effects on microvascular conductance, possibly resulting from the increased shear stress and accompanying increased NO release[30]. RBC aggregation may well be considered as a factor affecting wall shear stress in a blood vessel. If RBC aggregation is enhanced, the higher tendency for axial accumulation of RBC may result in decreased wall shear stress by decreasing the fluid viscosity in the marginal zone. Diminished wall shear stress would, in turn, cause decreased generation of NO by endothelial cells. Furthermore, it has been experimentally demonstrated that prolonged enhancement of RBC aggregation by exchange transfusion of polymer-coated RBC suspensions in rats resulted in down-regulation of eNOs expression in skeletal muscle and blunted flow-mediated dilation response in arterioles isolated from these tissues[32]. These findings strongly suggest that chronically enhanced RBC aggregation results in diminished wall-shear stress, most probably due to enhanced axial accumulation of RBC. An important consequence of these alterations was increased arterial blood pressure due to altered vasomotor status as a result of diminished local shear forces[32].

Conclusion

It is obvious from the above discussion that RBC aggregation may influence various aspects of *in vivo* hemodynamics: (1) The most straight forward *in vitro* observation that can be applied to *in vivo* flow conditions in large blood vessels is the relationship between enhanced RBC aggregation and increased

blood viscosity. This effect is valid in vasculature where blood can be regarded as a continuous fluid and increased viscosity of blood under low-shear conditions results from this relationship. (2) RBC aggregates are dispersed into individual RBC as blood approaches capillaries. Enhanced aggregation tendency would increase the energy cost for this dispersion, increasing overall hydrodynamic resistance. (3) Enhanced RBC aggregation is expected to increase axial accumulation of RBC and lead to decreased local viscosity and reduced frictional resistance. The anticipated result of this effect is decreased hydrodynamic resistance with higher degrees of aggregation. (4) The reduced local viscosity and consequent reduction in wall shear stress resulting from increased axial accumulation of RBC may affect vascular control mechanisms that are mediated by local shear forces (e.g., nitric oxide related mechanisms). The expected result of this effect may be increased vascular resistance with enhanced aggregation, due to decreased nitric oxide output of endothelium. (5) Another consequence of increased axial accumulation and more effective cell-poor layer formation is the promotion of plasma skimming and the Fahraeus effect, which in turn may result in decreased microvascular hematocrit values, thereby reducing blood viscosity and flow resistance.

While all of the five effects of enhanced RBC aggregation on *in vivo* blood flow seem probable, this complex picture with various conflicting mechanisms tends to make it difficult if not impossible to predict the exact net effect of RBC aggregation in a given vascular system. Rather, the net effect should be determined by the interplay of these conflicting mechanisms and may depend on the degree and mechanism of alteration in RBC aggregation as well as on the properties of the vascular system under investigation.

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