Graded alterations of RBC aggregation influence in vivo blood flow resistance

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Although red blood cell (RBC) aggregation is generally accepted as a major determinant of low-shear whole blood viscosity (11), the effects of RBC aggregation on in vivo flow resistance are less conclusive. Numerous in vitro studies have been conducted to explore the effects of RBC aggregation on tube flow. The earliest study was by Fahreaus, who indicated that increased RBC aggregation resulted in thickening of a marginal zone of cell-poor plasma and decreased hydraulic resistance (28). More recent studies (2–4, 15, 22, 35, 38, 39) have confirmed these observations and described the effects of shear rate and tube diameter and the time dependency of the phenomenon. The effects of RBC aggregation also depend on the orientation of the flow system, as follows: 1) in straight, horizontal tubes at low flow rates, aggregation-induced sedimentation of RBCs results in increased viscosity due to an elevation of tube hematocrit (Hct; Refs. 2, 4, 34); and 2) in straight, vertical tubes at low flow rates, RBC aggregation leads to the formation of a cell-poor layer at the tube wall and an irregular core of RBCs at the tube center (3, 22, 37, 38, 42), which yields decreased flow resistance and lower apparent viscosity (3).

Given the geometric complexity of the microcirculation (36), observations in straight tubes may not be directly applicable to in vivo flow conditions. Frequent branching is characteristic of microvessels, and thus the residence time of blood in an individual vessel may not be sufficient to allow phase separation (3, 15). Furthermore, the orientation of individual microvessels may affect the development of a cell-poor marginal zone (22), and vascular control mechanisms may additionally complicate predictions based on in vitro findings (12). In fact, classical isolated limb and muscle studies by Whitaker and Winton (47) and later by Djojosugito et al. (23) have shown that the apparent viscosity of blood in vivo is lower than that measured in vitro. Factors such as the Fahraeus-Lindqvist effect (47), inertial energy losses, altered vessel geometry (27), and phase separation related to RBC aggregation have thus been suggested to play roles in the differences between in vivo and in vitro measurements of blood viscosity.

Several microvascular studies aimed at determining the specific effects of RBC aggregation on in vivo blood flow resistance have been reported; however, the results are not always in concordance. Intravital microscopy studies indicate that intensified RBC aggregation increases microvascular flow resistance (16, 17, 25, 32, 46). The effects of RBC aggregation on venous blood flow dynamics have been extensively studied (15–20), and results indicate that shear forces are low enough to allow significant aggregation and hence a predicted reduction in flow resistance. However, the expected reduction of flow resistance after increasing RBC aggregation by infusing 500-kDa dextran was not observed, which leads to the suggestion that RBC transit times through the nonbranched lengths of venules are too short to allow development of sufficient axial migration (15, 17–19). Conversely, using rat mesentery and cremaster preparations, Drussel et al. (25) indicated that greatly enhanced RBC aggregation achieved via 500-kDa dextran infusion markedly increased microvascular flow resistance. These authors report that a 5-fold increase of aggregate strength resulted in a 13-fold increase of mesentery resistance and a 3-fold increase in cremaster resistance. The difference between the vascular beds was suggested to be due to the effectiveness of blood flow regulation mechanisms (25).
Studies of RBC aggregation effects in whole organ preparations are also not concordant and indicate that enhanced aggregation decreases, increases, or has no effect on flow resistance. Using a perfused isolated rat heart and 70-kDa dextran, Charanssonney et al. (21) indicated that mild aggregation reduced resistance, whereas greatly enhanced aggregation elevated flow resistance. However, increased RBC aggregation caused by high-molecular-mass dextrans either increased blood flow resistance in the liver (39) or had no effect on uteroplacental blood flow (44). In a cat muscle preparation, Cabel et al. (20) observed an inverse relation between venous conductance and blood flow for normal blood, and this effect was reduced or absent for either nonaggregating or highly aggregating RBC suspensions; they thus concluded that RBC aggregation significantly contributes to venous vascular resistance in resting muscle and plays an important role in vascular system homeostasis (45). Using an isolated perfused rat hindlimb preparation and viscosity-matched suspending media, Baskurt et al. (7) observed that flow resistance was lower for aggregating vs. nonaggregating RBC suspensions.

As noted above, the results of microcirculatory studies do not agree with the findings of whole organ perfusion studies. Vicaut (45) 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 might be counteracted by the higher energy cost at the entrance of capillaries for disaggregation of RBCs in aggregating blood samples. Obviously, this higher energy cost should be reflected by microcirculation studies, whereas the whole organ studies should reflect a balance between the two opposing effects (45). Another explanation for the contradictory findings of microcirculation and whole organ studies might be the orientation of blood vessels under investigation. Most microcirculation studies that employ intravital microscopy technique are performed using tissues spread on a microscope stage, and blood flow orientation is usually horizontal. RBC aggregation has been demonstrated to increase flow resistance in tubes with such an orientation due to the sedimentation of aggregates (22). However, it has been suggested that in the intact vascular system, the residence time of blood in the vascular segments is insufficient for meaningful development of this sedimentation effect (15).

It should be noted that essentially all of the above-mentioned studies regarding the in vivo effects of RBC aggregation have utilized either large proteins (e.g., fibrinogen) or high-molecular-mass polymers (e.g., 70- or 500-kDa dextran) infused into the circulation to enhance aggregation. The present study was designed to employ polymer-coated RBCs (5) in native plasma to study the effects of graded alterations of RBC aggregation on vascular resistance in a guinea pig isolated and perfused hindlimb preparation.

**MATERIALS AND METHODS**

**Animals.** Adult guinea pigs of either sex (400–500 g body wt) were used in this study. Blood for perfusion of the hindlimbs of these animals was obtained from donor animals under light ether anesthesia by cardiac puncture and was anticoagulated with EDTA (1.5 mg/ml). The experimental protocol was approved by the Animal Care and Usage Committee of Akdeniz University and was in accordance with the Declaration of Helsinki and International Association for the Study of Pain guidelines.

**Modification of RBC aggregation.** RBC aggregation was modified by employing a novel technique whereby copolymers that possess the ability to self-associate above a specific temperature are covalently attached to the RBC surface. These triblock copolymers, which are commercially known as Pluronics and commonly known as polyoxymers, are composed of a central hydrophobic block of polypropylene glycol (PPG) flanked by two identical hydrophilic polyethylene glycol chains (40). Pluronics exhibit a critical micellization temperature (CMT) at which a phase transition occurs from predominantly single, fully hydrated copolymer chains to micelle-like structures that are characterized by association of the hydrophobic PPG blocks (1, 6, 14). The CMT decreases with increasing mass of the PPG block, and by the appropriate selection of a Pluronic, RBC aggregation can be controllably and predictably enhanced or inhibited following covalent attachment of the Pluronic to the RBC surface (5). That is, at temperatures above the CMT, Pluronics attached to the RBC surface micellize with Pluronics on adjacent RBCs and thereby enhance RBC aggregation; the strength of the association is dependent on the temperature and the concentration of Pluronic used during the coating process. Conversely, below the CMT, Pluronics attached to the RBC surface inhibit RBC aggregation due to polymer-polymer repulsion (5).

**Copolymer derivatization.** Pluronic copolymers were a gift from BASF Performance Chemicals (Parsippany, NJ). These polyoxymers contained 80% polyethylene glycol by mass and were designated as Pluronic F-68 (8.4 kDa) and Pluronic F-98 (13.0 kDa). To facilitate covalent attachment of the Pluronics to the RBC surface, a reactive derivative of the copolymers was first prepared. This was achieved by converting the terminal hydroxyl groups of the copolymer chain to succinimidyl carbonate (SC) groups as previously described (13) using a modification of the method described by Miron and Wilchek (33).

**RBC coating.** The SC derivatives of Pluronic F-68 (F68-SC) and Pluronic F-98 (F98-SC) covalently attach to primary amines, principally lysine residues, on the RBC surface when incubated at an elevated pH (pH > 7.50) for a short time period (ca. < 1 h). Any unreacted SC derivative hydrolyzes during the incubation period to a nonreactive form that is simply removed during subsequent RBC washing steps. To achieve graded increases of RBC aggregation, cells were incubated with various concentrations of the reactive F98-SC. At 37°C, Pluronic F-68-coated RBCs in autologous plasma exhibit aggregation and low-shear viscosity essentially identical to uncoated RBCs (14), and thus Pluronic F-68 was selected as an appropriate control for the presence of covalently attached copolymer without enhanced RBC aggregation.

Blood was centrifuged at 1,400 g for 6 min, and the plasma was separated and saved. RBCs were washed three times with isotonic phosphate-buffered saline (PBS, pH 7.4) and resuspended in 30 mM triethanolamine buffer (290 mosM/kg, pH 6.60) at a Hct of ~0.1 l/l, and the suspension was cooled to 4°C. All Pluronic-coating procedures were performed at 4°C (i.e., below the CMT) to obtain even coating of Pluronic on RBCs and to avoid the formation of cross-linked aggregates of coated RBCs. Immediately before use, the Pluronic-SC was dissolved in a 4°C hypotonic phosphate buffer (50 mM NaH₂PO₄ and 60 mM NaCl, pH 5) at a concentration of 10 mg/ml. The Pluronic-SC solution was promptly added to RBCs suspended in triethanolamine buffer to obtain six final concentrations between 0.00125 and 0.5 mg/ml, and the suspension was incubated for 2 h at 4°C with continuous gentle mixing on a tube rocker. Coated RBCs were separated, washed three times with PBS by gentle centrifugation (400 g for 5 min), and then resuspended in plasma at 0.4 l/l Hct.

**Microscopic and rheologic studies.** Wet mount preparations of dilute RBC suspensions in plasma were examined and photographed using bright-field light microscopy. Sedimentation rates were measured using Westergren sedimentation tubes, and RBC-plasma suspensions were adjusted to a Hct of 0.1 l/l. Owing to blood volume
demands of the perfusion protocol, these sedimentation rates were determined with a separate group of samples (n = 4) prepared as described above.

Isolated hindlimb preparation. Guinea pigs were anesthetized with urethane (20% solution in isotonic saline, 1 g/kg). The lower abdomen was opened by a midline incision, and a 24-gauge cannula was inserted into the left iliac artery and advanced to the femoral artery. The ipsilateral iliac vein was also catheterized using the same size of cannula. Visible branches throughout the dissected tract of the femoral artery and vein (i.e., collateral and cutaneous vessels) were cauterized to minimize flow through such routes, but the hindlimb was not surgically isolated from the rest of the body. The left leg was cooled during the cannulation procedure by externally applied ice. After cannulation was complete, the animal was euthanized via an overdose of pentobarbital sodium. Just before we started the perfusion studies, the lower body of the animal was submerged into a 37°C water bath.

Perfusion of the hindlimb preparation. The arterial cannula was connected to a pressure servo-control system composed of a roller pump, a pressure transducer monitoring the inflow pressure, and a control unit (PS/200/Q, Living Systems Instrumentation). A microfiber array oxygenator (model OX, Living Systems Instrumentation) was used in the arterial line to oxygenate the perfusion fluids (PBS or RBC suspensions) with room air. The PO2 of the RBC suspensions used for perfusion was 130 ± 17 mmHg. RBC sedimentation problems in the perfusion system were minimized via a magnetic stirrer in the suspension reservoir and the use of vertically oriented small-bore tubing. The arterial perfusion pressure was set to 100 mmHg for all experiments; venous pressure was also recorded for the perfused limb. Arterial and venous pressures together with the volumetric flow rate obtained from the calibrated speed of the roller pump were recorded on a laboratory computer throughout the experiment. RBC suspensions were either uncoated or Pluronic-coated RBCs in plasma at a Hct of 0.4 l/l.

The protocol for the studies involved perfusing the hindlimb using the following sequence: 1) cell-free PBS for 30 min, 2) 5 ml of uncoated RBC suspension (control), 3) 10 ml of PBS, 4) 5 ml of coated RBC suspension, 5) 10 ml of PBS before perfusion with the next RBC suspension, and 6) repeat of steps 4 and 5 for each test RBC suspension. Typical washout volumes ranged from 0.5 to 0.8 ml, and thus pressure and volumetric flow data used for calculation of flow resistance for PBS and RBC suspensions were obtained during the last 60 s of stable perfusion. For cells coated with Pluronic, the order in which the six different coating concentrations (0.00125–0.5 mg/ml) were used was varied between different preparations (i.e., low to high and high to low). Vascular resistance was calculated from the arterial-venous pressure difference and the volumetric flow rate. Flow resistance values were corrected for differences in vascular hindrance between preparations by dividing the calculated values by the flow resistance obtained during perfusion with cell-free PBS.

Blockage of smooth muscle tone. In one series of experiments, the PBS used for the initial 30-min perfusion contained 10−4 M papaverin to inhibit smooth muscle tone. This level of papaverin was also added to the RBC suspensions and the PBS perfused following each RBC suspension.

Statistics. Data are presented as means ± SE. Multiple comparisons were done by one-way ANOVA followed by Newman-Keuls post test or two-way ANOVA followed by a Bonferroni post test. Statistical significance was accepted at P values <0.05.

RESULTS

Graded alteration of RBC aggregation. Microscopic observations of dilute wet mounts of cells in plasma indicated that RBC aggregation was enhanced in a graded manner after incubation with F98-SC at concentrations between 0.00125 and 0.5 mg/ml (Fig. 1). It should be noted that the degree of aggregation (i.e., number of RBCs per aggregate) increased with F98-SC concentration and that three-dimensional structures dominate at and above F98-SC levels of 0.05 mg/ml. RBC aggregation quantified by measuring the sedimentation rate of dilute suspensions (i.e., 0.1 l/l Hct) was consistent with the microscopic observations, with the increases of RBC sedimentation statistically significant at and above F98-SC levels of 0.025 mg/ml (Fig. 2). Treatment of RBCs with F68-SC at concentrations between 0.00125 and 0.5 mg/ml had no effect on RBC aggregation as judged by both microscopic observations and sedimentation rates (data not shown).

Flow resistance in hindlimb preparations. Hydraulic resistance as determined during cell-free PBS perfusion of hindlimbs from seven animals averaged 24.8 ± 5.6 peripheral resistance units (PRU); this value decreased by 35% (P < 0.05) in six hindlimbs in which smooth muscle tone was inhibited with papaverin (Fig. 3).

At the 100-mmHg constant perfusion pressure employed for all studies, average blood flow for uncoated RBCs in seven hindlimbs with intact local vascular control mechanisms was 559 ± 129 μl/min [coefficient of variation (CV) = 61%]. These blood flow data were characterized by a substantial variation of individual flow rates ranging between 183 and 1,171 μl/min; the calculated mean blood flow resistance in this group was 251.3 ± 60.5 PRU (Fig. 3). In a separate group of six hindlimbs perfused with uncoated RBCs, blockage of vascular smooth muscle tone by papaverin resulted in a 130% increase of average blood flow rate to 1,279 ± 79 μl/min (CV = 15%) and a lower variation between animals (range, 999–1,456 μl/min). The calculated mean blood flow resistance in the papaverin-treated group was 83.1 ± 5.7 PRU and was thus significantly lower (P < 0.001) than the group with intact vascular control (Fig. 3).

Altered flow resistance by increased RBC aggregation. Flow resistance values measured during perfusion with RBC suspensions treated with various concentrations of F98-SC are presented in Fig. 4. The values shown are corrected for differences in vascular hindrance in each hindlimb (i.e., flow resistance with cell-free PBS) and are expressed as the percentage of the value determined during perfusion with uncoated RBCs. In the experiments with intact vascular control mechanisms, flow resistance decreased slightly for RBCs treated with F98-SC at a concentration of 0.0125 mg/ml; this concentration also caused a significant 60% increase in sedimentation rate (see Fig. 2). With increasing F98-SC levels, flow resistance tended to increase and then decrease, although none of the alterations at any F98-SC level were significantly different from uncoated cells. However, in the papaverin-treated hindlimb preparations, there was an increment of flow resistance during perfusion with RBC suspensions treated with 0.0125 mg/ml F98-SC. At 0.025 mg/ml F98-SC, the increase was more profound and significantly greater than for uncoated RBCs (see Fig. 4); this F98-SC concentration resulted in a 97% increase in sedimentation rate (see Fig. 2). Additional increases in F98-SC concentration to 0.05 and 0.25 mg/ml, which correspond to 136 and 162% increments of RBC sedimentation rates, resulted in a decrease in flow resistance almost to uncoated cell values. At the highest F98-SC concentration (0.5 mg/ml, a 200% increase of sedimentation rate), flow resistance was again significantly increased (see Figs. 2 and 4). Note that in papaverin-treated hindlimb preparations perfused with F68-SC-treated RBCs, no significant differences in flow resistance vs. uncoated cells.
were observed at any concentration between 0.00125 and 0.5 mg/ml (Fig. 5).

DISCUSSION

The unique aspect of the present study is the novel method used to modify the aggregation behavior of RBCs when suspended in anticoagulated but otherwise unaltered and undiluted plasma. The method is based on the technique developed by Armstrong and co-workers in which nonionic Pluronic copolymers that possess the ability to self-associate (micellize) are covalently attached to the RBC membrane (5). This technique allows graded changes of aggregation by varying the concentration of the copolymer during the RBC coating process. Note

![Fig. 1. Photomicrographs of red blood cells (RBCs) in plasma for uncoated (control) cells and cells coated with Pluronic F-98 at concentrations between 0.00125 and 0.5 mg/ml.](image)

![Fig. 2. RBC sedimentation, measured as (millimeter) drop in interface after 30 min for 0.1 l/l hematocrit RBC-plasma suspensions before and after RBCs were coated with Pluronic F-98 at concentrations of 0.00125–0.5 mg/ml. *P < 0.05; **P < 0.01, differences from uncoated RBC suspensions (i.e., zero concentration).](image)

![Fig. 3. Flow resistance [in peripheral resistance units (PRU)] calculated using flow rates during perfusion with PBS and with uncoated RBCs in plasma for hindlimb preparations with intact vascular control mechanisms (control) and for papaverin-treated preparations. Values are means ± SE. *P < 0.05, difference from corresponding control value.](image)
resolution measured during perfusion with uncoated cells.

normal plasma is characterized by linear arrays of RBCs (i.e.,
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diffuses plasma proteins and reduces RBC, white blood cell, and
any macromolecular solution that is retained in the circulation
predicted simply by polymer concentration. Third, infusion of
ing plasma oncotic pressure would be much greater than
have very nonlinear van’t Hoff’s relations, and thus the result-
ance (36). Furthermore, high-molecular-mass dextrans
resistance by altering local shear forces and hence vascular
because F68-coated cells behaved like uncoated cells (Fig.
, it is possible to exclude rheological artifacts (e.g., decreased
RBC deformability) due to copolymer coating.

In contrast with the present study, almost all previous reports
designed to investigate hemodynamic consequences of RBC
aggregation have employed infusion of high-molecular-weight
macromolecules such as dextrans or starches (7, 17, 20, 25, 32,
39, 44–46). However, these polymer infusion approaches have
several disadvantages. First, it is not always possible to finely
vary the degree of RBC aggregation, and thus most studies
have employed two levels of aggregation (i.e., "normal" and
"enhanced") instead of a range of aggregation tendencies.
Second, high-molecular-weight molecules introduced into the
circulation not only modify RBC aggregation but also influence
the chemical and physical properties of plasma (e.g.,
increased viscosity and oncotic pressure). For example, 500-
kDa dextran plasma levels of 0.6 to 1.4 g/dl as used in
perfusion studies (16–19) result in 40–100% calculated in-
creases of plasma viscosity (43). In turn, elevated medium
viscosity may have significant effects on in vivo hydrodynamic
resistance by altering local shear forces and hence vascular
gemoetry (36). Furthermore, high-molecular-mass dextrans
have very nonlinear van’t Hoff’s relations, and thus the result-
ing plasma oncotic pressure would be much greater than
predicted simply by polymer concentration. Third, infusion of
any macromolecular solution that is retained in the circulation
dilutes plasma proteins and reduces RBC, white blood cell, and
platelet counts. Finally, appropriate control experiments using
lower-molecular-mass polymers (e.g., 40-kDa dextran) can be
difficult to design and undertake due to differences in plasma
viscosity at comparable polymer concentrations, in polymer
concentration at a constant plasma viscosity, in clearance route,
and in clearance rate over the time course of the experiment.

In addition to overcoming the abovementioned disadvantages, the
Pluronic-coating method may permit exploration of the effects of abnormally elevated "pathological" aggregation
of cells in native plasma. Aggregation at stasis for cells in
normal plasma is characterized by linear arrays of RBCs (i.e.,
RBC rouleaux) with some degree of side and end branching of
the rouleaux (26, 31, 41); such aggregation is observed for
uncoated guinea pig RBCs and, to a certain extent, for RBCs
treated with the lower concentrations of F98-SC (see Fig. 1).
However, RBC aggregation in pathophysiological states (e.g.,
poorly controlled diabetes, nephrotic syndrome, myocardial
infarction, and severe acute phase reaction) is much more
intense, and the structures consist of somewhat shorter rou-
leaux formed into irregular three-dimensional clumps (31) that
are more difficult to disperse by fluid shear forces (41). Al-
though F98-coated RBCs show nonrouleaux formation at stasis
(see Fig. 1), the aggregation is reversible with increasing shear
(5), and the aggregability is controllable with F98-SC concen-
tration. Intense, reversible aggregation can readily be induced
at elevated F98-SC levels (see Fig. 1), which leads to greatly
elevated low-shear blood viscosity (5) and may facilitate the
development of in vivo models that are relevant to those
conditions associated with greatly abnormal RBC aggregation.

The experimental results presented in Fig. 4 indicate that
alterations of RBC aggregation tendency can influence blood
flow resistance in the isolated hindlimb of guinea pigs. Two
important aspects of these results are that the changes of flow
resistance resulting from altered RBC aggregation intensity are
modulated by local vascular control mechanisms, and the
nature of the alterations in flow resistance does not vary
monotonically with RBC aggregation intensity. With regard to
the resistance results, note that although all visible branches of
the femoral artery and vein (i.e., collateral and cutaneous
vessels) were cauterized, the hindlimb was not surgically
isolated, and thus arterial blood flow could include contribu-
tions from noncauterized vessels. The proportion of this type of
flow was not determined due to technical limitations.

Dealing first with the influence of local vascular control
mechanisms, it is notable that in hindlimbs treated with papav-
erin, enhanced RBC aggregation resulted in significantly
increased flow resistance at two levels of aggregation intensity
(i.e., for cells treated with 0.025 and 0.5 mg/ml F98-SC).
Conversely, in control preparations with intact local vascular
control, the lower concentration yielded only a nonsignificant
increase, and no meaningful trend vs. uncoated cells was seen
at the higher level. The local physiological mechanisms (i.e.,

![Fig. 4. Flow resistance during perfusion with RBC suspensions treated with SC derivatives of Pluronic F-98 (F98-SC). Results are expressed as the percentage of flow resistance during perfusion with uncoated cells, and thus a value of 100 indicates equality with uncoated RBCs. Data are for seven hindlimb preparations with intact vascular control mechanisms (control) and for six papaverin-treated preparations. *P < 0.05, difference from flow resistance measured during perfusion with uncoated cells.](image)

![Fig. 5. Flow resistance during perfusion with RBC suspensions treated with SC derivatives of Pluronic F-68 (F68-SC). Results are expressed as the percentage of flow resistance during perfusion with uncoated cells, and thus a value of 100 indicates equality with uncoated RBCs. Data are for six papaverin-treated preparations.](image)
metabolic and myogenic responses) that regulate vascular geometry are very effective in maintaining adequate blood flow in a given vascular bed (12), and the metabolic demand of the tissue plays an important role. Any acute alteration that affects the balance between this demand vs. supply provided by the blood flow is usually compensated for by the alterations of vascular hindrance (24). Intravascular pressure changes due to altered perfusate viscosity could also affect vascular hindrance via the myogenic response, and it has been shown that compensation for alterations of hemorheological properties (e.g., increased Hct and decreased RBC deformability) can be achieved if the tissue has sufficient vasodilatory reserve (10, 12). The differences between the responses of the two types of preparations (see Fig. 4) thus most likely have their basis in the degree of vasodilatory reserve available in the control hindlimbs. Note that the vascular tone in the control group (i.e., the nonpapaverin-treated group) might be lower than for normal animals inasmuch as central nervous influences were not present in the isolated limb preparation. Therefore, the ability to compensate for hemorheological alterations should be even greater in normal animals with intact central nervous control of vascular tone. Conversely, the somewhat elevated PO2 value of the RBC suspensions used for perfusion and the resulting vasoconstriction could have augmented vasodilatory reserve in the control group.

Differences in the degree of vasodilatory reserve are also probably responsible for the variations (i.e., standard error) observed in the control data. Preparation to preparation differences of flow resistance for the control hindlimbs were large, especially at 0.025 and 0.5 mg/ml F98-SC, and were greater than for the corresponding concentrations in the papaverin series (see Fig. 4); flow resistance variations were also large when uncoated cells were used in the intact control preparations (see Fig. 3). These considerable variations most likely reflect the wide range of basal vascular tone in the intact preparations and could thus explain the large variations of flow resistance with enhanced RBC aggregation (see Fig. 4). That is, because the degree of vasodilatory reserve strongly depends on the level of vascular tone (12, 24), an initially high tone allows a large degree of compensation (i.e., vasodilation) for hemorheological abnormalities, whereas an initially low tone would have a smaller vasodilatory reserve available for compensation.

An interesting finding of this study is the nonmonotonic dependence of flow resistance alterations on RBC aggregation intensity: increasing intensity resulted in two regions of increased flow resistance separated by a region of essentially unaltered resistance (see Figs. 1, 2, and 4). Previous reports have primarily employed infusions of high-molecular-mass dextrans to alter aggregation with comparisons made between normal and hyperaggregating conditions (20, 44) or between nonaggregating and aggregating RBC suspensions (7, 39). However, Charansonney et al. (21) utilized two plasma concentrations of 500-kDa dextran in an isolated perfused rat heart preparation; rat RBCs in native plasma exhibit essentially no aggregation (8), and thus comparisons could be made with two levels of enhanced aggregation. They reported decreased flow resistance with the lower level of induced aggregation but increased flow resistance with the higher level (21). Guinea pig RBCs also exhibit low to moderate aggregation in native plasma (see Fig. 1), and increased aggregation first resulted in a significant increase in blood flow resistance in the papaverin series (see Fig. 4). Additional increases in aggregation then resulted in decreased flow resistance followed by another increase of flow resistance at the highest F98-SC level. Our results are thus conceptually consistent with the results of Charansonney et al. (21), although this study revealed three phases associated with graded increases of aggregation whereas their study revealed two.

The mechanisms underlying the observed effects of RBC aggregation on blood flow resistance (see Fig. 4) are most certainly complex and probably involve interactions between several theoretical and experimental considerations. In particular, RBC aggregation may affect in vivo flow resistance via the following mechanisms: 1) RBC aggregation is known to be the main cause of increased blood viscosity under low-shear conditions, and this effect is explained by the increased disturbance of flow-stream lines with increased particle size (11); 2) increased aggregation is expected to increase the energy cost for the breakdown of aggregates as the blood approaches the microcirculation and thereby increase flow resistance (45); 3) increased aggregation is expected to increase the axial accumulation of RBCs and thereby increase plasma skimming and lower tissue Hct (9, 29, 30); the extent of this effect is dependent on the orientation of the blood vessels vs. gravity and the shear rates within the vessels (22, 29), however, this effect might be minimized by the insufficient residence times in the vasculature (15); 4) increased axial accumulation of RBCs may result in decreased local viscosity at the marginal zone of blood vessels and, as has been demonstrated repeatedly in glass tubes (3, 22, 37, 42), decreased frictional resistance with the vessel wall; and 5) decreased local viscosity of the marginal layers in blood vessels might be associated with decreased pressure gradients and hence lower wall-shear stresses for some vessels, thereby affecting vascular control mechanisms that are modulated by shear stress (for example, we have demonstrated that increased RBC aggregation results in diminished nitric oxide (NO)-dependent vascular control and decreased endothelial NO synthase expression (13)); based on these considerations, increased RBC aggregation would result in increased vascular resistance due to inhibition of NO generation by the endothelium.

As is evident from the preceding paragraph, more than one process may affect the relations between RBC aggregation and vascular flow resistance. However, based on the results shown in Figs. 1, 2, and 4, one possible sequence with increasing RBC aggregation would involve an initial increase in flow resistance due to increased aggregate size and disturbed fluid stream lines followed by a decrease in flow resistance due to RBC axial accumulation, and then increased flow resistance related to the additional increment in aggregate size and strength, and, hence, increased energy costs for dispersion of RBC aggregates. Of course, other explanations are also possible, and thus a more detailed analysis of the origins of the complex relations between aggregation and vascular resistance is warranted.

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REFERENCES


