Microvascular perfusion upon exchange transfusion with stored red blood cells in normovolemic anemic conditions

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BACKGROUND: Transfusions are intended to augment oxygen-carrying capacity. The ability of fresh and stored red blood cells (RBCs) to maintain microvascular perfusion and oxygen delivery to the tissue has not been directly measured.

STUDY DESIGN AND METHODS: Microvascular responses to exchange transfusion with fresh and stored RBCs after acute isovolemic hemodilution with a plasma expander were investigated with the hamster window chamber model. In-vivo functional capillary density (FCD), blood flow, and high-resolution oxygen distribution in microvascular networks were measured by noninvasive methods.

RESULTS: Exchange transfusion with an RBC suspension after a 60 percent isovolemic hemodilution with dextran 70 (6% MW = 70 kDa) resulted in a hematocrit of 18 percent (5.6 ± 0.2 g/dL hemoglobin [Hb]). All other systemic variables were unchanged. Stored RBCs (28 days in citrate-phosphate-dextrose-adenine-1) resuspended in fresh frozen plasma matched to the Hct and Hb concentration were exchange transfused until 25 percent of the circulating RBCs were stored RBCs. Stored RBCs reduced microvascular flow and FCD by 63 and 54 percent, respectively, of the level achieved when fresh RBCs were exchange transfused. Microvascular oxygen extraction by the stored RBC was 54 percent lower than that of the fresh RBCs. The tissue oxygen levels were 3.5 and 14.4 mmHg for the stored and fresh RBCs, respectively.

CONCLUSION: Circulation of stored RBCs in a hemodiluted animal resulted in significantly malperfused and underoxygenated microvasculature that was not detectable at the systemic level.

Transfusion of blood to treat anemia in normovolemic patients is prescribed with the belief that it enhances oxygen-carrying capacity. Increasing hemoglobin (Hb) concentration by transfusion increases global oxygen delivery as measured by systemic variables, but little is known about whether transfusions provide an immediate increase in the oxygen delivery to tissue at the microvascular level thereby alleviating tissue hypoxia. Prior studies have shown that transfusion of stored blood is less efficacious than anticipated as critically ill patients were not able to augment their oxygen consumption.[1,2] Storage of red blood cells (RBCs) results in biochemical and physical changes that hinder their function during transfusion[3,4] and could increase risk factors. RBC 2,3-diphosphoglycerate acid (2,3 DPG) and adenosine triphosphate (ATP) concentrations become depleted during storage, which decreases their ability to off-load oxygen to the tissue and hinders their flexibility reducing local tissue perfusion.[5-7] RBC fragility increases during storage accelerating the release of cell-free Hb leading to nitric oxide scavenging and subsequent vasoconstriction.[8] All these factors tend to negate the positive effect of increasing the oxygen-carrying capacity by RBC transfusion.

Avoidance of unnecessary transfusion and reduction in the risk of transfusion-associated disorders requires a quantitative evaluation of the effectiveness of stored RBCs

ABBREVIATIONS: FCD = functional capillary density; HR(s) = heart rate(s); MAP = mean arterial pressure; p_aO_2 = arterial oxygen partial pressure.

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in oxygen delivery and tissue perfusion at the microvascular level. Functional capillary density (FCD), that is, the number of RBC-perfused capillaries per unit volume of tissue, an indicator of microvascular perfusion, has proven to be a critical variable for survival from hemorrhagic shock[9] and is very responsive to changes in oxygen-carrying capacity during extreme hemodilution.[10,11] These findings offer a link between microvascular and systemic parameters in terms of tissue perfusion and outcome; they suggest that it is critical to determine whether local tissue perfusion is maintained during transfusion in addition to the overriding concern for an increase in local tissue oxygen uptake.

In this investigation, moderate normovolemic hemodilution was performed with a plasma expander in the hamster window model to reduce the oxygen reserve. This procedure, which does not correspond to the clinical goal of transfusion of increasing hematocrit (Hct), was used to magnify potential changes in oxygen delivery and microvascular function by transfused RBCs. The objective of this study was to directly measure local perfusion and microvascular oxygen distribution when 28-day stored RBCs are introduced into an anemic normovolemic hemodiluted animal to examine whether these parameters are maintained as the Hb concentration is kept at a critical level but partially replaced by stored RBCs.

MATERIALS AND METHODS

Animal preparation

Investigations were performed in 55- to 65-g golden Syrian hamsters (Charles River Laboratories, Boston, MA). Animal handling and care were provided following the procedures outlined in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The study was approved by the institutional Animal Subjects Committee.

The hamster window chamber model is widely used to study the microvasculature in an unanesthetized state. The complete surgical technique is described in detail elsewhere.[12,13] A chamber consisted of two identical titanium frames with a 15-mm circular glass window. The animal was prepared for chamber implantation with a 50 mg/kg intraperitoneal injection of sodium pentobarbital anesthesia. After hair removal, the dorsal skin was lifted away from the animal with sutures and one frame of the chamber was positioned on the animal's back. With the aid of backlighting and a stereomicroscope, one side of the skin fold was removed following the outline of the window until only a thin layer of retractor muscle and the intact subcutaneous skin of the opposing side remained. A cover glass held in place by the other frame of the chamber was placed on the exposed skin thereby sealing the remaining intact skin of the other side from exposure to the ambient environment. The animal was allowed at least 2 days for recovery. If the chamber, assessed under the microscope, showed no signs of edema, bleeding, or unusual neovascularization, arterial and venous catheters (PE-50 with PE-10 tips), filled with a heparinized saline solution (30 IU/mL), were implanted in the carotid artery and jugular vein and then exteriorized at the dorsal side of the neck. Experiments were performed 24 to 48 hours after catheter implantation.

Inclusion criteria

Animals were suitable for the experiments if: 1) systemic variables were within normal range, namely, a heart rate (HR) of more than 340 beats per minute, mean arterial pressure (MAP) of more than 80 mmHg, systemic Hct level of more than 45 percent, and arterial oxygen partial pressure (p_aO_2) of more than 50 mmHg; and 2) microscopic examination of the tissue in the chamber observed under ×650 magnification did not reveal signs of edema or bleeding.

Systemic parameters

MAP was tracked periodically during the experiment via the arterial catheter and HR was determined from the pressure trace (Biopac MP150, Santa Barbara, CA). Systemic Hct was measured from centrifuged arterial blood samples taken in heparinized capillary tubes.

Blood chemistry

Arterial blood was sampled from the carotid artery catheter into a heparinized capillary tube and immediately analyzed for pO_2, pCO_2, and pH at 37°C (Blood Gas Analyzer, Model 248, Chiron Diagnostics, Tarrytown, NY). The blood and plasma Hb content was determined with a handheld photometer (B-Hemoglobin Photometer, Hemocue, Sweden) from a drop of blood and from fluid salvaged from the heparinized capillary tubes used for Hct measurement, respectively.

FCD

Detailed mappings were made of the chamber vasculature so that the same vessels were studied throughout the experiment. Capillary segments were considered functional if RBCs were observed to transit over a 30-second period. FCD is tabulated from the capillary lengths with RBC flow in an area composed of 10 successive microscopic fields ($420 \times 320\ \mu m^2$).[14]

Microhemodynamic variables

Arteriolar and venular blood flow velocity are measured online with the photodiode cross-correlation technique[15]

Fiber Optic Photo Diode Pickup and Velocity Tracker, Model 102B, Vista Electronics, San Diego, CA). The measured centerline velocity (V) was corrected according to vessel size to obtain the mean RBC velocity.[16] The video image shearing technique was used to measure vessel diameter (D).[17] Blood flow was calculated from the measured parameters as $Q = V \cdot \pi(D/2)^2$.

Microvascular pO_2 distribution

High-resolution microvascular pO_2 measurements were made with phosphorescence quenching microscopy.[18] This noninvasive method of measuring oxygen levels is based on the oxygen-dependent quenching of phosphorescence emitted by albumin-bound metalloporphyrin complex after pulsed light excitation. Phosphorescence microscopy is independent of the dye level within the tissue, and the decay time is inversely proportional to the pO_2 level. Interstitial pO_2 measurements made with this system have been compared to simultaneous measurements with recessed oxygen electrodes, and their differences were found not to be significant.[19] Animals received a slow intravenous injection of 15 mg per kg at a concentration of 10.1 mg per mL of a palladium-meso-tetra(4-carboxyphenyl)porphyrin (Porphyrin Products, Inc., Logan, UT). The dye is allowed to circulate for 20 minutes before pO_2 measurements.

In our system, intravascular pO_2 measurements are made by placing an optical rectangular window (5×40 μm) longitudinally within the vessel of interest. Tissue pO_2 was measured in regions void of large vessels within intercapillary spaces with an optical window size of approximately 10×10 μm. Thus exact localization of the pO_2 measurements is known, whether intravascular in an arteriole or a venule or in the interstitial tissue. Such precise localization is not possible with needle or surface array electrode techniques. Results from this type of measurement allow for detailed understanding of microvascular oxygen delivery and, most importantly, to assess oxygen uptake by the tissue. The phosphorescence decay due to quenching at a specific pO_2 yields a single decay constant and in vitro calibration has been demonstrated to be valid for in-vivo measurements.[19] Use of this technique with the window chamber model assures a high-resolution optical noninvasive method of assessing microvascular oxygen distribution in an intact tissue.

Tissue oxygen supply from microvascular data

Calculations of oxygen transport at the microvascular level are determined from the microvascular measurements. Oxygen delivery to the tissue is the difference between the oxygen content at the arterial (A) and venular (V) segment of the microvascular network times the average flow through the tissue and expressed as:

$$O_2 \text{ delivery to tissue} = \{([(\text{RBC Hb } \gamma\, S_A \text{ percentage}) + (1\text{—Hct})\alpha)\ pO_2A]) — ([(\text{RBC Hb } \gamma\, S_V \text{ percentage}) + (1 - \text{Hct})\alpha)\ pO_2V])\}^* Q,$$

where RBC Hb is the Hb concentration in RBCs (g/dL blood), γ is the oxygen-carrying capacity of Hb at 100% saturation or 1.34 mL O_2 per g Hb, S_A, V is the arteriolar oxygen saturation of RBCs (converted with hamster oxygen dissociation curve[20]), (1 – Hct) is the fractional plasma volume and converts the equation from per dL of plasma to per dL of blood, **????** is the solubility of oxygen in plasma, 3.14×10^{-3} mL O_2 per dL plasma mmHg, pO_2 is partial pressure of oxygen (mmHg), and Q is the volumetric blood flow calculated as the average arteriolar and venular flow.

Acute isovolemic exchange transfusion protocol

An acute anemic state was induced by lowering the systemic Hct to 40 percent of baseline with a two-step progressive isovolemic hemodilution with 6 percent dextran 70 (MW = 70 kDa), a colloid solution (Levels 1 and 2). Animals were then exchange-transfused in a third step (Level 3) with either fresh or stored RBCs. The progressive isovolemic blood exchange transfusion protocol is described in detail in our previous studies of hemodilution.[10,11] Briefly, the volume of each exchange transfusion step was calculated as percentage of the animal blood volume, estimated as 7 percent of the body weight. Level 1 exchange was 40 percent of the blood volume. Level 2 and 3 exchanges were both 35 percent of the blood volume. Level 1 and 2 exchanges were performed with dextran 70, and the Level 3 exchange was performed with an RBC suspension as the exchange medium.[10] A bidirection microinfusion syringe pump was used to withdraw blood from the carotid artery and simultaneously infuse the exchange solution into the jugular vein at a rate of 100 μL per second. The animal was given a 5-minute recovery period after the exchange transfusion before data acquisition.

Figure 1 shows the time course of the induction of acute anemia with the two stepwise isovolemic hemodilution exchanges followed by a one-step exchange transfusion with the test RBC suspension as a function of

	Baseline	Level 1	Level 2	Level 3
Time (hr)	0-	0	1	2
Exchange fluid		Dextran 70	Dextran 70	Fresh/stored RBCs
Hct (%)	47	28	19	18
[Hb] (g/dL)	15.0	9.0	6.2	5.9/5.7

Fig. 1. Experimental protocol. The time course of the normovolemic hemodilution (Levels 1 and 2) followed by the exchange transfusion (Level 3) with either fresh or stored RBCs.

relative Hct and total Hb content. Baseline systemic, microvascular, and hemodynamic characterizations were performed before the start of the exchange protocol. After each exchange and a stabilization period of 5 minutes, systemic and/or microvascular measurements were performed. Exchanges begin every hour, that is, the second exchange commences exactly 60 minutes after the start of the first exchange. After the exchange transfusion, the same measurements are taken and then the microvascular pO_2 distribution was determined with phosphorescence quenching microscopy.

Collection and storage of plasma and RBCs

Stored cells collection. Five donor hamsters (120-150 g) were anesthetized with 50 mL per kg intraperitoneally, and their left carotid artery was implanted with a PE-50 catheter per the protocol described above. The hamsters recovered from anesthesia before blood donation. The catheter was connected to a vacuum tube containing citrate-phosphate-dextrose-adenine-1 (CPDA-1) solution into which blood was collected. The blood sample was then centrifuged for 5 minutes, the plasma layer was collected from the supernate, and the RBCs were collected from the bottom. Samples were transferred into nonpyogenic, sterile polypropylene cryogenic vials (Corning, NY) and stored at –70 and 4°C, respectively.

Fresh cell collection. Fresh cells were collected from the study animal during the initial hemodilution steps. The sample was centrifuged, and then the supernate and RBCs were transferred into tubes and stored at room temperature until retransfusion. The RBCs were resuspended in fresh frozen plasma (FFP) taken from another animal to produce an 18 percent Hct or 5.6 g per dL solution. Care was taken to avoid removing samples from the layer between the plasma and RBCs that contained the white blood cells. The procedures for pipetting and transferring of materials were performed in a laminar flow hood for sterility.

Experimental groups and test solution preparation

RBCs were combined with thawed and filtered FFP to produce an RBC suspension of 5.6 g per dL. The mixture was gently inverted until mixed and the Hct and plasma Hb concentration was checked. Materials were prepared no more than 30 minutes before their infusion. In this study, p50, the partial pressure of oxygen at half-saturation, of fresh and 28-day stored blood with CPDA-1 was 28 and 12 mmHg, respectively (Hemox analyzer, TCS Corp., New Corp, PA). Methemoglobin levels of stored and fresh blood samples were not statistically different and ranged from 2 to 5 percent. Centrifugation of the stored RBC samples resulted in visually detectable levels of pink-tinged plasma which in a few cases corresponded to a measurable level with the Hemocue, which has a minimum resolution 0.2 g per dL. Samples with plasma Hb concentration greater than 0.2 g per dL were excluded from the study.

Animals were randomly divided into two experimental groups to receive the test RBC solution used during the final step of the hemodilution. Animals were then exchange-transfused in the third step (Level 3) with fresh autologous RBCs or 28-day stored donor RBCs.

Experimental setup

The unanesthetized animal was placed in a restraining tube that was stabilized by affixing the tube and the chamber to a plexiglass plate. The animal had free access to wet feed during the entire experimental period. The plexiglass stage holding the animal was placed on an inverted microscope (IMT-2 Olympus, New Hyde Park, NY) equipped with a 40× objective (Wplan 40×, n.a. = 0.7; water immersion; Olympus). The tissue image was projected onto a charge-coupled device camera (Model 4815-2000, Cohu, San Diego, CA) and viewed on a monitor (Model PVM-1271Q, Sony). The animal was allowed a 30-minute adjustment period to the tube environment before the baseline systemic variables (MAP, HR, arteriolar blood gases, and Hct) were measured. Microvascular fields of study were chosen by their visual clarity. FCD was assessed. Arterioles and venules (four to six of each type) were characterized by their diameter and blood flow velocity.

Statistical analysis

Results are presented with means ± SD unless otherwise noted. Data are presented as absolute values and ratios relative to baseline values. A ratio of 1.0 would signify no change from baseline while lower and higher ratios are indicative of changes proportionally lower and higher than baseline (i.e., 1.5 would mean a 50% increase from the baseline level). All measurements were compared with baseline levels obtained before the experimental procedure. The same vessels and functional capillary fields were followed so that direct comparisons to their baseline levels could be performed allowing for more robust statistics for small sample populations. For repeated measurements, time-related changes were assessed by analysis of variance for nonparametric repeated measurement and when appropriate post hoc analyses performed with the Dunn's multiple comparison test. Because microvascular oxygen tension measurements can only be performed once in each animal, these measurements are compared to a control group of animals. All statistics were calculated with computer software (Prism 4.0, GraphPad, San Diego, CA). Changes were considered statistically significant if the p value was less than 0.05.

RESULTS

Eleven animals that satisfied the inclusion criteria were entered into the study. All animals completed the protocols without any visible signs of discomfort. Animals were observed resting and periodically eating wet feed throughout the experimental period.

Animals were randomly assigned to the two experimental groups depending on the fluid used for the Level 3 exchange: stored RBCs (n = 5) or fresh RBCs (n = 6). One-way ANOVA of the systemic and microvascular data at Baseline and Level 2 in both experimental groups found no differences, and therefore the data were combined into one representative group (n = 11) for each state.

TABLE 1. Systemic variables before and after exchange protocol

Variable	Baseline (n = 11)	Level 2 (n = 11)	Level 3 Fresh RBCs (n = 6)	Level 3 Stored RBCs (n = 5)
Hct (%)	46.9 ± 2.5	18.8 ± 0.8*	18.0 ± 0.6*	17.6 ± 0.8*
		(0.39 ± 0.03)†	(0.39 ± 0.02)	(0.38 ± 0.01)
Hb (g/dL)	15.0 ± 0.9	6.2 ± 0.3*	5.9 ± 0.3*	5.7 ± 0.2*
		(0.41 ± 0.02)	(0.40 ± 0.03)	(0.38 ± 0.03)
Blood pressure (mmHg)	102.4 ± 6.6	90.5 ± 10.8*	84.4 ± 11.7*	83.2 ± 5.7*
		(0.88 ± 0.06)	(0.83 ± 0.10)	(0.81 ± 0.08)
HR (bpm)	431.5 ± 49.8	463.2 ± 27.3	444.7 ± 11.5	456.2 ± 19.5
		(1.09 ± 0.11)	(1.09 ± 0.15)	(1.06 ± 0.10)
p_aO_2 (mmHg)	60.3 ± 6.2	76.9 ± 8.2*	86.1 ± 6.6*	79.6 ± 6.2*
		(1.29 ± 0.20)	(1.50 ± 0.23)	(1.27 ± 0.09)
p_aCO_2 (mmHg)	60.9 ± 5.5	56.8 ± 7.9	59.7 ± 5.8	54.1 ± 3.7
		(0.93 ± 0.16)	(0.91 ± 0.14)	(0.93 ± 0.07)
pH	7.35 ± 0.03	7.35 ± 0.04	7.36 ± 0.04	7.39 ± 0.05

* $p < 0.05$ relative to baseline.
† The numbers in parentheses are changes presented normalized relative to baseline.
n = the number of animals in each group.

Systemic and blood gas parameters

Changes in the systemic variables and blood gas parameters are summarized in Table 1. Hct and total Hb after the hemodilution with dextran 70 to Level 2 followed by the Level 3 exchange transfusion with fresh and stored RBCs were both reduced as expected relative to baseline ($p < 0.05$), but were no different relative to each other.

The MAP was reduced after Level 2 hemodilution to 0.88 ± 0.06 of baseline ($p < 0.05$). Upon Level 3 exchange transfusion with fresh or stored RBCs the MAP was unchanged from Level 2 and both remained statistically lower than baseline. HRs after Level 2 hemodilution and RBC exchange transfusion were slightly increased relative to baseline, but the changes were not statistically significant.

The arterial blood gas analysis showed an statistically increase in pO_2 after hemodilution, but no additional changes were found after the Level 3 exchange transfusion in either group (all were $p < 0.05$ relative to baseline). Both arterial pCO_2 and pH were unchanged from baseline at Level 2 and after fresh or stored RBC exchange transfusion.

Physical properties of blood

No significant differences were found between the viscosity of the whole blood and plasma after fresh or stored RBC exchange transfusion. Similarly, there were no detectable differences between the plasma colloid osmotic pressure between the two groups.

Microhemodynamics

The changes in the diameter of arterioles (range, 24-108 µm; n = 80) and venules (range, 26-202 µm, n = 60) are shown in Fig. 2. The increase in arteriolar diameter after Level 3 exchange transfusion with fresh RBCs was significant whereas the changes with the stored RBCs were not different from Level 2. Figure 3 shows the changes in microvascular blood flow calculated from RBC velocity and vessel diameter. In both types of vessels, the flow was reduced as a consequence of Level 3 exchange transfusion in both groups, but to a much greater magnitude with the stored RBCs ($p < 0.05$).

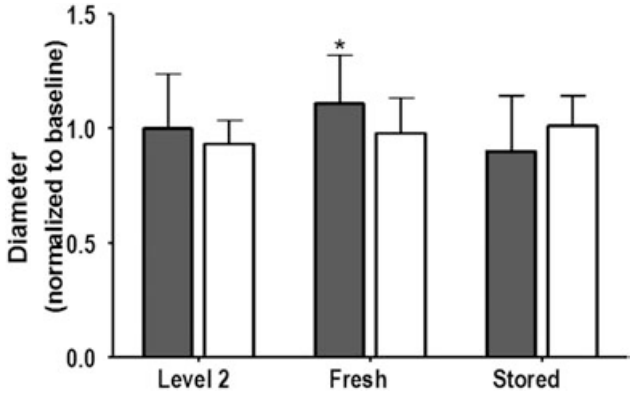

Fig. 2. Changes in arteriolar (shaded bars) and venular (unshaded bars) diameter after moderate hemodilution (Level 2) and in the two experimental groups: fresh and stored RBCs (Level 3). Significant vasodilation is observed when fresh RBCs are exchange transfused. Results are presented as means ± SD (*p < 0.05 relative to Level 2 and to stored).

FCD

The number of capillaries with RBC passage after Level 2 hemodilution with the dextran solution was 0.81 ± 0.09 relative to baseline ($p < 0.05$). Level 3 exchange transfu-

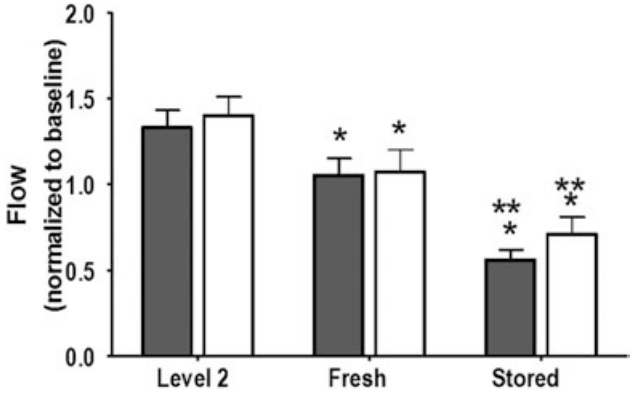

Fig. 3. Changes in arteriolar (shaded bars) and venular (unshaded bars) blood flow after moderate hemodilution (Level 2) and in the two experimental groups: fresh and stored RBCs (Level 3). In both experimental groups, fresh and stored RBCs, the blood flow was reduced from Level 2 (*) and was also significantly different from each other (**) ($p < 0.05$). Data are presented means ± SEM.

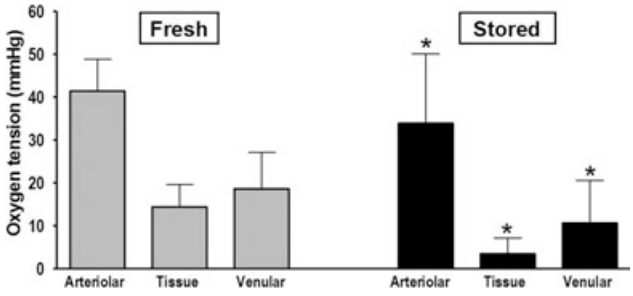

Fig. 4. Comparison of microvascular oxygen distribution in arterioles, venules, and tissue after Level 2 hemodilution and the two experimental groups: fresh (gray) and stored RBCs (black). Data are presented as means ± SD. In both experimental groups, the changes were significantly different from Level 2 and from each other (*) ($p < 0.05$).

sion with fresh RBCs changed FCD to 0.75 ± 0.14 relative to baseline, not significantly different from Level 2. Exchange transfusion of the stored RBCs caused a significant reduction to 0.51 ± 0.28 ($p < 0.05$ between all groups).

Microvascular oxygen distribution

Oxygen tension was measured with phosphorescence microscopy after the exchange transfusion with fresh or stored RBC suspensions. Figure 4 presents a comparison between intravascular pO_2 measurements in arterioles, venules, and interstitial tissue pO_2 levels after the exchange transfusion with fresh and stored RBCs. The oxygen levels at the three sites of the network studied were different between groups. Thus, the microvascular oxygen tension distribution in the stored RBC exchange transfusion group was found to be statistically lower than the values obtained in the fresh RBC group. In control conditions, the arteriolar and venular intravascular pO_2 was 57.0 ± 5.4 and 32.5 ± 8.8 mmHg, respectively,[21] with a tissue pO_2 of 21.3 ± 7.6 mmHg.[11]

DISCUSSION

Exchange transfusion with 28-day stored RBCs results in a significantly impaired microvascular perfusion that subsequently limits oxygen delivery to the tissue. Differences at the systemic level between the exchange transfusion of 28-day stored and fresh RBCs were unremarkable. The principal differences between fresh and stored RBCs at the microvascular level were that the latter reduced FCD and blood flow, leading to a maldistribution of microvascular pO_2, suggesting the potential development of focal ischemia. There were no discernible changes in the systemic parameters studied that could predict the existence and the magnitude of these microvascular imbalances.

The handling and storage conditions for RBCs and plasma in this study do not mimic the standard conditions used in the storage of human blood. In this study, blood is withdrawn from the donor animal into a vacuum tube filled with the anticoagulant CPDA-1. RBCs and plasma are then separated by centrifugation. Each is pipetted into polypropylene cryogenic vials and then stored at 4°C and –70°C for 28 days, respectively.

The intent of the protocol design in this study was to reduce oxygen-carrying capacity by an acute isovolemic hemodilution to reduce the oxygen reserve of blood, a condition that should in principle magnify changes that might occur when the RBCs are reintroduced into the circulation. Fresh and stored RBCs were introduced into the circulation by exchange transfusion to maintain Hb at the lowest level compatible with the maintenance of stable systemic and microhemodynamic parameters in this model.[11] Therefore, maintenance of Hb at this level by exchange transfusion should not alter microvascular or systemic conditions unless function of RBCs was impaired as was found in the case of stored RBCs. The results obtained from the fresh RBC group, when compared to Level 2, before their introduction, shows that manipulation of the RBCs and the exchange transfusion protocol has some effect on the microvasculature and oxygen distribution (Figs. 2-4).

Previous investigators focused on the systemic whole-blood oxygen uptake upon transfusion of stored and fresh RBCs after causing an oxygen supply dependency and then an increased oxygen demand such as: 1) isovolemic hemodilution and sepsis[22,23] or 2) hemorrhage and resuscitation.[24] Fitzgerald and coworkers[23] demonstrated that transfusion of 28-day stored RBCs in oxygen-supply-dependent (induced by hemodilution) septic and nonseptic rats failed to immediately improve tissue oxy-

genation,[22] which were very similar to findings of Sielenkämper and colleagues[23] in septic rats. Stored RBCs did not recover microvasculature oxygenation when compared to fresh RBCs upon resuscitation in a hemorrhagic shock resuscitation model in rats.[24] These studies collectively concluded that transfusions were a therapeutic maneuver that increased neither systemic nor individual organ oxygen utilization or uptake, suggesting that impaired RBC oxygen unloading or flexibility may have caused these negative findings.

This study allows a more direct examination of oxygen uptake at the microvascular level by stored RBCs determined from the measurement of microvascular flow and oxygen distribution. Calculations of the oxygen delivery parameters based on the microvascular measurements are presented in Table 2. In this calculation, the Hb for Level 2 and both Fresh and Stored RBC is assumed to be fully functional, able to load and off-load oxygen according to the oxygen dissociation curve.[25] In the case of fresh RBCs, lowered microvascular flow increased transit time and compensated for the reduced arteriolar oxygen content; thus the same amount of oxygen is extracted from the blood compared to Level 2. Stored RBCs caused a significant reduction in microvascular flow, which coupled with the concomitant reduction in arteriolar oxygen content, greatly reduced extraction relative to both Level 2 and fresh RBCs. As expected, exchange transfusion with fresh RBCs achieved a higher tissue pO_2 than with stored RBCs.

A shortcoming of our oxygen delivery and uptake analysis is the assumption that the oxygen transport characteristics of fresh and stored RBCs are no different from baseline. It is well documented in the literature that stored RBCs lead to a left shift in the oxygen dissociation curve owing to the decay of 2,3 DPG and ATP concentration over time depending on the storage media, which will gradually return to normal levels from 7[26] to 24 hours[27] after transfusion. The p50 of stored RBCs is significantly left shifted; thus upon transfusion they are able to load oxygen in the lungs, but will in principle be unable to release oxygen to the tissue unless the oxygen levels are very low.

An alternative explanation is that stored RBCs are not functional and do not transport oxygen. In this case, Hb oxygen-carrying capacity would be similar to that attained in a Level 3 exchange with a non-oxygen-carrying expander and results should be comparable to those obtained in a previous study with dextran 70 shown in the last column of Table 2.[11] It is notable that stored RBCs (assuming that all transfused RBCs are nonfunctional) and dextran 70 used in a Level 3 exchange produced similar levels of microvascular flow and oxygen extraction per gram of Hb. Stored RBCs, however, achieved higher venous oxygen content, tissue pO_2, and FCD.

The superiority of transfused stored RBCs versus non-oxygen-carrying plasma expanders in maintaining microvascular function may be primarily a consequence of RBCs increasing blood viscosity, which was demonstrated in studies by Tsai and associates[11] in extreme hemodilution. Viscosity was found to be a factor in maintaining a viable microcirculation independently of oxygen-carrying capacity and oxygen delivery. Furthermore, the left shift of the oxygen dissociation curve for Hb of stored RBCs may be highly beneficial in directing oxygen to only anoxic regions that otherwise do not receive oxygen from blood that preferentially unloads oxygen at normal tissue pO_2 levels.

In conclusion, Level 2 hemodilution provides a basis for comparing changes due to transfusion of test solutions because Level 3 exchange transfusion preserves the Hb and RBC concentration with 25 percent of the RBCs either

TABLE 2. Calculated oxygen transport parameters

	Level 2		Level 3					
Variable	Dextran 70		Stored RBCs		Fresh RBCs		Dextran 70	
Total Hb (g/dL)	5.73 (1.00)*		5.97 (1.04)		6.13 (1.07)		3.73 (0.65)	
Average microvascular flow (Q_{AVG} normalized to baseline)	1.20 (1.00)		0.65 (0.54)		1.06 (0.88)		0.69 (0.58)	
FCD (%)	81.2 (1.00)		51.3 (0.63)		74.7 (0.92)		38.0 (0.47)	
Tissue pO_2 (mmHg)	20.0 (1.00)		3.5 (0.18)		14.4 (0.72)		2.3 (0.12)	
	Arteriolar	Venular	Arteriolar	Venular	Arteriolar	Venular	Arteriolar	Venular
Intravascular pO_2 (mmHg)	54.5	30.1	33.9	10.6	41.3	18.6	34.6	5.1
S_A (fractional RBC saturation)	0.83	0.40	0.49	0.06	0.65	0.15	0.51	0.03
O_2 content (mL O_2/mL blood)	6.35	3.09	3.96	0.47	5.36	1.23	2.56	0.14
O_2 delivery ([mL O_2/mL blood])Q_{AVG})	7.62	3.71	2.57	0.31	5.65	1.30	1.75	0.10
O_2 delivery per g Hb	1.33	0.65	0.43	0.05	0.92	0.21	0.47	0.03
O_2 extraction or tissue uptake ([arteriolar – venular content]Q_{AVG})	3.91		2.27		4.35		1.66	
O_2 extraction or tissue uptake per g Hb ([arteriolar – venular content]Q_{AVG}/total Hb)	0.68 (1.00)		0.38 (0.56)		0.71 (1.05)		0.44 (0.65)	

* Numbers in parentheses are values normalized relative to Level 2 hemodilution.

fresh or stored cells. Results from the fresh RBC group show that the experimental procedure (i.e., manipulation of cells by centrifuge, exposure to dextran 70, Level 3 exchange transfusion) results in a lower tissue pO_2, decreased FCD, and blood flow, even though the circulating Hb concentration was maintained. Stored RBCs significantly reduced microvascular function relative to Level 3 with fresh RBC transfusion, indicating that the malfunctions observed are mainly due to the RBCs themselves rather than the exchange transfusion protocol. These findings suggest that microvascular oxygen delivery in the presence of stored RBCs is primarily due to the remaining normal RBCs, whereas the role of stored RBCs is that of releasing oxygen when tissue oxygen levels are low, similar to the concept of targeted delivery proposed for the design of oxygen therapeutics.[20] Thus increasing Hb concentration with the transfusion of stored RBCs may be beneficial because it is a mechanism for targeted oxygen delivery. Nevertheless, even a comparatively small introduction of stored RBCs in a transfusion (25 percent of the circulating total), and to a lesser degree with fresh blood, adversely affects microvascular function, a phenomenon not evidenced by the analysis of systemic parameters.

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