The effect of storage time of human red cells on intestinal microcirculatory oxygenation in a rat isovolemic exchange model*

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Objective: **To determine whether the storage time of human leukodepleted red blood cell concentrates compromises intestinal microvascular oxygen concentration oxygen (μPo_2) during isovolemic exchange transfusion at low hematocrit.**

Design: **Prospective, randomized, controlled study.**

Setting: **University research institute laboratory.**

Subjects: **Male Wistar rats.**

Interventions: **Intestinal μPo_2 was determined by Pd-porphyrin phosphorescence life-time measurements.**

Measurements and main results: **Rats were brought near to a state of oxygen supply dependency by hemodilution with a pasteurized plasma protein solution to a hematocrit of 14.3 ± 1.1% (n = 24). Subsequently, an isovolemic exchange transfusion with human leukodepleted red blood cells, stored for 2–6 days (fresh, n = 8), 2–3 wks (intermediate, n = 8), or 5–6 wks (old, n = 8), was performed to determine whether intestinal μPo_2 would be preserved. Immunologic reactions were avoided by washing the red blood cell concentrates three times before use. Isovolemic exchange with fresh and intermediate red blood cells maintained μPo_2 whereas old cells decreased μPo_2 with 26%. Subsequent transfusion with red blood cells (hematocrit ~60%) until reaching a hematocrit of 32.4 ± 2.1 % (n = 24) increased intestinal μPo_2 in all three groups to the same extent between 28% and 32%. No changes in red blood cell deformability, as determined by a Laser-assisted Optical Rotational Cell Analyzer, could be demonstrated during 5 wks of storage.**

Conclusion: **This study shows that at low hematocrit, the oxygen-delivering capacity of human red blood cells stored 5–6 wks is reduced compared with fresh cells and red blood cells stored for an intermediate period. Although red blood cells stored for 2–3 wks are completely devoid of 2,3-diphosphoglycerate, their oxygen-delivering capacity to the intestines was the same as fresh red blood cells. Our study showed that red blood cell deformability was preserved during storage, suggesting that other mechanisms may account for the observed decrease in oxygen delivery by red blood cells stored 2–3 wks. (Crit Care Med 2005; 33:39–45)**

KEY WORDS: blood transfusion; oxygen delivery; microvascular oxygen concentration; Pd porphyrin; red blood cell deformability

The practice of banking blood, or more specifically banking red blood cell concentrates (RBCCs), has improved red cell availability. However, as a consequence, red cell units can be up to 6 wks old at the time of transfusion. Although the mean storage time was limited to 19 days, we recently found that more than one third of the RBCCs issued in our hospital (a major university hospital) over a 5-yr period had been stored for >3 wks (1). Similar data were reported in two recent studies (2, 3). These findings support the need to determine whether such long-term stored red blood cells (RBCs) still maintain their functional capacity to transport oxygen to the tissue cells.

Several studies have demonstrated adverse effects of storage of RBCs on tissue oxygen variables, patient morbidity, and patient mortality (4–6). The development of infections during RBC storage, the release of cytokines by primed leukocytes, and storage-induced lesions of the RBCs themselves have been described as possible explanations for this storage effect (7–10). The current guideline for 24-hr posttransfusion survival of red cells in the circulation prescribes a minimum of 75% (11) and therefore mainly focuses on red cell integrity and viability instead of functional properties. Storage has also been shown to decrease oxygen release by hemoglobin due to the loss of the metabolic intermediate and allosteric factor 2,3-diphosphoglycerate (DPG) (12), although this level has been shown to recover within hours after transfusion (13). In addition to these effects, several studies have demonstrated a decrease in RBC deformability during storage (14–18) possibly impairing proper flow of red cells through the microcirculation (19, 20).

The primary goal of a blood transfusion is to treat or prevent tissue ischemia. It is therefore surprising that relatively few clinical and animal studies have investigated the effect of storage on tissue oxygenation (4, 21–23). The lack of these studies may be explained by the general difficulties of determining tissue oxygen requirement and local tissue oxygen delivery by red cells, particularly in the clinical setting. That is why surrogate variables like tonometry or changes in morbidity and mortality have been used (9). Most of these studies were performed in conditions of sepsis but have reported contradictory results (4–6, 9, 23, 24).

Comparable effects of storage time on oxygenation variables have been shown in animal models that allow measurements during more controlled conditions (21, 22). However, observations by d'Almeida

***See also p. 238.**

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et al. (25), as well our own initial results, question the relevance of rat RBC transfusions in rats as a model for oxygen delivery by human RBCCs. Not only do rat RBCs age much more rapidly during storage than human RBCs, but they also, unlike human RBCs, fail to regenerate 2,3-DPG when treated with a rejuvenation solution. We therefore developed a rat model that allowed the transfusion of stored human RBCs and investigated the hypothesis that transfusion of stored human RBCs affects their ability to transport oxygen to the gut microcirculation. We chose to investigate intestinal tissue due to its sensitivity to dysoxia and due to the importance of this organ in the development of sepsis and multiple organ failure (26). Several precautions were taken to prevent mutual reactions between human and rat RBCs, based on a previously developed model by Kurantsin-Mills et al. (27). The hematocrit of the rat was reduced to about 15% by hemodilution, and subsequent isovolemic exchange transfusion with human leukodepleted and washed RBCCs, stored for 2–6 days (indicated as fresh), 2–3 wks (indicated as intermediate), or 5–6 wks (indicated as old), was performed to determine whether intestinal microvascular oxygenation would be maintained. Leukodepleted RBCCs were chosen, as leukodepletion nowadays is the standard procedure in many European countries and has an increased use in the United States.

We first determined whether changes in microvascular P_{O_2} (μP_{O_2}) were associated with age. Subsequently, based on the findings of others using the Laser-assisted Optical Rotational Cell Analyzer (LORCA) technique (18), we investigated the hypothesis that a decrease in red cell deformability may affect tissue oxygen levels.

MATERIALS AND METHODS

Animals. This study was reviewed and approved by the ethical committee for animal subjects of the Academic Medical Center at the University of Amsterdam. Care and handling of the animals were in accord with the guidelines for Institutional and Animal Care and Use Committees. A total of 30 experiments with Wistar male rats (Charles River, The Netherlands) with a body weight of 340 ± 20 g (mean ± SD) were included in this study.

Human RBC Preparation. Standard RBCCs were prepared by centrifugation of whole blood (blood group 0^+) and removal of the plasma and buffy coat fraction, followed by addition of sodium chloride, adenine glucose, mannitol (SAGM) and leukocyte depletion over Biofil (Fresenius Hemocare, The Netherlands) inline RBCC filters. To counter the decrease in 2,3-DPG levels induced by this standard procedure, the "fresh" RBCCs were washed and resuspended in a chloride-free medium, consisting of 40 mM gluconate, 47.5 mM glucose, 1.44 mM adenine, 1.44 mM guanosine, 55 mM mannitol, and 16 mM sodium phosphate. For the other two groups of RBCCs studied (specified subsequently), the units were left in SAGM. After preparation, all RBCCs were stored at 2–6°C and used after 2–6 days of storage (mean, 2.5 ± 1.8 days), 14–21 days of storage (mean, 17.0 ± 5.1), or 35–42 days of storage (mean, 38.0 ± 2.9). Before infusion in the rats, all RBCCs were washed twice with SAGM containing 10% GPO (pasteurized human plasma 4%; Sanquin Plasmaproducts, The Netherlands) and 26 mM $NaHCO_3$, washed once with SAGM containing 50% GPO/26 mM $NaHCO_3$, and finally resuspended in 100% GPO/26 mM $NaHCO_3$ at a final hematocrit of around 15% or 60%.

Determination of RBC Compatibility Between Human and Rat RBCs. Human sera were tested with rat RBCs, and plasma from different rats was tested with human RBCs, using standard cross-match tests (indirect antiglobulin test). In case of positive reactions, we determined the titer of the sera to have an idea about the extent of washing needed.

Quantification of 2,3-DPG and Adenosine Triphosphate (ATP) Levels in Fresh and Stored Red Blood Cells. 2,3-DPG and ATP levels were determined in neutralized perchloric acid extracts of the human RBCs used for transfusion into rats, after dilution to a hematocrit of 30–40% (to ensure complete extraction). The 2,3-DPG content was determined using an enzymatic kit (Roche Applied Science, The Netherlands).

The ATP content was also determined enzymatically using the glucose/hexokinase method, coupled to reduced nicotinamide adenine dinucleotide phosphate formation by glucose-6-phosphate dehydrogenase (29).

Human RBC Quantification After Isovolemic Transfusion. To determine recovery of human RBCs in the rat circulation, samples withdrawn during isovolemic exchange were fixed with 1% (w/v) paraformaldehyde, washed twice in phosphate buffered saline containing 0.2% human serum albumin and 5 mM EDTA, and subsequently stained with fluorescein isothiocyanate-labeled Pelicluster Ery/1 (clustered as CD235a; Sanquin Reagents, The Netherlands), a monoclonal antibody directed against human glycophorin-A. After staining (30 mins at room temperature), samples were analyzed on a FACSCAN (BD Biosciences, San Jose, CA) flow cytometer. The antibody used showed no reactivity with rat RBCs, enabling the determination of the percentage human (Ery/1 positive) RBCs in the samples analyzed.

Red Cell Deformability. Red cell deformability was determined in blood samples that were taken aseptically at days 1, 8, 15, 22, 29, and 36 from six different blood bags that were stored under blood bank conditions at 4°C. The content of the blood bags was homogenized before sampling. RBC deformability was determined by LORCA (R&R Mechatronics, The Netherlands) as developed in our group (30) and as used by Izzo et al. (18) to demonstrate decreased RBC deformability of stored human RBCs. The sterility of the blood bags was checked afterward by the Sanquin Blood Supply Foundation (Amsterdam, The Netherlands), and no contaminations were found.

Surgical Preparation. Rats were anesthetized and mechanically ventilated, and four vessels were cannulated, as described in detail in a previous study (31), while body temperature was kept between 36.5 and 37.5°C. The right carotid artery catheter was cannulated for continuous monitoring of mean arterial blood pressure and heart rate, and the right jugular vein was cannulated for fluid support (15 mL/kg/hr Ringer's lactate; Freeflex, Fresenius Kabi, The Netherlands) and maintenance anesthesia. The right femoral artery was cannulated for blood withdrawal and arterial blood gas sampling, and the right femoral vein was cannulated for infusion of the GPO solution for hemodilution. Ventilation variables, like inspiratory phase (0.25–0.35) and respiration rate (50–75 breath per min), were adjusted to keep arterial P_{CO_2} values between 35 and 45 torr (4.7–6.0 kPa) during surgery and baseline and were left unmodified during the rest of the experiment.

A midline laparotomy was performed to position an ultrasonic flow probe (Transonic Systems) around the superior mesenteric artery for continuous measurement of blood flow that was expressed as mL/kg body weight. In addition, a loop of the ileum was exposed to measure the changes in oxygen-dependent quenching of Pd-porphyrin phosphorescence (32) in the intestinal wall with an optical fiber, which were converted to local μP_{O_2} values (31). The abdomen was covered with Saran wrap (Dowbrands) to prevent evaporation of body fluids, and the bladder was drained to prevent excessive extension.

Experimental Protocol. After infusion of the Pd-porphyrin solution and 45 mins of stabilization, a baseline blood sample (0.2 mL) was taken from the femoral artery for blood gas determination, and 0.25 mL of HAES 6% (Haes-steril, Fresenius Kabi, The Netherlands) was given back. Blood samples were analyzed in a blood gas analyzer (ABL 505, Radiometer, Denmark) and a hemoximeter (OSM3, Radiometer, Denmark).

Rats were hemodiluted to reduce hematocrit and limit oxygen delivery. Isovolemic hemodilution was conducted until a hematocrit of approximately 14% (range 11.7–16.2%) was achieved by withdrawal of blood from the femoral artery while at the same time GPO was administered through the femoral vein at the same rate. Infusion and withdrawal were performed at 60 mL/kg/hr using a double syringe

pump (Harvard 33 syringe pump, Harvard Apparatus). The last withdrawn blood sample was analyzed as described before. Hemodilution was followed by a 30-min stabilization period, and subsequently an isovolemic exchange transfusion (60 mL/kg/hr) was performed with washed human RBCs (mean hematocrit 14.4 ± 0.3%). The three age groups of human RBCs were evaluated for their capacity to maintain oxygenation of the gut, whereas the medium (GPO) in which the RBCs were suspended was used as a negative control group. Exchange transfusion was followed by a 30-min stabilization period after which 15 mL/kg of the same batch of washed human RBCs (mean hematocrit 61.6 ± 0.5%) were transfused at a rated of 30 mL/kg/hr. A blood sample (0.2 mL) for blood gas analysis was withdrawn 45 mins after transfusion, and the experiment was ended by infusion of 1 mL of 3 M KCl in the jugular vein, resulting in immediate cardiac arrest.

Statistical Analysis. Values are presented as mean ± SD, unless indicated otherwise. Differences between groups were analyzed using analysis of variance, and when indicated *post hoc* analysis was performed using a Scheffé test. A paired Student's *t*-test was used to compare differences within groups. We considered $p < .05$ to be significant.

RESULTS

Compatibility Between Rat and Human Red Blood Cells. To explore the feasibility of infusing human RBCs into rats, rat plasma from five different rats was tested for possible immunologic reactions against a panel of human red cells of five different blood groups. The sera of the tested rats all contained a very weak antibody reaction against all three age groups of human erythrocytes, but these were not suspected to disturb experiments, because no hemolysis was noted in the presence of either human plasma or rat plasma. Rat RBCs were tested for possible immunologic reactions against a panel of human serum samples from eight different persons with different blood groups. Only a dilution of the samples by a factor of >16 could eliminate reactions. In all subsequent experiments, human RBCs were washed three times, and this treatment was sufficient to avoid adverse immunologic reactions.

ATP and 2,3-DPG Levels in Human RBCCs Used for Rat Transfusions. To investigate the influence of storage time of human RBCCs on their ability to deliver oxygen, concentrates with three different storage periods were used: RBBCs stored for 2–6 days, RBCCs stored for 2–3 wks, or RBCCs stored for 5–6 wks. To ensure high levels of both ATP and 2,3-DPG in the fresh RBCs, these units were washed in a chloride-free buffer (28) to counteract the decrease caused by the blood component preparation. The actual values of ATP and 2,3-DPG in the three different groups are given in Table 1. In the intermediate group, ATP levels were not much different from the fresh RBCs, but the 2,3-DPG content had fallen to almost undetectable levels (Table 1), in agreement with earlier observations (33). In the old RBCs, ATP levels were decreased to about 63% of the level measured in the fresh RBCs.

Hemodynamics and Intestinal Oxygenation During Hemodilution

To enlarge the sensitivity for possible differences in oxygen delivery by fresh and old RBCCs, the rat was hemodiluted to reduce hematocrit and limit tissue oxygen delivery (22). An isovolemic hemodilution with a pasteurized protein solution down to a mean hematocrit of 14.2 ± 1.1 % (n = 28, Table 2) was performed and decreased mean arterial pressure and increased intestinal flow (Table 3, Fig. 1). No differences in blood gas variables (pH, arterial base excess, P_{O_2}, P_{CO_2}) were observed during hemodilution (Table 2). The mean μP_{O_2} decreased with approximately 37% during hemodilution (Table 4).

Hemodynamics and Intestinal Oxygenation During Isovolemic Exchange Transfusion

Subsequent isovolemic exchange transfusion with human RBCCs (mean hematocrit 14.7 ± 1.1%, n = 24) with different lengths of storage times did not alter mean arterial blood pressure, intes-

Table 1. Red blood cell 2,3-diphosphoglycerate (DPG) and adenosine triphosphate (ATP) concentrations

	2–6 Days	2–3 Wks	5–6 Wks
2,3-DPG, μmol/g Hb	8.10 ± 1.58	0.30 ± 0.18[a]	0.08 ± 0.10[a]
ATP, μmol/g Hb	3.74 ± 0.83	3.53 ± 0.63	2.35 ± 0.76[a]

Hb, hemoglobin.

[a] $p < .05$ vs. 2–6 days stored red blood cells (analysis of variance). Red blood cell 2,3-DPG and ATP concentrations during storage time. Values are expressed as mean ± SD and n = 6 for each group.

Table 2. Femoral artery blood gas values

Storage Time	BL	HD	IV	EN
pH				
2–6 days	7.41 ± 0.02	7.40 ± 0.03	7.41 ± 0.03	7.41 ± 0.06
2–3 wks	7.38 ± 0.05	7.33 ± 0.08	7.35 ± 0.06	7.33 ± 0.08
5–6 wks	7.39 ± 0.04	7.41 ± 0.04	7.41 ± 0.07	7.42 ± 0.04
BE, mM				
2–6 days	1.2 ± 1.3	0.8 ± 2.5	−0.2 ± 2.2	3.0 ± 2.0
2–3 wks	0.5 ± 1.1	−0.6 ± 3.7	−2.1 ± 2.7	0.3 ± 2.9
5–6 wks	1.2 ± 1.5	2.7 ± 1.2	−0.8 ± 3.0	3.2 ± 1.2
Pa_{O_2}, torr				
2–6 days	159 ± 9	168 ± 7	178 ± 20	161 ± 17
2–3 wks	148 ± 13	151 ± 29	161 ± 23	142 ± 28
5–6 wks	146 ± 15	169 ± 9	186 ± 7	157 ± 7
Pa_{CO_2}, torr				
2–6 days	42 ± 3	41 ± 5	38 ± 4	45 ± 8
2–3 wks	44 ± 6	49 ± 15	43 ± 10	52 ± 12
5–6 wks	45 ± 6	44 ± 4	38 ± 6	43 ± 5
Hct, %				
2–6 days	51.2 ± 3.4	14.6 ± 1.3[a]	13.5 ± 1.7[a]	31.8 ± 2.3[a]
2–3 wks	49.6 ± 4.4	13.6 ± 1.2[a]	12.0 ± 2.5[a]	32.5 ± 1.9[a]
5–6 wks	51.6 ± 2.3	14.5 ± 0.5[a]	13.4 ± 1.4[a]	33.0 ± 2.3[a]
Hb, g/dL				
2–6 days	16.7 ± 1.1	4.6 ± 0.5[a]	4.2 ± 0.6[a]	10.3 ± 0.8[a]
2–3 wks	16.9 ± 0.8	4.6 ± 0.2[a]	4.1 ± 0.5[a]	10.6 ± 0.6[a]
5–6 wks	16.2 ± 1.5	4.4 ± 0.5[a]	3.7 ± 0.8[a]	10.7 ± 0.8[a]

BL, baseline; HD, after hemodilution; IV, after isovolemic exchange transfusion; EN, end of experiment 45 mins after transfusion; BE, base excess; Hct, hematocrit; Hb, hemoglobin.

[a] $p < .05$ vs. BL (paired *t*-test). Values are expressed as mean ± SD and n = 8 for each group. $p < .05$ vs. 2–6 days stored RBC (analysis of variance).

Table 3. Hemodynamic variables

Storage Time	BL	HD	IV	TR	EN
MAP, mm Hg					
2–6 days	106 ± 8	70 ± 12[a]	64 ± 9[a]	94 ± 15[a]	81 ± 7[a]
2–3 wks	98 ± 16	62 ± 9[a]	57 ± 6[a]	81 ± 7[a]	73 ± 9[a]
5–6 wks	101 ± 14	65 ± 9[a]	59 ± 3[a]	84 ± 5[a]	73 ± 5[a]
Heart rate, beats/min					
2–6 days	264 ± 15	257 ± 24	247 ± 19	270 ± 22	268 ± 32
2–3 wks	263 ± 13	248 ± 9	254 ± 16	268 ± 19	266 ± 17
5–6 wks	265 ± 12	247 ± 9	250 ± 12	269 ± 19	274 ± 17
Intestinal blood flow, mL/min/kg					
2–6 days	11.6 ± 3.0	21.2 ± 8.1[a]	22.1 ± 7.6[a]	23.4 ± 8.6[a]	21.6 ± 9.1[a]
2–3 wks	13.7 ± 2.9	20.4 ± 5.3[a]	22.4 ± 3.9[a]	27.1 ± 5.7[a]	21.3 ± 4.3[a]
5–6 wks	14.8 ± 4.0	20.0 ± 5.5[a]	21.0 ± 5.8[a]	28.0 ± 6.6[a]	22.7 ± 4.1[a]

BL, baseline; HD, after hemodilution; IV, after isovolemic exchange transfusion; TR, transfusion; EN, end of experiment 45 mins after TR; MAP, mean arterial pressure.

[a] $p < .05$ vs. BL (paired t-test). Values are expressed as mean ± SD and n = 8 for each group. $p < .05$ vs. 2–6 days stored RBC (analysis of variance).

tinal flow (Table 3), and hematocrit (Table 2) significantly. No differences in blood gas variables (pH, arterial base excess, Po_2, Pco_2) between the three age groups were observed during isovolemic exchange transfusion (Table 2). There was no difference in intestinal μPo_2 before and after isovolemic exchange transfusion ($\Delta\mu Po_2$) for the fresh (−2.8 ± 3.1 torr, −0.3 ± 0.4 kPa, n = 8) and intermediate stored (−3.2 ± 4.4 torr, −0.4 ± 0.6 kPa, n = 8) RBCs (Fig. 2, Table 4). However, exchange transfusion with old human RBCCs did reduce $\Delta\mu Po_2$ significantly ($p < .05$) with 8.0 ± 2.6 torr (−1.1 ± 0.3 kPa, n = 8) and was significantly different ($p < .05$) from the fresh and intermediate RBCCs (Fig. 2, Table 4). Isovolemic exchange with a protein solution (GPO) without RBCs was performed as a negative control and decreased hematocrit to 8.3 ± 0.2%, whereas μPo_2 decreased with 13.2 ± 11.3 torr (−1.8 ± 1.5 kPa, n = 6). None of the animals from this group survived till the end of the experiment.

Number of Human RBCs Exchanged During Isovolemic Transfusion

To determine the final fraction of human RBCs exchanged against rat RBCs, the percentage of cells positively labeled with a monoclonal antibody directed against human glycophorin-A was measured by fluorescence-activated cell sorter analysis. The mean hematocrit for the RBCCs of the different age groups was similar, with 15.1 ± 0.7%, 14.0 ± 1.4%, and 15.1 ± 0.8% for fresh, intermediate, and old RBCs, respectively. Isovolemic exchange resulted in a contribution of human RBCs to total RBC numbers of 32.8 ± 4.2%, 30.1 ± 9.5%, and 30.0 ± 8.8 % for fresh, intermediate, and old RBCs, respectively. Hemodynamics and Intestinal Oxygenation During Transfusion.

Successive transfusion with human RBCs (hematocrit 59.4 ± 2.4 %, n = 23) increased the mean hematocrit in the three groups to 32.4 ± 2.1% (n = 22). The mean arterial blood pressure increased in all three groups, and an increase in intestinal flow was present in the intermediate ($p = .014$) and old groups ($p = .024$, Table 3). No differences in blood gas variables (pH, arterial base excess, Po_2, Pco_2) were observed during transfusion (Table 2). The intestinal μPo_2 increased in all three groups to the same extent, 9–12 torr (1.2–1.6 kPa, 28–32%; Fig. 3, Table 4).

RBC Deformability. To test the hypothesis that the observed decrease in oxygen delivery of 5–6 wks stored RBCs was caused by hemorheological alterations, RBC deformability was determined by ectacytometry using the LORCA (30). As displayed in Figure 4, no significant changes in the elongation index could be demonstrated during storage of the RBCCs for up to 5 wks at a shear stress of either 3 or 30 Pa.

DISCUSSION

The present study demonstrates the feasibility of using a rat model to evaluate the acute effects of storage time of human leukodepleted and washed RBCs on μPo_2, an important variable determining tissue oxygenation. Using this model we tested the hypothesis that aged stored RBCs are ineffective in transporting oxygen to the intestinal microcirculation during reduced oxygen delivery at low hematocrit (∼15%).

Isovolemic exchange transfusion with 5–6 wks stored human RBCs decreased intestinal microvascular oxygen levels by about 25%, whereas no significant changes were observed with fresh red cells stored for 2–6 days. The cells designated as fresh had a somewhat higher 2,3-DPG level than standard RCCs freshly prepared in SAGM (about 30%, see Table 1) due to our procedure of resuspending these cells in a chloride-free medium (28). Despite this effort to keep 2,3-DPG near the physiologic level of 10–12 μmol/g hemoglobin, the fresh cells behaved similarly as RBCCs stored for 2–3 wks, which had lost their 2,3-DPG during storage (Table 1), indicating that the differences between fresh and old RBCCs were not caused by the loss in 2,3-DPG. This latter result is supported by several studies showing that 2,3-DPG levels are less important for oxygen delivery by RBCs as previously assumed (9, 34, 35). Although the P50 of rat RBCs has been reported to be higher (35–38 mm Hg) (34, 35) than human RBCs (25–30 mm Hg), the human RBC still released enough oxygen to preserve microvascular oxygen levels during exchange in the rat. During storage, the P50 has been reported to decrease to 15–20 mm Hg, and therefore the difference between fresh and old human RBCs is twice the difference between fresh human and rat RBCs.

Another explanation for the decrease in intestinal microvascular oxygen concentration during exchange transfusion with stored RBCs might be a reduction in their deformability (9). We were unable to demonstrate a decrease in deformability using ectacytometry (LORCA) (30) during 5 wks of storage of leukodepleted human RBCCs in CPD/SAGM solution. Previous studies have reported a decrease in deformability in stored RBCs (15–17, 25, 36), but results are difficult to compare due to differences in experimental conditions and the lack of a proper calibration standard. These differences include the use of either whole blood (16, 18), RBCCs (37), or leukodepleted RBCCs and the use of differing storage media (38, 39). In addition, different techniques have been used to measure deformability including micropipette aspiration (14,

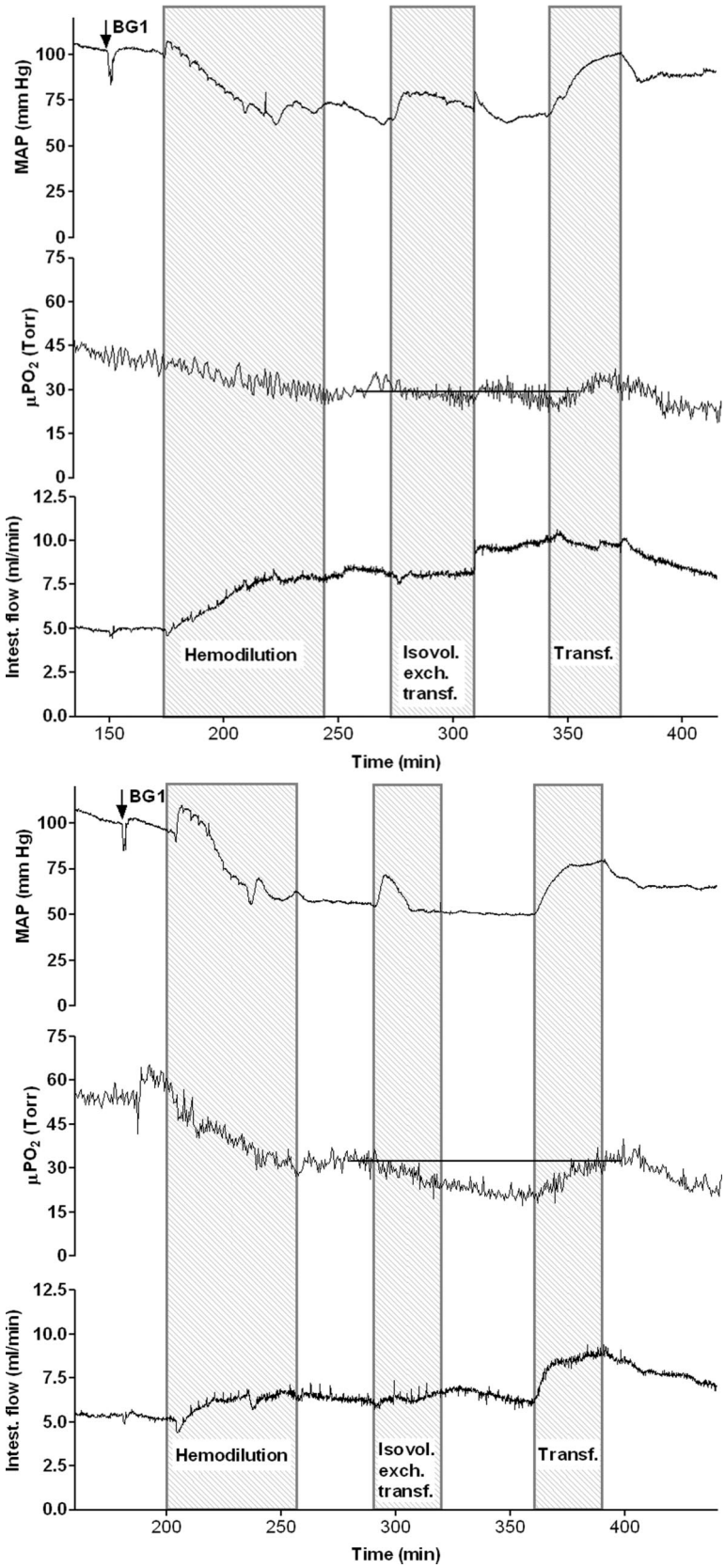

Figure 1. Typical example of the changes in mean arterial pressure (*MAP*), microvascular oxygen concentration (μPO_2), and intestinal (*Intest.*) flow after hemodilution with a pasteurized human protein solution (GPO) till a hematocrit of ~15% and subsequent isovolemic exchange transfusion (*Isovol. Exch. transf.*) and transfusion (*Transf.*) with (*top*) fresh (2–5 days) and (*bottom*) old (5–6 wks) washed red blood cell concentrates suspended in GPO. *BG*, blood gas.

This study shows that at low hematocrit, the oxygen-delivering capacity of human red blood cells stored 5–6 wks is reduced compared with fresh cells and red blood cells stored for an intermediate period.

25), micropore filtering (16, 17, 36, 38), and ectacytometry (18, 40).

Izzo et al. (18) reported a decrease in RBC deformability in whole blood stored for 5 wks in SAGM as measured with the LORCA technique. This result shows that the LORCA technique as used in the present study is sensitive enough to demonstrate decreases in RBC deformability in stored whole blood and suggests that the presence of leukocytes during storage may have an effect since we did not observe a decrease in RBC deformability with leukodepleted RBCCs. Based on the present study, we cannot exclude that changes in deformability are responsible for our observed decrease in microvascular oxygenation. No decrease in intestinal blood flow was observed during exchange with old RBCs, excluding massive and overall obstruction of the microcirculation by stored RBCs. A subfraction of rigid cells might arise during storage from RBCs that are already senescent at the time of blood donation. Since the LORCA measures RBC deformability in a whole population of cells, the presence of such a fraction of rigid RBCs could have been easily masked by the majority of the RBCs that maintained their deformability. Studies using filter techniques might have overestimated the effect of the presence of rigid cells on deformability and blood flow, as filtration times will increase when rigid cells block the filter pores. Microcirculatory shunting of a subfraction of rigid RBCs through larger vessels during exchange with stored cells could have bypassed capillary obstruction (41) and would explain our observed decrease in microcirculatory oxygen concentration.

Table 4. Oxygenation variables

Storage Time	BL	HD	IV	TR	EN
Do_{2int}, mL/min/kg					
2–6 days	2.6 ± 0.7	1.4 ± 0.6[a]	1.3 ± 0.4[a]	ND	2.9 ± 1.2
2–3 wks	3.0 ± 0.7	1.2 ± 0.3[a]	1.1 ± 0.2[a]	ND	2.8 ± 0.5
5–6 wks	3.3 ± 0.7	1.3 ± 0.4[a]	1.2 ± 0.4[a]	ND	3.2 ± 0.7
GPO	3.0 ± 0.7	1.1 ± 0.2	0.4 ± 0.3	ND	ND
Arterial O_2 content, mL O_2/dL					
2–6 days	22.3 ± 1.4	6.5 ± 0.6[a]	5.9 ± 0.7[a]	ND	13.3 ± 1.0[a]
2–3 wks	21.6 ± 2.0	6.1 ± 0.8[a]	5.2 ± 1.1[a]	ND	13.5 ± 0.8[a]
5–6 wks	22.8 ± 1.6	6.5 ± 0.2[a]	5.8 ± 0.6[a]	ND	13.9 ± 1.0[a]
GPO	23.0 ± 1.6	6.1 ± 0.3	2.6 ± 1.4	ND	ND
μPo_2, torr					
2–6 days	55 ± 6	34 ± 6[a]	29 ± 7[a]	41 ± 9[a]	35 ± 7[a]
2–3 wks	55 ± 11	28 ± 6[a]	25 ± 4[a]	37 ± 7[a]	34 ± 4[a]
5–6 wks	56 ± 11	34 ± 10[a]	23 ± 10[a]	31 ± 8[a]	26 ± 8[a]
GPO	50 ± 7	29 ± 9	18 ± 7	4.6 ± 0.8	ND

BL, baseline; HD, after hemodilution; IV, after isovolemic exchange transfusion; TR, transfusion; EN, end of experiment 45 mins after TR; DO_{2int}, intestinal oxygen delivery; ND, not determined; μPo_2, microvascular Po_2.

[a]$p < .05$ vs. BL (paired t-test). For μPo_2, the values for hemodilution and isovolemic exchange are given 30 mins after completion. Values are expressed as mean ± SD and n = 8 for each red blood cell group and n = 6 for the GPO group; only two animals survived till the TR time point. $p < .05$ vs. 2–6 days stored RBC (analysis of variance).

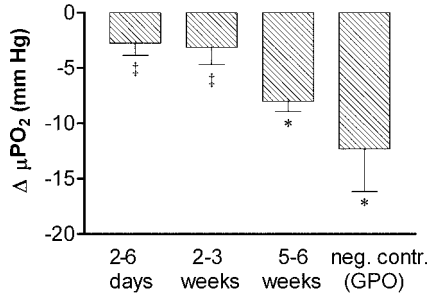

Figure 2. Difference between intestinal microvascular oxygen concentration (μPO_2) at the start and end of isovolemic exchange transfusion with washed human red blood cell concentrates suspended in pasteurized protein solution (*GPO*) that had been stored up to 6 wks. Exchange with GPO was used as a negative control (*neg. contr.*). Values are displayed as mean ± SEM, n = 8. *$p < .05$ vs. 2–6 days stored red blood cells (analysis of variance); ‡$p < .05$ vs. no change (zero) (one sample t-test).

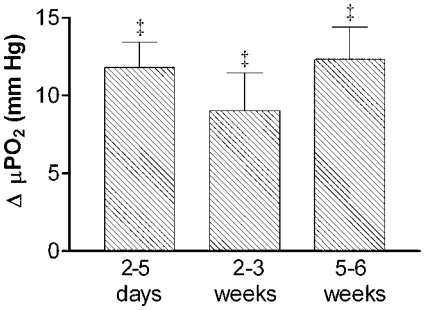

Figure 3. Difference in intestinal microvascular oxygen concentration (μPO_2) at the start and end of transfusion with washed human red blood cell concentrates (hematocrit ~60%) suspended in pasteurized protein solution (GPO) that had been stored up to 6 wks. Values are displayed as mean ± SEM, n = 8. ‡$p < .05$ vs. no change (zero) (one sample t-test).

The differences in tissue oxygenation observed between fresh and 5–6 wks stored RBCs during isovolemic exchange at low hematocrit were obtained while the hematocrit was kept constant. However, in the more clinically relevant procedure of transfusion, no differences in the intestinal μPo_2 were observed between stored and fresh cells. This observation suggests that only during limited tissue oxygen delivery, stored RBCs are less able to oxygenate the tissue as fresh cells. This finding corresponds to a study by Sielenkamper et al. (24) that showed a difference in outcome when young or old RBCs were transfused in oxygen supply-dependent animals. It is therefore important to investigate the underlying mechanism, as our study was performed in hemodiluted but otherwise healthy animals, and μPo_2 was measured in an organ with a relatively low need for oxygen. One could speculate that during conditions of shock or disturbed vascular autoregulation, mainly the hypoperfused and oxygen-depleted areas in oxygen-consuming tissue like heart and kidney would be less well oxygenated by stored cells. Hence, the initial state of local tissue oxygenation of a patient could determine whether a recovery in tissue oxygenation by stored cells is present.

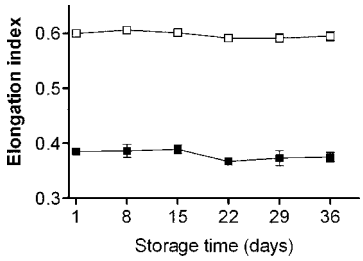

Figure 4. Change in elongation index as measure for red blood cell deformability in human red blood cell concentrate blood bags sampled up to 36 days of storage at a shear stress of 3 Pa (*filled squares*) and 30 Pa (*unfilled squares*). Values are displayed as mean ± SEM, n = 6.

In addition, in our rat model it was inevitable that we wash the human RBCs before transfusion, a procedure that is uncommon in clinical practice. Although previous studies have shown that cytokine levels are substantially reduced during leukodepletion, we cannot exclude that this procedure might have underestimated the observed differences in microvascular oxygen concentration. A final issue that needs mentioning is the possible effects of our experimental strategy in which we did not change ventilatory variables after baseline measurements to avoid confounding effects on hemodynamic properties between individual experiments. This approach caused some animals to have somewhat elevated expiratory Co_2 levels, resulting in a lower systemic blood pH, and this could possibly have affected oxygen off-loading characteristics of the RBCs.

CONCLUSIONS

Our study has introduced a rat model that can be used to evaluate the efficacy of stored human RBCs to deliver oxygen to the microcirculation. A decrease in μPo_2 was noticed during exchange with 5–6 wks stored cells and not with 2–3 wks stored and fresh cells. These results suggest that RBCs that are close to their expiration date are less capable of releasing their oxygen content during oxygen-limiting conditions that occur at low hemoglobin levels. Future measurements will be focused on organs with higher oxygen consumption such as the heart or kidney, under conditions of both oxygen dependency and induced sepsis. The presented model is expected to provide more details about the variables affecting the ability of stored RBCs to transport oxygen to the tissue and may result in the development of more optimal storage conditions.

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