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Effects of non-leukocyte-reduced and leukocyte-reduced packed red blood cell transfusions on oxygenation of rat spinotrapezius muscle

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Abstract

Leukoreduction of blood used for transfusion alleviates febrile transfusion reactions, graft *versus* host disease and alloimmunization to leukocyte antigen. However, the actual clinical benefit of leukoreduction in terms of microcirculatory tissue O₂ delivery after packed red blood cell (pRBC) transfusion has not been investigated. As such, the aim of this study was to determine the effects of non-leukoreduced (NLR) and leukoreduced (LR) fresh pRBC transfusion on interstitial oxygenation in anesthetized male Sprague-Dawley rats. Interstitial fluid PO₂ and arteriolar diameters in spinotrapezius muscle preparations were monitored before and after transfusion with NLR- or LR-pRBCs. The major findings were that (1) transfusion of NLR-pRBCs significantly decreased interstitial oxygenation whereas transfusion of LR-pRBCs did not, and (2) transfusion with LR-pRBCs elicited a substantially greater increase in arterial blood pressure (ABP) than did transfusion with NLR-pRBCs. These changes in PO₂ and ABP were not associated with changes in the diameters of resistance arterioles in the spinotrapezius muscle. These data suggest that transfusion of fresh NLR-pRBCs may negatively affect tissue oxygenation via enhanced leukocyte influx and decreased O₂ delivery. They also suggest that leukocytes diminish the capability of transfused pRBCs to increase cardiac output. As such, transfusion of LR-pRBCs may be less deleterious on tissue PO₂ levels than NLR-pRBCs although a concomitantly greater increase in ABP may accompany transfusion of LR-pRBCs.

Keywords

Red blood cell transfusion; leukoreduction; spinotrapezius muscle; interstitial fluid-tissue oxygenation; arteriolar diameter; arterial blood pressure; heart rate; arterial blood gas chemistry; Arterial-alveolar gradient; anesthetized rat

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Introduction

The transport of O₂ to tissues via the microcirculation is the key function of red blood cells (RBCs). Although the primary aim of blood transfusion is to improve tissue O₂ delivery, O₂ uptake and consumption frequently do not improve after blood transfusion of packed RBCs (pRBCs) (Shah et al., 1982; Gramm et al., 1996; Casutt et al., 1999). One potential reason for this is the presence of leukocytes, which impair tissue oxygenation by mechanisms that are yet to be fully determined (Ho et al., 2003; Vamvakas, 2003), but which may involve rheologic disturbances within (Powell et al., 1993; Baskurt et al., 1998) or occlusion of (Marik and Sibbald, 1993) the microcirculation. Indeed, transfusion of non-leukoreduced blood results in leukocyte rolling and adhesion within microvessels, which is likely to impede capillary blood flow and therefore tissue oxygenation (Chin-Yee et al., 2009). Removal of leukocytes from blood through leuko-filtration may prevent the formation of microaggregates that could contribute to the microvascular occlusion after transfusion (Bruil et al., 1995).

The clinical benefit of leukoreduction in terms of microcirculatory tissue O₂ delivery after leukoreduced pRBC transfusion has not been determined. Accordingly, the major aim of this study was to use the *in situ* rat spinotrapezius muscle preparation to compare the effects of transfusion of fresh non-leukoreduced pRBCs (NLR-pRBCs) with transfusion of fresh leukoreduced pRBCs (LR-pRBCs) on interstitial fluid PO₂ (index of tissue oxygenation) using phosphorescence quenching microscopy, and arteriolar diameter using intravital microscopy (Pittman et al., 2010).

Materials and methods

Rats

This study was approved by the *Virginia Commonwealth University Institutional Animal Care and Use Committee* and is consistent with the *National Institutes of Health* guidelines for the humane treatment of laboratory animals, as well as the *American Physiological Society's Guiding Principles in the Care and Use of Animals*. Male Sprague-Dawley rats (275–300 g) were used for experiments while 450–500 g rats were used as allogeneic donors for blood harvesting.

General surgeries

The rats were acclimatized for 2 days in our institutional facilities for animal storage. Four vessel cannulations and blood sampling were carried out following standard procedures. Briefly, animals were anesthetized with intraperitoneal injection of a mixture of acepromazine (5 mg/kg)/ketamine (75 mg/kg) for initial induction. Surgeries and experiments were performed under intravenous infusion of Alfaxan (Alfaxalone, 10 mg/ml) via the femoral vein. All vessel cannulations were done with polyethylene tubing (PE-90), while the trachea was cannulated with polyethylene tubing (PE-240) to maintain a patent airway. The right internal jugular vein was cannulated for transfusion of pRBCs. The right carotid artery was cannulated for blood sampling. Arterial blood pressure (ABP) was monitored via a femoral artery catheter (MP-150) and heart rate was derived from the ABP.

Rat temperature was kept at 37°C using a heating pad. Upon completion of experiments, animals were euthanized with Euthasol (pentobarbital, 390 mg/ml and phenytoin 50 mg/ml).

The spinotrapezius muscle preparation

The exteriorized spinotrapezius muscle was prepared as originally described by Gray (1973) and used for measurement of interstitial PO₂. The muscle was kept moist with a phosphate buffered salt solution during surgery and bleeding was managed by cauterization. The preparation leaves the incoming vasculature and nerves intact and microvascular function was maintained. Upon successful exteriorization, the rat and spinotrapezius muscle were put on a thermo-stabilized trans-illumination pedestal and animal pad for the microscope stage, both maintained at 37°C (Golub and Pittman, 2008). The muscle preparation with O₂ probe was covered with a gas barrier polyvinylidene chloride film to minimize desiccation and O₂ exchange with room-air.

Measurement of arteriolar diameter

Circular regions (600 µm diameter) of the spinotrapezius preparation were selected and intra-luminal diameters of arterioles (70–80 µm), were measured using computerized image analysis of video sequences (Golub et al., 1997, 2007; Golub and Pittman, 2008). A branch of the spinotrapezius artery was identified and the inner diameters of 6 branching arterioles (3 more proximal and 3 more distal to the major branch) were determined using a video-image measurement and marking system. The data from the 3 proximal arterioles were pooled, as were the data from the 3 distal arterioles, because of findings pertaining to changes in interstitial fluid PO₂ during pRBC transfusions (see Results).

Arterial blood sampling

Arterial blood samples were collected before and after NLR-RBC and LR-RBC infusions via a carotid artery. Blood pH, pO₂, PCO₂, PO₂, total hemoglobin (Hb) concentration, hematocrit, glucose and electrolytes were measured via an arterial blood gas analyzer (ABL700 or ABL800 FLEX, Radiometer, Denmark). The Arterial-alveolar (A-a) PO₂ difference measures differences between alveolar and arterial blood PO₂ and is an index of ventilation-perfusion mismatch in the lung (Stein et al., 1995). A-a gradients were determined by the following formulae. A-a gradient = P_AO₂ – P_aO₂, where P_AO₂ = alveolar PO₂ = F_iO₂ × (P_{atm} – P_{H2O}) – P_aCO₂/Respiratory quotient; P_aO₂ = arterial blood P_{O2}; P_aCO₂ = arterial blood P_{CO2}; F_iO₂ is the fraction of O₂ in inspired air; P_{atm} is atmospheric pressure; P_{H2O} is the partial pressure of water in alveoli. We took F_iO₂ of room-air to be 21% = 0.21; respiratory quotient to be 0.8; P_{atm} to be 760 mmHg; and P_{H2O} to be 100 mmHg (100% humidity).

Phosphorescence quenching microscopy

Instrumentation used for phosphorescence quenching microscopy consisted of an Axioplan 2 intravital microscope (Zeiss, Germany) combined with other optical equipment on an optical breadboard (Edmund Optics, Barrington, NJ). To observe changes in arteriolar diameter and leukocyte rolling through the tissue bed, the microscope was equipped with a video camera (MTI CCD72, Dage-MTI, Michigan City, IN) and a customized

photomultiplier unit. The phosphorescence signal was gathered by an Achroplan 20x/0.45 objective (Zeiss, Germany) and sent to an analog-to-digital converter (SCB-68, National Instruments, Austin, TX) to be analyzed with custom software written in LabVIEW (National Instruments, Austin, TX). To limit the contribution of O₂ inflow to the interstitial PO₂ within the illuminated tissue disc, a flash area of 600 μm was used. A xenon lamp (FX-249, PerkinElmer, Salem MA) was used to excite the area with an output of 167 mJ/μs at a rate of 1 Hz and 3 μs per flash. Phosphorescence decay curves (500 data points per curve) were sampled at a rate of 500 kHz and were analyzed by fitting curves non-linearly with the following rectangular PO₂ distribution model (Golub et al., 1997, 2007; Golub and Pittman, 2008):

$$I(t) = I(0) \exp[-(k_0 + k_q M)t] \times (\sinh(k_q \delta t)) / (k_q \delta t) + A \exp(-t/T) + B$$

where t (μs) is time of phosphorescence decay, $I(t)$ is the magnitude of the phosphorescence signal, $I(0)$ is the magnitude of the phosphorescence signal at $t = 0$, M (mmHg) is the mean pO₂, δ (mmHg) is the half-width of the PO₂ distribution, T (μs) is the lifetime of the fast post-excitation transient, and B is the baseline offset. k_0 , the phosphorescence decay rate in the absence of O₂ and k_q , the quenching coefficient for the particular probe were $18.3 \times 10^{-4} \mu s^{-1}$ and $3.06 \times 10^{-4} \mu s^{-1} \text{ mmHg}^{-1}$, respectively (Zheng et al., 1996). All experiments were performed in a dark room.

Pd-MTCPP O₂ probe preparation and administration

The O₂ sensitive, phosphorescent probe, palladium meso-tetra-(4-carboxyphenyl)-porphyrin (Pd-MTCPP, Oxygen Enterprises, Philadelphia, PA), was bound to bovine serum albumin (Vanderkooi et al., 1987). The probe solution was topically applied to the surface of the spinotrapezius muscle for 45 min using 41 grade filter paper. This provides sufficient penetration of the probe to the interstitial fluid throughout the tissue and prevents intravascular and intracellular penetration (Golub et al., 2007a,b).

Leukoreduction, blood storage and transfusion

Whole blood was collected from the carotid artery of donor rats into a 20 ml heparinized syringe. For leukoreduction, heparinized blood was filtered through a PALL filter (Leukotrap SC RC, PALL Medical, NY). Following filtration, packed RBCs were prepared by centrifugation (3000 x g) of leukoreduced whole blood in a sterile blood collection conical tube. pRBCs (60%–70% hematocrit) were stored at 4°C in 15 ml sterile conical tubes containing citrate-phosphate-dextrose with citrate as an anticoagulant (CPD-A, Terumo Corporation, Tokyo, Japan) at a 1.5:10 CPDA-blood ratio. A complete blood count (CBC) analysis (Sysmex XE 2100s, Sysmex America, IL, USA) was performed in NLR-pRBCs and in LR-pRBCs to confirm the effectiveness of the leukoreduction. Leukoreduced blood contained 0.67 ± 0.17 leukocytes/μL whereas non-leukocyte reduced blood contained $12,300 \pm 1,300$ leukocytes/μL, consistent with published values of 3,000–17,000 leukocytes/μL (Sharp and Villano, 2012). An amount estimated to be 10% of the rat's total blood volume [Estimated total blood volume (ml) = $0.06 \times$ body weight (grams); Lee and Blafox, 1985] was withdrawn and used for CBC and blood-gas analyses (Table 1). Similar

volumes of NLR-pRBCs and LR-pRBCs (within 4–6h of storage at 4°C) were infused into recipient rats. Measurements were taken immediately after muscle dissection to serve as baseline, and 20 min after transfusion of pRBCs.

Experimental Protocol

Once the preparation had stabilized following withdrawal of blood, an initial arterial blood sample was taken for blood-gas chemistry analysis. Interstitial fluid PO₂ was sampled at each of the sites and arteriolar diameters were determined. The volumes of NLR-pRBCs or LR-pRBCs (Table 1) were infused over a 5 min period (300–380 µl/min). After a 15 min equilibration period, arteriolar diameter and interstitial fluid PO₂ were sampled at each site and another blood gas sample was taken. Arterial O₂ saturation; systolic, diastolic and mean ABPs; and heart rate were sampled every 5 min. The spinotrapezius preparations remained viable during the entire procedure, including from topical application of the O₂-sensitive phosphorescent probe until after pRBC transfusion.

Statistical methods

The data are presented as mean ± SEM. The data was analyzed by one-way and two-way ANOVA using BMDP statistical soft-ware (Statistical Solutions, Saugus, MA) followed by Student's modified *t*-tests with Bonferroni corrections for multiple comparisons between means using the error mean square terms from the ANOVAs (Wallenstein et al., 1980) as described previously (Hashmi-Hill et al., 2007).

RESULTS

Hemoglobin, hematocrit, and blood O₂ saturation levels

Hb concentration, hematocrit, pH and SO₂ values were similar in donor NLR-pRBCs and LR-pRBCs (Table 2). Hb concentration and hematocrit did not change in rats that received NLR-pRBCs whereas they were elevated in rats that received LR-pRBCs (Table 3). pH, SO₂ and A-a gradients did not change after transfusion of NLR-pRBCs or LR-pRBCs. PCO₂ fell in rats that received NLR-RBCs but not LR-RBCs (Table 3). There were minimal changes in blood electrolytes and glucose in rats that received NLR-RBCs or LR-RBCs (Table 3).

Interstitial fluid oxygenation

Prior to infusions of NLR- or LR-pRBCs, the PO₂ of the interstitial fluid around the proximal and distal arterioles were similar to one another (Table 4). PO₂ around the proximal and distal arterioles fell substantially after transfusion of NLR-pRBCs, with the fall being greater in interstitial fluid around proximal than distal arterioles (Fig. 1, top panel). In contrast, interstitial fluid PO₂ did not change after transfusion of LR-pRBCs (Fig. 1, top panel).

Arteriolar diameters

Prior to RBC transfusion, the intra-luminal diameters of proximal and distal arteries were similar (Table 5). These diameters did not change after transfusion of NLR-pRBCs or LR-pRBC (Fig 1, bottom panel).

Blood pressure and heart rate

Prior to transfusion, the cardiovascular parameters were similar in the two groups of rats (Table 5). ABPs also rose after transfusion of NLR-pRBCs and remained elevated for 10–15 min. ABPs rose more substantially in rats that received LR-pRBCs than in those that received NLR-pRBCs although the increases were again of 15–20 min in duration. Heart rate changed minimally following transfusion of NLR-pRBCs or LR-pRBCs (Fig. 2).

DISCUSSION

Tissue oxygenation

This study demonstrated that oxygenation of interstitial fluid within the spinotrapezius muscle of anesthetized rats was greatly reduced after transfusion of NLR-pRBCs. This reduction in interstitial fluid (and presumably tissue) oxygenation, was not associated with decreases in arterial PO₂ or increases in A-a gradients, which would reflect adverse gas-exchange within the lungs (Stein et al., 1995). Moreover, the decreases in interstitial fluid PO₂ were not associated with decreases in the diameters of proximal or distal arterioles. It is likely that the studied arterioles (70–80 µm in internal diameter) in the spinotrapezius muscle bed would qualify as true resistance arterioles (Christensen and Mulvany, 2001), which account for the largest portion of vascular resistance in muscle beds (Heistad and Abboud, 1974). Accordingly, the lack of change in the diameter of the putative resistance arterioles would suggest that the reduction in interstitial fluid PO₂ after transfusion of NLR-pRBCs was not due to vasoconstriction and associated diminution of blood flow into capillary beds. It should be noted that the effects of RBC transfusions on microvascular blood flow may be bed-specific since transfusion of NLR-pRBCs improve sublingual microcirculatory perfusion in humans by elevating the number of perfused microvessels (Ayhan et al., 2013).

The transfusion of NLR-pRBCs elicits substantial (van Brommel et al., 2001) or only partial (Tsai et al., 2010) improvements in tissue oxygenation in rats that underwent prior withdrawal of large volumes of blood. As such it is evident that transfusions of NLR-pRBCs have beneficial effects on microvascular O₂ delivery under conditions of hemorrhagic shock, no doubt due to multi-factorial mechanisms including restoration of blood RBC-Hb concentrations and blood viscosity to normal levels (Cabrales et al., 2007). It is less clear that such transfusions are able to increase O₂ delivery to tissues under conditions of more moderate blood loss or in anemic patients (Shah et al., 1982; Gramm et al., 1996; Casutt et al., 1999; Harder and Boshkov, 2010). In our study, the withdrawal of approximately 10% of blood had minimal effects on resting arterial blood gas chemistry and cardiovascular values such that at 15 min post-withdrawal, these values were at normal levels. It should be noted that transfusion of NLR-pRBCs did not increase Hb concentration or hematocrit in recipient rats whereas transfusion of LR-pRBCs led to modest increases in these parameters. Identical volumes of donor blood per body weight were given to all rats, and the Hb and hematocrit values in the donor NLR-pRBCs and LR-pRBCs were very similar to one another (values tended to be higher, but not significantly, in the LR-RBCs). Since leukocytes represent 0.1% of the total cell volume in blood it is unlikely that the change in RBC numbers within LR-pRBCs would account for the differential effects of donor NLR- and LR-pRBCs on recipient

Hb concentrations and hematocrit values. It would seem possible that donor NLR-pRBCs may leave the general circulation of the recipient rat, or perhaps more likely, adhere to microvessel (e.g., capillary) walls (Chin-Yee et al., 2009; Harder and Boshkov, 2010) such that blood sampling underestimates the true RBC numbers. Since transfusion of LR-pRBCs increased Hb concentrations in the donor rats, it would appear that the presence of leukocytes in the transfused RBCs may have been responsible for the putative adherence of RBCs to microvascular walls. This would be consistent with the wealth of evidence that leukocytes have substantial effects on RBC function including adherence (Powell et al., 1993; Baskurt et al., 1998; Marik and Sibbald, 1993; Ho et al., 2003; Vamvakas, 2003; Harder and Boshkov, 2010). RBCs adhered to capillary walls would not only lack the capacity to transfer O₂ since they would not circulate through the lungs, but would also block access of O₂-laden RBCs to capillaries. Both effects, along with the adherence of leukocytes (see below) would contribute to a dramatic drop in PO₂ within the spinotrapezius muscle.

A key finding of the present study was that despite the increase in hematocrit and Hb concentrations, oxygenation status in the spinotrapezius muscle did not change after transfusion of LR-pRBCs. Nonetheless, the transfusion of LR-RBCs did not elicit a dramatic drop in interstitial fluid oxygenation as did transfusion of NLR-RBCs. Similar to transfusion of NLR-pRBCs, the transfusion of LR-pRBCs, was not associated with decreases in arterial PO₂, increases in A-a gradients, or decreases in the diameters of proximal or distal arterioles within the spinotrapezius muscle bed. Accordingly, it is evident that at rest, transfusion of NLR-RBCs did not effectively increase O₂ levels in the interstitial fluid within the muscle tissue. During our process of packing of donor NLR-RBCs, the plasma supernatant including the buffy coat, was suctioned off after packing via centrifugation. In contrast, during the process of packing donor LR-RBCs, the blood was initially flushed out from the PALL filter using normal saline followed by packing and removal of the supernatant. The process of filtration and washing with normal saline removes cellular debris as well as leukocytes from blood and is effective at reducing the risk of graft *versus* host disease (Armentrout and Getz, 1998). As such, the lack of precipitous fall in interstitial fluid PO₂ after transfusion of LR-pRBCs, suggests that leukocytes and/or cellular debris resulting from packing of blood were responsible for the marked drop in PO₂ after administration of NLR-pRBCs.

Leukocytes impair tissue oxygenation (Ho et al., 2003; Vamvakas, 2003) by mechanisms that are likely to involve rolling and adhesion to the endothelium of capillaries, thereby impeding capillary blood flow (and numbers of functional capillaries), and diminishing tissue PO₂ (Siemionow et al., 1991; Marik and Sibbald, 1993; Powell et al., 1993; Astiz et al., 1995; Fitzgerald et al., 1997; Purdy et al., 1997; Baskurt et al., 1998; Morisaki and Sibbald, 2004; Arslan et al., 2005; Gonzales et al., 2007; Chin-Yee et al., 2009). Moreover, leukocytes promote adherence of RBCs to the microvasculature (Chin-Yee et al., 2009). The removal of leukocytes from pRBCs may also prevent the formation of microaggregates that would also contribute to microvascular occlusion after transfusion (Bruil et al., 1995). With respect to interstitial fluid/tissue oxygenation, the increase in Hb concentrations and

hematocrit elicited by transfusion of LR-pRBCs would also tend to maintain arterial blood O₂ levels and PO₂ in spinotrapezius muscle.

Cardiovascular changes

Blood transfusion increases ABP via circulatory overload, especially in patients with compromised cardiac and/or pulmonary function and severe anemia (Agarwal et al., 2012; Alam et al., 2013; Murphy et al., 2013). Increases in ABP occur following transfusion of NLR-pRBCs in adult humans (Shoemaker and Wo, 1998; Saugel et al., 2013) and after transfusion of LR-pRBCs in premature and low birth-weight infants (Rankova and Beshinska, 1989; Bauer et al., 1993; Alkalay et al., 2003) and neonates (Rashid et al., 2013) but not always in children with severe anemia (Grant et al., 2003; Olgun et al., 2009). Transfusions of LR-pRBCs do not always impact ABP because of lower pRBC volumes and transfusion rates (Nelle et al., 1994, 1997) and because increases in total peripheral resistance are matched by decreases in cardiac output (CO) (Nelle et al., 1994).

A key observation was that transfusion of LR-pRBCs elicited more substantial and sustained increases in ABP than NLR-pRBCs. The exaggerated increase in ABPs may be the result of passing blood through the PALL leukocyte filters, leading to formation of vasoactive factor(s) or release of Hb, which is a powerful vasoconstrictor (Tsai, 2001; Solomon et al., 2012). However, it is unlikely that the exaggerated pressor response following transfusion of LR-RBCs involved contaminating vasoconstrictor factors, debris from filtration, or release of K⁺ or free Hb because (1) LR-RBCs were washed extensively prior to packing, which is likely to remove most contaminants including extracellular Hb, (2) blood levels of K⁺, a surrogate marker of hemolysis (Szpisjak et al., 2000), were not elevated in rats that received LR-pRBCs or NLR-pRBCs, and (3) the hypertension after transfusion of NLR- or LR-pRBCs was not associated with decreases in the diameters of resistance arterioles in spinotrapezius muscle (i.e., no vasoconstrictor influence).

Hematocrit rose slightly higher after transfusion of LR-pRBCs than NLR-pRBCs ($+4 \pm 1\%$ versus $0 \pm 1\%$). It is unlikely that this minor increase would affect blood viscosity or hinder blood flow (Bauer et al., 1993). The exaggerated increase in ABP after transfusion of LR-pRBCs may have involved enhanced CO rather than total peripheral resistance. Indeed, Bauer et al (1993) reported that the increase in ABP after transfusion of LR-pRBCs in pre-term infants was due to increased CO since peripheral vascular resistance fell slightly. Fluid shifts to the intravascular space after blood transfusion (Bauer et al., 1993; Harder and Boshkov, 2010) and so a lesser shift in plasma to the intravascular space after transfusion of LR-pRBCs would tend to elevate CO. Leukocytes may dampen the ability of pRBCs to enhance cardiac contractility and therefore CO. Indeed, monocytes secrete chemokines that suppress cardiac contractility and β -adrenoceptor responsiveness (Davani et al., 2004; Pyo et al., 2006; Gullestad et al., 2012; Ghasemzadeh and Hosseini, 2013).

Summary and conclusions

To the best of our knowledge, this is the first study to directly compare the relative effects of NL-pRBCs and LR-pRBCs on interstitial oxygenation and ABP. The key findings were that despite minimal changes in arterial blood-gas chemistry and A-a gradients, (1) perfusion of

NLR-pRBCs elicited a dramatic drop in interstitial PO₂ in rat spinotrapezius muscle whereas transfusion of LR-pRBCs did not, and (2) transfusion with LR-pRBCs elicited a substantially greater increase in ABP than transfusion with NLR-pRBCs, most likely via greater increases in cardiac output. These findings support evidence that microvascular O₂ delivery cannot be necessarily predicted from measurements of arterial O₂ and SO₂ levels (Trzeciak et al., 2007), and clearly suggest that the leukocytes play a critical role with respect to tissue O₂ delivery and cardiovascular consequences associated with the transfusion of pRBCs. The transfusion of NLR-pRBCs does not always elicit positive effects on microvascular oxygenation and consumption in patients with severe sepsis (Sadaka et al., 2011). Our studies raise the possibility that transfusion with LR-pRBCs may have a greater impact on tissue PO₂. Leukoreduction has been advocated to alleviate febrile transfusion reaction, graft *versus* host disease and alloimmunization to leukocyte antigen (Bordin et al., 1994; Singh and Kumar, 2009; Cata et al., 2013). Although, leukoreduction avoids unwanted effects associated with pRBC transfusions, the actual clinical benefit of leukoreduction on microcirculatory O₂ delivery has not been established. Our data suggest that transfusions of LR-pRBCs may have better effects on tissue oxygenation and provide a more robust increase in ABP, which may be problematic in normotensive patients but beneficial in patients with low ABPs. The present study examined the effects of RBC transfusion in rats with relatively normal levels of blood Hb and tissue oxygenation. In clinical practice, transfusions are often triggered by evidence of impaired O₂ delivery such as a raised blood lactate levels or decreased central venous oxygenation. Sadaka et al. (2011) provided evidence that the effects of transfusions on tissue oxygenation in humans may be dependent on prevailing pathophysiological conditions and that the worse the oxygenation the more beneficial are the effects of RBC transfusion. Our future studies will therefore determine the effects of transfusing NLR-pRBCs and LR-pRBCs on tissue oxygenation in rats with anemia/hypovolemia with clear signs of impaired O₂ delivery. Significant challenges will remain while extrapolating findings from animal blood transfusion models to humans under O₂ restricted conditions such as hypoxia or hypovolemia due to species differences in terms of physiology, metabolism and cell biology (Raat and Ince, 2007; Spinella et al., 2011a,b).

The potential mechanisms contributing to the decreased O₂ delivery following transfusion of NLR-pRBC are likely to involve adherence of leukocytes or RBCs to the microvessels. Although we did not directly assess this possibility, it is known that adhesion of these cells to vascular endothelium can disrupt local blood flow, decrease O₂ delivery to peripheral tissues and in severe cases, lead to occlusion of microvessels (Shiu and McIntire, 2003). It is also known that pre-storage leukocyte-depletion reduces RBC adhesion to the vascular wall (Chin-Yee et al., 2009). The mechanisms involved in the interaction between RBCs and endothelial cells are likely to involve the presence of adhesion molecules (e.g., CD44, CD47, CD58, CD147, CD239, CD242) in mature RBCs (Telen, 2005). In summary, our major findings were that (1) transfusion of NLR-pRBCs decreased interstitial fluid oxygenation in the spinotrapezius muscle preparation, whereas the fall in PO₂ was substantially smaller after transfusion of LR-pRBCs, and (2) ABP rose more dramatically after transfusion of LR-pRBCs than NLR-pRBCs.

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Fig. 1.

Change in interstitial fluid (ISF) PO₂ (top panel) and arteriolar diameter within the spinotrapezius muscle bed 20 min after transfusion of packed non-leukoreduced red blood cells (NLR-RBCs) or packed leuko-reduced red blood cells (LR-RBCs). The data are presented as mean $\pm$ SEM. There were 5 rats in each group. * $P < 0.05$, significant change. † $P < 0.05$, distal versus proximal. ‡ $P < 0.05$, LR-RBC versus NLR-RBC.

Fig. 2.

Percent changes in systolic arterial blood pressure (systolic ABP, top-left panel), diastolic ABP (bottom-left panel), mean ABP (top-right panel), and heart rate (bottom-right panel) following transfusion of packed non-leukoreduced red blood cells (NLR-pRBCs) or packed leuko-reduced red blood cells (LR-pRBCs). The data are presented as mean $\pm$ SEM. There were 5 rats in each of the NLR-pRBC and LR-pRBC groups. * $P < 0.05$, LR-pRBC *versus* NLR-pRBC.

Table 1

Body weights and blood volumes

Parameter	NLR-pRBC	LR-pRBC
N	5	5
Body weight, g	278 ± 2	318 ± 33
Estimated blood volume, ml	16.7 ± 0.1	19.1 ± 2.0
10% blood volume, ml	1.7 ± 0.1	1.9 ± 0.2

The data are presented as mean ± SEM. NLR-pRBCs, non-leukocyte-reduced packed red blood cells. LR-pRBCs, leukocyte-reduced packed red blood cells. There were no between-group differences for any parameter ($P > 0.05$, for all comparisons).

Table 2

Arterial blood chemistry values of packed RBCs from donor rats

Parameter	NLR-pRBC	LR-pRBC
Hb, g/L	214 ± 5	228 ± 9
Hct, %	65 ± 2	69 ± 3
pH	6.984 ± 0.038	6.973 ± 0.033
SO ₂ , %	93.5 ± 2.9	98.9 ± 1.7

The data are presented as mean ± SEM. NLR-pRBCs, non-leukocyte-reduced packed red blood cells. LR-pRBCs, leukocyte-reduced packed red blood cells. There were 5 rats in each group. There were no between-group differences for any parameter ($P > 0.05$, for all comparisons).

Table 3

Arterial blood chemistry values before and after transfusion of packed RBCs

Parameter	Non-leukocyte reduced pRBCs			Leukocyte-reduced pRBCs		
	Pre	Post	Arithmetic change	Pre	Post	Arithmetic change
Hb, g/L	137 ± 2	139 ± 3	+2 ± 5	143 ± 3	159 ± 5	+16 ± 4 *
Hct, %	42 ± 1	42 ± 1	0 ± 1	43 ± 1	47 ± 1	+4 ± 1 *
pH	7.196 ± 0.065	7.228 ± 0.084	+0.03 ± 0.02	7.248 ± 0.077	7.264 ± 0.074	+0.016 ± 0.007
PCO ₂ , mmHg	39 ± 2	30 ± 2	-9 ± 2 *	37 ± 1	34 ± 1	-3 ± 1
PO ₂ , mmHg	91 ± 3	98 ± 6	+7 ± 9	98 ± 4	94 ± 3	-4 ± 1 *
SO ₂ , %	91 ± 1	93 ± 1	+2 ± 1	92 ± 1	93 ± 1	+1 ± 1
A-a gradient	12.8 ± 2.0	15.0 ± 1.3	+2.2 ± 1.5	11.9 ± 1.0	12.5 ± 2.1	+0.6 ± 2.0
Na ⁺ , mmol/L	137	137	0	141 ± 1	142 ± 1	+0.7 ± 0.7
K ⁺ , mmol/L	3.7	4.0	0.3	4.3 ± 0.2	3.8 ± 0.1	-0.5 ± 0.1 *
Cl ⁻ , mmol/L	107	106	+1	107 ± 1	110 ± 1	+2.3 ± 1.4
Ca ²⁺ , mmol/L	2.2	2.1	-0.1	2.5 ± 0.1	2.4 ± 0.1	-0.1 ± 0.1
Glucose, mmol/L	13.3	12.9	-0.4	9.0 ± 0.5	8.6 ± 0.7	-0.4 ± 0.3

The data are presented as mean ± SEM. pRBCs, packed red blood cells. A-a gradient, Arterial-alveolar gradient. There were 5 rats in each group except for electrolytes and glucose in the NLR-pRBC group in which only one rat was sampled.

* $P < 0.05$, significant change.

Table 4

Changes in interstitial fluid PO₂ and arteriolar diameter following transfusion of RBCs

Parameter	Site	Non-leukocyte reduced pRBCs		Leukocyte-reduced pRBCs	
		Pre	Post	Pre	Post
Interstitial fluid PO ₂ , mmHg	Proximal	61 ± 7	24 ± 8*	68 ± 6	61 ± 14
	Distal	45 ± 9	25 ± 7*	62 ± 8	55 ± 10
Arteriolar diameter, µm	Proximal	72 ± 10	66 ± 11	73 ± 2	73 ± 3
	Distal	74 ± 10	80 ± 9	70 ± 6	63 ± 5

The data are presented as mean ± SEM. pRBCs, packed red blood cells. There were 5 rats in each group.

* $P < 0.05$, significant change.

Table 5

Resting cardiovascular parameters prior to infusion of RBCs

Parameter	NLR-pRBCs	LR-pRBCs
N	5	5
Systolic ABP, mmHg	143 ± 16	117 ± 7
Diastolic ABP, mmHg	98 ± 16	84 ± 3
Mean ABP, mmHg	114 ± 14	99 ± 3
Heart rate, beats/min	374 ± 26	359 ± 10

The data are presented as mean ± SEM. NLR-pRBCs, non-leukocyte-reduced packed red blood cells. LR-pRBCs, leukocyte-reduced packed red blood cells. ABP, arterial blood pressure. There were no between-group differences for any parameter ($P > 0.05$, for all comparisons).