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Impact of Transfusion of Autologous 7 versus 42 day old AS-3 Red Blood Cells on Tissue Oxygenation and the Microcirculation in Healthy Volunteers

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Abstract

Background—Stored RBCs accumulate biochemical and biophysical changes. Maximum storage duration is based on acceptable in vitro characteristics and 24-hour survival, but not RBC function. Relatively little is known about the impact of RBC-storage duration on oxygenation and the microcirculation.

Study Design and Methods—Eight healthy subjects donated a double RBC apheresis, which were prestorage leukocyte-reduced, and processed in AS-3. Subjects were transfused one unit of RBCs at 7- and 42-days following blood collection. Measurements of percent (%) tissue oxygenation in the thenar eminence muscle (StO₂) and brain (SctO₂) were recorded with FDA-cleared non-invasive devices. Sublingual microvascular blood flow (MFI) was quantified prior to and after RBC transfusion using a video microscope. Raw electronic data for all measurements were analyzed by a blinded observer at a core laboratory.

Results—The only pre-versus-posttransfusion change observed in measurements of SctO₂, StO₂, or MFI was a very small increase in SctO₂, from 70.4 to 71.8 (means, $p=0.032$) at 7 days. There was no significant difference in the amount of pre-post change at 7 days versus 42 days for any of the measures.

Conclusion—Transfusion of one unit of 42-day stored RBCs to healthy subjects has no overt detrimental effect on tissue oxygenation or the microcirculation assessed by clinically available monitors.

Keywords

transfusion; packed red blood cells (RBCs); tissue oxygenation; microvascular blood flow; near infrared spectroscopy (NIRS); microcirculatory flow index (MFI); age of blood

INTRODUCTION

Red blood cells (RBCs) are the most commonly transfused blood component in the United States. Currently, regulations permit RBC storage under controlled conditions for up to 42 days prior to transfusion. During storage RBCs accumulate biochemical and physiological abnormalities.^{1, 2} For example, during storage, 2,3-diphosphoglycerate (DPG) levels fall, which could impact on the ability of oxygen to be offloaded from hemoglobin in tissue.² Also, significant changes in RBC deformability have been reported with storage^{1, 3} which could affect the movement of RBCs throughout the microcirculation.

Some retrospective observational evidence has linked transfusion of RBCs stored for a longer period of time with increased morbidity, mortality, and length of stay compared with transfusion of fresher RBCs,^{4, 5} although some investigators have failed to find such an association.⁶ However, residual confounding is a possibility inherent in the design of these studies, therefore no causal relationship between adverse outcome and transfusion of older blood has been proven.⁷

To our knowledge there have been no studies testing the effect of storage duration of RBCs on tissue oxygenation and the microcirculation using clinically available FDA-cleared monitors. Therefore the primary objective of this pilot study was to test the impact of transfusion of 7 versus 42 day-old stored, autologous RBCs on tissue oxygenation and the microcirculation in healthy volunteers.

METHODS

After Institutional Review Board approval of this study, healthy volunteers meeting standard allogeneic blood donation eligibility criteria were enrolled after written, informed consent was obtained. Inclusion criteria were: age between 18 and 50 years and a calculated total blood volume of at least 4500 ml (the minimum blood volume required for donation of a double unit of RBCs via apheresis). Exclusion criteria were: significant medical problems requiring treatment, diabetes, current infection, recent blood donation, known hereditary thrombophilia or recent usage of anticoagulants, pregnancy, and nicotine consumption within 30 days of enrollment. After enrollment, eligibility was confirmed with a written questionnaire and lab measurements of hemoglobin, hemoglobin A1C, and a screen for sickle hemoglobin.

Blood Donation, Processing, and Storage

Two units of RBCs were collected in CP2D via apheresis (Trima Accel® Automated Blood Collection System, CaridianBCT, Lakewood, CO) and processed by Rex Blood Services (Raleigh, NC) using standard techniques. All collected RBC apheresis units were pre-storage leukocyte-reduced, tested for required infectious diseases, and stored in AS-3 additive solution. These units were transferred to Transfusion Services at Duke University Medical Center (DUMC) for storage under standard conditions at 1–6°C. Potential bacterial contamination of each stored unit was ruled out by Gram stain and culture prior to infusion.

Blood Transfusion

Each subject visited Duke Hospital at 7 and 42 days following blood collection for transfusion of one of their two units of autologous RBCs. An intravenous (IV) cannula was placed in an upper extremity vein. Pretransfusion testing, anti-human globulin crossmatching with a sample of blood from the donor subject, and product sterility confirmation were performed by the Duke Transfusion Service. One-unit of autologous RBCs was transfused over sixty minutes using an Alaris® infusion pump (CareFusion, San Diego, CA). Transfusions were administered in Duke Medical Center's Post Anesthesia

Care Unit and overseen by at least one physician investigator. The blood was not diluted or warmed prior to infusion. During the transfusion, vital signs were monitored every 15 minutes. Subjects were directly observed for adverse events during transfusion, as well as instructed to report any adverse symptoms.

Measurement of SctO₂ and StO₂

Before, during, and after the transfusion, each subject was monitored with a Fore-Sight[®] cerebral oximeter (SctO₂, CAS Medical Systems, Inc., Branford, CT) and an InSpectra[™] tissue oxygenation monitor model 1615 (StO₂, Hutchinson Technologies, Hutchinson, MN). These are FDA cleared devices that use near infrared spectroscopy (NIRS) to monitor percent (%) tissue oxygenation non-invasively in the brain and at the thenar eminence, respectively. Data was captured by these monitors from each subject prior to, during, and after the transfusion. These de-identified raw electronic data files were sent to the core lab (Dr. Shapiro, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA) where a blinded observer assessed the average SctO₂ and StO₂. A best-line fit was applied to describe average values for each of these variables approximately 5 minutes prior to and after each transfusion.

Measurement of Sublingual Microcirculatory Blood Flow

Video clips of sublingual microvascular blood flow were obtained prior to and after RBC transfusion using the Microscan[®] video microscope (Microscan, Microvision Medical, Amsterdam), which is an FDA cleared device that records microvascular blood flow. At each imaging time point, video clips, each approximately 45 to 60 seconds in length, were obtained from several sublingual sites. The images were captured using a customized video capture program (Studymaker, Newton, MA) that recorded the videos and performs an electronic file transfer to the core laboratory. De-identified unprocessed video clips were transferred via the internet to this core lab (Dr. Shapiro, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA) where a blinded observer quantified flow characteristics of the microvasculature using the microcirculatory flow index (MFI) as previously described.⁸ The observer was blinded to the duration of storage (7 versus 42 day), the subject's identity, and whether the data corresponded to the pre- versus post-transfusion period.

Measurement of RBC 2,3-DPG and ATP

2,3-diphosphoglycerate (DPG) was measured in deproteinized RBC lysates (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. RBC ATP content was assayed after hypotonic lysis of RBCs in deionized water containing the chelator DTPA (diethylenetetrapentaacetic acid, 0.1 mM) using a luciferin-luciferase assay. For both assays, measurements were made with fresh samples when possible, but measurements made from frozen samples were substituted when necessary. ATP and DPG standard curves generated daily were used to compute sample values. The results were expressed relative to undiluted packed RBCs (~70% hematocrit).

Statistics

The study was primarily descriptive in nature and given the lack of previous data and the innovative nature of this study, a power/sample size analysis was not deemed useful or reliable. Raw numeric results were checked for extreme outliers and normal distribution. Results are summarized as mean $\pm$ standard deviation. Changes from pre- to post-transfusion were tested as significantly different from zero with a paired t-test. The amount of pre-post change at 7 days was similarly compared to the change at 42 days with a paired t-test. Alpha of 0.05 was considered significant in all comparisons.

RESULTS

A total of 12 subjects were consented. One subject was a screen failure due to having donated blood recently. Two subjects withdrew from the study prior to donation of blood (one could not obtain transportation to the collection center, one withdrew for personal reasons). One subject felt nauseated during blood donation, which resulted in termination of his donation visit. A total of 8 subjects successfully donated 2 units of RBCs and received transfusion of autologous RBCs 7 and 42 days post-donation.

Subject hemoglobin (g/dL $\pm$ SD) prior to transfusion of 7 day stored blood was 12.6 ± 1.3 (n=5). The hemoglobin (g/dL $\pm$ SD) prior to transfusion of 42 day stored blood was 14.2 ± 1.2 in these same subjects. On average, the hemoglobin in subjects 42 days post-donation was 1.7 g/dL higher than 7 days post-donation ($p=0.004$). The values for StO₂, SctO₂, and MFI before and after transfusion of 7 and 42 day-old RBCs are shown in Table 2. The only significant change from pre- to post-transfusion was a very small increase in the SctO₂ following transfusion of 7 day-old RBCs. There was no significant difference in the amount of pre-to-post-transfusion change at 7 days compared to change at 42 days in any of the other measures. Raw data for tissue oxygenation and microcirculatory blood flow before and after transfusion for each subject are shown graphically in Figures 1–3. The difference in the 42 day SctO₂ change minus the 7 day SctO₂ change was: mean -0.94% , 95% Confidence Limits -2.57 to $+0.7$, $p=0.217$. The difference in the 42 day StO₂ change minus the 7 day StO₂ change was: mean 1.25 , 95% Confidence Limits -1.06 to $+3.56$, $p=0.242$. The difference in the 42 day MFI change minus the 7 day MFI change was: mean 0.104 , 95% Confidence Limits -0.189 to $+0.397$, $p=0.428$.

The Pearson correlation coefficient between hemoglobin and tissue oxygen saturation (StO₂) before transfusion was 0.308 , $R^2 = 0.095$, $p=0.356$. The Pearson correlation coefficient between hemoglobin and cerebral oxygen saturation before transfusion (SctO₂) was 0.348 , $R^2=0.121$, $p=0.2943$.

ATP concentrations in RBCs stored for 7 days averaged $218 \pm 32 \mu\text{M}$, and by 6 weeks of storage had declined to $105 \pm 56 \mu\text{M}$ ($p=0.005$). RBC 2,3-DPG concentration was very low in RBC units stored for 7 days ($177 \pm 373 \mu\text{M}$), and was close to zero ($26 \pm 73 \mu\text{M}$) in 42-day units ($p=0.364$).

Four subjects had transient pain at the site of the IV cannula (anterior forearm in all cases) during transfusion of the 42 day-old unit. In all cases the pain resolved immediately after temporary discontinuation of the RBC infusion to confirm that the IV was free flowing and not infiltrated. Replacement of the IV into a more proximal, larger vein in the antecubital fossa eliminated this discomfort in all four subjects. Subsequently, all IV lines were placed in the antecubital fossa which resulted in no subjects complaining of discomfort with transfusion of the 42 day-stored unit.

No other adverse transfusion reactions were observed in the subjects.

DISCUSSION

No differences were observed in peripheral tissue oxygenation or microcirculatory blood flow during transfusion of one unit of 7 and 42 day-old RBCs in healthy resting volunteers. A very small increase in cerebral oxygenation was noted following transfusion of 7 day-old RBCs, but not following transfusion of 42 day-old RBCs.

Previous studies assessing the effect of age of RBCs transfused on surrogates of tissue perfusion have reached conflicting results. Kiraly *et al.* observed a significant decrease in the

StO₂ of anemic, critically-ill, trauma patients following transfusion of old blood (21 days old) compared to patients who received a transfusion of new blood (21 days old),⁹ although the authors acknowledged that the absolute difference they observed may be of limited significance. In contrast, in a prospective, double-blind, randomized study comparing transfusion of two units of fresh (5 days old) versus old (20 days old) RBCs, Walsh *et al.* found that transfusion of old RBCs to euvolemic, anemic, critically ill patients had no clinically significant adverse effects on gastric tonometry or global indexes of tissue oxygenation.¹⁰ Weiskopf *et al.* found that RBCs stored for 3 weeks were as effective as RBCs stored for 3.5 hours in reversing the neurocognitive deficit of acute anemia in healthy volunteers.¹¹ Finally, Marik and Sibbald failed to demonstrate a beneficial effect of transfusion of 3 units of RBCs on measured systemic oxygen uptake in patients with sepsis, and showed that patients receiving RBCs stored for more than 15 days developed evidence of splanchnic ischemia.¹²

No rise in the StO₂ following transfusion of fresh or old RBCs was observed in this study or Kiraly *et al.*⁹ However, a very small rise in the SctO₂ was noted following transfusion of 7 day-old RBCs, but not with transfusion of 42 day-old RBCs. There are several potential reasons that this study failed to detect a difference in these measures of tissue oxygenation or microcirculatory blood flow following transfusion of 7 versus 42 day old blood. First, it is possible that despite morphological and biochemical differences, RBCs stored for 42 and 7 days have similar effects on oxygenation and the microcirculation. While subjects did not show effects of altered tissue oxygenation in the tissue beds evaluated in this study, other regions which were not monitored and which are characterized by greater O₂-dependence (such as the coronary microcirculation) may have experienced effects not captured by these methods.^{9, 12, 13} In addition to sufficient levels of oxygenated hemoglobin, adequate tissue oxygenation requires appropriate flow on the microcirculatory level.¹⁴ A decrement in microcirculatory blood flow could theoretically diminish the ability of hemoglobin to deliver oxygen to the microcirculation, however, no differences in the microcirculatory flow following transfusion of fresh or old RBCs were observed. Administering RBCs increases the hemoglobin content, which should hypothetically improve the oxygen carrying capacity of the blood. However, it should be noted that the administration of a single unit in at-rest subjects in our study represents a relatively small volume (approximately 330 ml) relative to the circulating blood volume (approximately 4,500 ml); therefore effects of the 42 day RBCs on the microcirculation may have been overwhelmed by the larger volume of endogenous RBCs already circulating. Additionally, RBCs were transfused over a one hour period; a more rapid transfusion may have helped expose potential differences in 7 versus 42 day units. Lastly, it is possible that the monitors used in our study were not sensitive enough to detect a difference in tissue oxygenation or indices of microcirculatory blood flow. However, we specifically choose these devices because they are FDA cleared and therefore are the monitors available to clinicians to measure oxygenation and microcirculation at the bedside.

Strengths of this study include the fact that each subject served as his/her own control and that two units of blood were removed simultaneously by apheresis so that the two units of RBCs transfused on days 7 and 42 were identical apart from their storage duration. FDA cleared clinically available monitors of tissue oxygenation and microcirculatory blood flow were used, making these data relevant to clinicians who use these monitors. Additionally, blood was collected and stored under controlled conditions and all raw data were sent electronically to a core lab (Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA) where it was analyzed by a blinded individual. Finally, use of healthy subjects allowed for an assessment of the storage duration on tissue oxygenation and microcirculatory blood flow without potential confounding from comorbid illness and recent surgery.

This study has several limitations. First, the subjects were healthy volunteers which may limit the generalizability of the results to acutely ill patients. Second, the hemoglobin prior to transfusion of 7 day old RBCs was lower than on day 42 because subjects received a transfusion of one unit of RBCs on day 7 and because the subject's hemoglobin level increased as expected over the intervening five weeks. It is possible that the small difference in pre-transfusion hemoglobin influenced the results. However, we observed no correlation between hemoglobin level and cerebral or tissue oxygen saturation in these non-anemic subjects, which suggests that the small difference in hemoglobin between the 7 and 42 day transfusion visits did not have a significant effect on the results. Third, subjects were not blinded to the purpose or intervention performed in the trial, but this was necessary to allow each subject to serve as his/her own control. The fact that analysis of the raw data occurred in a core laboratory by a blinded individual should mitigate the influence this limitation may have had on the results.

In conclusion, transfusion of one unit of RBCs stored for 7 days does not appear to affect tissue oxygenation or microcirculatory flow in healthy, resting, isovolemic subjects. In addition, transfusion of one unit of RBCs stored for 42 days does not appear to have any overt detrimental effects on oxygenation or the microcirculation. Although these data are reassuring in some respects, the lack of difference between 7 and 42 day stored RBCs does not rule out greater effects in patient who are sicker, more anemic, and/or who receive multiple RBC units.

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1a Cerebral Oxygenation (SctO₂) Before and After Transfusion of 7 day-old AS-3 RBCs. Each line represents a different subject.

1b Cerebral Oxygenation (SctO₂) Before and After Transfusion of 42 day-old AS-3 RBCs. Each line represents a different subject.

Figure 1.

1a and 1b shows cerebral oxygenation (SctO₂) before and after transfusion of 7 and 42 day-old AS-3 RBCs, respectively. Each line represents a different subject.

2a Tissue oxygenation (StO₂) Before and After Transfusion of 7 day-old AS-3 RBCs.
Each line represents a different subject.

2b Tissue oxygenation (StO₂) Before and After Transfusion of 42 day-old AS-3 RBCs.
Each line represents a different subject.

Figure 2.
2a and 2b shows tissue oxygenation (StO₂) before and after transfusion of 7 and 42 day-old AS-3 RBCs, respectively. Each line represents a different subject.

3a Microcirculatory Flow Index (MFI) Before and After transfusion of 7 day-old AS-3 RBCs. Each line represents a different subject

3b Microcirculatory Flow Index (MFI) Before and After transfusion of 42 day-old AS-3 RBCs. Each line represents a different subject

Figure 3. 3a and 3b shows microcirculatory flow index (MFI) before and after transfusion of 7 and 42 day-old AS-3 RBCs, respectively. Each line represents a different subject.

Table 1

Demographic Data

Variable	
Age (years)	31.5 ± 10.3
Gender	7 males, 1 female
Height (meters)	1.82 ± 0.06
Weight (kilograms)	90.2 ± 18.6
Screening hemoglobin (g/dl)	14.6 ± 1.1
Blood Type	O+ (3) A+ (4) A- (1)

Mean ± SD shown were appropriate.

Table 2

Peripheral tissue oxygenation (StO₂), cerebral oxygenation (SctO₂), and microcirculatory flow index (MFI) before and after transfusion of a single unit of 7 and 42 day stored AS-1 RBCs

	Pre-Transfusion	Post-Transfusion	P Value*
<u>StO₂, %</u>			
7 Day- Stored RBCs	79.1 ± 4.6	77.3 ± 5.8	0.140
42 Day- Stored RBCs	79.5 ± 8.6	78.9 ± 7.9	0.405
Change, 7 versus 42 day		1.3 ± 2.8	0.242
<u>SctO₂, %</u>			
7 Day- Stored RBCs	70.4 ± 3.7	71.8 ± 3.2	0.032
42 Day- Stored RBCs	71.7 ± 1.7	72.1 ± 1.9	0.231
Change, 7 versus 42 day		-0.9 ± 1.9	0.217
<u>MFI</u>			
7 Day- Stored RBCs	2.9 ± 0.1	2.8 ± 0.1	0.819
42 Day- Stored RBCs	2.8 ± 0.2	2.9 ± 0.1	0.261
Change, 7 versus 42 day		0.1 ± 0.4	0.428

Data shown as mean ± standard deviation (SD).

* P value is for comparison of pre and post-transfusion values.