Review Article

Perioperative Red Blood Cell Transfusion: What We Do Not Know

Chong Lei, Li-Ze Xiong

Department of Anesthesiology, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi 710032, China

Abstract

Objective: Blood transfusion saves lives but may also increase the risk of injury. The objective of this review was to evaluate the possible adverse effects related to transfusion of red blood cell (RBC) concentrates stored for prolonged periods.

Data Sources: The data used in this review were mainly from PubMed articles published in English up to February 2015.

Study Selection: Clinical and basic research articles were selected according to their relevance to this topic.

Results: The *ex vivo* changes to RBC that occur during storage are collectively called storage lesion. It is still inconclusive if transfusion of RBC with storage lesion has clinical relevance. Multiple ongoing prospective randomized controlled trials are aimed to clarify this clinical issue. It was observed that the adverse events related to stored RBC transfusion were prominent in certain patient populations, including trauma, critical care, pediatric, and cardiac surgery patients, which leads to the investigation of underlying mechanisms. It is demonstrated that free hemoglobin toxicity, decreasing of nitric oxide bioavailability, and free iron-induced increasing of inflammation may play an important role in this process.

Conclusion: It is still unclear whether transfusion of older RBC has adverse effects, and if so, which factors determine such clinical effects. However, considering the magnitude of transfusion and the widespread medical significance, potential preventive strategies should be considered, especially for the susceptible recipients.

Key words: Endothelium; Hemolysis; Iron; Nitric Oxide; Red Blood Cell; Storage Lesion

Introduction

Each year, approximately 80 million units of blood are collected worldwide, and 14 million units of blood are transfused in the US,[1] of which 70% was used for perioperative patients.[2] When transfusing red blood cells (RBCs), the timing and dose are two of the most important concerns for physicians. However, the quality or the duration of RBC that had been stored was seldom considered. During the *ex vivo* storage, RBC experienced biochemical, structural, and functional alternations, which are collectively referred to "storage lesion." Despite the well-investigated, apparent, and documented storage lesions, the clinical significance of transfusing the stored RBC remains unclear. It is still under hotly discussion currently and will continue to be a matter of intense investigation.

Pathophysiology Changes During Red Blood Cell *Ex Vivo* Storage

The development of RBC *in vitro* storage method represents one of the major achievements in the field of modern medicine, which allowed prolonged RBCs *ex vivo* storage while maintaining much of their function and viability. The technique development made it possible to delay transfusion after RBC was donated. However, the time interval between donation and transfusion is limited. Just like any other drugs, RBCs expires when storage time exceed the limitation of storage duration. Klein once stated in his book "Unlike wine and fine violins, red cells do not improve with age."

The maximum storage duration of RBC is dependent on the storage medium or preservative solution. In currently

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.163384

Address for correspondence: Dr. Li-Ze Xiong, Department of Anesthesiology, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi 710032, China
E-Mail: mzkxlz@126.com

Received: 25-02-2015 **Edited by:** Li-Min Chen

How to cite this article: Lei C, Xiong LZ. Perioperative Red Blood Cell Transfusion: What We Do Not Know. Chin Med J 2015;128:2383-6.

used additive solution, RBC can be stored for up to 42 days. The mean time of RBC storage duration was reported to be 17.9 days in the US.[3] The maximum duration of RBC storage is determined based on the criteria that the amount of hemolysis in the storage bag remains below 1% and at least 75% of the transfused cells persist in the recipient's blood at 24 h after transfusion.[4] However, these criteria do not reflect the complex pathophysiological changes that RBC experienced during the *ex vivo* storage. These biochemical, structural, and functional alternations are collectively referred to "storage lesion".

The biochemical changes include the alternation in the concentration of adenosine triphosphate (ATP), 2,3-DPG, NADH, and the intracellular/extracellular ion distribution. The reduced ATP concentrations impair the RBC metabolic activities. The depletion of 2,3-DPG lead to the left-shift of oxygen dissociation curve, and therefore, reduce oxygen delivery. The depletion of NADH impairs the antioxidant defense, and thereby aggravating oxidative stress. The increased intracellular sodium concentration affects cell volume and causes edema.[5] After prolonged storage, RBC change shape from a normal biconcave disk to echinocytes and Spheroechinocytes. These shape alternations reduce their deformability and increase aggregability, rigidity, and adhesion to endothelial cells, and in turn increase their likelihood of occluding the microcirculation.[6] Accumulating bioactive substances in the supernatant of stored RBC, including free hemoglobin (Hb), hemin, microvesicles, iron, cytokines, lipids, and enzymes may induce vascular dysfunction, oxidative stress, inflammation, and thrombosis.[7]

Clinical Relevance of Stored Red Blood Cell Transfusion

A landmark observational study demonstrated that transfusion of RBCs stored for more than 2 weeks was associated with increased risk of postoperative complications and jeopardized survival in patients undergoing cardiac surgery.[8] This alarm-pulled study provoked the attentions into the association of longer stored RBCs transfusion and poor patient outcomes. Since then, numerous clinical studies have been conducted to address this problem. Unfortunately, to generate a consensual answer to this conceptually simple question challenged the field of transfusion medicine.

There have been accumulating studies, include healthy volunteers, retrospective and prospective observational studies, and randomized controlled trials (RCTs), investigating the effects of stored RBC transfusion on clinical outcomes (e.g., infection, sepsis, transfusion-related acute lung injury, multi-organ dysfunction and failure, myocardial infarction, thrombotic complications, and mortality, etc.). Many recent reviews summarized the results of these studies.[3,5-7,9] However, the present information is too limited and conflicting to answer the oversimplified clinical question.

There are several possible explanations for the inconclusive results from aforementioned studies. In the healthy volunteer studies, volunteers are devoid of the comorbidities present in clinical patients and receive low-volume infusions of autologous blood, which cannot mimic the volume overload and immunomodulation in the clinical scenario.[5] Standard RBCs do not exist. Locally varying production procedures result in RBCs with widely varying storage properties. The production procedures differ in the timing of process (immediately processed versus processed after an overnight stored at room temperature), method of process (leukoreduced or not), and the storage medium.[10] Besides, there is also donor variation that one donor's blood are more pristine compared with those from another donor.[9] All these detail information and variations are seldom provided in retrospective observational studies. Most of the observational studies are underpowered small size, single-center, and retrospective studies. In many studies, confounding parameters were not adjusted, heterogeneity in the thresholds for fresh versus old/aged RBCs were used, and/or subjects were transfused with multiple units of RBCs with mixed storage times.[7]

Observational studies are fraught with a potential source of bias that impedes their ability to answer the question. Several prospective RCTs have been conducted in order to clarifying this issue. The Age of Red Blood Cells in Premature Infants (ARIPI, NCT00326924) trial[11] compared the effects of transfusing 5.1 days stored fresher blood and 14.6 days stored standard blood to neonates. No difference was demonstrated. However, it has been challenged that if the "standard" blood is old enough to testify the hypothesis. Another multicenter RCT,[12] the Age of Blood Evaluation (ABLE, ISRCTN44878718) compared the 90-day mortality of <7-day-old and standard issue RBC transfusion in 2420 critically ill patients. The preliminary results from this study also showed no difference in clinical outcomes associated with longer stored RBC transfusion. A recent published multicenter RCT,[13] the Red Cell Storage Duration Study[14] (RECESS, NCT00991341) investigated the outcomes of 1098 patients undergoing complex cardiac surgery who received leukocyte - reduced RBC stored for 10 days or less (shorter-term storage) or for 21 days or more (longer-term storage). It was demonstrated that transfusion of shorter-term stored RBC was not superior to that of longer-term stored RBC regarding the postoperative incidence of Multiple Organ Dysfunction Score. The attentions are now focused on the ongoing large-scale RCTs, hoping that they will shed a light on this tricky problem. These RCTs include the red cell storage duration and outcomes in cardiac surgery study (NCT00458783); the informing fresh versus old red cell management study (INFORM, ISRCTN08118744); the standard issue transfusion versus fresher RBC use in intensive care study (TRANSFUSE, NCT01638416).

The Underlying Mechanisms and Potential Prevention Strategies

Two-hit hypothesis

The simple question of whether prolonged storage RBC

influences clinical patient outcomes is now far from getting close to the answer. However, there is accumulating evidence that indicates the transfusion of aged RBC related adverse events are prominent in some susceptible patient populations, like critical care patients, trauma patients, cardiac surgery patients, and neonates, whereas other patient populations are immune to the stored RBC transfusion-induced bad prognosis.[15] A meta-analysis conducted by Wang *et al*.[16] supported the association between transfusion of older blood and adverse clinical outcome (mortality), especially in cardiac surgery, and trauma patients. On contrary, after reviewing 6994 noncardiac surgical patients, no evidence that increasing median storage duration was associated with a difference in the risk of postoperative mortality was found.[15] This phenomenon supports the "two-hit" or "two-insult" hypothesis that the first event (normally the existence of comorbidities or certain pathologic conditions in the recipients) predisposes to adverse outcome when a second potentially injurious event is introduced, such as stored RBC transfusion.

Nitric oxide theory and the benefit of implementation of exogenous nitric oxide

Thereafter, what is the first event for the vulnerable recipients, and is there anything in common for them? After reviewing these susceptible hosts, it was found that most of them with endothelial dysfunction, which is characterized with the impaired endothelial production of nitric oxide (NO). After transfusing of stored RBC, the accumulating of free Hb further scavenge NO and result in a decrease of NO bioavailability. The reduced NO bioavailability in RBC recipients causes alternation of vascular tones, platelet aggregation, and inflammatory responses, which lead to tissue ischemia and end-organ injury. In the subjects without endothelial dysfunction, the healthy endothelial cells produce NO to compensate for those scavenged by the free Hb after transfusing stored RBC, which explain why these patients are resistance to the adverse events related to aged RBC transfusion. Here rises the "NO theory." This theory was confirmed in animal[17-19] and in the volunteer[20] studies. For instance, it was reported that the syngeneic transfusion of aged RBCs induced adverse effects in diabetic recipients, whereas such responses were not evident in the healthy counterparts.

After transfusion, some of the biochemical changes of storage lesion, such as the reduction of ATP and 2,3-DPG levels are reversible. The irreversible changes of stored RBC, including the release of microvesicles, hemolysis and accumulating bioactive substance in the supernatant, induce the adverse clinical events. Under normal circumstance, approximately 1×10^{10} red cells are hemolyzed per hour. A single unit of blood stored for more than 28 days contains about 1.5×10^{12} RBC. Twenty-five percentage of them, or 4×10^{11} will be cleared from the circulation within 1 h following transfusion.[5] This process generates a large amount of extracellular free Hb. The released Hb is quickly bound to the haptoglobin (Hp) and then removed. The Hp scavenging capacity is about 4 μmol/L Hb. The concentration of Hb reaches to 22 μmol/L after a blood transfusion.[21] If the blood were stored for a longer time, the number would be higher. When the scavenging capacity of Hp is exceeded, the free Hb scavenges endothelial-derived NO to form met-Hb and nitrite. Normally, the RBC encapsulates the Hb and prevents the Hb-NO interaction. The rate of Hb-NO interaction increased by 1000-fold when Hb is released from RBC or in the microvesicles. The increased Hb-NO interaction after stored RBC transfusion reduces NO bioavailability and act as the second hit, and finally result in bad clinical prognosis. The correlation between hemolysis-mediated NO depleting, and transfusion-related adverse events, especially in the subjects suffering from endothelial dysfunction[17-20,22] has encouraged the potential strategies to improve NO bioavailability in the recipient circulation. The effectiveness of these strategies had been testified and demonstrated in animal and volunteer studies[17-20,22] that complement of NO bioavailability by inhalation of 80 ppm NO during stored RBC transfusion effectively attenuated or prevented aged blood transfusion-induced systemic[19] and pulmonary hypertension,[17,20,22] inflammation,[17,18] multi-organ injury,[18] and thus improving survival.[18]

Free hemoglobin and effect of haptoglobin

Free Hb is considered to be the offending molecule of transfusion-related side effects. Therefore, except for adding NO bioavailability in recipient's circulation, increasing host free Hb scavenging capacity may be workable. The aforementioned Hp is an acute phase reaction proteins, which is synthesized in the hepatocyte. Hp rapidly and irreversibly binds to the free Hb to form Hp-Hb complex and thereafter be cleared by the reticuloendothelial system. If the circulating Hp can be increased to a level that is potent to bind all free Hb released after aged blood transfusion, there will be no Hb-NO reaction. Therefore, the NO bioavailability sustains and devoid the transfusion-related adverse effects. On this basis, the effect of exogenetic Hp was tested in animal experiments. Boretti *et al*.[23] demonstrated the exogenous administration of Hp neutralize the vascular oxidative stress and prevent systemic hypertension by removing free Hb. Also, injection of 750 mg Hp at the time of the transfusion in guinea pigs alleviated acute hypertension, vascular injury, and kidney dysfunction associated with 28-day stored blood transfusion.[24]

Iron theory and iron chelator

Once scavenged, free Hb is catalyzed by heme-oxygenase-1 into carbon monoxide, biliverdin, and iron. The "iron theory"[25,26] stated that the acute delivery of a bolus of Hb-derived iron into the mononuclear phagocyte system by clearance of stored RBC is responsible for the harmful effects of transfusion. At steady state, adult human process approximately 1 ml of RBC each hour, which produces about 1 mg of iron to the mononuclear phagocyte system. In the circumstance of aged RBC transfusion, if 25% of the transfused cells were clear in 1 h, one unit of RBC would deliver 60 mg of iron (60-fold increase) into the mononuclear phagocyte system. When iron production

exceeds the binding capacity of its chaperone transferrin, the nontransferrin bound iron (NTBI) will be produced in the circulation. The NTBI induces oxidative stress, produces cytotoxicity, promotes the growth of ferrophilic bacteria, and increases the risk of posttransfusion infection.[26] This hypothesis was first tested in a mouse model.[27] It was demonstrated that transfusion of older, stored RBCs induce increasing levels of circulating NTBI. Also, the increased NTBI promotes the ferrophilic *Escherichia coli* proliferation. Iron chelator DFO (deferoxamine) and FO partly ameliorate the inflammatory response induced by stored RBCs transfusion.[27] The iron theory was also be tested in volunteers,[25] with similar results reported as demonstrated in the animal study.

Summary and Perspective

The storage lesion is a clear and obvious process that RBCs degrade over prolonged storage, which makes the differences between fresh and older blood. On the contrary, the question of "if these difference has clinical relevance" remains unclear. Due to the substantial number of transfusions annually (1 of every 70 Americans), even minor effects on clinical prognosis may have a substantial impact. Considering the magnitude of transfusion and the widespread medical significance, we should be more prudent in prescribing RBC transfusion. Using "younger" blood for certain susceptible patient populations may lead to a better prognosis for these patients. Currently, blood banks dispute blood on a first-in-first-out basis, with the oldest unit issued first. When "fresh" RBC is not available, preventive strategies (increasing Hp or NO level, using iron chelator) should be under consideration for the vulnerable recipients.

Financial support and sponsorship

This work was supported by a grant from the National Natural Science Foundation of China (No. 81370011).

Conflicts of interest

There are no conflicts of interest.

References

1. Klein HG, Spahn DR, Carson JL. Red blood cell transfusion in clinical practice. Lancet 2007;370:415-26.
2. Yu X, Huang Y, Qu G, Xu J, Hui S. Safety and current status of blood transfusion in China. Lancet 2010;375:1420-1.
3. Qu L, Triulzi DJ. Clinical effects of red blood cell storage. Cancer Control 2015;22:26-37.
4. Dumont LJ, AuBuchon JP. Evaluation of proposed FDA criteria for the evaluation of radiolabeled red cell recovery trials. Transfusion 2008;48:1053-60.
5. Orlov D, Karkouti K. The pathophysiology and consequences of red blood cell storage. Anaesthesia 2015;70 Suppl 1:29-37, e9.
6. Koch CG, Figueroa PI, Li L, Sabik JF 3rd, Mihaljevic T, Blackstone EH. Red blood cell storage: How long is too long? Ann Thorac Surg 2013;96:1894-9.
7. Cohen B, Matot I. Aged erythrocytes: A fine wine or sour grapes? Br J Anaesth 2013;111 Suppl 1:i62-70.
8. Koch CG, Li L, Sessler DI, Figueroa P, Hoeltge GA, Mihaljevic T, *et al.* Duration of red-cell storage and complications after cardiac surgery. N Engl J Med 2008;358:1229-39.
9. Zimring JC. Fresh versus old blood: Are there differences and do they matter? Hematology Am Soc Hematol Educ Program 2013;2013:651-5.
10. van de Watering LM. Age of blood: Does older blood yield poorer outcomes? Curr Opin Hematol 2013;20:526-32.
11. Fergusson DA, Hébert P, Hogan DL, LeBel L, Rouvinez-Bouali N, Smyth JA, *et al.* Effect of fresh red blood cell transfusions on clinical outcomes in premature, very low-birth-weight infants: The ARIPI randomized trial. JAMA 2012;308:1443-51.
12. Lacroix J, Hébert P, Fergusson D, Tinmouth A, Blajchman MA, Callum J, *et al.* The Age of Blood Evaluation (ABLE) randomized controlled trial: Study design. Transfus Med Rev 2011;25:197-205.
13. Steiner ME, Ness PM, Assmann SF, Triulzi DJ, Sloan SR, Delaney M, *et al.* Effects of red-cell storage duration on patients undergoing cardiac surgery. N Engl J Med 2015;372:1419-29.
14. Steiner ME, Assmann SF, Levy JH, Marshall J, Pulkrabek S, Sloan SR, *et al.* Addressing the question of the effect of RBC storage on clinical outcomes: The Red Cell Storage Duration Study (RECESS) (Section 7). Transfus Apher Sci 2010;43:107-16.
15. Saager L, Turan A, Dalton JE, Figueroa PI, Sessler DI, Kurz A. Erythrocyte storage duration is not associated with increased mortality in noncardiac surgical patients: A retrospective analysis of 6,994 patients. Anesthesiology 2013;118:51-8.
16. Wang D, Sun J, Solomon SB, Klein HG, Natanson C. Transfusion of older stored blood and risk of death: A meta-analysis. Transfusion 2012;52:1184-95.
17. Baron DM, Beloiartsev A, Nakagawa A, Martyn T, Stowell CP, Malhotra R, *et al.* Adverse effects of hemorrhagic shock resuscitation with stored blood are ameliorated by inhaled nitric oxide in lambs*. Crit Care Med 2013;41:2492-501.
18. Lei C, Yu B, Shahid M, Beloiartsev A, Bloch KD, Zapol WM. Inhaled nitric oxide attenuates the adverse effects of transfusing stored syngeneic erythrocytes in mice with endothelial dysfunction after hemorrhagic shock. Anesthesiology 2012;117:1190-202.
19. Yu B, Lei C, Baron DM, Steinbicker AU, Bloch KD, Zapol WM. Diabetes augments and inhaled nitric oxide prevents the adverse hemodynamic effects of transfusing syngeneic stored blood in mice. Transfusion 2012;52:1410-22.
20. Berra L, Pinciroli R, Stowell CP, Wang L, Yu B, Fernandez BO, *et al.* Autologous transfusion of stored red blood cells increases pulmonary artery pressure. Am J Respir Crit Care Med 2014;190:800-7.
21. Wang Y, Zhang Y, Zhao L, Yin Y, Wang Q, Zhou H. Addition of haptoglobin to RBCs storage, a new strategy to improve quality of stored RBCs and transfusion. Med Hypotheses 2014;82:125-8.
22. Baron DM, Yu B, Lei C, Bagchi A, Beloiartsev A, Stowell CP, *et al.* Pulmonary hypertension in lambs transfused with stored blood is prevented by breathing nitric oxide. Anesthesiology 2012;116:637-47.
23. Boretti FS, Buehler PW, D'Agnillo F, Kluge K, Glaus T, Butt OI, *et al.* Sequestration of extracellular hemoglobin within a haptoglobin complex decreases its hypertensive and oxidative effects in dogs and guinea pigs. J Clin Invest 2009;119:2271-80.
24. Baek JH, D'Agnillo F, Vallelian F, Pereira CP, Williams MC, Jia Y, *et al.* Hemoglobin-driven pathophysiology is an *in vivo* consequence of the red blood cell storage lesion that can be attenuated in guinea pigs by haptoglobin therapy. J Clin Invest 2012;122:1444-58.
25. Hod EA, Spitalnik SL. Stored red blood cell transfusions: Iron, inflammation, immunity, and infection. Transfus Clin Biol 2012;19:84-9.
26. Spitalnik SL. Stored red blood cell transfusions: Iron, inflammation, immunity, and infection. Transfusion 2014;54:2365-71.
27. Hod EA, Zhang N, Sokol SA, Wojczyk BS, Francis RO, Ansaldi D, *et al.* Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. Blood 2010;115:4284-92.