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Harmful effects of transfusion of older stored red blood cells: iron and inflammation

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Abstract

Retrospective studies suggest that the transfusion of older, stored red blood cells (RBCs) may be associated with increases in mortality, serious infections, multiorgan failure, thrombosis, and hospital length of stay. Our research is based on the overarching hypothesis that the adverse effects associated with transfusion of older, stored RBCs result from the acute delivery of hemoglobin iron to the monocyte-macrophage system. To test this "iron hypothesis," we are recruiting healthy human volunteers to donate double, leukoreduced, RBC units. We then transfuse them with one autologous fresh unit (i.e., after 3–7 days of storage) and one older, stored unit (i.e., at 40–42 days of storage). The primary study outcome will compare laboratory iron measures and proinflammatory cytokines after transfusion of fresh or older, stored RBCs. Similar studies using allogeneic RBC transfusions will be performed in chronically transfused patients with either sickle cell disease or β-thalassemia. Although prospective, randomized studies will ultimately determine the existence of adverse effects from transfusing older, stored RBCs, our goal is to determine the mechanism(s) for this potential effect.

CURRENT FDA STANDARDS ALLOW FOR SUBSTANTIAL CLEARANCE OF TRANSFUSED RED BLOOD CELLS

The Food and Drug Administration (FDA) mandates that the maximal allowable shelf life of stored red blood cells (RBCs) requires maintaining sufficient cellular integrity (i.e., free hemoglobin [Hb] <1% of total Hb in most units) and providing adequate 24-hour RBC recovery posttransfusion (i.e., mean recovery ≥75%); however, these are only surrogate markers of therapeutic benefit.[1] Although the RBC storage lesion is complex, and uncertainty remains regarding the mechanisms responsible for reduced RBC recovery post-transfusion, the end result is decreased 24-hour RBC recovery with increased storage time. Despite the FDA requirement regarding posttransfusion RBC recovery at outdate, the standard deviation in most studies is large and problematic.[2] Indeed, 24-hour recovery is often less than 75%.[2,3] In addition, most RBC clearance occurs within the first hour after

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CONFLICT OF INTEREST

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transfusion;[3] thus, this rapid clearance acutely delivers a substantial load of Hb iron to the monocyte-macrophage system. Finally, although studies for FDA licensure are performed in healthy volunteers, the 24-hour posttransfusion RBC recovery is even lower in critically ill patients.[3,4] Thus, we hypothesize that the adverse effects associated with stored RBC transfusions result from the acute delivery of Hb iron to the monocyte-macrophage system by clearance of the RBC subset that is irreversibly damaged during storage.

CONSEQUENCES OF INCREASED RBC CLEARANCE

We developed and validated a mouse model of leukoreduced RBC storage that is comparable to the current standard of practice for human RBCs.[5] In this model, transfused older, stored RBCs have decreased 24-hour RBC recovery and are cleared by the monocyte-macrophage system, resulting in significant iron delivery to the liver and spleen.[6] In addition, transfusions of older, stored RBCs, but not fresh RBCs, produce significant amounts of circulating plasma iron in the form of non–transferrin-bound iron (NTBI; i.e., iron not bound to transferrin, the physiologic iron transport protein). Transfusions of older, stored RBCs also induce a proinflammatory cytokine response.[6] This cytokine and NTBI response still occurs even if the stored RBCs are washed; however, it is not induced by transfusion of either the supernatant or the membrane “ghosts” derived from the older, stored RBCs. Taken together, these results suggest that these harmful effects of transfusing older, stored RBCs result from the intact donor RBCs and not from vesicles or other substances that accumulate in the supernatant during storage. Finally, the transfusion of older, stored RBCs exacerbates the inflammatory effects of subclinical endotoxinemia, producing clinical signs and symptoms. Other studies suggest that erythrophagocytosis by various mechanisms (e.g., IgG-coated RBCs, oxidatively damaged RBCs, or desialylated RBCs) decreases survival of rats and mice exposed to bacteremia.[7–10] Interestingly, phagocytosis of Hb-free RBC ghosts does not affect survival in this setting, suggesting that phagocytosis of RBC contents, but not phagocytosis itself, inhibits host defenses after RBC ingestion. In summary, these preclinical data support our hypothesis that the acute delivery of Hb iron to the monocyte-macrophage system by transfusion and phagocytosis of damaged, older, stored RBCs impairs host defenses and can synergize with underlying illness to produce more severe disease.

TRANSFUSION OF OLDER, STORED RBCS DELIVERS A SUBSTANTIAL BOLUS OF IRON TO THE MONOCYTE-MACROPHAGE SYSTEM

The net effect of the RBC storage lesion in vitro is rapid clearance of the transfused RBCs in vivo. In humans, although some RBC units demonstrate less than 75% 24-hour posttransfusion recovery, if we assume that 25% of each RBC unit is cleared at outdate, then the total iron load delivered to the monocyte-macrophage system is approximately 60 mg of iron per RBC unit, most of which is delivered in the first hour after transfusion.[3] To put this into perspective, in healthy adults at steady state, approximately 20 mg of iron (derived from approx. 20 mL of senescent RBCs) are cleared daily by the monocyte-macrophage system (i.e., approx. 1 mg/hr). Thus, transfusion of 1 unit of older, stored RBCs acutely delivers an approximately 60-fold increase in the hourly dose of iron. Therefore, even transfusions of relatively fresh RBCs, which may have an approximately 90% 24-hour recovery, can acutely

deliver a significant bolus of iron, and many patients receive multiple RBC units in rapid succession.

IRON, AN ELEMENT CRITICAL FOR LIFE, CAN ALSO BE TOXIC

Iron, an essential nutrient, plays a critical role in oxygen transport, electron transport, and various metalloenzyme-catalyzed reactions. However, iron's redox capacity is a proverbial "two-edged sword,"[11] providing the basis for its potential toxicity, which results from the Fenton chemistry reactions[12] shown below:

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet -}$$

$$2O_2^{\bullet -} + 2H^{+} \rightarrow H_2O_2 + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow OH^{\bullet} + OH^{-} + Fe^{3+}.$$

These reactions produce the potent hydroxyl radical ($OH^{\bullet}$), which can attack proteins, nucleic acids, carbohydrates, and lipids. Therefore, important homeostatic mechanisms exist to control the concentration of free iron. Intracellularly, increased "free" chelatable iron is sensed by a cytosolic iron regulatory protein, inducing ferritin synthesis, which oxidizes and sequesters excess ferrous iron.[13,14] Extracellularly, transferrin, the iron transport protein, prevents oxidative damage. Interestingly, iron remains bound to transferrin until it is imported (along with transferrin) into cells by the transferrin receptor, only being released when safely compartmentalized in endosomal vesicles.[11] Thus, iron regulation evolved to optimize its redox power for metabolic processes and to minimize its harmful effects.

EXCESS IRON PRODUCES ADVERSE EFFECTS

Iron overload from diet, chronic hemolysis, or inherited metabolic disorders predisposes patients to certain bacterial infections.[15–17] For example, infants receiving prophylactic intramuscular iron dextran were at increased risk for Gram-negative sepsis, which declined when supplementation was stopped.[18] As another example, children in a malaria-endemic area who received oral iron supplementation were at increased risk for hospitalization and mortality.[19] One proposed explanation for these findings implicated plasma NTBI levels. Although NTBI is undetectable in healthy adults, "when bolus doses of iron are administered parenterally or orally, especially without food, they may increase plasma iron concentrations and transferrin saturation and exceed the binding capacity of transferrin, leading to the appearance of NTBI. NTBI is potentially toxic because it may promote free radical formation and be more readily available to pathogens."[20,21] In addition, when plasma iron is not sequestered by transferrin, NTBI can participate in redox reactions producing oxidative damage, cytotoxicity, and increased adhesion molecule expression.[22,23] For example, in humans in vivo, high plasma NTBI levels correlate with high soluble

intercellular adhesion molecule-1 levels (a marker of activated endothelial cells).[24] Thus, if transfusions of stored RBCs increase plasma NTBI levels, the mechanism for increased risks of bacterial infections and thrombosis may be provided.

EXAMINING THE EFFECTS OF OLDER, STORED RBC TRANSFUSIONS IN HUMANS

The overall rationale of our ongoing studies is to extend our preclinical results from mice to humans, determining whether older, stored RBC transfusions increase plasma NTBI levels and induce proinflammatory responses in healthy volunteers and in patients with sickle cell disease and β-thalassemia.

The first phase of the study will determine the effects of older, stored RBC transfusions on clinical laboratory variables in healthy volunteers. The outcome variables will focus on iron-related laboratory measures and proinflammatory cytokine profiles. Each volunteer serves as his or her own control by initially donating a leukoreduced double RBC unit, with one autologous unit transfused “fresh” (i.e., on Storage Days 3–7) and the other transfused “old” (i.e., on Days 40–42). This design allows for paired comparisons between the differences between measures taken from blood samples before transfusion to those taken at multiple times after transfusion (e.g., 0, 1, 2, 4, 24, and 72 hr posttransfusion), comparing each participant’s fresh and old transfusion results. To control for the effect of blood donation on subsequent transfusion, all subjects are phlebotomized one whole blood unit 3 to 7 days before the old blood transfusion; thus, each donor is treated similarly before the fresh and old transfusions.

Using healthy volunteers for this phase eliminates confounding factors that complicated prior studies examining the adverse effects of stored RBC transfusions in critically ill patients. Although we do not expect to detect clinical symptoms induced by 1-unit autologous transfusions in healthy volunteers, we predict that the amount of iron delivered (i.e., up to approx. 60 mg) to the monocyte-macrophage system by transfusing 40- to 42-day-old RBCs will induce significant increases in serum iron, transferrin saturation, NTBI, and proinflammatory cytokine levels.

In the second phase of the study, we will determine whether older, stored RBC transfusions induce similar responses in patients with sickle cell disease or β-thalassemia who regularly receive simple transfusions. In addition, we will determine whether other standard products that are often used in this setting (i.e., washed RBCs and cryopreserved RBCs) induce similar effects. Because these patients routinely receive iron chelation therapy, and because acute iron delivery to the monocyte-macrophage system is central to our hypothesis, some of these transfusions will be performed after the patients temporarily halt chelation therapy for, at least, five terminal half-lives of the drug. This design will also allow examination of the effect of iron chelators on these outcome variables. Thus, these patients will receive transfusions of fresh and older, stored RBCs while on and off chelation therapy and these results will be compared, providing a direct test of whether modulating the effects of the acute delivery of iron ameliorates the acute response to the transfusion of older, stored RBCs.

To accomplish the second phase of the study, experienced, dedicated volunteer blood donors will repetitively donate RBCs for specific patients. Each paired donor and recipient will be recruited for a 2-year period involving eight transfusion events derived from four leukoreduced, apheresis-derived, double-RBC donations. This approach will decrease the experimental variability between different transfusion events and, as an added clinical benefit, will minimize donor exposure for the recipients. In addition, all transfusions will occur according to each patient's regularly defined schedule. Finally, by comparing the results in these two patient groups, we will determine whether the more severe ongoing chronic hemolysis in sickle cell disease patients, in comparison to β-thalassemia patients, modifies the acute effects resulting from RBC transfusion.

LIMITATIONS OF THE PLANNED STUDIES

There are several limitations to our study design. In the prior mouse studies, the equivalent of 2 units of RBCs was necessary to detect a robust proinflammatory response.[6] In addition, mouse transfusions were performed by intravenous push, rather than by slow infusion. In the human studies, only 1 unit of RBCs will be transfused into healthy volunteers and the transfusion rate will be no more than 150 mL/hr due to safety concerns (i.e., 1 unit over approx. 2 hr). Although the slower transfusion rate is not expected to affect the iron-related outcome measures, it may affect the inflammatory response. In addition, the mouse studies suggest that older, stored RBC transfusions synergize with underlying inflammation; therefore, the effects in ill hospitalized patients may differ and be more severe than those observed in either healthy volunteers or stable hemoglobinopathy patients.

Another theoretical design limitation is that recipients will always receive their fresh RBC transfusion before their old RBC transfusion, albeit after an approximately 5-week interval. Thus, any residual effects caused by transfusing fresh RBCs may affect the old RBC transfusion outcome. However, it is unlikely that there will be any lasting effects of the fresh RBC transfusion after an approximately 5-week interval.

IMPLICATIONS OF THE "IRON HYPOTHESIS"

These studies are not powered, nor are they intended, to determine whether older, stored RBC transfusions induce adverse effects in hospitalized patients. However, these studies will enhance understanding of the physiologic effects of transfusion. Only well-designed randomized, prospective studies will determine whether the effects we identify are pathophysiologically relevant in patients. In addition, by identifying a potential mechanism for the adverse effects, the "iron hypothesis" provides a target for improving the safety of transfusion. The simplest way, yet most devastating to the blood supply, would be to reduce the allowable storage period for RBCs. Nonetheless, we anticipate that more innovative approaches to improve the safety of the blood supply will be developed, for example, by inventing better storage solutions with improved RBC recovery, thereby decreasing iron delivery to the monocyte-macrophage system. Finally, pharmacologic approaches may be useful; for example, in a recent study, bacterial proliferation in macrophages correlated with intracellular iron levels and iron chelation inhibited bacterial growth, suggesting a promising therapeutic intervention.[25]

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ABBREVIATION:

NTBI	non–transferrin-bound iron

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