RED BLOOD CELL STORAGE LESION

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Abstract

The past two decades have witnessed increased scrutiny regarding efficacy and risk of the once unquestioned therapy of red blood cell (RBC) transfusion. Simultaneously, a variety of changes have been identified within the RBC and storage media during RBC preservation that are correlated with reduced tissue oxygenation and transfusion-associated adverse effects. These alterations are collectively termed the storage lesion and include extensive biochemical, biomechanical, and immunologic changes involving cells of diverse origin. Time-dependent falls is 2,3-diphosphoglycerate, intracellular RBC adenosine triphosphate, and nitric oxide have been shown to impact RBC deformability and delivery of oxygen to the end-organ. The accumulation of biologic response modifiers such as soluble CD40 ligand (sCD40L), lyso-phosphatidylcholine (lyso-PC), and Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES) have been associated with altered recipient immune function as well. This review will address the alterations occurring within the RBC and storage media during RBC preservation and will address the potential clinical consequence thereof.

KEY WORDS: red blood cell transfusion, storage lesion, transfusion efficacy, transfusion risks.

Introduction

Since the first successful attempt at blood storage almost a century ago, advances in extracorporeal red blood cell (RBC) preservation have incrementally prolonged the viability of stored RBCs. Persistent difficulties evaluating the efficacy of RBC transfusion has resulted in the exclusive reliance on post-transfusion 24-hour RBC survival when defining the acceptable RBC storage duration. Acceptable 24-hour RBC survival has been defined as 75%(1) and with contemporary preservative solutions, the storage duration for RBCs has been extended to 42 days.(2) Over the past two decades, there has been increased interest in the time-dependent changes in RBC quantity and quality during the storage period. The various changes that occur within both the RBC and storage media during ex vivo preservation have been collectively termed the RBC "storage lesion" (Figure 1). These alterations can be extensive and are primarily classified into three broad categories: biochemical, biomechanical, and immunologic. Importantly, the

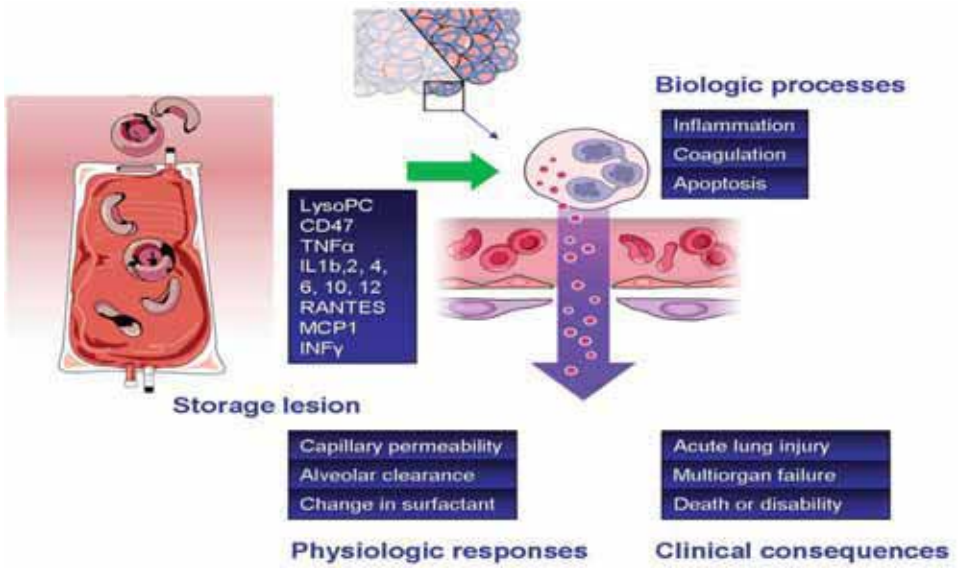

FIGURE 1. Putative biologic and physiologic consequences of storage lesion

alterations that occur during this storage process are believed responsible for many of the increasingly recognized adverse effects associated with RBC transfusion. In the present review, we will address the characteristic changes observed during the storage process. We will also address their potential clinical impact.

Biochemical Changes During RBC Storage

The biochemical changes within stored RBCs are principally related to alterations in energy metabolism with 2,3-diphosphoglycerate (DPG)(3,4) and adenosine triphosphate (ATP)(5,6) depletion. Our improved understanding of the role of the RBC in regional hypoxic vasodilation has also raised interest in the impact of RBC storage on nitric oxide (NO) metabolism(7,8). Additional time-dependent biochemical changes of potential significance include the accumulation of oxidized lipid and protein species (9) and loss of chemokine scavenging capacity(10).

2,3-DPG

One of the most notable changes during RBC storage is the rapid fall in 2,3-DPG.(3,4) 2,3-DPG is an allosteric modifier of hemoglobin which plays a critical role in the release of oxygen at the end-organ. Levels of 2,3-DPG have been shown to fall quickly during the storage of RBC, becoming undetectable within 2 weeks(11). This observation has raised concern that despite improved oxygen delivery with transfusion, stored RBCs may not release sufficient oxygen to the tissues (4). While biologically plausible, there appears to be little clinical consequence from this dramatic fall in 2,3-DPG as multiple authors have failed to find a meaningful effect from the transfusion of RBCs deplete in 2,3-DPG(12). In part, this lack of affect may result from the quick recovery of 2,3-DPG following transfusion. Normalization of 2,3-DPG levels begins within hours of transfusion and is completely restored within 48 to 72 hours (13).

ATP

A second well-described biochemical change of potential significance is a time-dependent reduction in intracellular RBC ATP.(5,6) Due to its central role in cellular metabolism, adequate levels of ATP are essential for innumerable cellular processes.(11) Examples include the maintenance of Na^+-K^+ ATPase activity, RBC membrane stability, glucose transport, oxidative stress defense mechanisms, membrane phospholipid distribution, and regional vasodilation under hypoxic conditions. Raat and colleagues have shown marked reductions in ATP levels during RBC storage (6). The fall in ATP levels appear most pronounced in blood stored for greater than 5 weeks. While ATP depletion can result in the characteristic deformation changes seen with prolonged RBC storage (see below), these morphologic changes are readably reversed with normalization of ATP levels (14). Importantly, these levels normalize quickly after RBC transfusion. Additionally, more gradual reductions in intracellular ATP levels do not appear to correlate well with these morphologic changes(5). The impact of RBC ATP depletion on the vasodilatory response to regional hypoxemia is an area of interest that has been inadequately explored and will require additional study.

Nitric Oxide

Nitric Oxide (NO) plays a critical role in vascular reactivity due to its potent vasodilatory affects (8,15). The critical role for RBCs in the oxygen-dependent regulation of blood flow has recently been described (7, 16). This oxygen-sensitive vasodilatory effect of RBCs is believed largely mediated by hemoglobin (Hb), which releases the vasodilator S-nitrosothiol (SNO) in proportion to the extent of regional hypoxemia(7,8). S-nitrosohemoglobin (SNO-Hb) forms within RBCs when a NO equivalent binds to the β93 Cys thiol residue of Hb(7). The RBC then releases SNO at the tissue level in proportion to Hb oxygen desaturation, matching regional perfusion and oxygen delivery to metabolic demand (17). The effect of RBC storage on this response has recently been characterized.(7,8) Specifically, SNO bioactivity within RBCs has been shown to fall rapidly with storage (within 3 hours ex vivo). It has been suggested that this may result in altered oxygen delivery within the microcirculation and adverse clinical outcomes even after "fresh" RBC transfusions (7, 8, 18). The rapidity of the effect obscures the impact of prolonged RBC storage on SNO-mediated hypoxic vasodilation. As with ATP-mediated hypoxic vasodilation, this too will require additional study for further characterization.

Biomechanical Changes During RBC Storage

As capillary diameter ranges from 3 to 8-μM, subtle alterations in the deformability of an 8-μ˝M RBC can have substantial impact on its ability to traverse the microcirculation. RBC deformability has been shown to rely greatly on surface area-to-volume ratio, membrane elasticity, and intracellular viscosity.(19) Notably, the biomechanical changes seen in RBCs during the storage process include: alterations in corpuscle shape, deformability, osmotic fragility, aggregability, and intracellular viscosity.(11) These changes have been shown to affect RBC transit through the microcirculation with a resultant counterintuitive decrement in tissue oxygenation (20,21). Specific changes in RBC morphology include a transition from a deformable biconcave disc to poorly deformable echinocytes with protrusions, and ultimately non-deformable spheroechinocytes (22,23). The proposed biochemical alterations in stored RBCs which result in these morphologic changes include: a depletion of ATP (5,6) and 2-3 DPG,(3,4) loss of membrane phospholipid with associated vesiculation, (14,23), protein rearrangement (9,22) and lipid oxidation (24). Additionally, Brunauer (25) and others (26) have described the internalization of membrane phospholipids in the setting of an oxidative load. The resultant loss of phospholipid asymmetry is believed to affect RBC deformability and survival in vivo as well. Recently, Karon et al. demonstrated that there may be irreversible morphological changes with loss of RBC function which occur early during storage, at day 12 (27). Using a more sensitive spectroscopic technique (TPA, or Time-resolved Phosphorescence Anisotropy), the authors found that Band 3 reorganized within intact RBC membranes before the loss of Band 3 from the RBC membrane and the appearance of Band 3 vesicles, both of which are known to occur later in storage (30 days and beyond). Additional studies are ongoing in the attempt to better characterize the morphologic changes which occur in the RBC membrane during cold storage(28,29).

Immunologic Changes During RBC Storage

The association between transfusion therapy and alterations in recipient immune function had not been recognized until 1973, when Opelz et al.(30) reported an intriguing observation that recipients of allogeneic blood transfusions had improved renal allograft survival compared to similar patients who did not receive a blood transfusion. Specifically, this work demonstrated the presence of an immunosuppressive or immunomodulatory effect of RBC transfusion on the recipient (later referred to as "TRIM", or Transfusion Related Immunodulation) (31-34). Subsequent observations have noted the potential for multiple adverse effects (e.g. nosocomial infections, transfusion-related acute lung injury, multiorgan failure) resulting from these immunomodulatory effects.

Leukocyte Contamination

Though profound immunologic changes have been noted during RBC storage, it remains unclear which substances play a primary role in the immune modulating process. Historically, emphasis has been placed on the role of leukocyte contamination in the RBC product (35). Though leukoreduction has mitigated the occurrence of specific adverse effects such as febrile non-hemolytic transfusion reactions, it appears to have had little effect on other adverse consequences such as transfusion-related acute lung injury (TRALI)(36) or nosocomial infections in trauma patients (37). Recent evidence has also failed to find a beneficial effect of leukoreduction on mortality (37,38) .

Soluble biologic response modifiers

An alternative explanation for the immunomodulatory effects of RBC transfusion relates to the presence of a variety of non-leukocyte derived biologic response modifiers. In a prospective evaluation of 22 units of leukocyte-reduced stored RBCs (LR-RBC), we recently characterized the time dependent changes of a variety of these substances. Progressive elevations in multiple pro-inflammatory mediators (e.g. cytokines, immunologically active phospholipids, CD-40 ligand) from diverse cellular origin were noted (Figure 1). Soluble CD40 ligand (sCD40L) and lysophosphatidylcholines (lyso-PCs) were two examples that may be of particular importance.

sCD40L is a platelet-derived, pro-inflammatory mediator which binds rapidly to CD40 expressed on neutrophils that adhere to the endothelium. In pre-clinical models, sCD40L induces neutrophil-mediated increases in pulmonary capillary permeability (39). We continue to explore the potential role of sCD40L in the adverse effects associated with RBC transfusions.

Lyso-PC's are the most abundant lysophospholipids in plasma and tissues and their levels increase in the setting of ischemia and inflammation. Lyso-PC shares a structural similarity to platelet activating factor. It is capable of priming and activating neutrophils and induces various proinflammatory actions in leukocytes, endothelial cells and smooth muscle cells. Unsaturated LysoPC can induce long-lasting superoxide production in neutrophils (40,41) and has been shown to increase alveolar-capillary permeability and pulmonary arterial pressure in pre-clinical models (42,43). Increased levels of this lipid have been found in patients after TRALI reactions and in the blood products associated with TRALI reactions as well (44). LysoPC-mediated neutrophil priming activity develops by the second week of routine RBC storage, with maximal priming activity by product outdate (42 days) (45).

Finally, although current pre-storage leukoreduction methods greatly diminish the concentration of leukocyte-derived products, our preliminary data suggest that some pro-inflammatory molecules may still accumulate in clinically relevant concentrations. For example, RANTES, or Regulated on Activation, Normal T-cell Expressed and Secreted, is a chemotactic cytokine, or chemokine, released from white blood cells (46). This cytokine functions as a pro-inflammatory mediator by actively recruiting T-cells, eosinophils, basophils, and monocytes to sites of inflammation. In addition, RANTES also induces the proliferation and activation of natural killer cells (NK cells) (46). We recently noted a progressive increase in RANTES levels during LR-RBC storage. The clinical significance of this finding remains under investigation.

Clinical Implications of RBC Storage Duration

Over the past decade, we have witnessed an extensive re-evaluation of transfusion strategies. Pre-clinical and clinical studies have begun to question the efficacy of RBC administration and increasingly expose the potential risk. Specific concerns associated with allogeneic RBC transfusions include an increased risk of infection, pulmonary complications such as TRALI, multiorgan failure, and mortality. Importantly, the duration of RBC storage is believed to impact both transfusion efficacy and the associated risks.

RBC Efficacy

Regarding efficacy, it should be noted that few studies outside the setting of acute hemorrhage have shown meaningful clinical benefit with RBC transfusion (47). Indeed, a landmark multicenter randomized controlled trial failed to find benefit with a more aggressive RBC transfusion strategy (48). Despite the intention to increase end-organ oxygen utilization, multiple evaluations have failed to identify an increase in oxygen utilization with the administration of allogeneic RBCs (20,49,50). To the contrary, loss of RBC membrane integrity and reduced red cell deformability, as occurs with RBC storage, has raised concern over the potential for microcirculatory occlusion and resultant tissue ischemia. In support of this theory, Murphy et al. (38) noted a substantially higher rate of ischemic events with RBC administration in their risk-adjusted analysis of cardiac surgery patients receiving RBC transfusion. Marik and colleagues (20) also reported a fall in gastric mucosal pH, an indicator of splanchnic hypoxia, after transfusion of RBCs stored for more than 15 days. Preclinical studies have suggested the possibility of reduced microcirculatory oxygenation in the setting of RBC transfusion as well (21). In contrast, Walsh and colleagues' recent randomized controlled trial failed to confirm this finding (50). Though conflicting data exist, available evidence provides remarkably little support for the once unquestioned benefit of RBC transfusion.

Risks associated with RBC transfusion

Concern over the potential transmission of blood-born pathogens such as human immunodeficiency virus (HIV) has led to a heightened awareness of the risks associated with allogeneic RBC transfusion. Although advances in blood banking strategies have markedly reduced the incidence of transfusion-transmitted infections, increased scrutiny of transfusion practices has identified several additional adverse outcomes associated with RBC administration. Chief among these concerns is the mounting evidence correlating RBC transfusion with risk-adjusted mortality (38,47,51,52). Importantly, multiple studies have suggested this association becomes stronger with increasing duration of RBC storage (53). Multiple additional adverse effects of RBC transfusion are believed influenced by the RBC storage lesion as well.

In 1999, the Transfusion Requirements in Critical Care investigators published results from their randomized controlled trial of restrictive versus liberal transfusion strategies in critically ill patients (48). A trend towards higher mortality was noted in the liberal transfusion group. More recently, Koch and colleagues performed a large, single-center, retrospective review of RBC transfusion in patients undergoing cardiac surgery (52). When compared to those who received fresh blood ($\leq$ 14 days old), patients who received older blood ($>$ 14 days old) had a higher rate of in-hospital mortality (1.7% vs. 2.8%, $p = 0.004$). Concerns over the unequal distribution of patients who received massive transfusion tempered the results of this trial and while multiple additional studies have suggested the presence of higher mortality with increasing RBC storage age, (54,55) findings to the contrary exist as well (56). At present, the conflicting data prevent definitive statements on the effect of an RBC storage lesion on mortality.

As noted, allogeneic RBC administration can have profound affects on recipient immune function. While some aspects of the immunomodulatory process appear short lived, others appear to have long term or potentially permanent impact (57). Mounting evidence associates these immunomodulatory effects with an increased risk for nosocomial infections (38,47,58). This association has been noted in multiple surgical populations (38,58) in addition to those who are critically ill (31,47). This risk of infection has also been associated with RBC storage duration (52,59,60) . While it has been suggested that the causative immunosuppressive factors arise from leukocyte contamination,(35) the impact of leukoreduction has been inconsistent(37,38,61). Though leukocytes and leukocyte-derived products (e.g. RANTES) likely have a role, additional non-leukocyted derived products such as the above mentioned CD-40 ligand and lyso-PC may be involved as well.

Conceptually, the lungs are particularly susceptible to the adverse effects of stored RBCs as the pulmonary microcirculation is the first exposed to the mediators of a storage lesion. Transfusion-related pulmonary complications, and in particular TRALI, have emerged as the most important group of complications resulting from transfusion (62). In addition to plasma transfusion from alloimmunized donors, biologic response modifiers that accumulate during storage of cellular blood products (e.g. sCD40L and Lyso-PC) are implicated in the pathogenesis of this syndrome(39,63). In contrast to febrile reactions, donor white blood cells are not believed instrumental in mediating the pulmonary vascular permeability seen in TRALI (36). In a recent experimental study, plasma from stored LR-RBCs induced a disruption of pulmonary endothelium and resulted in increased capillary permeability(64). This finding was abrogated by pre-transfusion washing, suggesting a soluble nature of biologic response modifiers. Notably, in our recent prospective study in critically ill medical patients, both the presence of alloantibodies in multiparous female donors and a higher LysoPC concentration (odds ratio 1.5 for each 10 μL, $p<0.01$), but not RBC storage age per se were associated with development of TRALI.

In addition to the adverse effects described above, RBC transfusion has also been associated with the development of multi-organ failure (MOF) (53,65). Zallen and colleagues evaluated RBC transfusions in trauma patients and demonstrated that the mean duration of storage of RBCs, the number of RBC units stored for longer than 14 days, and the number of RBC units stored for more than 21 days were all independent risk factors for MOF (53). In a more recent single-center retrospective evaluation of patients undergoing re-operative cardiac surgery, similar findings were reported (65). In addition to an association between RBC storage duration and mortality, a particularly strong correlation was noted between mean duration of RBC storage and postoperative acute kidney injury. While intriguing and hypothesis generating, this data must be interpreted with caution due to its retrospective nature and potential for multiple uncontrolled confounding variables.

Conclusion

The past two decades have witnessed an extensive re-evaluation of the risks and benefits of RBC transfusion. Accumulating evidence questions the efficacy of RBC administration while simultaneously exposing previously unrecognized risks. Multiple investigations have identified an array of biochemical, biomechanical, and immunologic changes which occur within RBCs and the associated storage media during the storage process. These alterations are collectively termed the "RBC storage lesion." Mounting evidence suggests a potential relationship of RBC storage lesion with transfusion-associated complications such as nosocomial infection, multiorgan failure, and mortality. Unfortunately, available data are conflicting and likely confounded. Prospective trials will need to confirm this relationship before strategies aimed at preventing or avoiding the RBC storage lesion are pursued.

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