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Editorial Adress:
d/a Laboratorium Patologi Klinik RSUD Dr. Soetomo, Gedung Diagnostik Center Lt. IV
Jl. Mayjend. Prof. Dr Moestopo 6–8 Surabaya, Indonesia
Telp/Fax. (031) 5042113, 085-733220600 E-mail: majalah.ijcp@yahoo.com, jurnal.ijcp@gmail.com
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LITERATURE REVIEW

QUALITY OF STORED RED BLOOD

(Kualitas Sel Darah Merah Simpan)

Anak Agung Wiradewi Lestari[1], Teguh Triyono[2], Usi Sukoroni[2]

ABSTRAK

Telah diketahui bahwa selama penyimpanan, sel darah merah mengalami sejumlah perubahan yang mempengaruhi viabilitas dan kemampuannya untuk membawa oksigen ke jaringan. Perubahan tersebut digolongkan menjadi perubahan biomekanik dan biokimia. Perubahan biomekanik yang terjadi adalah perubahan membran sel. Selama penyimpanan, sel darah merah mengalami perubahan morfologi secara pesat, dari bikonkaf menjadi echinocytes dengan tonjolan dan akhirnya menjadi spheroechinocytes. Hilangnya kesatuan sel darah merah tersebut menyebabkan pelepasan hemoglobin (hemolisis) dan pembentukan mikropartikel yang dapat menyebabkan komplikasi transfusi. Pelepasan hemoglobin (Hb) dan mikropartikel bebas menyebabkan peningkatan penggunaan Nitric Oxide (NO), sebuah molekul sinyal penting yang berperan dalam aliran darah dan dapat merangsang terjadinya inflamasi. Perubahan kimia lainnya yang dapat terjadi adalah penurunan glukosa dan penumpukan asam laktat, penurunan kalium, kepekatan adenosine triphosphate (ATP) dan 2,3-diphosphoglycerate (DPG). Tidak semua kerusakan sel akibat penyimpanan ini bersifat eryptotic. Penurunan kalium bersifat pasif (suhu yang dingin menyebabkan pompa pertukaran natrium/kalium menjadi tidak aktif). Penurunan DPG juga bersifat pasif, terkait dengan perubahan kekhasan enzim diphosphoglycerate mutase/diphosphoglyceratephosphatase dan penurunan pH. Penurunan NO terjadi karena larutnya NO bersama dengan Hb yang dilepaskan saat hemolisis. Hb plasma lebih cepat bereaksi dengan NO, dibandingkan dengan Hb dalam sel darah merah. Berkurangnya NO ini berperan dalam keadaan patologis yang terjadi sehubungan dengan pemberian darah simpan termasuk dalam hal pengangkutan oksigen oleh Hb. Perubahan akibat penyimpanan ini reversibel bila sel darah merah tersebut ditransfusikan kembali ke dalam peredaran. Tolok ukur utama yang dikontrol secara rutin untuk penyimpanan RBC adalah 0,8–1% hemolisis, 75% in-vivo survival dalam waktu 24 jam setelah transfusi, volume dan kadar hemoglobin sel darah merah. Tolok ukur tersebut memang sangat berguna, namun, perubahan biokimia yang berhubungan dengan fungsi vaskular juga harus dipertimbangkan. Perubahan yang terjadi selama penyimpanan tersebut akan reversibel melalui upaya peningkatan kualitas penyimpanan, atau menambahkan larutan additive.

***Kata kunci**: Kualitas, sel darah merah, simpan*

ABSTRACT

It has been shown that Red Blood Cells (RBC) undergo a number of changes during liquid storage which affect their viability and their ability to deliver oxygen to the tissues. The alterations can be classified in two major groups: biomechanical and biochemical. The first group of changes in red-blood-cell properties is membrane alteration. During storage, red cells undergo progressive morphological changes, from deformable biconcave disks to echinocytes with protrusions and finally to spheroechinocytes. The loss of RBC integrity results in release of cell-free Hb (hemolysis) and formation of microparticles that may contribute to complications associated with transfusion. Release of cell-free Hb and microparticles leads to increased consumption of NO, an important signaling molecule that modulates blood flow and may promote inflammation. The other chemical changes include consumption of glucose and accumulation of lactic acid, loss of potassium, and decreases in the concentrations of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (DPG). Not all aspects of the storage lesion are erytotic. RBC potassium loss is passive (cold temperature turns off the ATP-dependent sodium/potassium exchange pump). DPG loss is also passive, related to the changing enzyme specificity of diphosphoglyceratemutase/diphosphoglycerate phosphatase with decreasing pH. Nitric Oxide (NO) reduction occurs resulting in scavenging by cell-free Hb that is released upon hemolysis. Cell-free Hb reacts with NO much faster than that encapsulated in an RBC. Nitric oxide scavenging has been a major contributor to pathologic consequences of many blood substitutes that involve Hb-based oxygen carriers. These changes are reversible when stored RBCs are returned to the circulation. The major properties of

[1] Department of Clinical Pathology, Faculty of Medicine, Udayana University/Sanglah Hospital, Denpasar, Indonesia.
E-mail: aa_wiradewi@yahoo.com
[2] Department Clinical Pathology, Faculty of Medicine, Gadjah Mada University/Dr. Sardjito Hospital, Yogyakarta, Indonesia

blood routinely controlled are 0.8–1% hemolysis in stored units, 75% in-vivo survival within 24 hours after transfusions, volume and hemoglobin content of red blood cells. These are indeed very useful quality parameters; however, biochemical alterations of red-blood cells properties associated with vascular regulation should also be taken into consideration. The alterations which occur during storage appear to be at least partially reversible by use of improved storage conditions or additional solutions.

Key words: Quality, red blood cells, storage

INTRODUCTION

Oxygen is essential to most forms of life, but too much oxygen is harmful and can elicit tissue damage. Living creatures, therefore, have a tightly regulated system to deliver the necessary amount of oxygen to specific tissues at the right time. Utilizing the synergistic effects of hemoglobin, carbonic anhydrase and the anion exchange activity of band 3 protein, red blood cells play an important role in this system and provide an ideal vehicle for delivering oxygen to tissues, depending on their metabolic activity. Red blood cells can be stored for a relatively long period in liquid form, but their survival rate after transfusion and oxygen delivering capacity decreases.[1]

The safety of transfused Red Blood Cells (RBCs) is determined by the health of the donor, the needs and condition of the patient, accuracy of cross matching and quality of storage. Red blood cells are collected 'fresh' from donors. The individual cells vary from young reticulocytes and healthy cells in the middle of their normal lifespan to effete or damaged cells awaiting clearance. During storage, all cells get older, although at the reduced rates associated with low temperature and some will rupture or lose the ability to circulate. Some of these storage-related dysfunctions are corrected with return of the RBCs to the circulation whereas other defects persist. Storage-related changes in RBCs have both patient safety and regulatory consequences. Measurement of storage-related RBC changes vary in ease of performance and utility. A few measurement, such as estimating the hemoglobin concentration in RBC supernatant with a colour comparator chart or seeking evidence of bag leakage, are widely available and readily understood. Others, such as the potassium concentration of the suspending solution, rise predictably during storage but are only clinically significant in specific uncommon clinical contexts. Still, other storage-related changes, such as cell rigidity, increase during storage, but their clinical significance is largely unknown and their metrics are highly specialized.[2]

Besides being a cell without a nucleus and being responsible for oxygen and carbon dioxide transport between organs and lungs, new functions of red blood cells have been found which have led to the idea that red blood cells also play an important role in vascular regulation. Increasing numbers of studies have demonstrated that red blood cells induce vasodilation in the presence of hypoxia and promote oxygen transport. Two major compounds have been proposed in relation to this function: adenosine 5′-triphosphate (ATP) and Nitric Oxide (NO).[3]

Plastic bags are better than bottles and temperature is critical. Plastic bags are permeable to small gas molecules, so outward diffusion of CO2 provides about a quarter of the buffering with conventional RBC additive solutions, removing about 2 of the 8 mEq of protons formed in glycolysis. The di-ethylhexyl phthalate (DEHP) plasticizer of standard Poly-Vinyl Chloride (PVC) blood bags migrates from the bag into the RBCs membranes reducing hemolysis four fold. Leucocyte reduction shortly after collection prevents the accumulation of cytokines and the release of enzymes that contaminate or damage RBCs. The temperature of RBC storage is critical: rates of glycolysis and adenosine triphosphate (ATP) consumption fall by 10–15% per degree Celsius. RBCs stored at 25°C metabolize 10 times faster than those stored at 4°C. Measures of RBC quality are only useful in developmental studies when clearly tied to well-defined conditions of storage including bag type, degree of leukoreduction and temperature. The clinical effects of RBC storage and especially of the observed differences in storage quality are largely unknown. Regulatory science is limited by limited understanding of factors determining safety and efficacy.[2] Continued developments in storage techniques have resulted in improved storage times as well as red-blood-cells quality. In this context we refer to 'storage' as liquid preservation, as this is the most common blood preservation technique currently in use.[3] This review describes a number of components of the changes in red blood cells during storage.

STORAGE CONDITION AND DURATION

Red cells are stored at 1–6°C for 21–42 days depending on the anticoagulant–preservative solution used.[4,5] For optimum inventory management, most

blood centers use preservatives that allow 42-day storage. The end of the storage period is referred to as the expiration date or the "outdate." The cells must be stored in refrigerators with good air circulation and that are designed for blood storage. Household refrigerators are not suitable. The temperature in the refrigerator must be monitored and should be recorded periodically, at least every 4 hours. There should be an alarm system to warn staff if the temperature moves outside the acceptable limits. When blood is transported to the patient care area for transfusion, it may be allowed to warm to 10°C and still be suitable for return to the blood bank and reissue to other patients. Blood components must be maintained under proper storage conditions during transportation from the blood center to the hospital transfusion service. Various containers are available for this purpose and these processes are standard and work well in developed countries. However, in developing or undeveloped parts of the world usually these kinds of containers are not available and red cells may not be refrigerated or stored properly during this transportation. This is also an issue in military settings where it also important that these containers be light weight. At least one container has been reported that will maintain red cells at 1–10°C for up to 78 hours.[4]

THE CHANGES OF RED BLOOD CELLS DURING STORAGE

The increasing demand for allogeneic blood transfusions has resulted in millions of liquid-stored allogeneic red blood cell units being used annually for transfusions worldwide. This practice is based on the theoretical expectation that increasing the intravascular mass of red blood cells will increase oxygen delivery to the tissues. However, accumulating evidence is showing that this expectation may not be true and that there is a negative relationship between the storage time and red-blood-cells viability and function. Additionally, recent findings in observational studies on large populations showed that restrictive transfusion triggers were associated with a better patient outcome. Nevertheless, despite these new findings and the possibility of using allogeneic blood transfusion alternatives – such as peri/postoperative cell salvage, pre-donation and recombinant erythropoietin administration – liquid-stored allogeneic red blood cells are still the most favored transfused blood products. The increasing concerns about the efficacy of allogeneic blood transfusions force the question about the impact of storage on red-blood-cell function and hence on their use for blood transfusion. First, however, the issue of how the physical and biochemical properties of red blood cells are altered under conditions of storage should be discussed. Indeed, it has been shown that red blood cells undergo a number of changes during liquid storage which affect their viability and their ability delivering oxygen to the tissues the alterations can be classified in two major groups: biomechanical and biochemical.[3]

Biomechanical changes

The first group of changes in red-blood-cells properties is membrane alteration. The structure of the red blood cell is complex and membrane phospholipids and proteins, cytoskeletal proteins and cytoplasmic components are all related to each other. Hemorheological alterations–such red blood cell shape changes, decreased membrane deformability and surface/volume ratio, increased mean cell hemoglobin (Hb) concentration and osmotic fragility, increased aggregability and intracellular viscosity–can occur during storage and may possibly disturb the flow of red blood cells through the microcirculation and influence red-blood-cells transport of oxygen to the tissues.[3]

During storage, red cells undergo progressive morphological changes, from deformable biconcave disks to echinocytes with protrusions and finally to spheroechinocytes. In parallel with these changes, redistribution and loss of phospholipids in the red cell membrane by the formation of microvesicles are seen both in storage and in red cell aging and may contribute these changes during storage.[2,3,6] RBC membrane loss during storage can lead to substantial changes in rheologic properties. This loss of RBC integrity results in hemolysis and formation of microparticles that may contribute to complications associated with transfusion. There have been several studies that documented hemolysis as a function of time during storage. The levels of extracellular Hb reported in the literature, range from 28 mmol/L (in heme) after 35 days of storage in citrate phosphate-dextrose adenine to 80 mmol/L after 50 days of storage. Transfusion of just 1 unit of blood with this much hemolysis would result in plasma levels exceeding those of steady-state sickle cell disease.[6]

Hemoglobin, which is 98% of the non-water content of RBCs and intensely colored, serves as the obvious marker of RBC membrane failure. Extra-cellular hemoglobin can be observed in the suspending fluid when the RBCs settle, its amount determined spectrophotometrically and the resultant hemolysed fraction expressed as a percentage. It is

now understood that 25–70% of this extracellular hemoglobin is in microvesicles. Normally, there is no visible hemolysis in leukocyte reduced RBC units at the end of component processing and the measureable amount is <0.02%. This value rises on average to 0.30% at the end of 6 weeks of storage when it is visible as a red-tinged supernatant. Hemolysis at 6 weeks tends to be about 4 times the hemolysis at 3 weeks. This increasing rate of hemolysis with storage time comes about as the conditions for storage inside the blood bag are continuously deteriorating. The new Food and Drug administration (FDA) requirement is that, at the end of storage, 95% of RBC units have <1% hemolysis with 95% confidence effectively sets the required mean end-of-storage hemolysis at about 0.35%. The free hemoglobin in stored blood is present in many different forms including oxy-hemoglobin, deoxyhemoglobin and methemoglobin. Hemolysis remains important as a clinical quality measure in RBC storage because it is directly observable and accurately measureable.[2]

The current criteria for the quality of red blood cells for transfusions take biomechanical alterations into consideration as the basis for determining the in-vivo function of the cells.[3] The major properties of blood which are routinely controlled are 0.8–1% hemolysis in stored units, 75% in-vivo survival within 24 hours after transfusions and volume and hemoglobin content of red blood cells.[3,5,7-9] The alterations which occur during storage appear to be at least partially reversible by use of improved storage conditions, additional solutions, or rejuvenation.[3]

Biochemical changes

To maintain optimum viability, blood is stored in the liquid state between 1°C and 6°C for a specific number of days, as determined by the preservative solution(s) used. The loss of RBC viability has been correlated with the lesion of storage, which is associated with various biochemical changes (Table 1).[8] Chemical changes include consumption of glucose and accumulation of lactic acid, loss of potassium and gain of calcium, loss of hemoglobin-bound NO and decreases in the concentrations of ATP and 2,3-diphosphoglycerate (DPG).[2,6,7,10] Central to the storage lesion is the programmed cell death of damaged and effete RBCs. Erythrocyte apoptosis or eryptosis is largely held in check by high concentrations of RBC ATP. Not all aspects of the storage lesion are erytotic. RBC potassium loss is passive–cold temperature turns off the ATP-dependent sodium/potassium exchange pump. DPG loss is also passive, related to the changing enzyme specificity of diphosphoglyceratemutase/diphosphoglycerate phosphatase with decreasing pH. S-nitrosylhaemoglobin declines in concentration when RBCs are removed from the NO-secreting vasculature. These changes are reversible when stored RBCs are returned to the circulation, for example, DPG is typically restored in 5 hours, but have physiologic consequences before normal concentrations are restored. Conditions of storage markedly affect the quality of storage.[2]

It has long been known that oxygen affinity of blood increases when it is stored at 4°C. Figure 1 shows

Table 1. RBC storage lesion[4,8]

Characteristic	Change Observed
% Viable cells	Decreased
Glucose	Decreased
ATP	Decreased
Intracellular potassium	Decreased
pH	Decreased
2,3 DPG	Decreased
Oxygen dissociation curve	Shift to the left (increase in hemoglobin and oxygen affinity; less oxygen delivered to tissues)
Plasma potassium	Increased
Plasma hemoglobin	Increased
Lactic acid	Increased
Pyruvate	Increased
Ammonia	Increased
Intracellular sodium	Increased
Membrane vesicles	Increased

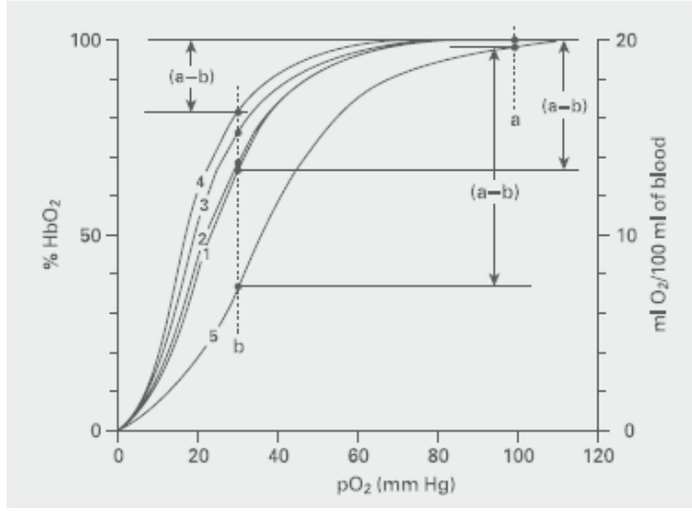

Figure 1. Oxygen dissociation curves of preserved red blood cells. Curve 1: oxygen dissociation curve of fresh blood; curve 2: oxygen dissociation curve of 1-week-old CPD blood; curve 3: oxygen dissociation curve of 2-week-old CPD blood; curve 4: oxygen dissociation curve of 3-week-old CPD blood; curve 5: oxygen dissociation curve of 3-week-old CPD blood after treatment with PEP. (a–b) is the oxygen- delivering capacity of red blood cells[1]

changes in the red blood cell oxygen dissociation curve during liquid preservation. As the preservation period is prolonged, the oxygen dissociation curve shifts to the left compared with that of fresh red blood cells. This indicates progressive increases in oxygen affinity of red blood cells during storage. An increase in oxygen affinity of red blood cells decreases their oxygen-delivering capacity.[1]

RBC DPG concentration

The concentration of erythrocyte 2,3-DPG in RBCs also decreases with storage. The decrease is dependent on pH. 2,3-DPG is a well-known molecule in red-blood-cell function, as its role in hemoglobin oxygen affinity regulation is crucial for tissues.[3] It was discovered that 2,3-DPG binds specifically with hemoglobin and reduces its oxygen affinity.[1] 2,3-DPG is normally present in RBCs in molar quantities slightly greater than hemoglobin to allow for 1:1 binding.[2] Therefore, any alteration of 2,3-DPG is believed to be very important and initial studies on the loss of oxygen-delivering ability of red blood cells during storage were focused mostly on 2,3-DPG. 2,3-DPG is a metabolite and allosteric modifier of hemoglobin and decreases quickly during the first 2 weeks of storage to almost undetectable levels. This decrease leads to an increase in hemoglobin oxygen affinity, which may be an explanation for the decrease of red blood-cells oxygen-delivering ability during storage. However the 2,3-DPG levels appear to start to recover within

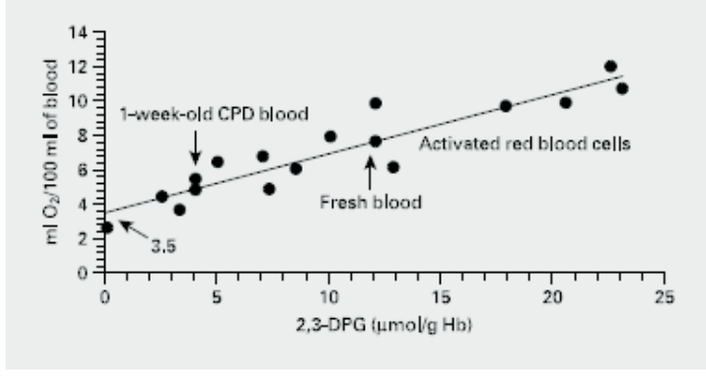

Figure 2. Correlation between the 2,3-DPG level of red blood cells and their oxygen-delivering capacity. The relationship between the 2,3-DPG level and the oxygen-delivering capacity is plotted over a 2,3-DPG range from 0 to 25 Ìmol/g Hb, which is approximately 200% of the normal 2,3-DPG level. The oxygen-delivering capacity is calculated by the equation $y=0.34x+3.5$[1]

several hours and this may take up to 72 hours after transfusion in vivo. Considering the fact that blood transfusions are often given to acute patients, waiting for 2–3 days to see the effects of blood transfusion is hardly acceptable. However, the clinical consequences of completely 2,3-DPG-depleted red-cells units do not seem to be that significant. Theoretically, if 2,3-DPG is not present in red blood cells stored longer than 2 weeks, then approximately two thirds or more of all stored red-cell units would be expected to be 2,3-DPG-depleted.[3] Oxygen affinity of red blood cells decreases as their 2,3-DPG content increases and oxygen affinity increases as 2,3-DPG content decreases. The progressive decrease in oxygen-delivering capacity of preserved red blood cells with prolongation of the preservation period is primarily due to decreases in 2,3-DPG in the preserved blood. For this reason, preventing or recovering the decrease of red blood cells 2,3-DPG during preservation is an important point in maintaining the quality of preserved red blood cells. Figure 2 shows the relationship between the 2,3-DPG level of red blood cells and their oxygen-delivering capacity in the 2,3-DPG ranges from 0 to 25 μmol/g Hb which is approximately 200% of the normal 2,3-DPG content.[1]

RBC ATP concentration

An additional biochemical change which occurs in stored red blood cells is the decrease in intracellular ATP levels. In addition to functioning as an intracellular energy source, ATP can serve as important extracellular signaling molecule. It is now known that red blood cells release ATP in response to hypoxia, pH and mechanical stress. ATP, besides playing a secondary role in membrane deformability, is crucial for red-blood-cell function due to its central role in cellular metabolism as an energy source. Sugar transport into the red cell, protective antioxidant mechanisms, membrane phospholipid distribution and all other functions are only possible if ATP is present or can be regenerated in the red blood cells. The newly discovered role of ATP as a vasodilator under hypoxic conditions has highlighted its importance for red-blood-cells function. The mechanical and hypoxia-induced ATP release is believed to be through a specific membrane-bound receptor, the Cystic Fibrosis Transmembrane Protein Receptor (CFTR). This function probably depends on a number of factors, including the intracellular adenosine pool, red cell cytoskeletal and membrane structure and partially 2,3-DPG presence, in order to detect hypoxia. Nevertheless, the complex regulation mechanism of oxygen-sensing and ATP-releasing functions is not very well understood and needs further studies. Adenosine triphosphate depletion and the adenine pool in the red cell do not determine the red cell survival directly, but certainly have an important role in red-blood-cells function. Adenosine triphosphate may contribute to oxygen delivery by red blood cells due to it's action as a vasodilator and its being released by red blood cells in the presence of hypoxia. This physiological property of ATP may be negatively affected by storage duration.[3]

The storage-related decrease in red-blood-cells membrane deformability is a crucial change in red-blood-cells properties and is associated with post-transfusional 24-hour survival. The decreased deformability was thought to be associated with reduced ATP levels.[3] While ATP depletion, as seen during storage, can produce many shape changes, a reduction in surface/volume ratio and increases in intracellular viscosity and post-transfusional 24-hour survival of red blood cells precede the decreases in ATP concentration. Only decreases beyond 50% of the ATP concentration can be shown to be associated with decreased mortality, suggesting that the role of ATP depletion in storage-related damage may be limited. Nevertheless, restoring ATP levels in red-blood-cell units appears to correct membrane alterations to a certain level. It is probable that a basal ATP level is necessary for the survival of red cells, and therefore the adenine pool (AMP, ADP and ATP) has more effect on cellular changes than ATP alone.[3]

It has been the measurement of choice for developmental studies testing new concepts and conditions of RBC storage in the laboratory. At the time of collection, the RBC ATP concentration typically averages 4±1 μM/g Hb. This value rises to 5±1 μM/g Hb in the first week of storage as DPG is broken down and falls thereafter as conditions for ATP synthesis become progressively worse. At the end of 6 weeks of storage in a conventional RBCs additive solution, the RBC ATP concentration ranged from as little as 1 and as much as 3 μM/g Hb. RBCs use ATP to drive the pumps and regulate the permeases on their membranes and to control cytoskeletal interactions. At the end of storage, re-infused, stored, RBCs use their remaining ATP to reinitiate glycolysis and the resulting new ATP to drive potassium and phospholipid pumping. For this reason, the end-of storage RBC ATP concentration also determines the rate of the recovery of some functions of transfused RBCs.[2] Storage lesion is one of the factors determining how long components may be stored (Table 2).[5]

Table 2. Biochemical changes in stored non leukocyte-reduced red blood cells[5,11]

Variable	CPDA-1		AS-1	AS-3	AS-5
Days of storage	0	35	42	42	42
% viable cells (24 hours after transfusion)	100	71	76	84	80
pH (measure at 37°C)	7.55	6.71	6.6	6.5	6.5
ATP (% of initial value)	100	45±12	60	59	68.5
2,3 DPG (% of initial value)	100	<10	<5	<10	<5
Plasma K+ (mmol/L)	5.1	78.5†	50	46	45.6
Plasma Hemoglobin	78	658†	N/A	386	N/A
% Hemolysis	N/A	N/A	0.5	0.9	0.6

Values for plasma hemoglobin and potassium concentrations may appear somewhat high in 35 day, stored RBC units; however, the total plasma in these units is only about 70 mL.
CPD=citrate-phosphate-dextrose; CPDA-1=citrate-phosphate-dextrose-adenine-1; AS-1=additive solution formula 1; ATP=adenosine triphosphate; 2,3-DPG=2,3-diphosphoglycerate; N/A=not applicable.

Nitric Oxide (NO)

Another possible mechanism which may account for alterations in the oxygen transporting capabilities of transfused red blood cells is their ability to generate nitric oxide under acidic and hypoxic conditions. An alternative route for hypoxia-induced nitric oxide has been proposed to be the presence of red blood cell-bound S-nitrosothiol.[3] Nitric oxide is a neutral, radical molecule that has several important roles in physiologic signaling. Nitric oxide is the endothelial derived relaxing factor; it is made in endothelial cells and plays a major role in controlling blood flow by effecting smooth muscle relaxation adjacent to the blood vessels. It is made by endothelial NO synthase (NOS) from arginine and diffuses to the smooth muscle where it activates soluble guanylyl cyclase to produce cGMP, initiating a signaling cascade leading to vasodilation. In addition, via this endothelial NOS, the two other isoforms (inducible NOS and neuronal NOS), or other mechanisms of formation, it plays a role in homeostasis through inhibition of platelet (PLT) aggregation, acts as a toxic agent in host defense, decreases expression of adhesion molecules, and has antioxidant properties. More recently, an RBCsNOS has been discovered. Importantly then, NO is seen to contribute to many functions that could be linked to the storage lesion including blood flow, inflammation and thrombosis.[2,3]

Given the many important functions of NO, it is not surprising that diminished NO bioavailability contributes to pathology in many diseases, as result from endothelial dysfunction that is often due to reduced NO synthesis by NOS. Besides reduced production, NO bioavailability can also be reduced by increased consumption; one way for this reduction to occur results from scavenging by cell-free Hb that is released upon hemolysis. Cell-free Hb reacts with NO much faster than that encapsulated in an RBCs. NO scavenging has been a major contributor to pathologic consequences of many blood substitutes that involve Hb-based oxygen carriers.[5] Several proposed mechanisms by which stored red blood cells can perpetuate microcirculatory perturbations and organ dysfunction. Red blood cells undergo hemolysis and microparticle formation. Free hemoglobin and microparticle hemoglobin scavenge NO, resulting in the loss of tonic vasodilation and generation of reactive oxygen species. The reduction in NO bioavailability promotes both platelet adhesion and aggregation in an already compromised microcirculation. Microparticles with exposed phosphatidyl serine on their surface may promote hemostatic activation and platelet-neutrophil aggregation (Figure 3).[6,12]

Nitric oxide consumption by the non-erythrocytic (plasma) fraction of older stored blood is dramatically greater than that from blood stored for only 1 week. Several studies have found that transfusions using older blood are associated with adverse clinical outcomes. It should be noted, however, that others have not found these types of associations. Although the impact of transfusion of old blood is a matter of debate, the fact that transfusion represents one of the most common medical therapies suggests that a further large-scale study of its impact is warranted and that the mechanisms involved should be elucidated.[6]

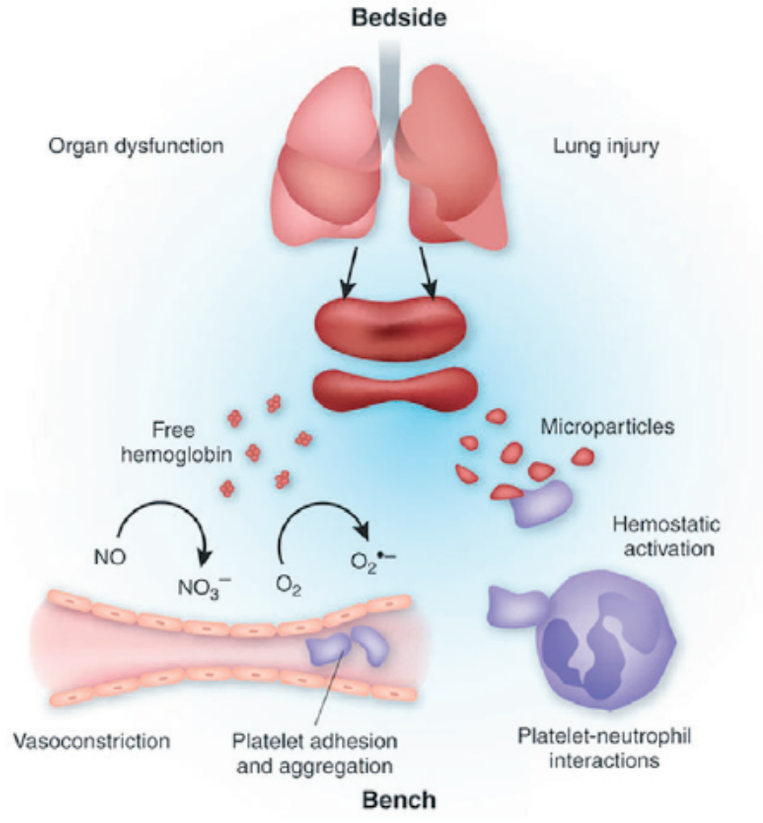

Figure 3. Proposed mechanisms contributing to the storage lesion. RBC breakdown leads to release of cell-free Hb and RBC microparticles. These scavenge NO, which leads to vasoconstriction, PLT activation and adhesion and inflammatory pathways[6,12]

Peroxidized lipids, damaged proteins and altered glycands

Red blood cells enter the circulation as anucleate end stage cells with largely normal proteins, lipids and carbohydrates.They circulate and sustain damage to these constituents until some critical level of injury is attained, and then they are removed from the circulation. Currently, removal of damaged or effete cells from the storage bag is not possible, so they and their damaged parts persist until they rupture.

Oxidation appears to drive much of this accumulating cellular damage. Hemoglobin, the major constituent of RBCs, is the ultimate source of most of this oxidative activity, which can go on to damage lipids, proteins and carbohydrates. Lipids can be damaged in at least three well-defined ways. In the simplest, oxidative injury breaks chemical bonds. By such a mechanism, tri-alkyl glycerols are broken down into di-alkyl glycerols and free fatty acids. Silliman and his colleagues have shown that such di-alkylglycerols accumulate in stored RBCs and have biological response modifier activity that can cause Transfusion Related Acute Lung Injury (TRALI).

In a second mechanism, unsaturated lipids arranged in parallel can undergo polymerization in an oxidative chain reaction. Glutathione is a major defence against this action in normal cells, but its concentration decreases during RBC storage.

A third mechanism involves the oxidative internal rearrangement of double bonds in alkene chains to create cyclic endo-peroxides with prostaglandin, prostacyclin and other biological activities. These activities can be measured as the evolution of specific breakdown products or by measures of general lipid peroxidation.

Proteins on the outer surface of the RBC, in its membrane, cytoskeletal components and cytosolic components can all be damaged in ways similar to lipids. Oxidative injury to spectrin slowly weakens the cytoskeleton. Covalent bonding of the beta-chain of hemoglobin to the cytosolic tail of Band 3 slowly weakens membrane to-cytoskeletal attachment. Oxidative cross-linking of cytosolic proteins is part of the slow inactivation of some RBC enzymes.

Carbohydrates are also subject to oxidative attack. Simple breakdown reactions such as the loss of sialic acid from cell surface glycolipids and glycoproteins can

expose deeper antigens. Attachment of sugars in their aldehyde form to protein amines leads to the formation of advanced glycation end products (AGEs). Such AGEs form at high rates in the high sugar environment of RBCs storage solutions. Products of these reactions can be detected by immunological techniques such as the counting of normally masked Forsmann antigen sites and assessing reactivity with the receptor of AGEs. An estimate of total metabolic depletion and peroxidative damage can be made with infrared spectroscopy. A simultaneous measure of total glucose content, ATP concentration, surface lipid and skeletal protein content was made in a single spectrum. Such an assay has the potential to be most useful if it can done non-invasively, through the wall of the plastic bag and can detect products at risk of poor recovery. At the current time, these measures are research tools.[2]

CONCLUSION

It has been shown that RBCs undergo a number of changes during liquid storage which affect their viability and their ability to deliver oxygen to the tissues. The alterations can be classified in two major groups: biomechanical and biochemical. The first group of changes in RBCs properties is membrane alteration. The loss of RBCs integrity results in release of cell-free Hb (hemolysis) and formation of microparticles that may contribute to complications associated with transfusion. Release of cell-free Hb and microparticles leads to increased consumption of NO, an important signaling molecule that modulates blood flow and may promote inflammation. The other chemical changes include consumption of glucose and accumulation of lactic acid, loss of potassium and decreases in the concentrations of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (DPG). Nitric oxide reduction occur which results from scavenging by cell-free Hb that is released upon hemolysis. Nitric oxide scavenging has been a major contributor to pathologic consequences of many blood substitutes that involve Hb-based oxygen carriers. These changes are reversible when stored RBCs are returned to the circulation. The major properties of blood which are routinely controlled are 0.8–1% hemolysis in stored units, 75% in-vivo survival within 24 hours after transfusions and volume and hemoglobin content of red blood cells. These are indeed very useful quality parameters; however, biochemical alterations of red-blood cells properties associated with vascular regulation should also be taken into consideration. The alterations which occur during storage appear to be at least partially reversible by use of improved storage conditions or additional solutions.

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