Aspects of the Quality of Red Blood Cells in Transfusion Therapy

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Introduction

The quality of red blood cells (RBC) suitable for transfusion involves factors both directly related to the red cells and to other blood cells as well as infectious agents, viruses in particular, potentially contaminating the product. In addition, substances added to the storage medium may have an effect on the transfusion recipient (**Table 1**). In this paper, emphasis will be put on the first-mentioned factors, since the important question of virus transmission is dealt with in other communications at this conference.

Viability of stored RBCs

The ability of RBCs to survive and circulate in the transfused recipient is related both to the arrest of the normal aging processes by the lowered temperature and to the measures taken to avoid or diminish damage caused by the storage process itself. At 2-6 °C the enzyme processes in the RBCs are slowed down. Under suitable storage conditions RBCs may have close to 100% viability even after 4 weeks of storage at 4 °C, instead of the 70-75% viability that could be expected at the normal rate of dying of RBC at 1% per day. This "hibernation," however, also has negative effects. It almost completely paralyzes the Na+/K+ membrane pump mechanism, which results in leakage of K+ out of and Na+ into the RBCs. The change is reversible, but full recovery in vivo takes several days. Unless adenine is incorporated in the storage medium, adenine groups will be lost due to irreversible decomposition of adenosine monophosphate (AMP) to hypoxanthine. The dose of adenine submitted to the RBCs should be just enough to replace that used by the cells for the maintenance of the adenine nucleotide pool. This is because a surplus of adenine, unused by the RBC, may, in a massive transfusion, cause formation of 2,8-dihydroxyadenine, a very poorly water-soluble metabolite formed mainly in the liver. It may precipitate in the kidney tubules of the recipient with potentially negative consequences. A suitable adenine dose is 0.1–0.2 mmol per red cell unit obtained from a 450 ml blood collection[1].

Table 1. Factors of major importance for the quality of RBCs intended for transfusion.

- Ability to survive and circulate after transfusion
- Maintain normal functional capacity with respect to membrane flexibility uptake and release of oxygen and carbon dioxide
- Possible negative effects of substances included in the storage medium
- Side-effects of other blood cells than RBCs or their products
- Transmission of infectious agents

Other storage changes are loss of the normal discoid shape (formation of echinocytes), loss of membrane deformability, and microvesiculation. The first-mentioned of these changes are reversible to a certain extent, the last-mentioned not. Generally, reversible changes are unrelated to the loss of viability, whereas an irreversible loss of membrane shape seems to be associated with it. The formation of microvesicles, commonly from strangulation and budding off spicules from echinocytes, is associated with an increase in extracellular hemoglobin (Hb). In fact, most of the measurable increase in plasma Hb occurring during liquid storage of whole blood or RBCs is due to Hb encapsulated in microvesicles[2]. It is still not known to what extent RBC membrane loss may cause non-viability.

There is evidence that the removal of aged RBC from the circulation is due to exposure of normally hidden antigenic receptors on the band 3 protein of the RBC membrane. The binding of autoantibodies, directed toward this receptor and normally present in the plasma, together with complement will create an "eat-me signal" to phagocytic cells in the recipient[3]. Another mechanism which may cause phagocytosis of RBC is externalization of phosphatidylserine, a phospholipid that is part of the RBC membrane double-layer, but normally turned toward the inside of the cell. It seems likely, although still unproven, that similar mechanisms as those mentioned are responsible for the removal of RBC damaged by liquid storage. In washed suspensions of RBCs, a phagocytosis-promoting signal seems to be transferrable from apoptotic leukocytes to RBCs [1]. This is unlikely to happen when plasma is present in the storage medium, even when the amount of plasma is small, such as when RBCs are stored in an additive solution without previous washing.

One of the great and so far unsolved problems in the preservation of RBC viability is the formation of lactate and the associated acidification of the cell mixture. A decreased pH has a depressing effect on several of the important metabolic steps in RBCs and eventually leads to impaired metabolic activity and loss of adenine nucleotides, in its turn associated with lowered in vivo recovery and survival of the transfused RBCs. Several attempts to improve the storage conditions have been reported [for review see 1].

In principle, it should be possible to improve RBC storage by a less acidic anticoagulant than ACD or CPD and an additive solution with buffering properties. In reality, a number of factors have to be considered: the fact that some RBC units will be used fresh, others stored; that the additive solution has to be sterilized, which normally means by heat; that substances used to promote RBC viability must not be

toxic to the recipient (neither they themselves nor their metabilites), etc.

An improved system has been designed and published by our group, using half-strength citrate CPD (0.5CPD[4]) as anticoagulant and a simple, non-toxic additive solution for the storage of red cells, RAS2 or Erythro-Sol®[5-7]. The major principle is to apply a slightly higher pH than normal in the whole blood and to elevate the intracellular pH by using a hypotonic additive solution containing citrate, phosphate, adenine, and mannitol at pH 7.4; glucose is submitted as a concentrated solution from a tubing. Although proven to work clinically, the system has not been used extensively due to hesitation from plasma fractionators to accept 0.5CPD plasma, with obvious difficulties for blood banks to deliver their 0.5 CPD plasma for fractionation. However, it seems likely that a useful system, without the above mentioned problem but with equally good or even better properties, can be designed along these principles[1].

Post-transfusion function of RBC

As already mentioned, some changes induced by the storage process are reversible, others irreversible. By simply incubating stored RBCs at 37 °C for an hour, part of the storage-induced changes of membrane shape and flexibility are reversed. However, this does not influence in vivo recovery. Another reversible change is the loss of 2,3-diphosphoglycerate (2,3-DPG). After transfusion this metabolite will be recovered in vivo, but regeneration, following a complete loss, is slower than the reversal of morphological changes; 50% recovery of RBC 2,3-DPG requires 6-8 h and full recovery about 24-48 h [for references see 1].

In order to be able to take up and deliver oxygen and carbon dioxide the RBC uses its flexible membrane to establish firm contact with the capillary walls, which promotes rapid exchange. Hydrogen ions and 2,3-DPG are potent regulators of the binding of oxygen to the RBC hemoglobin (Hb). Increasing concentrations of both of these decreases the oxygen affinity of the Hb, thus promoting the release of oxygen. The loss of 2,3-DPG implies an increased affinity with potentially lower release of oxygen from the Hb.

It has been a matter of debate to what extent these changes have practical importance, i.e. if stored RBC, in the circulation of a recipient, have an impaired capacity to deliver oxygen to the tissues. Recent experimental studies in rats show that storage of RBC for 28 days in CPDA-1 anticoagulant impaired their ability to improve tissue oxygenation when transfused into either control or septic rats placed into supply dependency of systemic oxygen uptake. In contrast, fresh RBC caused the expected increase of oxygen uptake and utilization[8]. The authors suggested that impaired membrane flexibility of the RBCs might have been the cause of poorly functioning stored cells, but the loss of 2,3-DPG offers an equally likely alternative explanation. In another study, the use of RBC with elevated levels of 2,3-DPG saved the brains of rats from ischemic metabolic changes during hemodilution. Transfusion of low-DPG RBC caused clearcut disturbances of brain function under identical conditions[9].

Although reversible storage-induced changes are probably of minor significance in patients who can use compensating mechanisms, for instance an increased blood flow through the capillaries, it seems likely that a suboptimal

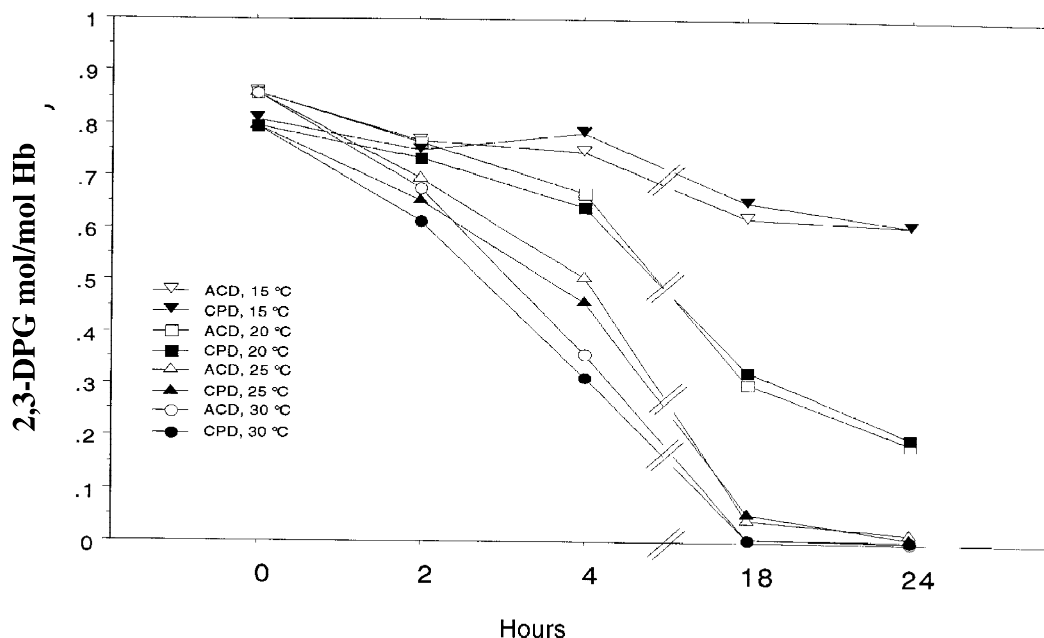

Figure 1. The concentration of RBC 2,3-DPG in whole blood collected in two anticoagulant solutions (ACD 7% and CPD 12%, respectively) and stored at temperatures between 20 °C and 30 °C for 24 hours (data from [10]).

capacity to release oxygen may be of vital importance in patients with high oxygen demand and blood flow restrictions. This indicates that the quality of RBCs, with respect to oxygen delivery, should attract more interest than has been the case in recent decades.

In the early days of transfusion medicine, the collected whole blood was rapidly cooled to 4 °C, the reason being partly metabolic, partly microbiologic. When separation into components became common practice, some time of hold at ambient temperature became routine in order to make it possible to harvest well-functioning platelets. However, little attention has been paid to the fact that the temperature at which the blood unit is kept has a strong influence on the quality of the blood in two respects; one is that, due to the association between temperature and pH, there is a rapid decrease in RBC 2,3-DPG at 25 °C and above, the other that lactate formation is increased at elevated temperatures, thus speeding up the rate of acidification of the blood unit[10]. Whole blood stored at 25 °C loses half of its 2,3-DPG within 4 h and all within 24 h (**Fig. 1**). In addition, the commonly used additive solutions, such as SAGM, AS-1 , and AS-3 solutions, do not promote the maintenance of 2,3-DPG. In practice, therefore, many RBC units produced in such systems are likely to have lost most of their 2,3-DPG within the first week of storage (**Fig. 2**).

It is advisable to cool all blood units to 20 °C directly after collection, which can be achieved with butane 1,4-diol as a heat absorber[11]. The new storage system mentioned above, 0.5CPD/ Erythro-Sol, is a clearcut improvement with respect to the maintenance of 2,3-DPG, as is illustrated in Figure 2.

From the user's point of view it may be concluded that patients with impaired circulation to vital organs (such as the brain and heart) and in need of transfusion should have short-stored units of red cells (≈5 days) under presently available storage conditions. Improved storage conditions should be given priority.

Immunomodulating effects of RBC transfusion

In a recent randomized study of patients with cardiac diseases who were less acutely ill and below 55 years of age, Hebert et al. observed a lower mortality in the group of patients exposed to a restrictive transfusion policy (transfusion Hb trigger 7 g/dL, maintenance of Hb 7-9 g/dL), than

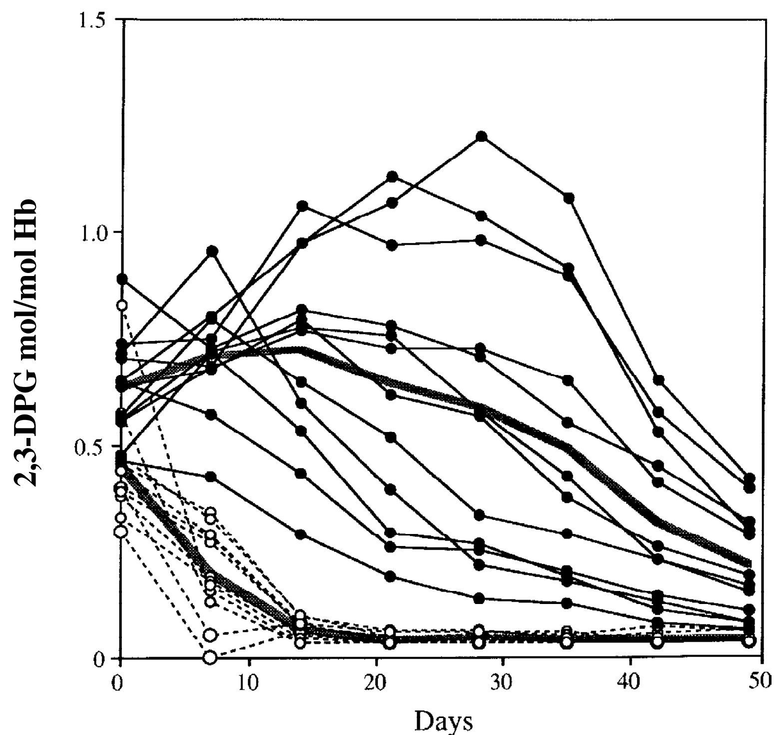

Figure 2. The concentration of human RBC 2,3-DPG in SAGM-suspended cells (dotted lines) and Erythro-Sol-suspended cells (straight lines). Thick lines represent means. The normal concentration is 0.95 mol/mol Hb. The whole blood was collected in CPD and 0.5CPD, respectively, and stored at ambient temperature for 8 hours without cooling before separation into components. Note that the 2,3- DPG concentrations at the beginning of storage were reduced to 47% (CPD) and 66% (0.5CPD) of normal concentration, respectively. In about half of the units stored in Erythro-Sol the concentration raised to normal levels or was maintained at initial level for 4-5 weeks. In SAGM-stored RBCs very low levels were found already at the end of the first storage week; after two weeks almost no 2,3-DPG remained in any of the blood units. Storage of the RBCs was performed at 4 °C (data from [5]).

in patients receiving RBC transfusion more liberally (transfusion Hb trigger 10 g/dL, maintenance of Hb 10-12 g/dL)[12].

It is now well established that transfusion of whole blood and RBC preparations can affect the immune system of the recipient in different ways. As indicated in the quoted study of cardiac patients, the disadvantages of transfusion may even outweigh the advantages. To a considerable degree these effects are associated with the presence of leukocytes in the RBC preparation.

The risk of alloimmunization is greater if the antigen-presenting cells and other leukocytes participating in the immunization process are viable and capable of producing cytokines[13]. This implies that the risk of primary HLA immunization from transfusion of RBCs contaminated with leukocytes is strongly diminished after 10-14 days of storage. Removal of buffy coat is particularly effective in diminishing the number of mononuclear cells (≈ 1% of cells remaining) and less effective in removing granulocytes (20-40% of cells remaining)[14]. The conclusion may be drawn that buffy-coat removal from RBC units in combination with 2-week storage most likely reduces the risk for the recipient of getting HLA alloimmunization.

The immunodepressive effect of transfusion is at least partly associated with contaminating leukocytes. Some of the discrepancies in the literature can be explained by the fact that buffy-coat removal is a regular routine in several European countries whereas this is not the case on the American continent. In a recent randomized study of cardiac surgery patients, it was shown that RBC, depleted of leukocytes by filtration, had a lower rate of postoperative infections and lower mortality than patients transfused with red cells leukoreduced by buffy-coat removal alone. The difference was particularly striking in patients receiving 10 units of RBCs or more, thus implying a quantitative effect [15]. However, in an earlier study from the same group it was shown that patients receiving transfusion, both autologous and allogeneic, had a higher incidence of postoperative infections[16]. The authors suggested that non-viable RBC, by occupying a considerable part of the phagocytosis machinery, may have an undesired competitive effect leading to an increase of infections.

For these and other reasons, in some countries the step has been taken to leuko-filter all units of RBC, reducing the leukocyte count to $<10^6$ per unit of RBC. This is obviously an important step toward improved quality of RBC preparations, but it comes at a high increase in cost. The benefit to the majority of recipients still remains controversial.

Concluding remarks

For a long time, the ability of RBCs to survive in the circulation of a transfused recipient has been the most important quality requirement. A loss of viability in about 25% of the RBCs is accepted as suitable for transfusion. However, the phagocytosis of approximately 10^{12} RBCs, which will occur after a 4-unit transfusion of RBCs at the end of their shelf life, puts a considerable burden on the reticuloendothelial system, which may compete with defense mechanisms against infections in the body[16]. In addition to the proven effects of contaminating white cells, this may explain the negative effects of transfusion that have been documented in randomized clinical trials[12,15] and may contribute to a fatal outcome. The best possible storage conditions and removal of at least the greater part of the leukocytes from the RBC units should thus be on the priority list of the blood banks.

The loss of RBC 2,3-DPG is unavoidable in all currently used systems for RBC preservation in the liquid state[1,14]. The fact that this change is reversible and probably of minor significance in patients who can compensate themselves for an immediate loss of oxygen releasing capacity seems to have overshadowed the likelihood that it may be detrimental in severely ill patients with a high oxygen demand and poor compensating capacity[8,9]. Cooling to 20 °C of the whole blood directly after collection and use of improved systems for RBC storage are relatively simple means of improving the quality in these respects.

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