Collection and storage of leukocyte depleted whole blood in autologous blood predeposit in elective surgery programs

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Abstract
Introduction: The aim of this investigation was to provide evidence that leukocyte depleted whole blood meets the requirements for transfusion of the European Council and thus may be an alternative to leukocyte and plasma depleted packed red blood cells in autologous blood predeposit for patients undergoing elective surgery programs.

Material and methods: Standard units of 450 mL blood were collected from 25 healthy male volunteers. Leukocyte depletion was done via inline filtration 4 h after collection. Storage lesion was assessed by measuring the release of K⁺, LDH, free hemoglobin, and lactate into the storage medium, as well as by the increase of hemolysis, the decrease of pH and consumption of glucose over a storage period of 35 days. As surrogate marker for red cell quality the intracellular concentrations of adenine nucleotides [ATP, ADP, AMP] were determined.

Results: The extent of storage lesion remained within the ranges of standard liquid storage conditions. Hemolysis was far below the threshold of 0.8% in all WB units at the end of their shelf life. Only minor changes of intracellular adenine nucleotide levels were measured indicating a preserved function of red blood cells in leukocyte depleted whole blood. At the end of shelf life 70% ± 18% of initial ATP levels were detected.
Conclusion: Based on our data we propose that leukocyte depleted whole blood, stored for 35 days can be an option in the autologous blood supply as it meets the requirements for transfusion of the European Council.

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Introduction

Preoperative autologous blood donation is a well established procedure in elective surgery programs.1,2 The production and storage of autologous blood components are recommended by the European Guidelines to follow the same rules as the production and storage of homologous blood components.3 Thereby collected whole blood is plasma and leukocyte depleted in order to minimise the occurrence of adverse reactions in the transfused patient, i.e. allergic reactions, febrile non-haemolytic transfusion reactions (FNHTR) and transfusion related lung injury, due to alloproteins, cytokines and antibodies against granulocytes or HLA.4–6 While FNHTR due to cytokines can occur in both, in the homologous and autologous setting adverse reactions caused by the plasma fraction are mainly a problem of the homologous setting. Production of Leukocyte depleted whole blood (LDWB) is a time and cost sparing procedure. After leukocyte depletion LD WB is stored without further manipulation.

Here we investigated whether LD WB units stored for 35 days meet the requirements for transfusion of the European Guidelines and can be an option in the autologous setting.7 The quality of stored red blood cells (RBC) was assessed by measuring the release of K⁺, LDH, and free hemoglobin (fHb) into the supernatant over time as well as by the increase of hemolysis and the decrease of glucose and pH till the end of shelf life. We paid special attention to the intracellular adenine nucleotide content considering the latter as surrogate marker for red cell quality.8

Donors and methods

Donors

Twenty five healthy male donors (median age 40 years, range 22–64 years) recruited from our donor pool underwent whole blood donation. All donors met the European criteria for eligibility for whole blood donors and written informed consent to participate in the study was obtained. Standard units of whole blood (450 mL) were collected from each donor into a double blood bag system [MacoPharma, Toucoing, France] with an integrated filter (Leucoflex, MacoPharma, Toucoing, France) between collection bag and final storage bag representing a closed system.

Collection and filtration procedure

The collection bag, containing 70 mL CPDA-1 anticoagulant/preservative solution, and the final storage bag were labelled before the beginning of collection. WB and anticoagulant were mixed thoroughly immediately after beginning and throughout the entire collection procedure using an automated blood mixer (Optimix, Baxter). All units were kept at room temperature for at least 4 h to ensure bacterial phagocytosis and were leukocyte depleted via inline filtration thereafter. The storage bag was disconnected from the filter using a sterile disconnection device (Composeal, NPBI International BV Fresenius HemoCare, Netherlands). WB units were stored at 4 °C for 35 days (blood refrigerator, Dometic, Medical Systems, U.S.A.). Blood bags, solutions and filter systems are CE marked (CE0123) and approved by the Paul Ehrlich Institute (German) for the use of blood products.

Laboratory analyses

Samples were drawn under aseptic conditions for the determination of red blood cell counts and parameters indicating storage lesion immediately after leukocyte depletion on day 0 and subsequently at weekly intervals until the end of the shelf life on day 35. K⁺, LDH, and free hemoglobin (fHb) contents in the supernatant were determined using a Hitachi 917 spectrophotometer (Boehringer Mannheim, Mannheim, Germany). Linear detection limits ranged between 0.5 mg/dl and 500 mg/dl for fHb and between 6 and 1200 U/L for LDH. Glucose was analyzed enzymatically by the hexokinase assay (Olympus System Reagent/Olympus Diagnostica, Ireland). Linear detection limits ranged between 10 and 800 mg/dl for serum and plasma. Lactate was determined using the lactate assay on a Roche/Hitachi 917. All analyses were done according to the manufacturer’s protocol.

Intracellular nucleotides (ATP, ADP and AMP) were determined by high performance liquid
Whole blood for autologous blood predeposit

Characteristics of whole blood units

The mean Hct and the mean hemoglobin levels were 38.3% (±3.6%) and 12.9 g/dL (±1.1 g/dL), respectively. Leukocyte contamination (mean 0.32 × 10^6 absolute, ±0.3) was less than 1 × 10^6 leukocytes in all units.

Results

Statistics

Results are expressed as means ± standard deviation for descriptive purposes in the text. Because of the non-normal distribution of data, all comparisons were made by non-parametric statistics. All statistical analyses were performed by using Friedman analysis of variance (ANOVA) test. A two tailed p value < 0.05 was considered significant. For comparisons between LD WB and the historical control group the Mann–Whitney-U test was applied.

Table 1 Metabolic parameters, decrease of pH and increase of hemolysis in CPDA-1 preserved whole blood

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺ (mmol/L)</td>
<td>3.8 (±0.8)</td>
<td>14.3 (±4.0)*</td>
<td>17.2 (±2.5)*</td>
<td>20 (±2.5)*</td>
<td>22.9 (±3.1)*</td>
<td>24.9 (±3.2)*</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>168 (±61)</td>
<td>172 (±60)</td>
<td>176 (±61)</td>
<td>167 (±48)</td>
<td>178 (±66)</td>
<td>185 (±65)</td>
</tr>
<tr>
<td>fHb (mg/dL)</td>
<td>16.6 (±19.2)</td>
<td>28.9 (±25.6)</td>
<td>29.3 (±26.8)</td>
<td>34.4 (±33.0)</td>
<td>35.4 (±33.2)</td>
<td>37.8 (±29.1)</td>
</tr>
<tr>
<td>Glucose (mg%)</td>
<td>482 (±24)</td>
<td>406 (±39)</td>
<td>370 (±33)</td>
<td>344 (±30)</td>
<td>314 (±32)</td>
<td>291 (±27)</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>3.18 (±0.8)</td>
<td>10.4 (±2.8)</td>
<td>13.9 (±2.1)</td>
<td>17.7 (±2.5)</td>
<td>20.8 (±2.4)</td>
<td>22.6 (±2.6)</td>
</tr>
<tr>
<td>pH</td>
<td>7.0 (±0.08)</td>
<td>6.84 (±0.08)</td>
<td>6.71 (±0.05)*</td>
<td>6.64 (±0.07)*</td>
<td>6.58 (±0.04)*</td>
<td>6.56 (±0.06)*</td>
</tr>
<tr>
<td>Hemolysis (%)</td>
<td>0.08 (±0.09)</td>
<td>0.11 (±0.1)</td>
<td>0.14 (±0.1)</td>
<td>0.16 (±0.175)</td>
<td>0.18 (±0.17)</td>
<td>0.20 (±0.15)</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard deviation. * Indicate statistical significant changes during the storage period with a p value < 0.05.
of stored blood is the increase of hemolysis (reflected by the increase of K⁺, LDH and fHb) and is limited to 0.8% at the end of shelf life. In LD WB units a RBC hemolysis of 0.2% was measured on day 35 which is clearly below the threshold but slightly higher than that in PRBCs (Table 2). In the process to obtain PRBCs, plasma containing free hemoglobin, is depleted after centrifugation and replaced by additive solution. So artificial lower initial values of fHb may account for this observation. During storage erythrocytes have to carry out certain energy requiring processes in order to maintain membrane stability. This energy is provided mainly from glycolysis and its sufficiency can be measured by the concentration of ATP in erythrocytes and the consumption of glucose. We found a significant initial increase of the intracellular adenine nucleotides ATP, ADP and AMP indicating a net synthesis of these nucleotides due to the presence of adenine in the storage medium and a high rate of glycolysis during the first two weeks. During further storage the ratio of production vs. consumption changed towards consumption and ATP levels decreased to 70% ± 18 of initial levels until the end of shelf life which indicates less degradation compared to conventionally produced PRBCs where 30% ± 4.9 of initial values were detected (Table 2). This can be explained by omitting the centrifugation step in order to split WB in its components. Shear stress during production may lead to membrane damage inducing enhanced energy requiring membrane repair processes. In apheresis derived PRBCs, which are also exposed to lower initial membrane damage higher ATP levels were found too at the end of shelf life.

A satisfying glucose reserve remained at this time. Sixty percent of glucose was utilized and lactate was increased to 8 fold of initial values which is also measured in PRBCs stored in additive solutions. Simultaneously ADP and AMP levels remained elevated. Enhanced loss of ADP was found in PRBCs stored after irradiation, which is known to induce membrane damage indicating a high phosphorylation rate. Taken together, elevated ADP and AMP values, well maintained intracellular ATP levels as well as acceptable hemolysis measured in stored LD WB at the end of shelf life may account for less initial membrane damage by omitting the centrifugation to split LD WB into its components. As described previously, the 24 h recovery of transfused PRBCs correlates strongly with red cell quality and is significantly affected when ATP concentrations fall below 10% of initial values.

In conclusion we observed that red blood cells stored as LD WB fulfil the requirements for transfusion warranted by the European Council and the in vitro assessed adequate quality of erythrocytes allow the assumption that the 24 h post-transfusion recovery will not be impaired by the storage of red blood cells as LD WB. Thus storage and transfusion of LD WB may be an option in the autologous blood predeposit especially in small hospitals without the technical and personnel equipment of a blood transfusion centre.

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**References**


