Transfusion Medicine and Hemotherapy

Original Article

Transfus Med Hemother 2015;42:46–51
DOI: 10.1159/000370140

Received: July 23, 2013
Accepted: February 10, 2014
Published online: December 22, 2014

Biochemical and Cellular Changes in Leukocyte-Depleted Red Blood Cells Stored for Transfusion

Diana Nogueira[a] Susana Rocha[b, c] Estela Abreu[d] Elísio Costa[b, c] Alice Santos-Silva[b, c]

[a] Serviço de Patologia Clínica, Laboratório de Hematologia, Centro Hospitalar S. João, Porto, Portugal;
[b] Departamento de Ciências Biológicas, Laboratório de Bioquímica, Faculdade Farmácia, Universidade do Porto, Porto, Portugal;
[c] UCIBIO @ REQUIMTEUniversidade do Porto, Porto, Portugal;
[d] Serviço de Imunohemoterapia, Centro Hospitalar S. João, Porto, Portugal

Keywords
RBC · Membrane proteins · Transfusion · Leukocyte depletion · Red cell aging

Summary
Background: To evaluate biochemical and cellular changes associated with the storage of leukocyte-depleted red blood cells (RBCs). **Methods:** We investigated 10 leukocyte-depleted RBC units, randomly chosen from volunteer donors. Every week an aliquot was collected for laboratorial evaluation, which included complete cell blood count, glucose-6-phosphate dehydrogenase (G6PD) activity, extracellular sodium, potassium and pH, membrane-bound hemoglobin (MBH), band 3 profile, and quantification of RBC membrane proteins composition. **Results:** We observed an increase in mean cell volume (from 91.86 ± 4.65 fl to 98.10 ± 5.80 fl, day 0 vs. day 21; $p < 0.05$), red cell distribution width, percentage of macrocytic RBCs, reticulocyte hemoglobin content and a decreased percentage of microcytic RBCs, mean cell volume concentration and G6PD activity. The extracellular concentration of sodium decreased, and that of potassium increased significantly over time. RBC membrane composition revealed an increase in spectrin/ankyrin ratio after 21 days (from 4.84 ± 0.99 to 5.27 ± 0.94, day 0 vs. day 21; $p < 0.05$). At day 35, a decrease in ankyrin (from 6.44 ± 1.70% to 5.49 ± 1.96%, day 0 vs. day 35; $p < 0.05$), in protein 4.1/band 3, protein 4.2/band 3, and ankyrin/band 3 ratios and in band 5 was observed. **Conclusions:** Our data show that leukocyte-depleted RBCs present changes in the RBC morphology, membrane protein composition, enzymatic activity, and extracellular electrolyte concentration and pH.

Introduction

Red blood cell (RBC) concentrates for transfusion are widely used for the treatment of patients with anemia of different etiologies. For this purpose it is important that the transfused RBCs preserve their metabolic capacity and mechanical functions. The mechanisms responsible for reduced in vivo viability after transfusion of stored RBCs have not definitively been determined; however, it is well documented that 24-hour RBC survival after transfusion decreases as storage time increases [1]. Moreover, many observational studies suggest that prolonged RBC storage increases mortality, the prevalence of serious infections, and multiorgan failure after transfusion in hospitalized patients [1, 2].

To reduce the risks associated with RBC transfusion, new anticoagulants, additive solutions, RBC membrane stabilizers, preservatives, and bags have been developed [3, 4]. Despite these improvements, a number of alterations in RBC concentrates have been described and called 'red cell storage lesions'. These include changes in RBC shape and metabolism, in loss of carbohydrates, lipids and proteins, in secretion and cell adhesion as well as in the oxygen transport [5, 6].

In most European countries, the administration of leukocyte-depleted RBCs for transfusion is now common practice, because of improved RBC functionality and persistence of RBCs [7]. However, little is known about the changes that occur during storage of leukocyte-depleted RBCs. As these changes could modify the functionality and persistence of RBCs, we investigated RBC membrane (protein composition, band 3 profile, membrane-bound hemoglobin, intracellular (hemoglobin concentration, glucose-6-phosphate dehydrogenase (G6PD) activity) as well as extracellular changes, e.g. those of electrolyte concentrations and pH.

KARGER

Fax +49 761 4 52 07 14
Information@Karger.com
www.karger.com

1660–3796/15/0421–0046$39.50/0

Accessible online at:
www.karger.com/tmh

Alice Santos-Silva
Serviço de Bioquímica – Departamento de Ciências Biológicas
Faculdade de Farmácia da Universidade do Porto
Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
assilva@ff.up.pt

Material and Methods

Sample Collection

We studied 10 leukocyte-depleted RBC concentrates, randomly chosen from volunteer donors of the Serviço de Imunohemoterapia, São João Hospital, Porto, Portugal. Whole blood (450 ml ± 10%) was collected into polyvinylchloride bags containing citrate-phosphate-dextrose anticoagulant (63 ml). After centrifugation, plasma was removed, and the RBCs were leukocyte-depleted (Leucored, Grifols S.A., Barcelona, Spain) and suspended in 100 ml of SAG-M preservative solution. The RBC concentrates were stored under standard blood bank conditions (2–6 °C). For laboratory evaluation an aliquot was aseptically removed every week, from day 0 to day 42 of storage.

Hematological and Biochemical Studies

We used an automated blood cell counter (Sysmex XE-5000; Sysmex Europe, Norderstedt, Germany) for determination of RBC and reticulocyte counts, RBC indices, reticulocyte hemoglobin content as well as the percentage of macro/microcytic and hypo/hypercromia RBCs. Plasma levels of Na^+ and K^+ and extracellular pH were analyzed by a direct potentiometric method (Spotlyte; A. Menarini Diagnostics, Madrid, Spain). The G6PD activity was measured by spectrophotometry, using a two-point kinetic reaction (Trinity Biotech Glucose 6-phosphate dehydrogenase; BioPortugal, Lisbon, Portugal).

Preparation of RBC Membrane Suspension for Electrophoretic Analysis

To assure the complete removal of leukocytes from the RBC aliquot (3 ml), we performed a centrifugation on a density gradient (Histopaque 1.119; Sigma-Aldrich, St Louis, MO, USA). After washing the isolated RBCs were exposed to hypotonic lysis, according to Dodge et al. [8]. The obtained membrane suspensions were washed with a Dodge buffer (phosphate buffer solution pH 8.0), adding phenylmethylsulphonyl fluoride, a protease inhibitor (final concentration 0.1 mmol/l), in the first two washes. The protein concentration of RBC membrane suspensions was determined by the Bradford method [9]. Briefly, 200 µl of Bradford reagent are added to 40 µl of RBC membrane suspension in 96 well plates, the plates are incubated for 5 min, and the absorbance was measured at 595 nm. A standard curve was developed using different concentrations of bovine serum albumin. The membrane suspensions were treated with an equal volume of a solubilization buffer (0,125 mol/l Tris-HCl pH 6.8, 4% sodium dodecyl sulphate (SDS), 20% glycerol, 10% 2-mercaptoethanol) and heat denatured.

Membrane-Bound Hemoglobin Measurement

Membrane-bound hemoglobin (MBH) was measured by spectrophotometry after protein dissociation with Triton X-100 (5%) in phosphate buffer, pH 8. The absorbance was measured at 415 nm, and this value was corrected by the background absorbance at 700 nm. These values and the protein concentration were used to estimate MBH percentage.

Identification and Quantification of RBC Membrane Proteins

The electrophoretic analysis of RBC membrane proteins was carried out on a discontinuous system of polyacrylamide gels in the presence of SDS (SDS-PAGE) using a 5–15% linear acrylamide gradient gel (8 µg protein/lane), according to Laemmli [10], and a 3.5–17% exponential acrylamide gradient gel (6 µg protein/lane), according to Fairbanks [11]. The proteins were stained with Coomassie brilliant blue and scanned (Darkroom CN UV/wl, BioCaptMW version 99; Vilber Lourmat, Eberhardzell, Germany). The relative amount of each major protein was quantified by densitometry (Bio1D++ version 99; Vilber Lourmat). This technique allowed the identification and quantification of the major protein constituents of the RBC membrane (α-spectrin, β-spectrin, ankyrin, band 3, protein 4.1, protein 4.2, actin, G3PD and stomatin/tropomyosin).

Immunobloting Analysis for Band 3

RBC membrane protein suspensions, previously prepared for the electrophoretic studies were submitted to SDS-PAGE in a 9% acrylamide gel (20 µg protein/lane), using the discontinuous Laemmli [10] system. Proteins were electrophoretically transferred from SDS-PAGE gels to a nitrocellulose membrane sheet with a porosity of 0,2 µm. Additional reactive sites on the nitrocellulose were blocked by incubation in low-fat dry milk (5% w/v) and 0.1% Triton-X 100 in PBS pH 7.0, overnight, under gentle rotation, at 4 °C. Band 3 immunoblotting was carried out by adding a primary murine anti-human band 3 monoclonal antibody (1:3,000, Sigma-Aldrich) and incubating for 4 h. Washing of the nitrocellulose was followed by incubation with a rabbit anti-mouse IgG linked to peroxidase (1:4,000) (Sigma-Aldrich). The incubations were carried out at room temperature; the dilutions of the antibodies were prepared with PBS pH 7.0, containing 0.1% Triton-X 100 and 0.5% low fat dry milk. The washes used the same buffer, without low-fat dry milk. The development of the immunoblot was performed by adding hydrogen peroxide and α-chloronaphtol (Sigma Aldrich). The immunoblots were scanned (Darkroom CN UV/wl, BioCaptMW version 99) and the relative amounts of Band 3 monomer (Bd3M), high molecular aggregates (HMWAg) and proteolytic fragments (Frag) were quantified by densitometry (Bio1Dqq version 99, Vilber Lourmat).

Statistical Analysis

For statistical analysis we used the Statistical Package for Social Sciences (SPSS) 20.0 for Windows (SPSS, Inc., Chicago, IL, USA). The results are presented as mean ± SD. The Kolmogorov-Smirnov test was used to evaluate normality. In cases of normal distribution, we used the paired-sample Student's t-test; in cases of abnormal distribution we used the Wilcoxon test. A $p < 0.05$ value was considered as statistically significant.

Results

The numbers of RBCs, the extracellular concentration of electrolytes, and the pH are shown in tables 1 and 2.

The RBC counts as well as the hemoglobin concentration kept unchanged throughout the 42 days of conservation (table 1). However, the mean hematocrit increased from day 0 (54.30 ± 2.43%) to day 14 (57.68 ± 2.06%) and remained stable afterwards. The mean cell volume (MCV) increased gradually, and significantly, from day 0 (91.86 ± 4.31 fl) to day 21 (98.10 ± 5.80 fl) and remained stable to the end of the storage period. In contrast, the mean cellular hemoglobin concentration (MCHC) decreased gradually from day 0 (34.03 ± 0.99 g/dl) to day 21 (31.93 ± 1.06 g/dl). This was associated with an increase in the proportion of macrocytic RBCs; the proportion of microcytic RBC decreased progressively after day 14; RBC distribution width (RDW) increased at day 7 and remained stable to the end of the storage period. Reticulocyte counts increased at day 21 and remained constant over time, while the reticulocyte hemoglobin content increased progressively after day 35. The activity of the RBC enzyme G6PD presented a significant reduction at day 28 and continued to decrease till the end of the storage period. The extracellular concentration of sodium decreased at day 7, showing a gradual and reduction throughout the remaining time of conservation. On the other hand, the extracellular potassium concentration increased significantly at day 7 and continued to increase throughout the 5 following weeks. Concerning to extracellular pH, we observed a reduction throughout the conservation period.

Concerning to RBC membrane protein composition, an increase was observed for band 7 at day 7, then this value remained constant till the end of the storage period. At day 14 several significant changes occurred: in particular, an increase in band 3, protein

Table 1. RBC studies, extracellular pH, electrolytes and G6PD activity, throughout storage of RBC units under standard blood bank conditions*

	D0	D7	D14	D21	D28	D35	D42
RBC studies							
Hb, g/dl	18.47 ± 079	18.52 ± 0.78	18.64 ± 0.71	18.4 ± 0.66	18.43 ± 0.69	18.47 ± 0.69	18.4 ± 0.66
RBC × 10^{12}/l	5.92 ± 0.21	5.94 ± 0.32	5.88 ± 0.43	5.91 ± 0.27	5.90 ± 0.27	5.89 ± 0.26	5.86 ± .032
Htc, %	54.3 ± 2.43	55.2 ± 2.06	57.68 ± 2.63[a.b]	58.02 ± 2.37[a.b]	58.15 ± 2.99[a.b]	58.17 ± 2.87[a.b]	58.2 ± 3.70[a.b]
MCH, pg	31.25 ± 1.78	31.2 ± 1.88	31.27 ± 1.65	31.29 ± 1.59	31.30 ± 1.76	31.41 ± 1.73[b]	31.42 ± 1.98
MCV, fl	91.86 ± 4.65	93.06 ± 4.31[a]	96.77 ± 4.90[a.b]	98.1 ± 5.80[a.b.c]	99.30 ± 6.10[a]	98.93 ± 6.2[a.b.c]	99.4 ± 6.30[a.b.c.d]
MCHC , g/dl	34.03 ± 0.99	33.54 ± 090[a]	32.31 ± 0.86[a.b]	31.93 ± 1.06[a.b.c]	31.77 ± 1.13[a.b.c]	31.78 ± 1.3[a.b.c]	31.67 ± 1.40[a.b.c]
RDW, %	13.26 ± 0.89	13.78 ± 0.95[a]	14.15 ± 1.25[a)]	14.36 ± 1.63[a]	14.47 ± 1.85[a]	14.55 ± 1.93[a]	14.67 ± 1.96[a]
Reticulocytes × 10^{9}/l	63.18 ± 12.10	68.04 ± 13.18	70.03 ± 12.14	72.34 ± 12.18[a]	70.40 ± 9.02[a]	70.87 ± 12.07[a]	69.64 ± 8.76[a]
Reticulocytes, %	1.07 ± 0.22	1.15 ± 0.23[a]	1.20 ± 0.24[a.b]	1.23 ± 0.23[a]	1.20 ± 0.17[a]	1.21 ± 0.23[a]	1.19 ± 0.19[a]
Reticulocyte Hb, pg	34.68 ± 2.41	35.47 ± 1.84	34.99 ± 2.13	35.0 ± 1.92	34.96 ± 1.52	36.1 ± 1.94[a.c.d.e]	37.31 ± 1.76[a.b.c.d.e.f]
Microcytic RBCs, %	1.13 ± 1.03	1.12 ± 0.98	0.82 ± 0.755[b]	0.76 ± 0.66[a.b]	0.71 ± 0.68[a.b.c]	0.7 ± 0.66[a.b.c]	0.66 ± 0.67[a.b.c.d.e]
Macrocytic RBCs, %	10.8 ± 3.67	12.1 ± 3.42[a]	16.55 ± 5.99[a.b]	18.53 ± 7.90[a.b.c]	19.58 ± 9.16[a.b.c]	20.03 ± 9.33[a.b.c.d]	21.18 ± 9.55[a.b.c.d.e.f]
Hypochromic RBCs, %	0.10 ± 0.03	0.10 ± 0.04	0.15 ± 0.03	0.25 ± 0.14	0.25 ± 0.13	0.10 ± 0.05	0.30 ± 0.44
Hyperchromic RBCs, %	1.1 ± 0.10	1.0 ± 0.09	0.80 ± 0.13	0.90 ± 0.11	0.90 ± 0.10	1.10 ± 0.13	0.90 ± 0.11
Extracellular pH and electrolytes							
Sodium, mmol/l	143.08 ± 1.43	131.15 ± 2.75[a]	124.92 ± 3.01[a.b]	119.8 ± 2.91[a.b.c]	114.36 ± 2.71[a.b.c.d]	10.34 ± 3.52[a.b.c.d.e]	107.1 ± 3.45[a.b.c.d.e.f]
Potassium, mmol/l	1.31 ± 0.46	14.1 ± 1.44[a]	19.36 ± 1.31[a.b]	23.09 ± 1.08[a.b.c]	25.84 ± 1.26[a.b.c.d]	28.09 ± 1.34[a.b.c.d.e]	29.84 ± 1.25[a.b.c.d.e.f]
pH	7.33 ± 0.06	7.15 ± 0.05[a]	7.012 ± 0.07[a.b]	6.874 ± 0.03[a.b.c]	6.723 ± 0.08[a.b.c.d]	6.592 ± 0.17[a.b.c.d.e]	6.528 ± 0.14[a.b.c.d.e]
Enzymatic activity							
G6PD, UI/g Hb	6.33 ± 0.58	6.79 ± 0.85	6.51 ± 0.40	6.51 ± 0.54	6.29 ± 0.59[b.d]	6.12 ± 0.58[b.c.d.e]	5.483 ± 0.58[a.b.c.d.e.f]

Hb = Hemoglobin; RBC =erythrocytes; Ht = hematocrit; MCH = mean cell hemoglobin; MCV = mean cell volume ; MCHC = mean cell hemoglobin concentration; RDW = red cell distribution width; G6PD = glucose-6-phosphate-dehydrogenase.

*Data presented as mean and standard deviation.

[a]$p < 0.05$, vs. D0.

[b]$p < 0.05$, vs. D7.

[c]$p < 0.05$, vs. D14.

[d]$p < 0.05$, vs. D21.

[e]$p < 0.05$, vs. D28.

[f]$p < 0.05$, vs. D35.

Table 2. RBC membrane protein composition, band 3 profile of and membrane-bound hemoglobin throughout the storage of RBC units under standard blood bank conditions

	D0	D7	D14	D21	D28	D35	D42
Band 3 profile							
HMWAg, %	12.26 ± 7.16	12.36 ± 7.84	10.98 ± 7.22	11.70 ± 7.11	11.09 ± 8.18	12.19 ± 8.14	11.88 ± 7.97
Band 3 monomer, %	75.43 ± 7.43	75.14 ± 8.88	75.93 ± 8.86	75.22 ± 6.85	75.02 ± 7.99	74.08 ± 8.48	76.10 ± 10.43
Pfrag, %	12.98 ± 9.45	13.11 ± 7.57	13.73 ± 5.95	13.98 ± 6.51	14.54 ± 7.63	14.46 ± 6.65	12.48 ± 8.82
HMWAg/Bd3	0.16 ± 0.10	0.17 ± 0.12	0.15 ± 0.11	0.16 ± 0.11	0.16 ± 0.13	0.17 ± 0.12	0.17 ± 0.12
Pfrag/Bd3	0.17 ± 0.16	0.17 ± 0.13	0.18 ± 0.12	0.18 ± 0.11	0.19 ± 0.13	0.19 ± 0.11	0.17 ± 0.15
RBC membrane protein composition							
Spectrin, %	29.96 ± 4.60	28.36 ± 5.89	26.72 ± 4.96 [a]	28.93 ± 4.26	28.62 ± 6.37	29.26 ± 6.29	28.79 ± 5.83
Ankyrin, %	6.44 ± 1.70	6.20 ± 1.57	5.91 ± 1.21	5.64 ± 1.25	5.66 ± 1.20	5.49 ± 1.96 [a,b]	5.59 ± 1.81 [b]
Band 3, %	29.29 ± 6.55	28.72 ± 6.87	30.87 ± 7.30 [b]	29.05 ± 6.82	30.20 ± 7.57	31.83 ± 5.92 [b]	30.69 ± 6.58
Protein 4.1, %	7.97 ± 1.59	7.84 ± 1.08	7.36 ± 2.17	7.73 ± 1.38	8.17 ± 1.39	7.30 ± 1.07	7.82 ± 1.06
Protein 4.2, %	6.01 ± 1.22	6.27 ± 1.59	6.86 ± 1.34 [a]	6.94 ± 2.53	6.27 ± 1.10	6.02 ± 1.05	6.37 ± 0.99
Band 5, %	9.18 ± 2.25	9.77 ± 1.22	9.46 ± 1.45	9.68 ± 1.93	8.93 ± ± 1.46	9.11 ± 0.99	9.10 ± 1.21 [b]
Band 6, %	9.14 ± 2.20	10.33 ± 2.61	10.06 ± 2.66	9.45 ± 1.91	9.65 ± 2.26	8.32 ± 1.59 [b]	9.29 ± 1.86
Band 7, %	2.02 ± 0.55	2.51 ± 0.91 [a]	2.77 ± 0.92 [a,b]	2.59 ± 0.86 [a]	2.50 ± 0.74 [a]	2.66 ± 1.17 [a]	2.34 ± 1.14
Protein 4.1/spectrin	0.27 ± 0.07	0.29 ± 0.08	0.28 ± 0.10	0.27 ± 0.06	0.30 ± 0.09	0.26 ± 0.09	0.28 ± 0.08
Protein 4.1/band 3	0.29 ± 0.10	0.29 ± 0.08	0.26 ± 0.13	0.28 ± 0.10	0.29 ± 0.08	0.24 ± 0.06 [b,e]	0.27 ± 0.10
Protein 4.2/band 3	0.21 ± 0.04	0.22 ± 0.04	0.23 ± 0.08	0.26 ± 0.14	0.21 ± 0.04	0.19 ± 0.03 [c,e]	0.22 ± 0.05
Spectrin/band 3	1.09 ± 0.36	1.10 ± 0.54	0.95 ± 0.48	1.06 ± 0.37	1.06 ± 0.54	0.98 ± 0.39	1.02 ± 0.45
Ankyrin/band 3	0.24 ± 0.10	0.24 ± 0.13	0.21 ± 0.10	0.21 ± 0.09	0.21 ± 0.09	0.19 ± 0.10 [a,b]	0.20 ± 0.12 [b]
Spectrin/ankyrin	4.84 ± 0.99	4.65 ± 0.50	4.59 ± 0.73	5.27 ± 0.94 [b,c]	5.19 ± 1.18	5.77 ± 1.74 [a,b,c]	5.45 ± 1.42
Membrane bound hemoglobin							
MBH × 10^{-4}, %	39.63 ± 26.92	31.00 ± 14.29	38.78 ± 18.00	35.11 ± 26.66	43.56 ± 28.09	41.67 ± 23.53	38.88 ± 24.21

HMWAg = High molecular weight aggregates; Pfrag = proteolytic fragments MBH = membrane-bound hemoglobin.*Data presented as mean and standard deviation.
[a]$p < 0.05$, vs. D0.
[b]$p < 0.05$, vs. D7.
[c]$p < 0.05$, vs. D14.
[d]$p < 0.05$, vs. D21.
[e]$p < 0.05$, vs. D28.
[f]$p < 0.05$, vs. D35.

4.2 and band 7, and a decrease in spectrin. However, these alterations did not affect RBC membrane structure, as suggested by the unchanged ratios between membrane proteins. Only at day 35 a new unbalance in RBC membrane protein composition occurred, with an increase in band 3 and a decrease in ankyrin and band 6. These changes in membrane protein composition triggered significant changes in membrane structure, as suggested by the significantly altered protein ratios of protein 4.1/band 3, protein 4.2/band 3, ankyrin/band 3 and spectrin/ankyrin. No statistically significant differences were found in MBH and in band 3 profile (table 2) during the storage period.

Discussion

The interest in leukocyte-depleted whole blood and blood component transfusion has increased in the recent years in order to minimize the risk of leukocyte-mediated immune reactions. There are several studies about the RBC changes during the storage period of whole blood and non-leukocyte-depleted RBCs for transfusion. Currently, little is known how the leukocyte depletion interferes with RBC membrane integrity and morphology.

No significant changes were found in RBC counts during the storage period, suggesting that the RBC integrity is not significantly affected. However, we observed some changes reflecting morphological alterations, such as an increase in hematocrit, in MCV and in RDW, as well as a decrease in MCHC. These changes have already been described by Bosman et al. [5], and seem to result from a deregulated mechanism of cell volume, which explains the increase in the volume of the RBCs, the increasing hypocromia, and the anisocytosis. Hence, the decrease in MCHC does not result from a reduction in hemoglobin concentration but from an increase in cell volume [5, 12, 13]. The transport of Na^+ and K^+ through the RBC membrane is also related to cell volume. According to Hogman and Meryman [19], the smaller the extracellular volume of the RBC concentrates, the faster the RBCs achieve their equilibrium and reduce the K^+ loss. To achieve this equilibrium, the RBCs release large amounts of K^+ (22 times higher than the ini-

tial concentration) in the first 3 weeks of storage. After that, we observed a less pronounced increase in extracellular K^+, which might result from microcytic RBC lysis, as the proportion of microcytic RBC showed a continuous reduction after day 14. The decreased concentration of extracellular Na^+ can be associated with water influx to the cytosol. This process and the absence of a selective mechanism of functionality, as performed in the spleen, could explain the increase of MCV in vitro with consecutive morphological RBC changes [5, 14–17].

We also observed an increase in the concentration of hemoglobin in reticulocytes. Apparently, the reticulocyte metabolism is slower during storage, presenting a peak of activity after day 35, when the concentration of hemoglobin in reticulocytes increased significantly. The reticulocyte counts increased significantly until day 21 and remained constant afterwards. This is probably due to a slight RBC hemolysis, as suggested by the trend towards lower values of RBC counts [18].

A progressive decrease in pH was found in the RBC concentrates, which is mainly related to the accumulation of lactate via anaerobic glycolysis with ongoing storage [19]. Moreover, the reduction in G6PD activity reflects a reduction in the activity of the glycolytic pathway and in the antioxidant defenses. This decrease in the antioxidant status could lead to an increase in methemoglobin, which binds to the membrane and could trigger its destabilization. However, no significant change in MBH during the storage process was found. This is in contrast to previous results of Bosman et al. [20] who reported an increased MBH during storage.

Concerning to RBC membrane protein composition, the first change appeared at day 7, in protein band 7 (tropomyosin and stomatin), which, according to Antonelou et al. [21], may be related to increased formation of vesicles containing stomatin and other membrane proteins. It has been hypothesized that stomatin is involved in the marked ionic imbalance observed during routine storage conditions, independently of its value in the membrane or conformational changes [22]. At day 14 other significant changes in RBC membrane protein composition were documented, such as a decrease in spectrin, and an increase in protein 4.2 and in band 3. However, at this time no significant changes in protein ratios were observed, suggesting that there were no relevant changes in membrane protein interaction and, therefore, in protein membrane structure. This is in accordance with the results D'Amici et al. [6], who report changes in RBC cytoskeleton and in band 3 protein during the first 2 weeks of storage. These alterations remained stable until day 35; at this day the RBC membrane protein changes observed at day 14 were enhanced and already associated with alterations in RBC membrane protein interactions, as shown by the significant decreases in protein 4.1/band 3, protein 4.2/band 3 and ankyrin/band 3 ratios, and an increase in spectrin/ankyrin ratio. It is likely that the increasing ionic intracellular concentration as well as the increasing metabolic degradation contribute to destabilize the membrane protein network, favoring membrane vesiculation and hemolysis. Actually, the changes in these proteins alter the normal vertical and horizontal protein interactions, modifying, therefore, the morphology and integrity of the membrane [20]. It has been reported that changes in the conformation of band 3, triggered by the linkage of denatured hemoglobin to its cytoplasmic domain, mark the RBCs for removal [5, 23]. In this study, no significant changes were observed in MBH, neither in band 3 protein profile throughout the storage period. It seems that the RBC membrane proteins in leukocyte-depleted RBC concentrates suffer less oxidative changes than those reported in previous studies with non-leukocyte-depleted RBC components [6, 20]. Leukocytes are important sources of ROS and proteases. Thus our data suggest that leucocyte depletion prevents RBC protein damage and may improve RBC viability after transfusion.

In conclusion, our data show that leukocyte-depleted RBC units stored for 42 days in CPD/SAG-M present several changes, e.g., in RBC morphology, RBC membrane protein composition, enzymatic activity, extracellular electrolyte concentration and pH, which can account for reduced in vivo recovery of the transfused patient.

Disclosure Statement

None of the authors have a conflict of interest.

References

1 Hod E, Zhang N, Sokol S, Wojczyk BS, Francis RO, Ansaldi D, Francis KP, Della-Latta P, Whittier S, Sheth S, Hendrickson JE, Zimring JC, Brittenham GM, Spitalnik SL: Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron inflammation. Blood 2010;115:4284–4292.

2 Lelubre C, Piagnerelli M, Vincent L: Association between duration of storage of transfused red blood cells and morbidity and mortality in adult patients: myth or reality? Transfusion 2009;49:1384–1394.

3 Kirby S, Lecak J, Acker J: Biopreservation of red blood cells: past, present, and future. Transfus Med Rev 2005; 19:127–142.

4 Tatsuro Y, Shevkoplyas S: Anaerobic storage of red blood cells. Blood Transfus 2010;8:220–236.

5 Bosman J, Werre J, Willekens F, Novotny V: Erythrocyte ageing in vivo and in vitro: structural aspects and implications for transfusion. Transfus Med 2008;18:335–347.

6 D'Amici G, Rinalducci S, Zolla L: Proteomic analysis of RBC membrane protein degradation during blood storage. J Proteome Res 2007;6:3242–3255.

7 Oksanen K, Elonen E: Impact of leucocyte-depleted blood components on the haematological recovery and prognosis of patients with acute myeloid leukaemia. Finnish Leukemia Group. Br J Haematol 1993;84:639–647.

8 Dodge T, Mitchell C, Hanahan D: The preparation and chemical characteristics of hemoglobin free ghost of human erythrocyte. Arch Biochem Biophys 1963;100: 119–130.

9 Bradford M: A rapid and sensitive method for the quantification of micrograms quantities of protein utilizing the principle of protein dye binding. Anal Biochem 1976;72:255.

10 Laemmli K: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 15:680–689.

11 Fairbanks G, Steck T, Wallack D: Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 1971;10:2606–2616.

12 Singh S, Kumar A: Leukocyte depletion for safe blood transfusion. Biotechnol J 2009;4:1140–1151.

13 Lichtman M, Beutler E, Kipps T, Seligsohn U, Kaushansky K, Prchal J: Williams Hematology, 7th ed. New York, McGraw-Hill Professional, 1999.

14 D'Alessandro A, D'Amici G M, Vaglio S, Zolla L: Time-course investigation of SAGM-stored leukocyte-filtered red blood cell concentrates: from metabolism to proteomics. Haematologica 2012;97:107–115.
15 Kanias T, Acker J: Biopreservation of red blood cells – the struggle with hemoglobin oxidation. FEBS J 2010; 277:343–356.
16 Leitner GC, Jilma-Stohlawetz P, Stiegler G, Weigel G, Horvath M, Tanzmann A, Höcker P, Fischer MB: Quality of packed red blood cells and platelet concentrates collected by multicomponent collection using the MCS plus device. J Clin Apher 2003;18:21–25.
17 Leitner GC, Neuhauser M, Weigel G, Kurze S, Fischer MB, Höcker P: Altered intracellular purine nucleotides in gamma-irradiated red blood cell concentrates. Vox Sang 2001;81:113–118.
18 Skadberg S, Brun A, Sandberg S: Human reticulocytes isolated from peripheral blood: maturation time and hemoglobin synthesis. Lab Hematol 2003;9:198–206.
19 Hogman C, Meryman H: Storage parameters affecting red blood cell survival and function after transfusion. Transfus Med Rev 1999;13:275–296.
20 Bosman J, Lasonder E, Luten M, Roerdinkholder-Stoelwinder B, Novotný V, Bos H, Grip W: The proteome of red cell membranes and vesicles during storage in blood bank conditions. Transfusion 2008;48:827–835.
21 Antonelou M, Kriebardis A, Stamoulis K, Economou-Petersen E, Margaritis L, Papassideri I: Red blood cell aging markers during storage in citrate-phosphate-dextrose-saline-adenine-glucose-mannitol. Transfusion 2010;50:376–389.
22 Delaunay J: The hereditary stomatocytosis: genetic disorders of red cell membrane permeability to monovalent cations. Semin Hematol 2004;41:165–172.
23 Pantaleo A, Giribaldi G, Mannu F, Arese P, Turrini F: Naturally occurring anti-band 3 antibodies and red blood cell removal under physiological and pathological conditions. Autoimmun Rev 2008;7:457–462.