Storage of whole blood overnight in different blood bags preceding preparation of blood components: *in vitro* effects on red blood cells

Hans Gulliksson[1], Pieter Ferdinand van der Meer[2]

[1] *Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden.*
[2] *Sanquin Blood Bank Region North West, Amsterdam, the Netherlands.*

Background. Routines for the storage of whole blood (WB) overnight for the preparation of blood components on the following day are of increasing interest primarily for logistic reasons. The present study focuses on in vitro effects during storage for 6 weeks on red blood cells (RBC) prepared in different blood containers after being held overnight.

Study design and methods. Five different blood collection systems were used with either inline leucocyte reduction red cell filters for the preparation of RBC, buffy coat (BC) and plasma or WB filters for the preparation of RBC and plasma. A new container with an integrated WB filter removing leucocytes but not platelets was also included for the preparation of leucocyte-reduced RBC, BC and plasma units. Standard CPD solution (63 or 70 mL) and SAG-M solution (100 or 110 mL) were used for the collection of either 450 or 500 mL blood. All WB units were stored at room temperature, either overnight for 18-24 hours (test groups, n=104) or for up to 8 hours (reference groups, n=20). In addition, five test units were stored overnight under refrigeration.

Results. In test groups (overnight storage at room temperature) we found significantly lower levels of extracellular potassium, 2,3-DPG and pH (up to day 28). During storage, higher levels of ATP (Terumo, CaridianBCT until day 35, Fresenius until day 14, Fenwal throughout storage) were seen in test groups than in reference groups. When WB was stored overnight at 2-6°C before WB filtration, the levels of ATP and haemolysis were higher than in the corresponding reference.

Conclusion. Significant differences in *in vitro* parameters were observed between RBC prepared within 8 hours and 18-24 hours after blood collection. The results were consistent irrespective of the blood container used. New alkaline solutions may decrease the differences.

Key words: red blood cells, overnight, storage, *in vitro*, ATP, 2,3-DPG

Introduction

Blood components are traditionally prepared from whole blood (WB) as soon as possible after blood collection, often within a maximum storage period of 8 hours at room temperature, i.e. 20-25°C. Routines for the storage of WB overnight for the preparation of blood components within 24 hours after blood collection were developed in the Netherlands in the 1980s [1]. Specific cooling plates were introduced to reduce the temperature rapidly from 37°C to room temperature after blood collection. This methodology is now used as a routine service in the Netherlands [2] and in other countries. The storage of WB overnight is associated with several logistic advantages: (i) all WB blood units are available the following morning, allowing very efficient routine production by avoiding periods of waiting for WB to be supplied from different blood collection sites; (ii) the staff for blood component preparation are basically needed only during routine working hours and the workload can

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be evenly distributed over time; and (iii) the number of transports of WB from collection sites may be significantly reduced. In addition, the risk of bacterial proliferation may be reduced after storage beyond 8 hours after collection[3,4] and the platelet yield from buffy coat (BC) for the preparation of pooled BC-derived platelets may be improved[5-8]. On the other hand, the quality of red blood cells (RBC) may be compromised due to prolonged storage at room temperature. In addition, plasma for fractionation with reduced levels of labile factors may be associated with lower financial compensation[1,6-8].

The RBC storage lesion is most usefully viewed as "the sum of all bad things" that happen to RBC in the course of storage and that limit their survival when they are returned to the circulation[9]. The RBC storage lesion include: (i) depletion of red cell ATP and 2,3-DPG; (ii) reduction in deformability associated with loss of membrane constituents and haemoglobin; and (iii) accumulation of bioreactive substances primarily released from white blood cells in non-leucocyte-reduced RBC units[9-11].

The present study focuses on the *in vitro* effects, in particular regarding ATP and 2,3-DPG, on leucocyte-reduced RBC in SAG-M additive solution prepared in different blood containers during storage for 6 weeks after storage of WB overnight preceding preparation of blood components.

Materials and Methods

This study was performed in two different sites (Stockholm, Sweden and Amsterdam, The Netherlands). Standard methods of the specific site for the collection of blood (450 mL, Stockholm or 500 mL, Amsterdam), preparation of blood components and *in vitro* analyses were used. Blood was collected from normal voluntary blood donors and generally drawn into quadruple-bag blood container systems. Four different blood containers were used: Terumo (Stockholm), Fresenius or CaridianBCT Atreus (Amsterdam) with inline leucocyte reduction red cell filters for the preparation of RBC, BC and plasma or alternatively Fenwal containers with WB filters for the preparation of RBC and plasma (Stockholm). In addition, a new variant of the Terumo container (triple bag) with an integrated filter removing leucocytes but not platelets was used in Amsterdam for the preparation of leucocyte-reduced RBC, BC and plasma units. The study design is outlined in table 1. Standard CPD solution (63 mL, Stockholm or 70 mL, Amsterdam) and SAG-M solution (100 or 110 mL, respectively) in one of the transfer packs was used as an additive for RBC. All WB units were stored at room temperature, either overnight for 18-24 hours (test groups) or for up to 8 hours (reference groups). The only exception was a number of units in one of the WB filtering test groups that were stored at 2-6°C overnight. Specific cooling plates were used to reduce the temperature rapidly from 37°C to room temperature after blood collection: Thermasure plates (Sebra, Tucson AZ, USA) in Stockholm and butane-1,4-diol plates (Fresenius HemoCare, Emmer-Compascuum, The Netherlands) in Amsterdam. All blood containers except CaridianBCT Atreus were centrifuged using hardspin centrifugation programmes. WB was processed into blood components using either T-ACE (Terumo, Leuven, Belgium) in Stockholm and Amsterdam (exclusively for Terumo containers), or Compomat (Fresenius) in Amsterdam. Blood units were leucocyte-reduced either before centrifugation using Fenwal (Stockholm) or Terumo (Amsterdam) containers with inline WB leucocyte reduction filters or alternatively after processing and addition of SAG-M solution to RBC for Terumo (Stockholm), Fresenius and CaridianBCT (Amsterdam) containers using inline leucocyte reduction red cell filters. CaridianBCT Atreus is new automatic equipment for the preparation of RBC, BC, and plasma. The equipment was used in accordance with the manufacturer's instructions. Following processing and leucocyte reduction, all RBC units were stored for 42 days at 2-6°C.

Measurements

Leucocyte count, pH (at 37°C), glucose, lactate, extracellular concentration of potassium and haemolysis in the RBC units were measured using routine methods[7,12]. ATP concentration was measured using a luminometric technique (Orion, Berthold, Pforzheim, Germany) based on methods described previously[13] (Stockholm). Either high-performance liquid chromatography or an enzymatic test was used in Amsterdam[1,7]. 2,3-DPG concentrations were measured with a spectrophotometer (Roche kit 148 334 001) in Stockholm and in Amsterdam. Test unit groups were all compared to the reference groups, respectively. Results are expressed as means ± 1

standard deviation. Comparisons were performed using a two-sample t-test at a 95% confidence level.

Results

In this study, two different blood component preparation alternatives were used (Table I): (i) blood containers with inline leucocyte reduction red cell filters for the preparation of RBC, BC and plasma including one reference (Terumo) and three test groups (Terumo, Fresenius or CaridianBCT Atreus). The specific Terumo container used in Amsterdam was assigned to this group, since BC was prepared from those units as well; (ii) blood containers with inline WB filters for the preparation of RBC and plasma including one reference (Fenwal) and two test groups (Fenwal stored as WB overnight either at room temperature or at 2-6°C). Reference units were stored as WB at room temperature for up to 8 hours after blood collection before the preparation of blood components. Test units were stored as WB overnight for 18-24 hours after blood collection, either at room temperature (5 groups) or at 2-6°C (1 group).

Results from RBC test groups were compared with those of the RBC reference group of each of the two blood component preparation methods.

The RBC composition and *in vitro* storage results are presented in tables I and II, respectively. Significant differences were found during storage in a number of biochemical and functional red cell parameters between the different test groups and the reference groups in question but the patterns were remarkably similar in both sites regardless of the blood containers used or the technique of preparation of the RBC. Our results indicated significantly lower levels of extracellular potassium, 2,3-DPG and pH (up to day 28) in test groups (overnight storage at room temperature). On the other hand, the levels of ATP (Terumo, CaridianBCT until day 35, Fresenius until day 14, Fenwal throughout storage) were higher in test groups during storage than in the reference groups. During the first 2-3 weeks of storage, the levels of extracellular potassium and 2,3-DPG were reduced by about 20% and 50%, respectively, in test groups as compared to reference. ATP levels were approximately 20% higher.

When WB was stored overnight at 2-6°C before WB filtration, the levels of ATP throughout storage were higher than in units held for up to 8 hours before

Table I - Outline of study design and composition of different red blood cell (RBC) preparations.

RBC preparation	WB storage time prior to processing	WB storage temperature	Component preparation method	Leucocyte reduction filter type	n	Volume (mL)	Haemoglobin (g/unit)	Haematocrit (%)	WBC (x10^6/unit)
Terumo (1; reference)	<8 hours	RT	manual	RBC filtration	10	258±19	50±6	60±3	<0.3
Terumo (2)	overnight	RT	manual	RBC filtration	10	254±13	47±4	58±2	≤0.7
Terumo (3)	overnight	RT	manual	WB filter removing leukocytes but not platelets	12	271±11	46±5	53±4	N.T.
Fresenius (4)	overnight	RT	manual	RBC filtration	51	277±23	50±7	54±3	<1.1
Caridian BCT Atreus (5)	overnight	RT	automated	RBC filtration	26	294±23	54±6	54±1	≤0.3
Fenwal (6; reference)	<8 hours	RT	manual	WB filtration	10	285±8	59±3	63±1	<0.3
Fenwal (7)	overnight	RT	manual	WB filtration	5	288±18	57±5	62±2	<1.1
Fenwal (8)	overnight	2-6°C	manual	WB filtration	5	296±16	61±5	64±2	<0.3

Results are expressed as mean ± standard deviation (SD). N.T.= not tested
RBC, red blood cells; WBC, white blood cells; RT, room temperature; WB, whole blood.

Table II - *In vitro* effects of 42-day storage of different red blood cell preparations

(1) Terumo reference at room temperature, Te, 8h, RT (preparation of blood components within 8 hours).
(2) Terumo overnight (o/n) at room temperature, Te, o/n, RT.
(3) Terumo overnight at room temperature, Te, o/n, RT.
(4) Fresenius overnight at room temperature, Fr, o/n, RT.
(5) CaridianBCT Atreus overnight at room temperature, Ca, o/n, RT.
(6) Fenwal reference at room temperature, Fe, 8h, RT (preparation of blood components within 8 hours).
(7) Fenwal overnight at room temperature, Fe, o/n, RT.
(8) Fenwal overnight in the cold, 2-6℃, Fe, o/n 4C.

In vitro parameter	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Haemolysis (%)							
Te, 8h, RT	0.09±0.04	0.11±0.06	0.14±0.05	0.23±0.12	0.23±0.08	0.29±0.11	0.39±0.13
Te, o/n, RT	0.08±0.03	N.T.	0.20±0.11	0.34±0.15	0.30±0.14	0.36±0.16	0.44±0.16
Te, o/n, RT	0.13±0.20	0.12±0.21	0.14±0.22	0.20±0.22	0.23±0.26	0.31±0.29	0.39±0.30
Fr, o/n, RT	0.05±0.04	0.04±0.01	0.05±0.02	N.T.	0.17±0.12	0.23±0.16	0.32±0.21
Ca, o/n, RT	0.05±0.02	0.08±0.04	0.12±0.04	N.T.	0.20±0.05	0.26±0.07	0.37±0.12
Fe, 8h, RT	0.08±0.06	0.13±0.08	0.18±0.11	0.25±0.18	N.T.	0.40±0.32	0.36±0.19
Fe, o/n, RT	0.07±0.02	0.20±0.07	0.18±0.08	0.26±0.13	0.31±0.14	0.41±0.18	0.54±0.22
Fe, o/n, 4C	0.07±0.02	0.24±0.17	0.26±0.18	0.35±0.19	0.54±0.41	0.53±0.36	0.76±0.45*
pH (37°C)							
Te, 8h, RT	7.02±0.03	6.86±0.03	6.71±0.03	6.58±0.02	6.50±0.03	6.44±0.04	6.40±0.05
Te, o/n, RT	6.91±0.03*	N.T.	6.69±0.03	6.54±0.03*	6.47±0.04	6.42±0.04	6.38±0.05
Te, o/n, RT	6.85±0.03*	6.72±0.04*	6.63±0.03*	6.53±0.03*	6.48±0.01	6.45±0.01	6.45±0.01
Fr, o/n, RT	6.89±0.04*	6.73±0.03*	6.61±0.03*	N.T.	6.49±0.06	6.42±0.04	6.37±0.04
Ca, o/n, RT	6.91±0.05*	6.74±0.05*	6.62±0.04*	N.T.	6.46±0.04*	6.40±0.04*	6.35±0.05*
Fe, 8h, RT	7.00±0.02	6.83±0.03	6.65±0.03	6.55±0.03	6.45±0.03	6.39±0.03	6.34±0.02
Fe, o/n, RT	6.86±0.03*	6.70±0.02*	6.59±0.02*	6.51±0.02*	6.46±0.02	6.40±0.03	6.35±0.03
Fe, o/n, 4C	6.97±0.04	6.78±0.04*	6.62±0.04*	6.52±0.04	6.45±0.04	6.39±0.04	6.34±0.04
Potassium, extracellular (mmol/L)							
Te, 8h, RT	3.7±0.5	23.9±6.3	30.9±5.9	36.8±5.2	41.5±4.8	44.8±4.4	46.8±4.1
Te, o/n, RT	1.5±0.1**	N.T.	16.8±2.3**	27.6±3.0**	33.1±3.3**	37.5±3.5**	40.7±3.4**
Te, o/n, RT	3.7±0.4	15.1±1.5**	23.5±2.1**	32.2±2.5**	38.5±3.3	44.8±3.3	49.3±3.5
Fr, o/n, RT	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
Ca, o/n, RT	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
Fe, 8h, RT	3.9±0.5	18.0±3.0	26.0±3.0	32.3±2.7	39.3±2.6	44.5±2.3	48.4±2.1
Fe, o/n, RT	1.7±0.1**	13.0±1.6**	21.3±2.5**	27.7±2.8**	34.2±3.2**	38.8±3.1**	42.8±2.9**
Fe, o/n, 4C	1.7±0.1**	15.6±1.8	24.8±3.0	32.0±3.6	38.9±4.2	43.4±4.4	47.7±4.4
ATP (mmol/g Hb)							
Te, 8h, RT	4.3±0.6	4.1±0.6	4.2±0.6	4.0±0.6	3.6±0.5	3.3±0.5	2.9±0.6
Te, o/n, RT	5.3±0.6**	N.T.	5.5±0.7**	4.8±0.7**	4.5±0.6**	3.9±0.6**	3.3±0.5
Te, o/n, RT	5.3±0.8**	5.3±0.7**	5.0±0.5**	4.7±0.6**	4.2±0.4**	3.7±0.3**	3.1±0.3
Fr, o/n, RT	5.5±0.6**	5.4±0.7**	4.7±0.6**	N.T.	3.8±0.6	3.4±0.5	2.9±0.4
Ca, o/n, RT	5.7±0.8**	6.0±0.8**	5.2±0.6**	N.T.	4.1±0.5**	3.7±0.5**	3.0±0.4
Fe, 8h, RT	5.2±0.5	4.9±0.5	5.3±0.5	4.6±0.3	3.8±0.3	3.3±0.3	2.9±0.2
Fe, o/n, RT	7.4±0.8**	6.7±0.8**	6.3±0.6**	5.4±0.7**	4.7±0.7**	4.0±0.5**	3.2±0.4**
Fe, o/n, 4C	6.7±0.7**	7.0±1.1**	6.4±2.4**	6.0±1.6**	5.1±1.6**	4.2±1.2**	3.0±1.0**
2,3-DPG (mol/mol Hb)							
Te, 8h, RT	0.85±0.13	0.75±0.20	0.36±0.17	0.19±0.05	0.06±0.02	N.T.	N.T.
Te, o/n, RT	0.42±0.15**	N.T.	0.18±0.04**	0.12±0.01**	0.07±0.02	N.T.	N.T.
Te, o/n, RT	0.57±0.13	0.37±0.16**	0.14±0.07**	0.03±0.02**	0.05±0.02**	N.T.	N.T.
Fr, o/n, RT	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
Ca, o/n, RT	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
Fe, 8h, RT	0.83±0.16	0.40±0.21	0.14±0.10	N.T.	N.T.	N.T.	N.T.
Fe, o/n, RT	0.20±0.03**	0.08±0.03**	0.04±0.01**	0.03±0.01	N.T.	N.T.	N.T.
Fe, o/n, 4C	0.77±0.07	0.36±0.09	0.18±0.17	0.08±0.06	N.T.	N.T.	N.T.

* Statistically different ($p<0.05$) from reference (either Terumo reference, Te, 8h, RT or Fenwal reference, Fe, 8h, RT. Cf. Table I. N.T.= not tested
** Statistically different ($p<0.05$) from reference (either Te, 8h, RT, or Fenwal reference, Fe, 8h, RT).

processing. Regarding haemolysis, generally higher values were found than in reference units including two RBC units showing haemolysis exceeding 0.8 % after day 28. Significant differences were only found at day 42. The levels of extracellular potassium, 2,3-DPG and pH were similar to those of the reference. However, this part of the study was based on very few units (five RBC units).

Discussion and conclusions

In some countries, WB is stored overnight at room temperature before the preparation of blood components, primarily for logistic reasons. The prolonged storage is generally performed at room temperature and is primarily justified as fulfilling the need to harvest platelets to be used for the preparation of BC-derived platelet units. A natural consequence is increased production of lactate from glucose by red cell glycolysis associated with a small drop in pH of about 0.1 unit as compared to the level in reference WB units processed within 8 hours and then stored at 2-6°C. This small drop seems to have a significant impact on red cell metabolism that can be discerned over several weeks. ATP levels are generally significantly higher and 2,3-DPG and extracellular potassium levels are lower in units stored overnight at room temperature. On the other hand, no effects were noted with regards to haemolysis except in individual RBC units prepared by WB filtration after storage overnight at 2-6°C.

In RBC units, 2,3-DPG is generally lost during the first 2 weeks of storage. The rate of synthesis of 2,3-DPG is associated with intracellular RBC pH with breakdown favoured at pH levels below 7.2[9]. Since that particular pH level will be reached earlier after WB storage overnight than in reference units, this small difference in pH may explain the rapid fall in 2,3-DPG[14]. The synthesis of 2,3-DPG is associated with a loss of ATP[9]. In the opposite situation, when the rate of 2,3-DPG synthesis is reduced, ATP synthesis should be favoured, which may explain the significantly higher levels of ATP after WB storage overnight. The increased level of ATP, reflecting an improved energy supply, may also explain the better maintenance of intracellular potassium.

A depletion of 2,3-DPG is associated with a left-shifted oxygen dissociation curve and with increased oxygen affinity and probably a less effective supply of oxygen to the tissues. After transfusion, RBC with low 2,3-DPG levels will generally normalise within a few days[11]. In a review from 1999, Högman and Meryman stated that reversal of the main part of the loss of 2,3-DPG requires from 12-48 hours and that there is clear evidence that recipients of multi-unit transfusions are poorly served by the administration of RBC units more than 10 days old[11]. On the other hand, Tinmouth and Chin-Yee indicated in their review from 2001 that transfusion of 2,3-DPG-depleted blood in human and animal subjects produced no impairment in work performance, mortality, or tolerance of hypoxic conditions[10]. They also concluded that while some blood banks advocate the use of fresh blood for massively transfused patients based on low levels of 2,3-DPG post-transfusion, there is little evidence to support impaired tissue oxygenation. However, both reviews were based mainly on studies published in the 1970s and 1980s and there seems to be room for further investigation in this field. New RBC additive solutions may improve the situation with better maintenance of 2,3-DPG after prolonged storage of WB preceding preparation of blood components. Indeed, new alkaline solutions were shown to give improved maintenance of 2,3-DPG and ATP[14-17] during storage. It is not known whether such solutions have a similar beneficial effect after addition to RBC units after storage of WB overnight. Further studies on such solutions and possibly modification of their composition may improve the situation for the future and make possible WB storage overnight with prolonged maintenance of 2,3-DPG and ATP levels.

In summary, the results of this study suggest that overnight storage of WB before the preparation of blood components is generally associated with significantly increased levels of ATP and reduced levels of extracellular potassium, 2,3-DPG and pH in RBC in SAG-M additive solution as compared to RBC prepared within 8 hours. The results were consistent irrespective of the blood container used.

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Correspondence: Hans Gulliksson
Department of Clinical Immunology and Transfusion Medicine C2 66
Karolinska University Hospital, Huddinge
SE-141 86 Stockholm, Sweden
e-mail: hans.gulliksson@karolinska.se