Quality and stability of red cells derived from gravity-separated placental blood with a hollow-fiber system

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BACKGROUND: Several studies show that donor red blood cells (RBCs) can be processed by gravity separation with a hollow-fiber filtration system. This study investigated whether fetal blood could be filtered in the same way.

STUDY DESIGN AND METHODS: Twelve newborns born after healthy pregnancies were included in the study. Placental blood was sampled with standard procedures. The sampled blood was separated with a specially designed hollow-fiber filtration system (Sangofer neonatal, Heim Group). The RBC bag contained 10 mL of saline, adenine, glucose–mannitol (SAG-M) for stabilization. After processing, the resulting RBC volume was estimated. Quality variables (blood counts, hemolysis rate) were measured before and after 35 days of storage at +4°C.

RESULTS: The 12 processed RBC units had a mean volume of 62.3 ± 13.5 mL and a mean hematocrit level of 0.56 ± 0.06. No white blood cell contamination could be detected in any of the RBC units tested. After 35 days of storage, the hemolysis was 0.1 ± 0.07 and the amount of free hemoglobin was 0.28 ± 0.017 mmol per L.

CONCLUSIONS: This study shows that it is possible to process placental blood to RBCs by gravity separation with a hollow-fiber system. The quality of the RBCs thus processed was suitable for 35 days storage. The use of placental blood in the treatment of children with anemia (e.g., malaria) in the underresourced world is widely discussed. Because the separation device used here needs no additional equipment or electrical devices, it is considered to be an ideal method for use in these countries.

The use of whole blood for the transfusion of patients with anemia has become an exception. Normally whole blood is processed to red cell (RBC) units before transfusion. The standard technique for separation of whole blood into plasma and RBCs is based on centrifugal force. Equipment for blood processing such as centrifuges and the subsequent processing of RBCs is expensive. The processing of RBCs with this method is often not possible because of the lack of an infrastructure (electrical devices, clean rooms, equipment), especially in the underresourced world. In a study with adult blood donors, it could be shown that it is possible to process RBCs without any other equipment with only a special hollow-fiber filtration system (Sangofer, Heim Group, Gladbeck, Germany). The quality of the RBCs processed by this method was comparable to RBCs processed by the classical method.[1] Fetal blood has a much higher mean corpuscular volume (MCV) than adult blood (100-125 fL vs. 85-95 fL). Because the MCV is a critical size in the filtration of blood, it was not known if this method would also work in processing placental blood. In this study, we evaluated the possibility of processing RBCs

ABBREVIATIONS: A-RBC(s) = adult red blood cell(s); CS-APB = centrifugation-separated autologous placental blood; GS-PB = gravity-separated placental blood; SAG-M = saline, adenine, glucose–mannitol.

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from placental blood of full-term newborns by gravity separation. The RBCs thus processed (gravity-separated placental blood [GS-PB]) were assessed for quality and storage stability to determine if they were suitable for later clinical use.

MATERIALS AND METHODS

The study was approved in its present form by the institutional review board of the University of Magdeburg and was performed in accordance with the Declaration of Helsinki. Both parents of all newborns gave their informed consent. The study was performed with placental blood of 12 healthy newborns born after healthy pregnancies at full term in the University Women's Clinic in Magdeburg. Exclusion criteria for the collection of placental blood were: birth weight <2500 g, rhesus incompatibility, hydrops fetalis, morbus hemolyticus neonatorum, hemoglobinopathies as well as β-thalassemia, and maternal viral or bacterial infections including suspected chorioamnionitis (green amniotic fluid, C-reactive protein >10 mg/L or an immature to total neutrophil ratio >0.15 in the mother).

Placental blood sampling

Directly after birth of the infants, the umbilical cord was disinfected carefully. The punction of the umbilical vein was performed near the insertion. As donor bag we used a standard blood sample bag with a reduced amount of only 20 mL of citrate phosphate dextrose (CPD) as anticoagulant. Directly before and after blood sampling, the bag was weighed and the sampled blood volume was calculated from the weight difference. To avoid a too low a RBC-to-storage medium ratio in the RBC blood, three blood samples with a volume below 60 mL were excluded from further processing.

Gravity separation and processing of the RBCs

After blood collection, the donation bag was connected under sterile conditions to the hollow-fiber filter system specially designed for the separation of placental blood (Sangofer neonatal, Heim Group). The details of the separation set are given in Fig. 1. The gravity separation procedure is performed as follows: All steps are performed at room temperature. First the donation bag (Fig. 1A) is hung on the upper hook of the main stand (Fig. 1B). The RBC bag (Fig. 1C) filled with 10 mL of extended storage medium (saline, adenine, glucose–mannitol [SAG-M]), and the separation filter (Fig. 1D) are hung on the middle attachments both exactly on the same level. After opening all clamps and inline break valves, the blood flows from the donation bag through the white blood cell (WBC) filter (standard 50-cm^2 WB filter, Fresenius HemoCare, Friedberg, Germany) (Fig. 1E) and subsequently into the hollow-fiber system (Fig. 1D). Here the leukoreduced blood is separated into plasma and RBCs. The plasma flows down into the plasma bag (Fig. 1F) and the RBCs to the RBC bag. Figure 2 shows the hollow-fiber filter in more detail. After a mean time of 20 minutes, the separation is finished. After completion of the separation, the RBC bag is sealed and disconnected from the separation set. For quality control (QC), we performed an automatic blood cell count (content of RBCs, thrombocytes, and WBCs; hemoglobin [Hb] content [Sysmex KS-1000a, Sysmex Corp., Kobe, Japan]) and determined the pH (AVL 984-S electrolyte analyzer, AVL Medical Instruments AG, Thurgau, Switzerland). Free Hb concentration was measured with a blood glucose analyzer (HemoCue, HemoCue AG, Wetzikon, Switzerland). Hemolysis was calculated according to the formula

$$100 - \text{Hct}(\%) \times \text{free Hb}(\text{mmol/L}) / \text{Total Hb}(\text{mmol/L}).$$

After processing, the 12 RBCs were stored for 35 days at +4°C.

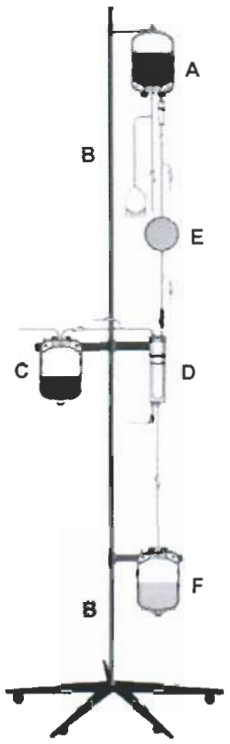

Fig. 1. Complete gravity separation system: (A) Donation bag filled with 20 mL CPD; (B) mean stand; (C) RBC bag filled with 10 mL of SAG-M; (D) hollow-fiber filter; (E) WBC filter; (F) plasma bag.

Storage stability

After 35 days of storage, the above-mentioned quality measures were performed again. In addition, microbio-

logic testing was performed with blood cultures of the 12 GS-PB units with a commercially available radiometric system (Bactec, Becton Dickinson, Heidelberg, Germany). The recommended blood volume (3 mL) was injected into aerobic-anaerobic 50-mL pediatric blood culture bottles (Bactec NR 660, Becton Dickinson). Seven days after inoculation, cultures were assessed for the presence of bacterial growth. The collected data were compared with QC data published by our group earlier and obtained from 12 RBC units processed from placental blood by centrifugation without WBC reduction (centrifugation-separated autologous placental blood [CS-APB]) and with 12 RBC units processed from adult blood by centrifugation with an inline WBC filtration set (A-RBC) stored under the same conditions.[2]

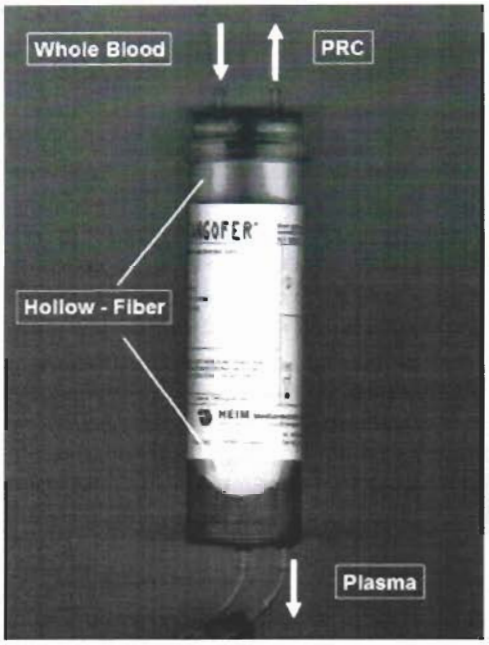

Fig. 2. Hollow-fiber filter in detail.

Statistical analysis

All results were investigated for Gaussian distribution and are given as means and standard deviations. A two-tailed t test for independent variables was used to compare the means.

RESULTS

The results of the QC of the 12 investigated GS-PB units as well as from the CS-PB and the A-RBC units are given in Table 1. There are significant differences between all three groups with regard to the contamination with thrombocytes and WBCs, with the lowest numbers being found in the group of GS-PB. Furthermore, directly after preparation, both placental blood preparations show significantly lower RBCs counts and a lower pH compared to A-RBCs. No significant differences between the groups could be detected in RBCs, WBCs, thrombocytes, Hb, hematocrit (Hct), and the hemolysis rate.

TABLE 1. Quality and storage data of RBCs from gravity GS-PB as well as CS-APB to A-RBCs

Variable	GS-PB	CS-APB	A-RBCs
Directly after preparation			
Volume (mL)	62.3 ± 13.5*†	23.4 ± 9.1	271.2 ± 7.2
RBCs/10^6 µL	5.2 ± 0.6*†	4.6 ± 0.9	6.6 ± 0.3
MCV (fL)	106.3 ± 2.8*†	114.3 ± 6.5	85.6 ± 4.8
WBCs/µL	0.5 ± 0.4*†	6,300 ± 2,600	2.1 ± 0.9
Thrombocytes/µL	958 ± 101*†	62,685 ± 24,100	3568 ± 1.193
Total Hb (mmol/L)	11.8 ± 1.1	10.4 ± 2.1	11.5 ± 0.2
Hct (%)	56.0 ± 5.6	52.6 ± 10.0	56.5 ± 1.3
Free Hb (mmol/L)	0.030 ± 0.010*†	0.012 ± 0.012	0.001 ± 0.000
Percentage hemolysis	0.01 ± 0.05	0.05 ± 0.03	0.004 ± 0.002
pH	6.7 ± 0.02*†	6.4 ± 0.1	7.6 ± 0.2
After 35 days of storage			
Volume (mL)	62.3 ± 13.5*†	23.4 ± 9.1	271.2 ± 7.2
RBCs/10^6 µL	5.1 ± 0.5†	4.7 ± 0.9	6.7 ± 0.3
MCV (fL)	111.1 ± 3.3*†‡	117 ± 8.6	96.4 ± 7.1
WBCs/10^3 µL	0.0 ± 0.0*‡	4,200 ± 200	0.0 ± 0.0
Thrombocytes/µL	0.0 ± 0.0*‡	54,666 ± 11,785	0.0 ± 0.0
Total Hb (mmol/L)	11.8 ± 1.1*	10.4 ± 0.2	11.7 ± 0.4
Hct (%)	57.1 ± 4.8†	55.2 ± 11.1	63.5 ± 1.4
Free Hb (mmol/L)	0.280 ± 0.017†‡	0.256 ± 0.152	0.051 ± 0.025
Percentage hemolysis	1.0 ± 0.7*‡	1.1 ± 0.7	0.2 ± 0.1
pH	6.4 ± 0.0*†‡	6.1 ± 0.1	6.8 ± 0.1

* $p < 0.05$, GS-PB vs. GS-APB.
† $p < 0.05$, GS-PB vs. A- RBCs.
‡ $p < 0.05$, directly after preparation vs. after 35 days of storage.

Earlier investigations by our group showed that by use of a closed collection system and extended storage medium (SAG-M), CS-APB meets the quality criteria for A-RBCs after 35 days.[2] Owing to the hemolysis of 1.1 percent, a longer storage of RBCs derived from placental blood does not appear to be possible. To make the results comparable, we chose the same storage time for this study. The QC data after 35 days of storage show that now all RBCs displayed significantly different values from the initial values before storage with respect to WBCs, thrombocytes, free Hb, hemolysis rate, and pH.

Compared to the initial value, the Hct was significantly increased in the A-RBCs as a sign of increased MCV. This effect was not so prominent in the RBCs derived from placental blood. Crucial for the osmotic resistance and the following lysis of the cells is the surface-to-volume ratio of the cells. The placental blood cells have a larger MCV and

a lower capacity to swell before lysis. This results in a lower difference of the Hct levels before and after storage but enhanced hemolysis. All 12 samples of the GS-RBCs examined after 35 days of storage were free of microbial contamination. In summary, after 35 days storage, the GS-PB showed quality data comparable to those of the A-RBCs.

DISCUSSION

Placental blood for the treatment of newborns with anemia has already been discussed for a long time.[2-14] Several studies were able to show that it is technically possible to process and store RBCs derived from placental blood.[2,5-14] Studies concerning the clinical experience after return of placental blood show an efficacy and safety comparable with those of adult RBCs. Up to now no transfusion-related side effects have been observed.[15-20] Allogeneic transfusions of RBCs processed from cord blood of mature newborns could be a valuable alternative treatment for children with anemia in the underresourced world. Particularly malaria is a reason for the development of anemia in these children. Without blood transfusions the patients frequently fail to survive this life-threatening situation.

Blood inventories are not available in most of the hospitals of these countries, but frequently, however, they have a high birth rate. The cord blood of one newborn would be enough to transfuse one anemic child once.[21-25] The processing of the sampled placental blood with the usual heavy cooling centrifuges in clean rooms is, however, too expensive for these countries. The use of special inline filter systems that separate plasma and blood cells only by gravity would make these expensive procedures unnecessary and the processing of the blood would be possible without any special equipment or power. This idea is not new. First attempts with integrated special plasma filters to separate the whole blood into its components were carried out at the beginning of the 1990s. The major problems with these filter systems (BBC, Asahi, Tokyo, Japan) were the long separation time and the insufficient concentration ability of the filter systems.[26-28]

With a newly developed special hollow-fiber filtration system (Sangofer, Heim Group), Hornsey and colleagues[1] showed that it is possible to process RBCs of adequate quality without any other equipment. In this study, we were able to show that by use of a modification of the above-mentioned system (Sangofer neonatal, Heim Group), it is also possible to separate placental blood into its compounds. After 35 days of storage, the SAG-M–stabilized RBCs thus processed showed quality data that are comparable to those of placental blood RBCs processed by the classical separation technique.[2] All examined GS-PB units were free of microbial contamination. The exclusion of children with a suspected amnion infection syndrome and the careful disinfection of the umbilical cord before the puncturing seem to be successful in avoiding microbial contamination during placental blood collection. This confirms the results of our earlier studies.[2,15,16]

This study shows that placental blood can be separated into its components by gravity with only a hollow-fiber filter system with a quality suitable for later clinical use. Because no other equipment is necessary and it is possible to use it without electric current, it is our view that this system would be ideal for use in the underresourced world.

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