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BRIEF COMMUNICATION

Others

Influence of the washing program on the blood processing performance of a continuous autotransfusion device

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Abstract The continuous autotransfusion system has been widely used in surgical operations. It is known that if oil is added to blood, and this mixture is then processed by an autotransfusion device, the added oil is removed and reinfusion of fat is prevented by the device. However, there is no detailed report on the influence of the particular washing program selected on the levels of blood components including blood fat after continuous autotransfusion using such a system. Fresh bovine blood samples were processed by a commercial continuous autotransfusion device using the "emergency," "quality," and "high-quality" programs, applied in random order. Complete blood count (CBC) and serum chemistry were analyzed to determine how the blood processing performance of the device changes with the washing program applied. There was no significant difference in the CBC results obtained with the three washing programs. Although all of the blood lipids in the processed blood were decreased compared to those in the blood before processing, the levels of triglyceride, phospholipid, and total cholesterol after processing via the emergency program were significantly higher than those present after processing via the quality and high-quality programs. Although the continuous autotransfusion device provided consistent hematocrit quality, the levels of some blood lipid components showed significant differences among the washing programs.

Keywords Continuous autotransfusion · Blood processing · Blood lipid · Hematocrit · Washing program

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Introduction

Intraoperative autotransfusion is performed to reduce problems with allogeneic transfusion, including blood-related diseases, immune reactions, cross-matching errors, and blood bank shortages. Donated blood transfusion is refused by some patients for religious or cultural reasons. Such patients can be given a transfusion of autologous blood at the operative site using an autotransfusion device. Autotransfusion devices have proven useful in cardiothoracic surgery, liver transplantations, and orthopedics [1].

The continuous autotransfusion system has been widely used in surgical operations in recent decades. The feasibility and efficacy of small-volume blood processing using the continuous autotransfusion device have been compared with those of the intermittent bowl-type device [2]. Because there is a major risk of fat embolism during

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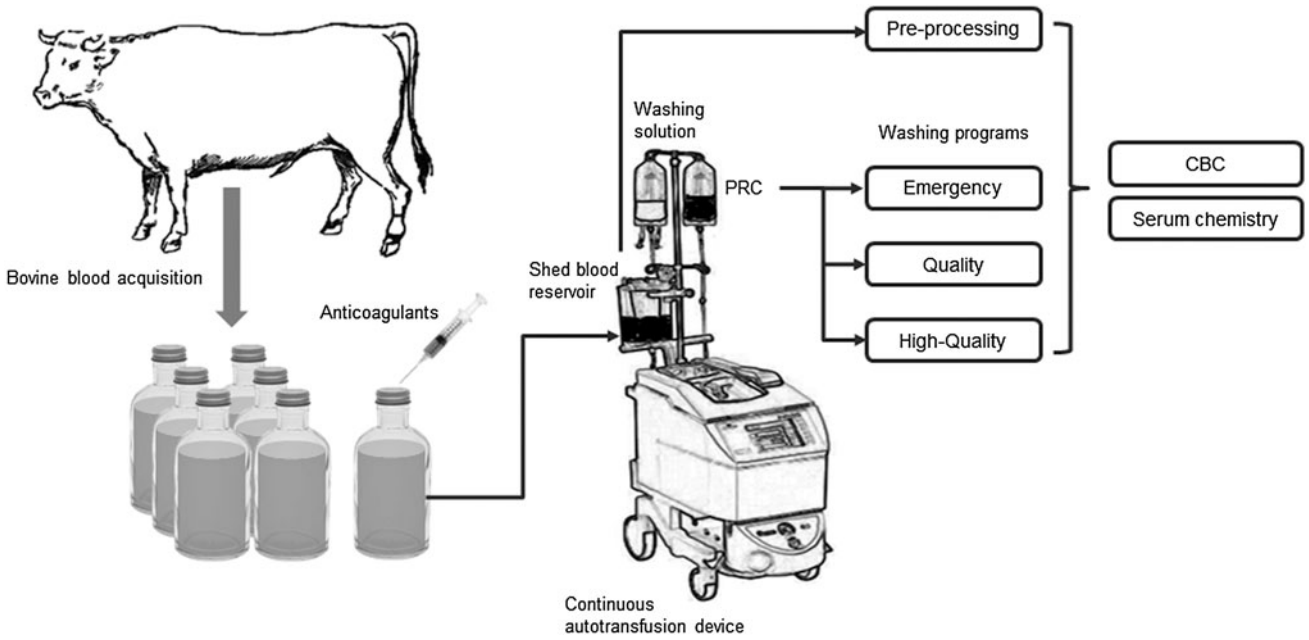

Fig. 1 Scheme showing the study design, from blood acquisition to measurement

autotransfusion, especially in orthopedic surgery and bone fracture [3], which can lead to critical dysfunction of the skin, brain, and lung [4, 5], several studies have focused on the fat elimination performance of the continuous autotransfusion system. The system was found to completely remove soya oil that had been pre-mixed with the blood, whereas the intermittent bowl-type device did not [6]. However, in this oil elimination study, only the "high-quality" washing program—which is the most time-consuming of the programs implemented by the continuous autotransfusion device—was used. Although the soya oil was easily eliminated, the elimination of fats that are naturally present in blood was not considered.

Although the continuous autotransfusion device provides a consistent hematocrit (Hct) range when various washing programs are performed [7, 8], there is no detailed report on the influence of the particular washing program implemented on the levels of blood components, including blood fat, following continuous autotransfusion with the device. Therefore, in the study described in the present paper, we investigated the effect of the washing program on the blood component levels after continuous autotransfusion of fresh blood.

Materials and methods

Discarded fresh bovine blood was acquired from a public slaughterhouse. In our preliminary experiments, we experienced clotting problems several times when using only heparin. These were due to the long transportation time from the site at which the blood was acquired to our laboratory. After several trials, a mixture of 75 ml of anticoagulant citrate dextrose solution USP (ACD) Formula A (Baxter Healthcare Corporation, Marion, NC, USA) and 1000 IU of heparin was chosen for use as the anticoagulant. Seven bottles containing blood were placed in an insulated box with frozen ice packs and transported to the laboratory for consecutive blood processing within 2 h after acquisition. No supplementary fat or oil was mixed with the blood (see Fig. 1).

The blood was placed into a blood collection reservoir incorporating a two-layer 120-μm polyurethane foam filter for a continuous autotransfusion device (C.A.T.S. Plus, Fresenius Kabi AG, Bad Homburg, Germany). The reservoir was connected to a blood line which in turn was connected to a washing chamber. Lactated Ringer's solution was used as the washing solution. Waste washing solution and superfluous blood components were collected in a waste bag. Processed packed red blood cells (PRC) were collected in a reinfusion bag. Before processing the blood in each bottle, 15 ml of it were sampled for the blood collection reservoir.

The device was set to the "emergency," "quality," and "high-quality" programs in random order to avoid any potential error caused by imposing a specific experimental sequence. The quality program is the standard washing program. The emergency program processes the blood more quickly than the other programs; it uses the maximum flow rate of shed blood and employs only 20 % of the washing saline used by the quality program. The high-quality program takes longer to get the same PRC volume

than the other programs and utilizes about 140 % of the washing saline used by the quality program. Before sampling the blood, the device was initialized by performing a priming mode to wash out the residue from the previous blood processing session using a fresh washing solution. The device was then operated according to the designated program for at least 5 min to wash out the primed solution and to get unmixed PRC. After 5 min of blood processing, the PRC (18 ml) were sampled at a three-way valve placed between the blood line and reinfusion bag. Half of the sampled PRC (9 ml) were saved in ethylenediaminetetraacetic acid (EDTA) tubes for anticoagulation. The remaining PRC (9 ml) were centrifuged for 10 min in 3000 RPM. Complete blood count (CBC) and serum chemistry tests, including those for blood lipids and potassium, were performed. The CBC was analyzed using an XE-2100 (Sysmex, Kobe, Japan). Plasma Hb was measured spectrophotometrically using a spectrometer (DU-730, Beckman Coulter, Brea, CA, USA). Potassium, triglyceride, and total cholesterol were measured using an automated analyzer (ADVIA 2400, Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Phospholipid, free fatty acid, and free cholesterol were measured using a Modular P analyzer (Roche–Hitachi, Kobe, Japan). Ester cholesterol was calculated as the difference between the total cholesterol and free cholesterol.

A one-way repeated-measures analysis of variance followed by Tukey's method was applied to parameters with Gaussian distributions, and a Friedman test followed by the Wilcoxon signed-rank test was used for nonparametric analysis. The level of significance was set as $p < 0.05$.

Results

All CBC and serum chemistry tests were performed successfully (Table 1). Slight dilution and mild hemolysis were observed in preprocessed blood. Except for the platelet count, all of the CBC measurements for the processed blood samples were markedly higher than those of the preprocessed blood. Although Hct gradually increased as the washing program was changed from emergency to quality to high quality, Hct did not differ statistically significantly among the programs. Similarly, Hb, RBC count, and plasma Hb gradually increased as the washing program was changed from emergency to quality to high quality, but they did not differ significantly among the programs. WBC and platelet count also did not differ statistically significantly among the programs.

All blood lipids of the processed blood samples were significantly decreased compared with those of the preprocessed blood samples. All of the blood lipids were

Table 1 Complete blood count (CBC) and serum chemistry test results for preprocessed blood and for blood following various washing programs of a continuous autotransfusion device

Measurement	Preprocessing ($n = 7$)	Emergency program ($n = 7$)	Quality program ($n = 7$)	High-quality program ($n = 7$)
CBC				
Hb (g dL^{-1})	9.8 ± 1.9	14.4 ± 1.5*	15.0 ± 2.4*	15.2 ± 3.9*
Hct (%)	31.2 ± 6.1	47.4 ± 5.3*	48.2 ± 7.1*	50.1 ± 11.6*
RBC count ($\times 10^9$ mL^{-1})	5.3 ± 1.1	7.7 ± 0.7*	8.1 ± 1.3*	8.1 ± 1.9*
WBC count ($\times 10^6$ mL^{-1})	2.0 ± 0.5	2.5 ± 0.3*	3.3 ± 0.8*	2.8 ± 0.7
Platelet count ($\times 10^6$ mL^{-1})	8.6 ± 5.1	5.1 ± 4.1	10.9 ± 9.6	20.6 ± 29.4
Plasma Hb (mg dL^{-1})	120.2 ± 106.4	251.9 ± 95.1*	283.4 ± 51.4*	302.8 ± 32.1*
Serum chemistry				
Total cholesterol (mg dL^{-1})	73.3 ± 21.7	43.0 ± 16.8*	30.0 ± 21.7*,†	29.0 ± 15.2*,†
Triglyceride (mg dL^{-1})	11.6 ± 5.4	4.7 ± 1.5*	2.4 ± 1.3*,†	3.0 ± 1.0*,†
Phospholipid (mg dL^{-1})	76.7 ± 19.6	45.6 ± 16.0*	31.6 ± 21.6*,†	30.9 ± 14.9*,†
Free fatty acid (uEq L^{-1})	96.4 ± 69.9	31.3 ± 38.2*	24.9 ± 25.5*	27.6 ± 27.2*
Free cholesterol (mg dL^{-1})	15.1 ± 4.3	7.0 ± 3.7*	6.0 ± 4.47*	6.0 ± 3.27*
Ester cholesterol (mg dL^{-1})	58.1 ± 17.4	36.0 ± 16.6*	24.0 ± 17.2*,†	23.0 ± 12.0*,†
Potassium (mEq L^{-1})	4.3 ± 0.59	4.3 ± 0.3	4.4 ± 0.2	4.4 ± 0.2

Values are presented as the mean ± standard deviation (SD)

Hb hemoglobin, *Hct* hematocrit, *RBC* red blood cell, *WBC* white blood cell

* $p < 0.05$ vs. preprocessing

† $p < 0.05$ vs. emergency program

higher after the emergency program among the three programs implemented. Triglyceride, phospholipid, and total cholesterol levels were significantly different between the emergency program and the quality program, and in the comparison between the emergency and high-quality programs, but this was not the case when the quality and high-quality programs were compared. Free fatty acid and free cholesterol did not vary significantly depending on the program used. Ester cholesterol was significantly different between the emergency program and the quality program, and between the emergency and high-quality programs, but not between the quality and high-quality programs. Potassium did not vary significantly among the different programs, or between the preprocessed blood and that obtained after any washing program.

Discussion

The continuous autotransfusion device provided a consistent Hct regardless of the washing program. This consistency was achieved by balancing the amount of washing solution used with the flow rate of shed blood, which was regulated by an optical sensor integrated in the system. The high-quality program used the largest amount of washing solution and the lowest flow rate of shed blood to produce the same amount of PRC, so the processing speed of the high-quality program was the slowest of the washing programs tested. There was also no significant difference in Hb, RBC count, WBC count, and potassium among the different programs. Although WBC should be eliminated after centrifugation in an autotransfusion system, some of the WBC were concentrated and extracted along with the PRC in all of the programs. This result is supported by a previous report that perfect elimination of WBC was not achieved in a continuous autotransfusion system [9]. Although the blood collection reservoir contained a two-layer 120 μm filter to screen out particulates or tissue debris, WBC or lipid would pass through the filter. Also, although hemolysis did not vary significantly among the programs, it was observed that plasma free Hb gradually increased as the washing program was changed from emergency to quality to high quality. This is thought to be because the duration of exposure to shear forces in the rotating chamber channel increased as the program was changed from emergency to quality to high quality.

Although CBC analysis did not highlight any significant difference among the results obtained using the three washing programs, the serum chemistry results did show significant differences in some blood lipid components among the programs. Some lipid components were significantly higher for the emergency program than for the other washing programs. Blood lipids such as cholesterol, free fatty acid, and triglyceride are commonly lighter than blood cell components such as RBC and WBC. When normal whole blood was centrifuged, the lipid floated on the plasma while the blood cell components sank according to the differences in specific gravity among the blood components. The emergency program required the shortest processing time and the smallest volume of washing solution to centrifuge the same amount of blood among the three washing programs. This result implies that the emergency program can provide a similar Hct to other programs but may lead to inadequate elimination of blood lipid components.

More lipid would be eliminated from shed blood if a faster rotation speed and a longer centrifugal duration than employed in the high-quality program were to be applied during blood processing. However, a very long centrifugal time would be required to remove all of the blood lipid from shed blood. This may reduce the practicality of the device. Moreover, a faster rotation speed (higher centrifugal force) and a longer centrifugal time may increase the risk of hemolysis. We think that the washing programs provided by the device are compromises with respect to processing time, washing performance, and risk of hemolysis.

Since the centrifugal processing performed by the autotransfusion device is unable to completely remove fat from autologous blood [10], it is important to use a suitable filter to remove sufficient fat. However, if a filter is used with continuous autotransfusion, we cannot rule out an unexpected increase in cerebral lipid microembolisms [11]. While large particles and fat globules would be removed by the filter [12], lipid would pass through the filter and remain in the blood. Although the use of a combination of autotransfusion with a surface filter instead of a depth filter is recommended to obtain the most fat-free blood [9], it is important to recognize that blood lipid is insufficiently eliminated during the operation of a continuous autotransfusion device.

In conclusion, some lipid remained in the blood after processing, and the amount of lipid that remained varied significantly depending on the washing program applied. While the emergency program of the continuous autotransfusion device has the advantage of reducing the processing time, it does pass more blood lipid than the quality and high-quality programs do. Physicians and medical personnel who utilize the continuous autotransfusion device should note that the performance of the device varies with the washing program, and should select the appropriate program for blood processing accordingly.

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Conflict of interest No conflict of interest is associated with the present study.

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