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Heparin concentration in cell salvage during heparinization: a pilot study

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ABSTRACT

Cell salvage is frequently used to avoid unnecessary allogeneic blood transfusions, which results in a reduction in blood transfusion volume and cost. The aspirated blood is washed with normal saline and centrifuged to recover only blood cells, salvaged blood is then made. In cardiovascular surgery, heparin is used to maintain activated clotting time over 400 seconds. Some practitioners believe that heparin remains in the salvaged blood. Therefore, we hypothesized that salvaged blood during cardiovascular surgery includes heparin. A pilot study was conducted to evaluate our hypothesis using three different salvage systems. This study was a prospective, observational, pilot study, with patients aged 20–85 years old who were scheduled for cardiovascular surgery from May 2018 to October 2018. The intent of this study was to evaluate whether salvaged blood with three different devices includes large enough quantities of heparin to influence activated clotting time in cardiovascular surgery. Between May and October 2018, 12 samples during heparinization were collected, and 12 samples of salvaged blood from 3 devices were collected after administrating protamine. The heparin concentration of the 24 samples was measured. All heparin concentrations in salvage blood sample from two devices was below the limit of measurement (0.10 IU/mL). Slightly measurable heparin was detected in salvaged blood sample from one device (mean 0.15 IU/mL). Salvaged blood during cardiovascular surgery intervention does not contain enough heparin to influence activated clotting time.

Keywords: cell salvage, heparin, heparinization, salvaged blood, concentration

Abbreviations:
ACT: activated clotting time
XTRA: XTRA®, manufactured by Liva Nova Japan K.K., Tokyo, Japan
ELITE: Cell Saver ELITE®, manufactured by HAEMONETICS Japan GK, Tokyo, Japan
AUTOLOG: AUTOLOG®, manufactured by Medtronic Japan Co. Ltd, Japan

INTRODUCTION

Cardiovascular surgery is associated with high rates of transfusion. There are multiple factors,

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such as hypothermia, acidosis, heparinization and induction of the inflammatory cascade, that are responsible for hemostatic disorders after bypass.[1-3] Cell salvage is frequently used to avoid unnecessary allogeneic blood transfusions. While allogeneic transfusion has minimal complications, it leads to an increased cost.[4-6] Cell salvage removes most of the unwanted components, such as free hemoglobin, and provides concentrated and washed red blood cell with a 50–60% hematocrit level. It is well known that the recovered blood contains almost no coagulation factors. However, because of using heparin to maintain activated clotting time (ACT) during bypass, some practitioners believe that heparin remains in the salvaged blood, and that heparin in salvaged blood causes bleeding. Therefore, we hypothesized that salvaged blood during cardiovascular surgery with heparinization includes heparin in large enough quantities to extend the ACT. Here, a pilot study was conducted to evaluate our hypothesis using three different salvage systems.

METHODS

This prospective study was approved by the Institutional Review Board of the Nagoya University Hospital (IRB # 2018–0084). Written informed consent was obtained from all subjects participating in the trial. The trial was registered prior to patient enrollment at the University Hospital Medical Information Network (UMIN000031125). Twelve patients aged 20–85 years who were scheduled for cardiovascular surgery with heparinization from May 2018 to October 2018 were enrolled. The twelve patients were randomly allocated by computer-generated randomization to one of 3 machines so that each machine was tested with 4 patients. The cell salvage machines used were as follows: XTRA®, manufactured by Liva Nova Japan K.K., Tokyo, Japan; Cell Saver ELITE®, manufactured by HAEMONETICS Japan G.K., Tokyo, Japan; AUTOLOG® manufactured by Medtronic Japan Co. Ltd., Japan. The AUTOLOG was employed only for aortic surgery in accordance with our facility usage agreement. The automatic washing mode for each device was used for washing. Each machines processing settings are shown in Table 1. The primary study endpoint was the heparin concentration in salvaged blood.

Non-invasive arterial blood pressure measurements, electrocardiography, pulse oximetry, bispectral index monitoring, and radial artery cannulation for blood pressure monitoring and sampling were performed in all patients. Fentanyl and midazolam were administered intravenously to induce general anesthesia. Remifentanil and rocuronium were additionally used to facilitate tracheal intubation. General anesthesia maintenance was performed using air, oxygen, remifentanil, and volatile anesthetics. In cardiac surgery (for XTRA, ELITE), porcine heparin (300 IU/kg) was administered before starting cannulation for cardiopulmonary bypass (CPB), and additional heparin boluses (50 IU/kg) were administered to maintain an ACT of at least 400 seconds. Ventilation of both lungs was initiated before separating from CPB. Protamine (3 mg/kg) was administered to antagonize the heparin effect. Red blood cell concentrates were transfused to maintain a hemoglobin level over 8 mg/dl during CPB. CPB was terminated with inotropic drug support, and the patient was intubated and admitted to the ICU. In aortic surgery (AUTOLOG), porcine heparin (300 IU/kg) was administered before aortic clamping, and additional heparin boluses (50 IU/kg) were administered to maintain an ACT of at least 400 seconds. Protamine (3 mg/kg) was administered to antagonize the heparin effect. Packed red blood cells were transfused to maintain a hemoglobin level over 8 mg/dl. In each patient, both the blood collected via radial artery catheter at the same time as measuring the first ACT and the completed cell salvage blood were collected and submitted for heparin concentration measurement and blood count. After blood collection, they were quickly centrifuged at 2500 rpm for 15 minutes, the serum was collected, and was stored at –80°C until heparin measurement.

Table 1 Summary of blood count and each device setting

Blood count	XTRA (n=4)		ELITE (n=4)		AUTOLOG (n=4)	
	Heparinization	Salvage	Heparinization	Salvage	Heparinization	Salvage
Hb (g/dl)	9.3 ± 0.6	18.2 ± 1.2	9.1 ± 0.7	19.8 ± 2.1	9.0 ± 0.7	20.0 ± 0.8
Ht (%)	29.0 ± 0.8	55.7 ± 1.2	29.2 ± 1.2	56.1 ± 1.7	28.7 ± 0.8	58.3 ± 1.7
Platelet ($\times 10^4/\mu l$)	8.5 ± 1.6	1.5 ± 5.1	8.6 ± 1.2	3.4 ± 6.2	7.2 ± 1.1	0.9 ± 3.2
Potassium (mmol/l)	4.2 ± 0.3	0.9 ± 0.2	4.2 ± 0.2	1.0 ± 0.2	4.1 ± 0.2	1.3 ± 0.3
Settings of Cell salvage devices	**XTRA**		**ELITE**		**AUTOLOG**	
Bowl size (ml)	125		125		135	
Washing quantity (ml)	500		500		250	
Washing flow rate (ml/min)	300		300			
Return flow rate (ml/min)	300		100		Not disclosed by the manufacturer*	
Concentration flow rate (ml/min)	300		250			
Heparin in normal saline for aspiration of blood (IU/ml)			30			
Dropping rate in normal saline for aspiration of blood (ml/h)			90			

Data are expressed as means ± standard deviation (SD), number of volume (ml), international unit (IU/ml), or flow rate (ml/min) and dropping rate (ml/h) (n = 12). Hb, hemoglobin; Ht, hematocrit; ml, milliliter; min, minutes; h, hours; Salvage, the blood collected in holding bag of salvaged blood. XTRA, XTRA®, manufactured by Liva Nova Japan K.K., Tokyo, Japan; ELITE, Cell Saver ELITE®, manufactured by HAEMONETICS Japan GK, Tokyo, Japan; AUTOLOG, AUTOLOG® manufactured by Medtronic Japan Co. Ltd., Tokyo, Japan. *We used the standard settings of this device.

Based on a measurement error less than 10% in quantitative measurement of heparin concentration, and minimum detection limit of 0.10 IU/mL, a pilot study was planned in which over three samples were measured and the power was compared. Parametric tests were used for statistical analysis because the Levene test showed that variances were homogeneous. Each group was compared using the one-way analysis of variance. A p-value of <0.05 indicated statistical significance and all p-values were two-tailed. All data were analyzed using SPSS software (version 26, IBM Japan Ltd., Tokyo, Japan).

RESULTS

Between May and October 2018, 12 patients were randomly allocated to each group, with 4 patients in XTRA, 4 in ELITE, and 4 in AUTOLOG groups, respectively. Demographic information for these patients is shown in Table 2. Data from 4 patients per group were included in the final analysis. Heparin concentrations of the 24 samples were measured.

All heparin concentrations in the salvaged blood from two devices (XTRA and ELITE) were below the lower limit of measurement (0.10 IU/mL). Slightly measurable heparin was detected in blood from one device (AUTOLOG, mean 0.15 IU/mL), $F(2, 9) = 4.3$, $p = 0.11$). Mean heparin concentrations of blood during heparinization were 3.78 IU/mL, 3.64 IU/mL, and 3.70 IU/mL, respectively, $F(2, 9) = 4.3$, $p = 0.90$). (Figure 1)

Table 1 shows the summary of blood counts in the salvaged blood and the blood collected via radial artery catheter. Potassium was almost completely removed. Interestingly, some platelets were also present in the washed salvaged blood.

Table 2 Patients demographic and surgical characteristics

Total n=12	**XTRA (n=4)**	**ELITE (n=4)**	**AUTOLOG (n=4)**
	mean ± SD	mean ± SD	mean ± SD
Demographic information			
Age (years)	62.1 ± 9.1	68.5 ± 3.9	63.5 ± 13.5
Height (cm)	165.2 ± 8.6	164.1 ± 5.3	167.4 ± 3.3
Body weight (kg)	66.1 ± 7.4	58.9 ± 5.5	64.1 ± 17.1
Body mass index (kg/m^2)	24.2 ± 1.3	21.1 ± 2.3	22.9 ± 6.3
Male:female	3:1	3:1	3:1
Surgical information			
On-pump CABG	1	0	0
Single valve	1	3	0
Aorta	2	1	4

Data are expressed as means ± standard deviation (SD) or number of patients. On-pump CABG, coronary artery bypass graft with cardio-pulmonary bypass. XTRA, XTRA®, manufactured by Liva Nova Japan K.K., Tokyo, Japan; ELITE, Cell Saver ELITE®, manufactured by HAEMONETICS Japan GK, Tokyo, Japan; AUTOLOG, AUTOLOG® manufactured by Medtronic Japan Co. Ltd., Tokyo, Japan.

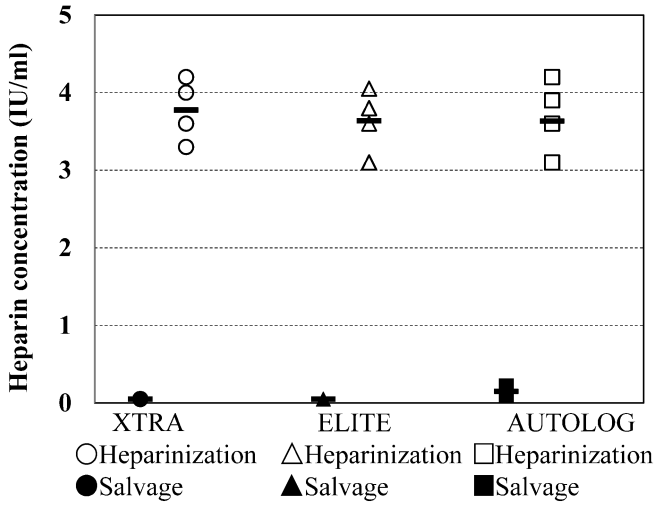

Fig. 1 Heparin concentration
Heparin concentration are indicated by IU/mL. The limit of measurement for heparin is 0.10 IU/mL. White markers indicate each concentration during heparinization (white circles, triangles, and squares), and black marlers indicate each concentration in salvaged blood (black circles, triangles, and squares). Mean concentrations were indicated by black bars. Mean concentration of heparin by AUTOLOG was 0.15 IU/mL. The others were less than 0.1 IU/ml. XTRA, XTRA®, manufactured by Liva Nova Japan K.K., Tokyo, Japan; ELITE, Cell Saver ELITE®, manufactured by HAEMONETICS Japan GK, Tokyo, Japan; AUTOLOG, AUTOLOG® manufactured by Medtronic Japan Co. Ltd., Tokyo, Japan.

DISCUSSION

Some practitioners believe that heparin remains in salvaged blood collected during CPB, and believe that it is one of the causes for massive transfusion and bleeding. This is sometimes controversial in clinical situation. This current study was a pilot study to analyze heparin concentrations in cell salvaged blood. The results of this study did not support our hypothesis which was that significant, residual heparin would be present in a salvaged blood product. Our results suggest that almost all of the heparin salvage blood is below the limit of measurement (0.10 IU/mL). Even if heparin is detected, it is only an average of 0.15 IU/mL which is well below that which would alter coagulation function. In comparison, mean heparin concentrations of blood during heparinization were 3.78 IU/mL, 3.64 IU/mL, and 3.70 IU/mL, respectively. In each cell salvage device, blood collected from the patient are washed in heparin-free normal saline, and are centrifuged. Cell salvage removes most of the unwanted components, such as free hemoglobin, and provides concentrated and washed red blood cells with a 50–60% hematocrit. It is safe to assume that there is almost no heparin contamination when blood is processed with standard cell salvage equipment and standard operating procedures. When 500 ml of salvaged blood is administered, only 0–100 units of heparin could be given which would be below the detection limit. It is well known that heparin less than 100 units is not involved in APTT prolongation.[7]

When red blood cell transfusion, crystalloid, and/or colloid are given to bleeding patients, it is likely causing a dilutional coagulopathy. In addition, hemorrhagic shock and associated hypothermia and acidosis exacerbate consumptive and dilutional coagulopathy, resulting in a vicious circle that impairs patient prognosis.[8] Therefore, at the time of cardiovascular surgery,

there are several factors that facilitate bleeding other than just surgical bleeding.[1-3] Previous reports indicated that salvaged blood collected from the surgical field during CPB increased postoperative bleeding and increased blood transfusion volume.[9] The main reasons that were proposed was that the salvaged blood prolonged activated partial thromboplastin time, prothrombin time and international normalized ratio, and decreased fibrinogen levels. It is highly unlikely that coagulation factors or fibrinogen were adequately replaced in this report. In addition, multiple factors such as hemorrhagic shock, associated hypothermia and dilutional coagulopathy could be related. Therefore, it was considered that the administration of salvaged blood was not the sole cause. Recently, the optimal administration of blood products according to the situation is recommended such as using TEG6s.[10-12] If larger amounts of salvaged blood without coagulation factors are transfused, it may result in a dilutional coagulopathy. In addition, the production of large amounts of salvaged blood suggests that bleeding is proportionately high. In order to replenish this, a large dose of salvaged blood, crystalloid or colloid also can cause dilutional coagulopathy. In order to solve this dilutional effect, it is absolutely recommended that appropriate amounts of plasma and platelets should be administered according to the results from point of care devices such as thromboelastography (TEG6s).[13-17] Thus, various factors are involved in bleeding, however, it can be concluded from this study that one of the causes is not heparin from salvaged blood.

This study has two major limitations that should be addressed. First, this study was a pilot study, and the number of cases was small. All of the heparin concentrations in the salvaged blood samples were too low to measure. As result, the power is sufficient to reject the hypothesis that cell salvage blood contains heparin. Thus, a larger study to demonstrate that heparin in salvaged blood is the cause of bleeding is unnecessary. Second, the results of this study may vary depending on the processing settings. Therefore, the processing settings for each facility should be adjusted and confirmed.

In conclusion, almost all of the heparin in salvaged blood as evaluated by quantitative measurements was below the lower limit of measurement (0.10 IU/mL). Salvaged blood during cardiovascular surgery intervention does not contain enough heparin to influence ACT.

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DISCLOSURE STATEMENT

None of the authors has any conflicts of interest to declare in relation to this work.

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