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ORIGINAL ARTICLE

INTRAOPERATIVE AUTOLOGOUS CELL SALVAGE CAN INDUCE COAGULOPATHY IN CARDIOPULMONARY BYPASS SURGERY: AN EX-VIVO STUDY

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Abstract Objectives: The aim of this study is to investigate the effect of washed salvaged red blood cells on hemostasis in open heart surgery using the cardiopulmonary bypass (CPB).
Methods: Nine patients were enrolled in this study. The cell salvage system was used throughout the surgery. Three blood samples (1mL each) were obtained from each patient one hour after the CPB for the measurements consisting of control blood sample (Control group), 1.0ml of blood added with 0.2mL salvage blood (CS group) or 0.2mL normal saline (NS group). Test samples were evaluated using rotational thromboelastometry (ROTEM®). Coagulation was triggered by ellagic acid.
Results: No significant differences were observed in the clotting time among the three groups. Clot formation time in the CS group and NS group was prolonged significantly compared with Control group (Control, 138 ± 51; CS, 103 ± 28; NS, 135 ± 48 sec). Amplitude of ROTEM® band at 10 minutes and maximum clot firmness in both the CS and NS group were significantly narrower than that of Control group.
Conclusions: This ex-vivo study using ROTEM® suggested that the perioperative administration of washed salvaged red blood cells might have a potential to induce coagulopathy in cardiac surgery using the CPB.

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Key words: washed salvaged red blood cells; coagulopathy; cardiopulmonary bypass; thromboelastometry

Introduction

The use of intraoperative salvaged red blood cells (RBCs) and autologous blood transfusion are considered as a useful method for blood conservation in cardiac surgery. Some randomized trials reported that there was no increase in bleeding or coagulopathy by auto-transfusion of salvaged RBCs [1-4], while other reports suggested that large volume auto-transfusion might be accompanied by coagulopathy [5]. These findings are still controversial because the effects of salvaged RBCs on coagulative function are not evaluated fully. Since there are various cascades or influences on hemostasis in vivo, ex vivo study is desirable for the investigation of the blood itself. Therefore, we investigated the effect of washed salvaged RBCs on hemostasis using rotational thromboelastometry in cardiac surgery using the cardiopulmonary bypass (CPB).

Methods

Nine patients who underwent cardiac surgery using the CPB were enrolled in this study. No one had refusal policy to receive blood or blood products. Patients with congenital heart disease, clotting disorders or systemic sepsis were excluded. We obtained informed consent for the study from all candidates. This study was performed with the approval of the local ethical committee of Hirosaki University

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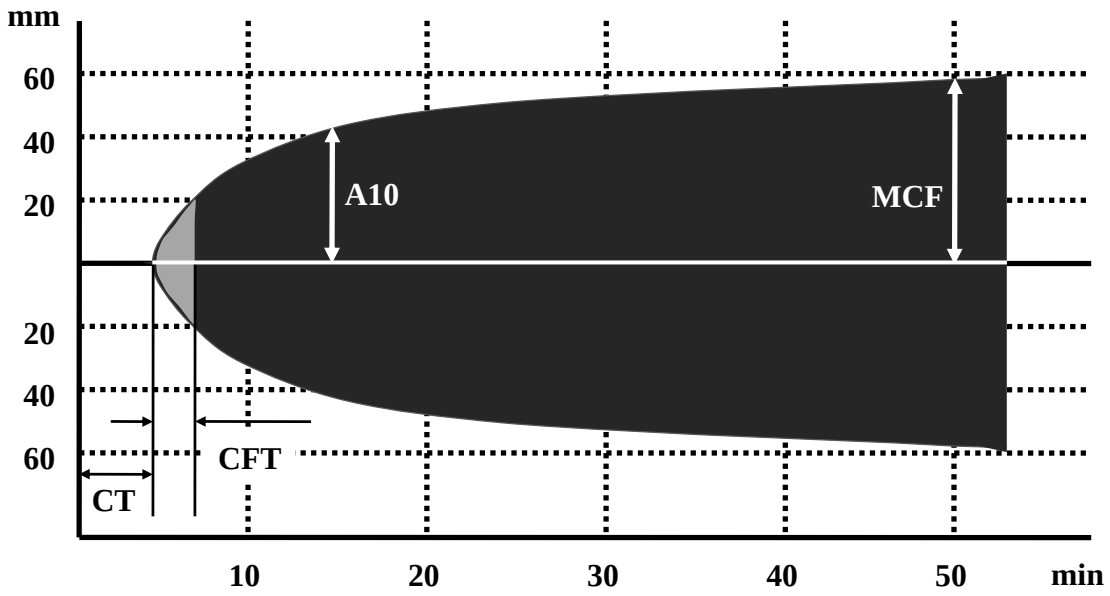

Fig. 1 Variables of rotation thromboelastometry (ROTEM). CT, clotting time (sec); CFT, clot formation time (sec); A10, amplitude at 10 minutes (mm); MCF, maximum clot firmness (mm).

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We performed the simulation supposing the cell salvaged blood transfusion during cardiac surgery using the CPB. The circulating blood volume (ml) of patients was calculated as 70 × body weight (Kg). The cell salvage ratio (%) was defined as the volume of salvaged transfused RBCs (mL) divided by circulating blood volume (mL). Regarding cell salvage ratio, we hypothesized the ratio to be 17% as a mock simulation of percent of cells salvaged from RBCs transfusion during standard cardiac surgery. The cell salvage device (Fresenius C.A.T.S *PLUS* Continuous Auto transfusion System Plus, TERUMO Co.ltd., Ann Arbor, MI, USA) was used entirely for surgical suction before and after CPB. Blood remaining in the CPB circuit was processed by the cell salvage device [3].

All measurements were performed using rotation thromboelastometry (ROTEM®, Tem International GmbH, Munich, Germany). Blood samples were taken from patient's arterial line one hour after the CPB and collected into citrate-containing tubes for the assessment of the following three sets of thromboelastometry analysis groups as the following: 1.0ml of control blood sample (Control group); 1.0ml of blood added with 0.2mL of salvaged RBCs (CS group); 1.0ml of blood added with 0.2ml of normal saline (NS group). Three hundred μL of each sample at the temperature of 37°C and recalcification by 0.2 mol/L $CaCl_2$ were analyzed using the INTEM test which reflects the ability of the intrinsic coagulation triggered by ellagic acid. The following variables were collected by INTEM analysis: clotting time (CT, sec) which corresponds to the lag time before clotting; clot formation time (CFT, sec); amplitude at 10 minutes (A10, mm) which reflects the amplitude at 10 minutes after CT; and maximum clot firmness (MCF, mm) as the maximal tensile strength of clot (Fig. 1)[6]. The other hematological values influencing hemostasis, such as blood hemoglobin, hematocrit, platelet number, serum fibrinogen, activated coagulating time, were corrected to normal range as closely as possible before collecting blood samples for the ROTEM® measurement.

Table 1. Patients Characteristics

Case	9
Age	68.6 ± 8.1
Sex (men/ women)	5 / 4
Body weight (Kg)	57.4 ± 9.9
Emergency operation	2
Extracardiac arteriopathy	2
Previous cardiac surgery	1
Chronic lung disease	0
Active endocarditis	0
Diabetes mellitus on insulin use	1
NYHA classification > 2	2
CCS class 4 angina	0
Left ventricular ejection fraction (%)	50.2 ± 13.8
Recent myocardial infarction	0
Pulmonary artery pressure (mmHg)	40.2 ± 21.6
Creatinine clearance (ml/min)	47.2 ± 19.4
Surgery on thoracic aorta	3
Logistic Euro II SCORE (%)	6.4 ± 4.3
Preoperative anticoagulants use	1
Preoperative antiplatelets use	1

NYHA, New York Heart Association; CCS, Canadian Cardiovascular Society grading of angina pectoris. Values are expressed as mean ± SD.

A repeated ANOVA following Bonferroni test was used for the analysis of the influences of CS or NS on ROTEM® variables. Data are expressed as mean ± standard deviation (SD) and $P<0.05$ was considered significant. All statistical analyses were performed using computer software Dr. SPSS II for Windows (SPSS, Inc., Chicago, IL, USA).

Results

The characteristics of the patients are shown in Table 1. Surgical data are described in Table 2. Cell salvaged RBCs were given as 19.8 ± 8.3% of their estimated circulating blood volumes.

No significant differences were observed in the clotting time (CT, sec) among the three groups (Control, 216 ± 33; CS, 252 ± 80; NS, 257 ± 66) (Table 3). Clot formation time (CFT/sec) in both the CS group (135 ± 48) and NS group (138 ± 51) was prolonged significantly more than that of Control group (103 ± 28). Amplitude at 10 minutes (A10, mm) in the Control group (50 ± 7) was within normal range, while A10 in the CS group (44 ± 7) and NS group (43 ± 8) were significantly narrower (Fig. 2). Maximum clot firmness (MCF, mm) in the CS group (55 ± 6) and NS group (53 ± 8) was also significantly narrower than that of Control group (59 ± 7).

Discussion

Historically, auto-transfusion has been associated with increased bleeding and coagulopathy which was the so-called salvaged-cell syndrome [7]. This has been addressed by modern cell-salvage and -washing systems, which effectively remove activated leukocytes, platelets, and inflammatory mediators from

Table 2. Operative details

Operative procedures (number)	
AVR	1
MVR + TAP	2
LVP + CABG	1
AVP + CABG	1
DVR + TAP	1
DVR + TAP + AAR	1
Hemiarch replacement	1
Total arch replacement	1
Operation time (min)	460 ± 97
Cardiopulmonary bypass time (min)	223 ± 42
Aortic cross clamp time (min)	141 ± 28
Lowest rectal temperature (℃)	30.9 ± 4.8
Intraoperative blood loss (ml)	1515 ± 1082
Preoperative estimated circulating blood volume (ml)	4015 ± 1509
RBCs salvage (ml)	813 ± 487
Cell salvage ratio (%)	19.8 ± 8.3

AVR, aortic valve replacement; MVR, mitral valve replacement; TAP, tricuspid valve replacement; LVP, left ventricular plasty; CABG, coronary artery bypass grafting; DVR, duoble valve replacement; RBCs, red blood cells. Values are expressed as mean ± SD.

Table 3. Thromboelastometry variables measured using the INTEM test

	Control	C S	N S
Clotting time (CT) (sec)	216 ± 33	253 ± 80	257 ± 66
Clot formation time (CFT) (sec)	103 ± 28	135 ± 48 *	138 ± 51 *
Amplitude at 10 minutes (A10) (mm)	50 ± 7	44 ± 7 *	43 ± 8 *
Maximum clot firmness (MCF) (mm)	59 ± 7	55 ± 6 *	53 ± 8 *

Values are expressed as mean ± SD. * $P < 0.01$ vs. Control

salvaged blood [8-10]. Concern remains, however, that even washed cells may have a coagulopathic effect. Similarly, retransfusion of washed salvaged RBCs from the residual extracorporeal circuit blood after the CPB has been shown to result in deranged coagulation on laboratory testing [9, 10]. A randomized study demonstrated that postoperative auto-transfusion of washed RBCs increased serum fibrinogen levels, as well as prothrombin and activated partial thromboplastin time (APTT), after coronary artery bypass grafting using the CPB [11]. In that study, Murphy et al. showed auto-transfusion was associated with a more significant derangement of APTT ratio than homologous blood: however, this was not associated with any clinical sequels after coronary artery bypass grafting using the CPB [11]. Klein et al. reported that CS group had the lower platelet count and fibrinogen level one hour postoperatively compared to the control group with direct transfusion of the blood remaining in the bypass machine tubing and reservoir, whereas there was no difference in postoperative bleeding between the two groups [12]. The larger dose of protamine required in the control group to neutralize

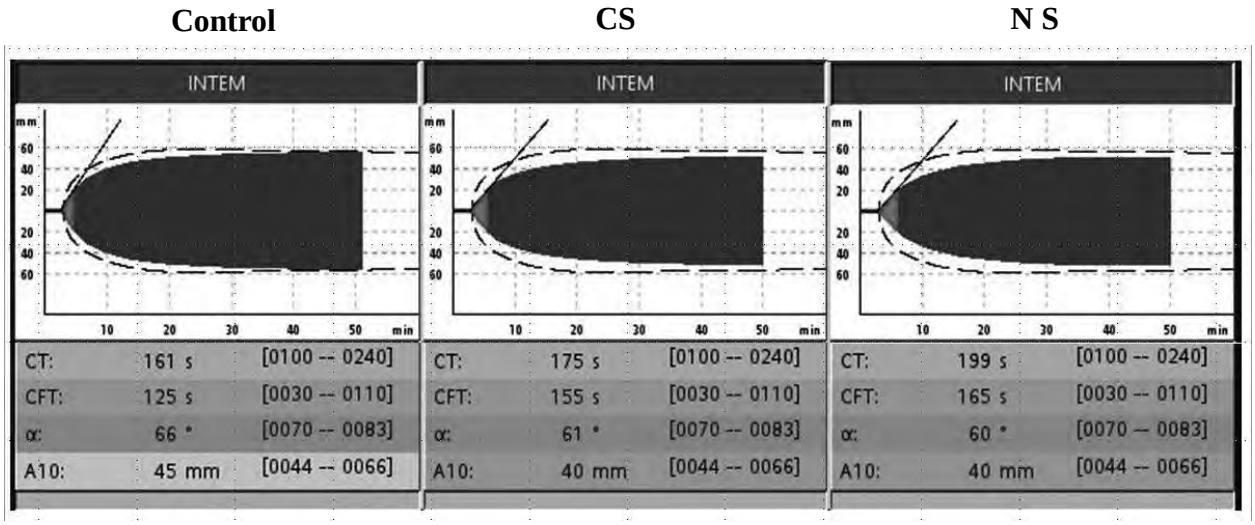

Fig. 2 An example of typical rotation thromboelastometry traces. Clotting time (CT) and clot formation time (CFT) in SC and NS were prolonged compared to those in Control, while amplitude at 10 minutes (A10) in SC and NS were narrower than that in Control.

heparin contained unprocessed blood from the CPB circuit [12]. These results appear to show the dilution by CS blood had an equivalent effect of remaining heparin in the unprocessed blood.

Some clinical studies have shown that a low fibrinogen concentration better predicts increased bleeding after prolonged CPB [13, 14]. In this ex-vivo study, we found that washed salvaged RBCs can potentially induce coagulopathy after cardiovascular surgery using the CPB. Although it will also be necessary to investigate whether this coagulopathy is caused by hemodilution or by the characteristic of washed salvaged RBCs itself, our current result showed equivalent changes after salvaged blood cells or saline. In terms of reducing risk of viral infection through the allogeneic blood transfusion, washed salvaged RBCs or crystalloid fluid infusion is safe. However, there is a potential of derangement of intrinsic coagulation pathway when crystalloid fluid is rapidly infused to correct circulatory blood loss. In such a case, simultaneous administration of colloid or fresh frozen plasma with RBCs will be recommended. We verified that patients had received cell salvaged blood equal to about 20% of patient's circulating blood volumes. In the case of massive bleeding, this ratio will become higher, and auto-transfusion of washed salvage RBCs may induce further coagulopathy. Klein et al. also showed hematological deterioration by cardiac surgery recovers over time in-vivo [12]. As previous reports had already shown, coagulopathy is different from bleeding and some in-vivo studies showed cell salvage and auto-transfusion was not associated with postoperative bleeding, because fibrinogen is an acute-phase protein, its level increases gradually after surgical procedures [13].

In the case in which a small volume of washed salvaged RBCs is given, the coagulopathy may be corrected by reduced concentration of coagulation factor resulting from urine outflow and self-replenishment of clotting factors. It has also been shown that reduced perioperative urine output was an important factor of mortality in the patients with massive hemorrhage [15]. Based on those findings, we should change the way to use salvaged blood after completion of hemostasis.

Limitations

In this study, we did not evaluate the influence of allogeneic RBCs transfusion on the blood coagulation system after the CPB. In cardiac surgery, auto-transfusion of washed salvage RBCs is safe in terms of avoiding viral infections that may occur by allogeneic blood transfusion, but there are many situations to use allogeneic blood product. So, we should have better to test and compare if allogeneic blood impairs the coagulation system as likely as washed salvage RBCs or saline.

Conclusion

This ex-vivo study using ROTEM suggested that the perioperative administration of washed salvaged RBCs might have a potential to induce coagulopathy in cardiac surgery using the cardiopulmonary bypass. Care should be taken to use washed salvaged RBCs before hemostasis.

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