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The *In vitro* Effects on Platelet Functions and Coagulation of Different Prime Solutions Used in Cardiopulmonary by Pass

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Authors' contributions

This work was carried out in collaboration between all authors. Author OB designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author FS managed the literature searches, analyses of the study and author CK managed the clinical process of study. All authors read and approved the final manuscript.

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ABSTRACT

Purpose: To investigate the in vitro effect of human albumin (HA) and fresh frozen plasma (FFP) added to prime solution on platelet functions and coagulation in patients undergoing cardiopulmonary bypass (CPB) surgery.
Methods: Sixty consecutive patients receiving elective cardiopulmonary bypass with open heart surgery were enrolled in the study. Patients were divided into three equal groups. Group 1: with 2 units of fresh frozen plasma added to prime solution. Group 2: With 100 cc 25% human albumins added to prime solution. Group 3: (control group) with no FFP or HA added to prime solution. PFA-100 platelet function analyzer and platelet aggregation tests were investigated pre-induction, during and after CPB and on the 1st day postoperatively.

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Results: Postoperative drainage was significantly higher in groups 2 and 3 compared to Group 1 ($p<0.01$). The compromise in platelet functions in groups 1 and 2 improved, while in Group 3 preoperative values were not attained at the end of the 1st day postoperatively. There was a significant difference between groups 2 and 3 in terms of erythrocyte suspension (ES) used in intensive care ($p<0.01$). Greater hemorrhage occurred in the postoperative period in Group 3 and more ES was used. In addition, lengths of stay in intensive care differed significantly between groups 2 and 3 ($p<0.01$).
Conclusion: FFP used in CPB causes reduced drainage in the postoperative period and necessitates less use of blood and blood products.

Keywords: Cardiopulmonary bypass; fresh frozen plasma; human albumin; platelet function analyzer.

1. INTRODUCTION

One of the most important complications of cardiopulmonary bypass (CPB) is intraoperative and postoperative bleeding. Disposition to bleeding occurs for reasons such as platelet function defect, coagulation activation despite heparin, inactivation of coagulation factors and fibrinolysis associated with facilitated release of tissue type plasminogen activator. Re-exploration due to bleeding is reported at levels of 3-14%, with a mean of 6.2% [1,2]. CPB can alter the normal hemostatic balance. In addition to the effects of hypothermia and hemodilution, activation of the fibrinolytic and inflammatory pathways can also lead to postoperative bleeding and other potential complications. The emphasis has been on pharmacological precautionary measures for postoperative bleeding due to CPB. Platelet function defect has been investigated with the PFA-100 (platelet function analyzer) system in several studies [3,4].

PFA-100 is an in vitro system developed by Kratzer and Born in order to identify platelet function defect. Platelet function in anticoagulated full blood makes it possible to obtain a quantitative measurement. The PFA-100 system determined time from beginning of the test to platelet occlusion [5].

The aim of this study is to investigate the in vitro effects of human albumin (HA) and fresh frozen plasma (FFP) added to prime solution on platelet functions and coagulation in patients undergoing cardiopulmonary bypass surgery.

2. MATERIALS AND METHODS

Sixty consecutive patients receiving elective CPB with open heart surgery at the cardiovascular surgery department and with no exclusion criteria were enrolled in the study with ethical committee approval. Patients receiving emergency surgery or reoperations, aged 75 or over, with left ventricular ejection fractions of 35% or less, with a history of unstable angina pectoris, requiring additional intra- or extra-cardiac, or with peripheral artery disease or chronic obstructive pulmonary disease, with myeloproliferative disorders, blood diseases such as thrombocytopenia, recent blood transfusion were excluded from the study. Patients enrolled were informed about the study and gave written consent. Patients were divided into 3 equal groups (n=20) such as to result in equivalence among preoperative data.

Group 1: Twenty cases with 2 units of FFP added to prime solution
Group 2: Twenty cases with 100 cc 25% HA added to prime solution.
Group 3: Twenty cases (control group) with no FFP or HA added to prime solution.

Surgery commenced with median sternotomy, and anticoagulation was induced with 350 iu/kg heparin. Cannulation for CPB began when activated coagulation time (ACT) rose 4 times or more above basal value (> 400 s). Heparin neutralization in patients weaned from CPB was established with 1.2 iu protamine for 1 iu heparin. Additional doses of protamine were administered when required on the basis of ACT values. Blood samples were collected from a peripheral vein from all patient before induction (phase1), during CPB (phase2), after CPB (phase3) and on the 1st postoperative day (phase4) for hemoglobin (Hb), hematocrit (Htc), white cell, platelet number, aPTT, prothrombin time (PT), international normalized ratio (INR) and platelet aggregation tests (Col/ADP and Col/epi). Patients were monitored in intensive care in the first 6 hours, and then at the 6th, 12th and 24thg hours. Levels of blood draining from mediastinum and chest tubes, levels of FFP, erythrocyte suspension (ES) and platelet suspension (PS) and HA given in intensive care,

length of time on mechanical ventilation and length of stay in intensive care.

2.1 Statistical Analysis

All statistical calculations in this study were performed on SPSS for Windows Version 11.5.0 software (SPSS Inc., Chicago, IL, USA). Constant variables were normally distributed according to the Kolmogorov-Smirnov compatibility with normal distribution test. ANOVA was therefore used to compare constant variables between groups, the Scheffe test was used for post-hoc two-way comparison of variables with homogeneous variations and the Tamhane test for variables with non-homogeneous variations. The chi square test was used to compare descriptive variables between the groups. Constant variables were expressed as mean and standard deviation and descriptive variables as number (n) and percentage (%). A p value less than 0.05 was regarded as significant.

3. RESULTS

The mean ages, gender distribution, body surface area, duration of cross clamp, duration of CPB and heparin protamine doses of the groups in the study are shown in Table 1. Blood and blood products used in postoperative intensive care are given in Table 2, recorded Hb, white cell and platelet values in Table 3, and aPTT, INR, Col/ADP and Col/epi values in Table 4. There was no statistically significant difference between the groups in terms of demographic characteristics ($p>0.05$). Group 1 consisted of 14 male and six female patients with a mean age of 53.92±21.29 years. Mean duration of CPB was 103.15±37.84 min. Pleura were opened in 16 patients after median sternotomy and during surgery, but not in the other four patients; 1.80±0.81 units of ES, and 4.75±2.39 units of FFP were used in postoperative intensive care. Eleven of the 20 patients in Group 2 were men and 9 were women, with a mean age of 41.95±24.49 years. Mean duration of CPB was 113.66±45.88 min. Pleura were opened in 10 patients after median sternotomy and during surgery, but not in the remaining 10; 1.25±0.83 units ES and 3.50±1.67 units FFP were used in postoperative intensive care. Twelve of the 20 patients in group 3 were men and 8 were women, with a mean age of 53.47±20.28 years. Mean duration of CPB was 138.42±50.26 min. Pleura were opened in 17 patients after median sternotomy and during surgery, but not in the remaining 3; 2.28±0.91 units ES and 3.80±0.78 units FFP were used in postoperative intensive care. This study investigated postoperative effects on coagulation and platelet functions of FFP and HA added to prime solution during CPB in patients undergoing open heart surgery; no difference was determined between groups 1, 2 and 3 in terms of age, gender, body surface area or duration of CPB. There was a statistically significant difference between groups 1 and 3 in terms of ES use in blood and blood products used in intensive care ($p<0.01$). There was no statistically significant difference between the three groups in terms of Hb, Htc and platelet numbers in all measurement phases ($p>0.05$). A significant difference occurred in platelet numbers and functions from beginning of CPB in all groups. Preoperative platelet numbers were not achieved in the early period in any of the groups. A significant compromise of platelet functions was observed during CPB in all three patient groups. However, the impairment of platelet functions in groups 1 and 2 improved in the early period, while in Group 3, preoperative values were not achieved at the end of the 1st postoperative day. Postoperative drainage levels were significantly higher in groups 2 and 3 compared to Group 1. A significant difference in terms of ES used in intensive care was determined between groups 2 and 3. Amount of drainage (amount of post-operative bleeding) was significantly lower in Group 1 than in groups 2 and 3. There was no difference between the groups in terms of time spent on mechanical ventilation in postoperative care. Length of time in intensive care differed significantly between groups 2 and 3. Col/epi values reached preoperative values after CPB in the HA and FFP groups, but this was only achieved in 1. day postoperatively in the control group. Col/ADP values in groups 1 and 2 returned to normal immediately after CPB, but had still not returned to normal at the end of the 1st postoperative day in the control group.

4. DISCUSSION

Various additional techniques and procedures have been shown to be quite useful in reducing coagulation impairments arising in CPB to a minimum. These include reducing fibrinolysis using a centrifugal pump instead of a roller pump, reducing complement activation and platelet breakdown using heparin-coated CPB systems and using heparin in lower doses and reducing dilution-related coagulopathy with modified ultrafiltration. Additionally, greater endothelial injury and platelet aggregation and

greater compromise of platelet aggregation have been shown in hypothermic CPB compared to normothermic [6-8]. The primary cause in the majority of bleeding is hemostasis deficiency. Diffuse systemic bleeding may also occur in some patients due to acute acquired hemostatic defect. The basic pathophysiology that compromises hemostasis is changes that cannot be controlled with CPB. These may be associated with surgical procedure, type of anesthesia, drugs used, blood products transfused, hypothermia, hemodilution and oxygenator type. Common anomalies are excess heparin and protamine, heparin rebound, low platelet number, platelet function defect, low fibrinogen and deficiency of other coagulation factors, primary fibrinolysis and disseminated intravascular coagulation. The most common cause of hemostasis anomaly among these is compromise of platelet functions [9-10]. A decrease in postoperative blood loss and transfusion requirement has been observed in heart surgery in recent years. This is due to use of fibrinolysis inhibitors together with intraoperative blood protection systems [11-12]. Variation in hemostatic changes such as hyperfibrinolysis associated with facilitated release of tissue type plasminogen activator (t-PA) has also been reported. Increased fibrinolytic activity during CPB is associated with a rise in t-PA. This leads to a greater disposition to bleeding [13-14]. Tigchelaar et al. investigated the effects on hemostasis of human albumin and non-natural plasma expanders with the PFA-100 system used in the current study. They showed that one of the non-natural expanders had similar effects to albumin while the other had adverse effects on hemostasis [15]. We did not use hydroxyethyl starch and similar artificial colloids, whose effects on bleeding were already known. Significantly less postoperative drainage occurred in the HA and FFP groups compared to the control group. Lasne et al. showed that platelet dysfunction was a cause of major bleeding in 146 patients undergoing CABG surgery using the PFA-100 system. The PFA-100 device is a system that best replicates injury in the vascular wall in vitro and that includes a biologically active membrane. Potential screening for platelet dysfunction using the PFA-100 system in patients at risk of bleeding in open heart surgery has acquired considerable importance [5]. In our study, too, platelet counts falling rapidly at the beginning of CPB remained below preoperative values in all remaining measurement phases. Platelet numbers being affected by CPB in this way is an important factor in development of postoperative coagulopathy, together with platelet function loss, consumption of coagulation factors through dilution and/or microvascular thrombosis and increased fibrinolytic activity [16-18]. Michelson et al. showed that prolongation of closure times identified using the PFA-100 system after hypothermic CPB led to hypothermia-induced platelet dysfunction [19]. Intraoperative hematocrit values in 74 patients undergoing CABG reached preoperative values using autologous erythrocyte suspension, but this was insufficient to improve prolonged closure times. This showed that platelet dysfunction associated with CPB following heparin neutralization contributes to increasing PFA-100 values [20]. Most studies investigating the effects on hemostasis of artificial colloids used in prime solutions have compared these with HA.

Table 1. Demographic and medical characteristics of patients

	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)
Mean age (years)	53.9±21.2	41.9±24.4	53.4±20,2
Sex (m/f)	14/6	11/9	12/8
BSA	1.7±0.3	1.6±0.4	1.6±0.4
Duration of CPB (min)	103.1±37.8	113.6±45.8	108.4±50.2
Heparin dose (u)	23493.3±10079.2	23203.3±6978.0	24792.8±3963.0
Protamine dose (u)	33005.0±7729.4	32000.0±8462.1	34500.0±5210.9

BSA: Body surface area, CPB: Cardiopulmonary by pass

Table 2. Blood and blood products used in postoperative intensive care

	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)
ES(u)	1.8±0.8	1.2±0.8	2.2±0.9*
FFP(u)	3.7±2.3	3.5±l.6	3.8±0.7
HA(u)	0.0±0,0	0.6±0.2	0.1±0.3

*ES: Erythrocyte suspension, FFP: Fresh frozen plasma, HA: Human albumin. * p<0,05*

Table 3. Hb, white blood cell and platelet values recorded in the study

		Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)
Hb	phase1	13.1±1.8	12.8±1.7	13.9±1.8
	phase2	8.1±0.9	7.9±0.6	7.9±0.7
	phase3	11.9±1.2	11.7±1.1	11.3±1.3
	phase4	11.5±1.1	11.6±1.3	11.9±0.8
Wbc	phase1	6981.3±2411.6*	10603.3±4039.9	8962.8±3401.9
	phase2	10525.6±21097.2	7209.3±4147.5	6727.8±3338.4
	phase3	12484.0±5148.7	12808.0±4825.6	11787.8±3069.6
	phase4	11153.3±3684.1	13400.6±3631.1	13740.7±3048.3
Plt	phase1	235266.6±62521.3	279133.3±86939.1	240785.7±73551.8
	phase2	111333.3±25750.6	116866.6±50264.8	116000.0±31189.2
	phase3	133266.6±34763.4	134733.3±54205.2	127428.5±39769.9
	phase4	120000.0±25991.7	136000.0±59505.3	119071.4±36686.7

*Phasel: Pre-induction, phase2: During cardiopulmonary by pass, phase3: Post-CPB, phase4: Postoperative 1st day. Hb (gr/dl): Hemoglobin, Wbc: White blood cell, Plt: Platelet. *p<0.02*

Table 4. aPTT, INR, Col/ADP and Col/epi values

		Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)
aPTT(sec)	phase1	28.5±4.3	31.9±5.5	29.9±3.5
	phase2	150.0±0.0	150.0±0.0	150.0±0.0
	phase3	32.3±6.3	31.3±4.1	29.9±3.5
	phase4	31.9±3.6	29.7±4.6	31.6±4.1
INR	phase1	1.0±0.1	1.1±0.1	1.1±0.1
	phase2	2.2±0.3*	1.7±0.4	1.8±0.6
	phase3	1.2±0.1	1.2±0.2	1.2±0.1
	phase4	1.1±0.1	1.2±0.1	1.2±0.9
Col/ADP	phase1	88.0±19.4	89.8±14.6	98.5±23.9
	phase2	191.2±51.0*	158.2±49,3	158.0±36.4
	phase3	119.8±52.5	106.4±24.6	124.8±28.6
	phase4	97.8±32.7	100.0±23.0	120.4±35.8
Col/epi	phase1	116.5±56.0	111.8±21.2	120.4±35.8
	phase2	213.8±54.0	201.1±57.0	190.0±57.1
	phase3	153.0±60.9	130.7±50.1	164.3±52.0
	phase4	135.3±70.1	134.0±42.0	143.8±55.9

*aPTT: Activated partial thromboplastin time, INR: International normalized ratio, Phase 1: Pre-induction, phase 2: During CPB, phase3: Post-CPB, phase 4: Postoperative 1st day. *p<0.04*

We obtained significantly better results in the FFP group compared to the albumin group. In our study, and in agreement with the literature, there was a decrease in platelet numbers and a significant worsening in platelet functions in all three groups following CPB and full-dose heparin. As also shown in several studies, we attribute this to low hematocrit values related to low hematocrit levels associated with hemodilution, high-dose heparin, hypothermia and the injurious effect of CPB.

5. CONCLUSION

Platelet dysfunction resolved in the early postoperative in the FFP and HA groups. Patients can leave intensive care earlier due to reduced bleeding.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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