Does Plasma Sequestration Reduce Post-Operative Bleeding?

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ABSTRACT

Hematologic profiles, postoperative blood loss and transfusion requirements were studied in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) in order to determine the effect of sequestration and reinfusion of platelet-rich plasma (PRP). Eighteen patients were randomly selected to have approximately one unit of PRP collected or not prior to CPB. Autologous PRP was reinfused in nine patients after reversal of anticoagulation. Evaluation of results was based upon thrombocyte counts, Hgb, Hct, and platelet index readings pre- and post-PRP reinfusion, amount of blood loss and volumes of homologous blood and banked blood products required postoperatively. There were no hemodynamic complications related to the sequestration process. PRP reinfused patients had significantly higher thrombocyte counts after reversal of anticoagulation ($p < 0.05$). Patients receiving PRP required approximately 2/3 less banked blood products, their bleeding being significantly reduced 24 hours postoperatively ($p < 0.05$). We concluded that plasma sequestration and reinfusion of autologous PRP post-bypass may serve as an effective and safe way in decreasing blood loss after cardiac operations necessitating extracorporeal circulation.

INTRODUCTION

Patients undergoing cardiac surgeries with cardiopulmonary bypass (CPB) are prone to developing serious hemorrhagic diathesis. Incomplete neutralization of heparin, [1,2] decreased plasma coagulation factors,[3,4] and excessive fibrinolysis,[3-6] remain some of the major derangements seen after CPB. Platelet activation and consumption during CPB, causing postoperative thrombocytopenia as well as platelet dysfunction, are recognized as strong factors which induce bleeding after cardiac surgery.[8,9] Methods for preparation and storage of homologous platelets are available, however, their randomly or selectively matched transfusion for correction of post-bypass bleeding is not without risks. Complications of platelet transfusions are similar to those of red cells. In addition, alloimunization to histocompatibility antigens and refractoriness to random donor platelets may occur.[10] Transfusion-transmitted diseases,[11,12] bacterial contamination,[13] non-hemolytic transfusion reactions,[14] graft-versus-host disease[15] and significant cost are only some of the potential problems related to platelet replacement from banked sources. In the era whereby concerns that demand of blood products may exceed the supply, interest in finding ways to counteract platelet disturbances after CPB has increased. Used initially in community blood centers to collect plasma, the Haemonetics Plasma Saver System has evolved as an instrument to collect Platelet-Rich Plasma (PRP) prior to CPB. When reinfused after anticoagulant neutralization, PRP may contain important clotting elements lost during CPB, their replacement resulting in improved hemostasis and a significant reduction of blood loss.

We undertook this study to establish the effects of platelet sequestration and reinfusion of PRP on hematologic parameters, postoperative blood loss and transfusion requirements following cardiac surgery with extracorporeal circulation.

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MATERIALS AND METHODS

This randomized prospective study was performed on 18 adult patients undergoing elective cardiac surgeries at Cooper Hospital/University Medical Center in Camden, New Jersey. The patients included in this study did not take any antiplatelet drugs for at least one week prior to their operation. All patients received standard anesthesia induced with fentanyl, ethomidate and pancuronium, maintained with oxygen and nitrous oxide, and supplemented with fentanyl. Ventilation with 100% oxygen was adjusted to maintain normocarbia. Standard CPB was instituted in all patients using a roller pump [a] with PVC tubing. Heparin (3mg/kg) was used prior to cannulation and activated clotting time (ACT) was frequently checked to measure the level of anti-coagulation. ACT was maintained over 480 seconds during CPB by supplemental heparin. CPB was conducted under moderate hypothermia. Non-pulsatile flow between 2.1 L (hypothermia)

and 2.4 L/min./m (normothermia) was employed. The extracorporeal circuit was primed with 2 liters of Ringer's injection[b] and 150 cc of 25% albumin.[c] One ampule of $NaHCO_3$ and 5,000 units of heparin were also added. A membrane oxygenator[d] was employed in all cases.

All patients signed a consent form to participate in the study, which was approved by the Institutional Review Board. In nine randomly selected patients, Haemonetics Plasma Saver System[e] was used to remove PRP. After induction of anesthesia, the amount of PRP removed was in accordance with the patient's weight, body build and hematocrit level. Blood was drawn from a large gauge internal jugular catheter.[f] Fluid was given prior to starting the collection of PRP and sufficient volume replacement (lactated Ringer's solution) was infused during the collection to replenish the lost volume. Haemonetics Plasma Saver System is composed of a blood pump, centrifuge assembly programmable to variable rates, red cell detector, anticoagulant pump, plasma weigher, four air detectors and a sensor to monitor the blood pressure. In accordance with the manufacturer's instructions, the actual centrifuge rate used in this study was 3000 rpm. Centrifugation of the PRP at 4000 rpm for 10 minutes results in platelet injury, while, 3000 rpm maintains platelet viability. PRP was collected, properly labeled, placed on a "rocker" and stored at room temperature. Blood pressure was regulated with sodium nitroprusside.

Upon completion of CPB, heparin was antagonized with protamine sulfate (1:1 with heparin), half given as bolus and half as a continuous infusion. When ACT returned to, or near baseline level, PRP was reinfused to the patient. Blood samples for hematological measurements were collected by venipuncture preoperatively (baseline), during CPB at hypothermia (28°C) and normothermia (34°C), 15 minutes upon termination of CPB (after PRP was reinfused), and at 24 and 48 hours postoperatively. Hematological assessments consisted of platelet counts, hemoglobin and hematocrit measurements and were assessed by standard laboratory techniques. A platelet index was calculated by dividing the platelet count by the hematocrit value. Postoperative blood loss was estimated by the volume of blood present in the chest drainage reservoir from the time of chest tube insertion intraoperatively through the first 48 hours. The content of the oxygenator was returned to each patient at the end of the procedure as centrifugated blood. Transfusion amounts of banked whole blood, packed red cells (PRC) and fresh-frozen plasma (FFP) were also recorded. No intra- or post-operative deaths occurred. All patients had an uneventful hospital course. In both groups, reoperation for bleeding was not necessary. SPSS PC Plus (version 2.0) was used for statistical analysis. Paired t-test and analysis of variance (ANOVA) were used where appropriate for comparison between variables at specific times. Values are presented as mean standard deviation. A p value of < 0.05 was considered statistically significant.

a. COBE Laboratories, Lakewood, CO
b. and c. Travenol Laboratories, Deerfield, IL
d. COBE Laboratories, Lakewood, CO
e. Haemonetics Corporation, Braintree, MA
f. Arrow International, Reading, PA

RESULTS

The demographic and hematologic profiles were similar between the two groups (**Tables 1 and 3**). The intraoperative conditions, including the time on CPB and the surgical procedures performed, were also comparable (**Tables 2 and 3**). During CPB, hypertension was controlled with sodium nitroprusside or phenylephrine and mean arterial pressure was maintained between 60 and 85 mm Hg. There were no major hemodynamic reactions to jeopardize or interrupt the collection of PRP. In both groups, CPB produced a statistically significant reduction in platelet count, Hct. and Hgb. level (**Table 1**). Significantly low hematological values were observed up to 48 hours postoperatively. In the control group, the platelet count decreased further after reversal of heparin with protamine sulfate (post-bypass); this was not seen in the PRP- reinfused group ($p < 0.05$). Twenty-four and 48 hours after surgery, platelet counts in the two groups were not statistically different (122.8 ± 70.9 vs 127.8 ± 31.5 and 128.4 ± 36.8 vs 131.6 ± 30.2 respectively, $p > 0.05$). At all times, comparison of the platelet index between the two groups did not reach statistical significance (**Table 1**). Comparable amounts of centrifuged blood retrieved from the oxygenator circuit were reinfused in both groups ($p > 0.05$). The plasma sequestrated group received a mean of 220.8 ± 61 ml PRP (range 121 to 300 ml.) representing 9% of the total platelet volume. The control group received none. However, there was a striking difference in blood loss between the control and PRP-reinfused group over the first 24 hours (630.0 ± 78.5 vs 407.8 ± 87.7 ml., $p < 0.05$). During the second postoperative day the differences in blood loss between the two groups did not reach statistical significance. However, PRP-reinfused patients had 25% less total blood loss at the end of 48 hours postoperatively (963.3 ± 80.5 vs 718.9 ± 99.0, $p < 0.05$). The need for transfusion of banked blood products was significantly different. Patients in the control group received 65% more blood products given as FFP and PRC than those receiving autologous PRP (total 24 units of FFP plus 15 units of PRC vs four units of FFP plus 12 units of PRC); 9,750 ml of blood products were used in the control group and only 3,500 ml were used in the PRP-reinfused group ($p < 0.05$).

DISCUSSION

The hemostatic alterations observed after CPB, explained in part by the hemodilution or displacement of the coagulation fibrinolysis balance toward a tendency of hemorrhage, is especially considered to be the effect of real depletion of the coagulation cascade due to trauma to the blood components and plasma denaturation. Triggered by the contact of blood elements with the extracorporeal circuit, this process may result in disseminated intravascular coagulation (DIC) and the activation of vasoactive kinines and complement systems [16-18]. Activation of the Hageman Factor XII commonly initiates the body's stress reaction system (coagulation, fibrinolysis, complement and kinine activation).[19] CPB produces thrombocytopenia which persists for several days post-operatively.[8,20] A loss of platelet function characterized by increases in plasma thromboxane B [21,22] plasma, beta-

TABLE 1

CHARACTERISTICS OF PLASMA SEQUESTRATED GROUP VS. CONTROL

Characteristics	Base Line	Bypass	
		Cold ($28^{o}C$)	Warm ($34^{o}C$)
Platelets Count ($x10^3$)			
Control Group	253.7 ± 65.9	118.3 ± 38.1	139.3 ± 46.2
PS Group	235.2 ± 64.1	93.1 ± 27.9	129.9 ± 36.5
Significance**	NS	NS	NS
Hematocrit (%)			
Control Group	33.2 ± 4.3	19.0 ± 2.8	20.1 ± 2.6
PS Group	34.3 ± 4.2	19.7 ± 2.5	21.7 ± 2.5
Significance**	NS	NS	NS
Hemoglobin (g/dl)			
Control Group	11.4 ± 1.5	6.5 ± 0.8	6.8 ± 0.9
PS Group	11.5 ± 1.5	6.7 ± 0.8	7.3 ± 0.7
Significance**	NS	NS	NS
Platelet Index ($x10^3$)			
Control Group	7.8 ± 2.3	5.9 ± 1.6	6.9 ± 1.9
PS Group	6.9 ± 2.1	4.3 ± 0.5	6.0 ± 1.3
Significance**	NS	NS	NS

Characteristics	Post Bypass (15 min.)	Postoperative Day 1	Day 2	Significance*
Platelets Count ($x10^3$)				
Control Group	95.4 ± 15.6	122.8 ± 70.9	128.4 ± 36.8	$p < 0.05$
PS Group	135.7 ± 28.1	127.8 ± 31.5	131.6 ± 30.2	$p < 0.05$
Significance**	$p < 0.05$	NS	NS	
Hematocrit (%)				
Control Group	22.5 ± 4.4	25.8 ± 3.2	27.8 ± 3.3	$p < 0.05$
PS Group	22.3 ± 1.9	26.4 ± 1.2	26.6 ± 3.0	$p < 0.05$
Significance**	NS	NS	NS	
Hemoglobin (g/dl)				
Control Group	7.5 ± 1.3	8.8 ± 1.0	9.5 ± 0.8	$p < 0.05$
PS Group	7.3 ± 0.6	8.9 ± 0.6	9.1 ± 0.9	$p < 0.05$
Significance**	NS	NS	NS	
Platelet Index ($x10^3$)				
Control Group	5.4 ± 1.1	6.3 ± 2.3	4.7 ± 1.6	
PS Group	6.0 ± 0.8	4.8 ± 1.1	4.2 ± 1.3	
Significance**	NS	NS	NS	

PS = plasma sequestrated; All values are mean ± standard deviation;
NS = not significant *ANOVA = between values in the same group
S**= t-test between mean values of the 2 groups

TABLE 2

TYPE OF OPERATIONS PERFORMED

TYPE OF OPERATION	NO. OF PATIENTS	
	CONTROL (%)	PLASMA SEQUESTRATION (%)
CABG	5 (55.6%)	4 (44.5%)
Valve Replacement (VR)	2 (22.2%)	3 (33.3%)
Combined Procedures*	2 (22.2%)	2 (22.2%)
TOTALS	9	9

CABG - Coronary Artery Bypass Grafting

* CABG plus VR

TABLE 3

GENERAL CHARACTERISTICS

Variable	Control (n=9)	PS (n=9)	Significance
AGE (yr)	58.2 ± 3.4	59.0 ± 1.8	NS
BSA (m^2)	1.9 ± 0.9	1.9 ± 0.2	NS
Perfusion Time (min.)	128.4 ± 4.5	125.8 ± 6.8	NS
Blood Loss (ml)			
Postop Day 1 (0-24 hrs.)	630.0 ± 78.5	407.8 ± 87.7	$p < 0.05$
Postop Day 2 (24-48 hrs.)	344.4 ± 72.3	311.1 ± 37.6	NS
TOTALS	963.3 ± 80.5	718.9 ± 99.0	$p < 0.05$
Blood Received			
Centrifugated Blood (ml)	525.0 ± 50.0	542.0 ± 35.0	NS
Autologous PRP (ml)	----------	220.8 ± 61.0	
Bank Blood (units)	24 FFP + 15 PRC	4 FFP + 12 PRC	$p < 0.05$
Total Bank Blood (ml)	9,750	3,500	$p < 0.05$

Values are presented as mean ± standard deviation

NS = Not Significant PS = Plasma Sequestrated Group

thromboglobulin and platelet factor IV, [8,21] and decreases in platelet beta-thromboglobulin and fibrinogen will occur. [4] Additionally, selective loss of alpha-granules,[8] reduction of thrombocyte aggregability,[3,23,24] and their adhesiveness[25] and increased bleeding time[8] have been extensively related to extracorporeal circulation. Also, neutralization of heparin with protamine sulfate at the completion of CPB produces a damaging dose-dependent effect on platelets.[26-28] In order to reverse some of the derangements seen after CPB and to try to restore clotting factors to levels required for normal hemostatic response, homologous banked blood products have been extensively used.[8,29-32] There are, however, potential complications related to crossmatching errors, allergic reactions, isoimmunization and contamination. Also a significant reduction of blood banked products have been signaled during recent years.

This study was initiated to assess the efficacy and safety of a commercially available Plasma Saving System. The Haemonetics Plasma Saver System has the capability to sequestrate a certain amount of PRP prior to the initiation of CPB and to reinfuse it after completion of heparin neutralization and to return it into the circulation as non-altered, viable thrombocytes dispersed into physiologically normal plasma. Our study demonstrated that sequestration of 9% of the total platelet volume does not produce any additional significant alterations of the hematologic course during CPB. Reinfusion of autologous PRP significantly increased thrombocyte count levels post-bypass, a time when further alteration in coagulation profile induced by heparin neutralization with protamine sulfate may eventually produce diffuse bleeding. There are several possible reasons for improved platelet function after administration of autologous PRP. First, PRP contains thrombocytes which are not damaged by storage as are those in banked platelet concentrates.[33-35] Second, fibrinogen, factor V and VIII (von Willebrand) which are decreased during CPB, and are inactive or destroyed in banked blood products [36] but fully active in freshly collected PRP, may be responsible for the better hemostatic effect of reinfused-PRP. Improvement in the post-bypass platelet counts with normal functional quality made possible a significant reduction in post-CPB bleeding at the end of 24 and/or 48 hours. In our study, patients receiving autologous PRP required 65% less homologous blood to control postoperative bleeding, thereby being exposed to considerably less risk of transfusion-transmitted diseases. In summary, our observations demonstrated that reinfusion of autologous PRP may significantly reduce the need for homologous blood requirement following CPB in a safe and tolerable manner.

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