Koroner Arter Bypass Cerrahisinde Akut Normovolemik Hemodilüsyonun Koagülasyon, Fibrinolitik Sistem, Protein C ve Protein S Üzerine Etkisi

THE EFFECT OF ACUTE NORMOVOLEMIC HEMODILUTION ON COAGULATION, FIBRINOLYTIC SYSTEM, PROTEIN C AND S IN CORONARY ARTERY BYPASS SURGERY

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Özet

Amaç: Kardiyopulmoner bypass (KPB) kullanılarak yapılan kalp cerrahisi, hemostatik sistemde çeşitli değişikliklere yol açar. Bu çalışmada koroner arter cerrahisi uygulanan hastalarda akut normovolemik hemodilüsyonun koagülasyon, fibrinolitik sistem ve protein S ve C gibi fizyolojik antikoagülanlar üzerine etkisini inceledik.
Materyal ve Metod: Akut normovolemik hemodilüsyon uygulanarak (Grup 1, n = 20) ve uygulanmayarak (Grup 2, n = 21) koroner arter cerrahisi yapılan 41 hasta çalışmaya alındı. Aktive edilmiş pıhtılaşma zamanı, protrombin zamanı, aktif parsiyel tromboplastin zamanı, fibrin yıkım ürünleri (d-dimer), fibrinojen, protein C ve S ile trombosit sayıları ölçüldü. Kan örnekleri anestezi indüksiyonunu takiben KPB'nin 20. dakikası, protamin uygulamasından 30 dakika sonrası ve KPB'den çıkıldıktan 24 saat sonra alındı.
Bulgular: Her iki grupta preoperative değerlerle kıyaslandığında diğer örneklerde d-dimer değerleri yüksek, protein C ve trombosit sayıları anlamlı derecede düşük bulundu. Gruplar arasında aktive pıhtılaşma zamanı, protrombin ve aktif parsiyel tromboplastin zamanları, d-dimer, fibrinojen, protein C ve protein S değerleri açısından farklılık tespit edilmedi. İki grup arasında kan ve kan ürünleri transfüzyonu ve drenaj miktarı açısından fark bulunmadı.
Sonuç: Koroner arter cerrahisinde akut normovolemik hemodilüsyon kan ve kan ürünleri ihtiyacını azaltmamakta ve hemostatik sistemdeki değişiklikleri etkilememektedir.

Anahtar kelimeler: Koroner bypass, hemodilüsyon, kan transfüzyonu, koagülasyon

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Summary

Background: Cardiac surgery performing with cardiopulmonary bypass (CPB) causes multiple alterations in the hemostatic system. In this study, we investigated the effect of acute normovolemic hemodilution on coagulation, fibrinolytic system and physiologic anticoagulants such as protein C and S among patients who underwent coronary artery bypass grafting (CABG).
Methods: Forty one patients who underwent CABG with (Group 1, n = 20) and without (Group 2, n = 21) acute normovolemic hemodilution were enrolled in this study. Activated clotting time (ACT), prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer (fibrin degradation product), fibrinogen, protein C and S, and platelet count were measured. Blood samples were collected after induction of anesthesia, 20 minutes after the beginning of CPB, 30 minutes after administration of protamine, and 24 hours after the weaning from CPB. Transfused blood products and mediastinal bleeding were recorded.
Results: Within both groups, D-dimer elevated, and protein C values and platelet count decreased significantly at all samples compared with preoperative values. The changes of ACT, PT, aPTT, D-dimer, fibrinogen, protein C and S and platelet count were similar in both groups. There were no statistically significant differences between both groups with respect to the blood transfusion and mediastinal bleeding.
Conclusions: Acute normovolemic hemodilution in CABG surgery does not significantly decrease blood products requirements and the changes in hemostatic system are not effected by this technique.

Keywords: Coronary artery bypass grafting, hemodilution, blood transfusion, coagulation

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Introduction

During cardiac surgery, the nonbiological surfaces of the extracorporeal circulation and mechanical pumping cause significant changes in the hemostatic system. In addition, use of homologous blood, pump prime fluids, heparinization and reversal with protamine introduce complex changes in hemostasis. These changes include platelet function, coagulation factors, activation of fibrinolytic system and physiologic inhibitors of coagulation [1-6]. Excessive bleeding and homologous blood transfusion due to these hemostatic system changes may constitute significant, but often preventable, complications. There are various techniques in order to avoid homologous blood transfusion, which is a significant factor that also alters hemostatic system [7-9]. Acute normovolemic hemodilution (ANH) and autotransfusion during cardiac surgery is one of these techniques. It was reported that this technique provides benefit in reducing blood loss and the need for blood products in the postoperative period [9-12]. In this study, we investigated the effect of ANH on coagulation and fibrinolytic system during and after cardiopulmonary bypass (CPB) and evaluated physiologic anticoagulants, such as protein C (PC), and protein S (PS) in patients undergoing coronary artery bypass grafting (CABG).

Materials and Methods

Patients

Forty one patients, undergone CABG who did not receive antiagregant, anticoagulant or fibrinolytic therapy, were enrolled in this study. Patients were assigned to acute normovolemic hemodilution group (Group 1; n = 20) or control group (Group 2; n = 21).

Standardized surgical and CPB techniques were used in all patients. Anesthetic induction consisted of fentanyl (5 mg/kg), thiopenthotal (2-3 mg/kg) and vencuronium (0.1 mg/kg) or pancronium for muscular relaxation. Anesthetic maintenance consisted of sevoflurone (%0.5-2) or isoflurone (%0.5-1.5) and additional fentanyl if needed. Before CPB, patients received a loading dose of heparin (300 U/kg) to achieve an activated clotting time (ACT) longer than 450 seconds (Hemochron 80, International Technidyne Corp., Edison N.J.) and additional heparin was given to maintain the ACT over 450 second. Cardiopulmonary bypass was performed by using a membrane oxygenator (Dideco, D708 Simplex III, Mirandola, Italy). Extracorporeal circuit was primed with ringer solution, mannitol, and heparin at a dose of 2500 U.

All patients were cooled to 28-32^{o}C during CPB and were subsequently warmed to a core temperature of 36^{o}C before weaning from the CPB. After CPB, the effect of heparin was reversed with protamine administration in order to return the ACT to preheparin levels. Following induction of anesthesia, in group 1 patients underwent hemodilution by withdrawal of whole blood and simultaneous infusion of equal volume hydroxyethyl starch (HEAS-steril %6, Fresenius Kabi Deutchland GmbH) (HES) solution. The average blood volume withdrawn was 9.26 ± 3.3 mL/body weight. We took account not to decrease hematocrit value below 35%, during determination of withdrawn blood amount. They had retransfusion at the termination of CPB and heparin reversal. Once sternotomy was closed, mediastinal bleeding was recorded hourly. Cell savers were not used in any patients. During the stay of the patients in the intensive care unit, packed red blood cells was given to the patients if hematocrit value decreased below 25%. The shed mediastinal blood through the chest tubes was not retransfused.

Samples and Analytic Method

Blood samples were obtained from intra-arterial catheters and collected in 3% trisodium citrate tubes. The samples were centrifuged at 2000 rpm for 15 minutes and frozen at –70^{o}C until assayed. The samples were collected as follows: after induction of anesthesia (preoperative), 20 minutes after the beginning of CPB (during CPB), 30 minutes after induction of protamine (postprotamine), and 24 hours after the weaning from CPB (postoperative). The samples were assayed for ACT, prothrombin time (PT), activated thromboplastin time (aPTT), D-dimer (fibrin degradation product), fibrinogen, PC, PS, and platelet count.

Statistical Analysis

Results were calculated as the mean value ± the standard deviation. Within each group, changes from baseline values and comparison between values were tested by using Wilcoxon signed-rank test and independent samples one way ANOVA. Mann-Whitney U test was used for comparison between both groups. *P* values □ 0.05 were considered significant.

Table 1. Demographic and operative data.

	Group 1 (n = 20)	Group 2 (n = 21)	*P*
Age (years)	55.07 ± 10.96	58.62 ± 11.53	0.345
Sex (male/female)	16/4	16/5	
Weight (kg)	75.07 ± 11.91	68.38 ± 9.12	0.092
Total heparin (U/kg)	505.19 ± 156.28	612.54 ± 135.41	0.053
Total protamine (U/kg)	581.28 ± 128.16	590.99 ± 108.04	0.698
Cardiopulmonory bypass time (min)	103.29 ± 28.8	112.24 ± 28.4	0.429
Aortic cross-clamp time (min)	65.43 ± 17.75	78.71 ± 24.41	0.113
Bleeding (mL/kg/24 h)	8.23 ± 4.27	11.22 ± 5.7	0.167
Fresh frozen plazma transfusion (mL/kg)	10.18 ± 4.3	12.1 ± 8.18	0.529
Blood transfusion (mL/kg)	6.23 ± 2.68	7.16 ± 4.73	0.614

Table 2. Coagulation parameters.

		Preoperative	During CPB	Post protamine	24 h postop
ACT (sec)	I	144.86 ± 17.24	597.14 ± 77.47 §	134.21 ± 15.41	145.36 ± 24.52
	II	147.86 ± 23.1	629.1 ± 198.97 §	130.1 ± 11.93 †	145.38 ± 23.05
PT (sec)	I	12.3 ± 1.2	120 ± 0 _	16.15 ± 1.62 §	13.24 ± 1.24 *
	II	12.32 ± 1.04	120 ± 0 _	16.23 ± 3.87 §	13.8 ± 1.23 §
APTT (sec)	I	31.19 ± 3.4	120 ± 0 _	49.14 ± 13.8 §	31.92 ± 4.97
	II	33.68 ± 7.01	120 ± 0 _	41.81 ± 12.31	34.18 ± 5.35
D-Dimer (ng/ml)	I	197.79 ± 101.12	236.57 ± 144.16 *	404.53 ± 191.71 §	269.34 ± 70.01 *
	II	232.98 ± 106.66	263.2 ± 120.93 *	520.84 ± 277.26 §	288.98 ± 130.57 †
Fibrinogen (mg/dl)	I	334.04 ± 84.47	160.62 ± 41.51 §	197.18 ± 44.86 §	502.24 ± 190.23 †
	II	389.65 ± 116.05	204.29 ± 104.54 §	239.32 ± 67.49 §	486.96 ± 108.38 †
Protein C (%)	I	110.74 ± 19.79	69.68 ± 16.78 §	61.84 ± 20.42 §	77.68 ± 21.15 §
	II	117.54 ± 25.52	88.11 ± 32.34 §	68.3 ± 18.76 §	82.02 ± 20.48 §
Protein S (%)	I	73.66 ± 25.08	176.02 ± 37.98 §	60.81 ± 24.65 *	68.49 ± 25.9
	II	79.51 ± 20.68	198.95 ± 29.02 §	65.55 ± 19.58 §	63.77 ± 22.96 †
Platelet (103/ml)	I	288.142 ± 66.74	146.785 ± 52.49 §	164.214 ± 44.94 §	177.428 ± 38.95 §
	II	248.428 ± 75.76	137.619 ± 60.73 §	147.095 ± 33.94 §	170.190 ± 90.83 §

ACT= Activated clotting time; aPTT = activated partial thromboplastin time; PT = prothrombin time;
* $p < 0.05$ compared with preoperative value
† p □ 0.01 compared with preoperative value
§ p □ 0.001 compared with preoperative value
_ : the upper limit of machine calculation capacity

Results

Patients

There were no statistically significant differences between both groups with respect to the age, weight, mean total heparin and protamine dose, CPB and aortic cross clamp time, fresh frozen plasma (FFP) and blood transfusion, and mediastinal bleeding (Table 1). Although, it was not found statistically significant, transfused FFP and blood, and mediastinal bleeding were lower in Group 1. There was no excessive mediastinal bleeding and thromboembolic event necessitating additional medical and surgical intervention. Neither of the patients died postoperatively.

Coagulation Parameters

All the coagulation parameters are outlined in the Table 2. The preoperative values of ACT, PT, aPTT, D-dimer, fibrinogen, PC, PS and platelet count in both groups were similar. The changes in the ACT, PT, and aPTT at the other samples point studied were also similar. Activated PTT and PT values were above the upper limit of machine calculation capacity (120 sec) in both groups at the 20 min of CPB.

Within both groups there was statistically significant elevation of D-dimer formation during CPB, at the postprotamine and 24 h postoperative samples with respect to preoperative values. The elevation was highest at the postprotamine sample. Although D-dimer value was lower at this sample in Group 1, it was not found statistically significant. Comparing with the preoperative values, fibrinogen levels significantly decreased during CPB and at the postprotamine sample in both groups. However, there was a significant elevation at 24 h postoperatively. No intergroup differences were observed in the fibrinogen values.

Protein C values were below the preoperative levels in two groups, and this was found statistically significant. There was no significant difference between groups. Protein S values significantly increased during CPB, but decreased at the postprotamine and 24 h postoperative samples in both groups and no intergroup difference was found. Platelet counts decreased significantly in all samples with respect to the preoperative values in both groups. No significant intergroup difference was observed.

Discussion

Despite a normal clotting mechanism before operation, CPB activates coagulation and fibrinolysis, consequently predisposes excessive bleeding and blood product infusion in cardiac surgery [13]. There are a number of problems with homologous blood transfusion. Some of these problems are rised cost, the risk of viral infection, and increased patient mortality and morbidity. On the other hand, there are several autotransfusion techniques to minimize the homologous blood transfusion requirements for open heart surgery [7-9]. Autotransfusion includes any techniques in which the patient's own blood is collected, processed and stored, followed by retransfusion. Acute normovolemic hemodilution, one of these techniques is recommended that reduces homologous blood transfusion requirements [9-12]. In this study, we aimed to investigate whether there were any perioperative blood product requirements, mediastinal bleeding, coagulation and fibrinolytic activity, PC and PS plasma levels difference between patients who underwent CABG with and without acute normovolemic hemodilution.

In this study, we used HES solutions providing a sterile, alternative colloidal fluid to albumin solutions or plasma in the

management of patients who need plasma volume expansion. Although solutions of HES are widely accepted internationally, there is still concern that HES may have adverse effects on hemostasis. However, recent studies showed that HES had minor effect on coagulation and platelet function remained within normal range in patients undergoing major surgery [14-15]. Therefore, we preferred to use HES solution as volume expander.

To inhibit the effect of CPB on coagulation and fibrinolytic system, administration of an anticoagulant agent is essential. Heparin is such an essential anticoagulant agent for CPB. Activated clotting time is used to monitor the level of heparin-induced anticoagulation. In this study, for achieving a suitable ACT level, both groups received similar mean total heparin dose and protamine dose which used for heparin reversal was also similar. No significant intergroup difference was observed according to PT and aPTT values.

D-dimer is the most specific method for determining the fibrinolytic activation [6-16]. Cardiopulmonary bypass-induced fibrinolysis leads to D-dimer formation after reversal of heparin [1-5-17-18]. Although, in the present study, we observed that D-dimer levels elevated significantly at all samples compared with preoperative levels in both groups, the elevation at postprotamine sample was highest in accordance with these reports. D-dimer formation was lower in Group 1 at this sample point, but no significant intergroup difference was found. This finding probably indicates a subclinical consumptive coagulopathic state. Enhanced anticoagulation during CPB results in better preservation of hemostasis, because of the lesser activation of coagulation and consumption [19-21]. Therefore formation of D-dimer is expected to be low in these circumstances. Consistent with these data, in the present study heparin dose or ACT and the changes in D-dimer formation were found to be similar in both groups. It was reported that fibrinolysis could be the primary etiology of excessive bleeding in postoperative period in some patients undergoing CPB [4]. Comunale and associates reported that patients with bleeding had significantly higher D-dimer levels than patients without bleeding [22]. Contrary to this report, Whitten and associates [5] reported that increased D-dimer levels did not predict excessive postoperative bleeding. In this study, D-dimer was not associated with postoperative mediastinal bleeding.

Protein C is an important physiologic anticoagulant that modulate coagulation at the blood-endothelial interphase [1,23]. It was reported that PC decreased in patients undergone CPB [1,24]. In the present study, we observed that PC values, compared with preoperative values, decreased at all intervals studied in both groups and no significant intergroup difference was observed. Protein S is a necessary cofactor for the action of PC. It participates in the formation of a complex containing protein S, activated protein C [23]. We measured the functional activity of PS. During CPB, PS activity increased significantly in two groups. However there was no significant difference between groups. Thus, it can be said that ANH does not alter the PC and PS levels. Cardiopulmonary bypass induces a reversible platelet dysfunction and reduction in platelet count. In the present study, the decrease in platelet counts was similar, and platelet counts also were not affected by ANH.

Although mediastinal bleeding, FFP and homologous blood transfusion were lower in Group 1, no significant intergroup difference was observed. In a study, reported findings support these results [25]. In addition, the similarities of coagulation parameters changes in both groups support similar mediastinal bleeding and blood product transfusion in two groups.

In summary, multiple significant changes in the hemostatic system occurred in both groups, but it was not found statistically significant between groups. Although, the 24 h postoperative mediastinal bleeding, amount of FFP and blood transfusion were lower in acute normovolemic hemodilution patients, no significant difference was observed between both groups. Acute normovolemic hemodilution in CABG surgery does not significantly decrease blood products requirements and the changes in hemostatic system are not effected by this technique.

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