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Levels of Protein C and Protein S, Tissue-Plasminogen Activator, and Fibrinogen During Cardiopulmonary Bypass

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Aim: The exposure of blood to foreign surfaces during cardiopulmonary bypass (CPB) leads to an activation of the coagulation system. The goal of this study was to evaluate the levels of protein C and protein S, tissue-plasminogen activator (t-PA) and fibrinogen before, during and after CPB.

Materials and Methods: Thirty-three patients undergoing elective coronary artery bypass grafting (CABG) and 11 patients undergoing non-cardiac surgery (control group) were included in this study. Blood samples were taken at different time intervals preoperatively, before, during and after CPB, and in the postoperative period. Protein C and protein S, t-PA and fibrinogen levels were measured before, during and after CPB.

Results: Protein C, protein S and fibrinogen levels were decreased during CPB ($p < 0.05$). As a marker of hyperfibrinolysis, levels of t-PA were higher during CPB, which might suggest ongoing subclinical hemostatic activation associated with CABG. Before release of aortic cross clamp, t-PA level had increased eight-fold. t-PA levels remained elevated throughout CPB and into the early postoperative period. Protein C, protein S and fibrinogen levels remained decreased throughout CPB, and the decrease continued until the end of CPB. There was no difference in the hemostatic markers in the control group. There was a statistically significant difference between the groups ($p < 0.01$) before the induction of anesthesia and during CPB for all parameters ($p < 0.01$).

Conclusions: As a marker of hyperfibrinolysis, levels of t-PA were increased during CPB and remained elevated in the early postoperative period. Protein C, protein S and fibrinogen levels were decreased during CPB. These results showed that thrombin generation is increased during CPB.

Key Words: Cardiopulmonary bypass, protein C and protein S systems, tissue plasminogen activator, fibrinogen

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Kardiyopulmoner Bypass Esnasında Protein-C ve Protein-S, Doku Plazminojen Aktivatörü ve Fibrinojen Düzeyleri

Amaç: Kardiyopulmoner bypass (KPB) esnasında kanın yabancı yüzeylerle teması, koagülasyon sisteminin aktive olmasına yol açar. Bu çalışmanın amacı KPB öncesinde, esnasında ve sonrasında protein C (PC) ve protein S (PS), doku plazminojen aktivatörü (t-PA) ve fibrinojen (F) düzeylerini değerlendirmektir.

Yöntem ve gereç: Bu çalışmaya elektif koroner arter bypass cerrahisi (KABC) yapılan 33 hasta ve kardiyak cerrahi yapılmayan 11 hasta (kontrol grubu) alındı. Kan örnekleri ameliyat öncesi, KPB öncesi, esnası ve sonrasında ve ameliyat sonrası olmak üzere farklı zamanlarda alındı. KPB öncesinde, esnasında ve sonrasında PC ve PS, t-PA ve F düzeyleri ölçüldü.

Bulgular: KPB esnasında PC ve PS ve F düzeyleri azaldı ($p < 0.05$). Bir hiperfibrinolizis markeri olan t-PA düzeyleri, KPB esnasında daha yüksekti. Bu durum KABC ile birlikte subklinik hemostatik aktivasyonun geliştiğini gösterebilir. KPB başlangıcında aorttaki kros klemp kaldırılmadan önce t-PA düzeyi 8 kat arttı, KPB esnasında ve ameliyat sonrası erken dönemde ise yüksek kaldı. KPB süresince PC ve PS ve F düzeyleri azaldı ve bu düşüş KPB'in sonuna kadar devam etti. Kontrol grubunda hemostatik markerler arasında anlamlı bir fark yoktu. Tüm parametreler için gruplar arasında anestezi indüksiyonundan önce ve KPB esnasında istatistikî olarak önemli fark vardı ($p < 0.01$).

Sonuç: Bir hiperfibrinolizis markeri olan t-PA düzeyleri, KPB esnasında yüksekti ve ameliyat sonrası erken dönemde de yüksek kaldı. Protein C ve PS ve F düzeyleri KPB süresince azaldı. Bu sonuçlar trombin generasyonunun KPB esnasında arttığını göstermektedir.

Anahtar Sözcükler: Kardiyopulmoner bypass, Protein C ve protein S sistemleri, Doku plazminojen aktivatörü, fibrinojen

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Introduction

Although cardiopulmonary bypass (CPB) is essential for most cardiac surgical procedures, it is not a physiological process because it activates the coagulation system. This is via a number of mechanisms, including alterations in the contact factor pathway, fibrinolysis, platelet function, complement and inflammation. Surgery itself also activates the tissue factor pathway of coagulation (1).

Intraoperative and postoperative bleeding is one of the main complications in patients undergoing CPB for cardiac surgery (1,2). During CPB, the blood is exposed to large foreign surfaces and materials, and flow conditions normally not found in the circulation (2). In addition, use of homologous blood, pump prime fluids and heparinization and reversal with protamine introduce complex changes in hemostasis. All of these factors affect the platelet function, coagulation and the fibrinolytic system (3). A variety of hemostatic changes such as platelet dysfunction, activation of blood coagulation despite anticoagulation with heparin, inactivation of clotting factors either by consumption or by residual effects of heparin after its neutralization by protamine, and hyperfibrinolysis due to an enhanced release of tissue-type plasminogen activator (t-PA) are reported (4-7).

Although routinely administered during CPB, heparin cannot completely prevent the activation of the coagulation cascades with a subsequent increase in thrombin generation (8,9). Prior studies have reported that CPB results in increased levels of active t-PA in blood during CPB. Elevated active t-PA during CPB is associated with an increased rate of fibrinolysis and excessive bleeding (10-14). Despite its central role in the regulation of blood coagulation, little is known about the behavior of the protein C system during CPB. The protein C system primarily consists of the plasma factors: protein C and S and the endothelium-bound thrombomodulin (15,16). Based on epidemiological studies revealing a significant correlation between thrombin activation and fibrinogen plasma levels in patients suffering from coronary artery sclerosis, a hypercoagulable state existing in ischemic heart disease has been postulated (9,17,18).

To assess whether there is a subclinical activation of the hemostatic system associated with open-heart surgery, in vivo molecular markers of hemostatic activation were determined preoperatively, during, and at

the end of CPB in operated patients. Therefore, we aimed to investigate in a randomized in vivo setting the behavior of the protein C and protein S system, t-PA and fibrinogen during CPB in patients with elective coronary artery bypass grafting (CABG).

Materials and Methods

Human Subjects

The study was carried out on 33 patients (27 male, 6 female, range 35-69 years of age) who underwent elective CABG and on 11 patients (7 male, 4 female, range 28-65 years of age) who underwent non-cardiac surgery in Atatürk University Cardiovascular Surgery Department. All the patients were informed and all provided written consent. Patients with bleeding diathesis and impaired renal function were not included in the study group. The control patients were free from arteriosclerosis.

In all patients, CPB was accomplished following general anesthesia, median sternotomy and heparinization. Before cannulation, bovine heparin (3 mg/kg) was administered. The CPB was maintained with a non-pulsatile blood flow with a rate of 2.2 L/m²/min during moderate hypothermia (30° to 32° C). Cardioplegia was achieved with ice-cold crystalloid cardioplegia (Plegisol, Abbot) infused in the ascending aorta after clamping the aorta. The CPB circuit consisted of a venous reservoir (Baxter, Bentley, Irvine, CA, USA), an arterial line filter (Affinity arterial filter), a roller-pump (Sarns 3M, Ann Arbor, Michigan, USA), and a membrane oxygenator (Oxim-II 34 and RV 40 Macchi, Braile Biomedica, Brazil). Heparin was neutralized with protamine sulfate in a 1:1 ratio with the initial heparin dose following completion of CPB.

Blood Sampling and Assays

Blood samples were taken from a central venous line in the preoperative period, after induction of anesthesia, 3 min after onset of CPB, after release of aortic cross-clamp (CC), after completion of CPB, and at the 1st and 24th hours after arrival in the intensive care unit in the study group. Blood samples were taken in the preoperative, operative and postoperative periods in the control group. The samples were collected in tubes containing sodium citrate. For the measurement of parameters, blood was collected in 10 ml tubes containing 1.5 mg/ml ethylenediaminetetraacetate and 50

U/ml aprotinin. The samples were centrifuged at 3,000 rpm for 10 min at 4 °C, and the plasma obtained was stored at -80 °C until assay.

Protein C was measured by enzyme-linked immunosorbent assay (ELISA) (Diagnostic Stago, France). Protein S was measured by ELISA. t-PA was measured by solid phase sandwich ELISA method (Diagnostic Stago, France). A nephelometric method was used to measure the concentration of fibrinogen (Fibriquik® kit) in plasma (Dade Behring); the functional fibrinogen levels were measured using Multifibrin U (Dade Behring).

Statistical Analysis

The statistical software package SPSS 9.0 for Windows (SPSS Inc.) was used for all analyses. Data were expressed as a mean \pm standard error of the mean. Two-way analysis of variance for repeated measures was used for comparisons of variables measured over time between the preoperative and postoperative parameters. Data were further calculated by one-way analyses of variance for each parameter and groups. The results were considered significant if the p value was less than 0.05.

Results

Patient Data

Thirty-three patients who underwent elective CABG were included in the study (27 male, 6 female, range 35-69 years of age). The characteristics of patients and operative parameters are presented in Table 1. There were 11 patients in the control group (7 male, 4 female, range 28-65 years of age) who were free from arteriosclerosis and underwent non-cardiac surgery. The perioperative course of the patients was uneventful. No major complications occurred.

Protein C, Protein S, t-PA and Fibrinogen Levels

Protein C levels decreased after heparinization and start of CPB. They remained low close to the end of CPB, and then rose postoperatively (Figure 1).

Table 1. Patient characteristics and operative parameters.

Age (years)	35-69
Sex (female/male)	6/27
BSA (m ²)	1.67-1.98
Cross-clamp (min)	39-97
CPB time (min)	80-164

BSA: Body-weight surface area. CPB: Cardiopulmonary bypass.

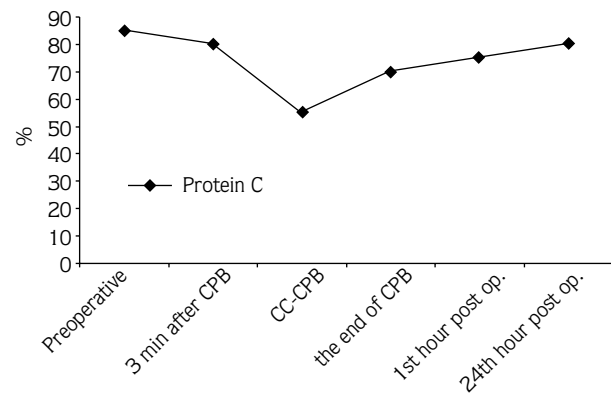


Figure 1. Plasma concentrations of protein C in the patients undergoing CPB surgery.

The course of protein S levels resembled that seen for protein C: a sharp decrease after heparinization and start of CPB, remaining low until the end of CPB, after which protein S began to rise but remained lower compared to baseline. Protein S levels began to increase at the end of CPB and continued to rise in the early postoperative period (Figure 2).

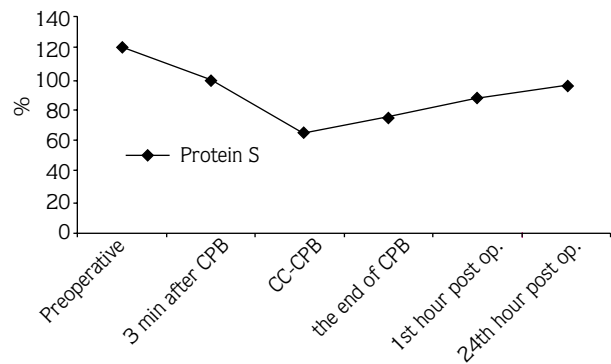


Figure 2. Plasma concentrations of protein S in the patients undergoing CPB surgery.

Within 3 min after starting CPB, t-PA levels began to increase. Before releasing aortic CC, t-PA levels had increased about eight-fold compared to baseline ($p < 0.001$). Peak t-PA levels occurred about 40 min into CPB. At the end of CPB, t-PA levels began to decrease and the decrease continued in the early postoperative period (Figure 3). Postoperative plasma concentrations of t-PA were higher in comparison to preoperative concentrations ($p < 0.05$).

After onset of CPB, fibrinogen levels decreased significantly. On average, fibrinogen levels remained low close to the end of CPB. During the postoperative period, a significant increase was observed in all patients (Figure 4).

The plasma levels of protein C, protein S, t-PA and fibrinogen were compared prior to starting CPB, during CPB, and in the postoperative period. Postoperative plasma levels of t-PA were higher, while postoperative plasma levels of protein C, protein S and fibrinogen were lower in comparison to their preoperative concentrations ($p < 0.01$). During the CPB, an increase in the plasma levels of t-PA was observed, while plasma levels of

protein C, protein S and fibrinogen decreased ($p < 0.05$, Figures 1-4).

Hematocrit (Htc) levels were 20 to 25% used during CPB, while they were normal in the control group. No differences were observed in hemostatic markers in the control group. There were statistically significant differences between the groups ($p < 0.01$) before the induction of anesthesia and during CPB for all parameters ($p < 0.01$).

Discussion

Despite extensive investigation, the underlying basis of hemorrhagic and thrombotic diatheses associated with CPB is not well characterized. Defective hemostasis during and following CPB is most likely caused by a combination of multiple factors, including a platelet function defect, thrombocytopenia, activation of coagulation, and a fibrinolytic state (1-3,6,7,12,13,19,20).

Fibrinolytic activity increases significantly during and following CPB and contributes to the hemostatic defect causing increased postoperative blood loss (2,13,20). This results from activation of fibrinolysis by systemic high-dose heparin as well as inhibition of α_2 -antiplasmin (an inhibitor of fibrinolysis) and the release of t-PA due to kallikrein production with the commencement of CPB (1).

A variety of hemostatic changes such as hyperfibrinolysis are due to an enhanced release of t-PA (6,7,10,14). Augmented fibrinolytic activity during CPB is related to the increased levels of t-PA (21). This situation is related to the high bleeding tendency.

The natural anticoagulant system, which is activated as a consequence of thrombin generation, represents the thrombomodulin-protein C system. Protein C, which modulates coagulation at the blood-endothelial interphase, is an important physiologic anticoagulant. Protein S is a necessary cofactor for the action of protein C. It participates in the formation of a complex containing protein S and activated protein C (3,5,7,9,15-18,22). A decrease in protein C and protein S levels with a subsequent rise in soluble thrombomodulin during CPB was described previously (3,9). In the present study, we observed that protein C and protein S levels decreased at all intervals studied during CPB. These results suggest that despite heparin administration during CPB, the

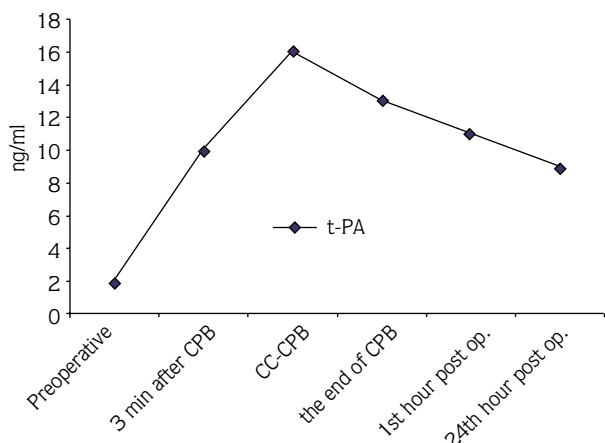


Figure 3. Plasma concentrations of tissue-plasminogen activator (t-PA) in the patients undergoing CPB surgery.

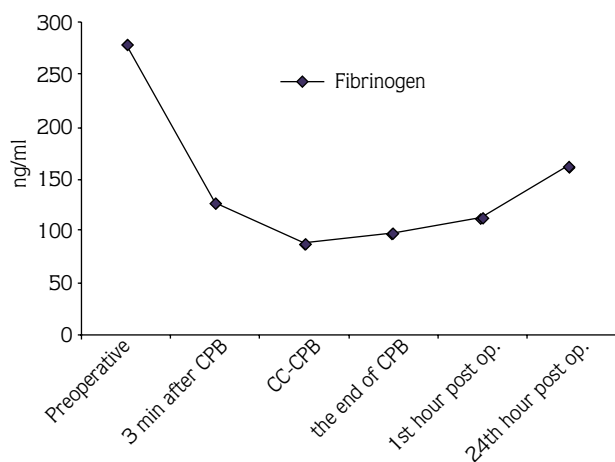


Figure 4. Plasma concentrations of fibrinogen in the patients undergoing CPB surgery.

protein C and protein S system is directly or indirectly activated by thrombin. Circulating thrombin rapidly binds to thrombomodulin expressed on the endothelial surface and enhances protein C activation more than 1000-fold (3,5,9,15). Maruyama (18) have shown that activated protein C not only inactivates clotting factors Va and VIIIa, but may also be involved in the induction of fibrinolysis.

This study was performed to elucidate the patterns of protein C, protein S, t-PA and fibrinogen activation during CPB in patients with CABG. The protein C, protein S, t-PA and fibrinogen levels were significantly different between the groups. Since Htc levels were 20 to 25% used during CPB, most of the reduction in the hemostatic markers appears to be due to dilution. However, hemostatic markers continued to differ at the end of CPB and into the postoperative period, while Htc levels were normal.

At the onset of CPB, we found sharp decreases in protein C and protein S levels, which persisted through the remainder of the procedure. These findings are in accordance with the results of Speekenbrink and colleagues (15). Protein S, protein C and fibrinogen values during the CPB showed an important decrease, as in other studies (3,5,7,9,21). Protein C showed its effect by rapidly removing the present thrombin from blood circulation (23). During the CPB, protein C plasma level showed an important decrease and this had an important role in the activation of the blood coagulation mechanism. t-PA levels were increased mildly during early surgery prior to the initiation of CPB, and then increased eight-fold during CPB, while protein S, protein C and fibrinogen levels remained low. t-PA levels remained elevated throughout the remainder of CPB, resulting in continued high levels of active t-PA. For the remainder of CPB and in the postoperative period, t-PA remained at high levels. Numerous studies have shown that t-PA values are increased during CPB (2,10,14,24), as in our study.

The cause of the increase in t-PA secretion during CPB is unknown. A variety of factors can stimulate t-PA secretion in vivo and in vitro including thrombin, bradykinin, epinephrine and vasopressin. Numerous studies have shown that thrombin generation is increased during CPB, and it has been suggested that increased t-PA secretion and hyperfibrinolysis during CPB might in part be due to increased thrombin generation (2,10,14,24,25).

While the early increase in t-PA and thrombin generation may be related, the continued high secretion of t-PA during the postoperative period appears to be due to some other mechanisms. The immediate early increase in t-PA levels may be due to stimulated release of stored t-PA from the endothelium, with the sustained increase in secretion due to an acute-phase increase in t-PA production (14).

In the Northwick Park Heart Study, a strong association between plasma levels of fibrinogen and clotting factors VII and VIII and the risk of coronary sclerosis was demonstrated. A significant correlation between thrombin activation and fibrinogen plasma levels was established (9,17,18). In accordance with these results, coronary artery bypass patients in our study demonstrated significantly lower fibrinogen levels during CPB.

In our study, t-PA levels after induction were significantly higher in the CABG group and remained at a higher level throughout the entire investigation period (9,26), and fibrinogen levels were markedly reduced. These changes reflect endothelial dysfunction in arteriosclerosis associated with impaired anticoagulant properties contributing to the hypercoagulable state observed in coronary artery disease.

The values of all parameters in this study showed a statistical difference ($p < 0.05$, Figures 1-4) compared to baseline. These results suggest that activation of coagulation was prior to the activation of fibrinolysis. Teufelsbauer and colleagues (2) concluded that patients undergoing CABG may be at a special risk for thromboembolic complications due to increased thrombin generation during CPB leading to hypercoagulability in the early postoperative period, which may be complicated by reactive hyperfibrinolysis.

Coronary artery bypass patients as well are threatened by thromboembolic complications such as myocardial infarction and stroke, which can partly be explained by the pre-existing hemostatic changes and an increased procoagulant activity in the perioperative period. Furthermore, based on the underlying disease, coronary artery bypass patients showed a higher degree of endothelial damage contributing to the impaired anticoagulant properties of the endothelium. A number of different techniques, including heparin-coated bypass circuits, off-pump heart surgery and drugs such as aprotinin and ϵ -amino caproic acid, may be used to

suppress enhanced fibrinolysis and reduce the inflammatory state associated with CPB.

In conclusion, the results from this study provide evidence for activation of the protein C and protein S system, t-PA and fibrinogen during CPB. Use of various

hemostatic markers to estimate the underlying rate of processes in vivo may be used to evaluate the effect of these activations in future studies. The effect of CPB on hemostasis requires further investigations.

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