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Complement (C3, C4) and C-reactive Protein Responses to Cardiopulmonary Bypass and Protamine Administration

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SUMMARY

Complement activation has been deemed responsible for the damaging effects of cardiopulmonary bypass (CPB) in patients undergoing open heart surgery. We studied C3, C4 and C-reactive protein (CRP) in 22 patients undergoing CPB. In Group 1 (11 patients), protamine was given intravenously and in Group 2 (11 patients), via the aortic root after CPB. Significant decreases were observed in C3 and C4 during CPB in both groups indicating complement activation primarily by the classic pathway. Protamine did not lead to further activation of the complement system. In both groups, C3 levels gradually returned toward baseline within 24 hours but C4 levels were still lower than baseline 24 hours postoperatively. CPB and protamine administration did not cause any significant changes in CRP levels, but CRP increased abruptly 24 hours after operation. Although activation of complement system during CPB is expected to invoke an acute phase response, we conclude that this period is not long enough to induce an increased production of CRP in response to tissue injury or inflammation.

Key Words: SURGERY, CARDIOVASCULAR, BYPASS: protamine, complement activation, C-reactive protein

The complement system which is the primary humoral mediator of antigen-antibody reactions, plays an important role in host defence and inflammation.[1] However, rapid and excessive activation of this system may cause tissue damage,[2,3] increased vascular permeability,[4] and also play a role in pathogenesis of multiple organ failure by inducing granulocyte aggregation.[5,6]

It has been reported that cardiopulmonary bypass (CPB) activates the complement system,[7-14] and this activation in turn may contribute to the development of the post-perfusion syndrome.[8,13,14] On the other hand, there are some studies indicating that protamine and in particular heparin-protamine complex may activate the complement system.[15-19] However, a recent study by Chiu and Samson[11] did not show complement activation after protamine administration. To our knowledge the effects of CPB on C-reactive protein (CRP) have not been studied. Siegel et al.[20] suggested that a protamine-CRP interaction may activate complement by the classic pathway.

In the present study, we assayed C3, C4, and CRP in 22 patients undergoing CPB to determine whether CPB and intra-aortic or intravenous administration of protamine activates the complement system and whether CRP might influence complement activation. We also investigated whether there is a correlation between C3 and C4 levels and postoperative subsystem dysfunction.

PATIENTS AND METHODS

This study was approved and supported by the Research Foundation of Ankara University and informed consent was obtained from each patient one day before operation.

Twenty-two consecutive patients undergoing coronary artery or valvular surgery with standard anaesthetic, operative and bypass procedures were studied. All patients had normal hepatic and renal function. Patients with preoperative anaemia, severe asthma or significant pulmonary disease and a history of recent steroid use were excluded from the study. The patients were divided into two groups of 11 patients. Patients in Group 1 received protamine intravenously at the usual interval (5 to 10 minutes) after CPB. Patients in Group 2 received protamine through the aortic root for reversal of heparin after CPB. Demographic data are presented in Table 1.

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TABLE 1
Demographic data of the patients

	Intravenous protamine (n:11)	Intra-aortic protamine (n:11)
Male to female ratio	6:5	5:6
Height (cm)	163.8 (9.4)	161.9 (8.7)
Weight (kg)	62.8 (13.4)	61.4 (13.0)
Age (yr)	33.5 (11.9)	38.0 (12.0)
Body surface area (m^2)	1.7 (0.2)	1.64 (0.2)
Cross-clamp time (min)	57.2 (16.5)	65.3 (39.0)
Bypass time (min)	81.7 (18.9)	91.3 (37.8)
Cardiac index ($l/min/m^2$)		
>2	10	9
1.5-2	1	2

All values except male to female ratio and cardiac index are mean (SD).

All patients received bovine lung heparin (350 units/kg of body weight) before institution of CPB to achieve an activated clotting time (ACT) greater than 450 seconds. ACT was periodically repeated during CPB and additional doses of heparin were administered as needed to keep ACT in this range. CPB equipment consisted of a Sarns roller pump, a Shiley cardiotomy reservoir, a Bentley BOS-10 bubble oxygenator, and a Bentley P-F 427 arterial filter. The CPB circuit was primed with 2000 ml of Ringer's lactate solution containing 5000 units of bovine lung heparin. An initial pump flow rate 2.1 to 2.5 litres per minute per square metre and moderate systemic hypothermia of 27°C to 30°C were employed. Myocardial protection was achieved with cold cardioplegic solution. None of the patients received steroids and whole blood was not added to the priming mixture in this study. After weaning from CPB, protamine was administered as 1.3 times the amount of residual heparin determined by ACT.

Protocol

Blood samples for analysis of C3, C4 and CRP were drawn at the following intervals: (0) before sternotomy and heparin administration (pre-CPB; baseline); (1) 20 minutes after CPB was commenced; (2) after weaning from CPB but prior to protamine administration; (3) 10 minutes after completion of a neutralising dose of protamine; (4) at skin closure; (5) 24 hours after operation.

Blood samples were drawn from an intra-arterial catheter (before and after protamine administration, at skin closure, and 24 hours after operation) and the right atrial catheter (during CPB). The samples were centrifuged within 30 minutes of collection and the plasma stored at −70°C until assay. The concentrations of C3, C4 and CRP were determined by rate immunonephelometry (automated immunochemistry system, Beckman Instrument Co, Fullerton, CA). Results were corrected for haemodilution occuring during the operation according to the following forumula:[16]

Corrected concentration = sample concentration x (initial haemoglobin/ sample haemoglobin).

The classic pathway was considered to be activated when there was a decrease in both C3 and C4 or only in C4 level. On the other hand, the alternative pathway was considered to be activated if C3 was decreased and C4 was in the normal range.

In this study we also measured total white blood cell (WBC) counts by automated counting methods and WBC differential counts in five patients of each group before sternotomy and heparinisation (baseline) and after establishment of pulmonary circulation during partial bypass. After establishment of pulmonary circulation blood samples were drawn from right and left atria to assess transpulmonary leukocyte sequestration.

Transpulmonary leukocyte sequestration was expressed as median cell difference (MCD) as previously described.[7]

MCD = WBC count in right atrium − WBC count in left atrium.

Differential counts were obtained from May-Grunewald/Giemsa stained smears. WBC counts and differential counts were also corrected for haemodilution as described above.

In all patients clinical and laboratory parameters including cardiac index (CI) measured by thermodilution method, arterial blood gas analysis, the concentration of serum creatinine, bilirubin, ALT, AST, presence or absence of inotropic support, weaning time from mechanical ventilation and urine flow were evaluated 24 hours postoperatively, and also on the seventh postoperative day (except CI) for subsystem functions.

Statistical analysis was performed using two-way repeated measures analysis of variance (ANOVA) and Newman-Keuls test for multiple comparisons. A P value of <0.05 was accepted as significant. The results are expressed as mean ± SD.

RESULTS

Patients in both groups were similar in terms of demographic data (age, height, weight, sex distribution, body surface area, cross-clamp time, bypass time) (Table 1).

Plasma C3 and C4 levels corrected for haemodilution at various periods of the study are shown in Figures 1 and 2. In both groups of patients the baseline blood

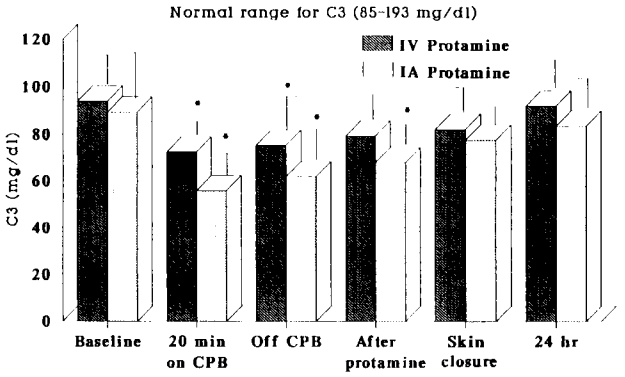


FIGURE 1: Plasma C3 levels. Abbreviations: IV, intravenous; IA, intra-aortic. Values are mean ± SD. *$P<0.05$ vs baseline.

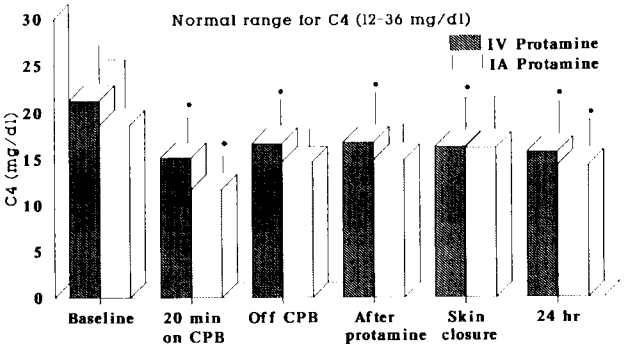


FIGURE 2: Plasma C4 levels. Abbreviations: IV, intravenous; IA, intra-aortic. Values are mean ± SD. *$P<0.05$ vs baseline.

samples revealed no complement activation and there was no statistically significant difference between groups. CPB was associated with a marked activation of the complement system in both groups. C3 and C4 were significantly reduced at 20 minutes of CPB when compared with baseline values in both groups ($P<0.05$). Compared with the levels after 20 minutes of CPB, C3 and C4 tended to increase both with the termination of CPB and after protamine administration. C3 levels were still lower than baseline values in both groups while C4 level was only significantly lower in Group 1 ($P<0.05$).

Intravenous or intra-aortic administration of protamine after CPB was not associated with further activation of the complement system. In both groups, C3 levels gradually returned toward baseline within 24 hours while C4 levels remained low ($P<0.05$) (Figure 2). This result indicates that the classic pathway of the complement system is activated 24 hours after operation.

As expected the institution of CPB was associated with rapid haemodilution with a significant decrease in haemoglobin concentration.

The levels of CRP in both groups are illustrated in Figure 3. Although CRP levels tended to remain below baseline values in both groups at 20 minutes of CPB, before and after protamine administration, and at skin closure, the decreases were not statistically significant. CRP levels were not affected significantly by intravenous or intra-aortic administration of protamine. CRP increased abruptly 24 hours after operation in both groups ($P<0.05$) and the increase was significantly greater in Group 2 than Group 1 ($P<0.05$).

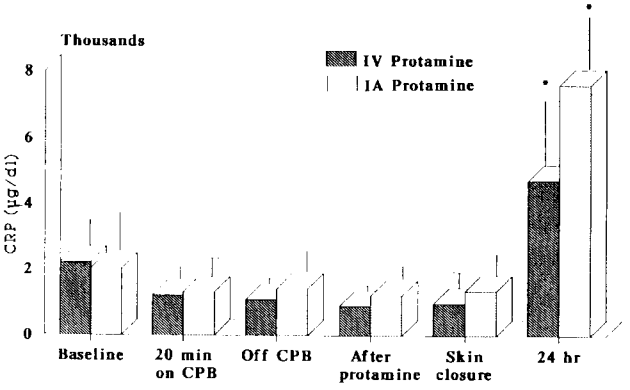


FIGURE 3: CRP levels. Abbreviations: IV, intravenous; IA, intra-aortic. Values are mean ± SD. *$P<0.05$ vs baseline.

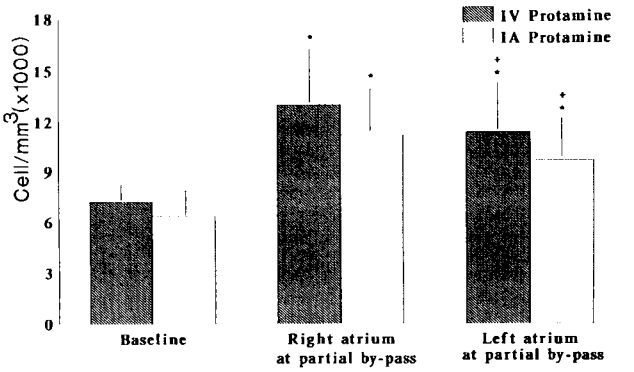


FIGURE 4: Changes in total white blood cell counts during study periods. Abbreviations: IV, intravenous; IA, intra-aortic. Values are mean ± SD. *$P<0.05$ vs baseline, +$P<0.05$ vs right atrium.

Right atrial blood samples taken at partial bypass revealed statistically significant increases in the total WBC counts compared with baseline values in both groups ($P<0.05$) (Figure 4). This leukocytosis was primarily due to an increase in PMNs. Establishment of pulmonary circulation at partial bypass resulted in marked transpulmonary leukocyte sequestration in Group 1 (MCD:1632 cells/mm^3) and Group 2 (MCD:1479 cells/mm^3) (Figure 5). MCD did not differ significantly in the groups.

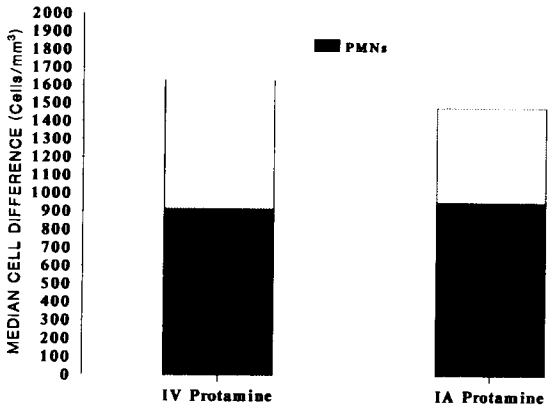


FIGURE 5: Transpulmonary leukocyte sequestration after establishment of pulmonary circulation at partial bypass. Abbreviations: IV, intravenous; IA, intra-aortic; PMNs, polymorphonuclear leucocytes. Transpulmonary leukocyte sequestration was expressed as median cell difference (MCD). (MCD = WBC count in the right atrium − WBC count in the left atrium.)

Postoperative subsystem functions were similar in both groups (Table 2). In Group 1, one patient required re-operation within 24 hours because of excessive bleeding and one patient died with low cardiac output syndrome on day 5 postoperatively. In Group 2, one patient required re-operation for bleeding.

TABLE 2
Postoperative subsystem functions

Function	Intravenous protamine (n:11)		Intra-aortic protamine (n:11)	
	24 hr p.o.	7th day p.o.	24 hr p.o.	7th day p.o.
Cardiac index (l/min/m²)				
>2	11	-	9	-
1.5-2	0	-	2	-
Inotropic support				
Yes*	1	0	2	0
No	10	10	9	11
Weaning from mechanical ventilation and extubation				
<24 hr	11	-	11	-
>24 hr	0	-	0	-
Hepatic				
Normal	11	10	11	11
Abnormal†	0	0	0	0
Renal				
Normal	11	10	11	11
Abnormal‡	0	0	0	0

* The administration of dopamine, dobutamine or epinephrine + calcium.
† Serum bilirubin level >2.0 mg/ml and elevation of SGOT and SGPT levels twice normal values.
‡ Serum creatinine level >2.0 mg/100 ml.

DISCUSSION

The present study demonstrates that the complement cascade is activated during CPB. Significant decreases in C3 and C4 during CPB indicate complement activation by the classic pathway. In fact as the levels of a given component reflect the balance between synthesis and metabolism, attempts to determine the exact complement pathway which is activated may be difficult. Classic pathway activation itself in turn may lead to alternative pathway activation.[1] Therefore it is likely that the decrease in C3 and C4 may indicate activation of both pathways. There are some studies[7,8,13] demonstrating pulmonary PMNs sequestration following CPB via the alternative pathway. In the present study, the pulmonary sequestration of PMNs seen in five patients of each group after establishment of pulmonary circulation at partial bypass may also indicate alternative pathway activation (C5a activation).

Our results are consistent with previous studies indicating that the complement system is activated during CPB.[7-13] Although it is currently accepted that the complement system may be activated during bypass, the involved pathway is controversial. In addition, the exact pathophysiological mechanisms responsible for this activation are still unknown. However, complement activation has been noted in patients undergoing renal dialysis with dialyzers equipped with cellophane membranes[21] and in patients undergoing nylon-fibre filtration leukapheresis.[22] During CPB synthetic material introduced into the blood stream, the contact of the blood with nylon, plastic, cellophane or the tubing of extracorporeal circuit as well as vigorous oxygenation of blood may cause complement activation.[7,8,14] Recent evidence indicates that factors other than extracorporeal circulation such as hypoperfusion, tissue injury, ischemia, and the aortic cross clamping itself may also activate complement during major operations.[3,23,24] Another possibility is the activation of the complement system by the heparin-protamine complex. The heparin-protamine complex has been shown previously to activate complement in man and an in vitro system.[15,17] Complement activation by the heparin-protamine complex has also been reported in a patient who had a severe anaphylactoid reaction following cardiac catheterisation.[18] In the present study, C3 and C4 levels did not change after intravenous or intra-aortic administration of protamine. This observation is consistent with the results of Chiu and Samson[11] suggesting that slow intravenous infusion of protamine after CPB does not lead to further consumption of C3 and C4. Protamine administration was also performed at a slow rate in our study.

Siegel et al[20] showed that the addition of protamine to sera from individuals with acute inflammatory diseases resulted in activation of the classic pathway while the addition of protamine to normal sera did not activate the complement system. They also observed a significant correlation between CRP levels and the amount of protamine-induced complement consumption. Therefore they concluded that CRP is considered to be an agent which can lead to "nonimmune" activation of the classic complement pathway.

CRP, the classical acute phase protein, is a potent activator of the classic complement pathway.[20,25,26] Its circulating concentration increases dramatically in response to tissue injury, inflammation and a wide variety of infectious processes.[27-30] In our study, no immediate increase of CRP levels was seen after the institution of CPB despite complement activation. We hypothesise that this period is not long enough to induce an increased production of CRP in response to tissue injury or inflammation. However, CRP levels increased dramatically 24 hours after operation while complement activation was evident with low C4 levels. These results are consistent with a previous study reporting increased CRP levels within six hours of major surgery and peaking 48 hours postoperatively.[31] In addition, our findings support the hypothesis of Kirklin et al[14] that the damaging effect of CPB may be the result of the inflammatory reaction and complement activation. The decreases in the levels of C4 observed 24 hours after operation may be related to a whole-body inflammatory reaction, the transfusion of stored blood deficient in complement or other unknown phenomena. Whole-body inflammatory reaction and complement activation may cause subsystem dysfunctions after CPB.[14] Significant subsystem dysfunctions were not observed at 24 hours nor on the seventh postoperative day in our small number of patients. It is possible that the degree of complement activation observed in this study is not sufficient to produce subsystem dysfunction. However, rapid and excessive activation of the complement system may cause subsystem dysfunction.

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